*Electronic Supplementary Information for*

# Specific recognition towards proteins and peptides via controllable oriented surface imprinting of boronate affinity-anchored epitope

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# MATERIALS AND METHODS

## 1. Reagents and Materials

$\beta_2$-Microglobulin (B2M), horseradish peroxidase (HRP), bovine serum albumin (BSA), ribonuclease A (RNase A), ribonuclease B (RNase B), adenosine, deoxyadenosine, 2,4-difluoro-3-formyl-phenylboronic acid (DFFPBA), trypsin, trifluoroacetic acid (TFA), sinapic acid (SA) and α-cyano-4-hydroxycinnamic acid (CHCA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Myoglobin (Mb) was purchased from abcam (Shanghai, China). KIVKWDRDM, KIVKWDRDMK-Fru, NYKELGFQG and NYKELGFQGK-Fru were synthesized by Shanghai Top-Peptide Biotechnology Co. Ltd. (Shanghai, China), and their purities (HPLC) were above 98%. Aminopropyltriethoxysilane (APTES), 3-ureidopropyltriethoxysilane (UPTES), isobutyltriethoxysilane (IBTES), tetraethyl orthosilicate (TEOS) and sodium cyanoborohydride were purchased from J&K scientific (Shanghai, China). Human serum was purchased from Shuangliu Zhenglong Chemical and Biological Research Laboratory (Sichuan, China). Ferric trichloride hexahydrate, 1,6-hexanediamine, anhydrous sodium acetate, glycol, silver nitrate ($AgNO_3$), ammonium bicarbonate, sodium dihydrogen phosphate, sodium hydroxide, sodium chloride (NaCl), acetic acid (HAc) and anhydrous ethanol were purchased from Nanjing Reagent Company (Nanjing, China). Methanol and acetonitrile (ACN) were purchased from Shanghai Macklin Biochemical Co. Ltd. (Shanghai, China). Trisodium citrate and ammonium hydroxide (28%) were purchased from Shanghai Lingfeng Chemical Reagent Co. Ltd. (Shanghai, China). All other reagents used were of analytical grade or higher. Water used in all the experiments was purified by a Milli-Q Advantage A10 water purification system (Millipore, Milford, MA, USA).

## 2. Instrumentation

Transmission electron microscopic (TEM) characterization was carried out on a JEM-1011 system (JEOL, Tokyo, Japan). Fourier transform infrared (FT-IR) spectrometry was carried out on a Nicolet 6700 FT-IR spectrometer (Thermo Fisher, MA, USA).

Ultraviolent (UV) spectral analysis was performed with a NanoDrop 2000/2000C spectrophotometer (Thermo Fisher, MA, USA).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometric (MALDI TOF MS) analyses were carried out on a 4800 plus MALDI TOF/TOF Analyzer (Applied Biosystems, Framingham, MA, USA) with a pulsed nitrogen laser operated at 337 nm. The laser energy was adjusted to slightly above the threshold to obtain good resolution and signal-to-noise ratio (S/N). All mass spectra reported were obtained in the positive ion mode. The instrument was operated in linear modes for intact protein detection and reflectron mode for peptide detection. External calibration was applied to the instrument before data collection. Protein standards (insulin: $M+H^+$ = 5734; ubiquitin: $M+H^+$ = 8565; cytochrome C: $M+H^+$ = 12,361 and $M+2H^{2+}$ = 6181) and peptide calibration standards (bradykinin fragment $M+H^+$ = 757.3997; angiotensin II: $M+H^+$ = 1046.5423; $P_{14}R$: $M+H^+$ = 1533.8582; ACTH fragment 18-39: $M+H^+$ = 2465.1989; insulin oxidized B: $M+H^+$ = 3494.6513) were used as external calibrations for intact protein and peptide detection, respectively. A typical spectrum was obtained by averaging 3000 laser shots from 30 positions within the sample well. The accelerating voltage was 20 kV. The whole process was controlled by the 4000 Series Explorer Software V3.7.0. Data were processed using Data Explorer Software Version 3.7 (Applied Biosystems, Framingham, MA, USA). The matrixes for MALDI-TOF MS were 15 mg/mL CHCA (for peptides analysis) and 10 mg/mL SA (for proteins analysis) dissolved in 50% ACN containing 0.1% (v/v) TFA. Equivalent amounts (1 μL) of the sample and matrix were sequentially dropped onto the MALDI plate for MALDI-TOF MS analysis.

### 3. Preparation of $Fe_3O_4$ magnetic nanoparticles (MNPs)

The $Fe_3O_4$ MNPs were synthesized according to a previously reported method.[1] Briefly, 2.0 g of ferric trichloride hexahydrate, 13.0 g of 1,6-hexanediamine and 4.0 g of anhydrous sodium acetate were mixed with 60 mL glycol in a PTFE-lined autoclave and reacted at 198 °C for 6 h. The resulting $Fe_3O_4$ MNPs were washed with water and

anhydrous ethanol for three times each, and then dried at 50 °C in a vacuum overnight.

## 4. Preparation of DFFPBA-functionalized $Fe_3O_4$@$SiO_2$ MNPs

DFFPBA-functionalized $Fe_3O_4$@$SiO_2$ MNPs were synthesized according to the route shown in Fig. S1b, which was comprised of three steps: 1) synthesis of $Fe_3O_4$@$SiO_2$ MNPs; 2) preparation of amino-functionalized $Fe_3O_4$@$SiO_2$ MNPs; 3) preparation of boracic acid-functionalized $Fe_3O_4$@$SiO_2$ MNPs. The detailed procedures are described below.

### *4.1. Synthesis of $Fe_3O_4$@$SiO_2$ MNPs*

200 mL of anhydrous ethanol, 7.5 mL of ammonium hydroxide (28%) and 1.4 mL of TEOS was added into a 500-mL three-neck round-bottomed flask, and then mechanically stirred at 400 rpm for 20 min at 40 °C. 200 mg of MNPs were dispersed into 20 mL of anhydrous ethanol by ultrasonication. The obtained suspension was added into the above flask, and then mechanically stirred at 400 rpm for 20 min at 40 °C. The resulting $Fe_3O_4$@$SiO_2$ MNPs were collected by a magnet, washed with water and anhydrous ethanol for three times each, and then dried at 50 °C in a vacuum overnight.

### *4.2. Preparation of amino-functionalized $Fe_3O_4$@$SiO_2$ MNPs*

The obtained $Fe_3O_4$@$SiO_2$ MNPs were dispersed into 100 mL of anhydrous ethanol by ultrasonication in a 250-mL three-neck round-bottomed flask. Then 3 mL of APTES was added into the flask, and mechanically stirred at 400 rpm for 12 h at 80 °C in a water bath. The resulting amino-functionalized $Fe_3O_4$@$SiO_2$ MNPs were collected by a magnet, washed with water and anhydrous ethanol for three times each, and then dried at 50 °C in a vacuum oven overnight.

### *4.3. Preparation of boracic acid-functionalized $Fe_3O_4$@$SiO_2$ MNPs*

200 mg of amino-functionalized $Fe_3O_4$@$SiO_2$ MNPs were added to 80 mL of methanol

containing 400 mg of DFFPBA and 1% (w/w) sodium cyanoborohydride in a 250-mL three-neck round-bottomed flask, then the mixture was mechanically stirred at 400 rpm for 24 h at room temperature. The obtained $Fe_3O_4$@$SiO_2$@DFFPBA MNPs were collected by a magnet, washed with water and anhydrous ethanol for three times each, and then dried at 50 °C in a vacuum overnight. The obtained $Fe_3O_4$@$SiO_2$@DFFPBA MNPs were stored in a dry and sealed tube at room temperature for further use.

## 5. Preparation of glycated epitope-imprinted MNPs

The preparation procedure is shown in Fig. S1c, which was composed of three steps: 1) immobilization of glycated epitope; 2) oriented imprinting; 3) removal of glycated epitope. The detailed procedures are described below.

### *5.1. Immobilization of glycated epitope*

2 mg of glycated epitope template was dissolved in 2 mL of ammonium bicarbonate buffer (50 mM, pH 8.5) containing 500 mM NaCl. Then 20 mg of $Fe_3O_4$@$SiO_2$@DFFPBA MNPs were dispersed in the resulting solution by ultrasonication, and then shaken at room temperature for 2 h. The obtained glycated epitope-immobilized $Fe_3O_4$@$SiO_2$@DFFPBA MNPs were magnetically collected and washed with ammonium bicarbonate buffer (50 mM, pH 8.5).

### *5.2. Oriented imprinting*

The collected glycated epitope bound $Fe_3O_4$@$SiO_2$@DFFPBA MNPs were dispersed into 150 mL of anhydrous ethanol containing 4.5 mL of ammonium hydroxide (28%), and 10 mL of water was added, the resulting suspension was mechanically stirred for 5 min. Then different ratios of APTES, UPTES, IBTES and TEOS in 40 mL of anhydrous ethanol were added to the above suspension, and then the resulting solution was mechanically stirred at room temperature for a period of time. In order to obtain the best recognition performance, the imprinting time was set at 50, 60, 70 or 80 min under different ratios of monomers (shown in Table S1). The obtained glycated

epitope-imprinted MNPs were collected by a magnet, washed with anhydrous ethanol for three times, and then dried at 40 °C in a vacuum oven overnight.

### *5.3. Removal of glycated epitope*

The obtained glycated epitope-imprinted MNPs were dispersed into 2 mL of ACN:$H_2O$:HAc = 50:49:1 (v/v) and shaken for 20 min at room temperature for three times. After removing the glycated epitope templates, the prepared glycated epitope-imprinted MNPs were magnetically collected, washed with water and anhydrous ethanol for three times each and then dried at 40 °C in a vacuum overnight. Non-imprinted MNPs were prepared using the same procedure except for the absence of glycated epitope templates.

## 6. Selectivity of $Fe_3O_4$@$SiO_2$@DFFPBA MNPs

The selectivity of $Fe_3O_4$@$SiO_2$@DFFPBA MNPs was performed using adenosine and deoxyadenosine as test compounds. 2 mg of $Fe_3O_4$@$SiO_2$@DFFPBA MNPs was dispersed into 200 μL of 1.0 mg/mL adenosine or deoxyadenosine in ammonium bicarbonate buffer (50 mM, pH 8.5) containing 500 mM NaCl, then the mixture was shocked on a rotator at room temperature for 2 h. The $Fe_3O_4$@$SiO_2$@DFFPBA MNPs were magnetically collected and rinsed with 200 μL of ammonium bicarbonate buffer (50 mM, pH 8.5) containing 500 mM NaCl and ammonium bicarbonate buffer (50 mM, pH 8.5) for three times each. Finally, the $Fe_3O_4$@$SiO_2$@DFFPBA MNPs were re-suspended and eluted in 20 μL of 100 mM HAc solution for 1 h on a rotator. The $Fe_3O_4$@$SiO_2$@DFFPBA MNPs were magnetically separated and the eluates were collected. The amount of adenosine or deoxyadenosine bound by the $Fe_3O_4$@$SiO_2$@DFFPBA MNPs were determined by measuring the amount of adenosine or deoxyadenosine in the eluates in terms of UV absorbance at 260 nm. The measurement was repeated for three times. As shown in Fig. S3a, the $Fe_3O_4$@$SiO_2$@DFFPBA MNPs had good selectivity to captured adenosine (*cis*-diol compound) but excluded deoxyasdenosine (non *cis*-diol compound), which proved that

the $Fe_3O_4$@$SiO_2$@DFFPBA MNPs had been successfully modified with boronic acid group. Subsequently, B2M epitope and glycated B2M epitope were selected as test compounds. The extraction procedure was the same as above except that the eluates were measured in terms of UV absorbance at 230 nm. As shown in Fig. S3b, $Fe_3O_4$@$SiO_2$@DFFPBA MNPs selectively bound to glycated B2M epitope, which suggested the B2M epitope was successfully glycated with fructose. The glycated B2M epitope as templates could be imprinted on $Fe_3O_4$@$SiO_2$@DFFPBA MNPs. To further demonstrate the selectivity of $Fe_3O_4$@$SiO_2$@DFFPBA MNPs, RNase A, RNase B, HRP, BSA were used as test compounds. The extraction procedure was the same as above except that the eluates were measured in terms of UV absorbance at 214 nm and further analyzed by MALDI-TOF MS. For control experiment, all the procedures were the same as described above except the absence of target analytes in the extraction buffer. The results of UV absorbance are shown in Fig. S3c, which suggested that $Fe_3O_4$@$SiO_2$@DFFPBA MNPs possessed good selectivity to *cis*-diol compound such as RNase B, and HRP. The results of MALDI-TOF MS are shown in Fig. S4, which were consistent well with the UV absorbance results.

## 7. Optimization of monomer composition and imprinting time for glycated epitope-imprinted MNPs.

The monomer composition and imprinting time of the prepared glycated epitope-imprinted MNPs were optimized according to the obtained imprinting factor (IF).

2.0 mg of glycated epitope-imprinted MNPs and non-imprinted MNPs prepared with 10 different compositions of the monomers of APTES, UPTES, IBTES and TEOS (See Table S1 for the compositions) for certain imprinting time were added to 1 mL of 0.1 mg/mL epitope dissolved in phosphate buffer (10 m, M pH 7.4), respectively. After incubation at room temperature for 20 min, the MNPs were magnetically collected and rinsed with 1 mL of phosphate buffer (10 mM, pH 7.4) for three times. The MNPs were re-suspended and eluted in 50 μL of ACN:$H_2O$:HAc = 50:49:1 (v/v) for 10 min on a rotator. Finally, the MNPs were magnetically separated and the eluates were collected.

The amount of epitope was determined by measuring the amount of epitope in the eluates in terms of UV absorbance at 230 nm. The measurement was repeated for three times. For control experiment, all the procedures were the same as described above except the absence of epitope in the extraction buffer. The results for glycated B2M epitope-imprinted MNPs and non-imprinted MNPs prepared by different proportions monomers under optimal imprinting time are shown in Fig. 2. Their IF values were plotted against the monomer ratio, which suggested that imprinting with 60 min at monomer ratio (APTES/UPTES/IBTES/TEOS = 10:10:20:60) obtained the best performance for glycated B2M epitope-imprinted MNPs.

## 8. Characterization of glycated epitope-imprinted MNPs

The size and morphology of the prepared glycated epitope-imprinted MNPs were characterized by TEM. As shown in Fig. S6, the particles size of glycated epitope-imprinted MNPs and non-imprinted MNPs were approximately 150 nm, and a thin silica layer could be seen on the surface of the particles. FT-IR spectroscopy was also used to characterize the glycated epitope-imprinted MNPs and non-imprinted MNPs, and the spectra are shown in Fig. S7. A strong adsorption peak at 580 $cm^{-1}$ was attributed to Fe-O vibration band. Three observed peaks at 3400 $cm^{-1}$, 1380 $cm^{-1}$ and 1048 $cm^{-1}$ were ascribed to the vibration band of N-H, C-H and C-N, respectively. Three adsorption peaks were increased, which indicated that MNPs were successfully modified with the amino group. The vibration bands at 1100 $cm^{-1}$ and 1090 $cm^{-1}$ were attributed to Si-O and C-F, respectively. The vibration bands at 2940 $cm^{-1}$ and 1630 $cm^{-1}$ were attributed to C-H and C=O, respectively. The adsorption peaks at 3400 $cm^{-1}$, 2940 $cm^{-1}$, 1630 $cm^{-1}$ 1100 $cm^{-1}$ and 1090 $cm^{-1}$ were gradually enhanced, which indicated that the MNPs were functionalized with DFFPBA, and then coated imprinting layer with different monomers (APTES, UPTES, IBTES and TEOS), respectively.

## 9. Measurement of adsorption isotherm

Equivalent glycated B2M epitope-imprinted MNPs and non-imprinted MNPs (2 mg

each) were added to 1 mL of different concentrations of B2M epitope solution in 1.5-mL centrifugal tubes, respectively. The tubes were shaken on a rotator for 20 min at room temperature. The MNPs were magnetically collected and rinsed with 1 mL of phosphate buffer (10 mM, pH 7.4) for three times. After washing, the MNPs were re-suspended and eluted in 50 μL of ACN:$H_2O$:HAc = 50:49:1 (v/v) for 10 min on a rotator. Finally, the MNPs were magnetically separated and the eluates were collected. The amount of B2M epitope captured by the MNPs were determined by measuring the B2M epitope in the eluates in terms of UV absorbance at 230 nm.

## 10. Scatchard analysis

To estimate the binding strength of glycated B2M epitope-imprinted MNPs, the amount of B2M epitope bound to the glycated B2M epitope-imprinted MNPs was plotted according to the Scatchard equation as given below:

$$\frac{Q_e}{S} = \frac{Q_{max}}{K_d} - \frac{Q_e}{K_d}$$

where $Q_e$, $S$, $Q_{max}$ and $K_d$ are the amount of B2M epitope bound to the glycated B2M epitope-imprinted MNPs at equilibrium, the free concentration at adsorption equilibrium, the saturated adsorption capacity and the dissociation constant, respectively. By plotting $Q_e/S$ versus $Q_e$, $K_d$ and $Q_{max}$ can be calculated from the slope and the intercept. The obtained Scatchard plot of the glycated B2M epitope-imprinted MNPs exhibited one straight line (shown in Fig. 3b), indicating that $K_d$ and $Q_{max}$ value were (2.08 ± 0.11) × $10^{-7}$ M and (169.03 ± 5.50) nmol/g, respectively.

## 11. Imprinting efficiency

Equivalent glycated B2M epitope-imprinted MNPs and MNPs@$SiO_2$@DFFPBA (2 mg each) were dispersed into 1 mL of 0.1 mg/mL glycated B2M epitope dissolved in ammonium bicarbonate buffer (50 mM, pH 8.5) containing 500 mM NaCl, and then the mixture was shaken on a rotator at room temperature for 2 h. The MNPs were magnetically collected and rinsed with 1 mL of ammonium bicarbonate buffer (50 mM, pH 8.5) containing 500 mM NaCl and ammonium bicarbonate buffer (50 mM, pH 8.5)

for three times each. After washing, the MNPs were re-suspended and eluted in 50 μL of ACN:$H_2O$:HAc = 50:49:1 (v/v) for 1 h on a rotator. Finally, the MNPs were magnetically separated and the eluates were collected. The amount of glycated B2M epitope bound by the MNPs were determined by measuring the amount of glycated B2M epitope in the eluates in terms of UV absorbance at 230 nm. The imprinting efficiency value was calculated by dividing the bound glycated B2M epitope amount of glycated B2M epitope-imprinted MNPs by that of the $Fe_3O_4$@$SiO_2$@DFFPBA MNPs. The measurement was repeated for three times. The imprinting efficiency value was measured to be 54.2%.

## 12. Extraction equilibrium

The incubation time between the B2M epitope and the glycated B2M epitope-imprinted MNPs was investigated. First, 0.1 mg/mL B2M epitope solution was prepared with phosphate buffer (10 mM, pH 7.4). Then, 2 mg of glycated B2M epitope-imprinted MNPs prepared by the monomers proportion of APTES/UPTES/IBTES/TEOS = 10:10:20:60 with 60 min of imprinting time was added to 1 mL of the B2M epitope solution in 1.5-mL centrifugal tubes. The tubes were shaken on a rotator at room temperature from 5 to 30 min for incubation between B2M epitope and glycated B2M epitope-imprinted MNPs. The MNPs were magnetically collected and rinsed with 1 mL of phosphate buffer (10 mM, pH 7.4) for three times. Second, the MNPs were re-suspended and eluted in 50 μL of ACN:$H_2O$:HAc = 50:49:1 (v/v) for 10 min on a rotator. Finally, the MNPs were magnetically separated and the eluates were collected. The amount of B2M epitope on the glycated B2M epitope-imprinted MNPs were determined by measuring the amount of B2M epitope in the eluates in terms of UV absorbance at 230 nm. The measurement was repeated for three times. The incubation time between the B2M intact protein and the glycated B2M epitope-imprinted MNPs was also investigated. The experimental procedure was the same as above except that the analyte was replaced by the B2M intact protein and the eluates were measured in terms of UV absorbance at 214 nm. The results are shown in Fig. S8, which indicates

that the extraction equilibrium times for the epitope and intact protein were both 20 min.

### 13. Stability test

The stability of glycated epitope-imprinted MNPs was evaluated in terms of the extracted target protein amount within three months. First, a solution of 0.1 mg/mL B2M was prepared with phosphate buffer (10 mM, pH 7.4). Then, 2 mg of glycated B2M epitope-imprinted MNPs prepared with the monomers proportion of APTES/UPTES/IBTES/TEOS = 10:10:20:60 for 60 min of imprinting time was added to 1 mL of the B2M solution in 1.5-mL centrifugal tubes. The tubes were shaken on a rotator at room temperature for 20 min. The MNPs were magnetically collected and rinsed with 1 mL of phosphate buffer (10 mM, pH 7.4) for three times. Second, the MNPs were re-suspended and eluted in 50 μL of ACN:$H_2O$:HAc = 50:49:1 (v/v) for 10 min on a rotator. Finally, the MNPs were magnetically separated and the eluates were collected. The amount of B2M on the glycated B2M epitope-imprinted MNPs were determined by measuring the amount of B2M in the eluates in terms of UV absorbance at 214 nm. The measurement was repeated for three times. The result is shown in Fig. S9, which indicates that the glycated B2M epitope-imprinted MNPs could be stably stored for at least three months in a dry and sealed tube at room temperature.

### 14. Reusability test

The reusability of the glycated B2M epitope-imprinted MNPs was evaluated in terms of the extracted amount of B2M, BSA, HRP, RNase A and RNase B for six consecutive uses. First, 0.1 mg/mL protein solutions were separately prepared with phosphate buffer (10 mM, pH 7.4). Then, 2 mg of glycated B2M epitope-imprinted MNPs prepared by the monomers proportion of APTES/UPTES/IBTES/TEOS = 10:10:20:60 with 60 min of imprinting time was added to 1 mL of the protein solutions in 1.5-mL centrifugal tubes, respectively. The tubes were shaken on a rotator at room temperature for 20 min. The MNPs were magnetically collected and rinsed with 1 mL of phosphate buffer (10 mM, pH 7.4) for three times. Second, the MNPs were re-suspended and eluted in

50 μL of ACN:$H_2O$:HAc = 50:49:1 (v/v) for 10 min on a rotator. Finally, the MNPs were magnetically separated and the eluates were collected. Subsequently, the MNPs were further washed with 1 mL of phosphate buffer (10 mM, pH 7.4) for three times and directly used for the next use. The amount of the proteins on the glycated B2M epitope-imprinted MNPs were determined by measuring the amount of the proteins in the eluates in terms of UV absorbance at 214 nm. The measurement was repeated for three times. The result is shown in Fig. S10, which indicates that the glycated B2M epitope-imprinted MNPs could endure 6 consecutive uses with cross-reactivity less than 30%.

## 15. Protein digestion by trypsin

The procedure for the tryptic digest of proteins used in the experiment was prepared as follows: 1.0 mg/mL of protein was dissolved in ammonium bicarbonate buffer (100 mM, pH 8.5) in a 1.5-mL centrifugal tube, and heated in a water bath of 95 °C for 10 min. After the protein solution was cooled to room temperature, trypsin was added to the above tube at 37 °C for 18 h (50:1, w/w). Finally, the resulting mixture was heated again to 95 °C for 10 min. The obtained protein digest solution was store at -20 °C.

## 16. Selectivity test

### *16.1. Selectivity of glycated epitope-imprinted MNPs in protein level*

The selectivity of glycated B2M epitope-imprinted MNPs was evaluated using B2M, RNase A, BSA, RNase B and HRP. First, 0.1 mg/mL each protein solution was separately prepared with phosphate buffer (10 mM, pH 7.4). Then equivalent glycated B2M epitope-imprinted MNPs and non-imprinted MNPs prepared by the monomers proportion of APTES/UPTES/IBTES/TEOS = 10:10:20:60 with different imprinting time (50, 60 and 70 min) (2 mg each) were added to 1 mL of the protein solutions in 1.5-mL centrifugal tubes, respectively. The tubes were shaken on a rotator at room temperature for 20 min. The MNPs were magnetically collected and rinsed with 1 mL of phosphate buffer (10 mM, pH 7.4) for three times. Second, the MNPs were re-

suspended and eluted in 50 μL of ACN:$H_2O$:HAc = 50:49:1 (v/v) for 10 min on a rotator. Finally, the MNPs were magnetically separated and the eluates were collected. The amount of proteins on the glycated B2M epitope-imprinted MNPs were determined by measuring the amount of proteins in the eluates in terms of UV absorbance at 214 nm. The measurement was repeated for three times. For control experiment, all the procedures were the same as described above except the absence of proteins in the extraction buffer. The results for glycated B2M epitope-imprinted MNPs and non-imprinted MNPs are shown in Fig. 4a-c. The IF was plotted against the imprinting time (see Fig. 4d), which suggested that imprinting with 60 min under optimal monomer ratio (APTES/UPTES/IBTES/TEOS = 10:10:20:60) provided the best performance. This was in accordance with the above result of the optimized imprinting time for B2M epitope, which further proved that the imprinting process was precisely controllable. The eluates were also analyzed by MALDI-TOF MS. The results are shown in Fig. S11-S13, which were consistent well with the UV detection results.

### *16.2. Selectivity of glycated epitope-imprinted MNPs in peptide level*

The selectivity of the glycated B2M epitope-imprinted MNPs in the peptide level was evaluated with the mixtures of B2M epitope and tryptic digest of HRP and BSA at mole ratios of 1:1:1. First, 1 mL of mixture solution of B2M epitope (0.01 mM) and tryptic digest of HRP (0.01 mM) and BSA (0.01 mM) was prepared by phosphate buffer (10 mM, pH 7.4). Then equivalent glycated B2M epitope-imprinted MNPs and non-imprinted MNPs (2 mg each) were added to the mixture solution in 1.5-mL centrifugal tubes, respectively. The tubes were shaken on a rotator at room temperature for 20 min. The MNPs were magnetically collected and rinsed with 1 mL of phosphate buffer (10 mM, pH 7.4) for three times. Second, the MNPs were re-suspended and eluted in 50 μL of ACN:$H_2O$:HAc = 50:49:1 (v/v) for 10 min on a rotator. Finally, the MNPs were magnetically separated and the eluates were collected. The eluates were analyzed by MALDI-TOF MS. The results are shown in Fig. S14, the glycated B2M epitope-imprinted MNPs exhibited excellent binding selectivity toward target epitope from

tryptic digest of HRP and BSA with strong interference.

## 17. Preparation of $Ag@SiO_2$ for thickness controllability characterization

As the thickness of the imprinting layer on the glycated epitope-imprinted MNPs is difficult to directly measure by TEM, $Ag@SiO_2$ were prepared as a substitute for the thickness controllability characterization.

The Ag nanoparticles (AgNPs) were prepared as described by Lee and Meisel.[2] In brief, 36 mg of $AgNO_3$ was dissolved in 200 mL of water in a 500-ml three-neck round-bottom flask and brought to boil under continuous stirring. Then, 4 mL of 1% (w/v) trisodium citrate was added to the above flask. The mixture was boiled with stirring for about 1 h and then cooled down to room temperature naturally. The obtained AgNPs colloidal solution was stored at 4 °C before use.

To coat an imprinting layer on the AgNPs surface, 10 mL of the Ag colloidal solution was added to 150 mL of anhydrous ethanol containing 4.5 mL of ammonium hydroxide (28%), and the suspension was stirred for 5 min. Then APTES (2 mM), UPTES (2 mM), IBTES (4 mM) and TEOS (12 mM) in 40 mL of anhydrous ethanol were added to the above suspension, and mechanically stirred at room temperature for a period of time. To obtain various thickness of imprinting layer, the stirring time of the mixture was increased from 30 to 80 min at 10 min per step. Finally, each suspension of $Ag@SiO_2$ was centrifuged at 10,000 rpm for 8 min and washed with anhydrous ethanol three times followed by re-dispersing in 10 mL of anhydrous ethanol. The thickness of the resulting imprinting layer was roughly estimated by TEM (shown in Fig. 5a). The estimated thickness was plotted against the polymerization time. As shown in Fig. 5b, the thickness increased linearly as increasing the polymerization time under monomers proportion of APTES/UPTES/IBTES/TEOS = 10:10:20:60.

## 18. Real sample application

### *18.1. Specific extraction of B2M from human serum*

To demonstrate the potentials of glycated epitope-imprinted MNPs for real-world

applications, extraction of B2M from human serum by glycated B2M epitope-imprinted MNPs was performed. First, human serum was diluted 20 times with phosphate buffer (10 mM, pH 7.4). Then equivalent glycated B2M epitope-imprinted MNPs or non-imprinted MNPs (2 mg each) were added to 1 mL of diluted human serum solution in 1.5-mL centrifugal tubes, respectively. The tubes were shaken on a rotator for 20 min at room temperature. The MNPs were magnetically collected and rinsed with 1 mL of phosphate buffer (10 mM, pH 7.4) for three times. After washing, the MNPs were re-suspended and eluted in 50 μL of ACN:$H_2O$:HAc = 50:49:1 (v/v) for 10 min on a rotator. Finally, the MNPs were magnetically separated and the eluates were collected. The eluates were directly analyzed by MALDI-TOF MS. For human serum sample, only B2M was extracted by the glycated B2M epitope-imprinted MNPs (Fig. S15).

### *18.2. Selective extraction of B2M epitope spiked in human serum*

To evaluate the selectivity of the glycated B2M epitope-imprinted MNPs in peptide level of complex samples, tryptic digest of human serum spiked with B2M epitope was investigated. First, 0.1 mM B2M epitope was spiked to the tryptic digest of human serum diluted 20 times in phosphate buffer (10 mM, pH 7.4). Equivalent glycated B2M epitope-imprinted MNPs or non-imprinted MNPs (2 mg each) were added to 1 mL of the above spiked human serum solution in 1.5-mL centrifugal tubes, respectively. The tubes were shaken on a rotator for 20 min at room temperature. The MNPs were magnetically collected and rinsed with 1 mL of phosphate buffer (10 mM, pH 7.4) for three times. After washing, the MNPs were re-suspended and eluted in 50 μL of ACN:$H_2O$:HAc = 50:49:1 (v/v) for 10 min on a rotator. Finally, the MNPs were magnetically separated and the eluates were collected. The eluates were directly analyzed by MALDI-TOF MS. For the B2M epitope spiked tryptic digest of human serum sample, only the B2M epitope was extracted by the glycated B2M epitope-imprinted MNPs (Fig. 6).

## 19. Preparation of glycated Mb epitope-imprinted MNPs

For synthesis of glycated Mb epitope-imprinted MNPs, the imprinting procedure was the same as that for glycated B2M epitope-imprinted MNPs except the template was replaced with the glycated Mb epitope. The corresponding non-imprinted MNPs were synthesized by using the same procedure except for the absence of glycated Mb epitope template.

## 20. Selectivity test of glycated Mb epitope-imprinted MNPs

### *20.1. Selectivity of glycated Mb epitope-imprinted MNPs in protein level*

The selectivity of glycated Mb epitope-imprinted MNPs in the protein level was evaluated as the same as the glycated B2M epitope-imprinted MNPs. The results of the UV detection are shown in Fig. S16 and the corresponding results of MALDI-TOF MS detection are shown in Fig. S17. These results indicate that the glycated Mb epitope-imprinted MNPs possessed excellent specificity toward Mb.

### *20.2. Selectivity of glycated Mb epitope-imprinted MNPs in peptide level*

The selectivity of glycated Mb epitope-imprinted MNPs in the peptide level was evaluated with a mixture of Mb epitope (0.01 mM) and the tryptic digest of HRP (0.01 mM) and BSA (0.01 mM). The extraction procedure was all the same as the glycated B2M epitope-imprinted MNPs. The results of MALDI-TOF MS detection are shown in Fig. S18. The results indicate that the glycated Mb epitope-imprinted MNPs exhibited excellent specificity in the peptide level.

**References:**

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

(2) P. C. Lee and D. Meisel, *J. Phys. Chem.*, 1982, **86**, 3391-3395.

# SUPPLEMENTARY DATA

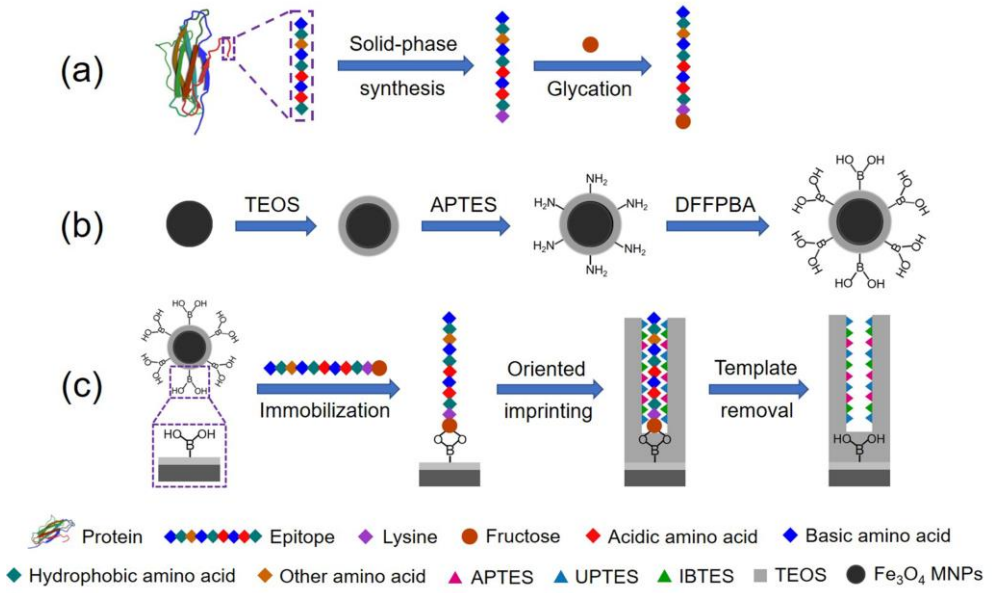


**Fig. S1** Synthesis routes of glycated epitope (a), $Fe_3O_4$@$SiO_2$@DFFPBA MNPs (b), and glycated epitope-imprinted MNPs (c).

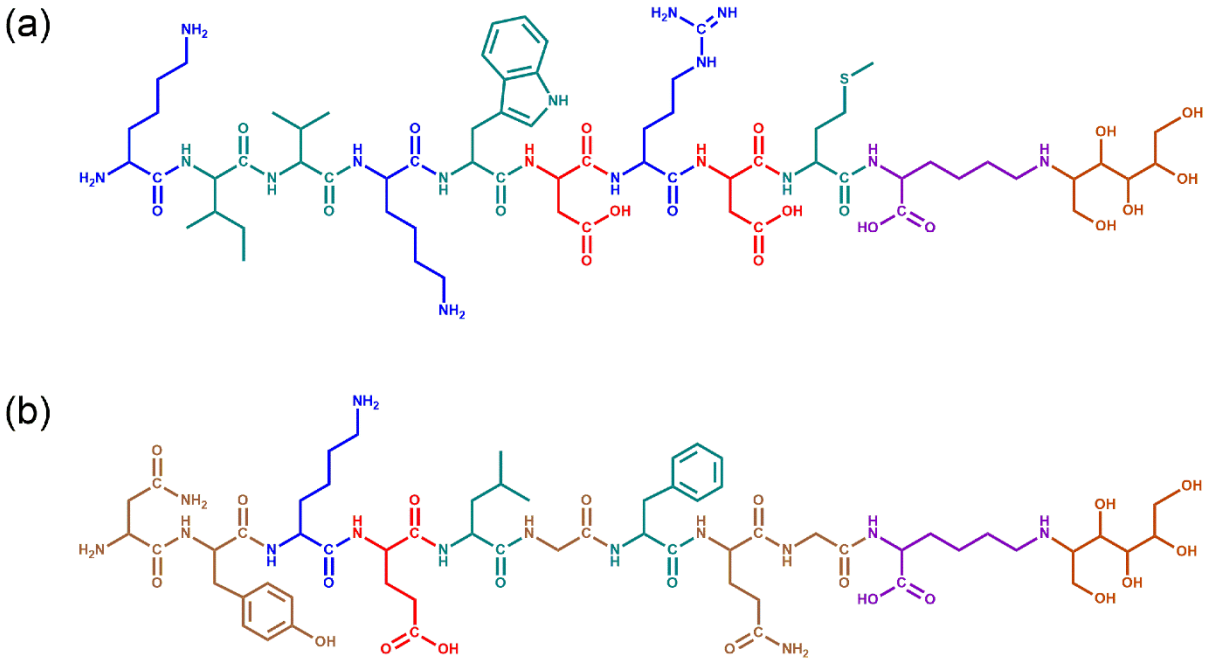


**Fig. S2** The structures of glycated B2M epitope (a) and glycated Mb epitope (b).

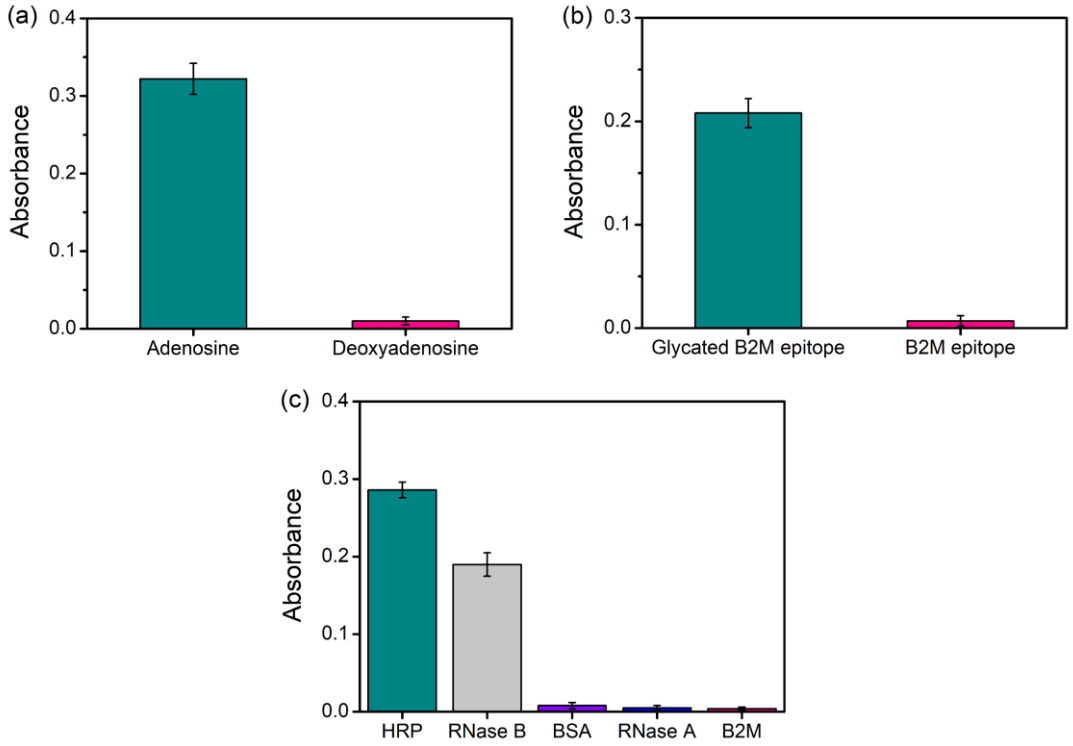


**Fig. S3** Comparison of the amount of different analytes captured by $Fe_3O_4$@$SiO_2$@DFFPBA MNPs. 1.0 mg/mL of adenosine or deoxyadenosine (a), glycated B2M epitope or B2M epitope (b) and B2M, RNase A, RNase B, HRP or BSA (c) dissolved in binding buffer. Binding buffer: ammonium bicarbonate (50 mM, pH 8.5) containing 500 mM NaCl; elution solution: 100 mM HAc. The error bars represent standard deviation of three parallel experiments.

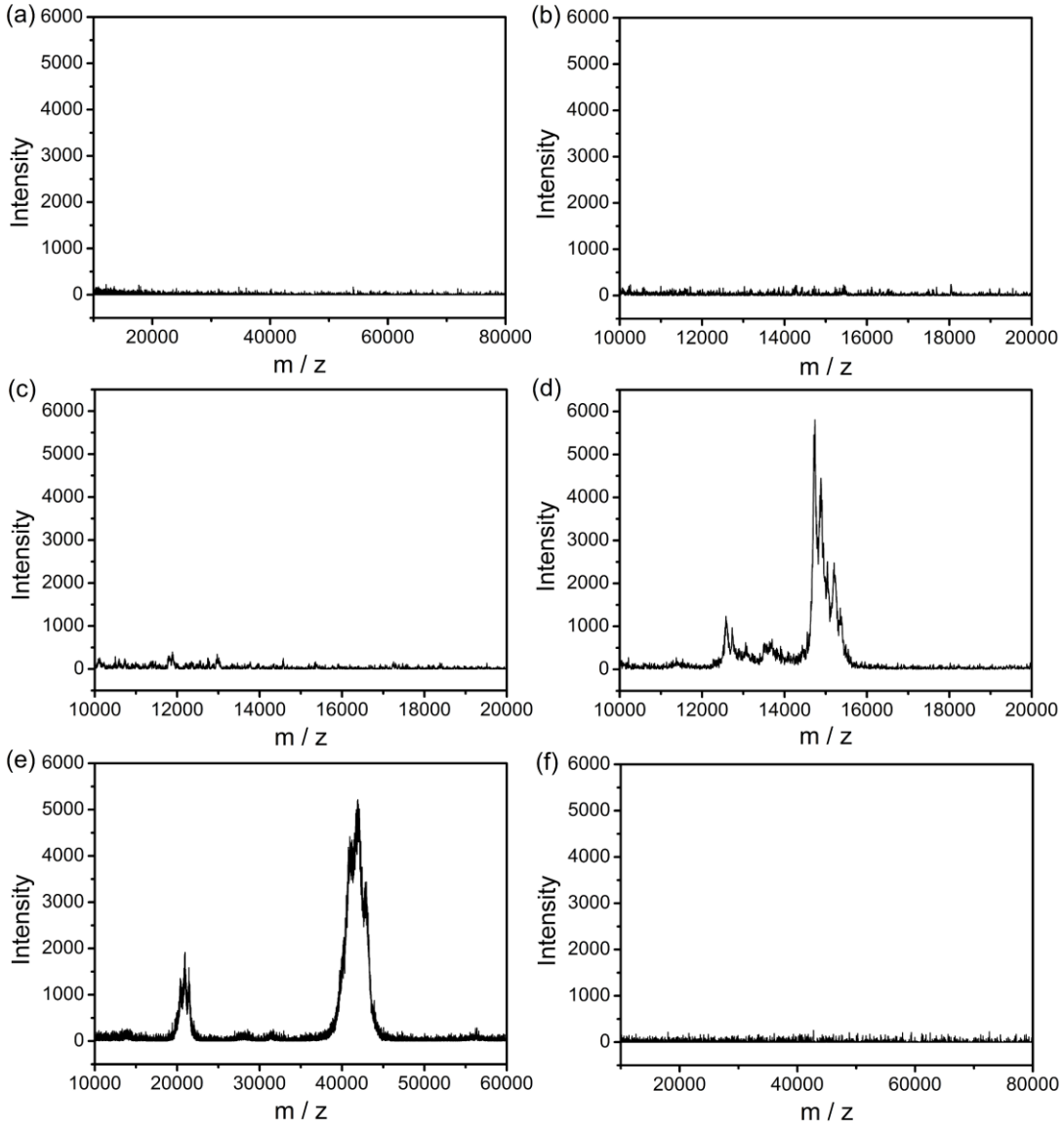


**Fig. S4** MALDI-TOF MS spectra for proteins extracted by $Fe_3O_4$@$SiO_2$@DFFPBA MNPs from different solutions. binding buffer (control) (a), 1.0 mg/mL of B2M (b) or RNase A (c) or RNase B (d) or HRP (e) or BSA (f) dissolved in binding buffer. Binding buffer: ammonium bicarbonate (50 mM, pH 8.5) containing 500 mM NaCl; elution solution: 100 mM HAc. These results show that RNase B and HRP were extracted by $Fe_3O_4$@$SiO_2$@DFFPBA MNPs, indicating the boronate affinity of the MNPs.

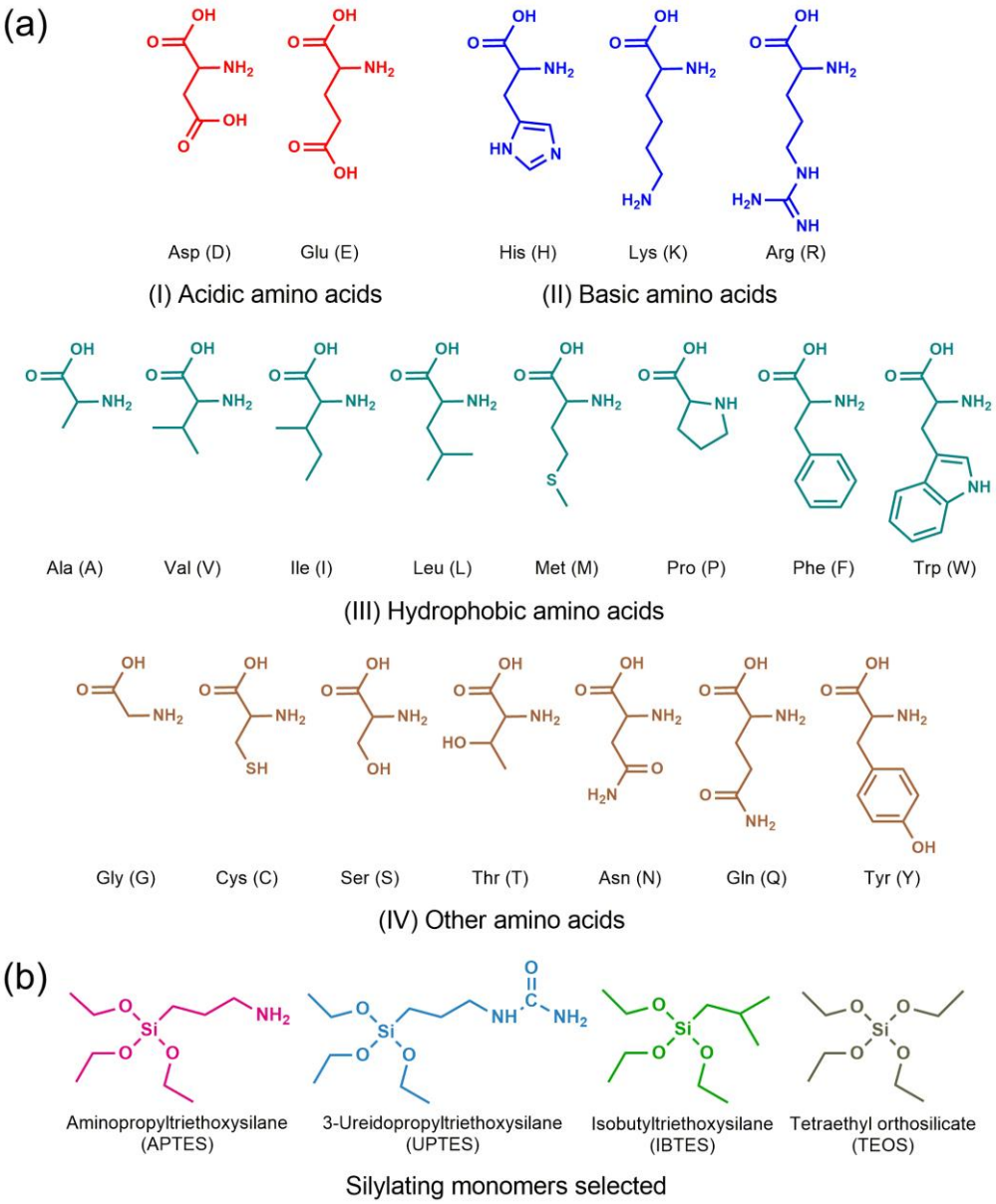


**Fig. S5** Classification of amino acids in terms of their structure (a) and the silylating monomers selected for the imprinting (b).

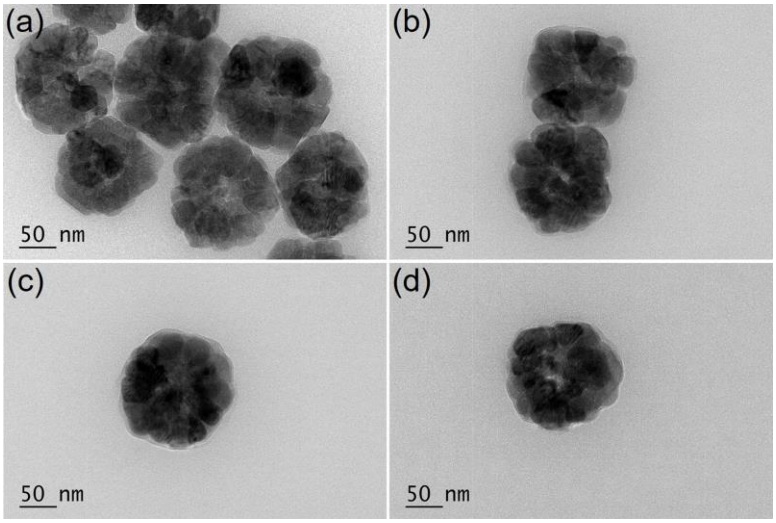


**Fig. S6** TEM images for $Fe_3O_4$ MNPs (a), $Fe_3O_4$@$SiO_2$@DFFPBA MNPs (b), glycated epitope-imprinted MNPs (c), and non-imprinted MNPs (d).

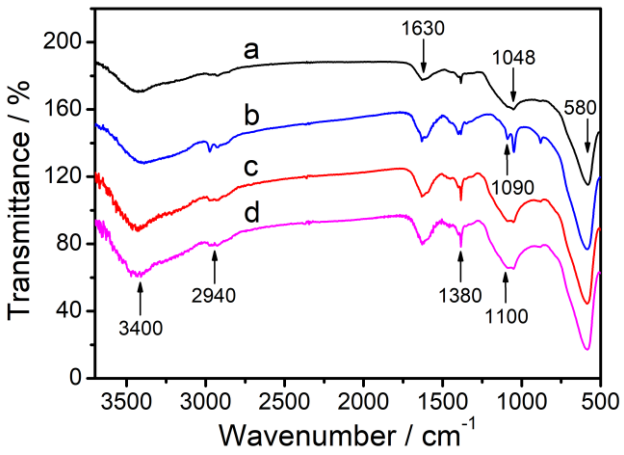


**Fig. S7** FI-IR spectra for $Fe_3O_4$ MNPs (a), $Fe_3O_4$@$SiO_2$@DFFPBA MNPs (b), glycated epitope-imprinted MNPs (c), and non-imprinted MNPs (d).

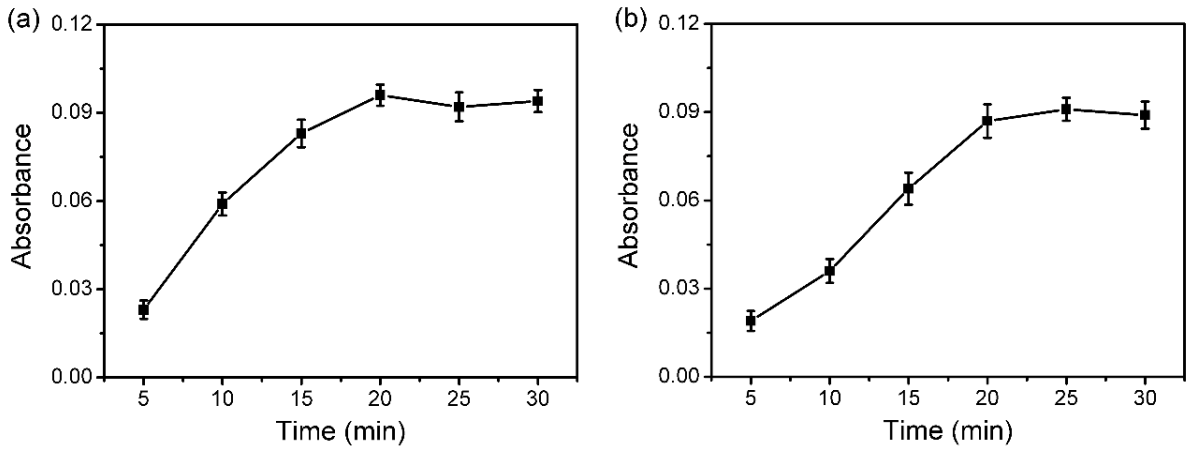


**Fig. S8** Dependence of the amount of B2M epitope (a) and B2M (b) extracted by glycated B2M epitope-imprinted MNPs on the extraction time.

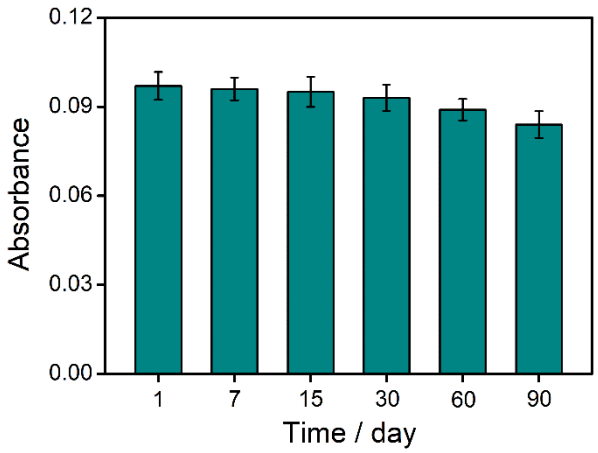


**Fig. S9** The amount of B2M captured by the glycated B2M epitope-imprinted MNPs at different storage time.

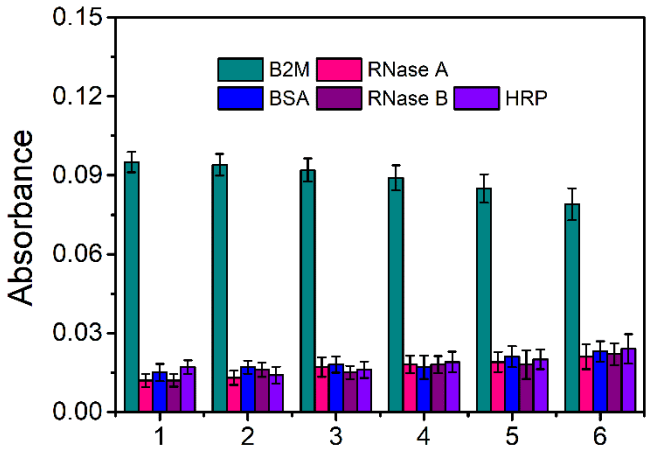


**Fig. S10** The amount of different proteins (B2M, RNase A, BSA, RNase B and HRP) extracted by glycated B2M epitope-imprinted MNPs for six consecutive uses.

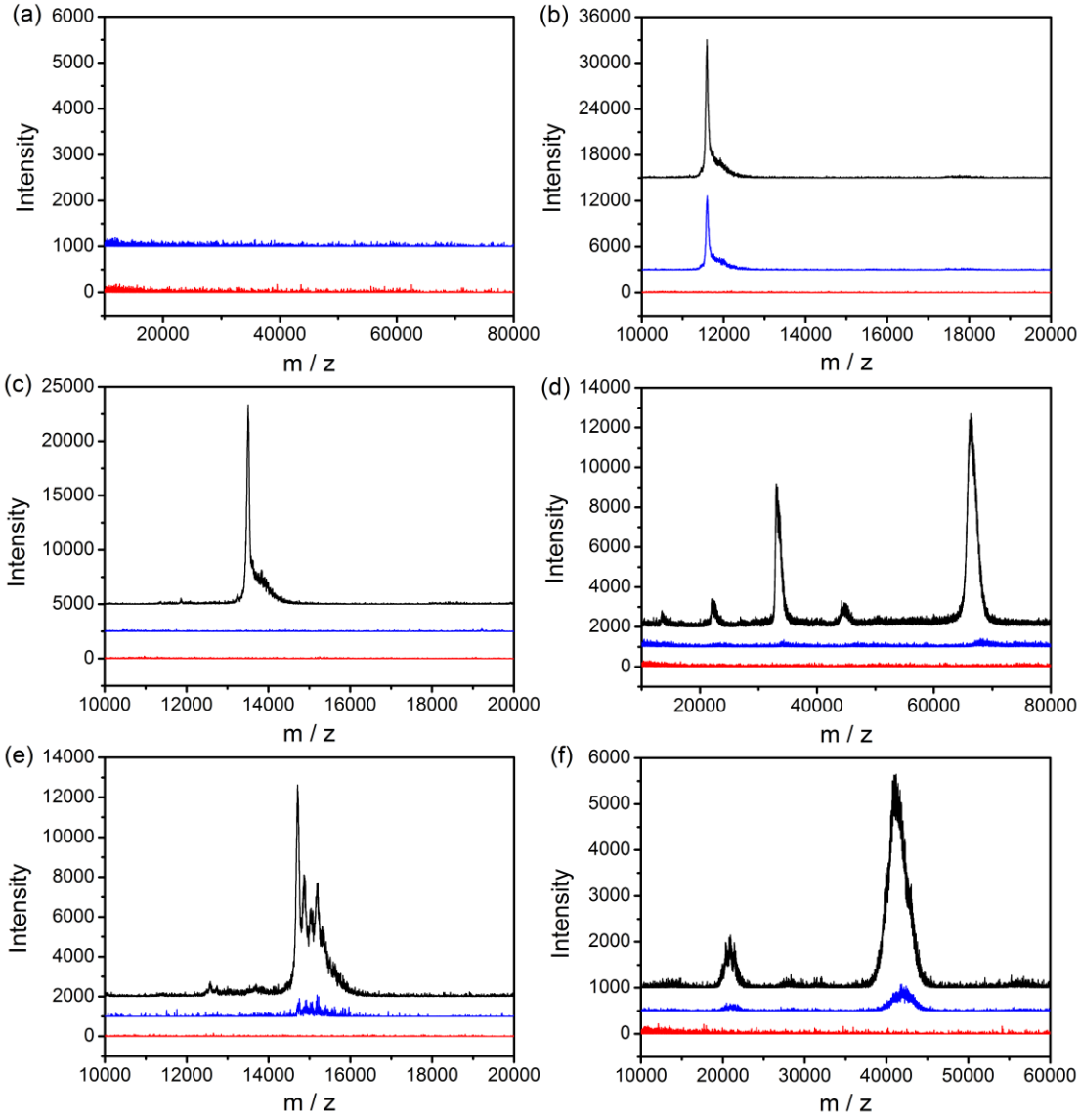


**Fig. S11** MALDI-TOF MS spectra of direct analysis of proteins (black line), proteins extracted by glycated B2M epitope-imprinted MNPs prepared under the ratio of APTES/UPTES/IBTES/TEOS at 10:10:20:60 with 50 min of imprinting time (blue line) and by corresponding non-imprinted MNPs (red line) from different samples: binding buffer (control) (a); 1.0 mg/mL B2M (b) or RNase A (c) or BSA (d) or RNase B (e) or HRP (f) dissolved in binding buffer. Binding buffer: phosphate buffer (10 mM, pH 7.4); elution solution: ACN:$H_2O$:HAc = 50:49:1 (v/v).

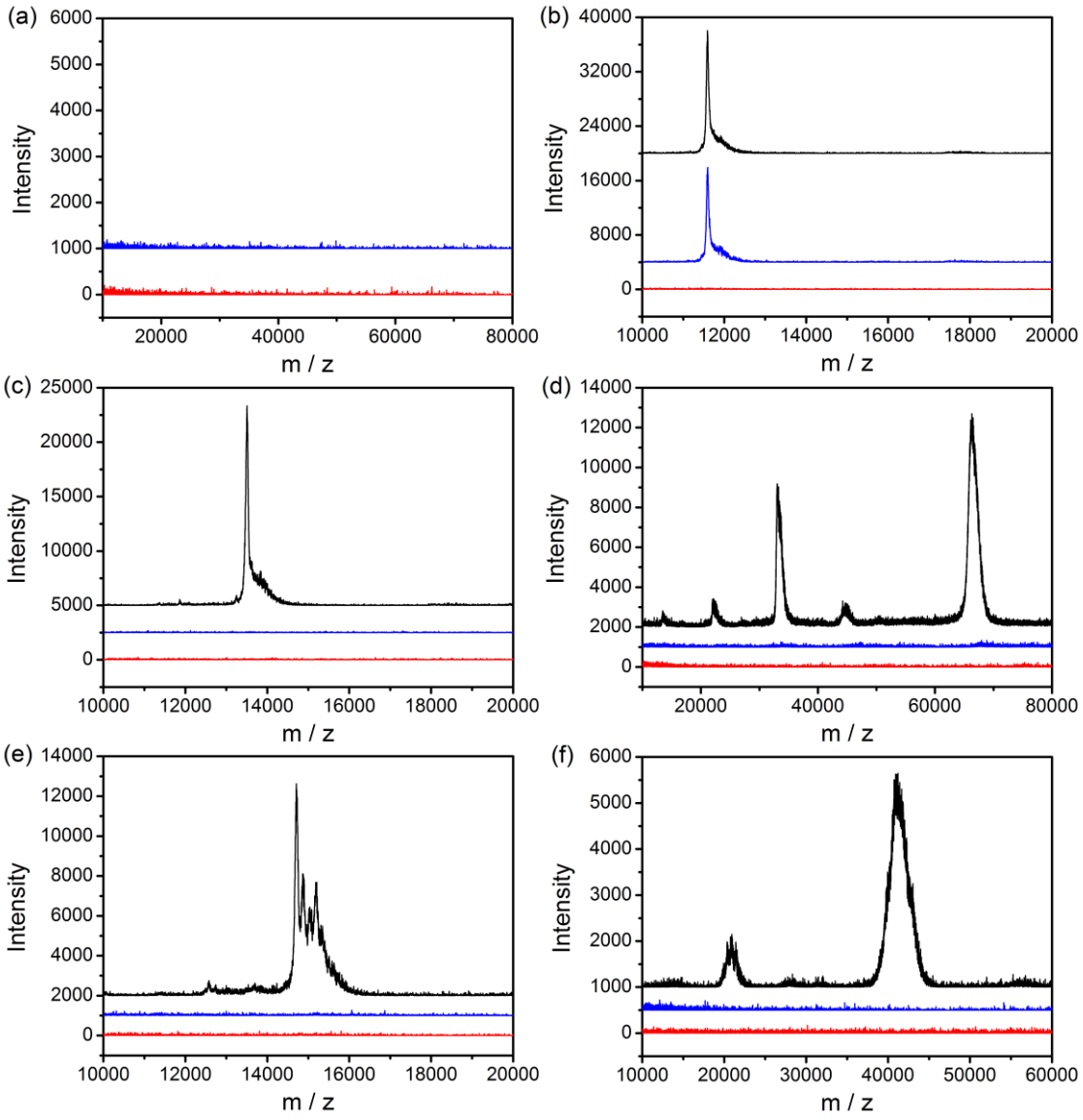


**Fig. S12** MALDI-TOF MS spectra of direct analysis of proteins (black line), proteins extracted by glycated B2M epitope-imprinted MNPs prepared under the ratio of APTES/UPTES/IBTES/TEOS at 10:10:20:60 with 60 min of imprinting time (blue line) and by corresponding non-imprinted MNPs (red line) from different samples: binding buffer (control) (a); 1.0 mg/mL B2M (b) or RNase A (c) or BSA (d) or RNase B (e) or HRP (f) dissolved in binding buffer. Binding buffer: phosphate buffer (10 mM, pH 7.4); elution solution: ACN:$H_2O$:HAc = 50:49:1 (v/v).

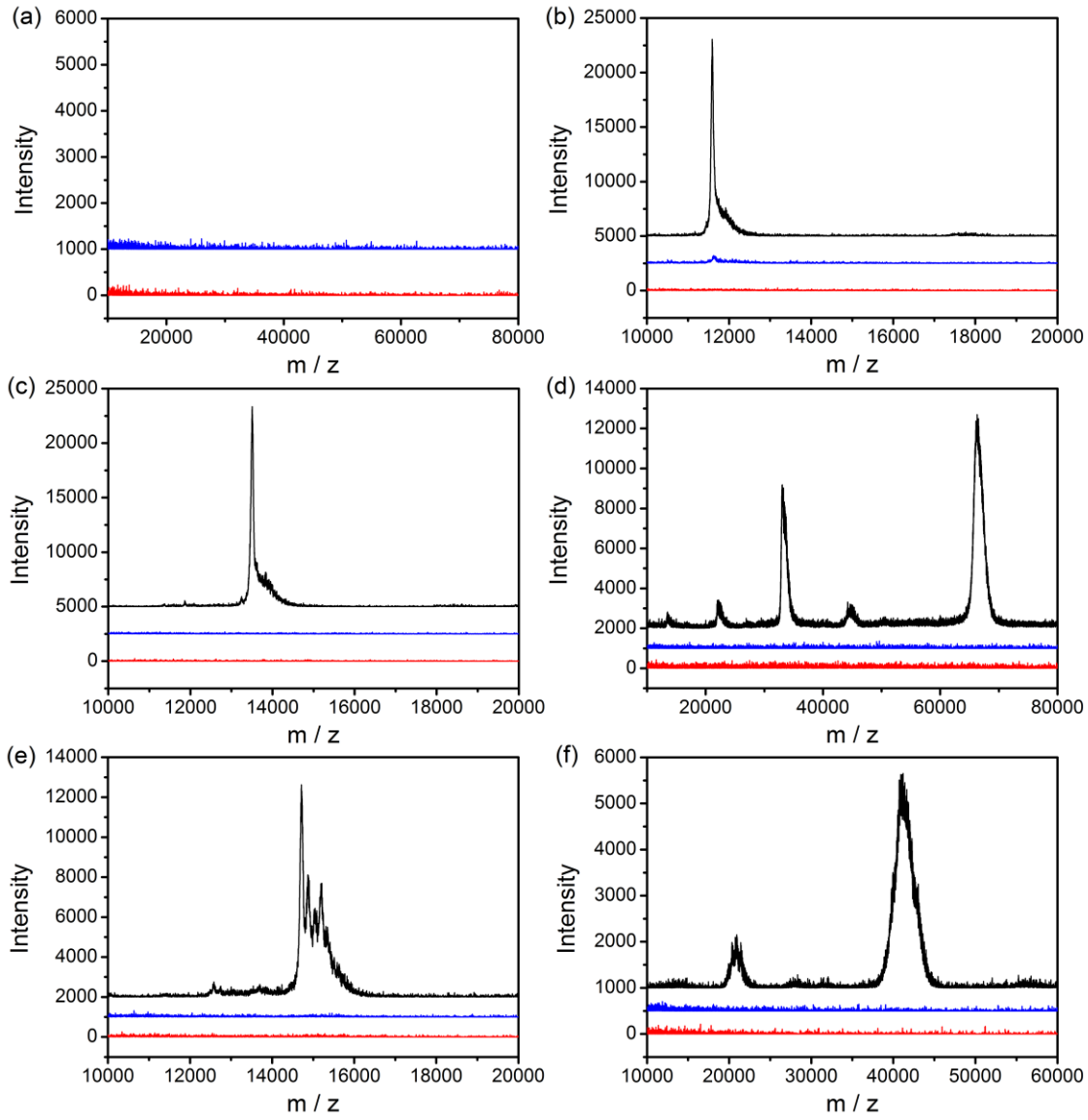


**Fig. S13** MALDI-TOF MS spectra of direct analysis of proteins (black line), proteins extracted by glycated B2M epitope-imprinted MNPs prepared under the ratio of APTES/UPTES/IBTES/TEOS at 10:10:20:60 with 70 min of imprinting time (blue line) and by corresponding non-imprinted MNPs (red line) from different samples: binding buffer (control) (a); 1.0 mg/mL B2M (b) or RNase A (c) or BSA (d) or RNase B (e) or HRP (f) dissolved in binding buffer. Binding buffer: phosphate buffer (10 mM, pH 7.4); elution solution: ACN:$H_2O$:HAc = 50:49:1 (v/v).

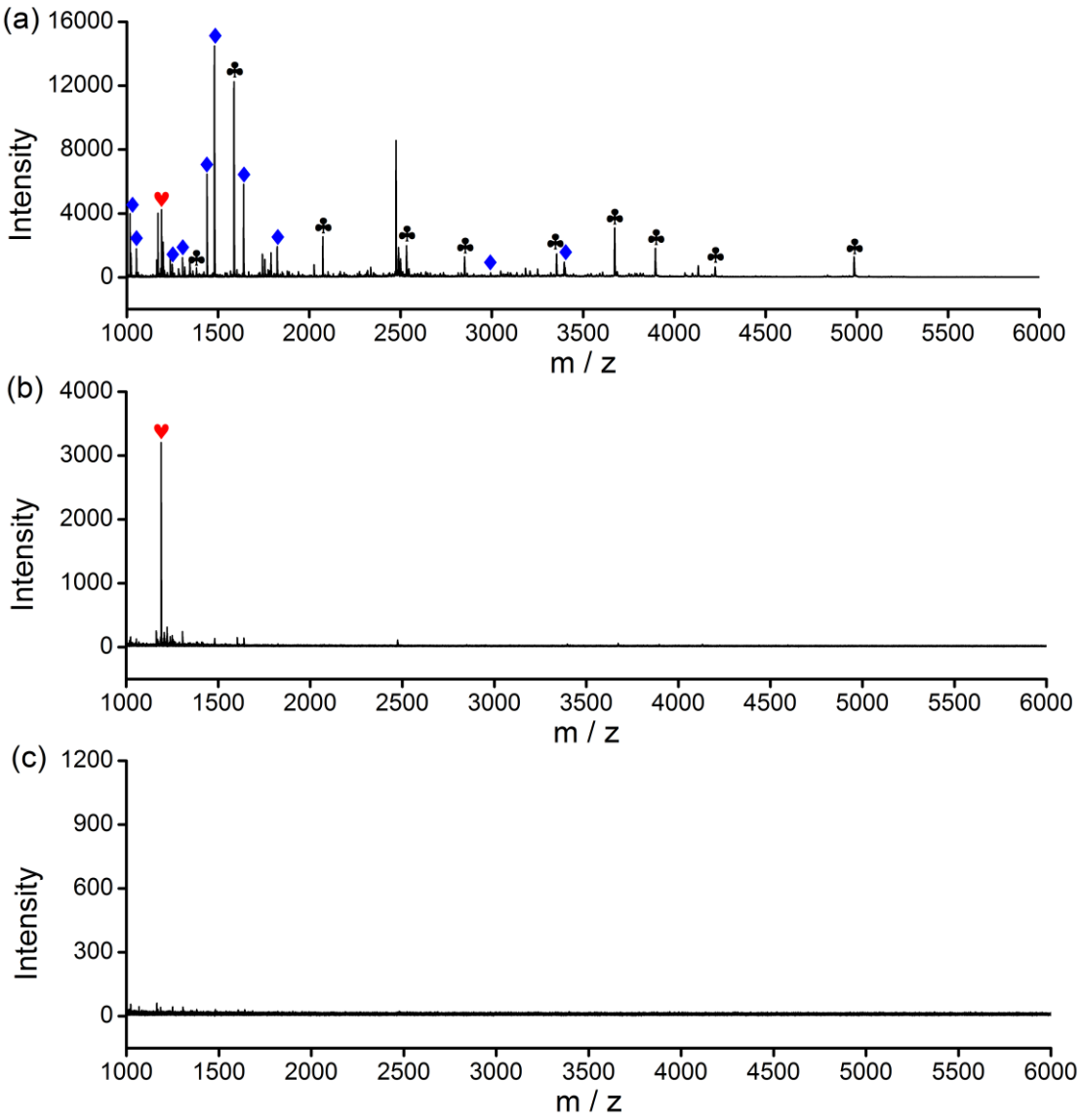


**Fig. S14** MALDI-TOF MS spectra for the analysis of the mixtures of B2M epitope and tryptic digests of HRP and BSA with the mole ratio 1:1:1. (a) direct analysis; (b) analysis after extracted by glycated B2M epitope-imprinted MNPs; (c) analysis after extracted by non-imprinted MNPs. (♥: B2M epitope; ♣: peptides from tryptic digest of HRP; ♦: peptides from tryptic digest of BSA).

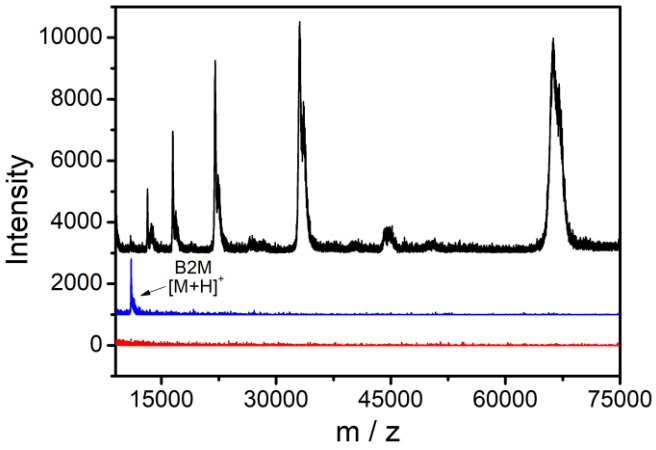


**Fig. S15** MALDI-TOF MS spectra for the direct analysis of human serum (black trace), analysis after extracted by glycated B2M epitope-imprinted MNPs (blue trace) and analysis after extracted by non-imprinted MNPs (red trace).

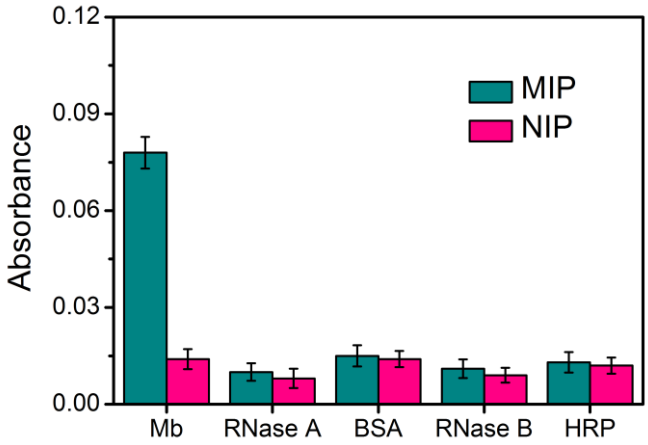


**Fig. S16** The amount of different proteins captured by glycated Mb epitope-imprinted MNPs and non-imprinted MNPs prepared by the monomers proportion of APTES/UPTES/IBTES/TEOS = 10:10:20:60 with 60 min of imprinting time. Samples: 1.0 mg/mL of Mb, RNase A, BSA, RNase B or HRP dissolved in binding buffer. Binding buffer: phosphate buffer (10 mM, pH 7.4); elution solution: ACN:$H_2O$:HAc = 50:49:1 (v/v). The error bars represent standard deviation of three parallel experiments.

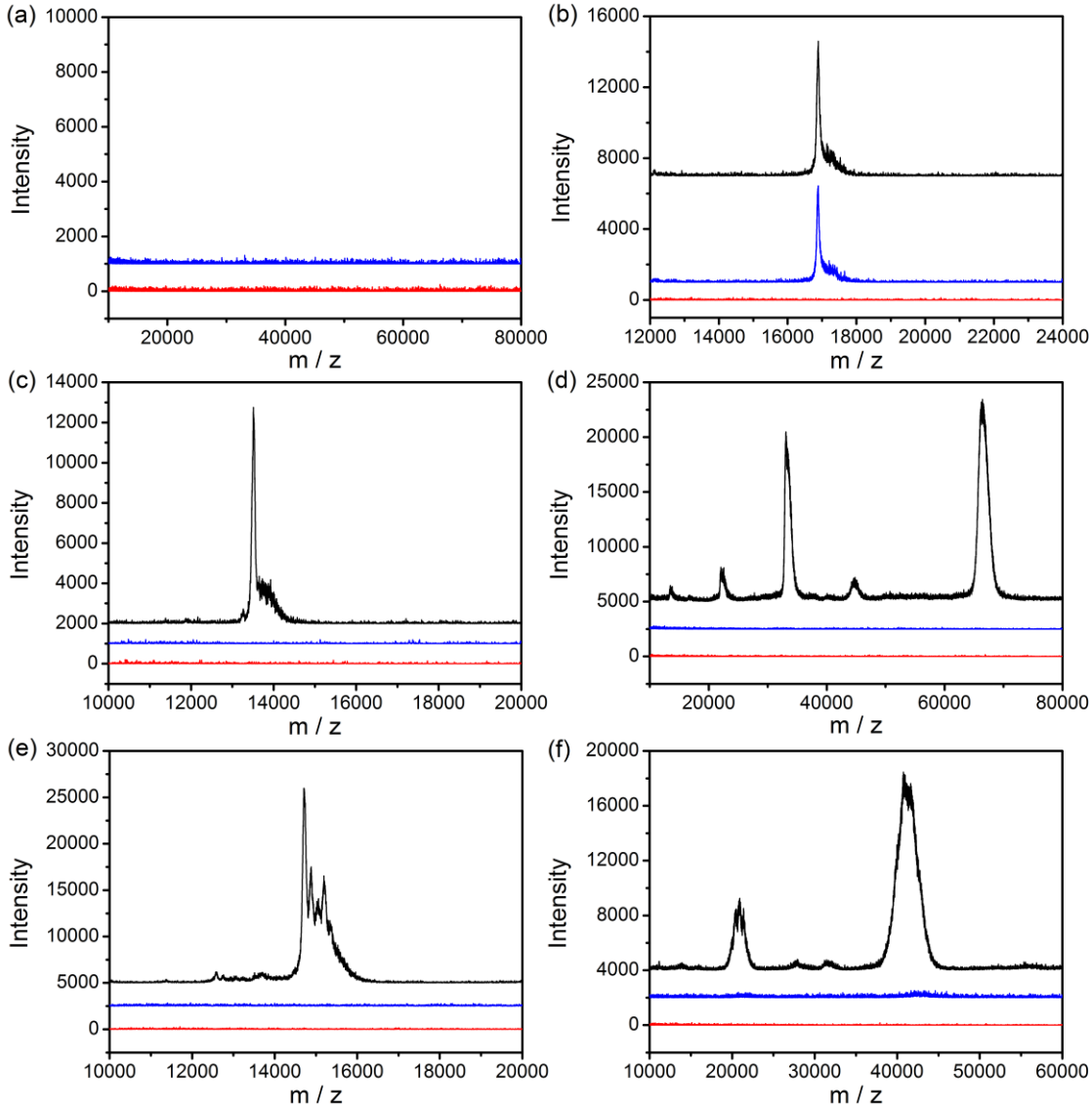


**Fig. S17** MALDI-TOF MS spectra of direct analysis of proteins (black line), proteins extracted by glycated Mb epitope-imprinted MNPs (blue line) and corresponding non-imprinted MNPs (red line) prepared by the monomers proportion of APTES/UPTES/IBTES/TEOS = 10:10:20:60 with 60 min of imprinting time from different samples: binding buffer (control) (a), 1.0 mg/mL of Mb (b) or RNase A (c) or BSA (d) or RNase B (e) or HRP (f) dissolved in binding buffer. Binding buffer: phosphate buffer (10 mM, pH 7.4); elution solution: ACN:$H_2O$:HAc = 50:49:1 (v/v).

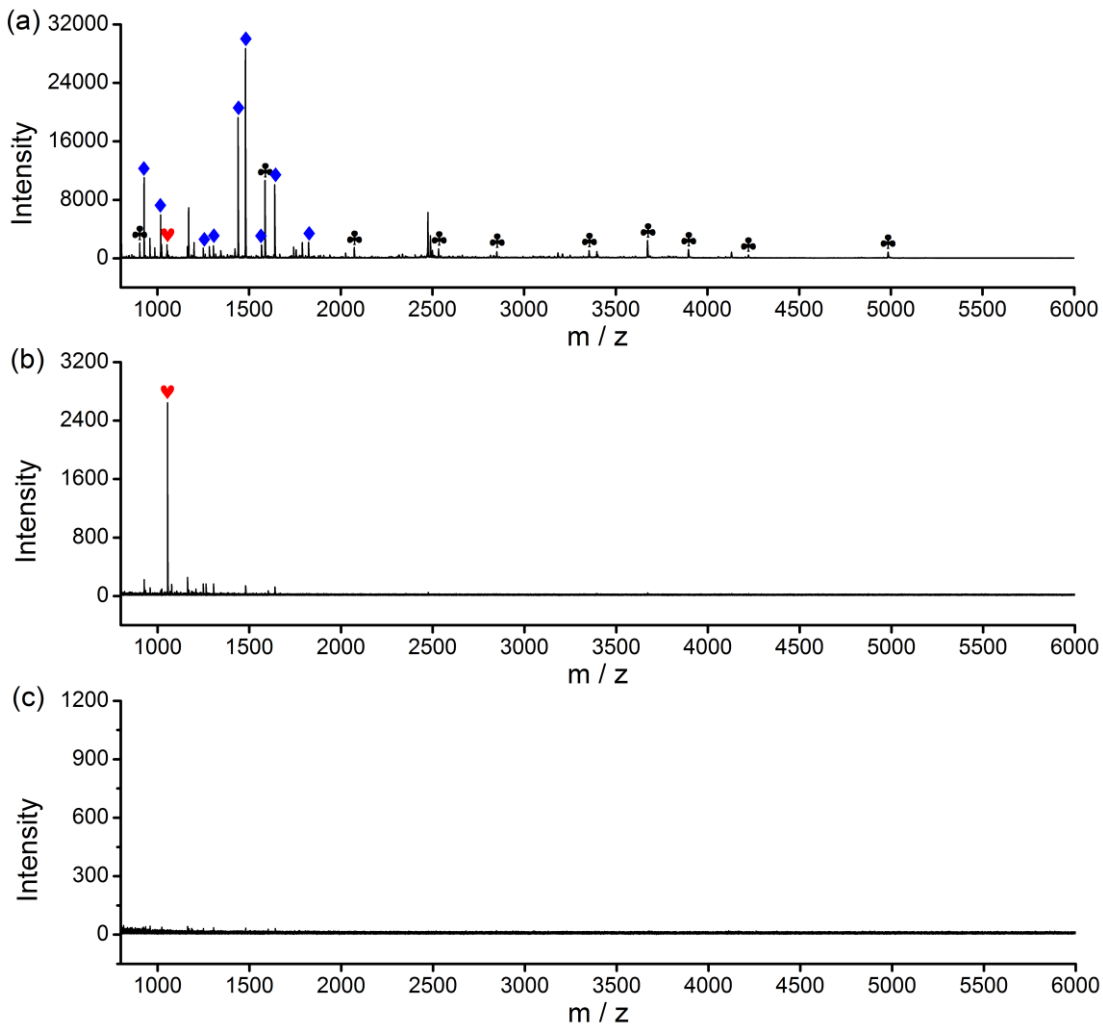


**Fig. S18** MALDI-TOF MS spectra for the analysis of the mixtures of Mb epitope and tryptic digests of HRP and BSA with the mole ratio 1:1:1. a) direct analysis; b) analysis after extracted by glycated Mb epitope-imprinted MNPs; c) analysis after extracted by corresponding non-imprinted MNPs. (♥: Mb epitope; ♣: peptides from tryptic digest of HRP; ♦: peptides from tryptic digest of BSA).

**Table S1.** Different types and proportions of monomers for the preparation of glycated B2M epitope-imprinted MNPs or corresponding non-imprinted MNPs, and their resulting imprinting factors at different imprinting times.

| Composition | Molar percentage of monomers | | | | Imprinting factor | | | |
|---|---|---|---|---|---|---|---|---|
| | APTES | UPTES | IBTES | TEOS | 50 min | 60 min | 70 min | 80 min |
| 1 | 0 | 0 | 10 | 90 | 1.02 | 1.07 | 1.13 | 1.01 |
| 2 | 0 | 10 | 0 | 90 | 1.56 | 1.91 | 1.22 | 1.03 |
| 3 | 10 | 0 | 0 | 90 | 1.12 | 1.28 | 1.10 | 1.05 |
| 4 | 10 | 10 | 0 | 80 | 1.51 | 2.12 | 1.19 | 1.04 |
| 5 | 0 | 10 | 10 | 80 | 1.28 | 2.14 | 2.50 | 1.08 |
| 6 | 0 | 10 | 20 | 70 | 1.36 | 2.48 | 3.61 | 1.14 |
| 7 | 10 | 0 | 20 | 70 | 1.21 | 1.76 | 2.59 | 1.13 |
| 8 | 10 | 10 | 20 | 60 | 3.81 | 5.81 | 1.40 | 1.10 |
| 9 | 15 | 15 | 30 | 40 | 1.96 | 3.54 | 1.58 | 1.07 |
| 10 | 20 | 20 | 40 | 20 | 1.17 | 1.98 | 2.63 | 1.10 |

**Table S2.** The detailed information of identified peptides from B2M epitope and tryptic digests of HRP and BSA.

| Protein | m/z | Sequence | Glycans |
|---|---|---|---|
| B2M | 1190 | KIVKWDRDM | —— |
| BSA | 1016 | SHCIAEVEK | —— |
| | 1052 | CCTKPESER | —— |
| | 1250 | FKDLGEEHFK | —— |
| | 1306 | HLVDEPQNLIK | —— |
| | 1440 | RHPEYAVSVLLR | —— |
| | 1480 | LGEYGFQNALIVR | —— |
| | 1640 | KVPQVSTPTLVEVSR | —— |
| | 1824 | RPCFSALTPDETYVPK | —— |
| | 2990 | DAFLGSFLYEYSRRHPEYAVSVLLR | —— |
| | 3392 | DLGEEHFKGLVLIAFSQYLQQCPFDEHVK | —— |
| HRP | 1381 | TEKDAFGNANSAR | —— |
| | 1587 | MGNITPLTGTQGQIR | —— |
| | 2072 | DSFRNVGLN#R | $Man_3GlcNAc_2$ |
| | 2533 | SFAN#STQTFFNAFVEAMDR | FucGlcNAc |
| | 2851 | GLIQSDQELFSSPN#ATDTIPLVR | FucGlcNAc |
| | 3355 | SFAN#STQTFFNAFVEAMDR | $XylMan_3FucGlcNAc_2$ |
| | 3671 | GLIQSDQELFSSPN#ATDTIPLVR | $XylMan_3FucGlcNAc_2$ |
| | 3895 | LHFHDCFVNGCDASILLDN#TTSFR | $XylMan_3FucGlcNAc_2$ |
| | 4223 | QLTPTFYDNSC(AAVESACPR)PN#VSNIVR | $XylMan_3FucGlcNAc_2$ |
| | 4984 | LYN#FSNTGLPDPTLN#TTYLQTLR | $XylMan_3FucGlcNAc_2$<br>$XylMan_3FucGlcNAc_2$ |

**Table S3.** The detailed information of identified peptides from Mb epitope and tryptic digests of HRP and BSA.

| Protein | m/z | Sequence | Glycans |
|---|---|---|---|
| Mb | 1055 | NYKELGFQG | —— |
| BSA | 928 | YLYEIAR | —— |
| | 1016 | SHCIAEVEK | —— |
| | 1250 | FKDLGEEHFK | —— |
| | 1306 | HLVDEPQNLIK | —— |
| | 1440 | RHPEYAVSVLLR | —— |
| | 1480 | LGEYGFQNALIVR | —— |
| | 1568 | DAFLGSFLYEYSR | —— |
| | 1640 | KVPQVSTPTLVEVSR | —— |
| | 1824 | RPCFSALTPDETYVPK | —— |
| HRP | 903 | AAVESACPR | —— |
| | 1587 | MGNITPLTGTQGQIR | —— |
| | 2072 | DSFRNVGLN#R | $Man_3GlcNAc_2$ |
| | 2533 | SFAN#STQTFFNAFVEAMDR | FucGlcNAc |
| | 2851 | GLIQSDQELFSSPN#ATDTIPLVR | FucGlcNAc |
| | 3355 | SFAN#STQTFFNAFVEAMDR | $XylMan_3FucGlcNAc_2$ |
| | 3671 | GLIQSDQELFSSPN#ATDTIPLVR | $XylMan_3FucGlcNAc_2$ |
| | 3895 | LHFHDCFVNGCDASILLDN#TTSFR | $XylMan_3FucGlcNAc_2$ |
| | 4223 | QLTPTFYDNSC(AAVESACPR)PN#VSNIVR | $XylMan_3FucGlcNAc_2$ |
| | 4984 | LYN#FSNTGLPDPTLN#TTYLQTLR | $XylMan_3FucGlcNAc_2$<br>$XylMan_3FucGlcNAc_2$ |