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# **Supporting Information**

# Negative Correlation between Water Content and Protein Adsorption on Polymer Brushes

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#### 1. Synthesis of Thiol-Terminated PMPC, PHEMA, and PHFBMA.

Dithioester terminated poly(2-(methacryloyloxy)ethyl phosphorylcholine) (PMPC), poly(2-hydroxyethyl methacrylate) (PHEMA,) and poly (2,2,3,4,4,4-hexafluorobutyl) methacrylate (PHFBMA) were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization at 70 °C, using ACVA as the initiator and CTP as the chain transfer agent. In a typical protocol, MPC (1.0 g, 3.4 mmol), CTP (0.015 g, 0.05 mmol), and ACVA (0.0075 g, 0.026 mmol) were dissolved in 3 mL of methanol in a 5 mL Schlenk tube. HEMA (2.0 g, 15.4 mmol), CTP (0.017 g, 0.06 mmol), and ACVA (0.0017 g, 0.006 mmol) were dissolved in 5 mL of N, N-dimethylformamide in a 10 mL Schlenk tube. HFBMA (5.0 g, 20.0 mmol), CTP (0.028 g, 0.10 mmol), and AIBN (0.0050 g, 0.026 mmol) were dissolved in 3 mL of methylbenzene in a 5 mL Schlenk tube. The solution was then degassed via three freeze-vacuum-thaw cycles and placed in an oil bath at 70 °C for 11 h. The polymer was obtained by precipitation in a large quantity of acetone (for PMPC), or hexane (for PHFBMA). The PHEMA polymer was obtained by dialyzed. Dithioester terminated PMPC and PHEMA methanol solutions (20 mL, 1.5 mg mL<sup>-1</sup>) were mixed with 2.0 mL of NaBH<sub>4</sub> methanol solution (1.0 M). The mixture was stirred at room temperature for 5 days so that the end groups of the polymer chains were completely reduced into thiols. For PHFBMA, similar reaction was conducted except methylbenzene used as solution. The molecular weight and polydispersity were measured by gel permeation chromatography (GPC) with aqueous eluent (for PMPC and PHEMA) or tetrahydrofuran eluent (for PHFBMA).

# 2. Characterization of PNIPAM Polymer Brush Surfaces.

PNIPAM polymer brush surfaces were prepared by surface-initiated atom transfer radical polymerization. The static contact angle of water in air and the surface elemental composition of the polymer brush surface was listed in Table S1. The significant contents of the C, N, O, elements on the gold surfaces confirm the formation of PNIPAM polymer layers on the gold chips. The decease of the static water contact angle of PNIPAM polymer brush surface compared to bare-Au chip further confirms the PNIPAM polymer layers on the gold chips.

Samplas	contact angle	Relativ	e molar	e molar ratio of surface element (mol %)					
Samples	(deg)	Au 4f	C 1s	O 1s	N 1s	Р 2р	F 1s		
Bare Au	87 ±1	72.57	20.78	6.66	0	0	0		
Au graft by PNIPAM	$58 \pm 1$	16.87	60.5	10.92	11.71	0	0		

Table S1 Static water contact angle and surface elemental composition of polymer brush surface

2-Bromoisobutyrate was adopted as the "sacrificial initiator". Free initiator was introduced into

the reacting vessel together with the substrates pre-coated with initiator monolayers. Characterization of brush thickness and molecular weight of PNIPAM in solution was conducted with ellipsometer and gel permeation chromatography (GPC), respectively. The grafting density  $\sigma$  was calculated from the equation  $\sigma = N_A h \rho / M_n$ ,<sup>1</sup> where  $N_A$  is the Avogadro's number, *h* the dry thickness of brushes,  $\rho$ the density of polymer (the density of PNIPAM was taken as 1.76 g cm<sup>-3.2</sup>), and  $M_n$  the number-average molecular weight of the polymers. The results are shown in Table S2. As expected, the thickness and the graft density of the PNIPAM are well controlled by tuning the ratio between SAM-Br and SAM-CH<sub>3</sub>: an increase of the thickness and the graft density of the polymer brushes was observed with the increase of the SAM-Br content. The smallest graft density studied is 0.12 chains nm<sup>-2</sup>, corresponding to an average inter-chain distance of 2.91 nm. Obviously, this inter-chain distance is smaller to the value of root-mean square end-to-end distance calculated by freely rotating chain model of the grafted polymer (5.50 nm), indicating the formation dense polymer brushes on the gold surfaces.

Samplag	Effective initiator ratio	$\mathbf{M}_{\mathbf{n}}$	Dry thickness	Graft density	$S = \sigma^{-0.5}$
Samples	(concentration %)	(g mol <sup>-1</sup> )	(nm)	(chains nm <sup>-2</sup> )	( <b>nm</b> )
PNIPAM20	20%		$4.03 \pm 0.9$	0.12	2.91
PNIPAM40	40%		$5.90 \pm 0.8$	0.17	2.40
PNIPAM60	60%	36,100	$6.99 \pm 0.9$	0.21	2.21
PNIPAM80	80%		$9.45~\pm1.1$	0.28	1.90
PNIPAM100	100%		$15.73 \pm 0.8$	0.46	1.47

Table S2 Parameters of the PNIPAM polymer brushes

The AFM images of PNIPAM brushes are shown in Figure S1. The results indicated the formation of uniform distribution of the PNIPAM polymer chains on the gold surfaces and a low surface roughness: the value of random mean square roughness in a range of  $5 \times 5 \ \mu\text{m}^2$  smaller than 4.9 nm.



Figure S1 AFM images of PNIPAM brushes with different graft density. The AFM images were obtained at 25  $^{\circ}$ C. The values of random mean square roughness were marked on the images.

#### 3. Characterization of PMPC, PEG, PHEMA, and PHFBMA coated Surfaces.

Chemical composition and hydrophilicity of the polymer layer were characterized by XPS spectroscopy and water contact angle measurement, respectively. As shown in Table S3, the existence of C, N, O, S, P, and F elements on gold surfaces confirm the PMPC, PEG, PHEMA, and PHFBMA polymer layers. The changes of the water static contact angle after coating further confirm the formation of the polymer layers.

Samples	contact angle	Relative molar ratio of surface element (mol %)						
	(deg)	Au 4f	C 1s	O 1s	N 1s	P 2p	F 1s	
Bare-Au	87±1	72.57	20.78	6.66	0	0	0	
Au-graft to PMPC	14±1	30.37	35.9	28.14	2.13	3.46	0	
Au-graft to PEG	35±1	32.49	38.93	28.58	0	0	0	
Au-graft to PHEMA	53±2	20.12	58.3	21.58	0	0	0	
Au-graft to PHFBMA	101±1	28.54	20.67	14.39	0	0	36.4	

Table S3 S	Static water of	contact angle a	ind surface e	elemental	compositio	n of po	lymer	brush s	urface
		U			1		2		

The AFM images of PMPC, PEG, PHEMA, and PHFBMA coated surfaces are shown in Figure S2. The results indicated the uniformity the PEG-, PHEMA-, and PHFBMA-coated surfaces on gold with a low surface roughness: the value of random mean square roughness in a range of  $5 \times 5 \ \mu m^2$  smaller than 2.31. For PMPC-coated surfaces, the substrate's surface cannot be fully covered by

polymer chains due to the very low graft density, and therefore the surface roughness is rather large (2.31) compared to its small thickness (0.83 nm).



**Figure S2** AFM images of PMPC, PEG, PHEMA, and PHFBMA coated Surfaces. The images were obtained at 25  $^{\circ}$ C. The values of random mean square roughness were marked on the images.



4. Adsorption amounts of BSA onto PNIPAM brushes measured by SPR

**Figure S3** (a) Typical BSA adsorption curves on PNIPAM brushes (0.46 chains  $nm^{-2}$ ) measured by SPR. (b) Adsorption amounts of BSA onto different PNIPAM at various temperature measured by SPR. N.D. means the adsorption of protein is undetectable.

### 5. Hydrodynamic radius of BSA protein at different temperatures measured by DLS



**Figure S4** The distribution (a) and its variation with temperature (b) of hydrodynamic radius of BSA protein, measured by dynamic light scattering.

# References

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