Supporting Information

Experimental

Reagents and chemicals

1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) and 4-carboxyphenyl boronic acid (CPBA) were purchased from J&K scientific (Shanghai,China). 3-Aminopropyltriethoxysilane (APS), tetraethyl orthosilicate (TEOS), N-cetyltrimethylammonium, bromide (CTAB), and mesitylene (TMB) were purchased from Shanghai Chemical Reagent Co., Ltd. Sodium hydroxides, chlorhydric acid, acetic acid, Na_2HPO_4, NaH_2PO_4, $Na_2B_4O_7$, anhydrous methanol, dimethyl sulfoxide (DMSO) and anhydrous toluene were purchased from Nanjing Reagent Company (Nanjing, China). Benzamidine hydrochloride monohydrate and heparin were obtained from Shanghai Sangon Biotech Co., Ltd. Healthy human serum samples were kindly provided by the Gulou Hospital (Nanjing, China). All experiments were conducted in accordance with protocols approved by the Animal Ethic Committee of Nanjing University of Chinese Medicine. All the chemicals were analytical grade and used without further treatment. Water used in all the experiments was purified by a milli-Q-RO4 ultrapure water purification system (Millipore, Milford, MA, USA).

Apparatus and characterization

Transmission electron microscopy (TEM) images were obtained on a JEOL JEM 2100 F transmission electron microscope. X-ray diffraction (XRD) measurements were performed on a Shimadzu XRD-6000 powder X-ray diffractometer, using Cu Ka as the incident radiation. The surface analysis was performed using nitrogen sorption isotherms, which were obtained at 77 K using a micromeritics ASAP2020 sorptometer. The surface areas were calculated using the Brunauer-Emmett-Teller (BET) method, and the pore size distributions were calculated using the Barrett-Joyner-Halenda (BJH) method. UV-vis absorption spectra were obtained using a UV-3600 spectrophotometer (Shimadzu). The fluorescence spectrum was performed on a LS-55 fluorimeter (Perkin-Elmer). LC separation was performed on a UFLC 20ADXR LC system on-line (Shimadzu Corporation, Kyoto, Japan) coupled with hybrid quadrupole time-of-flight tandem mass spectrometer Q-TOF MS (TripleTOFTM5600 MS system, AB Sciex Corporation).

Preparation of boronic acid functionalized-MSN.

Mesoporous silica nanospheres (MSNs) were synthesized by a method by Li and co-workers [RS1] with slight modifications. Briefly, 1.0 g CTAB and 7.0 mL TMB were firstly added into 480 mL water, and 3.50 mL of sodium hydroxide aqueous solution (2.00 M) was introduced to the above solution. After vigorous stirring at 80 ℃ for 4 h, 5.0 mL of TEOS was quickly added into the mixture under vigorous stirring. The mixture was vigorously stirred at 80 ℃ for another 2 h. The solid crude product was filtered, washed with water and methanol, and dried under high vacuum. The structure-template CTAB and TMB were removed by refluxing in ethanol solution of ammonium nitrate (NH_4NO_3/C_2H_5OH, 8 mg· mL^{-1}) for 6 h at 80 ℃. The surfactant-free product was filtrated and dried in a vacuum at 45 ℃ for 12 h to remove the remaining solvent from the mesopores to get MSN as a white powder.

400 mg of prepared MSN was refluxed for 20 h in 80 mL of anhydrous ethanol with 1.00 mL (5.67 mmol) of APS to yield the 3-aminopropyl functionalized MSN, and then followed by centrifugation, washing with ethanol for several times, and drying overnight in a vacuum at 45 ℃

for 12 h to give APS-MSN as white powder.

400 mg of the purified APS-MSN was dispersed in 20 mL DMSO. 0.15 g (0.90 mmol) CPBA was reacted with 0.10 g (0.87 mmol) NHS and 0.20 g (1.04 mmol) EDC in 5.0 mL DMSO, stirring at room temperature for 30 min before adding to the APS-MSN suspension. The mixture was stirred at room temperature for another 24 h, followed by filtration and washing with DMSO, water and methanol and dried to get boronic acid-functionalized MSN.

Solid-phase enrichment and tagging procedures.

For solid-phase enrichment, typically, 1 mL solution of 1 μg/mL heparin was heated to 60 ℃by water bath and then 50 mg boronic acid-functionalized mesoporous silica material was added. And the tube was shaken on a rotator for 60 min at room temperature for the enrichment. The heparin loaded MSN was then collected by centrifuge and rinsed with 200 μL of 50 mM phosphate buffer (pH 8.0) for 3 times each.

For solid-phase tagging, the heparin loaded MSN was re-suspended and mixed with 100 μL 0.1 M benzamidine at 95 ℃ for 6 min. After reaction, the material was collected by centrifuge and rinsed with 200 μL of deionized water for 3 times each. And then the material was re-suspended and eluted in 100 μL 100 mM acetic acid solution for 1 h on a rotator. Finally, the material was centrifuged again and the eluates were collected by pipetting carefully. The eluates were used for fluorescence measurement.

Liquid-phase tagging procedures.

The standard heparin solution (1μg/mL) and 0.1 M benzamidine were prepared with 50 mM phosphate buffer (pH 8.0). For liquid-phase tagging, 100 μL heparin solution was mixed with 100 μL benzamidine solution in a clean tube and then the mixture was allowed to react at 95 ℃ for 6 h.

HPLC-MS analysis

The mobile phase for tagging products separation was the mixture of acetonitrile (A:20%) and ultrapure water (B: 80%) at a flow rate of 1 mL/min. The separation was performed on a Waters Xbridge™ Amide column (3.5 μm, 4.6×250 mm) with 20 ℃. The injection volume was 5 μL.

The HPLC analysis was performed by diode array detection, and the peaks were recorded at 226 nm.

MS and MS/MS data were acquired by analyst 1.6@software using Triple TOF TM 5600 mass spectrometer with MS scan and information dependent acquisition (IDA) MS/MS scans. MS and MS/MS data were analyzed by PeakView 1.2@Software. The mass spectrometer was operated in positive ion mode with electrospray ionization (ESI) by a source temperature of 550℃. The temperature was obtained by optimization of intensity with increasing the ion temperature from 250 ℃ to 650 ℃ using lycorine as reference. High pure nitrogen gas was used as ion source gases and collision gas. The mass analysiswas conducted in the range of m/z 50-1000, with the following parameters: ion source gases 1 and 2 (GS1 and GS2), 50 psi; declustering potential, 60 V; entrance potential, 13 V and collision energy (CE) 10 V. MS/MS data were acquired using IDA methods. Most intense ten peaks for ions with m/z 50-1000 trigger IDA criteria using real time mass defect filtering (MDF) and dynamic background subtraction (DBS). External mass calibration was performed automatically using calibrate delivery system before every sample was analyzed.

Fluorescence measurements.

The fluorescence emission spectra of tagging sample were measured, with excitation

wavelength at 407 nm, emission wavelength at 488 nm. The slit width was 8 nm.

1. The amino-groups functionalization and further bond of CPBA in MSN nanospheres' mesopores was confirmed by the Fourier transform infrared (FT-IR) spectra as shown in Fig. S1. In contrast to that of the pure MSN nanospheres, the FT-IR spectra of the APS-layer coated MSN displays a new peak at 3478 cm^{-1}, ascribed to the N–H stretching vibration, which could prove the successful bond of APS onto the MSN nanospheres. After bonding subsequently with CPBA, two new adsorption bands at 1549 cm^{-1} and 1632 cm^{-1}, assigned to the C=O stretching vibration in the amide group, and the C=O stretching vibration in the carboxyl group, respectively, indicated the successful fabrication of CPBA-layer onto the MSN. After the heparin binding onto the MSN, the FT-IR showed the peak at 3500 cm^{-1} owing to the O-H stretching vibration and the new peak at 1411 cm^{-1} due to O-H bending vibration, and these indicated successful bond of heparin onto the MSN.
2. **The binding capacity of boronic acid-functionalized MSNs**. For measurement of the binding capacity, 5.0 mg/mL glucuronic acid was prepared and successively diluted to 2.00, 1.00, 0.50, 0.05, 0.025 mg/mL. The absorbance of the five solutions was detected to obtain the standard curve. 50 mg boronic acid-functionalized MSNs were added to 1 mL of 2.0 mg/mL glucuronic acid solution and the solution was shaken on a rotator for 60 min at room temperature. The glucuronic acid loaded MSNs was then collected by centrifuge and rinsed with 500 μL of 50 mM phosphate buffer (pH 8.0) for 3 times each. The material was resuspended and gradually dissloved in 1 mL aqueous solution of 0.5 M sodium hydroxide for 1 h. Finally, the obtained solution was collected by pipetting carefully. The absorbance of the solution at 216 nm was measured. According to the obtained solution absorbance and standard curve, the binding capacity was calculated. The binding capacity of boronic acid-functionalized MSNs was measured to be 123.6±4.9 μmol/g.
3. **The selectivity of boronic acid-functionalized MSNs**. For the evaluation for the selectivity of the boronic acid-functionalized MSNs, 5.0 mg/mL glucuronic acid was prepared and successively diluted to 2.00, 1.00, 0.50, 0.05, 0.025 mg/mL, and 5.0 mg/mL deoxyadenosine was also prepared and successively diluted to 2.00, 1.00, 0.50, 0.05, 0.025 mg/mL. The absorbance of the five solutions was detected to obtain the standard curve. 50 mg boronic acid-functionalized MSNs were separately added to 1 mL of 2.0 mg/mL glucuronic acid and deoxyadenosine solution and the the mixtures were shaken on a rotator for 60 min at room temperature. The glucuronic acid or deoxyadenosine loaded MSNs was then collected by centrifuge and rinsed with 500 μL of 50 mM phosphate buffer (pH 8.0) for 3 times each. The two materials were resuspended and gradually dissloved in 1 mL aqueous solution of 0.5 M sodium hydroxide for 1 h. Finally, the obtained solution was collected by pipetting carefully. The absorbance of the glucuronic acid solution at 216 nm was measured, and loaded deoxyadenosine solution was measured at 260 nm. According to the obtained solution absorbance and standard curve, the binding quantity of glucuronic acid and dexoadenosine were calculated. As shown in Fig S3, the loaded quantity of glucuronic acid onto the boronic acid-functionalized MSNs was measured to be 6.18 μmol, while the corresponding loaded quantity of dexoadenosine was only 0.0689 μmol in the same condition.
4. **Affinity of the MSNs toward derivatization reagent benzamidine (BZM).** The same solid-phase tagging experiment had carried out without adding the glucuronic acid solution. Then the standard benzamidine solution and the solution after the material absorbed benzamidine

dissolved in 0.5 M sodium hydroxide for 1 h were collected for UV absorbance measurement (Fig. S4).

5. Tagging reaction time for both liquid-phase and solid-phase tagging within CPBA-functionalized MSNs. We took 100 μL solution from the reaction mixture for HPLC measurement at 0.5, 1.5, 2.5, 4.0, 6.0, 8.0,10 h during the reaction For liquid-phase tagging. For solid-phase tagging, the reaction time to be tested was changed to 0.5, 1.5, 3.0, 4.0 min, 6.0 min, 8.0 min and 10.0 min, while other procedures were the same as liquid-phase tagging. All the eluates were collected for HPLC experiments after the tagging.

6. To evaluate the functionality of mesopores for the performance of enrichment and solid-phase tagging, CPBA-functionalized nonporous silica nanospheres with an average diameter of 150 nm (TEM image is shown in Fig.S6) were synthesized as an alternative sorbent and nanoreactor. The boronic acid-functionalized nonporous silica nanospheres exhibited relatively low binding capacity with 33.6±3.8 μmol/g toward glucuronic acid, compared with 123.6±4.9 μmol/g of MSNs. It took 15 min to reach reaction equilibrium by using nonporous silica nanospheres as nanoreactor as shown in Fig.S7, while only 6 min in the MSNs nanoreactors. These results suggested that the MSNs with mesoporous structure could perform a much more efficient enrichment and nanoreactor.

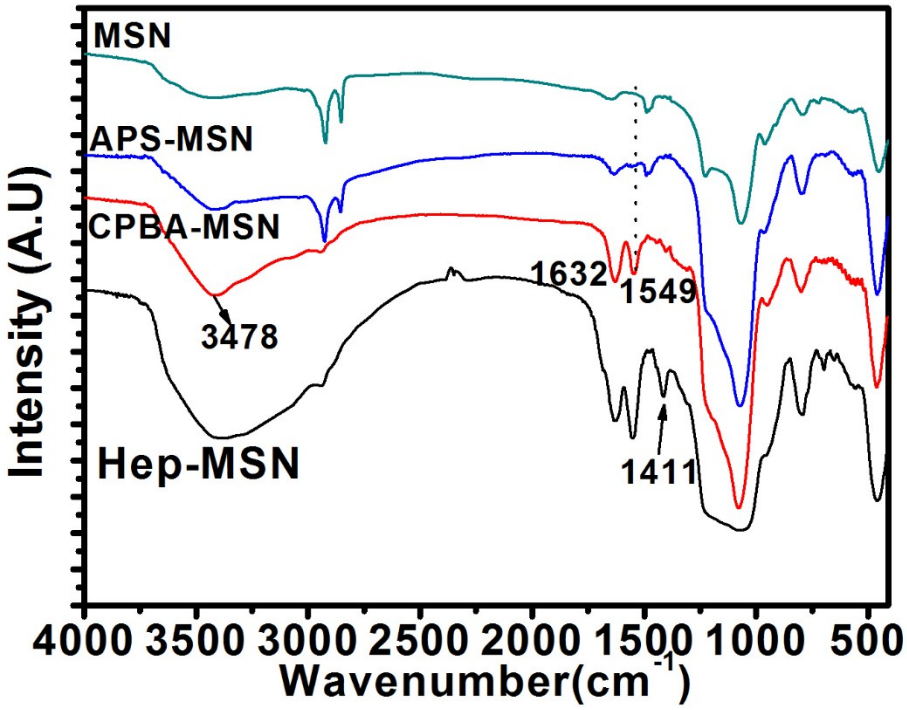

Fig. S1 FT-IR spectra of MSN, APS-MSN, CPBA-MSN and Heparin-MSN.

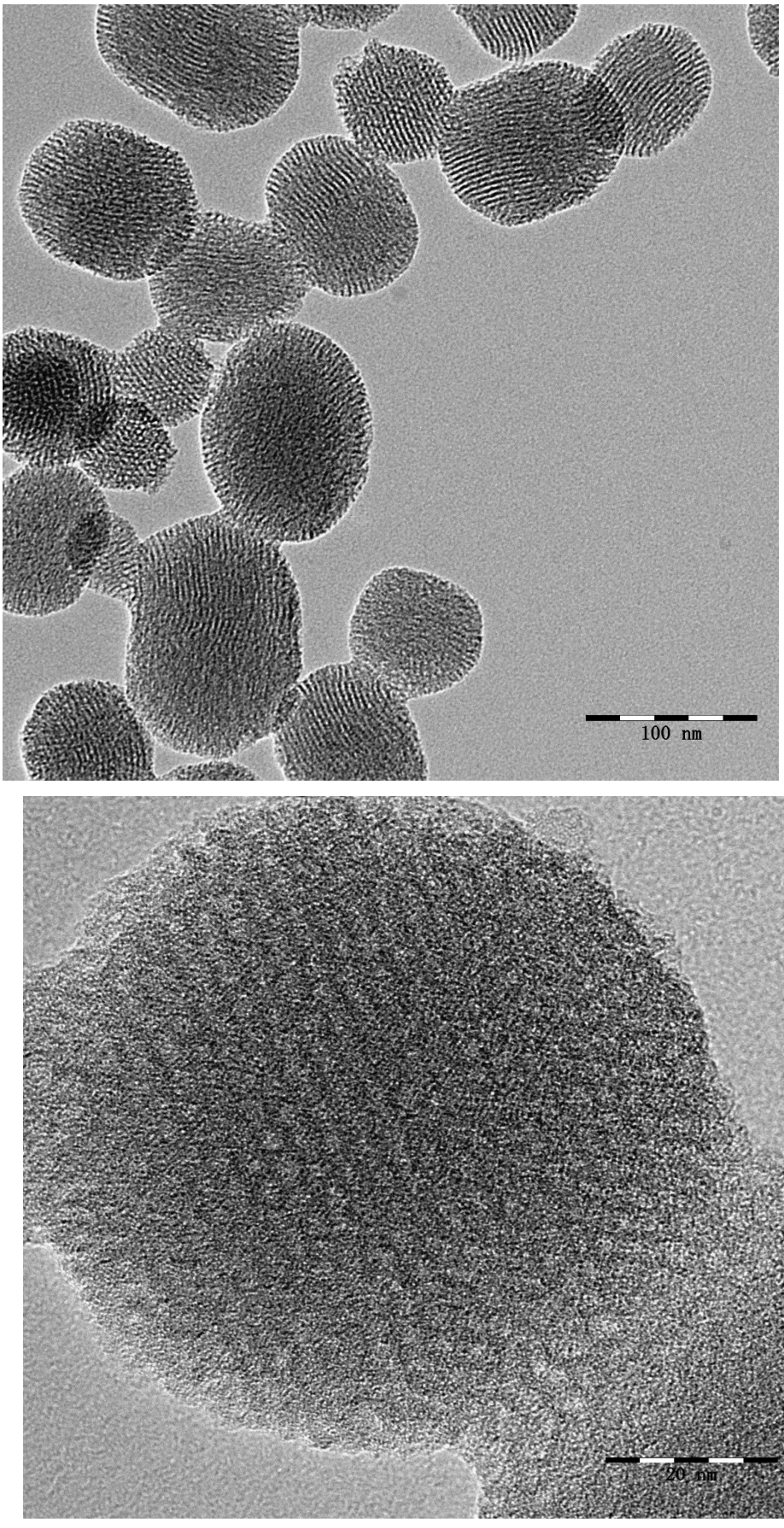

Fig. S2 The high-magnification TEM of mesoporous silica.

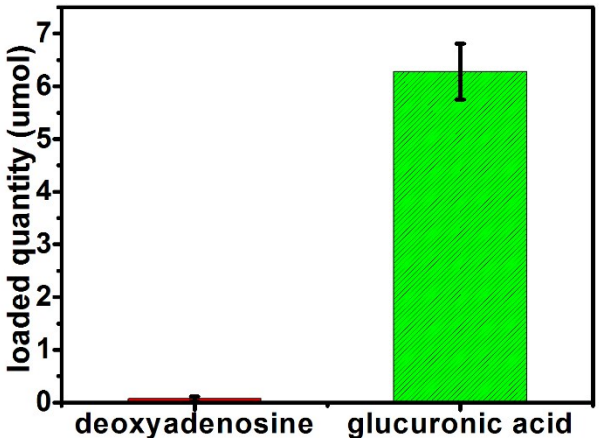

Fig. S3 The loaded quantities of deoxyadenosine and glucuronic acid onto the boronic acid-functionalized MSNs

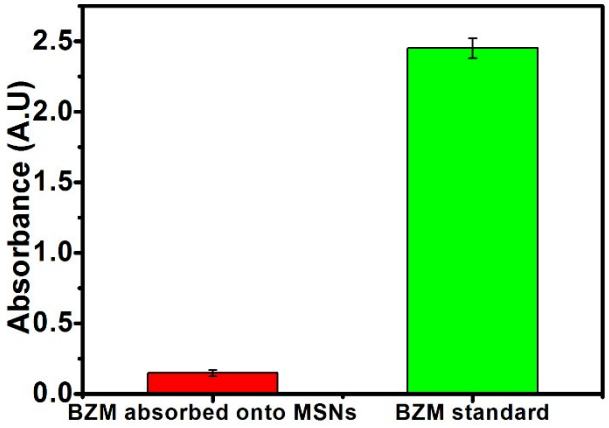

Fig. S4 Comparison of the absorbance for the benzamidine (BZM) standard solution and for BZM molecules absorbed by the functionalized MSNs.

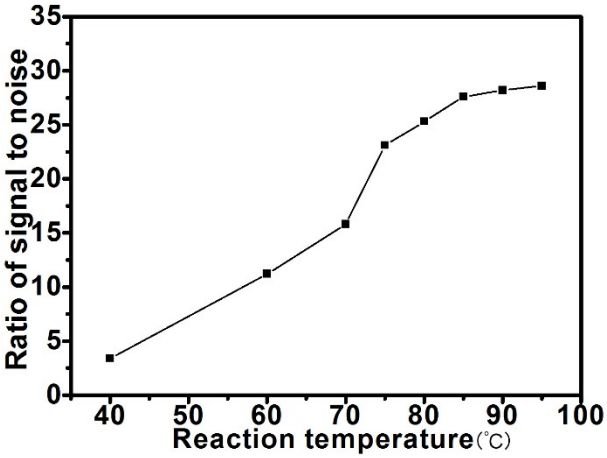

Fig. S5 Effects of reaction temperature on the ratios of signal-to-noise of the solid-phase tagging product on the glucuronic acid.

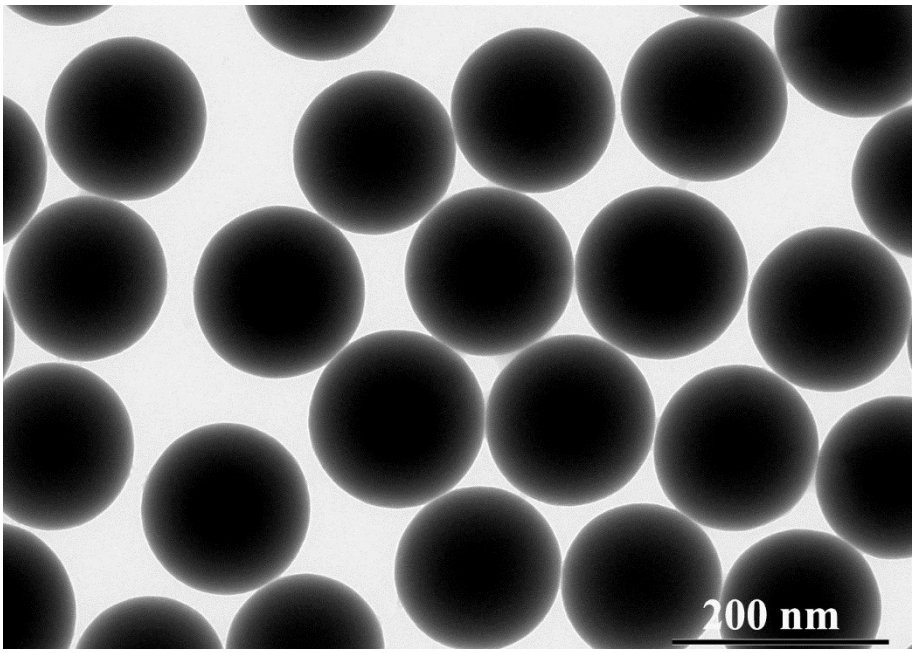

Fig. S6 TEM of nonporous silica nanospheres

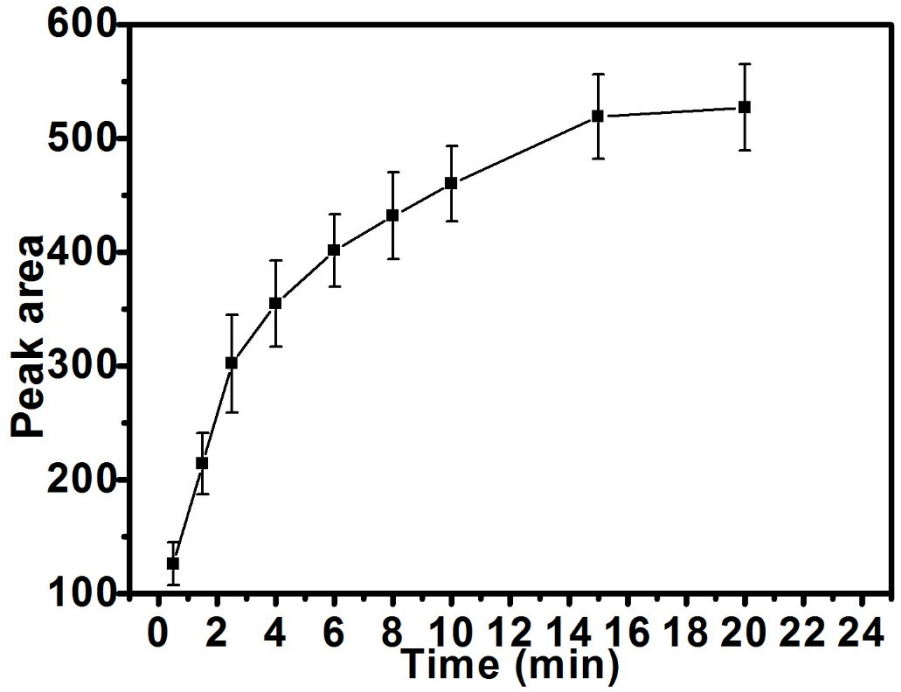

Fig.S7 The peak area of the tagging products on the reaction time by using nonporous silica nanospheres.

Table S1 Recovery (mean ±SD; n = 3) for determination of heparin in human serum by using the specific enrichment combined with highly efficient solid-phase tagging approach.

Sample	Spiked concn (μg/mL)	Recovery (%)
human serum	1.0	92±5
human serum	3.0	97±3
human serum	9.0	107±4

Table S2 Comparison of the proposed method with other methods

Methods	LOD (ng/mL)	Ref.
SERS assay	43.74	RS2
Resonance Rayleigh scattering	50.4	RS3
Polycation-induced Benzoperyleneprobe	600	RS4
Fluorescence quenching	20	RS5
Enrichment combined with tagging	25	Present method

RS1 L. Yuan, Q. Q. Tang, D. Yang, J. Z. Zhang, F. Zhang and J. Hu, *J. Phys. Chem. C*, 2011, **115**, 9926-9932.

RS2 Y.Zeng, J-J.Pei, Li-H.Wang, Ai-G.Shen and J.-M.Hu, *Biosensors and Bioelectronics,*2015, **66**,55-61

RS3 Y. Ling, Z. F. Gao, Q. Zhou, N. B. Li, and H. Q. Luo, *Anal. Chem*. 2015, **87**, 1575−1581

RS4 M.D. Yang, J.C, H.P.Zhou, W.Y.Li, Y.Wang, J.M.Li, C.Y.Zhang, C.B.Zhou and C.Yu, *Biosensors and Bioelectronics,* 2016, **75**, 404-410.

RS5 Y.Li, H.C.Sun, F.P.Shi, N.Cai, L.H.Lu and X.G.Su, *Biosensors and Bioelectronics*, 2015, **74**, 284–290