Electronic Supplementary Information For

Molecularly Imprinted and Cladded Polymers for Constructing a Portable Plasmonic Immunoassay for Peptides in Biofluids†

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Contents:

Supplementary Experimental Section

Reagents and materials. Ribonuclease A (RNase A), ribonuclease B (RNase B), horseradish peroxidase (HRP), bovine serum albumin (BSA), adenosine, deoxyadenosine, and 2,4-difluoro-3-formyl-phenylboronic acid **(**DFFPBA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Transferrin receptor **(**TfR), alpha fetoprotein (AFP) and carcinoembryonic antigen **(**CEA) were purchased from abcam (Shanghai, China). LSGDVWDIDNEF, RTLHRNEYGIAS, KLTIESTPFNVA, SLQPLALEGSLQ, EAEDLQVGQVEL, SLQPLALEGSLQK-Fru, Fru-EAEDLQ-VGQVEL, and C-peptide sequence (EAEDLQVGQVELGGGPGAGSLQPLALE-GSLQ) were synthesized by Shanghai Top-Peptide Biotechnology (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). Ferric trichloride hexahydrate, 1,6-hexanediamine, anhydrous sodium acetate, glycol, ammonium bicarbonate, sodium dihydrogen phosphate, sodium hydroxide, sodium chloride (NaCl), acetic acid (HAc), silver nitrate, trisodium citrate, hydrochloric acid (HCl) and anhydrous ethanol were purchased from Nanjing Reagent Company (Nanjing, China). Methanol and acetonitrile (ACN) were purchased from Shanghai Macklin Biochemical (Shanghai, China). Ammonium hydroxide (28%, v/v) was purchased from Shanghai Lingfeng Chemical Reagent

(Shanghai, China). Glutaraldehyde (GA, 25%, v/v) was purchased from Sinopharm Chemical Reagent (Shanghai, China). ELISA kit for the detection of human C-peptide was purchased from MLBIO (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). Urine samples from diabetes patients and healthy individuals were obtained from Taikang Xianlin Drum Tower Hospital (Nanjing, China) and approved by the Institutional Ethics Committee of Taikang Xianlin Drum Tower Hospital. All experiments were performed in compliance with relevant laws or guidelines and followed institutional guidelines.

Instruments. Transmission electron microscopic (TEM) characterization was carried out on JEM-2800 systems (JEOL, Tokyo, Japan). Ultraviolent (UV) spectral analysis was performed with a NanoDrop 2000/2000C spectrophotometer (Thermo Fisher, MA, USA). The Raman spectroscopy uses an i-Raman@Plus portable Raman spectrometer (B&W TEK, Shanghai, China); the laser light source is a He-Ne laser (λ_0 = 785 nm; working power: 50 mW); the Raman signal is passed through high-efficiency thin back-illuminated CCD detector; when measuring a sample, the exposure time is 10 s and 1 integration.

Preparation of Fe_3O_4 magnetic nanoparticles (MNPs). 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 of glycol in a PTFE-lined autoclave and reacted at 198 ºC for 6 h. The resulting Fe_3O_4 MNPs were washed with water and anhydrous ethanol three times each, and then dried at 50 ºC in a vacuum overnight.

Preparation of boronic acid-functionalized Fe_3O_4@SiO_2 MNPs. The synthesis of boronic acid-functionalized Fe_3O_4@SiO_2 MNPs was comprised of three steps: 1) preparation of Fe_3O_4@SiO_2 MNPs, 2) preparation of amino-functionalized Fe_3O_4@SiO_2 MNPs, and 3) preparation of boronic acid-functionalized Fe_3O_4@SiO_2 MNPs.

1) Preparation 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 were added into a 500-mL three-neck round-bottomed flask, and mechanically stirred at 300 rpm for 20 min at 40 ºC. 200 mg of Fe_3O_4 MNPs was dispersed into 20 mL of anhydrous ethanol by ultrasonication. The obtained suspension was added into the above flask, and then mechanically stirred at 300 rpm for 20 min at 40 ºC. The resulting Fe_3O_4@SiO_2 MNPs were collected by a magnet, washed with water and anhydrous ethanol three times each, and then dried at 50 ºC in a vacuum overnight.

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 300 rpm for 12 h at 80 ºC 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 three times each, and then dried at 50 ºC in a vacuum overnight.

3) Preparation of boronic acid-functionalized Fe_3O_4@SiO_2 MNPs. 200 mg of amino-functionalized Fe_3O_4@SiO_2 MNPs was 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 300 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 three times each, and then dried at 50 ºC 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.

Preparation of C-peptide N- or C-teminal epitope-imprinted and cladded MNPs. The preparation of C-peptide N- or C-teminal epitope-imprinted and cladded MNPs was composed of four steps: 1) template immobilization, 2) imprinting, 3) cladding, and 4) template removal.

1) Template immobilization. 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 was 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) three times.

2) 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 then 10 mL of water was added to resulting suspension and mechanically stirred for 5 min. After that, different molar ratios of APTES, UPTES, IBTES and TEOS (the total volume of silylating reagents was kept at 200 μL) dissolved in 40 mL of anhydrous ethanol were added to the above suspension, and then mechanically stirred at 25 ºC for an appropriate period time. In order to obtain the best recognition performance, the imprinting time was set at 40, 50, 60, 70 or 80 min under different ratios of monomers and the best imprinting time and monomer ratio were optimized. The prepared epitope-imprinted MNPs were collected by a magnet.

3) Cladding. The above prepared epitope-imprinted MNPs were re-dispersed into 160 mL of anhydrous ethanol containing 2.8 mL of ammonium hydroxide (28%). Then 40 mL of 10 mM TEOS in anhydrous ethanol was added to the above suspension and mechanically stirred at 25 ºC for 10 min. The prepared epitope-imprinted and cladded MNPs were collected by a magnet, washed with anhydrous ethanol three times, and then dried at 40 ºC in a vacuum overnight.

4) Template removal. The obtained epitope-imprinted and cladded MNPs were dispersed into 2 mL of ACN:H_2O:HAc = 50:49:1 (v/v) and shaken for 20 min at room

temperature. The above elution process was repeated three times. After removing the glycated epitope template, the prepared epitope-imprinted and cladded MNPs were magnetically collected, washed with water and anhydrous ethanol three times each and then dried at 40 ºC in a vacuum overnight.

Selectivity of boronic acid-functionalized Fe_3O_4@SiO_2 MNPs. In order to investigate the selectivity of boronic acid-functionalized Fe_3O_4@SiO_2 MNPs, adenosine and deoxyadenosine were selected as test compounds. An amount of 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) 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 at room temperature for 1 h on a rotator. The Fe_3O_4@SiO_2@DFFPBA MNPs were magnetically separated and the eluate was collected. The amounts of adenosine or deoxyadenosine bound by the Fe_3O_4@SiO_2@DFFPBA MNPs were determined by measuring the UV absorbance of the eluate at 260 nm. The measurement was repeated three times. Subsequently, HRP, Rnase B, BSA and RNase A were further selected as test compounds. The extraction procedure was the same as described above

except that the eluates were measured in terms of UV absorbance at 214 nm. For control experiments, all the procedures were the same as described above except the absence of test compounds in the test sample.

Optimization of monomer ratio and imprinting time. The monomer ratio and imprinting time of C-peptide N- or C-teminal epitope-imprinted and cladded MNPs were optimized according to the obtained imprinting factor (IF). 2.0 mg of the epitope-imprinted and cladded and non-imprinted but cladded MNPs prepared by different kinds and molar ratios of monomers (APTES, UPTES, IBTES and TEOS) at different imprinting time were added to 200 μL of 0.1 mg/mL dodecapeptide epitope dissolved in phosphate buffer (10 mM pH 7.4), respectively. After incubation at room temperature for 30 min, the MNPs were magnetically collected and rinsed with 200 μL of phosphate buffer (10 mM, pH 7.4) three times. The MNPs were re-suspended and eluted in 20 μL of ACN:H_2O:HAc = 50:49:1 (v/v) at room temperature 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 UV absorbance at 214 nm of the eluates. The measurement was repeated three times. For control experiments, all the procedures were the same as described above except the absence of epitope in the test sample.

Selectivity of epitope-imprinted and cladded MNPs. The selectivity of C-peptide N- or C-terminal epitope-imprinted and cladded MNPs at the peptide level was evaluated

using C-terminal epitopes of C-peptide and TfR, and N-terminal epitopes of C-peptide, AFP and CEA. First, solutions of 0.1 mg/mL of each epitope were separately prepared with phosphate buffer (10 mM, pH 7.4). Then, equivalent epitope-imprinted and cladded MNPs and non-imprinted but cladded MNPs (2 mg each) were added to 200 μL of the epitope solutions in 250-μL microcentrifugal tubes, respectively. The tubes were shaken on a rotator at room temperature for 30 min. The MNPs were magnetically collected and rinsed with 200 μL of phosphate buffer (10 mM, pH 7.4) three times. After that, the MNPs were re-suspended and eluted in 20 μL of ACN:H_2O:HAc = 50:49:1 (v/v) at room temperature for 10 min on a rotator. The MNPs were magnetically separated and the eluates were collected. The amounts of epitopes captured by the epitope-imprinted and cladded MNPs were determined by measuring the UV absorbance at 214 nm of the eluates. The measurement was repeated three times. For control experiments, all the procedures were the same as described above except the absence of epitopes in the test sample.

Preparation of silver nanoparticles (AgNPs). Briefly, 36 mg of $AgNO_3$ was dissolved in 200 mL of DI water in a 500 mL three-neck round bottom flask and the mixture was heated to boil under continuous stirring. Then, 4 mL of 1% (w/v) trisodium citrate was added. The mixture was boiled under stirring for about 40 min and then cooled down to room temperature. The obtained AgNPs colloidal solution was stored at 4 °C before use.

Preparation of DFFPBA-functionalized Ag/PATP@SiO_2 NPs. DFFPBA-functionalized Ag/PATP@SiO_2 NPs were prepared according to our previously reported method with slight modifications. The preparation route was comprised of three steps: 1) preparation of Ag/PATP@SiO_2 NPs, 2) preparation of amino-modified Ag/PATP@SiO_2 NPs, and 3) preparation of boronic acid-functionalized Ag/PATP@SiO_2 NPs with DFFPBA.

1) Preparation of Ag/PATP@SiO_2 NPs. First, 20 μL of 1 mM PATP dissolved in anhydrous ethanol was added dropwise to 10 mL of Ag colloidal solution under rapid stirring for 40 min. Then 40 mL of anhydrous ethanol and 0.7 mL of 28 % ammonia solution was added to the above solution respectively, and the mixture was stirred for 5 min. After that, 10 mL of 10 mM TEOS dissolved in anhydrous ethanol was added to the above suspension, and the mixture was stirred at room temperature for 50 min. The resultant Ag/PATP@ SiO_2 NPs were centrifuged at 8,000 rpm for 10 min and washed with anhydrous ethanol three times. The products were redispersed in 10 mL of anhydrous ethanol.

2) Preparation of amino-modified Ag/PATP@SiO_2 NPs. 100 μL of APTES was added to 10 mL of Ag/PATP@SiO_2 NPs suspension dissolved in anhydrous ethanol under stirring and kept stirring at room temperature for 1 h. The resulting mixture were centrifuged at 8,000 rpm for 10 min, and then washed with anhydrous ethanol three

times. The amino-modified Ag/PATP@SiO_2 NPs were redispersed in 30 mL of methanol.

3) Preparation of boronic acid-functionalized Ag/PATP@SiO_2 NPs with DFFPBA. 300 μL of 5 mg/mL DFFPBA and 300 μL of 5 mg/mL $NaBH_3CN$ were added respectively into 30 mL of amino-modified Ag/PATP@SiO_2 NPs suspension, and allowed to react for 24 h. The obtained mixture was centrifuged at 8,000 rpm for 10 min, and then washed with anhydrous ethanol and water three times each. The DFFPBA-functionalized Ag/PATP@SiO_2 NPs were redispersed in 9 mL of phosphate buffer (10 mM, pH 7.4).

Preparation of C-peptide N-terminal epitope-imprinted and cladded Ag/PATP@SiO_2 NPs. The preparation procedure was composed of three steps: 1) template immobilization, 2) imprinting, 3) cladding, and 4) template removal.

1) Template immobilization. 1 mL of 1.0 mg/mL glycated C-peptide N-terminal epitope dissolved in phosphate buffer (10 mM, pH 7.4) was added to 9 mL of a 2,4-difluoro-3-formyl-phenylboronic acid (DFFPBA)-functionalized Ag/PATP@SiO_2 NP suspension. After incubating at room temperature for 2 h, the obtained glycated C-peptide N-terminal epitope-immobilized Ag/PATP@SiO_2 NPs were centrifuged at 8000 rpm for 10 min and washed with phosphate buffer (10 mM, pH 7.4) three times.

2) Imprinting. The glycated C-peptide N-terminal epitope-immobilized Ag/PATP@SiO_2 NPs were dispersed into 15 mL of anhydrous ethanol containing 0.45

mL of ammonium hydroxide (28%), and 1 mL of water was added to the resulting suspension and stirred for 5 min. Then monomers (APTES, UPTES, IBTES, and TEOS) of the desired ratio dissolved in 4 mL of anhydrous ethanol (the volume of APTES was kept at 2 μL) were added to the above suspension and stirred at 25 °C for an appropriate period time. The resulting C-peptide N-terminal epitope-imprinted Ag/PATP@ SiO_2 NPs were collected by centrifugation at 8000 rpm for 10 min.

3) Cladding. The prepared C-peptide N-terminal epitope-imprinted Ag/PATP@ SiO_2 NPs were re-dispersed into 160 mL of anhydrous ethanol containing 2.8 mL of ammonium hydroxide (28%). Then 40 mL of 10 mM TEOS in anhydrous ethanol was added to the above suspension, and then mechanically stirred at 25 ^oC for 10 min. The prepared C-peptide N-terminal epitope-imprinted and cladded Ag/PATP@ SiO_2 NPs were collected by centrifuged at 8,000 rpm for 10 min, and then washed with anhydrous ethanol three times.

4) Template removal. The obtained C-peptide N-terminal epitope-imprinted and cladded Ag/PATP@ SiO_2 NPs were dispersed into 20 mL of ACN:H_2O:HAc = 50:49:1 (v/v) and shaken for 20 min at room temperature. The above elution process was repeated three times. After removing the glycated C-peptide N-terminal epitope templates, the prepared C-peptide N-terminal epitope-imprinted and cladded Ag/PATP@ SiO_2 NPs were centrifuged at 8000 rpm for 10 min and washed with anhydrous ethanol and water three times each. Finally, the C-peptide N-terminal epitope-imprinted and cladded Ag/PATP@SiO_2 NPs were re-dispersed in phosphate

buffer (10 mM, pH 7.4). For non-imprinted Ag/PATP@ SiO_2 NPs were prepared using the same procedure except for the absence of the glycated epitope template.

Selectivity of dual cMIPs-based C-peptide assay. The selectivity of dual cMIPs-based C-peptide assay was investigated with C-peptide, RNase A, RNase B, BSA and HRP. 50 μL of 10 mg/mL C-peptide C-terminal epitope-imprinted and cladded MNPs were taken in a 200 μL centrifuge tube and sonicate. Then 50 μL of protein solution (1 μg/mL) dissolved in phosphate buffer (10 mM, pH 7.4) and 30 μL of C-peptide N-terminal epitope-imprinted and cladded Ag/PATP@SiO_2 NPs were added and shaken at room temperature for 20 minutes. The unbound C-peptide N-terminal epitope-imprinted and cladded Ag/PATP@SiO_2 NPs were removed by magnetic separation and washed with 100 μL of phosphate buffer (10 mM, pH 7.4) three times. The obtained sandwich-structured nanocomposite products were dispersed into 10 μL of phosphate buffer (10 mM, pH 7.4). Then 1 μL of the sandwich-structured nanocomposite solution was taken on a glass slide covered with aluminum foil. Each sample has 5 droplets. After drying at room temperature, collect the Raman spectrum at the center of each droplet 3 times.

Determination of C-peptide in human body fluid. The 24-hour collected urine samples collected were first mixed together. Then a small amount of samples was taken to adjust the pH to 7.4 and centrifuge at 3000 rpm for 10 minutes. The supernatant for

was used for experiments. 50 μL of 10 mg/mL C-peptide C-terminal epitope-imprinted and cladded MNPs were taken in a 200 μL centrifuge tube and sonicated. Then 50 μL of urine samples and 30 μL of C-peptide N-terminal epitope-imprinted and cladded Ag/PATP@SiO_2 NPs were added and shaken at room temperature for 20 minutes. The unbound C-peptide N-terminal epitope-imprinted and cladded Ag/PATP@SiO_2 NPs were removed by magnetic separation and washed with 100 μL of phosphate buffer (10 mM, pH 7.4) three times. The obtained products were dispersed into 10 μL of phosphate buffer (10 mM, pH 7.4). 1 μL of the products solution was taken on a glass slide covered with aluminum foil. Each sample has 5 droplets. After drying at room temperature, collect the Raman spectrum at the center of each droplet 3 times.

Supplementary Figures

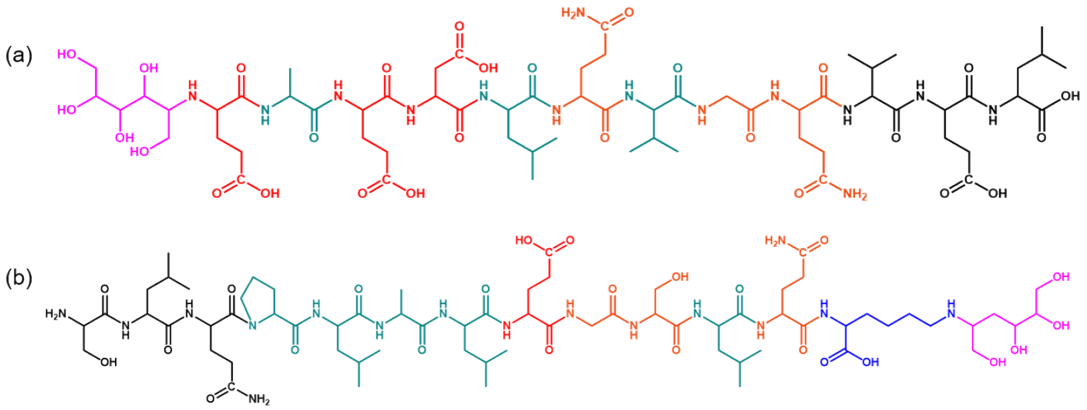

Figure S1. The chemical structures of glycated C-peptide N- (a) and C- (b) terminal epitopes.

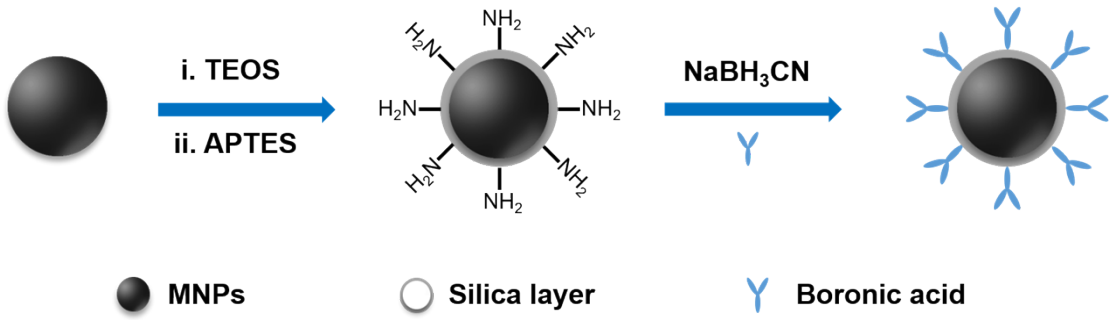

Figure S2. Schematic of the synthesis route of boronic acid-functionalized Fe_3O_4@SiO_2 MNPs.

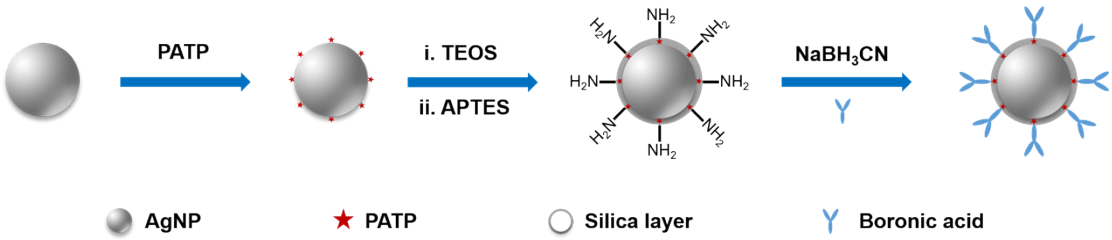

Figure S3. Schematic of the synthesis route of boronic acid-functionalized Ag/PATP@SiO_2 NPs.

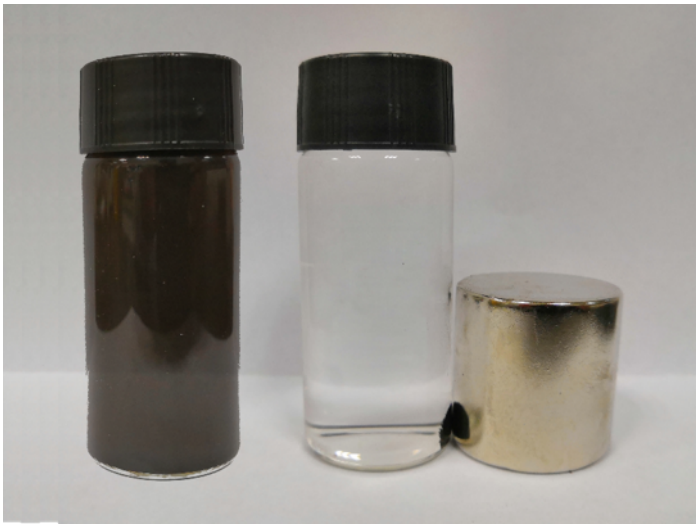

Figure S4. A photo of MNPs solution and MNPs separated by magnetic.

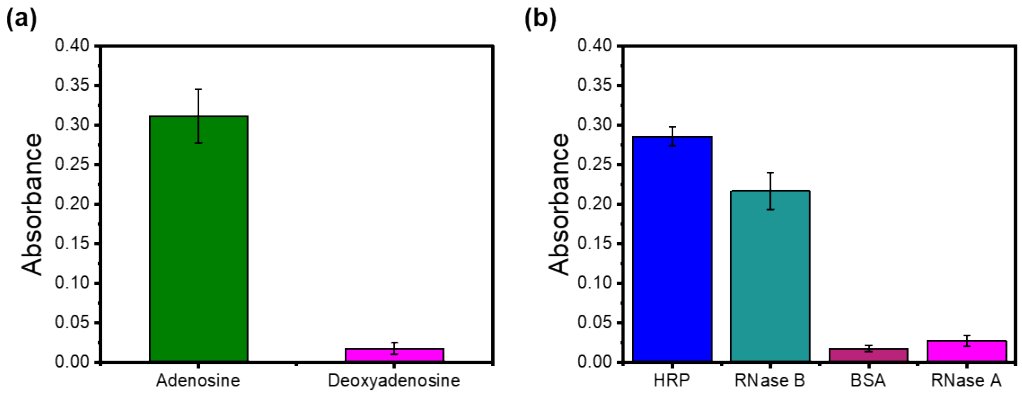

Figure S5. Comparison of the amounts of different analytes captured by boronic acid-functionalized Fe_3O_4@SiO_2 MNPs. (a) 1.0 mg/mL of adenosine and deoxyadenosine; (b) 1.0 mg/mL of HRP, BSA, RNase A and RNase B. Binding buffer: ammonium

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.

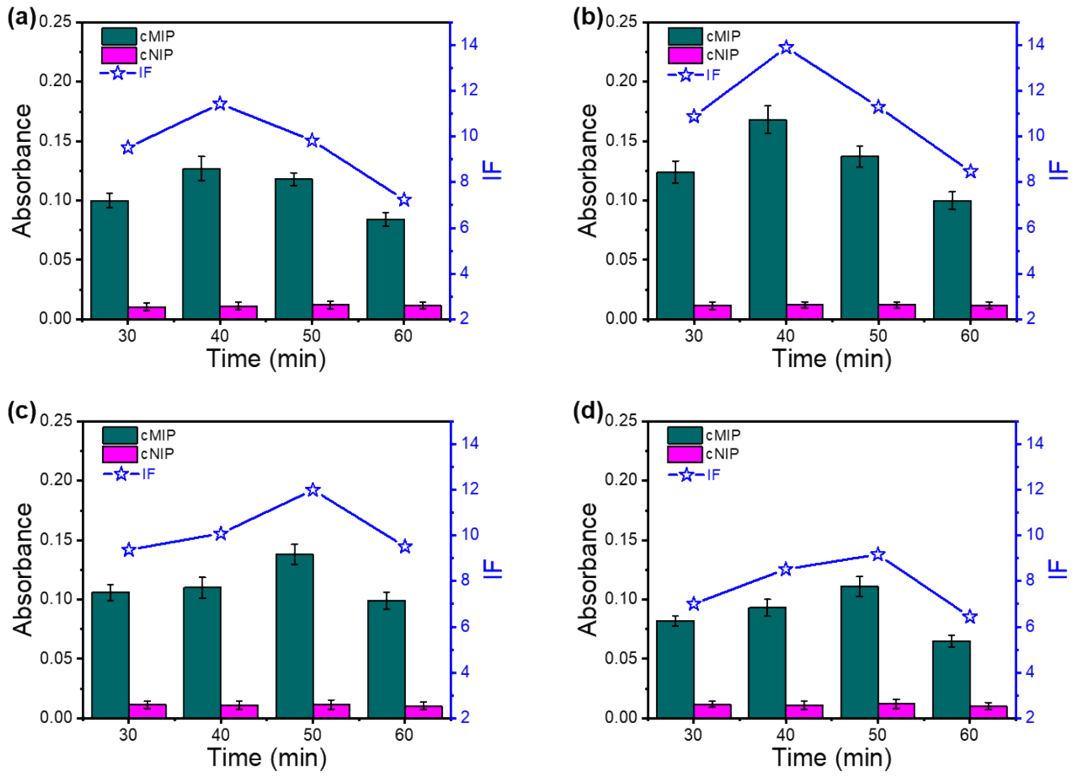

Figure S6. Optimization of the monomer ratio and imprinting time according to IF for imprinting and cladding of C-peptide N-terminal epitope imprinted and cladded MNPs. The monomer ratio of APTES/UPTES/IBTES/TEOS: (a) 40:10:30:20; (b) 30:20:30:20; (c) 20:20:40:20; (d) 20:20:30:30. The IF values are the ratio of the amount of C-peptide N-terminal epitope captured by C-peptide N-terminal epitope-imprinted and cladded MNPs over the amount by the corresponding non-imprinted but cladded MNPs.

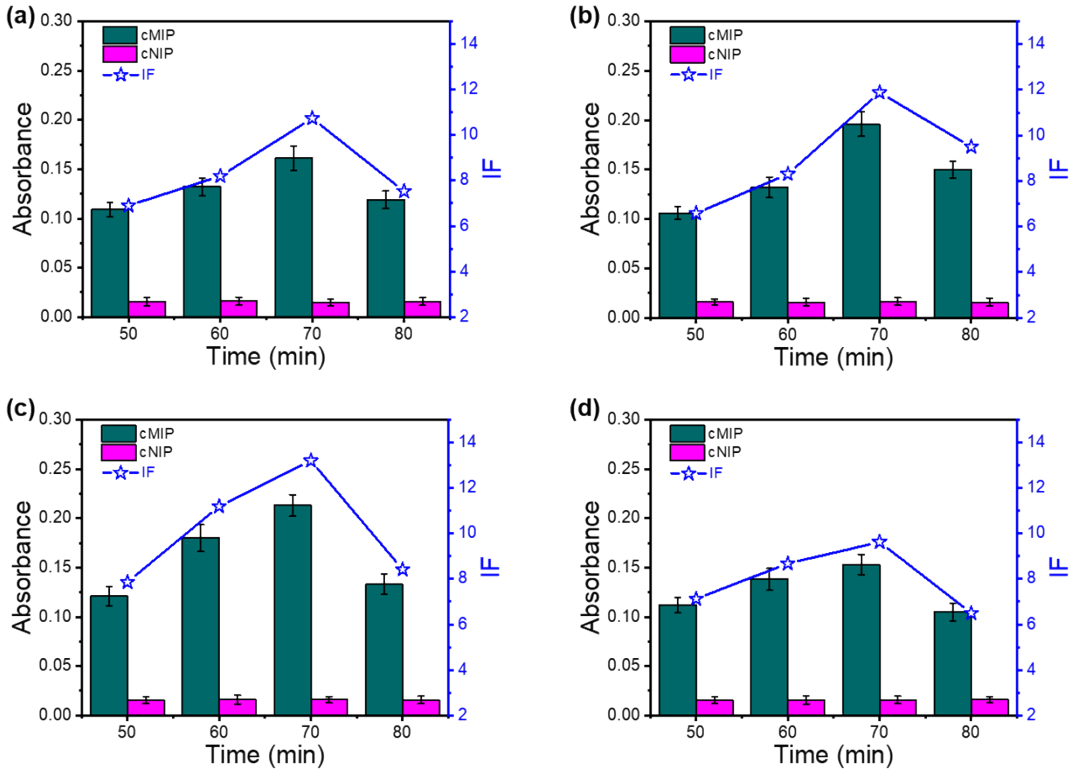

Figure S7. Optimization of the monomer ratio and imprinting time according to IF for imprinting and cladding of C-peptide C-terminal epitope imprinted and cladded MNPs. The monomer ratio of APTES/UPTES/IBTES/TEOS: (a) 20:10:50:20; (b) 10:10:60:20; (c) 10:20:50:20; (d) 10:10:50:30. The IF values are the ratio of the amount of C-peptide C-terminal epitope captured by C-peptide C-terminal epitope-imprinted and cladded MNPs over the amount by the corresponding non-imprinted but cladded MNPs.

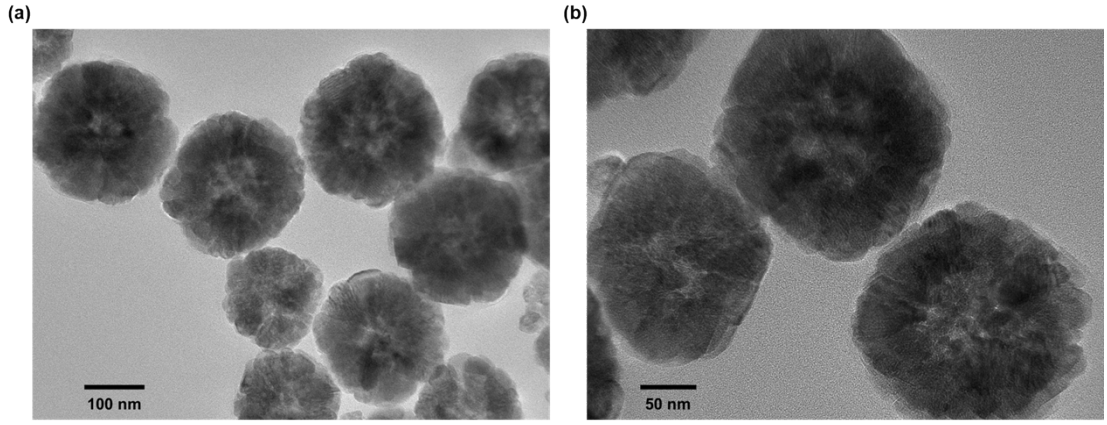

Figure S8. TEM images of (a) MNPs and (b) C-peptide C-teriminal epitope-imprinted and cladded MNPs.

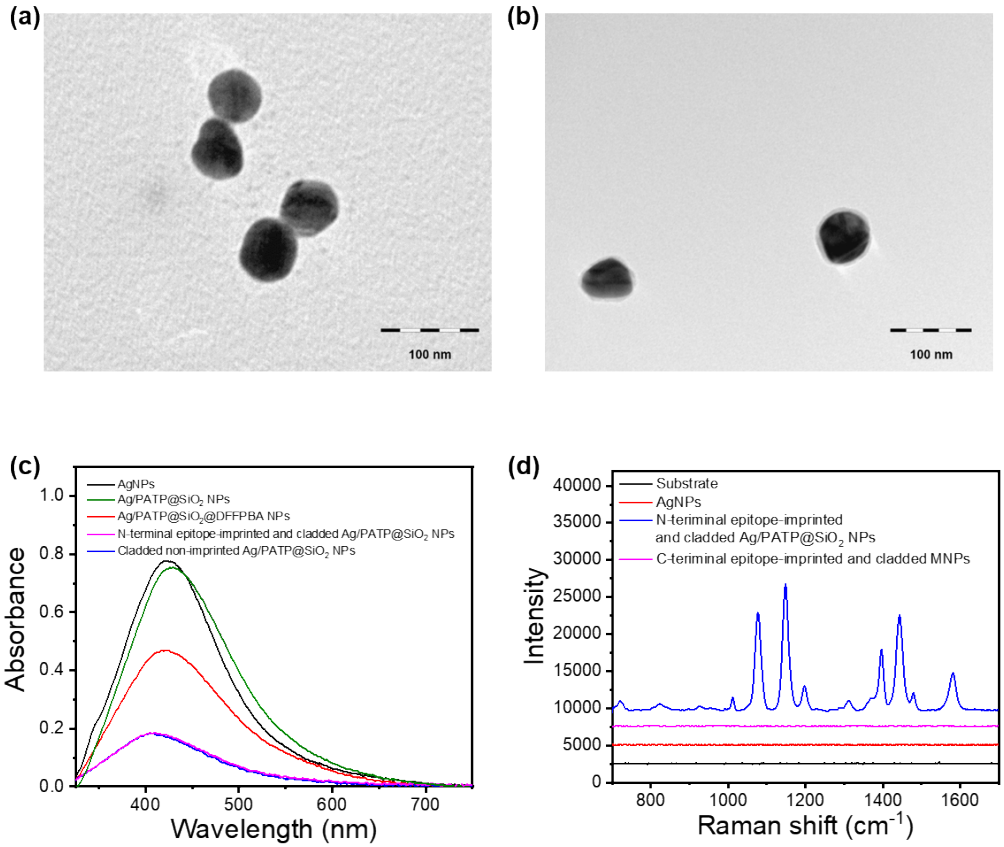

Figure S9. TEM images of (a) AgNPs and (b) C-peptide N-teriminal epitope-imprinted and cladded Ag/PATP@SiO_2 NPs. (c) UV-visible extinction spectra for AgNPs, Ag/PATP@SiO_2 NPs, Ag/PATP@SiO_2@DFFPBA NPs, C-peptide N-terminal epitope-imprinted and cladded Ag/PATP@SiO_2 NPs and cladded non-imprinted Ag/PATP@SiO_2 NPs. (d) Raman spectra of substrate, AgNPs, C-peptide N-terminal epitope-imprinted and cladded Ag/PATP@SiO_2 NPs and C-peptide C-terminal epitope-imprinted and cladded MNPs.

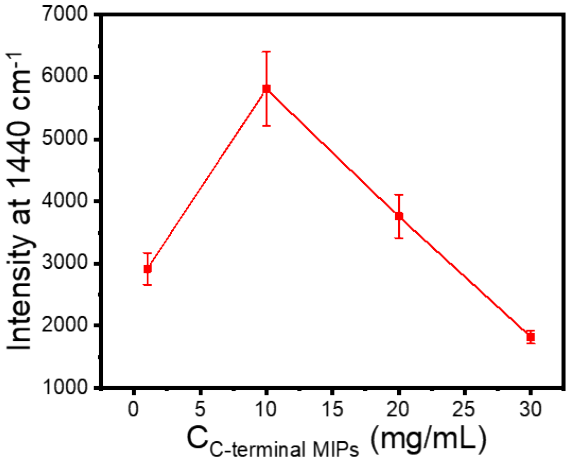

Figure S10. The concentration of C-peptide C-terminal epitope-imprinted and cladded MNPs used in the dual cMIPs-based C-peptide assay.

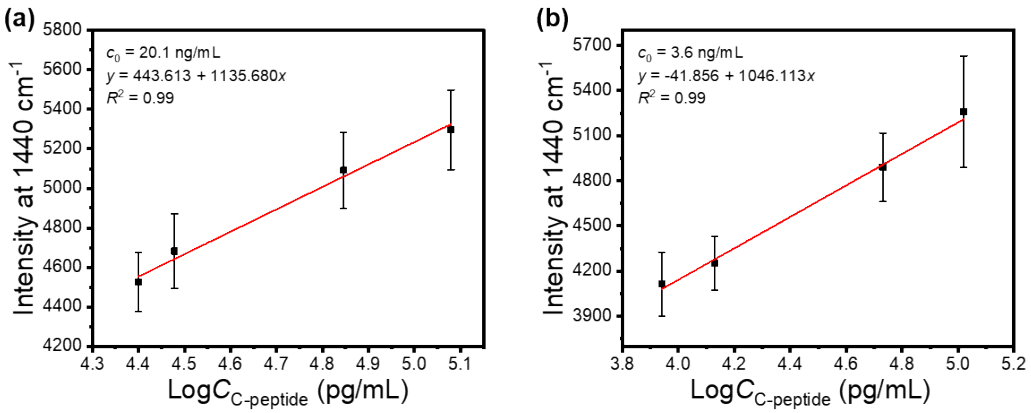

Figure S11. Linear dependence of the Raman intensity at 1440 cm^{-1} with the logarithmic value of the total concentration of C-peptide in the (a) spiked urine samples for a healthy individual and (b) spiked urine samples for a diabetes patient. C_0 was calculated according to the linear calibration curve shown in Fig. 2b. Error bars represent standard deviations for three parallel experiments.

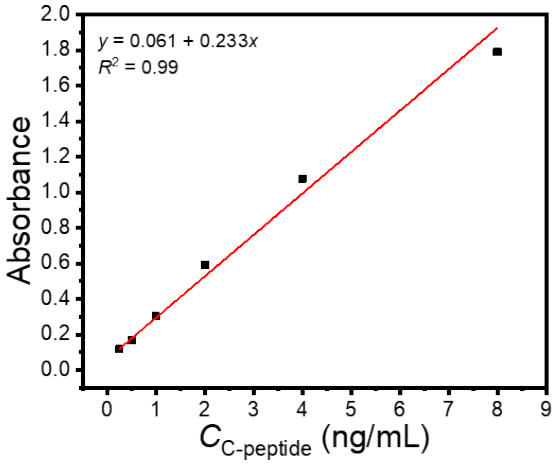

Figure S12. ELISA kit for linear relationship (y = 0.061 (± 0.001) + 0.233 (± 0.011) x， R^2 = 0.99) between the value of OD at 450 nm and of the concentration of C-peptide in standard solution. Error bars represent standard deviations for three parallel experiments.

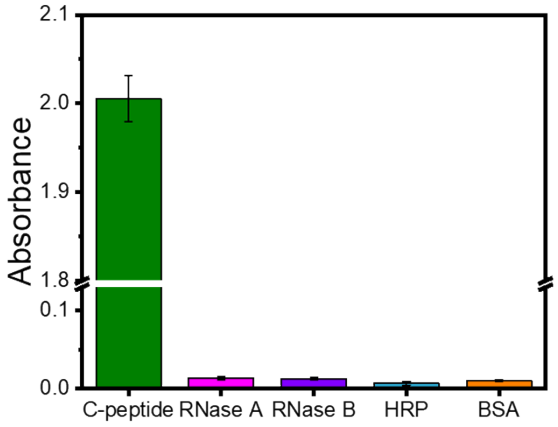

Figure S13. The selectivity of ELISA kit.

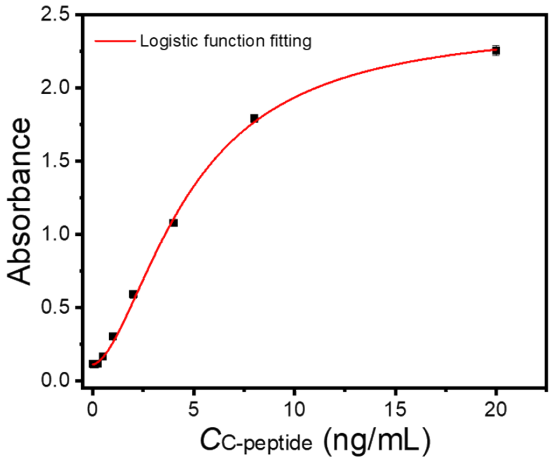

Figure S14. Dependence of UV-visible absorbance for C-peptide on the concentration by ELISA kit. Error bars represent standard deviations for three parallel experiments.