# Supplementary Information

## A thermally switchable chromatographic material for selective capture and rapid release of proteins and nucleotides

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## Experimental section

### Materials

Both *N*-isopropylacrylamide (NIPAAm) and 2-(diethylamino)ethylmethacrylate (DEAEMA) were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). NIPAAm was purified by recrystallization from n-hexane and dried under vacuum prior to use. *N*-tert-butylacrylamide (tBAAm), 2-bromoisobutyryl bromide (97%), and acetyl chloride (98%) were all provided by Acros Organics (USA). tBAAm was purified by recrystallization from acetone. 3-Aminopropyltriethoxysilane (APTES, 98%) and *N,N,N',N'',N''*-pentamethyldiethylenetriamine (PMDETA, 99%) were purchased from J&K Chemical Ltd. (Beijing, China). Silica particles (5 μm; pore size, 300 Å; specific surface area, 0.06 $m^2$/mg) were kindly provided by the Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences (Lanzhou, China). Hydrocortisone and dexamethasone were kindly provided by the National Institute for Food and Drug Control (Beijing, China). Adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP) were purchased from Beijing Bio-LAB Materials Institute (Beijing, China). All the standard proteins were purchased from Sigma-Aldrich (St. Louis, MO, USA): bovine serum albumin (BSA, pI 4.7 and MW=66kD), cytochrome c (pI 9.8-10.1 and MW=13kD), carbonic anhydrase (pI 5.9 and MW=29kD), human serum albumin (HSA, pI 4.64 MW=66kD), lysozyme (pI 11.0-11.35 and MW=14kD), myoglobin (pI 6.99 and MW=16.7kD), ribonuclease A (Rnase A, pI 7.8 and MW=13.7kD), and α-glucosidase (pI 4.5 and MW=67kD). All other chemicals were obtained from Beijing Chemical Reagent Corp. (Beijing, China) and were of analytical grade. Cuprous chloride (CuCl) was purified by stirring in acetic acid, washed with ethanol and acetone, and finally dried under vacuum. Triethylamine (TEA), tetrahydrofuran (THF), and 2-propanol were all dried by calcium hydride.

### Preparation of amino-functionalized silica particles

Silica particles were pretreated in 0.1 M hydrochloric acid for 6h at ambient temperature, washed repeatedly with large amounts of water, and dried at 110 ºC for 20 h. Silica particles (10.0 g) were immersed in anhydrous toluene (100 mL) and 3-Aminopropyltriethoxysilane (APTES; 10 mL, 42.5 mmol) was added to the mixture. The mixture was reacted at 110 ºC for 10 h. The obtained silica was washed with toluene, ethanol and water, and then dried under vacuum at 100 ºC overnight. Elemental

analyses: C, 2.11; H, 0.46; N, 0.50.

**Preparation of 2-bromoisobutyrate-functionalized silica particles**

The anhydrous amino-functionalized silica (0.8 g) was dispersed into a solution of TEA (0.5 mL) in THF (20 mL). After cooling to 0 ºC, the mixture was degassed three times under vacuum and nitrogen. A solution of 2-bromoisobutyryl bromide (240 μL, 1.92 mmol) and acetyl chloride (401 μL, 4.48 mmol) in anhydrous THF (8 mL) was added dropwise into the mixture under nitrogen. The reaction was carried out at 0 ºC for 2 h and then at ambient temperature for 12 h. 2-bromoisobutyrate-functionalized silica was collected by centrifugation, rinsed with ethanol, water, and acetone, and dried under vacuum at 50 ºC for 8 h. The above processes were repeated six times. Elemental analyses: C, 2.71; H, 0.50; N, 0.48.

**Surface modification of silica with cationic copolymers via SI-ATRP**

2-bromoisobutyrate-functionalized silica (0.6 g), NIPAAm (2.598 g, 22.9 mmol), DEAEMA (232 μL, 1.15 mmol), tBAAm (0.292 g, 2.29 mmol) (the feed ratios of comonomer to NIPAAm were 5% and 10% for DEAEMA and tBAAm, respectively), and PMDETA (183 μL, 0.87 mmol) were placed in a 85% 2-propanol aqueous solution (24 mL). The reaction mixture was deoxygenated under vacuum and flushed three times by nitrogen at 0 °C. Subsequently, CuCl (43 mg, 0.44 mmol) was added under the protection of nitrogen flow. The reaction was allowed to proceed at ambient temperature under continuous stirring. After 12 h, the flask was exposed to air to terminate the polymerization. The copolymer-grafted silica particles were washed with ethanol, disodium EDTA aqueous solution (20 mM), and finally water. The resulting silica particles were dried under vacuum at 60 ºC for 8 h. The above processes were repeated six times. Elemental analyses: C, 14.22; H, 2.41; N, 2.67.

Parts of the modified silica were treated with hydrofluoric acid and then neutralized with sodium carbonate to cleave grafted copolymer for further characterizations, such as gel permeation chromatography (GPC), $^{1}$H NMR, and optical transmittance measurements. The cleaved copolymer was purified by dialysis in water using a cellulose membrane (MWC: 3500) for 7 d, and water was changed every 12 h. The copolymers were finally obtained by freeze-drying under vacuum. $^{1}$H NMR

(600 MHz, $CDCl_3$): δ (ppm) 4.00 (s, 2H, $OCH_2$), 3.05 (s, 1H, CH), 1.16–1.34 ($8CH_3$).

**Assays for characterization of copolymer grafted silica and grafted cationic copolymer**

Elemental analyses were carried out using an Elemental Vario MICRO CUBE analyzer (Germany). To determine the grafting amount of copolymer on silica surfaces, thermogravimetric analysis (TGA) was performed with a METTLER TOLEDO TGA/DSC STAR$^e$ system at a heating rate of 10 ºC/min from ambient temperature to 800 ºC under nitrogen atmosphere. The amount of grafted copolymer on silica surfaces ($m_c$, mg/m$^2$) can be calculated using the following equation: [8d]

$$m_c\left(\frac{mg}{m^2}\right) = \frac{\Delta W_{SC} - \Delta W_{SI}}{W_{750} \times S} \qquad (1)$$

where $\Delta W_{SI}$ and $\Delta W_{SC}$ are the weight loss ratios of initiator-and copolymer-grafted silica ranging from 100 to 750 °C, respectively. $W_{750}$ is the residual weight ratio of copolymer-grafted silica at 750 °C, and S is the specific surface area of the silica support (m$^2$/mg) (from the manufacturer's data). Both the molecular weight and polydispersity index (PDI) were determined by GPC equipped with a Waters 1515 pump and a Waters 2414 differential refractive index detector. A series of three linear Styragel columns HT3, HT4, and HT5 were used at an oven temperature of 50 °C. The eluent was DMF containing 0.06 wt% LiBr at a flow rate of 1.0 mL/min, and polystyrene (PS) standards were employed for GPC calibration. The grafting density of copolymer was calculated using the following equation:[9c,9d,12]

$$\text{Grafting density}\,(\text{chains/nm}^2) = \frac{m_c \times N_A}{M_n \times 10^{21}} \qquad (2)$$

where $m_c$ denotes the amount of grafted copolymer per unit area (mg/m$^2$), and $M_n$ representes the number-average molecular weight of grafted copolymer (g/mol). $N_A$ is Avogadro's number. Thermosensitivity of grafted copolymers was evaluated by optical transmittance measurement. The optical transmittance of copolymer solution (5 mg/mL) was determined at 450 nm by a UV-visible spectrometer (TU-1810, Pgeneral, China). Low critical solution temperature (LCST) was defined as the

temperature where 50% optical transmittance of copolymer aqueous solution was observed. Comonomer contents in copolymers were determined by $^1$H NMR using a Bruke 600 MHZ NMR Spectrometer in $CDCl_3$.

**Chromatographic assay for separation and enrichment of steroids, adenosine nucleotides, and proteins**

Copolymer grafted silica particles were suspended in methanol and then packed into a stainless steel column (50 mm × 1.0 mm i.d.) under a maximum pressure of 50 MPa. High performance liquid chromatography (HPLC) was performed with a system consisting of an Elite P230 pump and a DAD 230 detector with variable wavelength. The column temperature was controlled with a deviation of ±0.1 °C using a Yataikelong YT-15A thermostated water bath (Beijing, China). Hydrophobic hydrocortisone (2 mg) and dexamethasone (2 mg) were dissolved in ethanol (1.5 mL), and then water (10 mL) was added to yield a concentration of 0.174 mg/mL. The 10 mM phosphate buffer at pH 7.0 was employed as a mobile phase for thermoresponsive elution of steroids with a flow rate of 0.05 mL/min, and the UV detection was at 254 nm. A mixture of adenosine, AMP, ADP, and ATP was prepared by dissolving them in the mobile phase. Two different pH values of 10 mM phosphate buffer (pH 7.3 and pH 7.0) were used as mobile phases for the temperature-induced adsorption and release of adenosine nucleotides with a flow rate of 0.05 mL/min and UV monitoring at 260 nm. Solutions of protein samples were prepared at concentrations of 1 mg/mL and 2 mg/mL for standard proteins and crude human serum, respectively, by dissolvingthem in the mobile phase of phosphate buffer (10 mM, pH 7.0). Temperature-modulated protein caputure and release was monitored at 280 nm with a flow rate of 0.05 mL/min. Elutions of crude human serum were collected for mass spectrum (MS) identification.

**Protocol for MS identification of proteins**

Protein samples were denatured and reduced by a solution containing 8 M urea, 10 mM DTT and 50 mM $NH_4HCO_3$, at 37 ºC for 4 h. Alkylation was carried out with 50 mM iodoacetamide at room temperature for 1 h in the dark, and then diluted with 50 mM $NH_4HCO_3$ (pH 8.0) to reduce the urea

concentration below 1 M. Tryptic digestion was performed at a concentration ratio of 50:1 (protein totrypsin, w/w) by incubation for 20 h at 37 °C in a water bath. Digested samples were lyophilized and then desalted by passing through C18 SPE cartridges. The peptides were first separated by strong cation exchange (SCX) chromatography carried out by an Agilent 1100 series device (Agilent, Palo Alto, CA) at a flow rate of 0.2 mL/min. The mobile phase consisted of solution A, 10 mM ammonium formate containing 25% acetonitrile at pH 3.0, and solution B, 500 mM ammonium formate containing 25% acetonitrile at pH 6.8. The gradient elution was programmed as follows: an isocratic condition at 0% B for 10 min, followed by a gradient from 0 to 50% of B over 50 min, and from 50 to 100% of B over 10 min, and finally using 100% B for another 10 min. Eluted peptides were monitored by UV at 280 nm, and fractions were collected every 2 min. After lyophilization, the fractions were analyzed by nanoflow LC/MS/MS.

The MS identification of peptides was performed on an Agilent HPLC-Chip/Q-TOF MS system. The HPLC-Chip configuration included a 160 nL on-line enrichment column and a 150 mm × 75 μm analytical column (Zorbax 300SB-C18). The mobile phases consisted of solution A, 0.1% formic acid in water, and solution B, 0.1% formic acid with acetonitrile. An 80 min long gradient method was used for the LC separation: from 0 to 60 min, solution B increased from 3 to 40%; from 60 to 65 min, B increased from 40 to 95%; from 65 to 75 min, B was kept at 95%; and from 75 to 80 min, B returned from 95 to 3%. The column was equilibrated for 5 min before each run. Samples were loaded at 4 μL/min of flow rate and eluted at 400 nL/min. Mass spectrometric analysis was done on a Q-TOF with ESI source in the positive ion mode. MS parameters were as follows: gas temperature at 300 °C, drying gas at 3 L/min, capillary voltage at 1700 V, fragmentor at 175 V, skimmer at 65 V, and OCT 1 RFVpp at 750 V. Mass data within mass ranges of 300-2000 m/z were set up at 3 spectrum/s.

MS/MS data were searched against the SwissProt database using the G2721AA Spectrum Mill software.

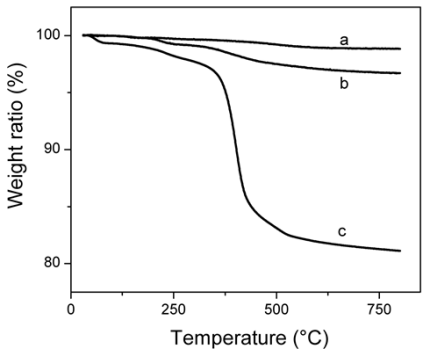


**Fig. S1** Determination of the grafting amount of copolymers on silica surfaces by thermogravimetric analysis (TGA) of silica: a) Amino group-functionalized silica, b) 2-Bromoisobutyrate group functionalized silica, and c) Poly (NIPAAm-co-DEAEMA-co-tBAAm) polymer grafted silica. TGA was performed in nitrogen atmosphere at a heating rate of 10 °C/ min.

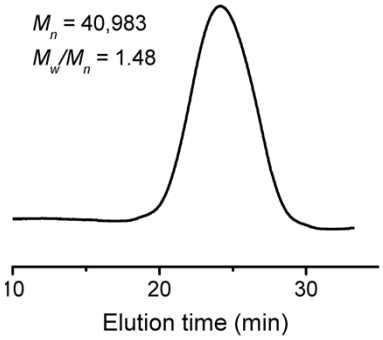


**Fig. S2** GPC traces of poly (NIPAAm-co-DEAEMA-co-tBAAm) cleaved from silica surfaces using DMF containing 0.06 wt% LiBr as the eluent with polystyrene standards at 50 °C.

The chemical shift of one proton of CH $(CH_3)_2$ in NIPAAm units is found to be located at ~3.0 ppm. The peaks, 0.8~1.1 ppm are assigned to six protons of $N(CH_2CH_3)_2$ chain ends and methyl protons adjacent to main chain in DEAEMA units, and nine protons of $C(CH_3)_3$ in tBAAm units. The chemical shift of six protons of CH $(CH_3)_2$ in NIPAAm units is at 1.2~1.4 ppm. The peaks, 1.4~1.7 ppm are methylene protons in backbone. The chemical shift of six protons of methylene adjacent to nitrogen in DEAEMA units and CH protons in backbone appears at 2.0~2.5 ppm. The chemical shift of 4.0~4.5 ppm corresponds to methylene protons adjacent to oxygen in DMAEMA.

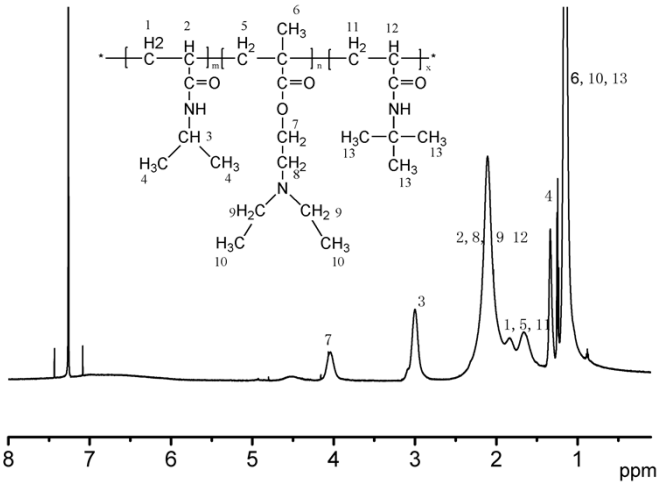


Fig. S3 [1]HNMR spectrum of the poly (NIPAAm-co-DEAEMA-co-tBAAm) cleaved from silica surface in $CDCl_3$.

**Table 1** Characterization of poly(NIPAAm-co-DEAEMA-co-tBAAm) polymers cleaved from silica surfaces.

| NIPAAm/ DEAEMA/ tBAAm (mol ratio) | | Mn[b] | Mw/Mn[b] | Grafted copolymer (mg/m$^2$) | Grafting density (chains/nm$^2$) | LCST (ºC)[c] | |
|---|---|---|---|---|---|---|---|
| In feed | In copolymer[a] | | | | | At pH 7.0 | At pH 7.3 |
| 87.0/ 4.3/ 8.7 | 82.0/ 16.6/ 1.4 | 40983 | 1.48 | 3.04 | 0.045 | 37.4 | 35.2 |

[a]Determined by [1]H NMR measurement. [b]Measured by GPC measurements using DMF containing 0.06 wt% LiBr as the eluent with polystyrene standards. [c]Defined as the temperature at 50% transmittance in 10 mM phosphate buffer solution.

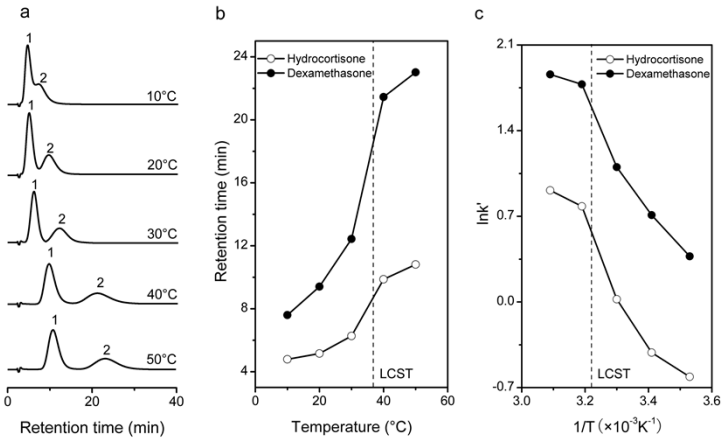


**Fig. S4** a) Chromatograms of steroids at various temperatures; b) Temperature-dependent retention time changes of steroids; c) Van't Hoff plots of steroids. Column packing material, poly (NIPAAm-co-DEAEMA-co-tBAAm) grafted silica; mobile phase, 10 mM phosphate buffer solution (pH 7.0); flow rate, 0.05 mL/min; detection at 254 nm. Peak 1: hydrocortisone; peak 2: dexamethasone.

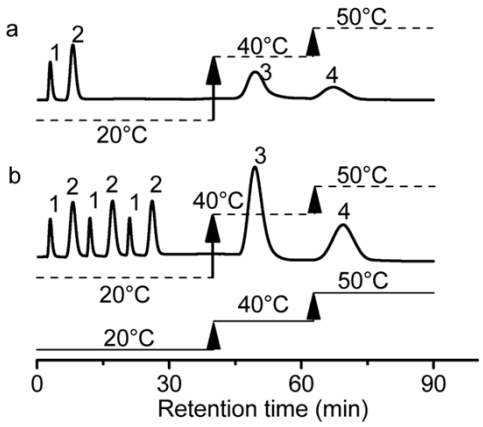


**Fig. S5** The separation and enrichment of ADP and ATP from the mixture of adenosine, AMP, ADP, and ATP via thermally controlled capture and release on poly (NIPAAm-co-DEAEMA-co-tBAAm) grafted silica column. a) One injection; b) Three injections. Mobile phase, 10 mM phosphate buffer solution (pH 7.0); flow rate, 0.05 mL/min; detection at 260 nm. Peaks: 1) adenosine; 2) AMP; 3) ADP; 4) ATP.

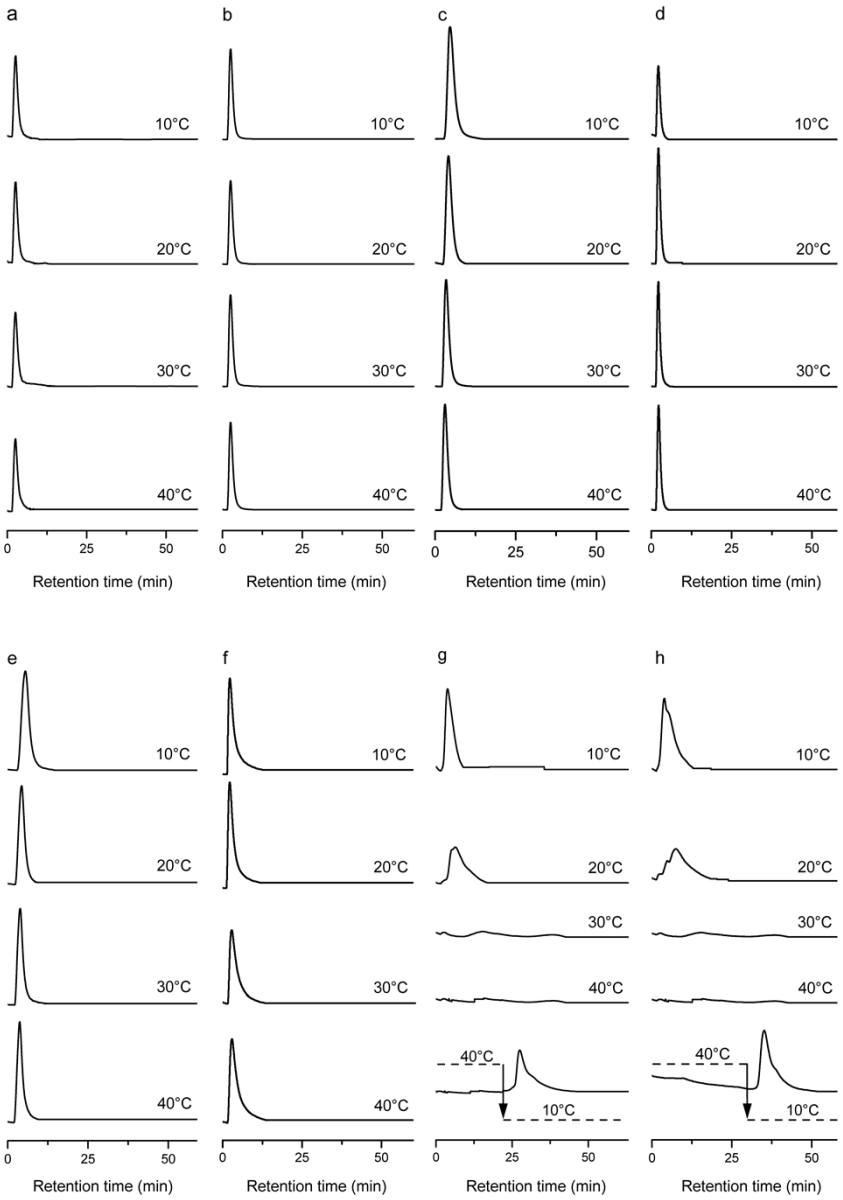


**Fig. S6** Temperature-responsive elution profiles of various proteins on poly(NIPAAm-co-DEAEMA-co-tBAAm) grafted silica column at various temperatures: a) cytochrome c; b) lysozyme; c) Rnase A; d) myoglobin; e) carbonic anhydrase; f) α-glucosidase; g) HSA; h) BSA. The step temperature gradient for HSA at 22 min and for BSA at 30 min was from 40 ºC to 10 ºC. Mobile phase, 10 mM phosphate buffer solution (pH 7.0); flow rate, 0.05 mL min-1; detection at 280 nm.

**Table S2.** The scores and coverages of identified proteins from pretreated with poly(NIPAAm-co-

DEAEMA-co-tBAAm) grafted silica columns and untreated human serum.

| Captured protein hits | score | coverage (%) | Serum protein hits[a] | score | coverage(%) |
|---|---|---|---|---|---|
| P02768\|Serum albumin | 736.85 | 66 | P02768\|Serum albumin | 367.65 | 42 |
| P01024\|Complement C3 | 293.06 | 17 | P01024\|Complement C3 | 242.31 | 12 |
| P02647\|Apolipoprotein A | 156.63 | 31 | P02647\|Apolipoprotein A | 124.42 | 27 |
| P00738\|Haptoglobin | 154.62 | 29 | P00738\|Haptoglobin | 83.37 | 13 |
| P02774\|Vitamin D-binding protein | 83.02 | 17 | P02774\|Vitamin D-binding protein | 21.56 | 4 |
| Uncaptured protein hits | | | | | |
| P02787\|Serotransferrin | 512.48 | 47 | P02787\|Serotransferrin | 172.47 | 20 |
| P01857\|Ig gamma-1 | 253.85 | 47 | P01857\|Ig gamma-1 | 130.99 | 29 |
| P01834\|Ig kappa chain C region | 118.77 | 81 | P01834\|Ig kappa chain C region | 82.61 | 79 |
| P02790\|Hemopexin | 69.18 | 14 | P02790\|Hemopexin | 44.05 | 6 |
| P02749\|Beta-2-glycoprotein | 43.7 | 8 | P02749\|Beta-2-glycoprotein | 30.64 | 8 |

[a] Identified from untreated human serum.