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Supporting information
For

One-pot synthesis of phenylboronic acid-functionalized core-shell magnetic nanoparticles for selective enrichment of glycoproteins†

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EXPERIMENTAL SECTION

Reagents and Materials. Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), sodium acetate(CH3COONa, NaOAc), ethylene glycol (EG), 1,6-hexamethylenediamine (HDA), acetonitrile (ACN), isopropanol and ethanol were obtained from Sinopharm Chemical Reagent Shanghai, Co., Ltd. (Shanghai, China). Tetraethoxysilane (TEOS) and 2,2-azobis-(isobutyronitrile) (AIBN) were obtained from Aladdin reagent Co., Ltd (Shanghai, China). γ-methacryloxypropyltrimethoxysilane (γ-MAPS) were purchased from the Hubei Wuhan University Silicone New Material Co., Ltd (Wuhan, China). 4-vinylphenylboronic acid (VPBA) and ethylene glycol dimethacrylate (EDMA) were obtained from AlfaAesar (Beijing, China). All reagents above were of analytical grade or better. Human IgG was purchased from Fuzhou Lanhao Bio-pharma Co., LTD (Shanghai, China). Ovalbumin (OVA), bovine serum albumin (BSA), human serum albumin (HSA) and lysozyme (Lyz) were purchased from Shanghai Lanji Co. Ltd. (Shanghai, China). Partially purified mucin from the porcine stomach (Muc, M1778) was purchased from Sigma (St. Louis, MO). Deionized water was prepared with a Milli-Q water purification system (Millipore, Milford, MA).

Syntheses of Fe_3O_4 MNPs. Fe_3O_4 MNPs were prepared by following the method described in the literature [1]. Briefly, $FeCl_3 \cdot 6H_2O$ (1.0 g), NaOAc (4.0 g), HDA(3.6 g) were dissolved in EG (30 mL). The obtained homogeneous yellow solution was transferred to a Teflon-lined stainless-steel autoclave, sealed and heated to 200℃. After reaction for 6.5h, the autoclave was cooled to room temperature. The obtained

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Fe_3O_4MNPs were washed several times with water, ethanol and isopropanol orderly.

One-Pot Synthesis of Fe_3O_4@SiO_2@VPBAMNPs. Fe_3O_4@SiO_2@VPBA MNPs were synthesized as follows. Briefly, the as-synthesized Fe_3O_4 were resuspended in 60 mL isopropanol with the help of ultrasonication, and then 5mL ammonia solution (15 wt %) was added. Subsequently, 20 mL isopropanol solution containing TEOS (3.0 mL) and γ-MAPS (0.50 mL） was injected into the above suspension at a rate of 2 mL/h under mechanical stirring and the reaction was allowed to proceed at 40 ℃. After all of the solution has been added, the reaction was continued for 7 h. Then, 30ml ethanol containing EDMA (0.6 mL), VPBA (0.3 g) and AIBN (0.3 g) was added dropwise within 1.5 h at 70 ℃. After another 10 h of heating, the product is collected by magnet and washed several times with ethanol, diluted hydrochloric acid, and water several times dried under ambient conditions.

Characterizations. The TEM images were taken using a FEI (Netherlands) Tecnai-20 transmission electronic microscopy (TEM) instrument. The vibrating sample magnetization (VSM) curves of Fe_3O_4 and Fe_3O_4@SiO_2@VPBA MNPs were analyzed by using a LDJ9600 vibrating sample magnetometer (LDJ Electronics, Troy, MI). Fourier transform infrared (FT-IR) spectra of MNPs were conducted with a FT-IR spectrophotometer (Nicolet 6700, Waltham, MA, USA). XPS spectra were obtained using X-ray photoelectron spectrophotometer (Thermo Scientific Escalab 250, USA). The crystal structure of the MNPs was determined by X-ray powder diffraction (XRD) (D/Max-2500 diffractometer, Shimadzu, Japan). Thermogravimetric analysis (TGA) was performed for power samples with a heating

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rate of 10℃/min using Simultaneous Thermal Analyzer (Netzsch STA 449, Germany) under nitrogen atmosphere up to 800℃. The data of adsorption were obtained by using UV/VIS Spectrophotometer (PerkinElmer Lambda 800, USA). Electrophoresis for protein separation was carried out by regular SDS-PAGE with 10% polyacrylamide gel and 5% stacking gel according to the manual introduction (Bio-Rad, Hercules, CA, USA).

Protein adsorption isotherm. Protein adsorption was performed with the binding buffer as described by Borlido [2] with some modifications, where the binding buffer was consisted of 25 mM phosphate buffer (pH 9.0) and 110 mM NaCl. The MNPs were incubated for 3 h with 1 mL of each protein solutions prepared in binding buffer. Then, the MNPs were decanted by applying an external magnetic field and the supernatant was determined by using a UV/Vis spectrophotometer. The capacity (Q mg/g) was calculated according to the difference in protein concentration before and after adsorption, the volume of aqueous solution and the weight of the nanoparticles according to

$$Q=(C_0-C_t)V/m \quad (1)$$

where C_0 is the initial protein concentration (mg/mL), C_t is the supernatant protein concentration (mg/mL), V is the volume of protein solution (mL) and m is the weight of the nanoparticles (g).

To obtain the maximum adsorption capacity of MNPs for proteins, Langmuir regression equation was adopted as follow:

$$Q=Q_{max}\, C_{eq}/(K_d+ C_{eq}) \quad (2)$$

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Where Q is the amount of adsorption capacity of proteins on the MNPs (in mg/g); C_{eq} is the equilibrium protein concentration in the solution (in mg/mL); K_d represents the dissociation constant; and Q_{max} is the maximum adsorption capacity (in mg/g).

Protein adsorption kinetics. To evaluate the binding kinetics of the Fe_3O_4@SiO_2@VPBAMNPs, 1 mg MNPs were incubated with 1 mL of the equilibrium concentration of protein solution for different times. The adsorption capacity was quantified as mentioned above.

Enrichment of glycoproteins by Fe_3O_4@SiO_2@VPBA MNPs. In order to further demonstrate the specific adsorption of the phenylboronic acid-functionalized MNPs to glycoprotein, enrichment of glycoprotein from a binary proteins mixture (BSA and IgG) was performed. For glycoprotein capture, about 3 mg of MNPs were incubated in 0.9 mL a solution of BSA (300 μg /mL) and IgG (600 μg /mL) in the binding buffer. After reaction, the MNPs were washed several times with 50% acetonitrile and then incubated with 50 μL, 2 × reducing elution buffer, which was consisted of 2.0 mL Tris-HCl (0.5M, pH 6.8), 4.0 ml SDS (10%), 2.0 mL glycerol, 1.0mL b-mercaptoethanol, 0.5mL bromophenol blue (0.1wt%) and 0.5 mL H_2O, After the enrichment procedure, original protein mixture and eluent were analyzed by 12% SDS-PAGE gels. Quantification of the protein bands was carried out with Quantity One Software (Bio-Rad).

Real sample analysis. 6 mg Fe_3O_4@SiO_2@VPBAMNPswere immersed in 1 mL human serum sample that had been diluted 50-fold with binding buffer. After

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incubation for 3 h at room temperature under gentle shaking, the MNPs were washed with 50%(v/v) ACN/H_2O solution several times to remove nonspecific binding proteins. Then, the bound glycoproteins were eluted by 40 μL 1×SDS-PAGE elution buffer. Finally, the supernatant and the eluted proteins were collected and analyzed by SDS-PAGE.

Reference

[1] L.Y. Wang, J. Bao, L. Wang, F. Zhang and Y.D. Li, Chem. Eur. J. 2006, 12, 6341-6347.

［2］ L. Borlido, A.M. Azevedo, A.C.A. Roque, M.R. Aires-Barros, J. Chromatogr. A. 2011,1218, 7821-7827.

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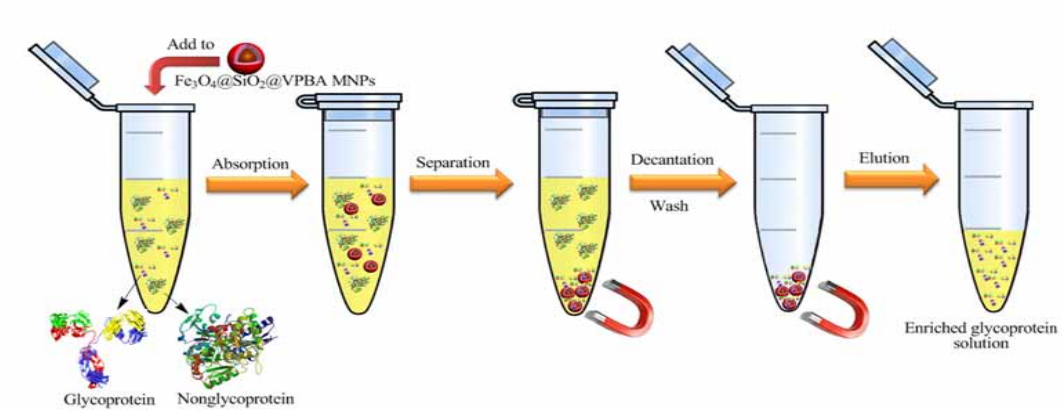
Supporting Figures



Scheme S1. VPBA-functionalized MNPs for the isolation and enrichment of glycoproteins with the help of magnet.

Scheme S1

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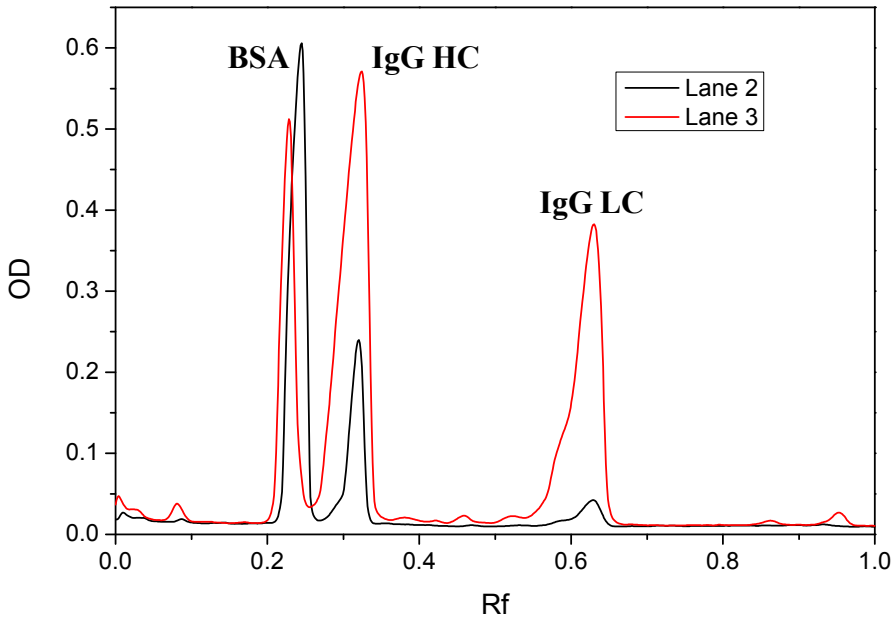

Fig. S1

Gel images were obtained using a GS-800 Calibrated Densitometer (Bio-Rad) and quantification of the protein bands was carried out with Quantity One software (Bio-Rad).

Optical density (OD) measures were corrected for background (via subtraction).

Units of protein quantity(OD*mm^2) were obtained by recording the average OD for each band minus a modal background density, and then multiplying by the area occupied by the band.

Rf = distance migrated/gel length