Supporting Information

## **Epitope-imprinted mesoporous silica nanoparticles for specific recognition of tyrosine phosphorylation**

Guiyuan Zhang<sup>a</sup>, Liyan Jiang<sup>a</sup>, Juntao Zhou<sup>a</sup>, Lianghai Hu\*<sup>a</sup>, Shouhua Feng<sup>b</sup>

aNational Engineering Laboratory of AIDS Vaccine, Key Laboratory Molecular

Enzymology and Engineering, the Ministry of Education, School of Life Sciences,

Jilin University, Changchun 130023, China

<sup>b</sup>State Key Laboratory of Inorganic Synthesis and Preparative Chemistry, College of Chemistry Jilin University, Changchun 130012, China.

\*Corresponding authors.

E-mail addresses: <u>lianghaihu@jlu.edu.cn</u> (L. Hu)

#### **EXPERIMENTAL SECTION**

#### Reagents and materials.

acid (BA), Benzoic Phenylphosphonic acid (PPA) were provided by Aladdin(Shanghai, China). Trifluoroacetic acid (TFA), acetonitrile (ACN) of chromatography grade, 2, 5-dihydroxybenzoic acid (DHB), bovine  $\beta$ -casein and trypsin were obtained from Sigma (St. Louis, MO, USA). Angiotensin II (pY : DRVpYIHPF) and angiotensin II mutant analog (pS : DRVpSIHPF) were obtained from Scilight Biotechnology LLC (Beijing, China). Tetraethoxysilane (TEOS), 3ureidopropyltriethoxysilane (UPTES), N-cetyltrimethylammonium bromide (CTAB) was obtained from Beijing J&K scientific. (Beijing, China). All other chemical reagents were of analytical grade and obtained from Sinopharm Chemical Reagent (Shanghai, China). All of the other reagents were of analytical grades and used without further purification. Water used in all the experiments was purified by a Milli-Q Advantage A10 ultrapure water purification system (Millipore, Milford, MA).

#### Instruments

JEM-2100 transmission electron microscopy JEOL (Tokyo, Japan), was used to characterize the size, structure and morphology of the sorbents. The operation voltage of SEM and TEM is 10 kV and 200 kV, respectively. The Fourier transform infrared (FTIR) spectrometer was from Thermo Fisher Scientific (Massachusetts, USA) to characterize the materials with KBr pellets at room temperature using an accumulation of 32 scans and a resolution of 4 cm<sup>-1</sup> in the range of 4000-500 cm<sup>-</sup> <sup>1</sup>. The X-ray diffraction (XRD) patterns of samples were acquired on a D/MAX2550 (Rigaku Corporation, Japan) in the  $2\theta$  range of  $1-4^\circ$ . The UV absorbance measurement was performed on a NanoDrop 2000/2000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA), and the wavelength was adopted at 265 nm for all the analytes. Nitrogen adsorption-desorption measurements were conducted at 77 K on an ASAP2020 instrument (Micromeritics, Norcross, GA). The surface areas were calculated by the Brunauer-Emmett-Teller (BET) method, and the pore size distributions were calculated by the Barrett-Joyner-Halenda (BJH) method. MALDI-TOF mass spectrometry analysis was performed on a AB SCIEX time-of-flight mass spectrometer (AB SCIEX, USA).

#### Synthesis of phosphate-imprinted materials

Firstly, 170 mg NaOH and 0.6 g CTAB were dissolved in distilled water (288 mL) simultaneously. Then, the solution was transferred into a 500 mL three-necked round-bottomed flask and was stirred at 80 °C for 15 min at a speed of 800 rpm. Subsequently, 63.3 mg of template PPA were added dropwise to the mixture and then reacted at 500 rpm for 30 min. Then a mixture of TEOS (9.6 mmol), UPTES (0.4 mmol), and methanol (200  $\mu$ L) was added dropwise to the solution under vigorous stirring, and the resulting mixture was allowed to react for 2 hrs to produce white precipitate. Finally, the obtained white powder was washed with ethanol for several

times and evaporated under vacuum at 40 °C for further use. Additionally, the complete removal of CTAB and PPA from MIMs was implemented with Soxhlet extraction method by mixing HCl (37%, 3 mL) and methanol (300 mL). After that, these imprinted materials were dried under vacuum at 60 °C for 24 hrs to remove residual solvent in the mesoporous materials. In parallel, non-imprinted mesoporous materials (NIMs)was prepared by the same procedure, but in the absence of template PPA.

# Evaluation of the binding ability of the MIMs Static and kinetic adsorption

MIMs and NIMs were mixed with PPA solution (1 mL, 0.01-0.5 mg/mL) separately. The mixtures were shaken on a rotator for 2 hrs at room temperature. After adsorption, the molecularly imprinted materials were collected by centrifugation and then washed three times with 200  $\mu$ L of 75% ACN/5% TFA, and 200  $\mu$ L of 50% ACN/1% TFA in turn. Finally, the bound PPA was eluted with 100  $\mu$ L of 0.4 M NH<sub>3</sub>·H<sub>2</sub>O under sonication for 10 min. After centrifugation at 15000 g for 10 min, the supernatant was dried by speed vacuum and redissolved in 50  $\mu$ L of 0.1% TFA and measured at the wavelength 265 nm by NanoDrop.

Kinetic adsorption tests were conducted using the following way. MIMs and NIMs were mixed with 1 mL of an aqueous PPA solution (1 mg/mL<sup>-1</sup>). The mixtures were incubated at room temperature with agitation at 750 rpm for different time. After adsorption, the molecularly imprinted materials were then collected by centrifugation and the washing and elution procedures were the same as those described above. Finally, after centrifugation at 15000 g for 10 min, the supernatant was dried by speed vacuum and redissolved in 50  $\mu$ L of 0.1% TFA and measured by NanoDrop. The adsorption capacity (Q) of PPA bound to the materials can be established using the following equation:

Q=CV/W (mg/g)

where C is the concentration of PPA (mg/mL), V is the volume of the solutions (mL), and W is the mass of the materials added to the solutions.

### Selectivity test

The benzoic acid (1 mg) and phenylphosphonicacid (1 mg) were dissolved in 1.0 mL of an aqueous solution separately. The solution was enriched with 2 mg MIMs and NIMs. The mixtures were incubated at ambient temperature with agitation at for 2 hrs. After adsorption, the molecularly imprinted materials were then collected by centrifugation and the washing and elution procedures were the same as those described above. Finally, after centrifugation at 15000 g for 10 min, the supernatant was dried by speed vacuum and redissolved in 50  $\mu$ L of 0.1% TFA and measured by NanoDrop.

#### Application of the MIMs for tyrosine phosphopeptides recognition

The peptides pY and pS (10  $\mu$ g of each) were dissolved in 100  $\mu$ L 75% ACN/5% TFA solution with 1 mg of molecularly imprinted MIMs and NIMs separately. The mixtures were shaken at room temperature for 30 min. After adsorption, the molecularly imprinted materials were then collected by centrifugation and the washing and elution procedures were the same as those described above. After centrifugation at 15000g for 10 min, the supernatant was dried by speed vacuum and redissolved in 10  $\mu$ L of 0.1% TFA before MALDI-TOF MS analysis.

#### Selective enrichment of the pTyr from protein digest

1 mg of  $\beta$ -casein is dissolved in 1 mL of NH<sub>4</sub>HCO<sub>3</sub> (50 mM, pH 8.2), and then digested for 18 h at 37 °C with trypsin at an enzyme-to-protein ratio of 1:100 (w/w). A mixture of tryptic digestion of pY and  $\beta$ -casein (w/w, 1:100) was used, the mixture was dissolved in 100  $\mu$ L 75% ACN/5% TFA solution with 1 mg of molecularly imprinted MIMs and NIMs separately. The enrichment, washing and elution procedures were the same as those described above. After centrifugation at 15000 g for 10 min, the supernatant was dried by speed vacuum and redissolved in 10  $\mu$ L of 0.1% TFA before MALDI-TOF MS analysis.



**Fig. S1** Schematic diagram for the synthesis of molecular imprinted mesoporous silica nanoparticles and enrichment/detection of tyrosine phosphorylated peptides.



**Fig. S2** Selective capability of the MIMs and NIMs to PPA and its analogue BA. The error bars are mean standard deviations from experiments performed in triplicate.



Fig. S3 TEM images for non-imprinted mesoporous silica nanoparticles.



**Fig. S4** BET nitrogen adsorption/desorption isotherms and BJH pore size distributions for NIMs.



**Fig. S5** FTIR spectra of (a) PPA, (b) MIMs before template removal, (c) MIMs and (d) NIMs.



Fig. S6 MALDI-TOF-MS obtained from the mixture of pY peptide and tryptic digestion of  $\beta$ -casein (1:100,w/w) enrichment from NIMs.



Fig. S7 Scatchard plot for the absorption of PPA on the MIMs and NIMs sorbents. (A):MIMs. (B):NIMs. Scatchard equation:  $Q_e/[S] = (Q_{max} - Q_e)/K_d$  where  $Q_e$  is amount of targets bound to material at equilibrium, [S] is the free concentration of targets at equilibrium,  $K_d$  is the dissociation constant,  $Q_{max}$  is the maximum specific binding capacity,  $R_2$  is the correlation coefficient.

No.	Amino acid sequence	Number of	Observed
		phosphoryl groups	m/z
□1	FQ[pS]EEQQQTEDELQDK	1	2063.1
□2	FQ[pS]EEQQQTEDELQDKIHPF	1	2555.9
	RELEELNVPGEIVE[pS]L[pS][pS][pS]E	4	3121.8
	ESITR		

**Table S1.** Detailed information of the identified phosphopeptides extracted from tryptic digestion of  $\beta$ -casein by MIMs.