Supporting Information

Synthesis of polydopamine-coated magnetic graphene for Cu^{2+} immobilization and application to the enrichment of low-concentration peptides for mass spectrometry analysis

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1. Chemicals. Graphene was purchased from Shanghai Boson Technology Co., Ltd. Dopamine hydrochloride was purchased from Aladdin Chemistry *Co*. Ltd. Bovine serum albumin, horse heart myoglobin and cytochrome c were obtained from Bio Basic (Toronto, Canada). The human urine and serum sample originated from a healthy man were offered by Shanghai Zhongshan Hospital. α-Cyano-4-hydroxy-cinnamic acid (α-CHCA) was purchased from Sigma. Sequencing grade-modified trypsin was purchased from Promega (Madison, WI). Acetonitrile (ACN) and trifluoroacetic acid (TFA) were purchased from Merck (Darmstadt, Germany). Distilled water was purified by a Milli-Q system (Millipore, Bedford, MA). All other chemicals and reagents are of the highest grade and used as received.

2. Synthesis of magG@PDA@Cu^{2+}. Firstly, the Fe_3O_4-modified graphene sheets (magG) were prepared via a hydrothermal reaction. In brief, graphene (400mg) was dispersed in 50 ml of HNO_3 at 60 ºC under magnetic stirring for 7 hours. The graphene treated by HNO_3 was washed with deionized water for several times and then dried in vacuum at 50 ºC. Then the pretreated graphene (150mg) and $FeCl_3 \cdot 6H_2O$ (405mg) were dispersed in 40 ml of ethylene glycol solution containing trisodium citrate (0.15 g), sodium acetate (1.8 g) and poly(ethylene glycol)-20000 (1.0 g) by magnetic stirring for 2 hours. The mixture was sealed in a Teflon-lined stainless-steel autoclave and was heated at 200 ºC for 10 hours. The product was collected by magnetic separation and washed with water. The resulting magG was dried in vacuum at 50 ºC.

10 mg of the obtained magG sheets were then dispersed in 35 mL of aqueous solution containing 80 mg dopamine hydrochloride by 30 min ultrasonication. After that, under continuous mechanical stirring, 10 mL of Tris buffer (10 mM, pH 8.5) solution was quickly added into the aqueous dispersion of magG, and the resulting dispersion was stirred for 6-20h at room temperature. The obtained product was isolated by magnetic separation, and washed with deionized water and ethanol several times. Eventually, the magG@PDA composites were dried in vacuum at 50 ºC.

After mildly stirring the obtained magG@PDA composites with 100 mM $Cu(Ac)_2$ solution at room temperature for 2-3 hours, the solution was still blue in color. And the desired magG@PDA@Cu^{2+} was gathered by magnetic separation and washed with deionized water.

3. Measurements and characterizations. Transmission electron microscopy (TEM) images were taken on a JEOL 2011 microscope (Japan) operated at 200 kV. Samples were firstly dispersed in ethanol and then collected by using carbon-film-covered copper grids for analysis. Scanning electronic microscope (SEM) images and energy dispersive X-ray (EDX) spectra were recorded on a Philips XL30 electron microscope (Netherlands) operating at 20 kV. A thin gold film was sprayed on the sample before measurements. Fourier transform infrared spectra (FT-IR) were collected on Nicolet Fourier spectrophotometer using KBr pellets (USA). The Raman spectra were recorded at room temperature on a LabRam-1B Raman spectrometer with a laser at an excitation wavelength of 632.8 nm. Zeta potential measurements were carried out on a Nano ZS90 zeta analyzer (Malvern Instruments Ltd.). Powder X-ray diffraction (XRD) patterns were recorded on a Bruker D4 X-ray diffractometer with Ni-filtered Cu K_R radiation (40 kV, 40 mA).

4. Preparation of tryptic digests of BSA, MYO and Cytc. Bovine serum albumin (BSA), horse heart myoglobin (MYO) and cytochrome c (Cytc) were dissolved in 25 mM ammonium bicarbonate buffer (NH_4HCO_3) at pH 8.3 (the final concentration was 0.2 mg/mL) respectively and treated with trypsin 40:1 (w/w) for 16 h at 37 °C. And the obtained peptide mixture was diluted with 25 mM NH_4HCO_3 for enrichment and MS analysis.

5. Enrichment of peptide samples. 10 mg of magG@PDA@Cu^{2+} composites were suspended in 1 mL of deionized water with the help of a vortex. In a typical enrichment process, 10 μL of magG@PDA@Cu^{2+} aqueous dispersion was added to 200 μL of peptide solution (the concentration ranging from 10 nM to 10 pM) in a 0.6 mL centrifuge tube, and the mixture was then vibrated in a shaker at 37 °C for 30 min to ensure equilibrium. After magnetic separation and removal of the supernatant, the magnetic particles were rinsed with deionized water three times. Then, 10 μL of 0.4

M ammonia was added into the tube and vibrated for 10 min to elute the adsorbed peptides. Eventually, the supernatant was pipetted onto a MALDI sample target for MALDI-TOF MS analysis. Later on, 0.5 μL of CHCA matrix was pipetted on it. The sample target was left at room temperature for evaporation of the solvent. And then the substrates were subjected to MALDI-MS for further analysis. For protein digest samples and peptides in human urine and serum, similar enrichment and separation procedures were conducted.

6. Enrichment of human urine and serum samples. To enrich the human urine sample, firstly we prepared the urine solution containing 20 nM or 10 nM Angiotensin II (a 1 mM Angiotensin II solution diluted 50,000 and 100,000 fold with human urine) and the urine solution containing 20 nM or 10 nM insulin (a 0.3 mM insulin solution diluted with human urine). Similar enrichment and washing protocol was followed. After that, the obtained adducts were eluted by 0.4 M ammonia. Peptides were deposited on the MALDI target using dried droplet method. 1 μL of the above eluent was deposited on the plate and dried, and then 0.5 μL of CHCA matrix was introduced.

Human serum contains a complex array of proteolytically derived peptides, which might be biomarkers of preclinical screening and disease diagnosis. To enrich the human serum sample, it was diluted with deionized water 8 fold in advance. Then, 1 mM Angiotensin II solution was diluted 50,000 and 100,000 fold with human serum dilution and its concentration was adjusted to 20 nM and 10 nM. Before enrichment, no peptides in the serum solutions could be identified with poor spectrum. After treatment with magG@PD@Cu^{2+}, the peak of Angiotensin II was successfully indentified with S/N ratio of 145.9 and 42.4 in 20 nM and 10 nM serum dilutions.

7. MALDI-TOF-MS analysis. Mass spectra were acquired in positive reflective mode on a 5800 Proteomics Analyzer (Applied Biosystems, Framingham, MA, USA) with the Nd: YAG laser at 366 nm, a repetition rate of 200 Hz and an acceleration voltage of 20 kV. The MASCOT server was used to interpret the MALDI-TOF MS data by searching the species of mammals for identification of three standard proteins with peptide fingerprint mass spectra. For the identification of insulin, mass spectra

were acquired in linear mode. Vertical scale was 0.1, input bandwidth was 20 MHz and bin size was 0.5 ns.

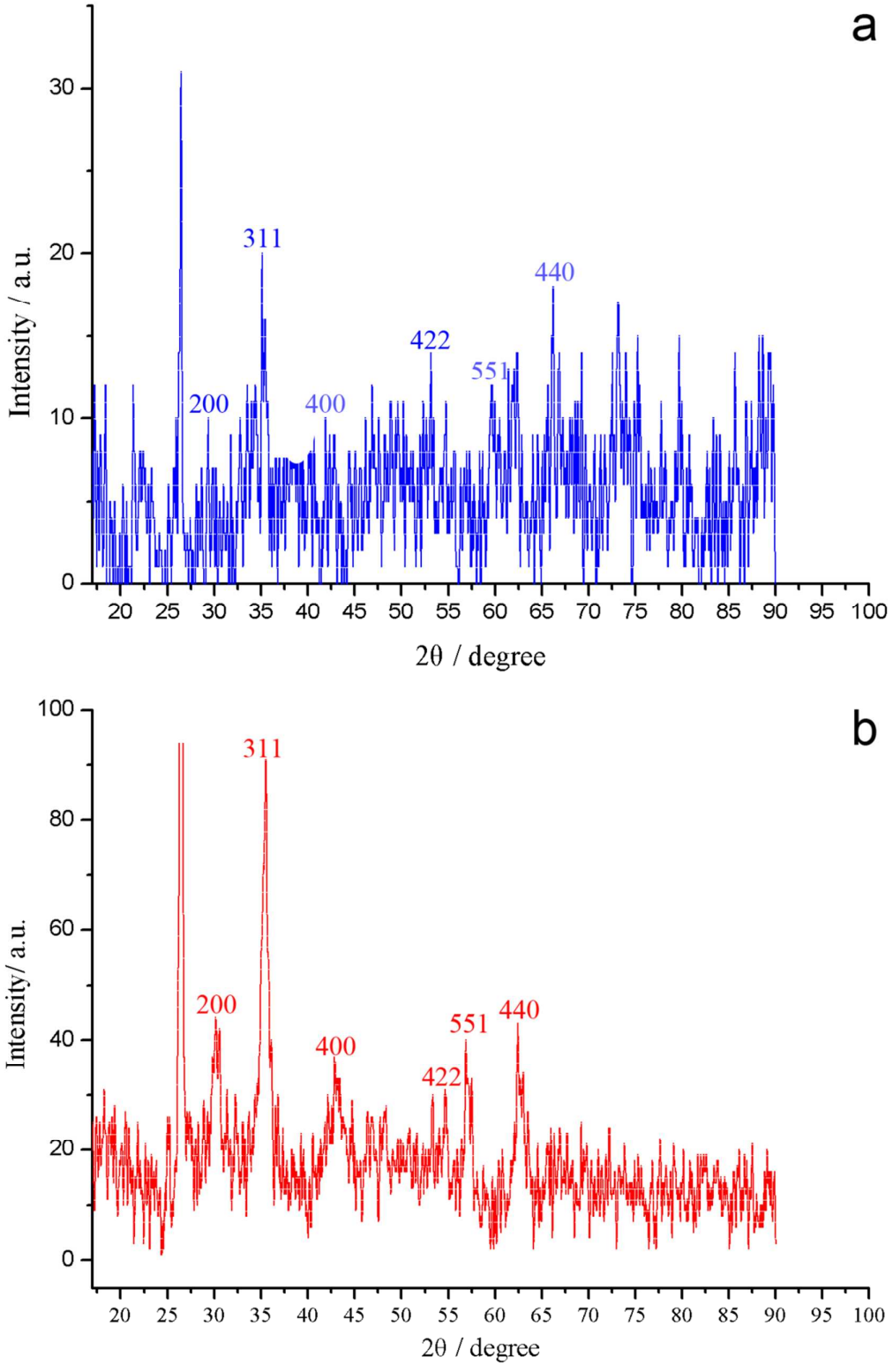

Figure S1. XRD patterns of a) magG and b) mag@PDA.

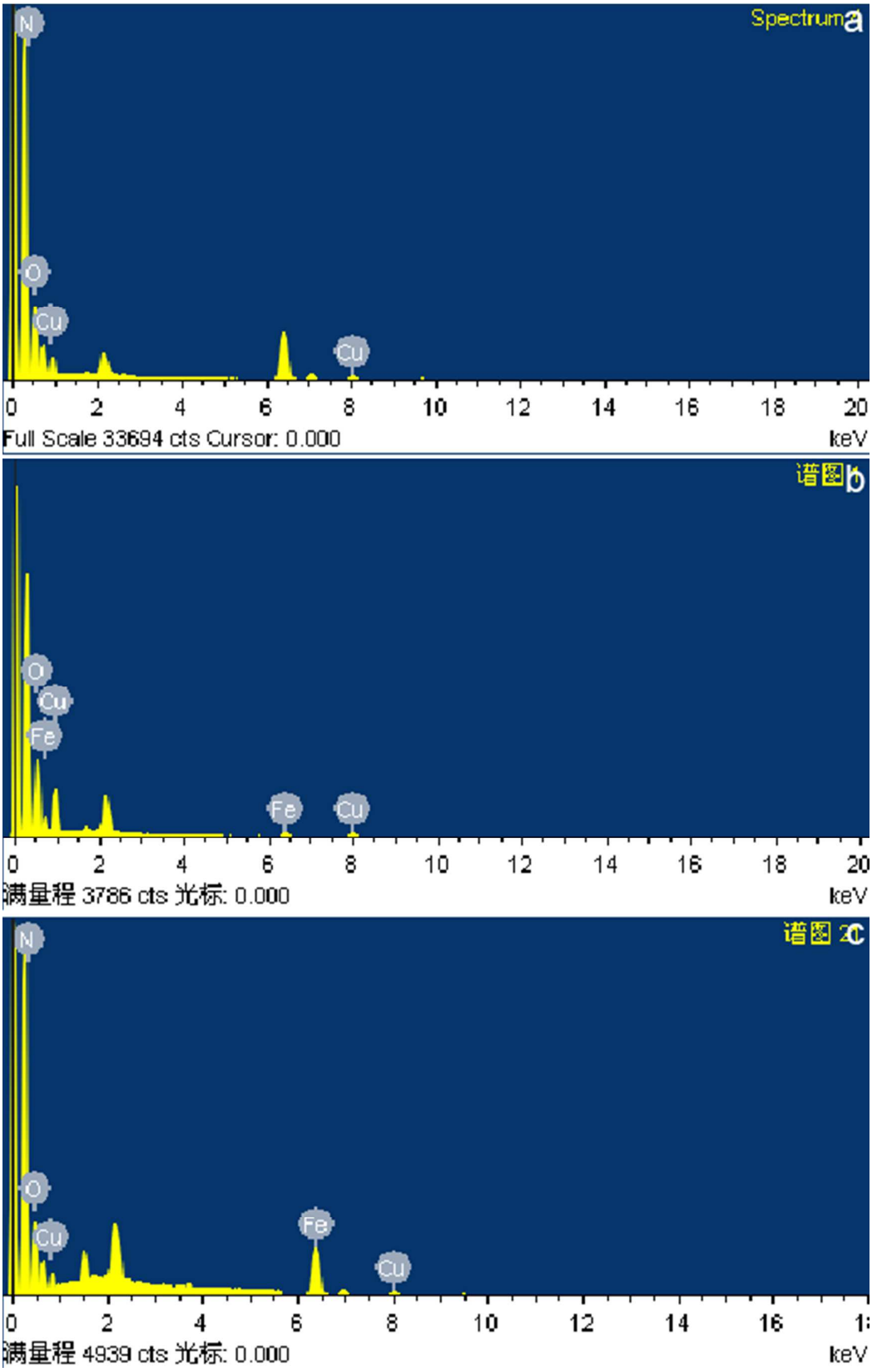

Figure S2. The energy dispersive X-ray (EDX) spectrum of the as-synthesized mag@PDA@Cu^{2+} composites (spots acquired in different regions on the surface).

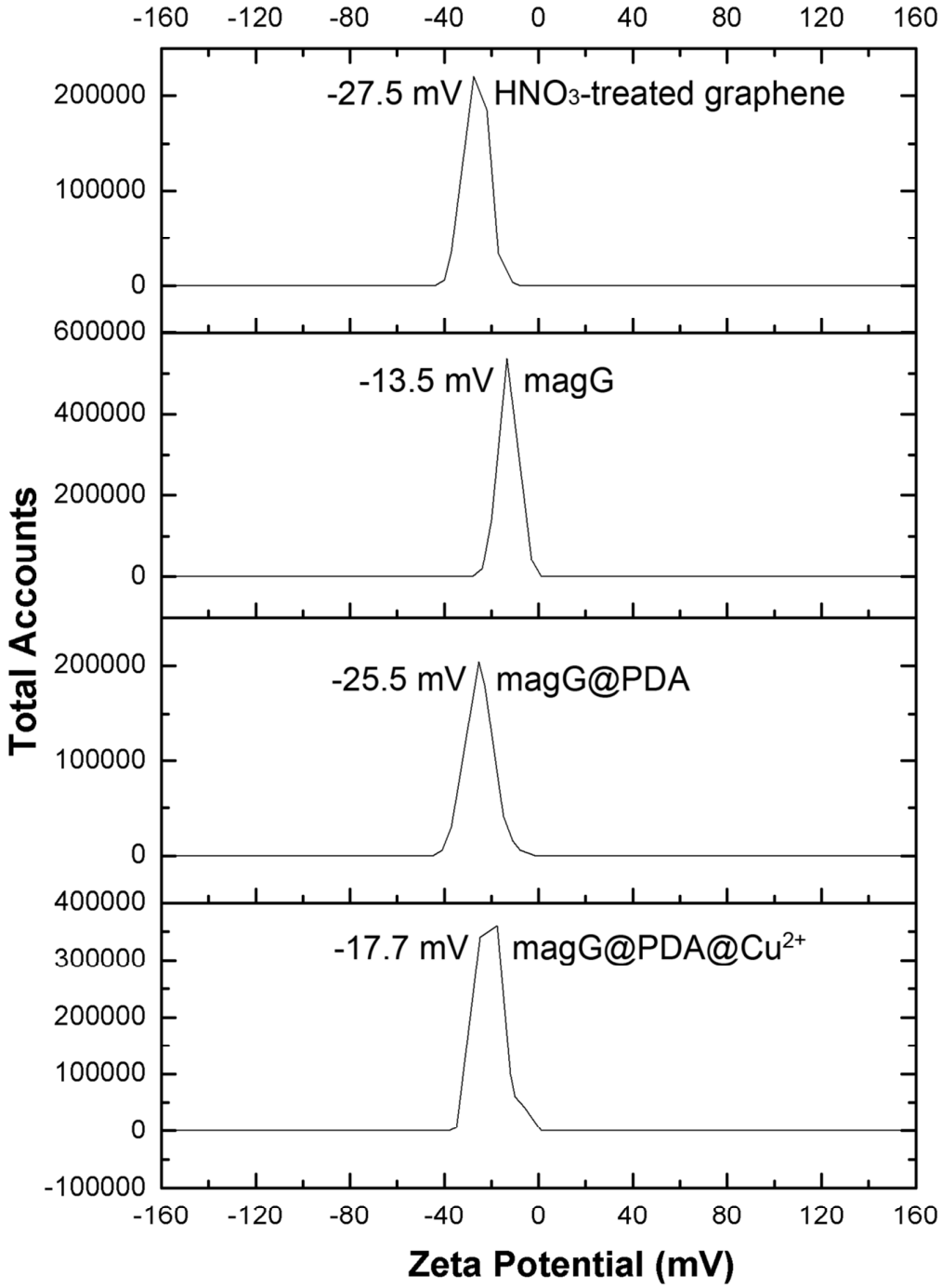

Figure S3. The zeta potential distributions of HNO_3-treated garphene, magG, mag@PDA and magG@PDA@Cu^{2+}.

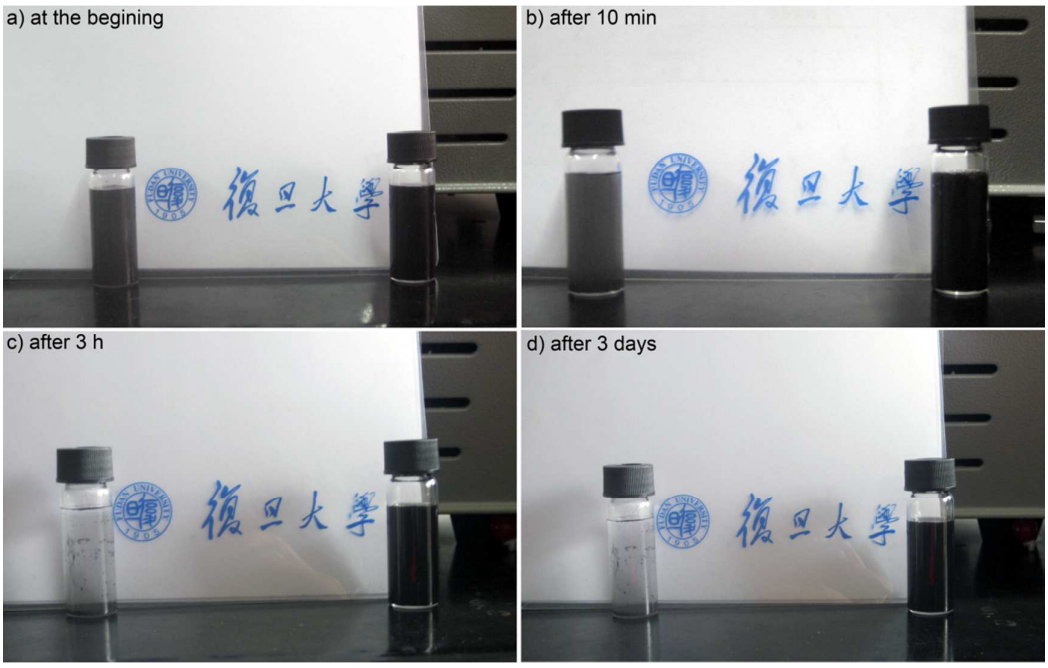

Figure S4. The photos of aqueous dispersion of untreated graphene and magG@PDA@Cu^{2+} composites after different periods of time.

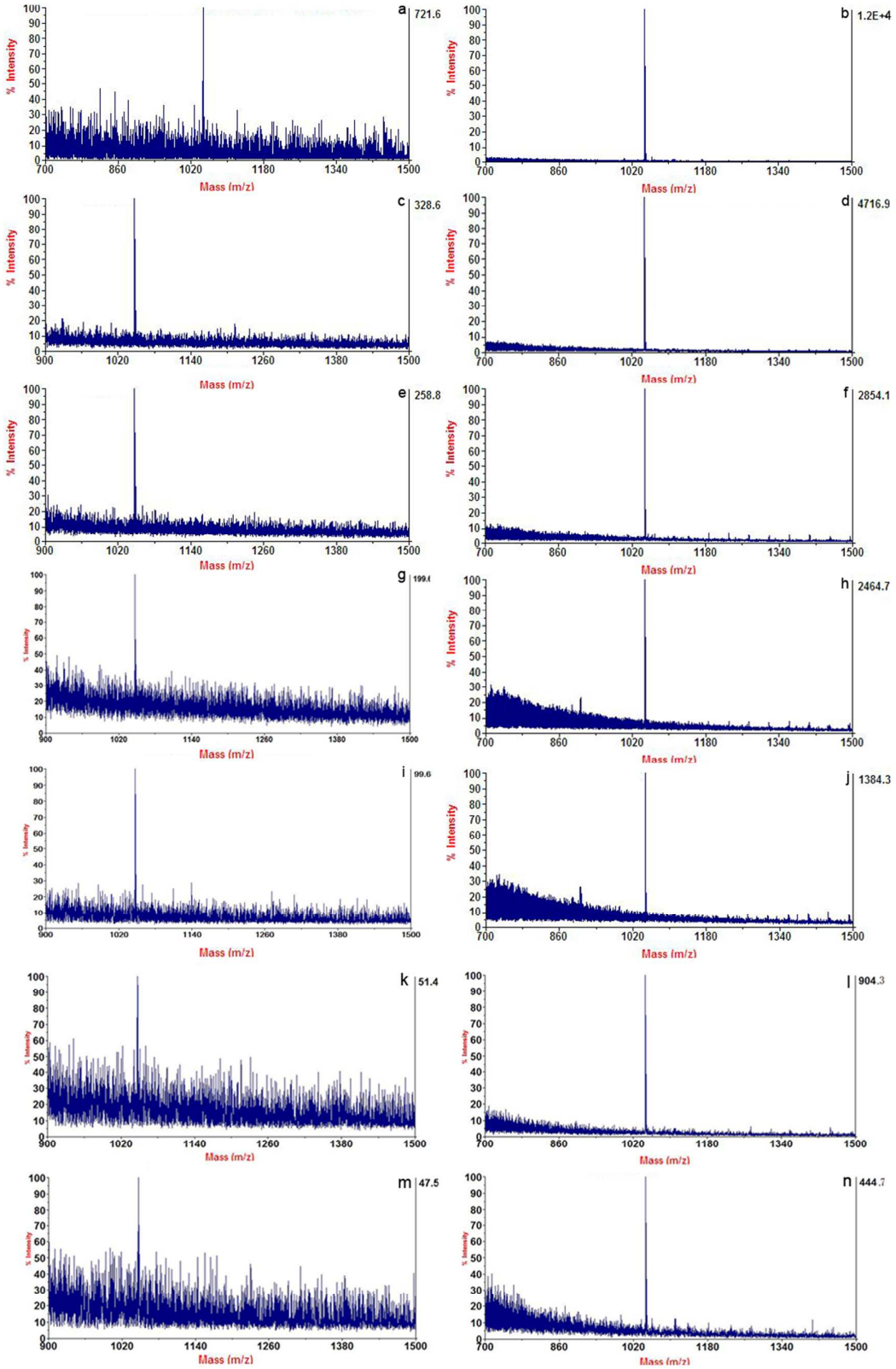

Figure S5. MALDI-TOF mass spectra of Angiotensin II aqueous solution at the concentration of: (a) 10 nM, (c) 5 nM, (e) 2 nM, (g) 1 nM, (i) 0.5 nM, (k) 200 pM and

(m) 100 pM with no treatment, (b) 10 nM, (d) 5 nM, (f) 2 nM, (h) 1 nM, (j) 10 pM, (l) 200 pM and (n) 100 pM after enrichment with magG@PDA@Cu^{2+}.

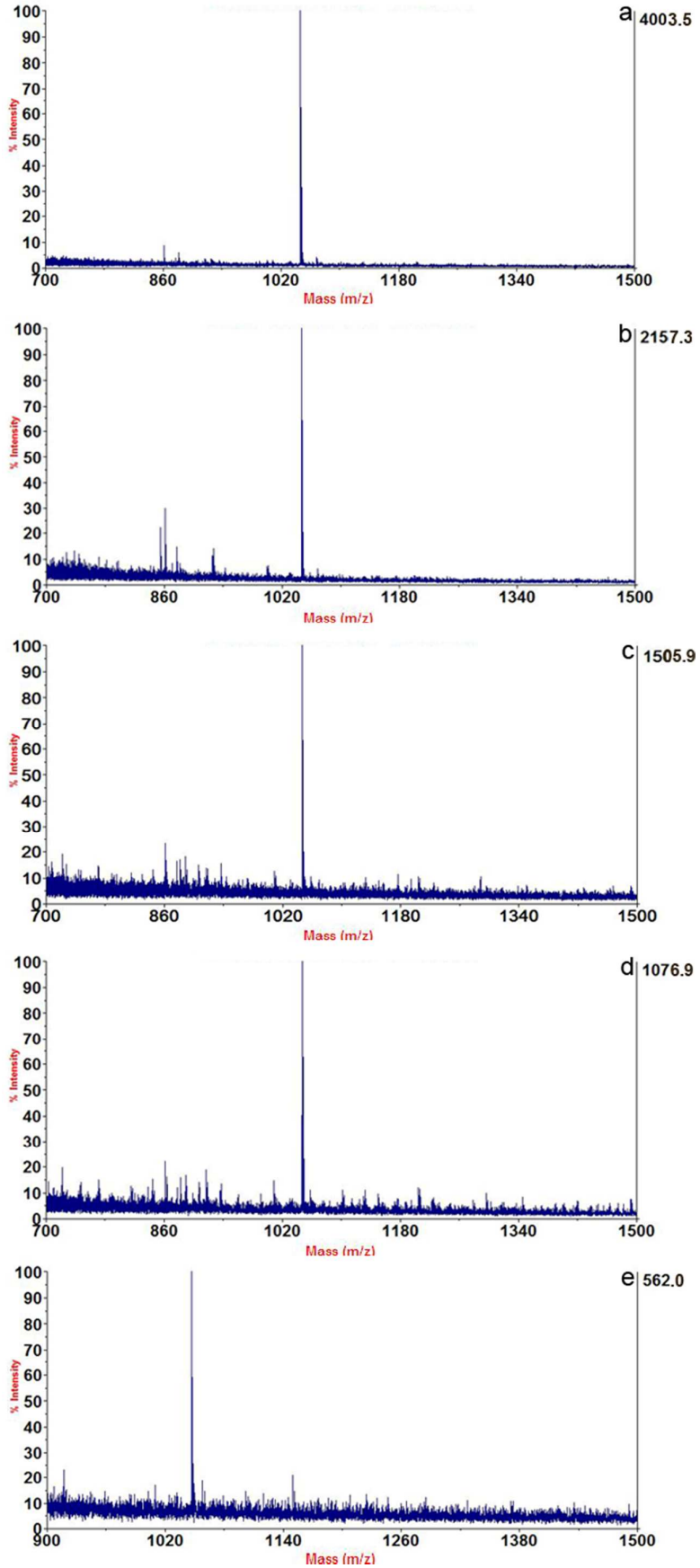
a
4003.5
b
2157.3
c
1505.9
d
1076.9
e
562.0
% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
700
860
1020
1180
1340
1500
900
1140
1260
1380

Figure S6. MALDI-TOF mass spectra of Angiotensin II aqueous solution concentrated by magG@PDA. (a) 10 nM, (b) 5 nM, (c) 2 nM, (d) 1 nM and (e) 0.5 nM.

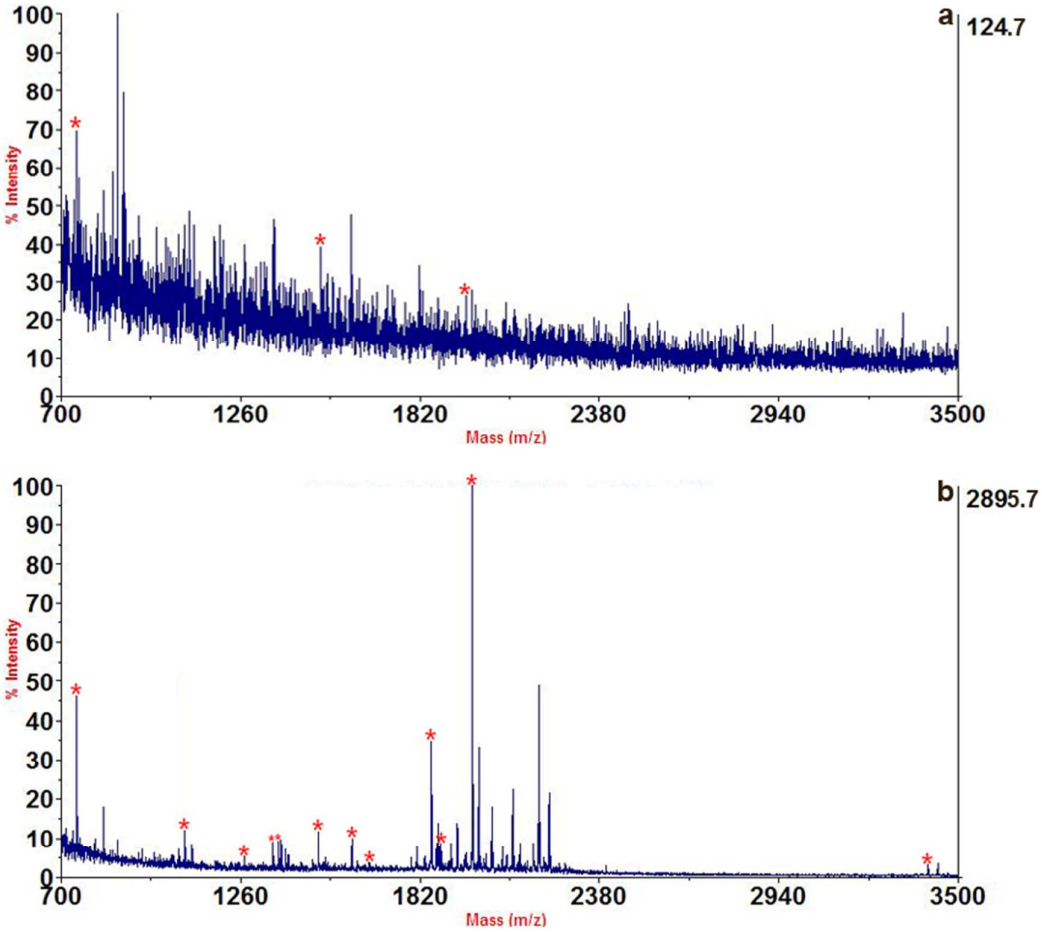

Figure S7. MALDI-TOF mass spectrum of 10 nM MYO digest: (a) without any enrichment, (b) after enrichment with magG@PDA@Cu^{2+}. The peak marked with asterisks represent peptides of MYO digest.

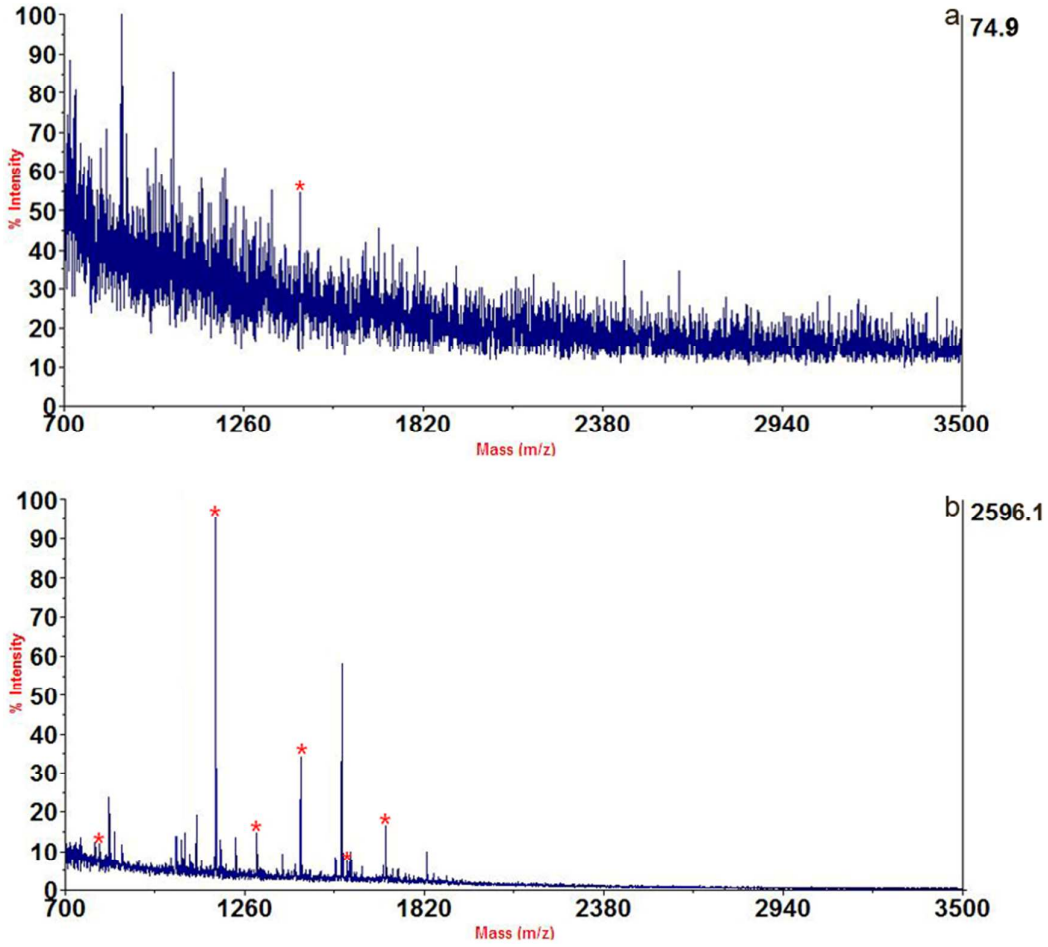

Figure S8. MALDI-TOF mass spectrum of 10 nM Cytc digest: (a) without any enrichment, (b) after enrichment with magG@PDA@Cu^{2+}. The peak marked with asterisks represent peptides of Cytc digest.

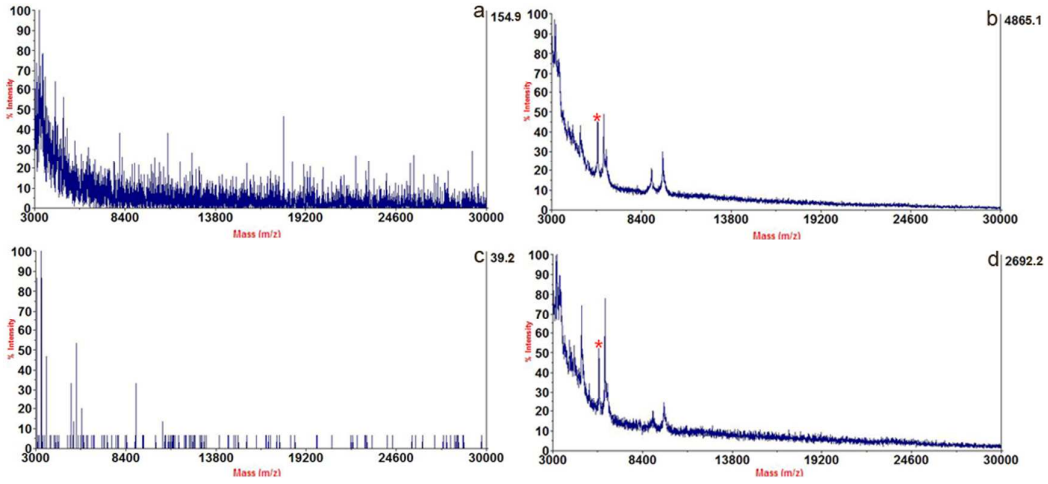

Figure S9. MALDI-TOF mass spectra of 20 nM and 10 nM insulin urine solution: (a), (c) without any treatment, (b), (d) after enrichment with magG@PDA@Cu^{2+}. The peaks of insulin were marked with asterisks.

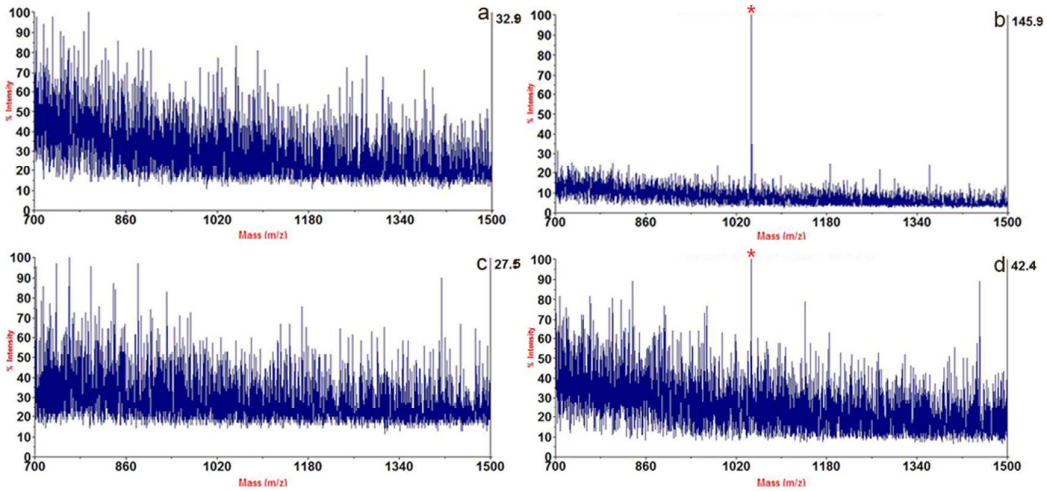

Figure S10. MALDI-TOF mass spectra of 20 nM and 10 nM Angiotensin II serum solution: (a), (c) without any treatment, (b), (d) after enrichment with magG@PDA@Cu^{2+}. The peaks of Angiotensin II were marked with asterisks.

Table S1. The Zeta potential changes throughout the formation of magG@PDA@Cu^{2+}

Sample	Zeta potential (mV)
HNO_3- treated graphene	-27.5
magG	-13.5
magG@PDA	-25.5
magG@PDA@Cu^{2+}	-17.7

Table S2. The search results of a BSA solution digested by trypsin after enrichment with magG@PDA@Cu^{2+}[a]

Calculated m/z	Database sequence	GRAVY	pI
927.4318	K.YLYEIAR.R	-0.071	6.00

1083.5253	K.YLYEIARR.H	-0.625	8.59
1193.5337	R.DTHKSEIAHR.F	-1.700	6.92
1249.5410	R.FKDLGEEHFK.G	-1.250	5.45
1439.7231	R.RHPEYAVSVLLR.L	-0.133	8.75
1479.7288	K.LGEYGFQNALIVR.Y	0.292	6.00
1567.6929	K.DAFLGSFLYEYSR.R	-0.085	4.37
1639.8641	R.KVPQVSTPTLVEVSR.S	-0.067	8.75
1823.7930	R.RPCFSALTPDEYTVPK.A	-0.537	6.06
Peptides matched	9		
Sequence coverage (%)	15		

[a] Search parameters: databse: SwissProt; digest used: Trypsin; maximum of missed cleavages:1; mass tolerance:±0.2 Da, Mascot from Matrix Science Ltd.(London, U.K.) was used to search all of the mass spectra.

'GRAVY' (grand average of hydropathicity) scores and theoretical isoelectric point (pI) were calculated with the ProtParam tool (http://ca.expasy.org/tools/ protparam. html).

Table S3. The search results of a MYO solution digested by trypsin after enrichment with magG@PDA@Cu^{2+}

Calculated m/z	Database sequence	GRAVY	pI
748.4350	K.ALELFR.N	0.700	6.05
1086.5586	K.HLKTEAEMK.A	-1.244	6.76
1271.6615	R.LFTGHPETLEK.F	-0.645	5.40
1360.7651	K.ALELFRNDIAAK.Y	0.117	6.11
1378.8431	K.HGTVVLTALGGILK.K	1.171	8.76
1502.6707	K.HPGDFGADAQGAMTK.A	-0.733	5.21
1606.8552	K.VEADIAGHGQEVLIR.L	0.153	4.65
1661.8573	R.LFTGHPETLEKFDK.F	-0.836	5.45
1853.9640	K.GHHEAELKPLAQSHATK.H	-1.082	7.03
1885.0186	K.YLEFISDAIIHVLHSK.H	0.606	5.99

1982.0593	K.KGHHEAELKPLAQSHATK.H	-1.239	8.52
Peptides matched	10		
Sequence coverage (%)	62		

Table S4. The search results of a Cytc solution digested by trypsin after enrichment with magG@PDA@Cu^{2+}

Calculated m/z	Database sequence	GRAVY	pI
806.4147	K.KYIPGTK.M	0.400	9.70
1168.5320	K.TGPNLHGLFGR.K	-0.391	9.44
1296.6184	K.TGPNLHGLFGRK.T	-0.683	11.00
1433.6600	K.HKTGPNLHGLFGR.K	-0.877	11.00
1584.7065	R.KTGQAPGFSYTDANK.N	-1.187	8.50
1698.6620	R.TGQAPGFSYTDANKNK.G	-1.331	8.17
Peptides matched	6		
Sequence coverage (%)	35		