Supporting Information

Functionalized Polyoxometalate Microspheres Ensure Selective Adsorption of Phosphoproteins and Glycoproteins

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Chemicals and Reagents

Sodium tungstate dihydrate ($Na_2WO_4 \bullet 2H_2O$, >99.5%) and tetrabutylammonium bromide were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). 3-isocyanatopropyltriethoxysilane and 3-aminophenylboronic acid (>98%) were achieved from Aladdin (Shanghai, China). Acetonitrile (ACN) and diethyl ether were obtained from Tianjin YongDa Chemical Reagent Co., Ltd. (Tianjin, China). Tris(hydroxymethyl)aminomethane (Tris, >99.5%) was the product from Dalian Meilun Biotechnology Co. Ltd. (Dalian, China).

β-casein from bovine milk (β-ca, >98%, Mr 26.6 kDa, pI 4.6-4.8), ovalbumin from chicken egg white (OVA, >98%, M_r 44.3 kDa, pI 4.7), γ-globulin from bovine serum (γ-Glo, M_r 150 kDa, pI 6.3-7.3), immunoglobulin G from human serum (IgG, >95%, M_r 150.0 kDa, pI 6.95), transferrin human (Trf, >98%, M_r 78 kDa, pI 5.2-5.9), bovine serum albumin (BSA, >98%, M_r 66.4 kDa, pI 4.7-4.9), and cytochrome c (cyt-c, Mr 12.4 kDa, pI 10.7) were obtained from Sigma-Aldrich (St Louis, USA). The protein molecular weight marker (Board, 3597A) was purchased from Takara Biotechnology Co., Ltd. Human serum sample of healthy volunteer was collected from the Hospital of Northeastern University.

All the reagents are at least of analytical-reagent grade and used without further purification. Deionized water of 18 MΩ cm was used throughout the experiments. In addition, all the experiments with human blood and serum samples were carried out in accordance with the current guidelines for the care of laboratory animals and were approved by Animal and Medical Ethics Committee of Northeastern University.

Instrumentations

UV-vis absorption spectra were collected on a U-3900 UV-vis spectrophotometer (Hitachi High Technologies, Japan) and microplate reader (BioTek Instruments, Inc., USA). Fourier transform infrared spectra (FT-IR) were recorded in the solid state using potassium bromide pellets on a VERTEX 70 FT-IR spectrophotometer (Brooker, Germany) from 4000 to 400 cm^{-1}. Thermogravimetric analysis (TGA) was carried out and recorded on a TGA/DSC3+ analyzer with a heating rate of 10 °C per min (25-800˚C) under a continuous N_2 flow (TGA, METTLER TOLEDO, Switzerland). X-ray diffraction (XRD) patterns were obtained on an Empyrean (BRUKER, Germany) with Cu Kα radiation at λ 1.54 Å. Scanning electron microscopy (SEM) images and energy

dispersive spectrum (EDS) were operated on a SU8010 scanning electron microscope (Hitachi High Technologies, Japan). Transmission electron microscopy (TEM) image was operated on a JEM2100PLUS transmission electron microscope (JEOL, Japan). X-ray photoelectron spectroscopy (XPS) measurements were progressed on a Thermo Scientific ESCALAB 250Xi electron spectrometer (Thermo Electron, America). Circular dichroism (CD) spectra were obtained on a MOS-450 (Bio-Logic, France) automatic recording spectropolarimeter with nitrogen protection.

Preparation of Si-APBA and PW_9-Si-APBA microspheres

The organosilicon reagent modified by boronic acid groups was first prepared. Shortly, 0.30 g of 3-aminophenylboronic acid (APBA) was dispersed in 50 mL acetonitrile (ACN) and then 500 μL of 3-isocyanatopropyltriethoxysilane was added. The reaction mixture was stirred for 24 h at 80°C. Afterwards, the boronic acid modified silylating linker Si-APBA was obtained by centrifugation at 8000 rpm for 10 min followed by washing with ethyl alcohol.

The lacunary polyoxometalate $Na_9[\alpha\text{-}PW_9O_{34}]\cdot xH_2O$ (PW_9) was prepared by following a previous procedure [1]. Thereafter, 1.27g of PW_9 was dispersed to 22 mL of H_2O/ACN (1:10 v/v), and 0.62 g of tetrabutylammonium bromide was added under vigorous stirring. Then 475 μL of the above prepared silylating linker Si-APBA and 1.44 mL of HCl (4 mol L^{-1}) were added consecutively. The reaction mixture was allowed to stand for 12 h at room temperature under vigorous stirring. Afterwards, the insoluble material was removed by filtration. The filtrate was evaporated to dryness at 50°C and re-dispersed in 2.0 mL acetonitrile. The product precipitated by adding 40 mL DI water and then collected by centrifugation at 8000 rpm for 5 min. After washing with DI water and diethyl ether alternatively for three times, the final product was obtained by natural drying.

The synthesis process of PW_9-Si hybrid complies with the PW_9-Si-APBA microspheres. The difference is that 3-isocyanatopropyltriethoxysilane was used directly instead of Si-APBA.

Protein adsorption by PW_9-Si-APBA microspheres

20 μL of PW_9-Si-APBA microspheres aqueous suspension (10 mg mL^{-1}) was added into a mixture containing 20 μL of protein solution (1.0 mg mL^{-1}) and 160 μL of Britton−Robinson (BR) buffer (40 mmol L^{-1}, pH 5.0). The adsorption progress was carried out under oscillating for 20 min at room temperature. PW_9-Si-APBA microspheres with adsorbed proteins were collected by centrifugation at 6000 rpm for 5 min. 200 μL of Tris-HCl buffer (pH 8.0, 0.1 mol L^{-1}) was used as stripping reagent to recover the retained proteins with shaking for 20 min, and then the recovered solution was obtained followed by centrifugation at 6000 rpm for 5 min. The quantification of recovered protein was performed by UV-vis absorption spectrophotometry and microplate reader by recording the characteristic absorption of proteins (410 nm for cyt-c, 595 nm for β-ca, OVA, IgG, γ-Glo, Trf, and BSA, staining with Coomassie brilliant blue).

The adsorption isotherms of proteins

To evaluate the enrichment capacity of PW_9-Si-APBA microsphere toward phosphoproteins and glycoproteins, 0.10 mg of PW_9-Si-APBA was used for adsorption of proteins in a range of 60-700 μg mL^{-1} (200 μL, 40 mmol L^{-1} BR buffer, pH 5.0). The adsorption capacity Q_e (mg g^{-1}) was calculated according to the follow equation. C_0 (mg mL^{-1}) is the initial concentration of protein, C_e (mg mL^{-1}) is the equilibrium concentration of protein after extraction, V (mL) is the volume of adsorption system and m (mg) is the mass of PW_9-Si-APBA microspheres.

$$\boldsymbol{Q_e = \frac{(C_0 - C_e)\,V}{10^{-3}\,m}} \quad \textbf{(1)}$$

Simultaneous adsorption of phosphoproteins and glycoproteins from protein mixture

The model protein mixture with a mass ratio of β-ca/OVA/IgG/BSA ranging from 1:1:1:1 to 1:1:1:200 was prepared. The concentration of β-ca, OVA and IgG kept constant at 200 μg mL^{-1}, and the concentration of BSA varied from 200 μg mL^{-1} to 40 mg mL^{-1}. 30 μL of PW_9-Si-APBA microspheres aqueous suspension (10 mg mL^{-1}) was taken to mix with 170 μL of different mass ratios of protein mixture (40 mmol L^{-1} BR buffer, pH 5.0). The adsorption and stripping of proteins are performed by following the above procedure. All the supernatant fractions after adsorption and stripping/elution were lyophilized for further analysis by Sodium Dodecyl Sulfate

Polyacrylamide Gel Electrophoresis (SDS-PAGE).

Simultaneous adsorption of phosphoproteins and glycoproteins from human serum

3 μL of human serum was diluted 100-fold with 222 μL BR buffer (40 mmol L^{-1}, pH 5.0) and 75 μL standard β-ca solution (2 mg mL^{-1}). The above mixture solution was incubated with 1.0 mg PW_9-Si-APBA microspheres and oscillated for 20 min. After centrifugation at 6000 rpm for 5 min, the supernatant was collected. The loosely retained proteins on the surface of PW_9-Si-APBA microspheres were eliminated by washing for 2 times with 50 μL of BR buffer (40 mmol L^{-1}, pH 5.0). Subsequently, 200 μL of Tris-HCl buffer (0.1 mol L^{-1}, pH 8.0) was added to release the retained phosphoproteins and glycoproteins from PW_9-Si-APBA by vibration for 20 min. After centrifugation at 6000 rpm for 5 min, the stripped protein solution was collected, lyophilized and redissolved in 150 μL water for SDS-PAGE analysis.

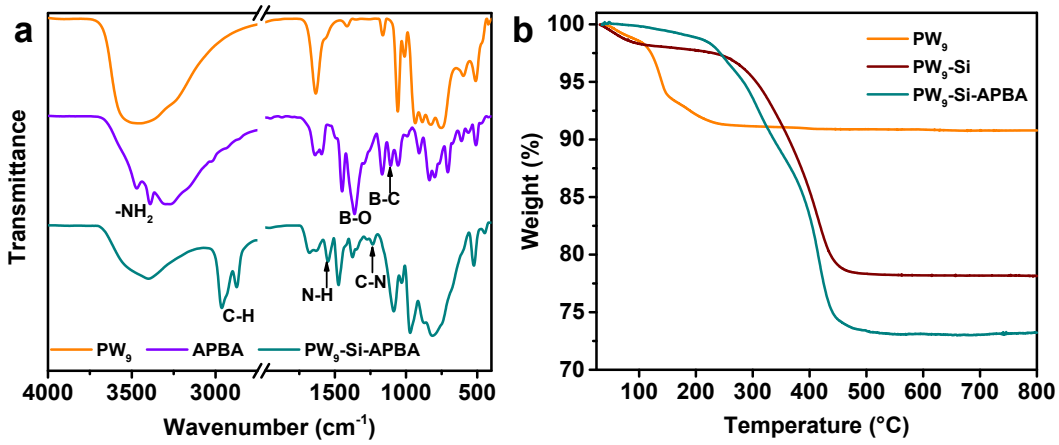

Figure S1. FT-IR spectra of PW_9, APBA, and PW_9-Si-APBA microspheres (a); TGA curves of PW_9, PW_9-Si, and PW_9-Si-APBA microspheres (b)

TGA analysis indicated 8.7% weight loss of crystallization water in PW_9 within 30-250°C ($Na_9[\alpha\text{-}PW_9O_{34}]\bullet xH_2O$, x = 13 based on TGA analysis) (Figure S1b). The formation of PW_9-Si & PW_9-Si-APBA reduced the crystallization water to 2.8% (30-250°C) and 1.5% (30-220°C), respectively. Decomposition of organosilicon & organic ammonium increased weight loss to 18.5% (250-460°C) for PW_9-Si. 22.9% weight loss for PW_9-Si-APBA at 220-470°C is the decomposition of organosilicon, organic ammonium and APBA.

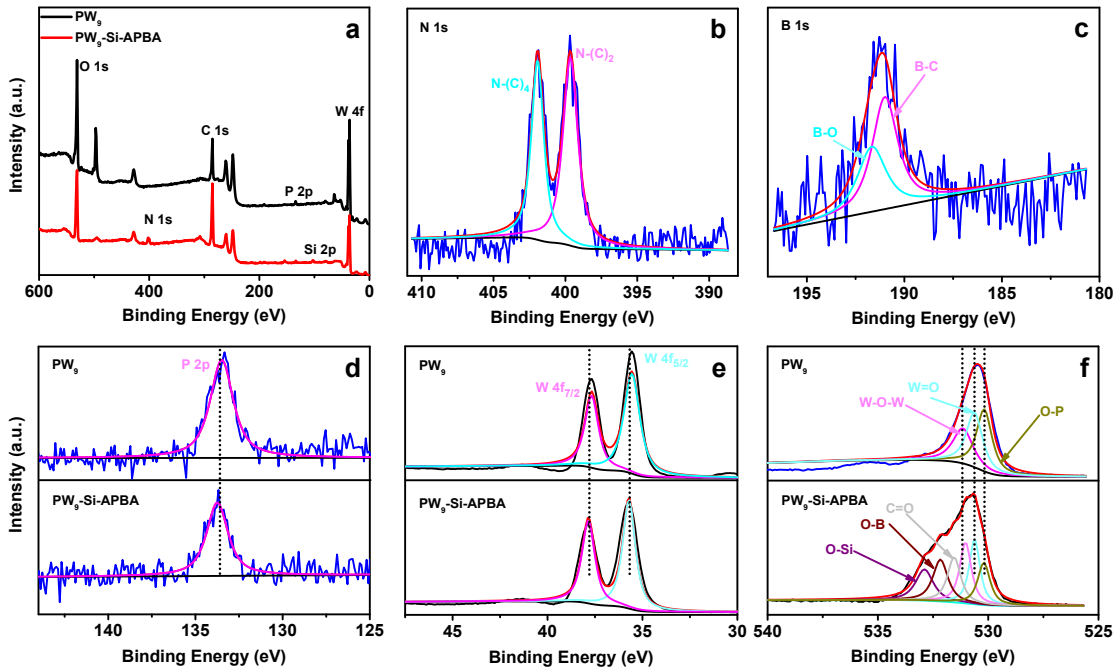

Figure S2. The full range XPS spectra of PW_9 and PW_9-Si-APBA microspheres (a); The narrow range high-resolution XPS spectra of N 1s (b) and B1s (c) of PW_9-Si-APBA microspheres, P 2p (d), W 4f (e) and O 1s (f) of PW_9 and PW_9-Si-APBA microspheres.

X-ray photoelectron spectra (XPS) in Figure S2a indicated two new peaks for Si 2p (102.1 eV) and N 1s (400.1 eV) for PW_9-Si-APBA compared to PW_9. Figure S2b showed that N 1s spectrum is deconvoluted into two peaks at 401.9 eV ($N-(C)_4$) and 399.7 eV ($N-(C)_2$) [2], due to the binding of organosilicon with PW_9. Figure S2c exhibited two peaks of B 1s for B−C (190.9 eV) and B−O (191.7 eV) in PW_9-Si-APBA [3]. With respect to O 1s spectrum of PW_9, PW_9-Si-APBA produces two new peaks of O−Si (532.9 eV) and O−B (531.2 eV) (Figure S2f). P 2p and W 4f showed no change after binding with Si-APBA [4,5], implying the maintainance of the primary structure of PW_9 moiety in PW_9-Si-APBA (Figure S2d,e). This further verified the integration of boronic acid modified organosilicon linker with PW_9 moiety.

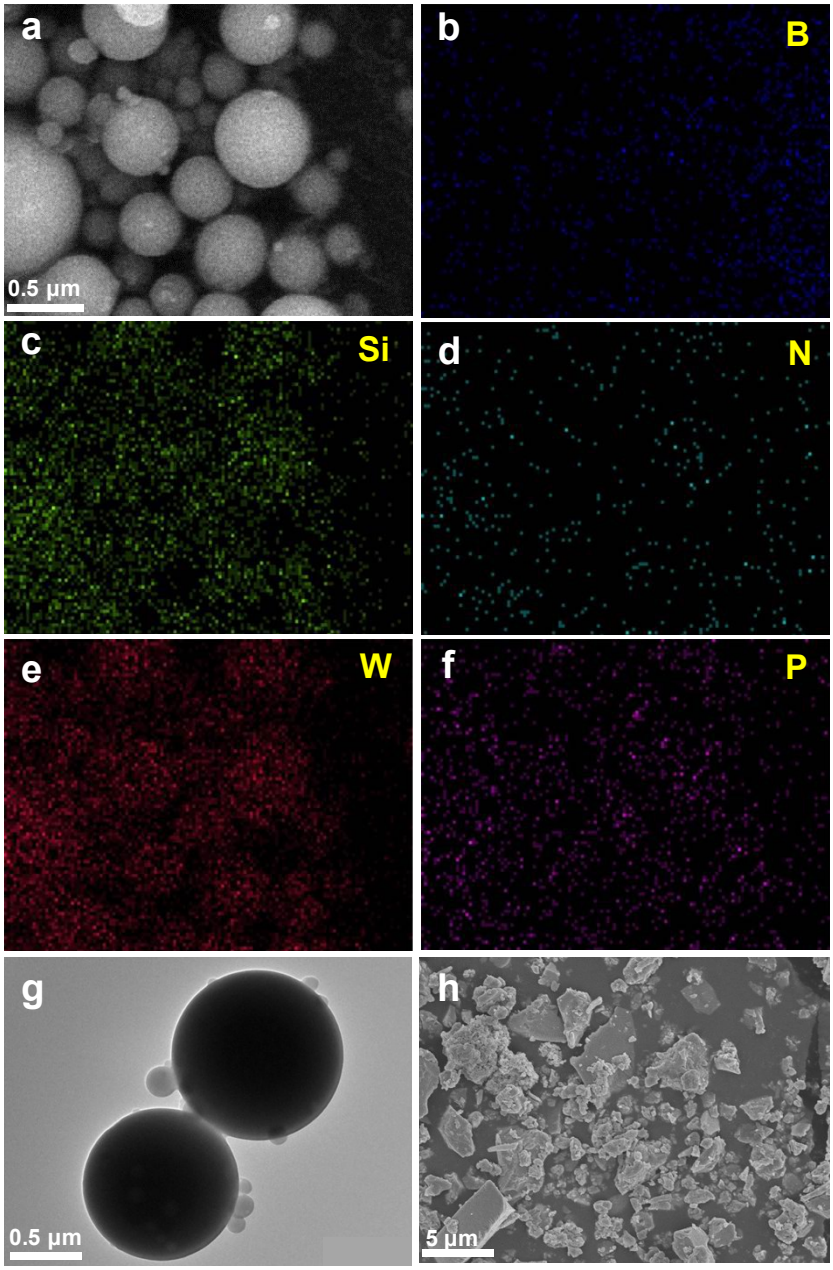

Figure S3. SEM image of PW_9-Si-APBA (a) and the corresponding EDS elemental mapping results of (b) B, (c) Si, (d) N, (e) W, and (f) P. TEM image of PW_9-Si-APBA (g). SEM image of PW_9-Si (h).

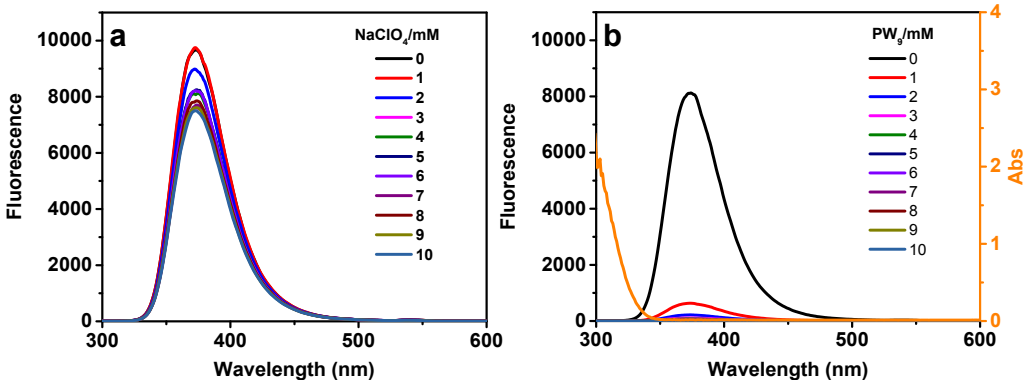

Figure S4. Fluorescence spectra at $\lambda_{ex}/\lambda_{em}$=270/370 nm for a 10 mM APBA solution with the presence of $NaClO_4$ (a) or PW_9 (b) within a range of 0-10 mmol L^{-1}. The orange color line in (b) is the adsorption spectrum of 1.0 mmol L^{-1} PW_9 solution.

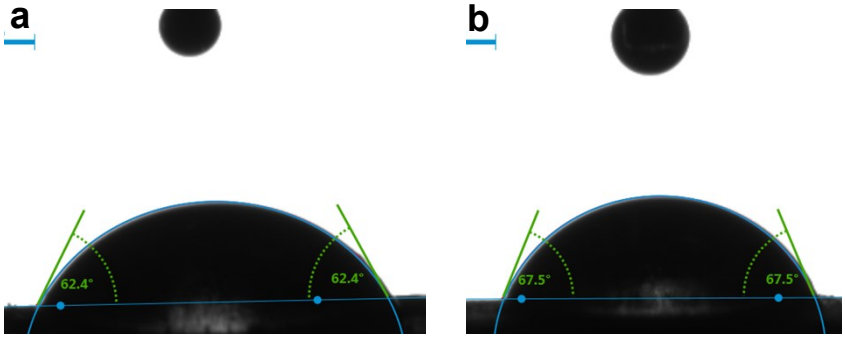

Figure S5. The contact angles of PW_9-Si (a) and PW_9-Si-APBA (b).

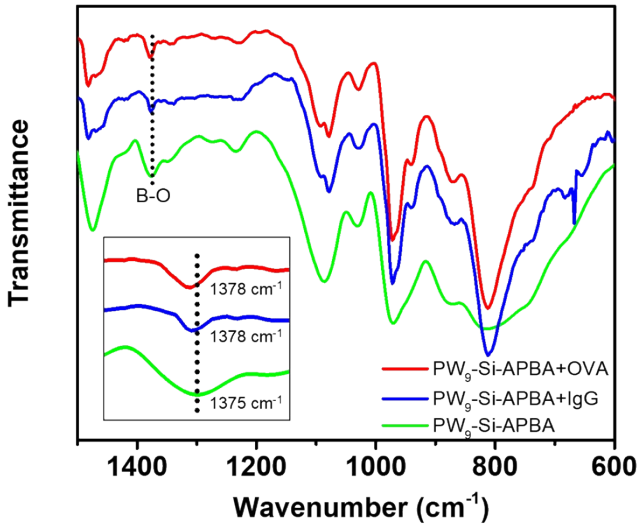

Figure S6. FT-IR spectra of PW_9-Si-APBA before and after the adsorption of glycoproteins, i.e., OVA and IgG.

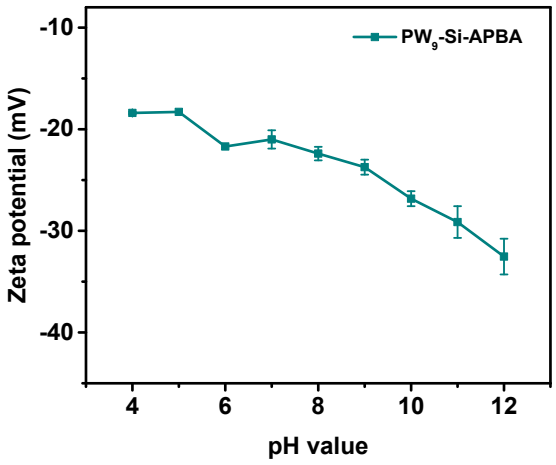

Figure S7. The zeta potentials of PW_9-Si and PW_9-Si-APBA within a range of pH 4.0-12.0. Mass of the material: 1.0 mg mL^{-1}, BR buffer 40 mmol L^{-1}.

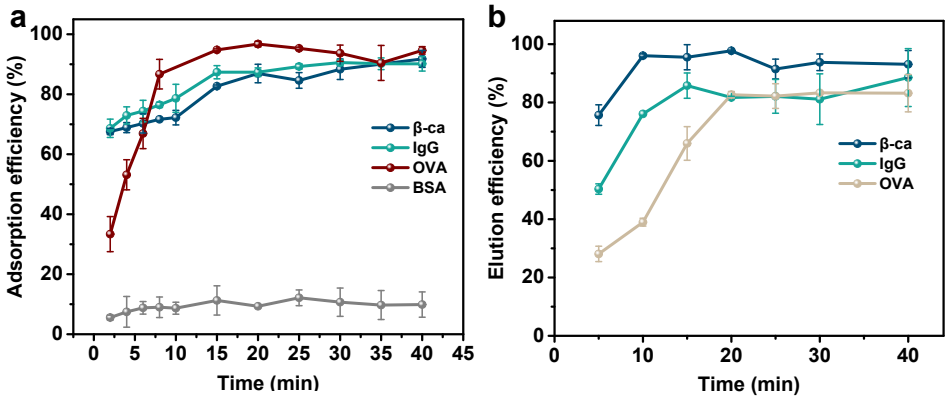

Figure S8. (a) Adsorption kinetics of β-ca, OVA, IgG and BSA on PW_9-Si-APBA microspheres. (b) The elution kinetics for β-ca, OVA and IgG from PW_9-Si-APBA microspheres. Protein solution: 100 μg mL^{-1}, 200 μL; PW_9-Si-APBA: 0.2 mg; BR buffer: 40 mmol L^{-1}, pH 5.0; Adsorption time: 2-40 min (a), 20 min (b); Stripping agent: 200 μL. Stripping time: 5-40 min.

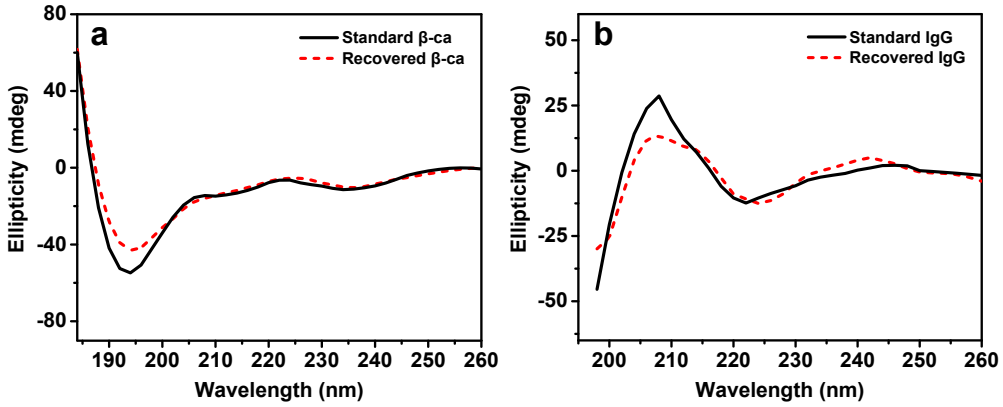

Figure S9. CD spectra of β-ca (a) and IgG (b). The recovered proteins were compared with their native counterparts in Tris-HCl buffer (0.1 mol L^{-1}, pH 8.0).

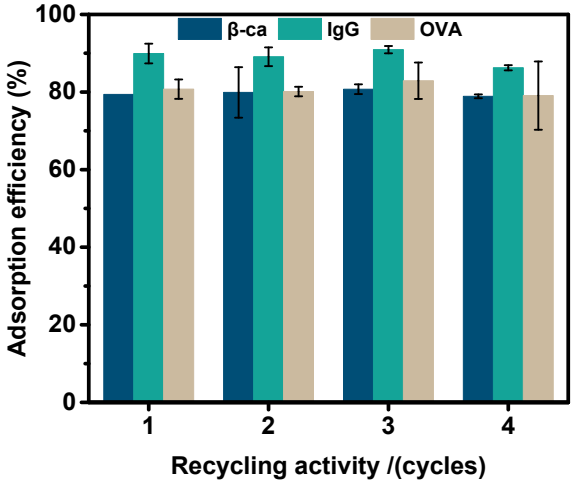

Figure S10. The recyclable performance of PW_9-Si-APBA for the adsorption of phosphoproteins and glycoproteins. Protein solution: 100 μg mL^{-1}, 200 μL; PW_9-Si-APBA: 0.2 mg; Adsorption time: 20 min; BR buffer: 40 mmol L^{-1}, pH 5.0; The stripping agent: Tris-HCl buffer (200 μL, 0.1mol L^{-1}, pH 8.0); Stripping time: 20 min.

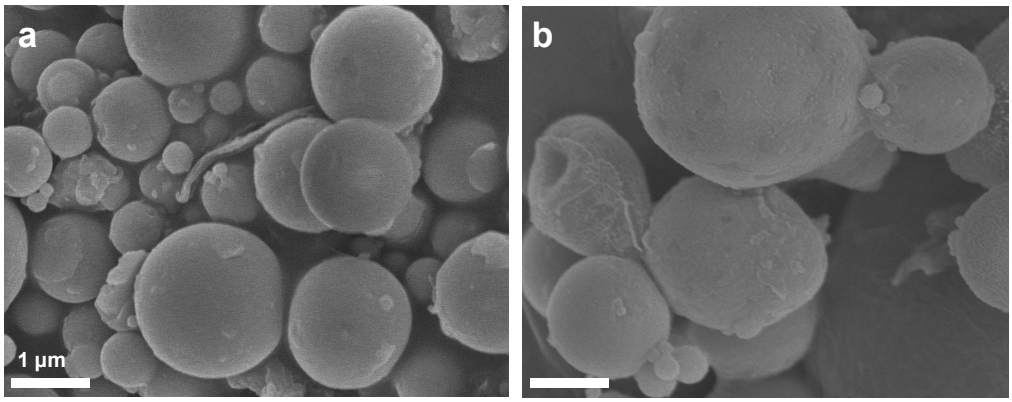

Figure S11. SEM images illustrating the spherical morphology of the PW_9-Si-APBA after the first (a) and the second (b) run of adsorption for the model protein of OVA serving as both phosphoprotein and glycoprotein.

Table S1. A comparison of the adsorption capacities and adsorption time for phosphoproteins and glycoproteins by PW_9-Si-APBA microspheres with respect to various adsorbents.

Adsorbent	Target protein	Sorption capacity (mg/g)	Sorption Time	Ref
Si@pNIPAm-b-pBA	OVA	98.0	4 h	6
GO-APBA/MIPs	OVA	278	40 min	7
B-TiO_2 NPs	OVA	53.0	2 h	8
P(AAPBA-AM@NIPAM-St)CCS	OVA	150	20 min	9
M-β-CD@PDES	OVA	151.62	100 min	10
pA-Mag(SiO_2)	IgG	30	1 h	11
SPSDVB-PAA-PEI-P_5W_{30}	IgG	110.2	30 min	12
Co-MOF-OH	IgG	158.0	30 min	13
Alg-SSF	IgG	175	1 h	14
CeO_2	β-ca	82.4	30 min	15
BT-PolyNIPAm-co-GMA-OH-Fe	β-ca	664	30 min	16
UiO@CmeimCl,UiO-66-NH_2	β-ca	588, 625	30 min	17
PW_9-Si-APBA	OVA	684.4	20 min	This work
	IgG	393.5		
	β-ca	654.3		

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