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*Electronic Supplementary Information*

# Protein Separations *via* Thermally Responsive Ionic Block Copolymer Brush Layers

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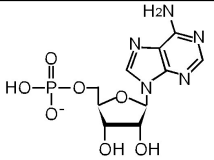
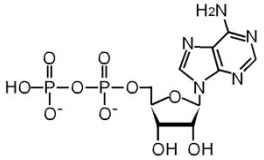
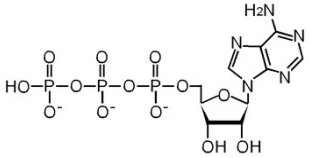
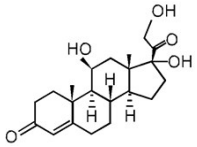
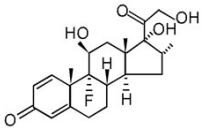
**Table S1** Properties of adenosine nucleotides and hydrophobic steroids

| Compounds | Structure | Molecular weight | LogP [a] | p*K* |
|---|---|---|---|---|
| Adenosine monophosphate (AMP) |  | 347.06 | | 3.3, 6.1 |
| Adenosine diphosphate (ADP) |  | 427.20 | | 3.95, 6.3 |
| Adenosine triphosphate (ATP) |  | 507.18 | | 4.00, 6.5 |
| Hydrocortisone |  | 362.46 | 1.61 | |
| Dexamethasone |  | 392.46 | 1.83 | |

a) Partition coefficient in an n-octanol/water system.[S35]

**Table S2** Properties of proteins in milk serum

| Analyte | Molecular weight [a)] | p*I* [a)] | Remarks |
|---|---|---|---|
| α-Lactalbumin | 14.2 kDa | 4.5 | Major protein in milk serum, Related to production lactose |
| β-Lactoglobulin | ~18.4 kDa | 5.1 | Major whey protein, Allergic property |
| Lactoferrine | 83.1 kDa | 8.2-8.9 | Transferrin family, Antimicrobial activity |
| Bovine serum albumin (BSA) | 66.4 kDa | 4.7 | Regulation of colloidal pressure of blood |
| γ-globulin | 155 kDa | 6.85 | Main protein for immunoreaction |

a) From manufacturer's data.

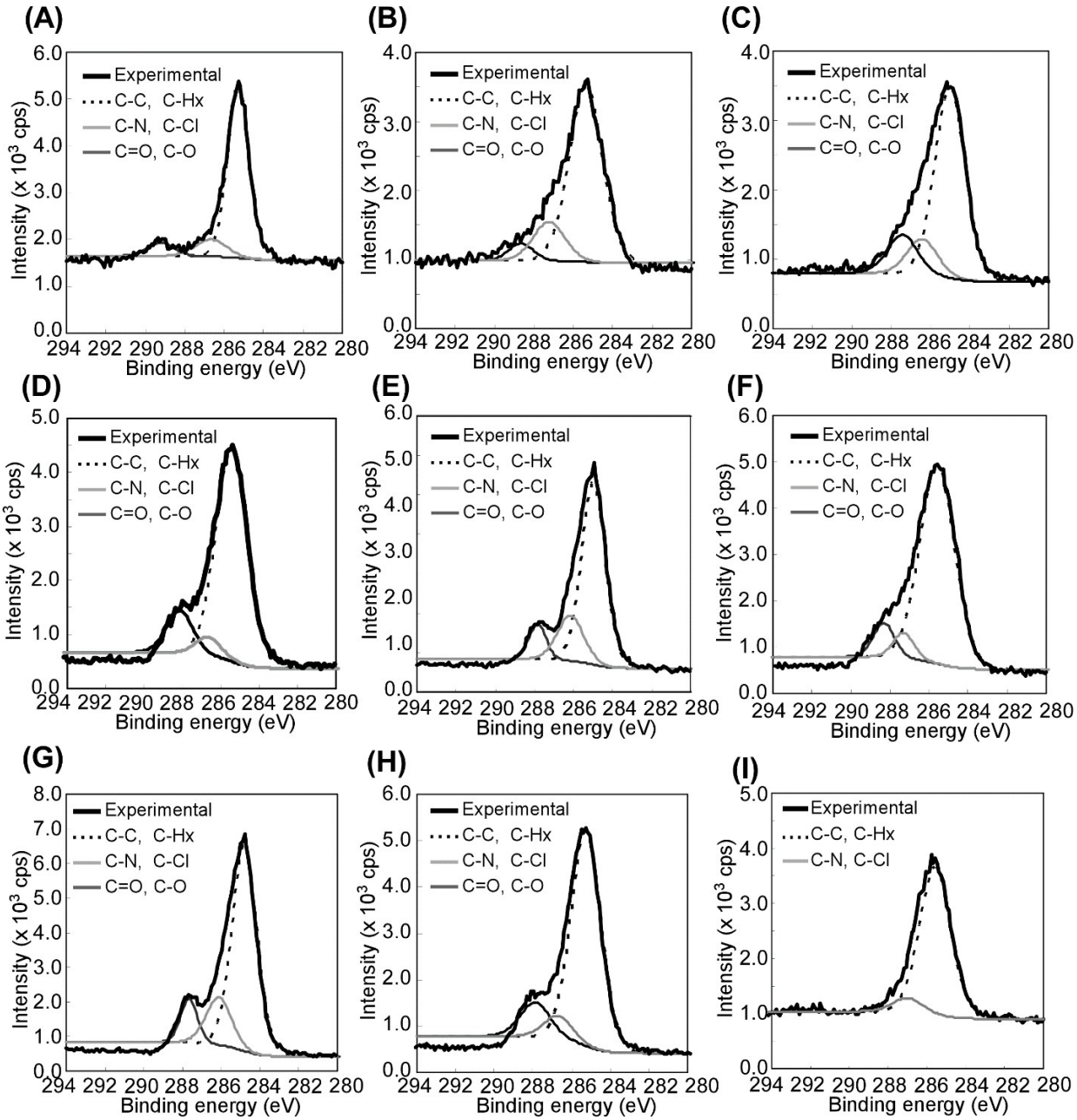



**Figure S1** Peak de-convolution of C1s peaks in X-ray photoelectron spectra of prepared beads (A) 10A, (B) 15A, (C) 20C, (D) 10A-1000IP, (E) 15A-1000IP, (F) 20A-1000IP, (G) 1000IP-*r*-15A, (H) 1000IP, and (I) ATRP-initiator modified silica beads (see Table 3). For copolymer-grafted silica bead surfaces, an additional peak was observed at 288 eV that corresponds to C=O and C-O bonds of the copolymer. There were no peaks in the spectrum of the ATRP-initiator-modified silica surfaces.

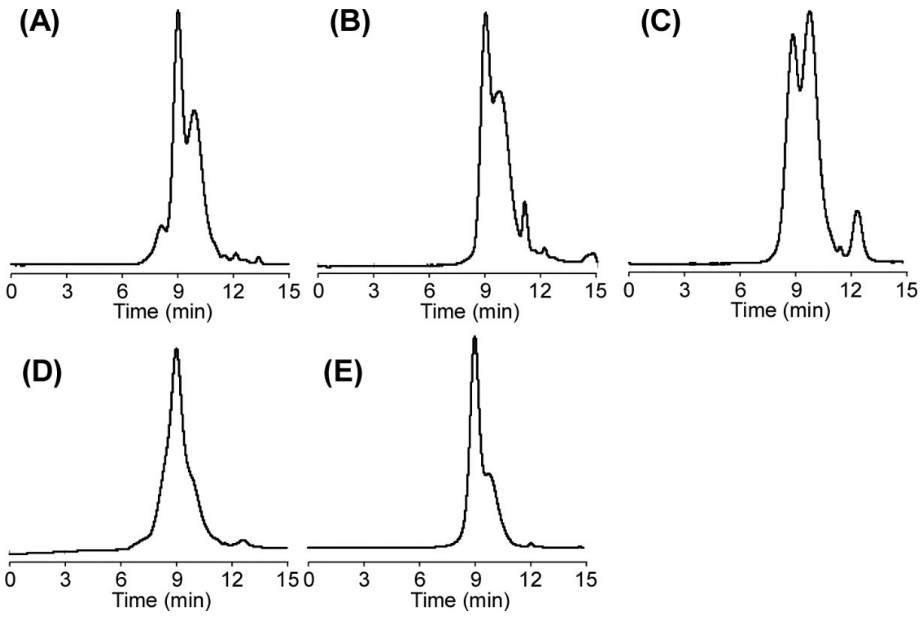



**Figure S2** Gel permeation chromatograms of PAPTAC-*b*-PIPAAm retrieved from silica bead surfaces to determine molecular weights: (A) 10A-1000IP, (B) 15A-1000IP, (C) 20A-1000IP, (D) 1000IP-*r*-15A, and (E) 1000IP (see Table 3).

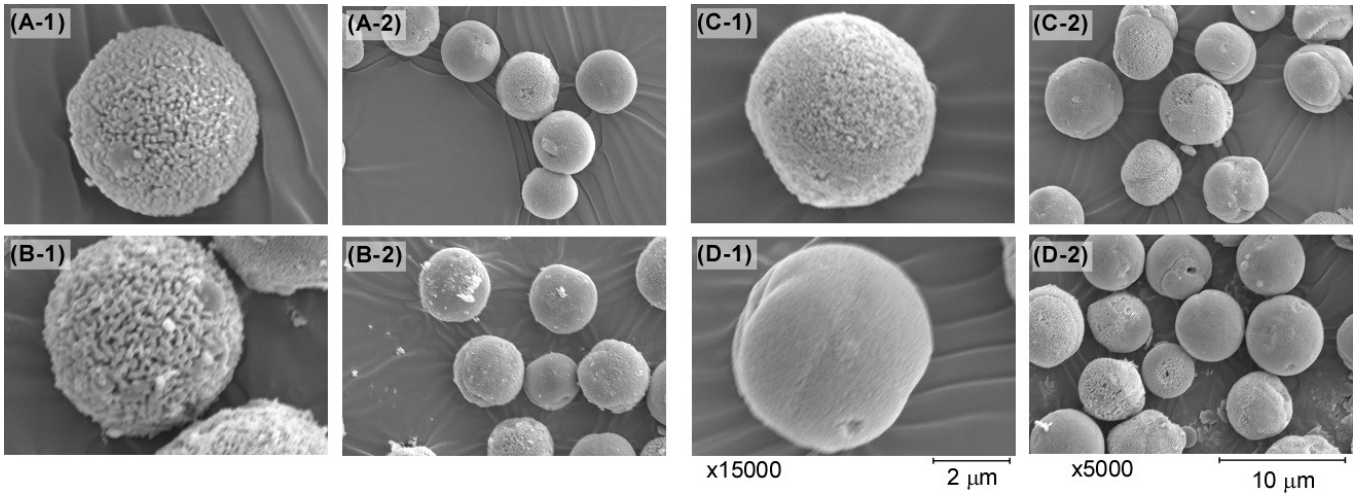



**Figure S3** (A-1, A-2) Scanning electron microscopy (SEM) images of non-modified silica beads; (B-1, B-2) SEM images of initiator-modified silica beads; (C-1, C-2) SEM images of PAPTAC brush-modified beads (15A in Table 1); (D-1, D-2) SEM images of PAPTAC-*b*-PIPAAm brush-grafted silica beads (15A-1000IP in Table 1). Background patterns in the images are double-sided tape that was used to affix the samples to stages. Images numbered (A-1), (B-1), (C-1), and (D-1) are high-resolution (×15 000 magnification).

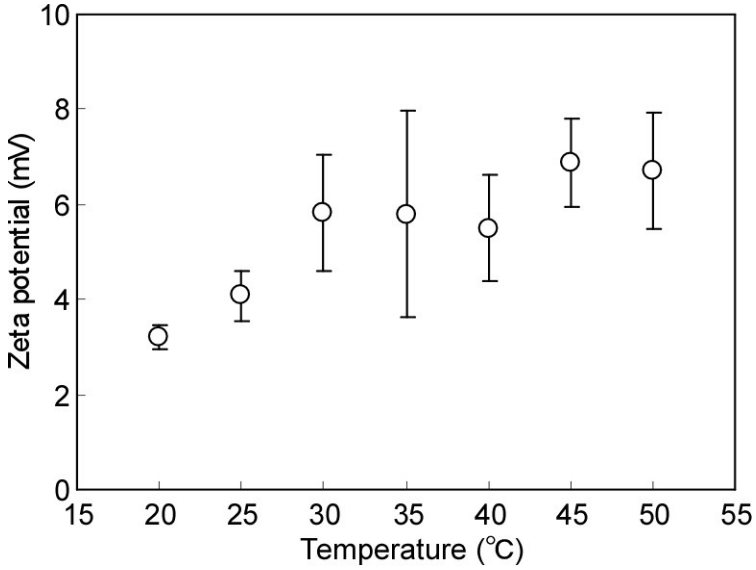



**Figure S4** Zeta potential of the PAPTAC-*b*-PIPAAm brush-grafted silica beads (15A-1000IP in Table 1) at various temperatures in a 5 mM KCl aqueous solution.

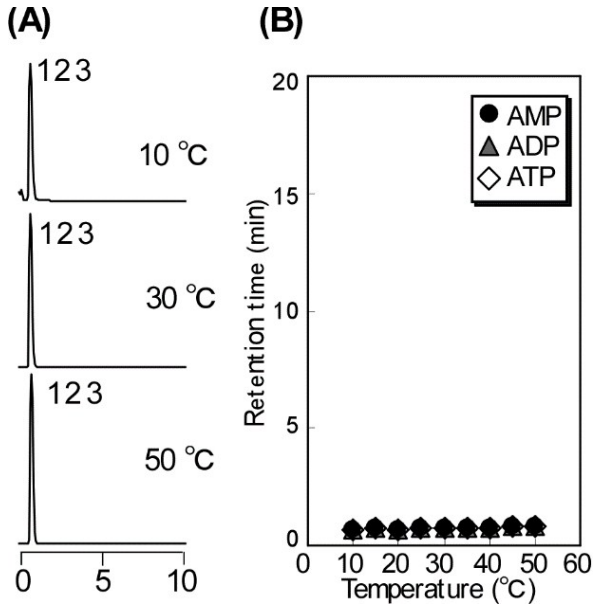



**Figure S5** Elution of adenosine nucleotides from a PI1000 column at various temperatures (A), and temperature-dependent retention times of adenosine nucleotides on PI1000 (B). Mobile phase is 66.7 mmol/L PB (pH 7.0). Peak 1 represents AMP; 2, ADP; 3, ATP.

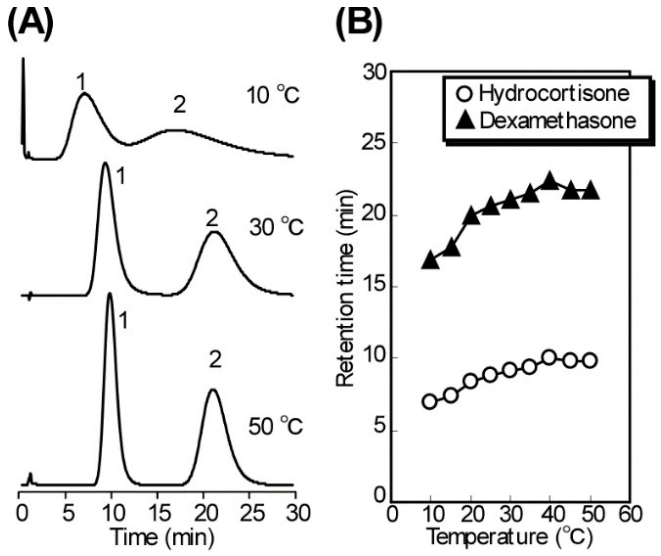



**Figure S6** Elution of steroids from a PI1000 column at various temperatures (A), and temperature-dependent retention times for steroids on PI1000 (B). Mobile phase is 66.7 mmol/L PB (pH 7.0). Peak 1 represents hydrocortisone; 2, dexamethasone.

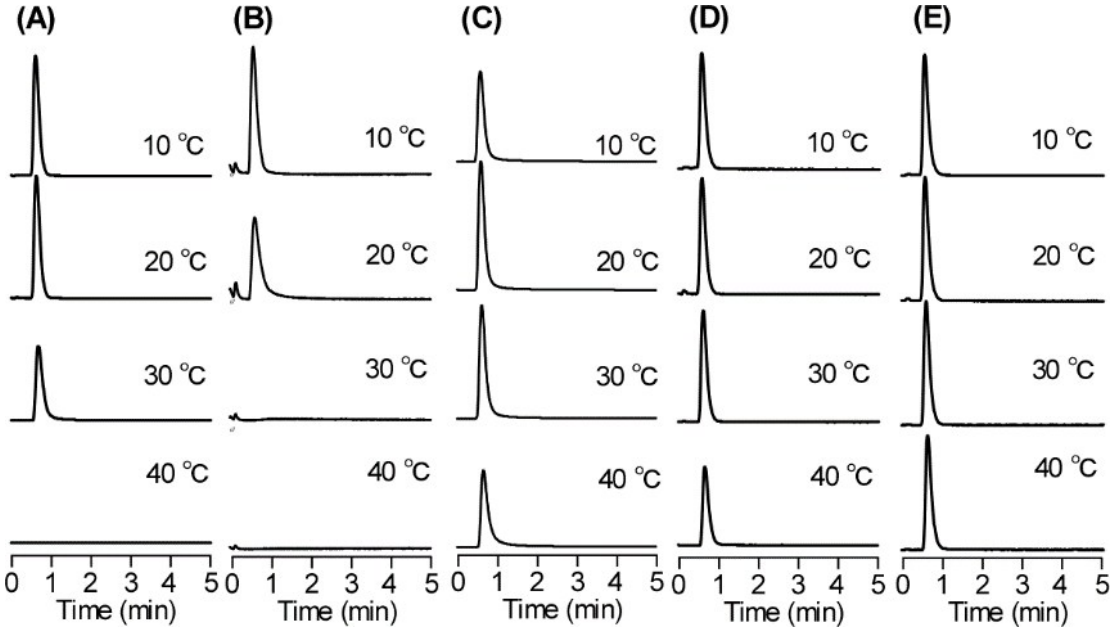



**Figure S7** Temperature-dependent elution of milk serum proteins from a 10A-1000IP column; (A) α-lactalbumin, (B) β-lactoglobulin, (C) lactoferrin, (D) bovine serum albumin, and (E) γ-globulin. Mobile phase is 66.7 mmol/L PB (pH 7.0).

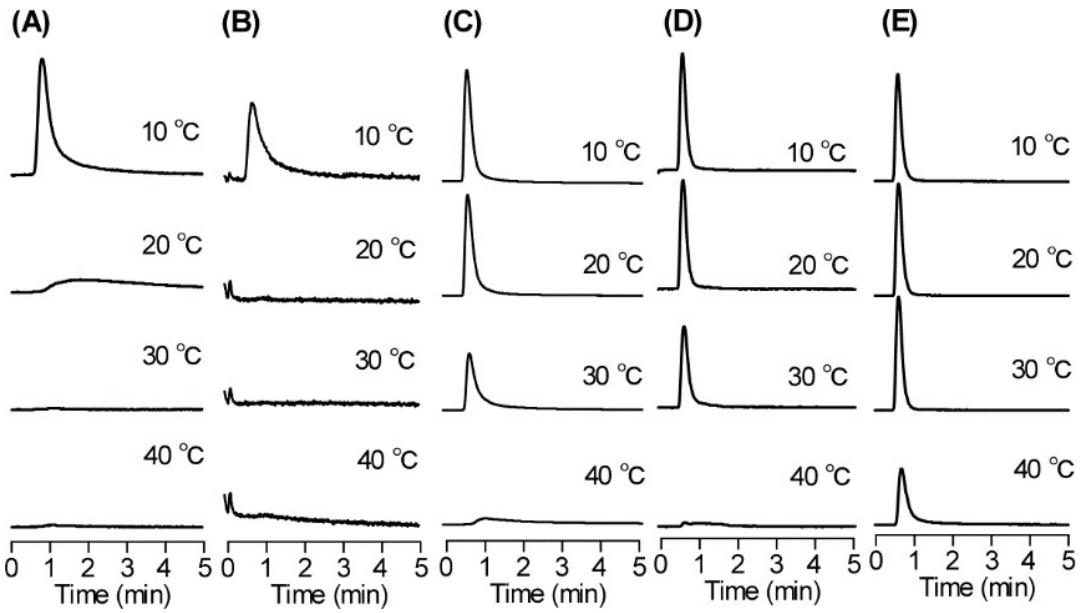



**Figure S8** Temperature-dependent elution of milk serum proteins from a 20A-1000IP column; (A) α-lactalbumin, (B) β-lactoglobulin, (C) lactoferrin, (D) bovine serum albumin, and (E) γ-globulin. Mobile phase is 66.7 mmol/L PB (pH 7.0).

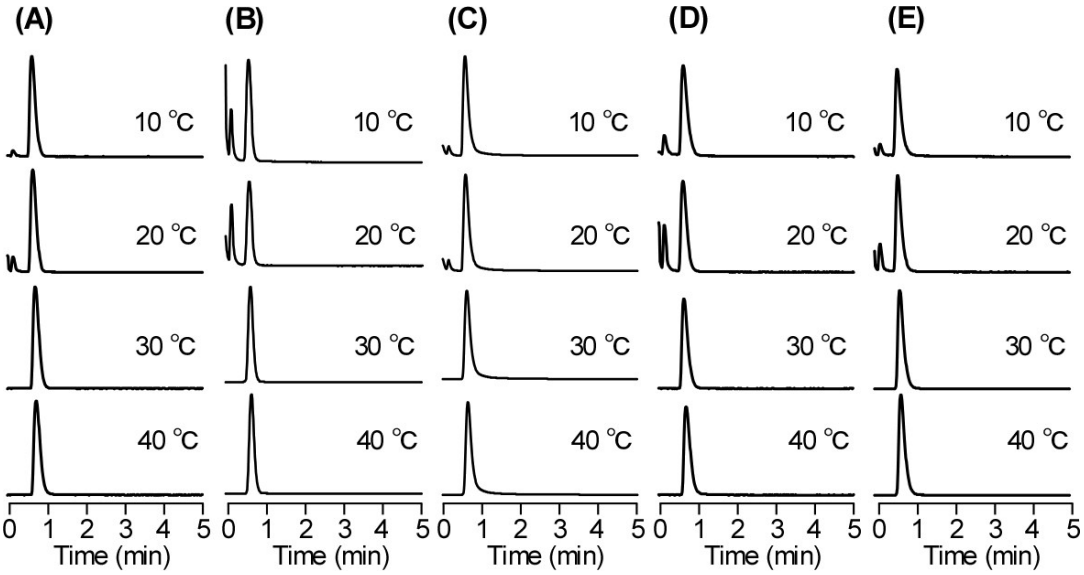



**Figure S9** Temperature-dependent elution of milk serum proteins from a 1000IP column; (A) α-lactalbumin, (B) β-lactoglobulin, (C) lactoferrin, (D) bovine serum albumin, and (E) γ-globulin. Mobile phase is 66.7 mmol/L PB (pH 7.0).

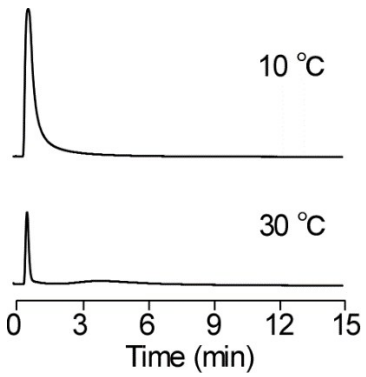



**Figure S10** Temperature-dependent elution of a mixture of milk serum proteins from 15A-1000IP column. Mobile phase is 66.7 mmol/L PB (pH 7.0).

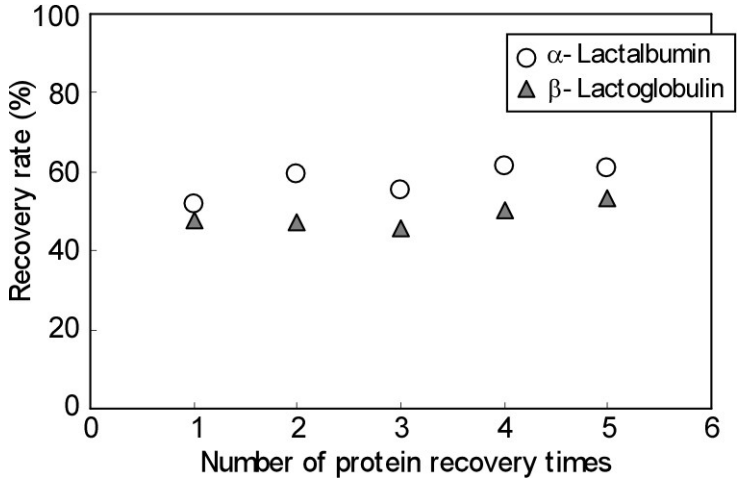



**Figure S11** Recovery rates for proteins with multiple thermally-modulated protein adsorption and desorption steps.

**Reference** (S1) Hansch, C.; Albert, L.; Hoekman, D., *In Exploring QSAR: Hydrophobic, Electronic and Steric Constant* American Chemical Society: Washinton DC, **1995**; p.174, 178.