Supporting Information

Integrating thermoresponsive copolymer with host-guest interactions for fabricating molecular recognition surfaces

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Results and discussions of the complexation of CD(mannose) onto poly(NIPAAm-co-Ada) surfaces

The successful surface modification by poly(NIPAAm-*co*-Ada) with different feed ratios were confirmed by transmittance FTIR spectra (**Fig. S1**) and the shift of its wettability transition behaviors (**Fig. S2**). After complexation with CD(mannose), for copolymer surface of 4.76% Ada feed ratio, the surface hydrophilicity and the LCST of polymer increased (**Fig. S2)**; The phase images characterized by AFM showed that larger domains were found connected and the R_q increased (**Fig. S3, e**), whilst surface before modification was homogeneous (**Fig. S3, d)**; In addition, the increase of both the carbon and oxygen content due to the incorporation of CD(mannose) was confirmed by XPS analysis (**Table S1)**. These results demonstrated the successful inclusion of CD(mannose) on poly(NIPAAm-*co*-Ada) layer. It should be noted that for copolymer surface of low Ada feed ratio, *i.e.* 1.64% Ada, the changes in surface property after CD(mannose) integration were not clearly detected by these techniques **(Fig. S2, S3, and Table S1)**. Nevertheless, the specific protein ConA was selectively adsorbed on CD(mannose)-complexed surfaces where the ConA adsorption was about five times higher than that on copolymer surfaces without mannose **(Fig. S4)**, indicating the successful inclusion of CD(mannose) on copolymer surfaces with low Ada feed ratios.

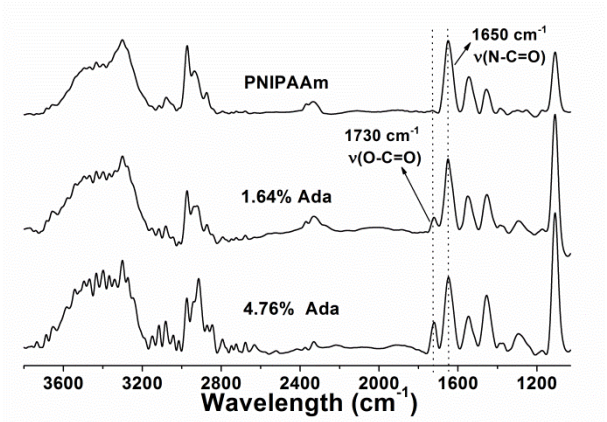

Fig. S1 Transmittance FTIR spectra of poly(NIPAAm-*co*-Ada) surfaces with different Ada feed ratios. The dotted lines referred to the ester group (1730 cm^{-1}) and amide I group (1650 cm^{-1}), which belongs to Ada group and NIPAAm group, respectively.

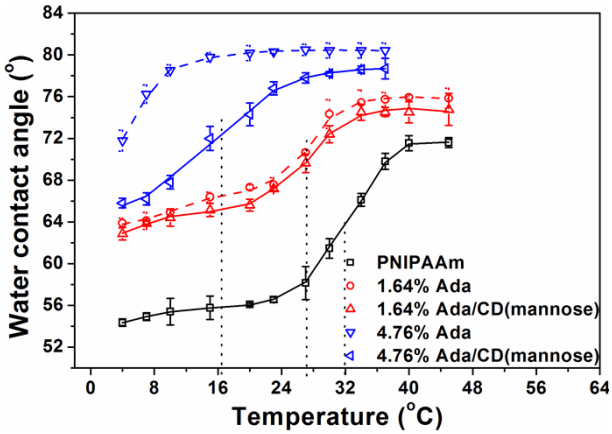

Fig. S2 Wettability transition behaviors of poly(NIPAAm-*co*-Ada) surfaces with different Ada feed ratios before (solid line) and after (dashed line) complexation with CD(mannose). The dotted vertical lines indicated the LCST. Data are means ± standard error (n = 3).

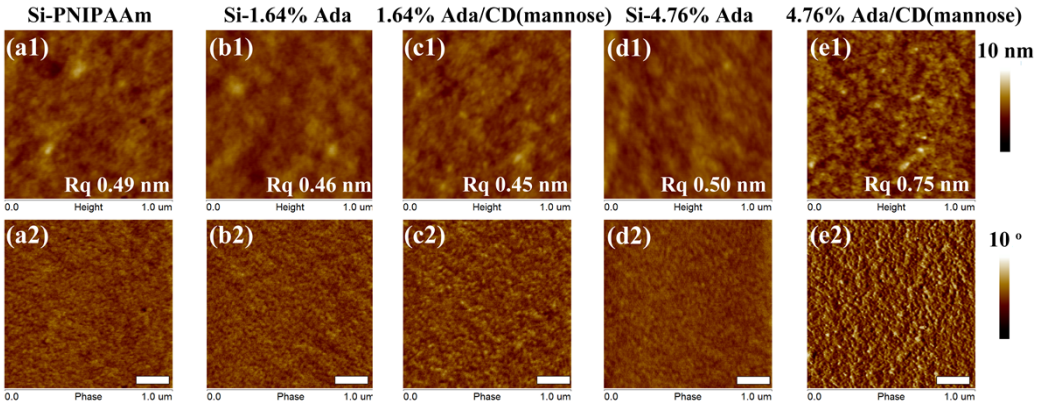

Fig. S3 AFM height (1) and phase (2) images of Si-poly(NIPAAm-*co*-Ada) with different Ada feed ratios before (b, d) and after (c, e) complexation with CDm. (a) Si-PNIPAAm. Scale bar is 200 nm.

Table S1 Element ratios on amino- and initiator-modified silicon surfaces, Si-PNIPAAm surface and Si-poly(NIPAAm-*co*-Ada) surfaces before and after complexation with CD(mannose) obtained by XPS (90° takeoff angle).

Surfaces	C (%)	O(%)	N (%)	Cl (%)	Si (%)	C/O/N
Si-NH_2	26.56	31.36	2.90	0.00	39.16	-
Si-Cl	21.76	28.68	2.38	2.70	44.46	-
PNIPAAm	70.19	13.04	11.09	0.33	5.34	6.33:1.18:1.00
1.64% Ada	70.22	13.36	10.60	0.34	5.50	6.62:1.26:1.00
1.64% Ada/CD(mannose)	69.44	13.34	10.56	0.44	6.20	6.58:1.26:1.00
4.76% Ada	64.06	14.22	9.70	0.64	11.38	6.60:1.47:1.00
4.76% Ada/CD(mannose)	67.36	16.06	8.86	0.48	7.24	7.60:1.81:1.00

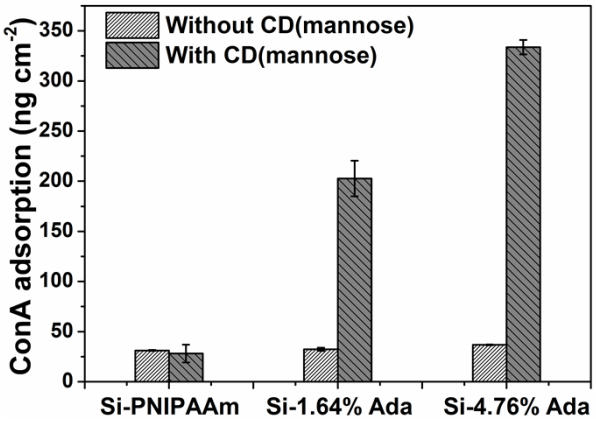

Fig. S4 ConA adsorption on Si-poly(NIPAAm-*co*-Ada) surfaces with different Ada feed ratios before and after complexation with CD(mannose). 0.3 mg/mL ConA in PBS (10 mM, pH 7.4, containing 1 mM Ca^{2+} and 1 mM Mn^{2+}) was adsorbed at 37 °C for 3 h. Data are means ± standard error (n = 3).

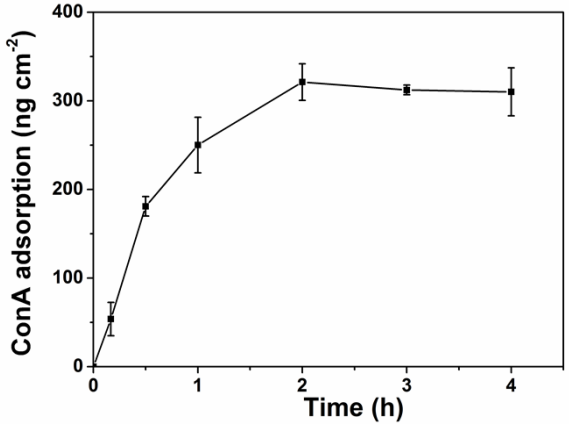

Fig. S5 Dynamic adsorption of ConA on CD(mannose)-modified surfaces with incubation time. 0.3 mg/mL ConA adsorbed on 4.76% Ada/CD(mannose) surfaces at 25 °C. Data are means ± standard error (n = 3).

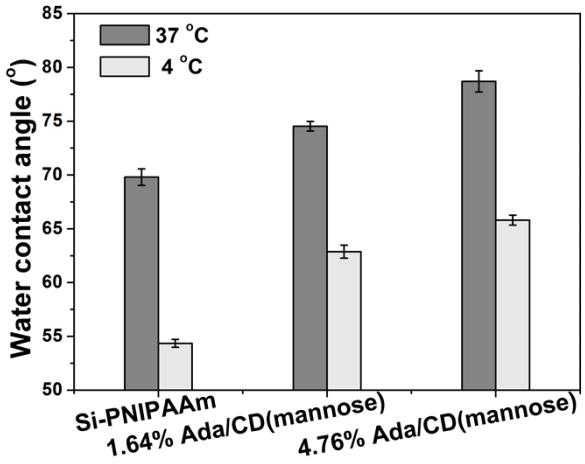

Fig. S6 Surface wettability of Si-PNIPAAm, 1.64% Ada/CD(mannose) and 4.76% Ada/CD(mannose) surfaces at 37 and 4 °C. Data are means ± standard error (n = 3).

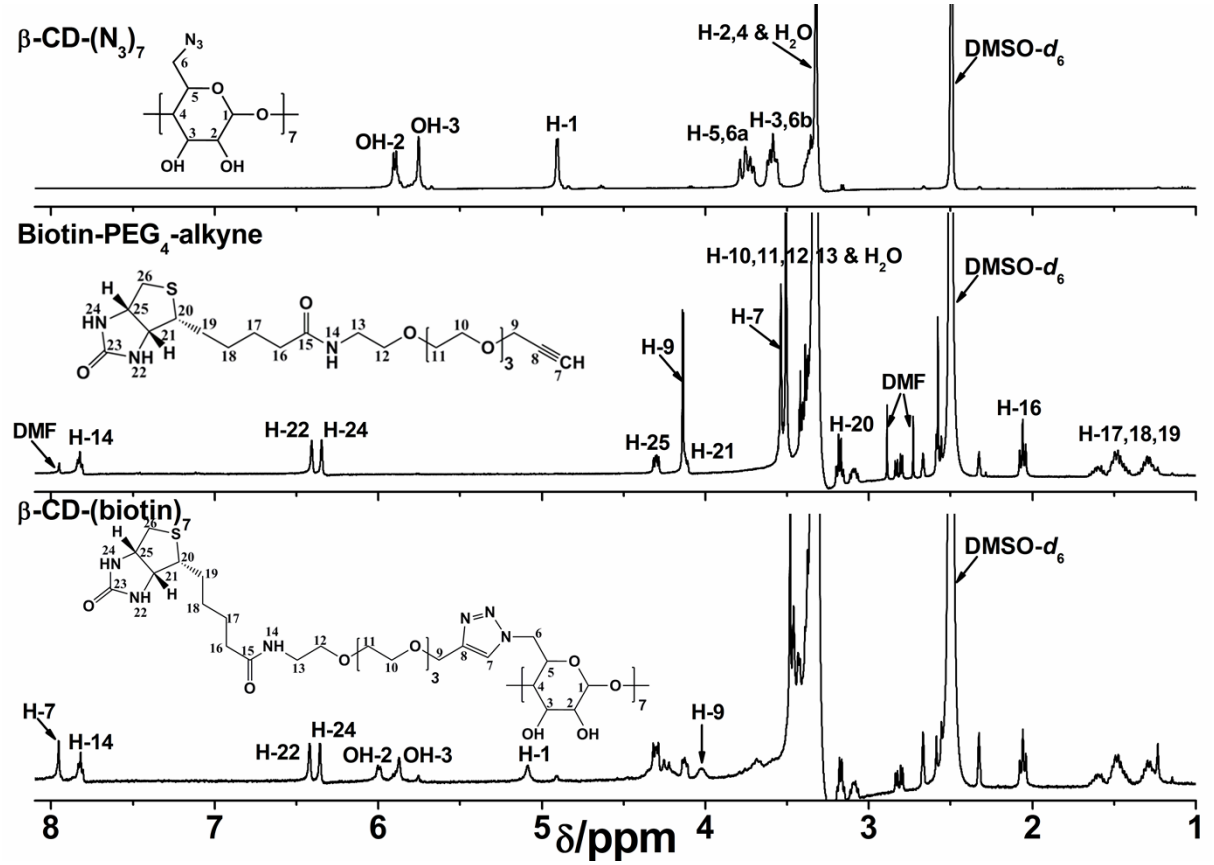

Fig. S7 ^{1}H NMR spectra of β-CD-$(N_3)_7$ (top), biotin-PEG_4-alkyne (middle), and β-CD-$(biotin)_7$ (bottom).

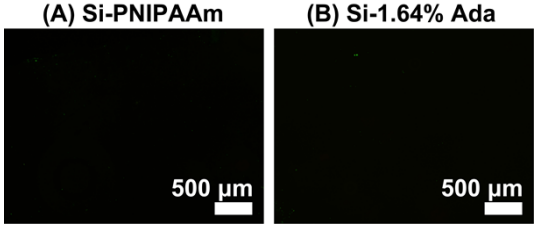

Fig. S8 Avidin-FITC adsorption on (A) Si-PNIPAAm and (B) Si-1.64% Ada surfaces at 37 °C.

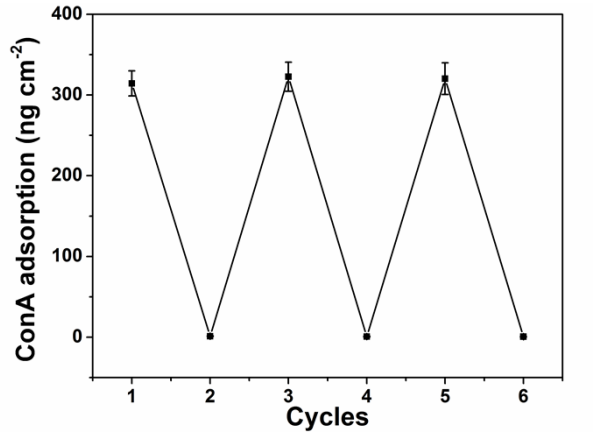

Fig. S9 The cycles of regeneration/reuse of CD(mannose)-modified surfaces for ConA adsorption. 0.3 mg/mL ConA adsorption was followed by 2% SDS wash. Data are means ± standard error (n = 3).

Experimental section

1. Materials

N-Isopropylacrylamide (NIPAAm, 99%, Acros) was recrystallized from a toluene/hexane solution (v/v = 1:2) and dried under vacuum prior to use. Copper(I) chloride (CuCl, 98%, Sinopharm Chemical Reagent Co., Ltd.) and copper(I) bromide (98%, Sigma-Aldrich) were washed sequentially with acetic acid and ethanol and dried under vacuum. β-Cyclodextrin (β-CD, 97%, Sigma-Aldrich) was recrystallized twice from water and dried in a vacuum oven at 100 ^{o}C for two days prior to use. 2-Chloropropanoyl chloride (97%, Sigma-Aldrich), methyl 2-chloropropionate (97%, Sigma-Aldrich) and 3-aminopropyltriethoxysilane (APTES, 99%, Sigma-Aldrich) were used as received. Hexamethylated tris(2-aminoethyl)amine (Me_6-TREN) was synthesized according to a literature procedure.[1] The preparation of 1-adamantan-1-ylmethyl acrylate, β-CD-$(N_3)_7$, and β-CD-(mannose)$_7$ were described in our previous work.[2] Biotin-PEG4-alkyne was from Sigma-Aldrich (St. Louis, MO). Concanavalin A (ConA, Mw = 104 - 112 kDa, type VI, from Canavalia ensiformis) and human serum albumin (HSA, Mw = 66.5 kDa) were from Sigma-Aldrich. Fluorescein isothiocyanate (FITC) was purchased from Amresco (Solon, USA). Avidin-FITC (2 - 4 mol FITC per mol avidin, from egg white) was from Sigma-Aldrich. Albumin bovine V (BSA) was from Solarbio. $Na^{125}I$ was from the China Isotope Corporation. All other solvents, of analytical reagent grade, were from Sinopharm Chemical Reagent Co. (Shanghai, China), and purified according to standard methods before use. Silicon wafers [*p*-doped, (100)-oriented, 0.45 mm thick, 100 mm diameter] were purchased from the laboratory of Guangzhou Semiconductor Materials (Guangzhou, China). The silicon wafers were cut into square chips of about 0.5 cm × 0.5 cm. All aqueous solutions were prepared in 18.2 MΩ·cm purified water prepared by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Nitrogen gas was of high-purity grade.

2. Synthesis of persubstituted cyclodextrin-based biotin via CuAAC

β-CD-(biotin)$_7$ was synthesized via Cu(I)-catalyzed azide–alkyne cyclization (CuAAC). DMSO (5 mL) in a Schlenk tube was deoxygenated by three freeze-pump-thaw cycles. In a glovebox purged with nitrogen, bpy (7.5 mg, 48 μmol) and CuBr (3.5 mg, 24 μmol) were dissolved in 4 mL deoxygenated DMSO. After dissolution, 400 μL catalyst solution were transferred into a small round-bottom flask containing β-CD-(N_3)$_7$ (4.2 mg, 3.2 μmol) and biotin-PEG4-alkyne (11.5 mg, 25 μmol). The resulting solution was stirred at 50 ^{o}C for 36 h. When the reaction was completed, the reaction mixture was dialyzed for 2 days using a dialysis tubing membrane (MWCO 1000, Spectrum Laboratories, Inc., USA). The product was acquired as white powder after freeze drying for 1 day (13.8 mg, yield: 95.8%).

3. Preparation of statistical copolymers and CD(X)-complexed surfaces

Surface-initiated SET-LRP of NIPAAm and Ada copolymers were prepared as described in our previous work.[2] Briefly, NIPAAm and Ada monomers with different ratios (total 4.42 mmol) and CuCl (7.3 mg, 0.074 mmol) were weighed in a round-bottom flask, then 4 mL bubbled solvent (4:1 mixture of isopropanol and water) and 20 μL Me_6-TREN (0.074 mmol) were added into the flask in a glovebox purged with nitrogen. The reaction solution was stirred vigorously allowing the Cu(I)Cl and Me_6-TREN to proceed to full disproportionation and then added to the glass vessels in which initiator functionalized wafers were placed. Polymerization was carried out at 25 ^{o}C under a nitrogen atmosphere for 6 h, and then the copolymer-grafted silicon wafers were taken out of the solution, rinsed with abundant isopropanol and deionized water to remove unreacted monomers, dried under a flow of nitrogen.

The poly(NIPAAm-*co*-Ada) copolymer surface was immersed in 0.5 mM CD(X) aqueous solution for 12 h at 4 ^{o}C to form CD(X)-complexed surfaces, and rinsed with deionized water to remove the unconjugated CD(X), and then blow-dried with N_2.

4. Characterization

The surface morphology of surfaces was probed by atomic force microscopy (AFM, Nanoscope V, Bruker, Santa Barbara, California) in tapping mode in air, with a rectangular silicon cantilever RTESP (Bruker, nominal spring constant of 40 N/m and resonance frequency of 300 kHz). The chemical composition of surfaces was determined using an ESCALAB MK II X-ray photoelectron spectrometer (XPS) (VG Scientific). The dry thickness of polymer grafts on the silicon substrate was measured with a M-2000 V spectroscopic ellipsometer within a spectral range of 371-1000 nm (J. A.Woollam Co., Inc.). Fourier transform infrared (FTIR) transmittance spectra were obtained from a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific Inc., Wilmington, USA) with a mercury cadmium telluride (MCT) detector. Typically, 256 scans and 4-nm resolution were collected for each spectrum. The peak area was computed with OMNIC 8.0 (Thermo Fisher Scientific Inc., Wilmington, USA) software. ^{1}H NMR spectra were recorded on an INOVA 400 MHz nuclear magnetic resonance (NMR) instrument, using DMSO-d_6 as solvent.

5. Contact angle measurement

The static water contact angles of surfaces were measured using the sessile drop method on a SL-200C optical contact angle meter (USA Kino Industry Co., Ltd.) under an atmospheric humidity less than 40%. Firstly, the copolymer surfaces for contact angle measurement were placed in deionized water for about 12 h at 4 ^{o}C, and blow-dried with N_2. Deionized water droplets (about 1 μL) were dropped carefully onto the surfaces with three replicate samples. The contact angles of the substrates were measured from 4 to 45 ^{o}C, which were controlled by

a DC-0506 Low Constant Temp Bath (Shanghai Sunny Hengping Scientific Instrument Co. Ltd). Before taking the measurement of the subsequent temperature, samples were blow-dried with N_2 and kept at the required temperature for 15 minutes. LCST of the surface obtained was defined as the specific temperature producing a 50% decrease in WCA.

6. Protein adsorption measured by ^{125}I- radiolabeling method

Human serum albumin (HSA) was dissolved in phosphate-buffered saline (PBS, 10 mM, pH 7.4) and labeled with ^{125}I by the iodine monochloride (ICl) method.[3] Concanavalin A (ConA) was labeled using the same method as HSA. Unbound radioactive iodide was removed by ion exchange chromatography on an AG-1-X4 resin column (Bio-Rad Laboratories, Hercules, CA). Protein concentrations were measured by a spectrophotometer (UV-7504, Shanghai Xinmao Instrument Co., Ltd., China). ConA concentration was determined using an extinction coefficient of 1.37 at λ = 280 nm for 0.1% solution (pH = 7.4).[4, 5] For studies of protein adsorption from buffer, labeled proteins were mixed with the respective unlabeled proteins (1:19, labeled: unlabeled) at a total concentration of 0.3 mg/mL for ConA and 0.5 mg/mL for HSA. ConA solution was prepared in PBS containing 1 mM $CaCl_2$ and 1 mM $MnCl_2$. In all cases, samples were equilibrated in PBS for 3 h prior to the adsorption experiments. Then, samples were incubated with 200 μL of ^{125}I-labeled protein solution for 3 h at different temperature and rinsed three times (10 min each time) with 200 μL PBS at the same temperature as the protein-adsorbing condition. The samples were dried by blotting with filter paper and transferred to clean tubes for radioactivity determination (Wallace 1480 Wizard gamma counter, PerkinElmer). The radioactivity was converted to the adsorbed amount of protein by comparing to a labeled protein solution of known concentration.

7. FITC- labeled protein adsorption

BSA was labeled with FITC following the procedure described in the literature.[6] Avidin-FITC and BSA-FITC were dissolved in PBS with the same concentration of 0.1 mg/mL. Samples were incubated with 250 μL of FITC-labeled protein solution at 37 or 4 °C for 3 h in dark and rinsed three times (10 min each time) with 250 μL PBS at the same temperature as protein-adsorbing condition. After samples were blotted with filter paper and dried with N_2, pictures were taken by fluorescence microscopy (IX-71, Olympus) using ImageJ software (public software from the National Institutes of Health; http://rsbweb.nih.gov/ij/).

8. Regeneration and reuse of poly(NIPAAm-co-Ada) surfaces

After the adsorption of avidin-FITC, 1.64% Ada/CD(biotin) surfaces were immersed in 1 mL of 2% SDS at 50 °C for 12 h in dark and rinsed three times (5 min each time) with 250 μL of PBS and three times with 250 μL of Milli-Q water at 4 °C. The regenerated samples were then immersed in 200 μL of 0.5 mM CD(biotin) aqueous solution for 3 h at 4 °C to form CD(biotin)-complexed surfaces. After the samples were rinsed three times with Milli-Q water, 250 μL of 0.1 mg/mL avidin-FITC solution was added and held at 37 °C for 3 h in dark. Following rinse with 250 μL PBS three times (10 min each time) at 37 °C, the surfaces with re-adsorbed avidin-FITC were washed with 2% SDS again as the steps described before. Pictures were taken by fluorescence microscopy after samples were blotted with filter paper and dried with N_2. The mean intensity of fluorescence was obtained by analyzing these images using ImageJ software. The procedure of regenerating ConA-adsorbed surfaces was the same as that for avidin-adsorbed surfaces.

References

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