Supporting information

Magnetic mesoporous carbon nanocomposites derived from bimetallic metal-organic framework for enrichment of low-abundance peptides

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Experimental Section

Materials. Cobalt nitrate ($Co(NO_3)_2 \cdot 6H_2O$) and nickel nitrate ($Ni(NO_3)_2 \cdot 6H_2O$) were purchased from Strem Chemicals (Newburyport, MA, USA) and J&K Chemicals (Beijing, China), respectively. 2-Methylimidazole (2-MIM), cobalt (Co) and cobalt oxide (Co_3O_4) nanopowders were purchased from Aladdin Reagent (Shanghai, China), and ammonium solution ($NH_3 \cdot H_2O$) (28%, w/w) was from Nanjing Chemical Reagent (Nanjing, China). Sequencing grade modified trypsin was purchased from Promega (Madison, WI, USA). Bovine serum albumin (BSA, MW = 67 kDa), angiotensin II (sequence is Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, MW = 1045.5 Da), α-cyano-4-hydroxycinnamic acid (CHCA, Puriss p.a.), acetonitrile (ACN, HPLC grade), trifluoroacetic acid (TFA, HPLC grade) and phosphoric acid (85%, w/w) were purchases from Sigma-Aldrich (St. Louris, MO, USA). ZipTipC18 pipette tips were brought from Millipore (Billerica, MA, USA). Ultrapure water from Milli-Q system (Millipore) was used throughout. All of other chemicals were analytical grade unless otherwise noted. Human serum and urine samples were provided by healthy volunteers, which was approved by the Medical Ethical Committee of the First Affiliated Hospital of Nanjing Medical University.

Material Characterization. The crystal phases of the prepared materials were characterized by a D8 Advance X-ray powder diffractometer (Bruker, Karlsruhe, German) with Cu Kα radiation ($\lambda = 1.5406$ Å) and operating at 40 kV and 40 mA.

Scanning electron microscope (SEM) images were gained with an S-3400N II SEM (Hitachi, Tokyo, Japan) at an acceleration voltage of 50 kV. The transmission electron microscope (TEM) images were obtained on a JEM-2100F TEM (JEOL, Tokyo, Japan) at an acceleration voltage of 200 kV coupled with EDS (Thermo Fisher Scientific, Waltham, MA, USA). Fourier-transform infrared (FT-IR) spectra of materials were collected on a Nicolet 6700 FT-IR spectrometer (Nicolet, Madison, WI, USA) using KBr pellets. The elemental composition and speciation of material surface was through a PHI5000 Versa Probe photoelectron spectrometer (ULVAC-PHI) XPS system (Kanagawa, Japan). Thermogravimetric (TG) curve of Co/Ni-MOFs was obtained by a STA-499C thermal analyzer (Netzsch, Bavaria, Germany) at 10 ^{o}C min^{-1} from room temperature to 800 ^{o}C in N_2 flowing. To characterize surface area and pore volume, N_2 sorption/desorption isotherms were measured at 77 K under a relative pressure (P/Po) ranging from 0.0001 to 0.99 using an ASAP 2020 volumetric sorption analyzer (Micromeritics, Atlanta, GA, USA). Magnetization of Co/Ni-MCNs was measured using a superconducting quantum interference device (SQUID) vibrating sample magnetometer (Quantum Design, San Diego, CA, USA) at room temperature.

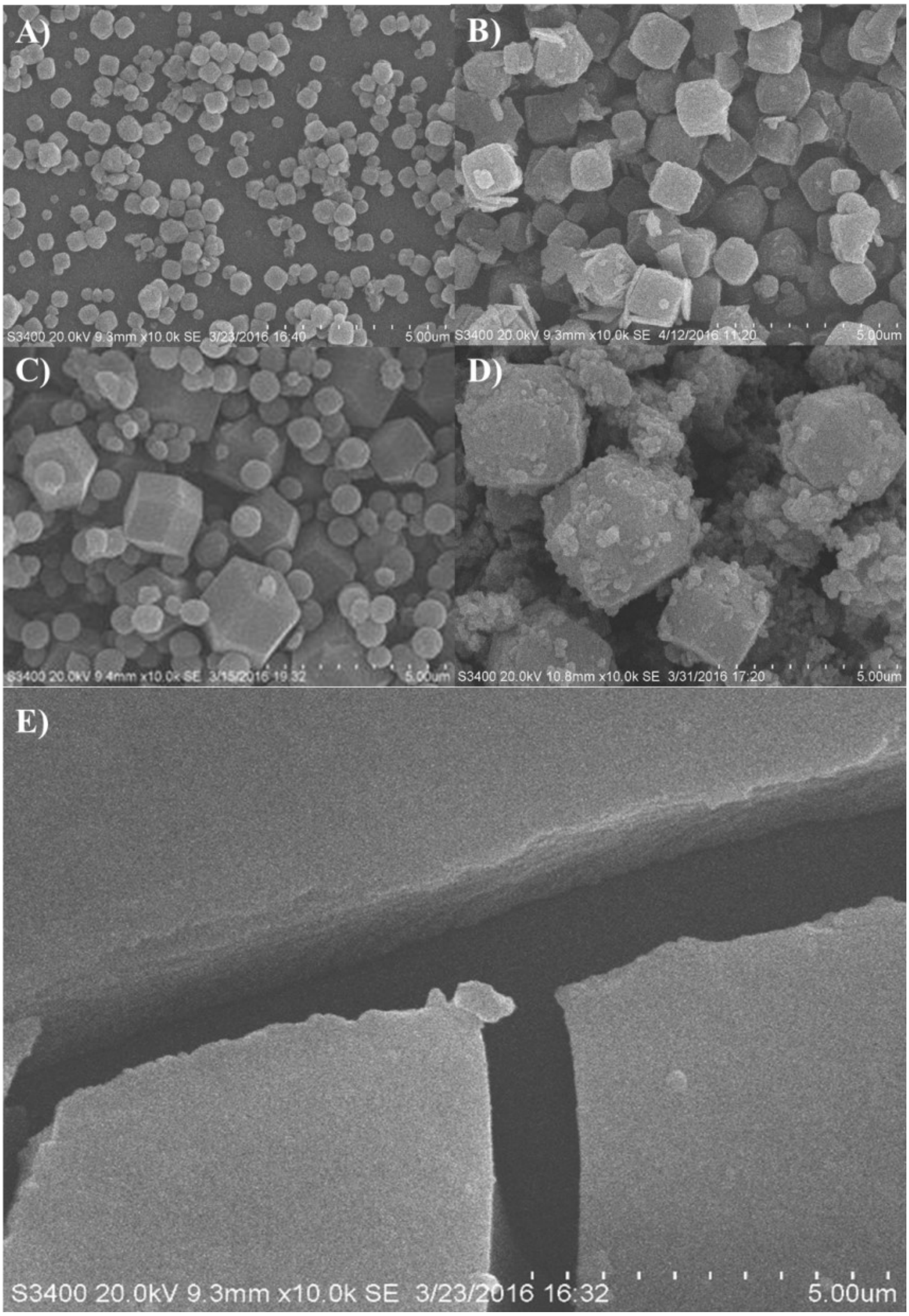

Figure S1. SEM images of Co/Ni-ZIF precursors prepared at different mass ratios of $Co(NO_3)_2\cdot 6H_2O$ to $Ni(NO_3)_2\cdot 6H_2O$. A. 5:0 (ZIF-67); B. 2:1; C. 1:1 (Co/Ni-ZIF); D. 1:2; E. 0:5.

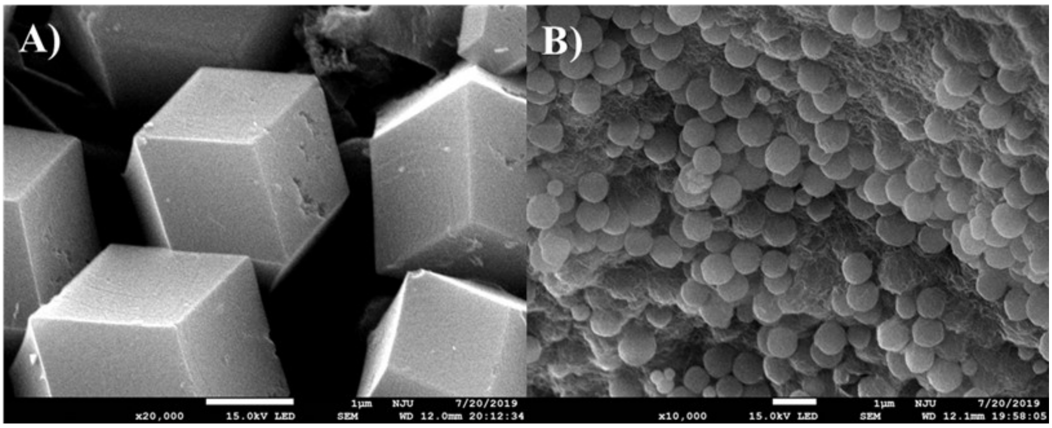

Figure S2. SEM images of rhombic dodecahedral (A) and spherical nanoparticles (B) separated from one-pot Co/Ni-ZIF products at Co/Ni mass ratio of 1:1.

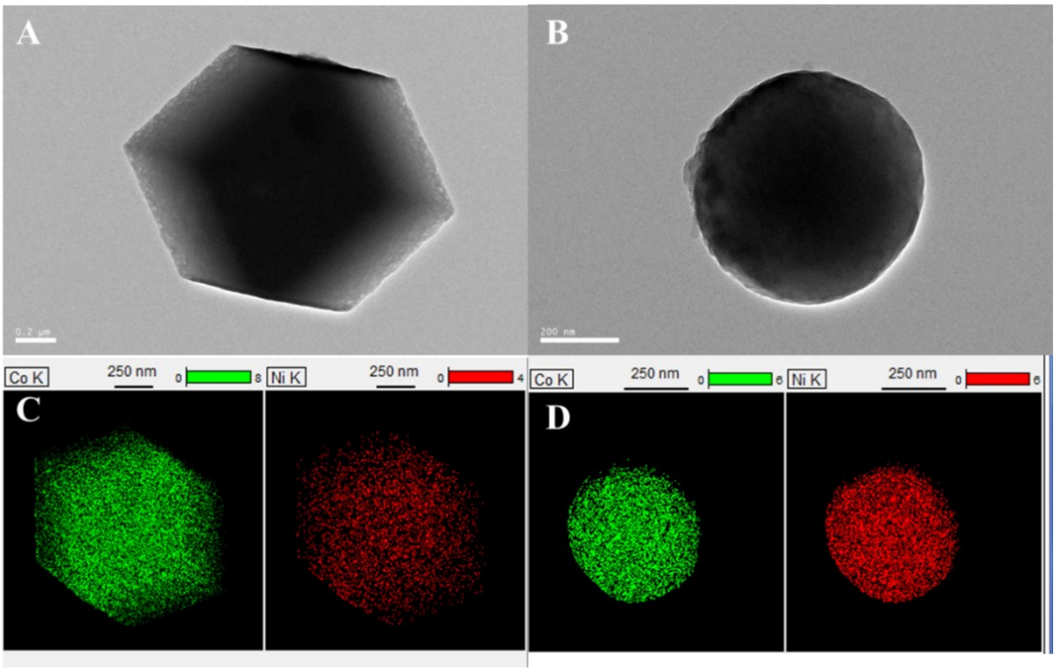

Figure S3. TEM images of dodecahedral (A) and spherical structures (B) of Co/Ni-ZIF, as well as their elemental mapping (C and D, respectively). Co/Ni-ZIF: $Co(NO_3)_2$:$Ni(NO_3)_2$, 1:1.

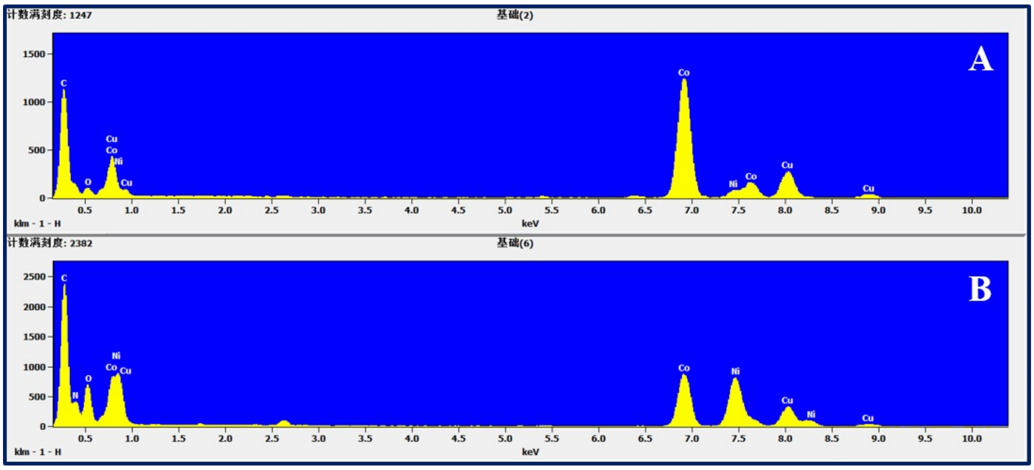

Figure S4. EDS of dodecahedrons (A) and spheres (B) from Co/Ni-ZIF ($Co(NO_3)_2$:$Ni(NO_3)_2$, 1:1).

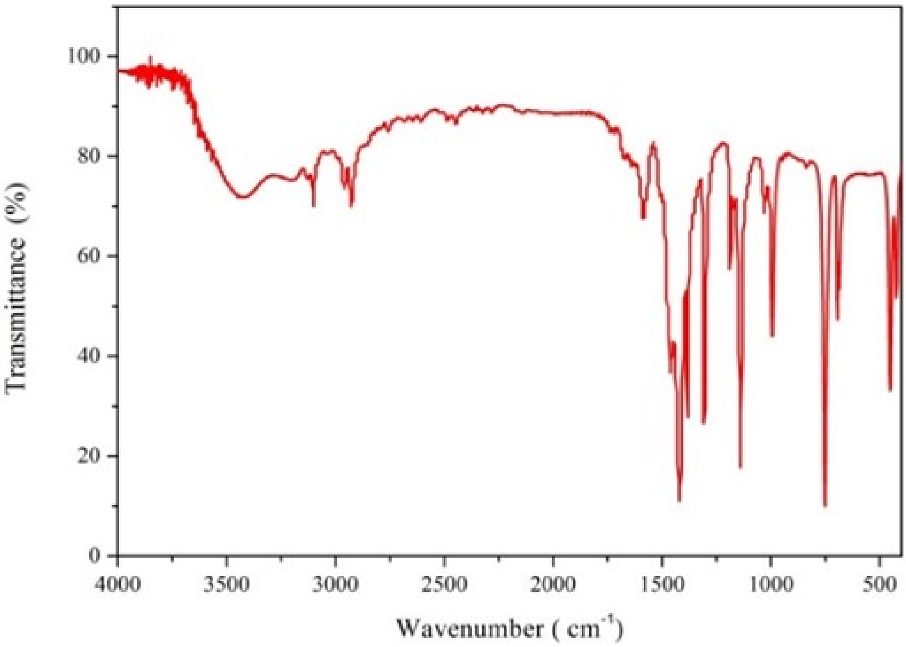

Figure S5. FT-IR spectrum of Co/Ni-ZIF ($Co(NO_3)_2$:$Ni(NO_3)_2$, 1:1).

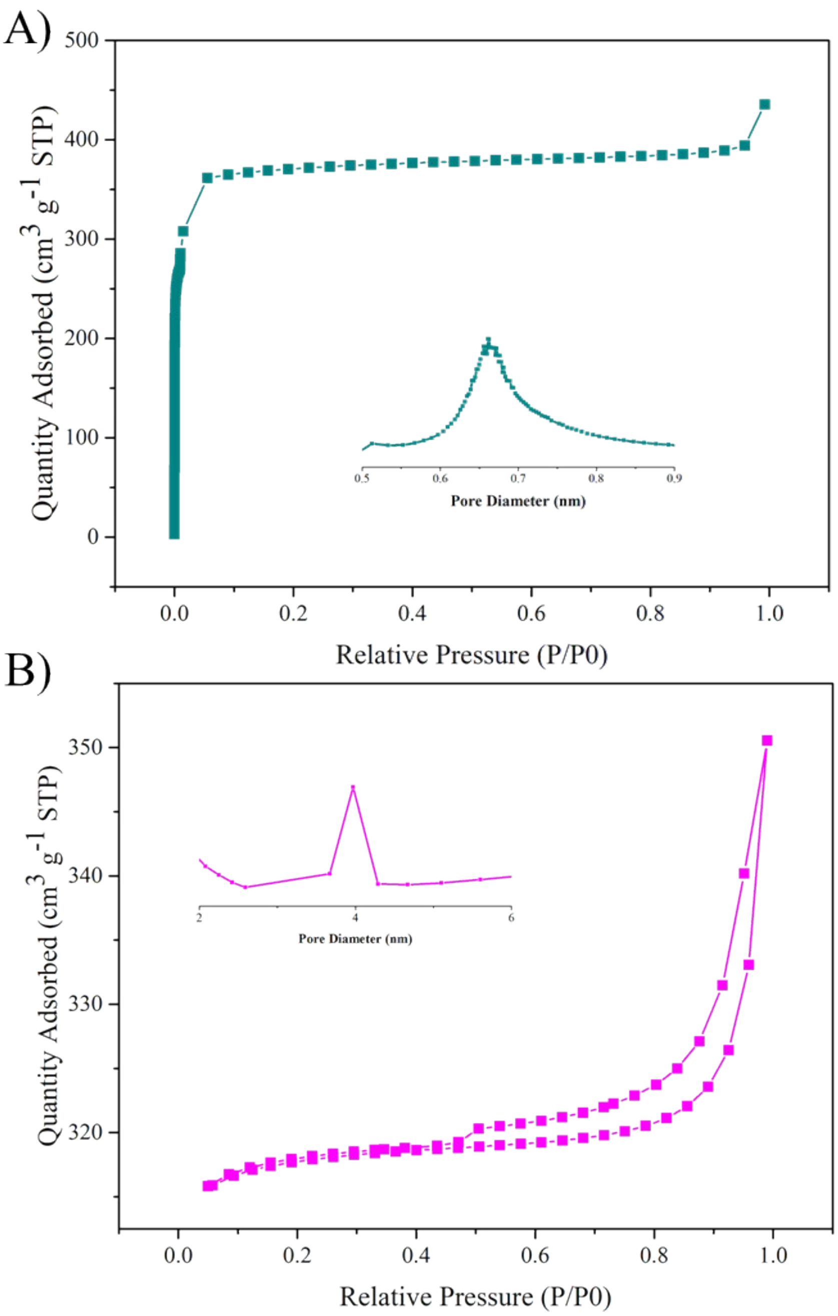

Figure S6. A. N_2 absorption-desorption isotherms of ZIF-67 measured at 77 K, and the inset shows the corresponding pore size distribution analyzed using Horvath-Kawazoe (HK) method; B. N_2 absorption-desorption isotherms of Co/Ni-ZIF, and the inset shows the corresponding pore size distribution analyzed using Barrett-Joyner-Halenda (BJH) method (B).

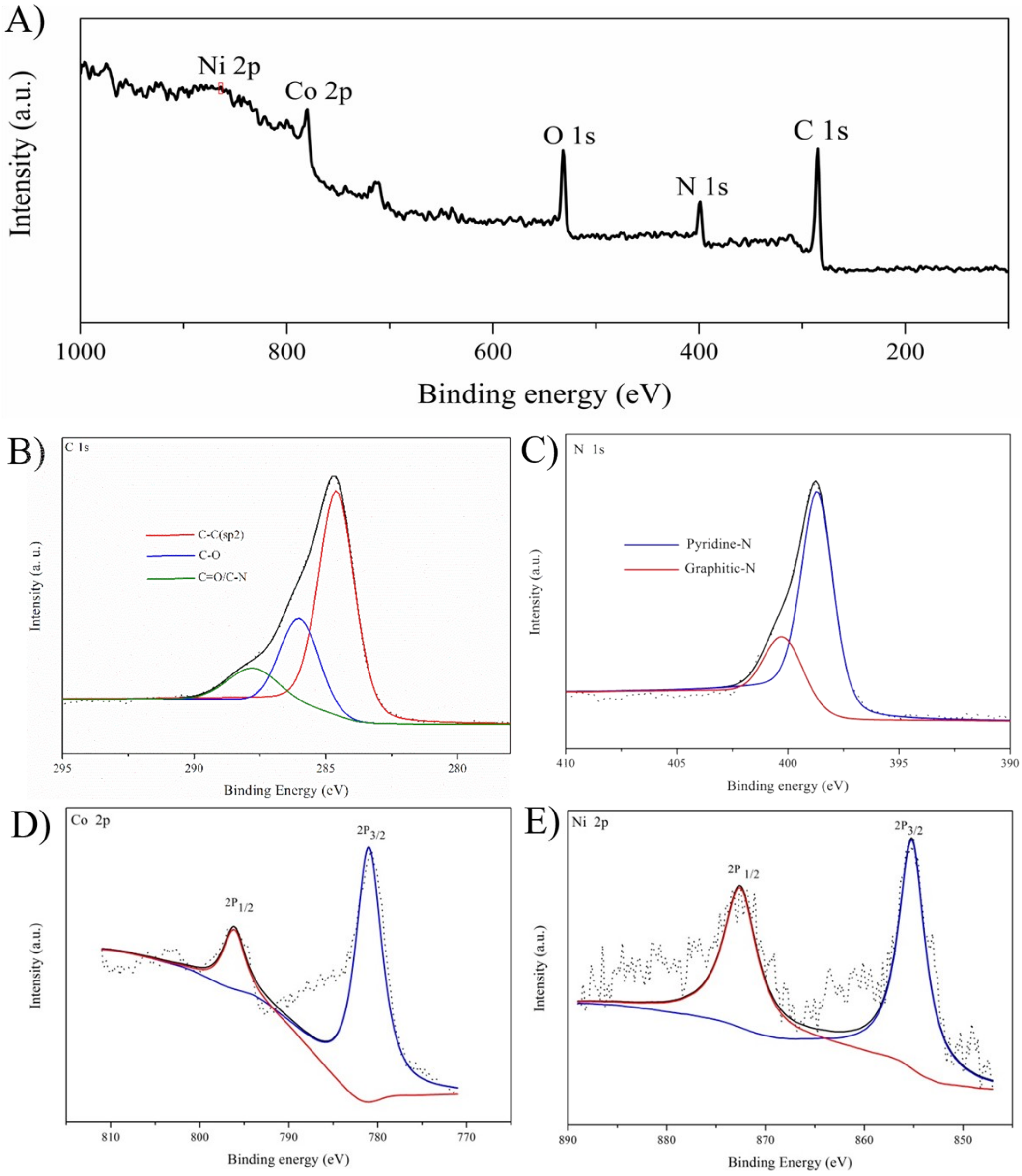

Figure S7. A. XPS full spectrum of Co/Ni-MCNs; C 1s (B), N 1s (C), Co 2p (D) and Ni 2p spectra (E) of Co/Ni-MCNs.

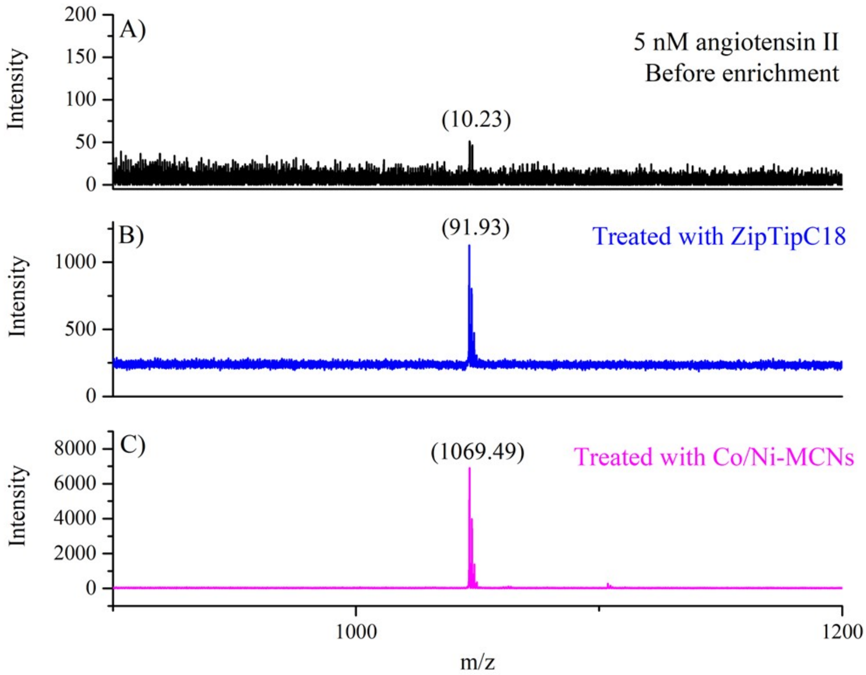

Figure S8. MS spectra of angiotensin II (5 nM) before (A), and after enrichment with ZiptipC18 (B) and Co/Ni-MCNs (C).

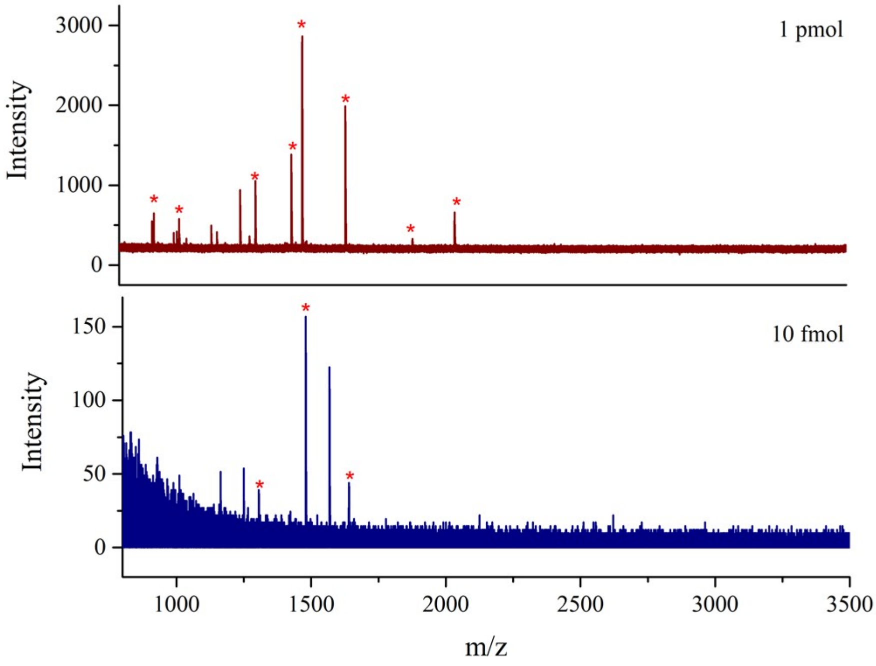

Figure S9. Mass spectra of BSA trypsin digests of 1 pmol (A) and 10 fmol (B) treated with Co/Ni-MCNs.

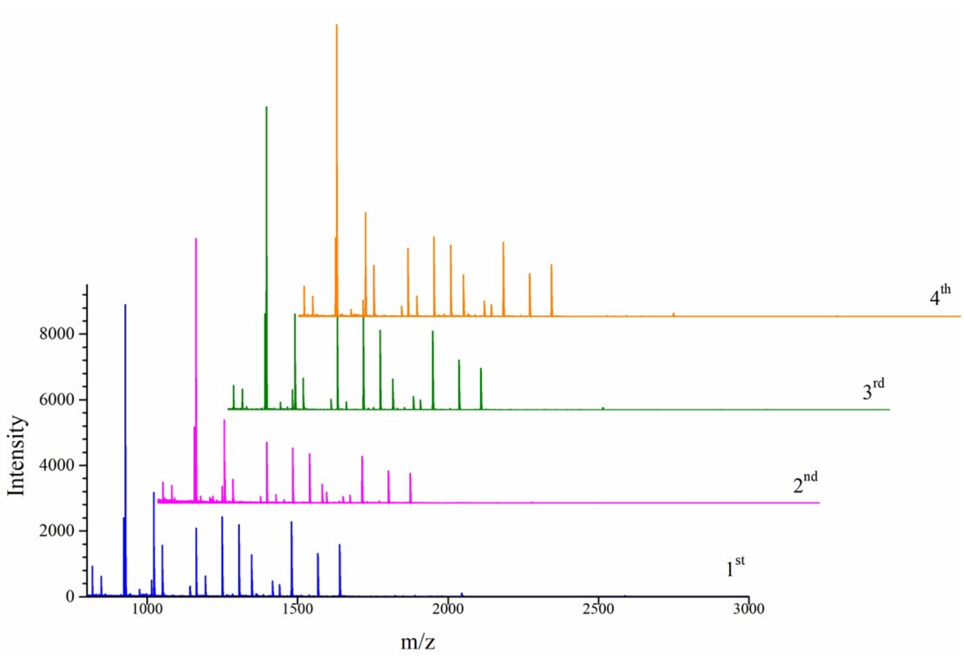

Figure S10. Recyclability test of Co/Ni-MCNs in enrichment of low-abundance peptides from BSA tryptic digests (10 nM).

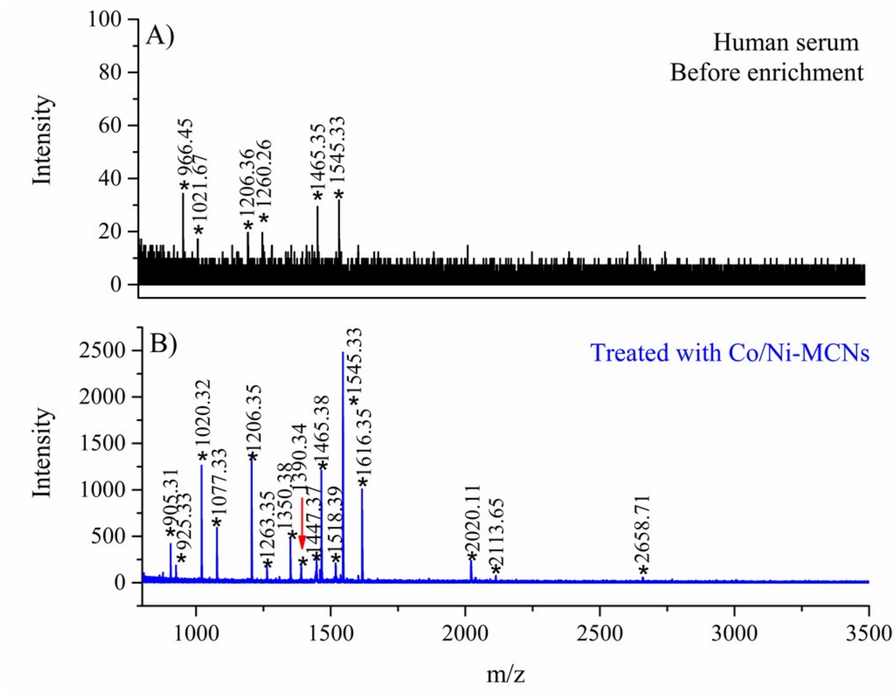

Figure S11. MS spectra of human serum before enrichment (A) and after enrichment with Co/Ni-MCNs (B).

Table S1. The search results of 10 nM BSA tryptic digest before and after enrichment with Co/Ni-MCNs affinity probes.

Position	Calc. m/z	Peptide sequences	Before	After (ZipTip C18)	After (Co/Ni-MCNs)
483-489	840.5	RLCVLHEKT			+
132-138	885.4	DDSPDLPK		+	
204-211	905.5	IETMREK		+	
249-256	921.5	AEFVEVTK	+		
161-167	927.5	YLYEIAR	+	+	+
37-44	973.5	DLGEEHFK			+
312-318	1014.5	SHCIAEVEK		+	+
501-507	1023.4	CCTESLVNR		+	
588-597	1049.5	EACFAVEGPK		+	+
66-75	1162.6	LVNELTEFAK	+	+	+
35-44	1248.6	FKDLGEEHFK	+	+	+

361-371	1282.7	RHPEYAVSVLLRL			+
402-412	1304.7	HLVDEPQNLIK		+	+
79-88	1348.5	TCVADESHAGCEK		+	
92-100	1361.7	SLHTLFGDELCK		+	
360-371	1438.8	RHPEYAVSVLLR			+
421-433	1478.8	LGEYGFQNALIVR			+
437-451	1510.8	VPQVSTPTLVEVSR		+	+
350-359	1566.7	DAFLGSFLYEYSR			+
427-451	1638.9	KVPQVSTPTLVEVSR		+	+
508-523	1822.9	RPCFSALTPDETYVPK			+
169-183	1887.9	RHPYFYAPELLYYANKY			+
168-183	2044.0	RRHPYFYAPELLYYANK			+
	Match		4	13	17

Coverage	5%	19%	29%

Table S2. Comparison of enrichment performance of the prepared Co/Ni-MCNs with other magnetic affinity probes for low-abundance peptides.

Typical materials	**Amount (μg)**	**Enrichment factor**	**Number of peptides from BSA digests**	**Samples**	**Ref.**
C60-f-MS	1×10^2	100	10	BSA, Urine	[S1]
Fe_3O_4@[$Cu_3(btc)_2$]	1×10^2	13	7	Angiotensin II, BSA, Myo, Urine	[S2]
Fe_3O_4@MIL-100(Fe)	5×10^2	-	22	BSA, Human serum	[S3]
Fe_3O_4@SiO_2@G	4×10^2	-	7	BSA, Myo, Cyt c, Saliva	[S4]
Fe_3O_4@mSiO_2	10-50	62	20-21	Angiotensin II, BSA, Human plasma	[S5]
Co/Ni-MCNs	1×10^2	100	17	Angiotensin II, BSA, Human serum; Human urine	This work

MATRIX SCIENCE MASCOT Search Results

Database: contaminants

Score: 25

Protein sequence coverage: 5%

Matched peptides shown in ***bold red***.

1	**MKWVTFISLL**	**LLFSSAYSRG**	**VFRRDTHKSE**	**IAHR**FKDLGE	EHFK**GLVLIA**
51	FSQYLQQCPF	DEHVK**LVNEL**	**TEFAK**TCVAD	ESHAGCEKSL	HTLFGDELCK
101	VASLRETYGD	MADCCEKQEP	ERNECFLSHK	DDSPDLPKLK	PDPNTLCDEF
151	KADEKKFWGK	**YLYEIAR**RHP	YFYAPELLYY	ANKYNGVFQE	CCQAEDKGAC
201	LLPKIETMRE	KVLASSARQR	LRCASIQKFG	ERALKAWSVA	RLSQKFPK**AE**
251	**FVEVTK**LVTD	LTKVHKECCH	GDLLECADDR	ADLAKYICDN	QDTISSKLKE
301	CCDKPLLEKS	HCIAEVEKDA	IPENLPPLTA	DFAEDKDVCK	NYQEAKDAFL
351	GSFLYEYSRR	HPEYAVSVLL	RLAKEYEATL	EECCAKDDPH	ACYSTVFDKL
401	KHLVDEPQNL	IKQNCDQFEK	LGEYGFQNAL	IVRYTRKVPQ	VSTPTLVEVS
451	RSLGKVGTRC	CTKPESERMP	CTEDYLSLIL	NRLCVLHEKT	PVSEKVTKCC
501	TESLVNRRPC	FSALTPDETY	VPKAFDEKLF	TFHADICTLP	DTEKQIKKQT
551	ALVELLKHKP	KATEEQLKTV	MENFVAFVDK	CCAADDKEAC	FAVEGPKLVV
601	STQTALA				

Start	–	End	Observed	Mr(expt)	Mr(calc)	Delta	M	Peptide
35	–	44	1249.1000	1248.0927	1248.6139	-0.5212	1	R.FKDLGEEHFK.G

66	–	75	1163.1600	1162.1527	1162.6234	-0.4706	0	K.LVNELTEFAK.T
161	–	167	927.1100	926.1027	926.4861	-0.3834	0	K.YLYEIAR.R
249	–	256	922.1100	921.1027	921.4807	-0.3780	0	K.AEFVEVTK.L

Database: contaminants

Score: 39

Protein sequence coverage: 19%

Matched peptides shown in *bold red*.

1	MKWVTFISLL	LLFSSAYSRG	VFRRDTHKSE	IAHRFKDLGE	EHFKGLVLIA
51	FSQYLQQCPF	DEHVKLVNEL	TEFAKTCVAD	ESHAGCEKSL	HTLFGDELCK
101	VASLRETYGD	MADCCEKQEP	ERNECFLSHK	DDSPDLPKLK	PDPNTLCDEF
151	KADEKKFWGK	**YLYEIAR**RHP	YFYAPELLYY	ANKYNGVFQE	CCQAEDKGAC
201	LLPK**IETMRE**	**K**VLASSARQR	LRCASIQKFG	ERALKAWSVA	RLSQKFPKAE
251	FVEVTKLVTD	LTKVHKECCH	GDLLECADDR	ADLAKYICDN	QDTISSKLKE
301	CCDKPLLEK**S**	**HCIAEVEK**DA	IPENLPPLTA	DFAEDKDVCK	NYQEAKDAFL
351	GSFLYEYSRR	HPEYAVSVLL	RLAKEYEATL	EECCAKDDPH	ACYSTVFDKL
401	K**HLVDEPQNL**	**IK**QNCDQFEK	LGEYGFQNAL	IVRYTR**KVPQ**	**VSTPTLVEVS**
451	**R**SLGKVGTRC	CTKPESERMP	CTEDYLSLIL	NRLCVLHEKT	PVSEKVTK**CC**
501	**TESLVNR**RPC	FSALTPDETY	VPKAFDEKLF	TFHADICTLP	DTEKQIKKQT
551	ALVELLKHKP	KATEEQLKTV	MENFVAFVDK	CCAADDK**EAC**	**FAVEGPK**LVV

601 STQTALA

Start	–	End	Observed	Mr(expt)	Mr(calc)	Delta	M	Peptide
35	–	44	1249.5308	1248.5235	1248.6139	-0.0904	1	R.FKDLGEEHFK.G
66	–	75	1163.5559	1162.5486	1162.6234	-0.0747	0	K.LVNELTEFAK.T
76	–	88	1347.4401	1346.4328	1348.5387	-2.1059	0	K.TCVADESHAGCEK.S
89	–	100	1362.5192	1361.5119	1361.6649	-0.1530	0	K.SLHTLFGDELCK.V
131	–	138	883.8901	882.8828	885.4080	-2.5252	0	K.DDSPDLPK.L
161	–	167	926.8820	925.8747	926.4861	-0.6114	0	K.YLYEIAR.R
161	–	167	927.8757	926.8685	926.4861	0.3823	0	K.YLYEIAR.R
205	–	211	908.9102	907.9029	905.4640	2.4389	1	K.IETMREK.V
310	–	318	1017.5236	1016.5163	1014.4804	2.0359	0	K.SHCIAEVEK.D
402	–	412	1305.6154	1304.6081	1304.7088	-0.1007	0	K.HLVDEPQNLIK.Q
437	–	451	1639.8079	1638.8006	1638.9305	-0.1299	1	R.KVPQVSTPTLVEVSR.S
438	–	451	1511.7302	1510.7229	1510.8355	-0.1126	0	K.VPQVSTPTLVEVSR.S
499	–	507	1022.3800	1021.3727	1023.4477	-2.0750	0	K.CCTESLVNR.R
588	–	597	1050.3645	1049.3572	1049.4852	-0.1279	0	K.EACFAVEGPK.L

Database: contaminants

Score: 126

Protein sequence coverage: 29%

Matched peptides shown in *bold red*.

1	MKWVTFISLL	LLFSSAYSRG	VFRRDTHKSE	IAHR**FKDLGE**	**EHFK**GLVLIA
51	FSQYLQQCPF	DEHVK**LVNEL**	**TEFAK**TCVAD	ESHAGCEKSL	HTLFGDELCK
101	VASLRETYGD	MADCCEKQEP	ERNECFLSHK	DDSPDLPKLK	PDPNTLCDEF
151	KADEKKFWGK	**YLYEIARRHP**	**YFYAPELLYY**	**ANK**YNGVFQE	CCQAEDKGAC
201	LLPKIETMRE	KVLASSARQR	LRCASIQKFG	ERALKAWSVA	RLSQKFPKAE
251	FVEVTKLVTD	LTKVHKECCH	GDLLECADDR	ADLAKYICDN	QDTISSKLKE
301	CCDKPLLEK**S**	**HCIAEVEK**DA	IPENLPPLTA	DFAEDKDVCK	NYQEAK**DAFL**
351	**GSFLYEYSRR**	**HPEYAVSVLL**	**R**LAKEYEATL	EECCAKDDPH	ACYSTVFDKL
401	K**HLVDEPQNL**	**IK**QNCDQFEK	**LGEYGFQNAL**	**IVR**YTR**KVPQ**	**VSTPTLVEVS**
451	**R**SLGKVGTRC	CTKPESERMP	CTEDYLSLIL	NR**LCVLHEK**T	PVSEKVTKCC
501	TESLVNR**RPC**	**FSALTPDETY**	**VPK**AFDEKLF	TFHADICTLP	DTEKQIKKQT
551	ALVELLKHKP	KATEEQLKTV	MENFVAFVDK	CCAADDK**EAC**	**FAVEGPK**LVV
601	STQTALA				

Start	–	End	Observed	Mr(expt)	Mr(calc)	Delta	M	Peptide
35	–	44	1249.4928	1248.4855	1248.6139	-0.1284	1	R.FKDLGEEHFK.G

37	–	44	974.3676	973.3603	973.4505	-0.0902	0	K.DLGEEHFK.G
66	–	75	1163.5106	1162.5033	1162.6234	-0.1200	0	K.LVNELTEFAK.T
161	–	167	927.4045	926.3973	926.4861	-0.0889	0	K.YLYEIAR.R
168	–	183	2044.7227	2043.7154	2044.0206	-0.3052	1	R.RHPYFYAPELLYYANK.Y
169	–	183	1888.6434	1887.6361	1887.9195	-0.2834	0	R.HPYFYAPELLYYANK.Y
310	–	318	1015.3990	1014.3917	1014.4804	-0.0887	0	K.SHCIAEVEK.D
347	–	359	1567.5642	1566.5569	1566.7354	-0.1785	0	K.DAFLGSFLYEYSR.R
360	–	371	1439.6469	1438.6396	1438.8045	-0.1648	1	R.RHPEYAVSVLLR.L
361	–	371	1283.5560	1282.5487	1282.7034	-0.1546	0	R.HPEYAVSVLLR.L
402	–	412	1305.5619	1304.5546	1304.7088	-0.1542	0	K.HLVDEPQNLIK.Q
421	–	433	1479.6295	1478.6222	1478.7881	-0.1659	0	K.LGEYGFQNALIVR.Y
437	–	451	1639.7103	1638.7030	1638.9305	-0.2275	1	R.KVPQVSTPTLVEVSR.S
438	–	451	1511.6542	1510.6469	1510.8355	-0.1886	0	K.VPQVSTPTLVEVSR.S
483	–	489	841.3783	840.3710	840.4527	-0.0817	0	R.LCVLHEK.T
508	–	523	1823.6403	1822.6330	1822.8924	-0.2593	0	R.RPCFSALTPDETYVPK.A
588	–	597	1050.3268	1049.3195	1049.4852	-0.1656	0	K.EACFAVEGPK.L

References

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