Supporting Information

CO_2-Switchable Polymer Brush for Reversible Capture and Release of Proteins

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1. Synthesis and Characterization

1.1 Materials

2-(Diethylamino)ethyl methacrylate (Reagent plus, 99 % Aldrich), was passed through an activated basic alumina column to remove the inhibitory substances. 3-Aminopropyltrimethoxysilane (APTES, Aldrich, 98 %), 2-Bromoisobutyryl bromide (2-BiBB; 97 % Aldrich), 11-Mercapto-1-undecanol (97 % Aldrich) *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (PMDETA; 99 % Aldrich), Copper$^{(I)}$ bromide ($Cu^{I}Br$, 99.9 %), and phosphate buffered saline (PBS; 10 × liquid concentrate, EMD) were used as received, without further purification. HPLC grade acetonitrile (J. T. Baker), anhydrous methanol (99.8 %, Aldrich) and anhydrous Toluene (99 %, Aldrich) solvents were used for the polymerizations. Milli-Q water with resistivity of 18 MΩ was used for the release experiments and polymerizations. Proteins bovine serum albumin (BSA) and Lysozyme (LYS) were purchased from Sigma-Aldrich and used as received.

1.2 Instrumentation

X-ray photoelectron spectroscopy (XPS) was carried out on an Axis Ultra DLD (Kratos Analytical Ltd.) instrument with photoelectrons generated by nonmonochromatic Al KR irradiation (1486.6 eV). The spectra were recorded in ultra high vacuum ($<10^{-9}$ Torr) and analyzer having pass energy of 20 eV. The data were collected for a takeoff angle of 60° with respect to the surface normal. No charge neutralization current was applied. Peak fitting and quantification analysis were performed using the software package Casa XPS to deconvolute the spectral envelopes into their constituent chemical states. Fourier transform infrared (FTIR) spectra of surface grafted polymer brushes were recorded on a Nicolet Avatar 370 DTGS (Thermo) on silicon wafer. Atomic force microscopy (AFM) images were recorded in water under ambient conditions on a Multimode 8 AFM instrument equipped with a NanoScope V controller (Veeco/Digital Instruments, Santa Barbara, CA). Both *ex situ* (in air) and *in situ* (in fluid) AFM imaging were done in tapping mode or ScanAsyst mode at room temperature. In situ imaging was performed using a commercial fluid cell and silicon nitride (force constant 0.7 N/m) cantilever tips under ScanAsyst mode. Polymer brush samples were incubated in gas purged ultrapure water for 30 min before mounting on AFM imaging area in which the fluid cells were filled with purged gas ultrapure water. All the height values were determined manually from the images using the NanoScope Analysis software version 1.4. Quartz crystal microbalance (QCM) measurements were made using a home-built Ward oscillator and 10 MHz, AT-cut quartz crystals (International Crystal Manufacturing Company) resonator (Au = 0.340"). An HPLC system (Agilent 1100 series) fitted with Symmetry C18 (5μm 4.6×150mm) column was employed to detect the protein. The mobile phase used was 0.1% TFA in water. The solvent flow rate was 1 mL/min and the injection volume was 50 μL. A UV detector at a detection wavelength of 220 nm was employed. Circular dichroism (CD) spectra of proteins were measured on a JASCO J-720 spectropolarimeter with quartz cuvettes of 1 mm path length at 20 °C. The spectra were recorded from 190 to 260 nm as an average of three scans at a rate of 20 nm/min. Static contact angle measurements were performed by using a First Ten Angstrom, model FTA 200 instrument, using the sessile drop method. The ζ potentials of proteins in PBS buffer were measured on a MALVERN Zetasizer Nano ZS instrument. Three rounds of analysis have been performed, and the average values were reported. For the High-performance Liquid Chromatography (HPLC) Analysis,

the CO_2 induced release of protein from PDEAEMA brushes were analyzed using high-performance liquid chromatography to determine the protein concentrations. The protein samples collected after several cycles were reduced to certain volume before injecting in to the HPLC. The retention time for LYS was observed at 9.6 min while for BSA it was at 10.8 min. Standard solutions of BSA and LYS 0.001 mg/mL were used to calibrate the peak position.

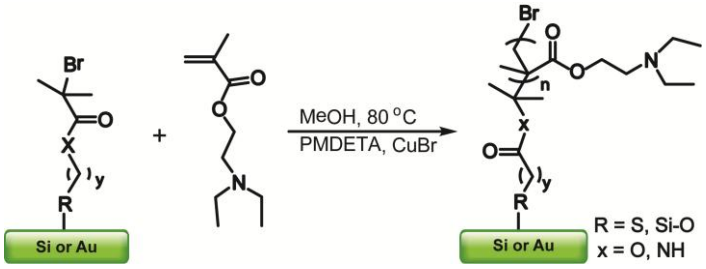
1.3 Surface-Initiated ATRP

1.3.1 ATRP initiator monolayer synthesis

Polymer brushes were grafted on silicon wafer (~ $1cm^2$), indium tin oxide (ITO), glass or gold coated quartz resonator (diameter 0.5 cm) substrates. Si wafer or glass substrates were cleaned in a mixture of H_2O_2:H_2SO_4, 3:7 (v:v) at 80°C ("piranha solution") for 30 min and washed thoroughly with Milli-Q grade water. **(Caution: *Piranha solution is extremely reactive and should be handled with great care*).** The gold coated resonator substrates were cleaned in RCA solution [H_2O_2:NH_4OH:H_2O, 1:1:5 (v:v)] at 80°C for 30 min. The synthesis of the ATRP initiator was adapted from previously published procedures. Briefly, a freshly cleaned surface (Si wafer, or glass) was placed into a round bottom flask containing a solution of 3-(trimethoxysilyl)propyl 2-bromo-2-methylpropionate[S1] (0.3 %), in dry toluene and heated at 80°C for 12 h. After deposition, the substrate was thoroughly cleaned with toluene and methanol and then dried under N_2 flow. Precleaned Au-coated quartz crystal microbalance resonator was soaked in a 1 mM anhydrous ethanol solution of ω-mercaptoundecyl bromoisobutyrate[S2] at room temperature for 24 h, and then the substrates were washed sequentially with THF, ethanol, and dried in a stream of nitrogen before use. The initiator-grafted substrates were immediately used for polymerization. Low density initiator surfaces were synthesized by mixing triethoxy(hexyl)silane or hexane-1-thiol with ATRP initiators.

1.3.2 Preparation of PDEAEMA brush

A 2.8 g sample of DEAEMA was dissolved in 5 mL of MeOH in a flask and then bubbled with N_2 for 30 min. Next, 0.062 g of Cu^IBr and 0.435 g of PMDETA were added, with vigorous stirring. The monomer catalyst solution was degassed by three freeze/vacuum/nitrogen cycles and then transferred to a round bottom flask containing initiator substrate. The reaction was continued at 80°C for 5 h. After polymerization (**Scheme S1**), the samples were removed, washed with water, then methanol, and dried under a stream of N_2.



Scheme S1. Synthesis of surface-initiated ATRP of PDEAEMA brush.

2. X-ray Photoelectron Spectroscopy (XPS) Analysis

Changes in the chemical composition of the silicon surfaces after grafting were determined from XPS data. **Figure S1a** shows that survey spectra of PDEAEMA brush. The C 1s peak was at 285 eV, the N 1s peak was at 400 eV, and the O 1s peak was at 532 eV. The high-resolution O 1s

(**Figure S1b**) signals can be fitted with the expected peak area ratios using five model Gaussian/Lorentzian curves, which correspond to the carbonyl oxygen at (C=O) 532 eV and carboxyl oxygen (O-C=O,) at 534 eV. The high resolution C 1s peak (**Figure S1c**) correspond to the aliphatic carbon atoms of the polymer backbone (C-C) at 285 eV, the secondary carbons of the ester groups (C-C=O) at 285.6 eV, the C-N moieties at 286.7 eV, and the carbonyl (C=O) ester group at 289 eV). The high resolution N 1s peak (**Figure S1d**) corresponding to the tertiary amine (C-N-C) group at 399 eV.

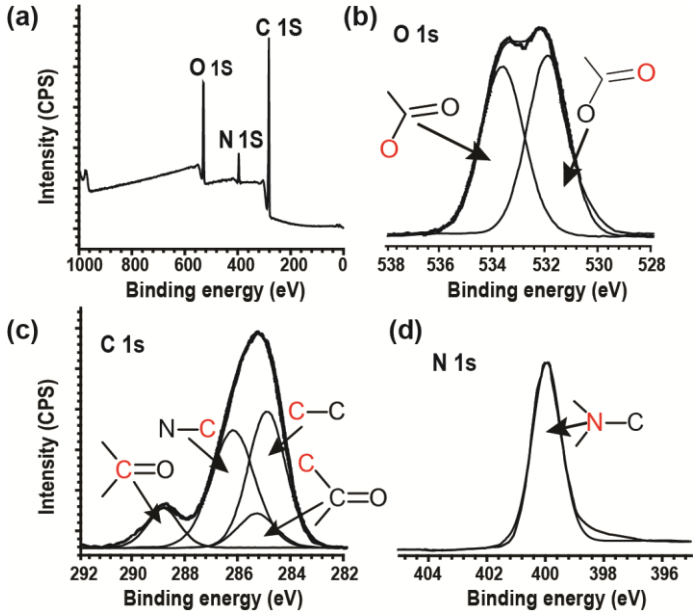


Figure S1. XPS survey (a) and high-resolution O 1s (b), C 1s (c), and N 1s (d) spectra of PDEAEMA brushes.

3. Infrared Spectrum of PDEAEMA Brush

The functional group of the PDEAEMA brush grafted on silicon wafer was determined from FTIR data (**Figure S2**). The characteristic absorption peaks of PDEAEMA, such as carbony group (–C=O) appeared clearly at 1733 cm^{-1}, C-N stretching at 1155–1181 cm^{-1}, aliphatic peaks from 2900–3000 cm^{-1}, and N-CH_2 at 1067 cm^{-1}.

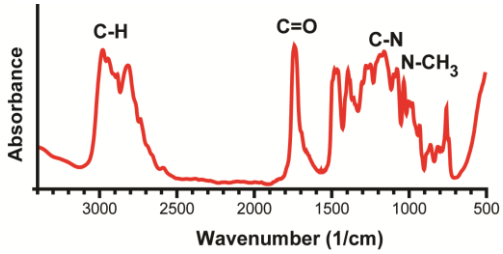


Figure S2. FTIR spectrum of PDEAEMA brush on Si wafer.

4. Quartz Crystal Microbalance (QCM) Measurements

QCM measurements were performed with a home build QCM equipped with a laminar flow cell made of Teflon. The schematic of an experimental setup for a 10 MHz QCM with a flow-type system is shown in **Figure S3**. The PDEAEMA immobilized AT cut quartz crystal resonator was

placed into the cavity formed by bringing together the flow cell housings (upper and lower housings) made of Teflon; using an elastic O-ring around the oscillator edge to fix it flexibly onto the solid support with out any leakage. A peristaltic pump (81 μL min^{-1}) was used to drive liquid flow from a reservoir bottle to a flow cell. The QCM liquid chamber was temperature-stabilized to 25°C. For CO_2 induced switching studies, at first ultrapure water (pH = 7) was injected to the flow cell to equilibrate the polymer-functionalized QCM crystal. Then solution saturated with CO_2 in ultrapure water was introduced. After CO_2 bubbling, the pH of the solution decreased from 7 to 4.9, as a result of the reaction between tertiary amine groups of PDEAEMA with CO_2 leading to the formation of carbonic acid. Subsequently, upon removal of CO_2 by passing N_2 through the solution, its pH recovered to 7. The frequency was measured by means of a frequency counter (Agilient 53131A). Home made computer program was used to obtain the frequency change. Finally, saturated solution of N_2 purged ultrapure water was introduced to switched back polymers original state.

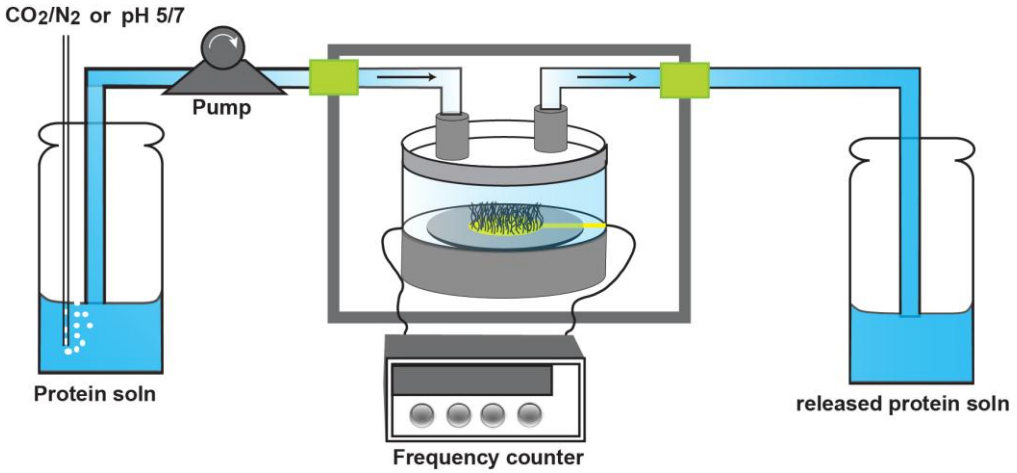


Figure S3. Graphic showing the setup used to monitor the reversible switching of polymer brush as well as protein "capture" and "release" on/from the polymer brush immobilized on QCM resonator.

4.1. BSA and LYS Proteins Adsorbance on PDEAEMA Brush

The frequency change Δf of the quartz resonator due to the scale deposition is proportional to the mass change (Δm) according to the Sauerbrey relation: $\Delta f = -C_f \Delta m$ (the mass sensitivity constant, $C_f = 5.4 \times 10^{-9}$ g cm^{-2} Hz^{-1} at 10 MHz). Monitoring the adsorption of proteins (BSA and LYS) was conducted as follows. At first, the baseline was completely stabilized with PBS buffer (pH = 7.2) for ~10 min, then 1mg mL^{-1} protein in PBS buffer was introduced into the flow cell for ~80 min. The frequency shit was monitored in real time. After the frequency shift attained platue, the surface was rinsed with PBS for 10-20 min to remove weakly bounded proteins. Once Δf is leveled off, the saturated solutions of CO_2 purged PBS solution (pH = 4.9) were introduced to the release of bounded proteins on the PDEAEMA brush surface. The released proteins were collected (several cycles) for HPLC analysis. The PDEAEMA brush was reverse back to hydrophobic state by incubating in to PBS buffer, purged with N_2 gas for ~30 min. This surface was directly used further adsorption of protein. Reversibility of protein adsorption and release was repeated for several cycles. Similarly, instead of CO_2, pH =5 was introduced into the incubating solution and the experiment was conducted as explained before. After the protein release at pH 5, the adsorption cycle was continued by incubating the PDEAEMA brush surface with same BSA solution of pH 7. Several cycles of pH induced adsorption and release of BSA was also conduced for direct comparison with

CO_2 switching. We note that the apparent amount of adsorbed BSA of ~0.11 μg/cm^2 corresponds to 37 monolayers, assuming a BSA monolayer thickness of 3 nm.[s3] As mentioned above, the pH value was 4.9 before adsorption of BSA and 7 after adsorption of the protein. As explained in the paper, the results suggest that the resulting conformational switch of PDEAEMA brushes was the main cause of the reversible adsorption and desorption of the protein. However, it cannot be rule out that the pH change could affect the conformation of the proteins and thus contribute to the absorption and desorption. In the present study, this contribution, if there is any, could not be separated from the effect of polymer brush's conformational change.

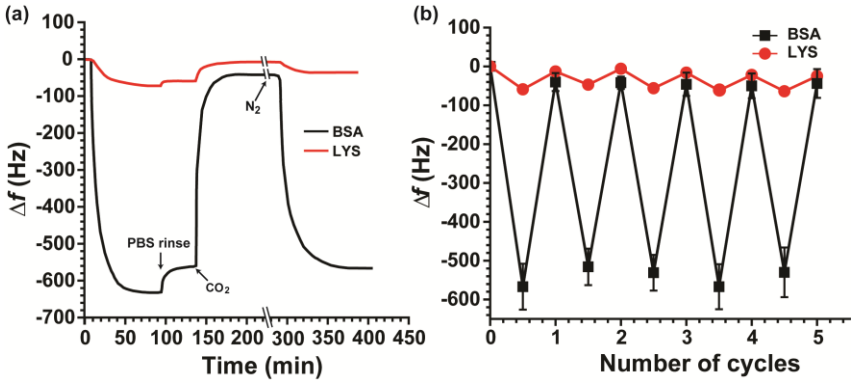


Figure S4. Quartz crystal microbalance response: (a) time depended frequency changes for the adsorption of BSA (—) and LYS (—) on a PDEAEMA brush surface at 25 °C. Arrows indicate where solutions were switched; (b) Dependence of frequency change (Δ*f*) upon successive capture and release of BSA (■) and LYS (●) for five cycles.

4.2. Selective Adsorption of BSA from Binary Mixture

The selective adsorption of BSA and subsequent release using mixtures of the two proteins (BSA/LYS molar ratios of 1:1, 1:5 and 1:10) in PBS buffer solutions under the same conditions as for the single-protein experiments. The purpose was to see if the competing adsorption of BSA and LYS on the PDEAEMA brush would "discriminate" between the two proteins of different isoelectric points and separate BSA from the mixture. Figure S5a shows the QCM results for the three BSA/LYS mixtures while keeping the total concentration of proteins identical at 1 mg mL^{-1}. Changes in frequency of ~ 410 Hz (BSA/LYS = 1:1), 180 Hz (BSA/LYS = 1:5) and 97 Hz (BSA/LYS = 1:10), which correspond to adsorption quantities (Δ*m*) of about 0.076, 0.033, and 0.018 μg cm^{-2}, respectively, were observed. On the basis of the adsorption behavior of single proteins (Figure 3 and Figure S4) and the fact that the changing adsorption quantity for the mixture reflects the actual concentration of BSA, the adsorption should predominantly arise from BSA. It can be seen that as compared to BSA alone, rinse with fresh PBS solution removed a more important amount of proteins, suggesting that the presence of LYS in the mixture could affect the adsorption state of BSA. Using 5-10 mL elution of CO_2-purged PBS buffer leads to complete release of proteins. This elution was collected and used for HPLC analysis to assess the CO_2-induced release of proteins from PDEAEMA brushes. Figure 4b shows the HPLC chromatogram of the solution containing released proteins from the mixture of BSA/LYS (1:1). The peak at 9.6 min corresponds to LYS, while the one at 10.8 min is attributed to BSA. From the normalized peak areas, the relative amounts of the two proteins released from the PDEAEMA brush by CO_2 could be determined. The results obtained from the three BSA/LYS mixtures are reported in the inset of Figure S5b. In all cases, even with the mixture containing 10 times LYS (BSA/LYS, 1:10), a much greater amount of BSA was adsorbed by and released from the PDEAEMA brush. From the three

mixtures with BSA/LYS molar ratios of 1:1, 1:5 and 1:10, the released proteins contain ~ 92 %, 85% and 74% of BSA. This gas-controlled and protein-specific capture and release by PDEAEMA brushes without adding chemicals in the solution or changing the solution temperature is of interest for possible applications.

Future studies may explore the use of CO_2-responsive polymer surfaces for separation of proteins by developing columns formulated for the separation of a certain protein whose isoelectric point favors adsorption on the surface. For example, it is conceivable that CO_2-responsive polymer brushes could be grafted onto silica particles for use as the stationary phase for HPLC column. By introducing a mixture of proteins into the column, the protein of interest can be selectively retained by the particles; afterwards, it can be released and collected by simply purging the eluent (aqueous solution) with CO_2. After reconditioning the column by passing N_2 through the eluent, with the polymer brush recovered to the dehydrated state, the separation process can be repeated. The cycles can easily be done for many times thanks to the absence of any chemical contaminations of the surface.

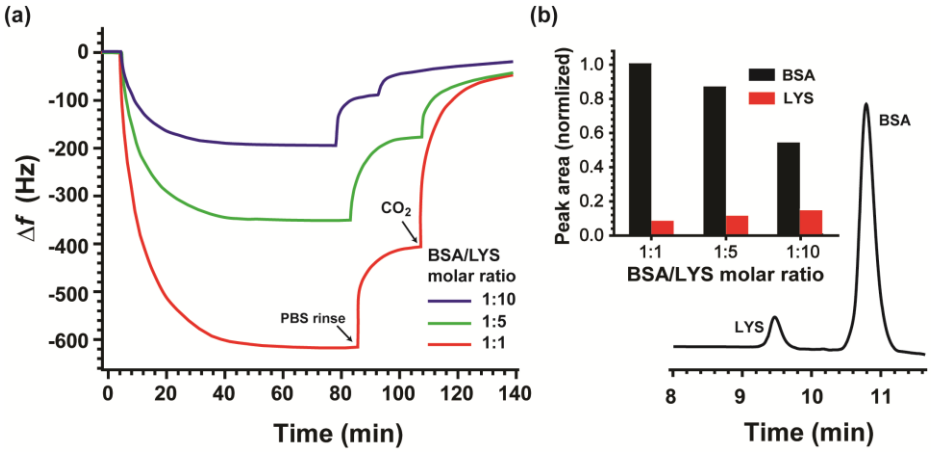


Figure S5. Quartz crystal microbalance response: (a) time dependent frequency changes for the adsorption of mixture of BSA/LYS solutions at molar ratio of 1:1, 1:5, and 1:10; arrows indicate where solutions were switched. (b) HPLC chromatogram (BSA/LYS 1:1) and corresponding peak area as a function of molar ratios of binary proteins (inset).

4.3. Adsorption of Proteins with respect to Polymer Brush Grafting Density

It is well known that the density of polymer brush affects the protein adsorption due to the fact that low density mushroom type polymer brush normally include larger amount of proteins than high density one. In order to compare the protein adsorption, we have prepared PDEAEMA brush having density ($\sigma_{PDEAEMA}$) ≈ 0.18 chains nm^{-2}. Six milliliters of BSA or LYS (PBS buffer) were pumped through the QCM setup using a peristaltic pump, and the grafted surface was rinsed with large quantity of PBS buffer (pH = 7.4); after allowing CO_2 purged solution to flow into the system, proteins were desorbed and the permeate was then collected (several cycles) for high performance liquid chromatography (HPLC) analysis. As shown in **Figure S6**, larger adsorption of both proteins, eg: - 0.113 $\mu g \cdot cm^{-2}$ of BSA and 0.072 $\mu g \cdot cm^{-2}$ of LYS were observed on the low density PDEAEMA compared to high density brush (**Figure S4**). Only ~ 65 % of BSA and 59 % of LYS were released by introduction of CO_2 purged PBS buffer, suggesting that sufficiently dense brushes

were needed for efficient reversible adsorption and release of proteins as well as for selective adsorption of BSA with respect to LYS.

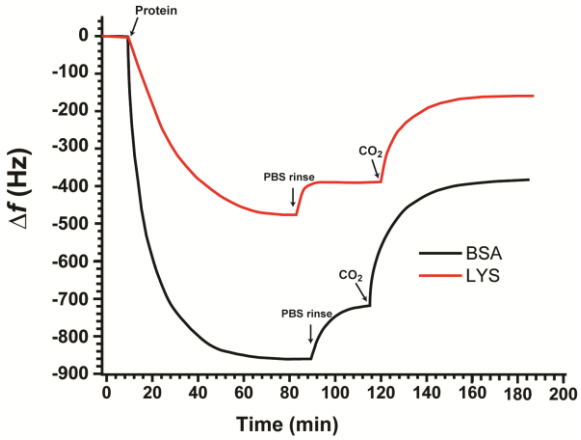


Figure S6. QCM results obtained with a PDEAEMA brush of low grafting density ($\sigma_{PDEAEMA} \approx$ 0.18 chains nm^{-2}).

4.4. Control Test with Bare Gold Resonator and Non-stimuli-responsive Polymer Brushes

For the sake of comparison, control protein adsorption test with bare gold resonator surface and polymer brushes having non-stimuli-responsiveness were also performed. A hydrophobic poly methacrylate (PMMA) brush having contact angle (CA) 86±1.7^{o} and hydrophilic poly(2-hydroxyethylmethacrylate) (PHEMA) having CA 26±1.2^{o} were prepared using the ATRP technique. At first, BSA adsorption on gold resonator surface (CA~ 78±2^{o}) was evaluated by using QCM, with which the adsorbed mass of protein could be analyzed *in situ* through the frequency shift (Δf). The BSA protein adsorption conditions were identical to those in the PDEAEMA system. The frequency shift resulting from BSA adsorption reached a plateau after ~ 60 min (**Figure S7**). After PBS rinse, the frequency change of ~ 186 Hz corresponds to an apparent adsorption quantity (Δm) of about 0.034 μg cm^{-2}. After introduction of purged CO_2 solution, no signs of protein desorption similar to the PDEAEMA brush were observed. However, after 50 min of introduction of CO_2 solution a slight BSA release (~60 Hz) was noticed. This small desorption is likely to be caused by the pH decrease in the presence of CO_2 (about 5) which brings BSA to close to its isoelectric point (p*I*=5.1–5.5), where the reduced repulsion between the protein molecules could result in a desorption.

Then we performed BSA adsorption measurements with the hydrophobic PMMA and hydrophilic PHEMA brushes. **Figure S8** compares the results. It is seen that the adsorption behavior of BSA on PMMA is similar to that obtained with the PDEAEMA brush, but with a smaller amount. After PBS rinse, the frequency change of ~ 322 Hz corresponds to an apparent adsorption quantity of about 0.06 μg cm^{-2} obtained. The protein adsorption is mainly due to the hydrophobic nature of both the protein and PMMA brush. On introduction of purged CO_2 solution, no sign of protein desorption was observed. This is due to the fact that the hydrophobicity of the PMMA brush cannot be switched by reaction with CO_2, hence the adsorbed protein remains on the PMMA brush. In the case of PHEMA, the QCM data show very little adsorption of the protein by the hydrophilic brush as compared to the PMMA brush. Again, on introduction of the purged CO_2 solution, no protein desorption occurred. These results are consistent with the large change in the amount of adsorbed BSA on the PDEAEMA brush switching between the hydrophobic and hydrophilic nature. They

also confirm that the responsiveness of PDEAEMA to CO_2 is the origin of the reversible adsorption and adsorption of the protein.

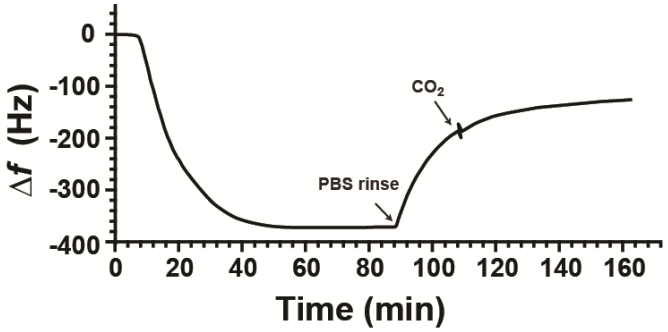


Figure S7. Time-dependent frequency changes (Δ*f*) for the adsorption of BSA on bare gold resonator at 25 °C. Arrows indicate where solutions were switched.

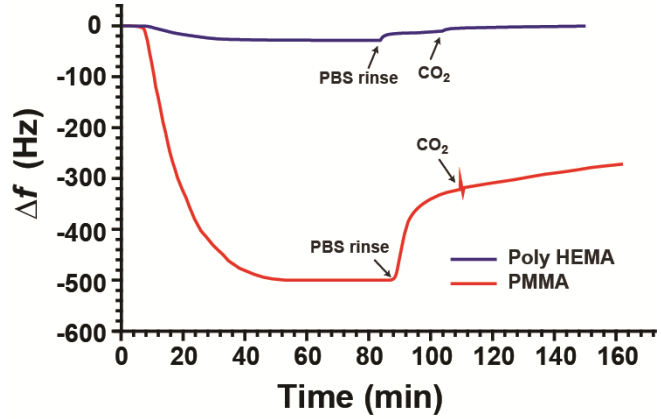


Figure S8. Time-dependent frequency changes (Δ*f*) for the adsorption of BSA on hydrophobic PMMA and hydrophilic poly HEMA brush surface at 25°C. Arrows indicate where solutions were switched.

5. Circular Dichroism of Proteins

Circular dichroism (CD) absorption spectrum was obtained for released BSA and LYS solutions and compared to a initial sample. The spectra (**Figure S9**) shows two strong negative bands at 208 and 222 nm as well as a strong positive band at 195 nm are attributed to a high content of α-helix structure in proteins. Released proteins showed decrease in α-helix (BSA ~14 % and LYS ~6 %) structure; suggesting that the BSA and LYS is slightly altered from its native state.

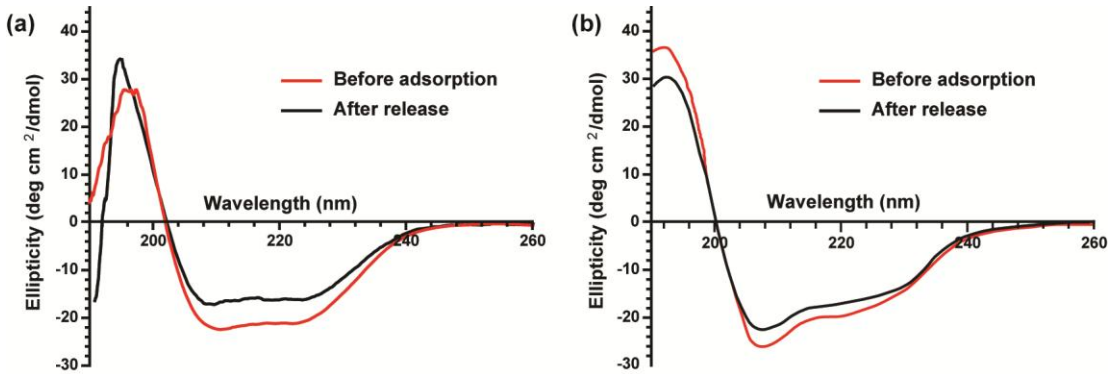


Figure S9. CD spectra of: (a) BSA and (b) LYS, before adsorption and after release on/from PDEAEMA brush at 25°C.

6. Zeta Potential Measurements

Zeta potential of the particles was determined with a Malvern Zetasizer Nano-ZS. 0.3 mg/mL of proteins in 10 mM PBS solution were transferred into a 1 mL clear zeta potential cuvette (DTS1060, Malvern). The electrophoretic mobility of the sample was measured and converted into the ζ potential by applying the Henry equation. The data were collected and analyzed with the Malvern zetasizer 6.3 software producing diagrams for the ζ potential as a distribution versus total counts. At first ζ potential of BSA in PBS was analyzed and found to be -39 mV (**Figure S10a**); then after purging CO_2 for 30 minutes, ζ potential was analyzed again (**Figure S10b**) and found to be -0.5 mV. The similar experiment also used for LYS protein as well. After purging CO_2 the ζ potential of LYS in PBS solution was shifted from +16 mV to +23 mV (**Figure S10c&d**). This indicates that the LYS (p*I* = 11) still maintained its cationic character even after purging with CO_2 gas.

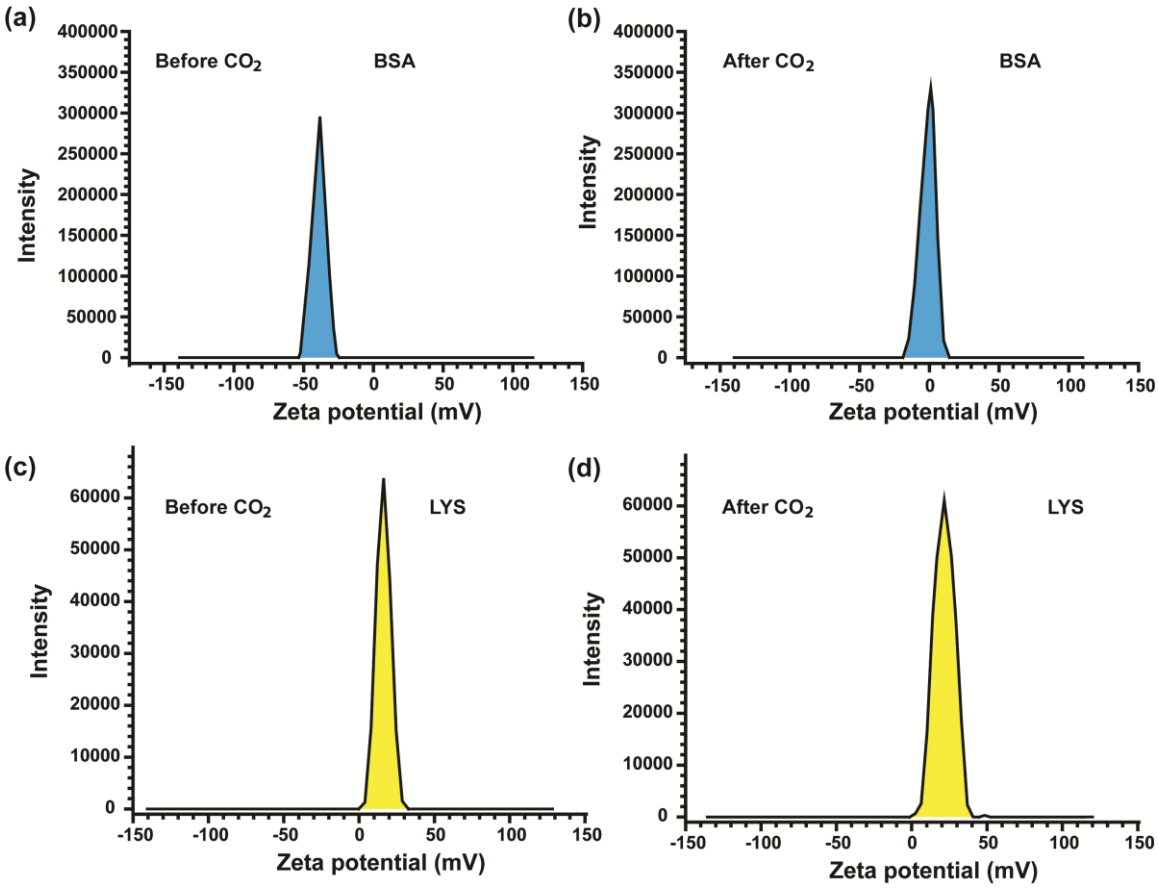


Figure S10. Zeta potentials of: (a) BSA before CO_2 purging, (b) BSA after CO_2 purging, (c) LYS before CO_2 purging and (d) LYS after CO_2 purging.

Reference:

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S2. Jones, D. M.; Brown, A. A.; Huck, W. T. S. *Langmuir* **2002**, *18*, 1265.

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