Polyoxometalate-functionalized macroporous microspheres for selective separation/enrichment of glycoproteins

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Electronic Supplementary Information

Materials and reagents

Styrene (St), polyvinylpyrrolidone (PVP, average Mw~55000), immunoglobulin G from human serum (IgG, I4506, pI 8.0, Mr 150 kDa), transferrin human (Trf, T3309, pI 5.3-5.5, Mr 80 kDa), albumin from bovine serum (BSA, V900933, pI 4.9, Mr 66.4 kDa) and α-Lactalbumin (α-La, L5385, pI 4.2-4.5, Mr 14.2 kDa) were purchased from Sigma-Aldrich. Anhydrous ethanol (C_2H_5OH), aluminum oxide (Al_2O_3), sodium dodecyl sulfate (SDS), acrylic acid (AA), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC HCl), coomassie brilliant blue G250 and hexadecyl trimethyl ammonium bromide (CTAB) were purchased from Sinopharm Chemical Reagent Co., Ltd. Sulfuric acid (H_2SO_4) was acquired from Damao Chemical Co., Ltd. 2,2'-azoisobutyronitrile (AIBN) were obtained by Shanghai Sihewei Chemical Co., Ltd. 1-chlorodecane (CD) were ordered from J&K Scientific Ltd. Divinyl benzene (DVB, 80%) and 2-morpholineethane sulfonic acid (MES) were purchased from Macklin Biochemical Co., Ltd. Polyvinylalcohol (PVA, 1788 low-viscosity), N-hydroxysuccinimide (NHS) and polyethyleneimine (PEI, Mw~10000) were obtained by Aladdin Bio-Chem Technology Co., Ltd. All reagents were used as received except for St and DVB which were treated with aluminum oxide prior to use. Deionized water (18 MΩ cm) was used throughout the experiment. Human serum was acquired from the Hospital of Northeastern University.

Characterizations

Fourier transform infrared (FT-IR) spectrum of SPSDVB-PAA-PEI-P_5W_{30} was measured with a Nicolet 6700 spectrometer (Thermo Electron, USA) using a KBr disk from 500 to 4000 cm^{-1}. Surface charges of SPSDVB-PAA, SPSDVB-PAA-PEI and SPSDVB-PAA-PEI-P_5W_{30} were analyzed at pH 7.0 by Zetasizer NanoZS90 (Malvern, UK). The surface morphology of SPSDVB-PAA-PEI-P_5W_{30} was observed on a ZEISS Ultra/Plus scanning electron microscope (SEM, Zeiss, Germany). Transmission electron microscopy (TEM) images of SPSDVB-PAA-PEI-P_5W_{30} were obtained by a Tecnai G2 F30 (FEI, USA). Before TEM characterization, the particles were embedded in epoxy resin, cut into frozen sections (thickness: ~100 nm) and stained with phosphotungstic acid. Nitrogen adsorption/desorption experiment of SPSDVB-PAA-PEI-P_5W_{30} was measured by Autosorb-IQ-MP-C (Quantachrome, America). X-ray photoelectron spectroscopy (XPS) of SPSDVB-PAA-PEI-P_5W_{30} was recorded on an ESCALAB 250 surface analysis system (Thermo ESCALAB 250Xi, USA).

Preparation of PS and SPS particles

PS particles were synthesized according to the literature.[1] The sulfonation of PS particles (SPS) was conducted as follows. 1 g of PS particles was dispersed in 30 mL of H_2SO_4 under ultrasonication for 30 min. The suspension was stirred at 40°C for 2 h and then diluted with a mixture of deionized water and anhydrous ethanol (1:1 in V/V). After cooling to room temperature, the SPS particles were collected by washing several times with a mixture of deionized water and anhydrous ethanol until neutral pH, and then freeze-drying.

Preparation of SPSDVB-PAA

0.2 g of SPS particles was dispersed in 20 mL of SDS aqueous solution (0.25% w/w), and 0.1 mL of CD was dissolved in 10 mL of SDS aqueous solution (0.25% w/w). After ultrasonication for 5 min, the latter was added to the former solution dropwise and the mixture was stirred at 40°C for 20 h. Subsequently, 0.5 mL of St, 2 mL of DVB, 1 mL of AA and 40 mg of AIBN were dissolved in 10 mL of SDS aqueous solution (0.25% w/w) and sonicated for 5 min to form emulsion. The emulsion was quickly added to the SPS particle suspension and kept stirring for 6 h. Then 5 mL of PVA aqueous solution (1% w/w) was added and bubbled with nitrogen for 5 min. The polymerization reaction was carried out at 70°C for 14 h in the absence of oxygen. The final product was washed with ethanol and deionized water for several times and then freeze-dried to obtain SPSDVB-PAA.

Preparation of SPSDVB-PAA-PEI-P_5W_{30}

P_5W_{30} was prepared according to the literature.[2] 0.1 g of SPSDVB-PAA microspheres was dispersed into 30 mL of 0.1 mol L^{-1} MES. After adding 0.3 g of NHS and 0.3 g of EDC HCl, the mixture solution was stirred for 30 min at room temperature. 3 mL of PEI aqueous solution (10% w/w) was added into the above solution and incubated for 12 h at room temperature. After centrifugation (15000 rpm, 10 min) and rinse with deionized water for several times, the SPSDVB-PAA-PEI was acquired. The product was redispersed in 5 mL of deionized water, and then 20 mL of P_5W_{30} aqueous solution (1% w/w) was added to the above suspension and incubated for 5 h at room temperature. The product SPSDVB-PAA-PEI-P_5W_{30} was rinsed several times with deionized water until there was no absorption peak in the supernatant, and then obtained by freeze-drying.

Adsorption/desorption of proteins by SPSDVB-PAA-PEI-P_5W_{30}

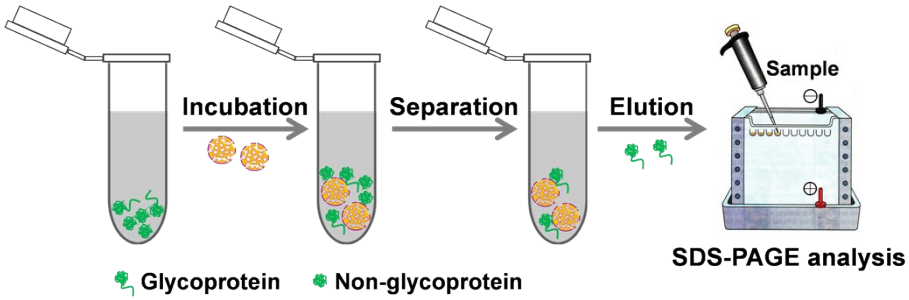
The adsorption performance of SPSDVB-PAA-PEI-P_5W_{30} to proteins was studied at 4 mmol L^{-1} Britton-Robinson (B-R) buffer within the pH ranged from 5.0 to 9.0. Typically, 2 mg of SPSDVB-PAA-PEI-P_5W_{30} was incubated with 1 mL of 100 μg mL^{-1} protein solution and the mixture was vibrated for 30 min. The supernatant was collected by centrifuging at 8000 rpm for 5 min and the absorbance was measured at 595 nm with coomassie brilliant blue dye to determine residual protein concentration. The material was pre-washed with 1 mL of pH 7.0 B-R buffer and then 1 mL of 0.01% CTAB was added and oscillated for 30 min to strip the captured proteins. The eluent was collected by centrifugation (8000 rpm, 5 min) to evaluate the recovery of proteins.

Separation/enrichment of glycoproteins from protein mixture by SPSDVB-PAA-PEI-P_5W_{30}

Protein mixture solution containing 100 μg mL^{-1} Trf, IgG, BSA and α-La at pH 7.0 B-R was prepared to obtain a mass ratio of Trf: IgG: BSA: α-La=1:1:1:1. Furthermore, protein mixtures of BSA and IgG with high mass ratios of 20:1, 50:1, 100:1 and 150:1 were employed to assess the selectivity of SPSDVB-PAA-PEI-P_5W_{30} to glycoproteins. The concentration of β-ca remained constant at 200 μg mL^{-1}, and the BSA concentrations were 4 mg mL^{-1}, 10 mg mL^{-1}, 20 mg mL^{-1} and 30 mg mL^{-1}, respectively. 4 mg of SPSDVB-PAA-PEI-P_5W_{30} was mixed with 1 mL of above protein mixture samples. The adsorption-washing-elution process was the same as above mentioned except that 0.5 mL of 0.01% CTAB was added, and the elution step was repeated three times. The elution fractions were combined, and concentrated to 200 μL by ultrafiltration. Due to the high content of BSA, different proportions of the original protein mixtures were diluted before SDS-PAGE assay, and the loading quantity of BSA was kept at 3 μg. Thus, the diluted protein sample, supernatant, and eluate were collected for further analysis by SDS-PAGE, respectively.

Separation/enrichment of glycoproteins from complex biological samples by SPSDVB-PAA-PEI-P_5W_{30}

The human serum sample was 100-fold diluted with pH 7.0 B-R. The *E. coli* DH5α lysate was prepared according to the literature reported.[3] 0.5 mL of *E. coli* DH5α was added into 200 mL of fresh sterile lysogeny broth (LB) medium and incubated at 37°C and 250 rpm for 12 h until OD_{600}=1.6. 6 mL of *E. coli* bacteria was collected by centrifugation at 4°C (8000 rpm, 5 min) and rinsed with deionized water for three times. The precipitate was redispersed in 6 mL of pH 7.0 B-R and sonicated for 50 times at 200 W. After centrifugation at 4°C (8500 rpm, 30 min), the supernatant was collected. After measurement of the protein concentration by coomassie brilliant blue dye, the standard IgG was added to obtain spiked *E. coli* lysate with a mass ratio of IgG: *E. coli*=1:10. 4 mg of SPSDVB-PAA-PEI-P_5W_{30} was mixed with 1 mL of biological samples. The adsorption-washing-elution process was the same as above mentioned except that 0.5 mL of 0.01% CTAB was added, and the elution step was repeated three times. The original sample, supernatant, and eluate were collected for further analysis by SDS-PAGE, respectively.



Scheme S1 The general procedure of glycoprotein separation.

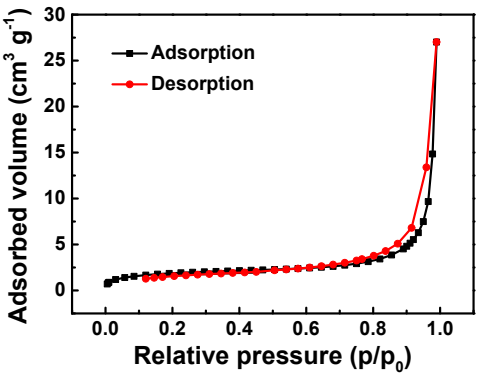


Fig. S1 Nitrogen adsorption-desorption isotherm of SPSDVB-PAA-PEI-P_5W_{30}.

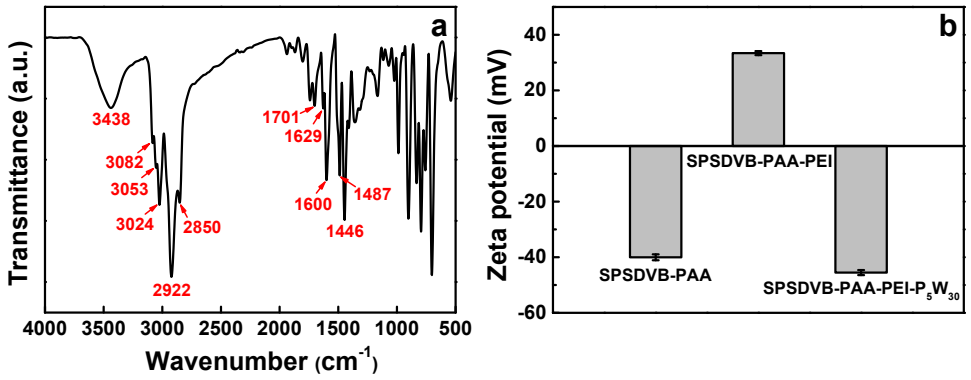


Fig. S2 (a) FT-IR spectrum of SPSDVB-PAA-PEI-P_5W_{30} and (b) Zeta potentials of SPSDVB-PAA, SPSDVB-PAA-PEI and SPSDVB-PAA-PEI-P_5W_{30} at pH 7.0.

In FT-IR spectrum, the peaks at 3082 cm^{-1}, 3053 cm^{-1} and 3024 cm^{-1} are assigned to -CH= stretching vibration of benzene,[4] and the peaks at 2922 cm^{-1} and 2850 cm^{-1} correspond to the stretching vibration of -CH_2- in benzene ring. The characteristic band at 1701 cm^{-1} is C=O stretching vibration of PAA. The peaks observed at 1600 cm^{-1}, 1487 cm^{-1} and 1446 cm^{-1} are ascribed to bending vibration band of C=C in benzene ring. These peaks demonstrate that the synthesized particles are composed of poly (St-DVB) and PAA. The peaks at 3438 cm^{-1} and 1629 cm^{-1} are attributed to N-H band stretching and bending vibration of PEI, respectively, indicating the successful coupling of PEI with SPSDVB-PAA.

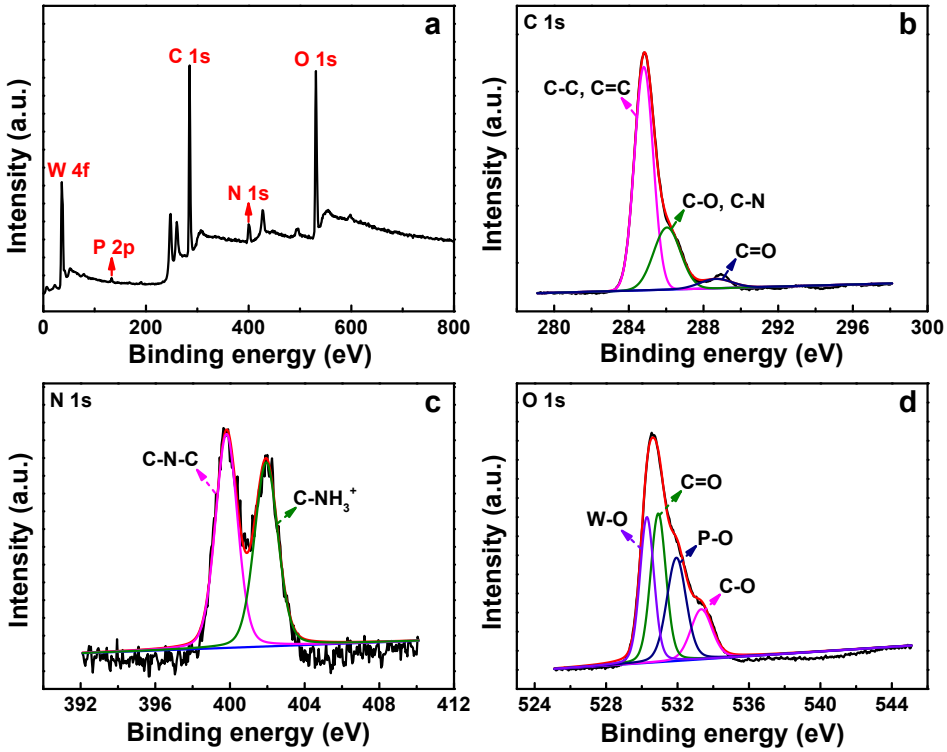


Fig. S3 (a) XPS spectrum of SPSDVB-PAA-PEI-P_5W_{30} and high resolution (b) C 1s, (c) N 1s and (d) O 1s XPS spectra.

SPSDVB-PAA-PEI-P_5W_{30} possesses W 4f, P 2p, C 1s, N 1s and O 1s peaks, indicating that the product is mainly composed of W, P, C, N and O elements (Fig. S3a). In high resolution C 1s XPS spectrum (Fig. S3b), the peak at 284.8 eV is attributed to C-C and C=C bonds in benzene ring.[5] The peak at 286.0 eV corresponds to C-O of PAA and C-N of PEI. The peak observed at 288.7 eV is ascribed to C=O of PAA. In high resolution N 1s XPS spectrum (Fig. S3c), the peaks at 399.8 eV and 401.9 eV are derived from branched chains C-N-C and terminal amino groups C-NH_3^+ in PEI.[3] The terminal amino groups can bind with the oxygen atoms in P_5W_{30} by electrostatic interaction. In high resolution O 1s XPS spectrum (Fig. S3d), the

peaks at 530.9 eV and 533.3 eV correspond to C-O and C=O,[6] and the peaks observed at 530.3 eV and 531.9 eV are derived from W-O and P-O bonds in P_5W_{30}.[7]

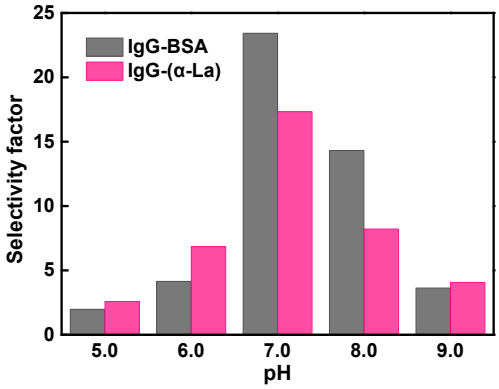


Fig. S4 The selectivity factors of IgG to BSA and α-La.

Selectivity factor is defined as the relative ratio of capacity factor, reflecting the selectivity of stationary phase (material) to target analyte (glycoproteins). In sample pretreatment, the capacity factor is calculated as follows: Capacity factor (k)=the amount of proteins adsorbed on the material / the amount of proteins dissolved in the mobile solvent under equilibrium conditions. Thus, the capacity factors of IgG, BSA and α-La at different pH values are obtained according to Fig. 2a, and selectivity factors of IgG to BSA and α-La are shown in Fig. S4. It is known that the higher selectivity factors leading to the better separation effects. Therefore, the present material can achieve the separation of IgG from BSA and α-La, and the optimal separation effect is acquired at pH 7.0.

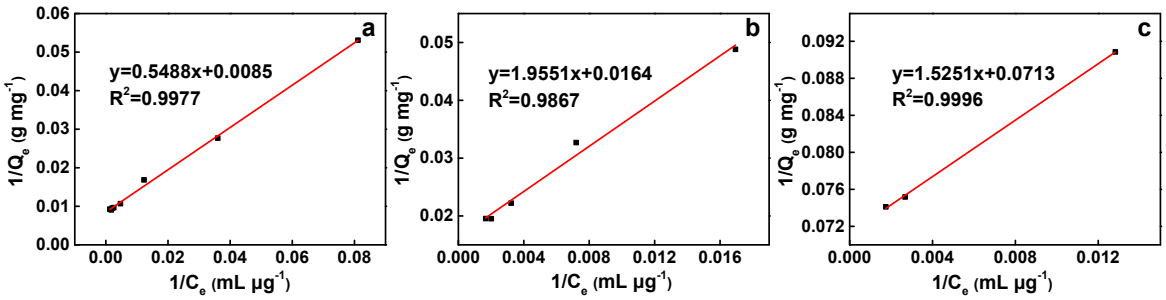


Fig. S5 *Langmuir* models of (a) IgG, (b) Trf and (c) BSA on SPSDVB-PAA-PEI-P_5W_{30}.

Adsorption isotherms of proteins are fitted well with *Langmuir* model as expressed in the following equation:

$$\frac{1}{Q_e} = \frac{K_d}{Q_{max} \cdot C_e} + \frac{1}{Q_{max}}$$

Where Q_e, Q_{max}, C_e and K_d represent the equilibrium adsorption capacity, the maximum adsorption capacity, the equilibrium protein concentration and dissociation constant, respectively. Theoretical maximum adsorption capacities of IgG, Trf and BSA were concluded to be 117.6 mg g^{-1}, 61.0 mg g^{-1} and 14.0 mg g^{-1}, according with the experimental results.

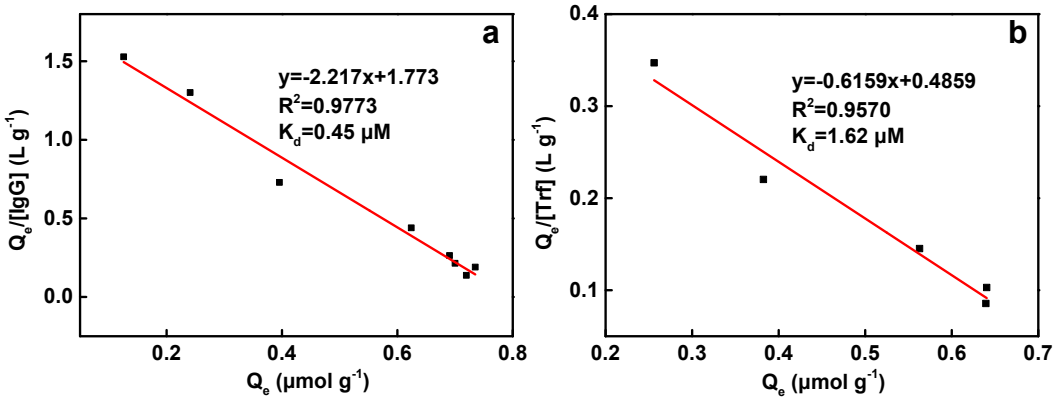


Fig. S6 Scatchard plots for the binding of SPSDVB-PAA-PEI-P_5W_{30} with (a) IgG and (b) Trf.

Scatchard analysis was performed as previously reported.[8] The Scatchard relationship can be established by the following equation:

$$Q_e/[S]=(Q_{max}-Q_e)/K_d$$

Where Q_e is the amount of glycoprotein adsorbed on SPSDVB-PAA-PEI-P_5W_{30} at equilibrium; [S] is the free concentration at adsorption equilibrium; Q_{max} is the maximum adsorption capacity and K_d is the dissociation constant. By plotting Q_e/[S] versus Q_e, K_d can be obtained from the slope. The Scatchard plots of SPSDVB-PAA-PEI-P_5W_{30} binding with glycoproteins showed a straight line, demonstrating that there was only a single type of binding site between the material and glycoproteins. The binding affinities of SPSDVB-PAA-PEI-P_5W_{30} with IgG and Trf give K_d values of 0.45 μM and 1.62 μM, respectively. Therefore, the material functionalized by polyoxometalates exhibited dissociation constants of 10^{-6} M towards glycoproteins, which are 3-4 orders of magnitude higher than the affinity of single boronic acid binding with glycoproteins.

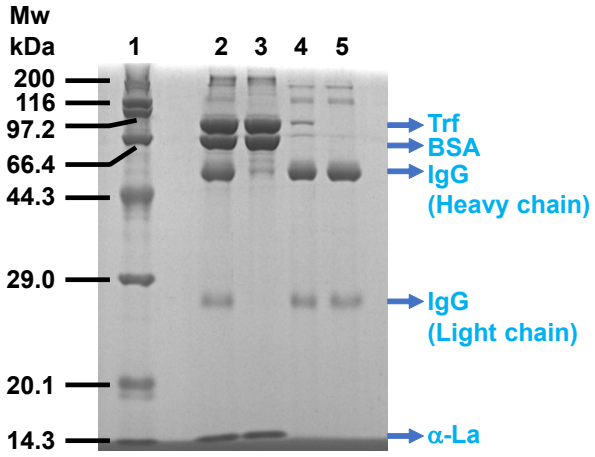


Fig. S7 SDS-PAGE assay of standard protein mixture before and after treatment with SPSDVB-PAA-PEI-P_5W_{30}. Lane 1: molecular weight marker (kDa); Lane 2: protein mixture (Trf: BSA: IgG: α-La=1:1:1:1, 100 μg mL^{-1}); Lane 3: the supernatant of protein mixture after treatment; Lane 4: the eluent of protein mixture after treatment; Lane 5: standard IgG solution (100 μg mL^{-1}).

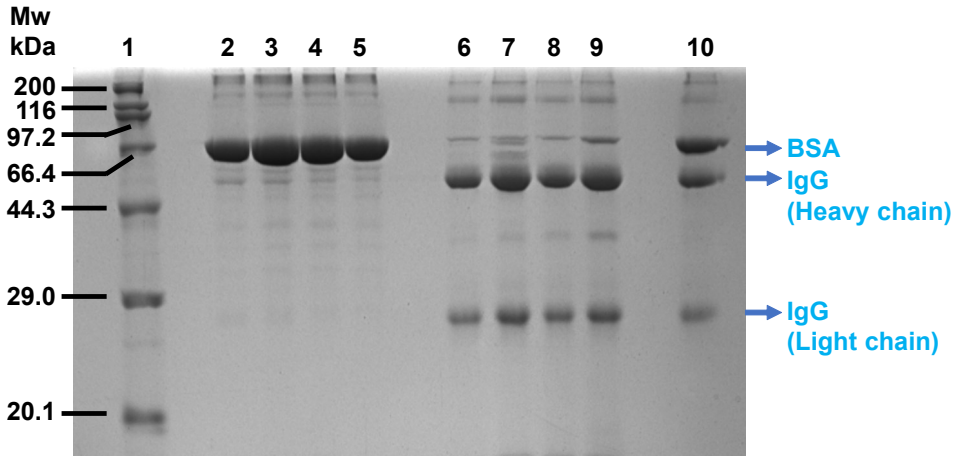


Fig. S8 SDS-PAGE assay of different mass ratios of BSA and IgG before and after treatment with SPSDVB-PAA-PEI-P_5W_{30}. Lane 1: molecular weight marker (kDa); Lane 2-5: 20, 50, 100 and 150-fold diluted protein mixture (BSA: IgG=20:1, 50:1, 100:1 and 150:1, IgG at 200 μg mL^{-1}); Lane 6-9: the eluent of protein mixture (BSA: IgG=20:1, 50:1, 100:1 and 150:1) after treatment; Lane 10: protein mixture of BSA: IgG=1:1 (200 μg mL^{-1}).

Table S1 Comparisons of the enrichment performance of various materials to glycoproteins.

Materials	Mass ratio of BSA/IgG	Real samples	Ref.
PHEMA/PGMA-IDA-Cu^{2+}	—	Human serum	9
His-MWNTs	20:1	Human serum	10
PW_{12}@TiO_2–Si(Et)Si/Pba	—	Human serum	11
Fe_3O_4@PEI@POM1 NPs	—	Human serum	12
CPBA-Ni_6PW_9/SA	—	Human serum	13
SPSDVB-PAA-PEI-P_5W_{30}	150:1	Human serum; IgG-spiked *E. coli* lysate	This work

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