Electronic Supplementary Material

Super-assembled sandwich-like Au@MSN@Ag nanomatrices for high-throughput and efficient detection of small biomolecules

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INFORMATION ABOUT ELECTRONIC SUPPLEMENTARY MATERIAL

Reagents and materials

Glucose, D-glucose-6-^{13}C, Gly-^{15}N, Ala-^{15}N, sucrose, metabolites (guanine, glutamine, and inosine), flavone (kaempferol, puerarin, and quercetin), resveratrol, caffeine, fatty acids (C12:0, C16:0, C18:0, C20:0, C22:0), $HAuCl_4 \cdot 3H_2O$, α-cyano-4-hydroxycinnamic (CHCA), 2,5-dihydroxybenzoic acid (DHB), triethanolamine (TEA), trifluoroacetic acid (TFA), polyvinylpyrrolidone (PVP, wt 40,000), trisodium citrate, cetyltrimethy-lammonium chloride (CTAC) solution (25 wt % in H_2O) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tetraethyl orthosilicate (TEOS) and 1-octadecene were purchased from Aladdin Reagent (Shanghai, China). Methanol, ethanol, and acetonitrile, both HPLC-grade, were purchased from Merck (Darmstadt, Germany). Amino acids (Gly, Ala, Val, Hyp, Ile, Leu, Thr, Lys, Glu, His, Phe, Arg, Tyr, Cys, Try, Ser, Pro), four polypeptides (including donkey derived peptide A1, donkey derived peptide A2, horse derived peptide A, and bovine derived peptide A1), four ginsenosides (including Rg1, Re, Rb1, and Notoginsenoside R1), and Ginkgo leaf total lactones reference extract were obtained from NIFDC (Beijing, China). Other chemicals and reagents were all analytical grade and provided by MACKLIN (Shanghai, China). All reagents were used without purification. Ultrapure water was produced by a Milli-Q Gradient water system (Millipore, MA, USA).

Material characterization and measurements

The images of TEM and HRTEM, including elemental mapping images, were recorded on an FEI Talos™ F200s instrument (Thermo Fisher Scientific Inc., USA) microscope operated at 200 kV. The materials' water suspension was deposited on a copper grid before observation. EDX spectra and SEM images were obtained on SUPRATM 55 instrument (ZEISS, Japan) by dropping the materials suspensions on the aluminum foil. Dynamic light scattering (DLS) size and Zeta potential was measured by a Nano-ZS90 instrument (Malvern, Worcestershire, UK) in the water at 25°C. To obtain porosity information of materials, Nitrogen adsorption isotherms were measured with a Kubo X1000 (Bjbuilder, China) system at 77 K and the samples were degassed before measurement. Absorption spectra of Ultraviolet-visible light (UV-vis) were collected using a UV-2700 spectrophotometer (Shimadzu, Japan) at a wavelength of 250-700 nm in room temperature. The crystal structures of the materials were obtained by X-ray diffraction (XRD) by EMPYREAN (PANalytical, Holland) using Cu Kα radiation (λ = 1.54056) in the 2θ range of 10°-80°. Fourier transform infrared (FTIR) spectra were recorded by the KBr method on a VERTEX 70 FTIR instrument (Bruker, Germany). The thermogravimetric analysis (TGA) were detected by a STA 449F3 simultaneous TG/DSC analyzer (Netzsch, Germany) in the range of 30℃ to 600℃. Inductively coupled plasma optical emission spectrometry (ICP-OES) were obtained by an IRIS Advantage instrument (Thermo Fisher Scientific Inc., USA).

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Supporting Figures

Figure S1 The photo picture of the reactor for the synthesis of the Au@MSN by the biphase stratification approach. The heterogeneous oil-water stratification reaction system shown in the figure contains the tetraethyl orthosilicate (TEOS) solution as a silica source in hydrophobic organic solvent as the upper oil phase, while the lower pink phase is the solution of the products, which contains monodisperse gold suspensions as core, cetyltrimethylammonium chloride (CTAC) as a template, and triethanolamine (TEA) as a catalyst.

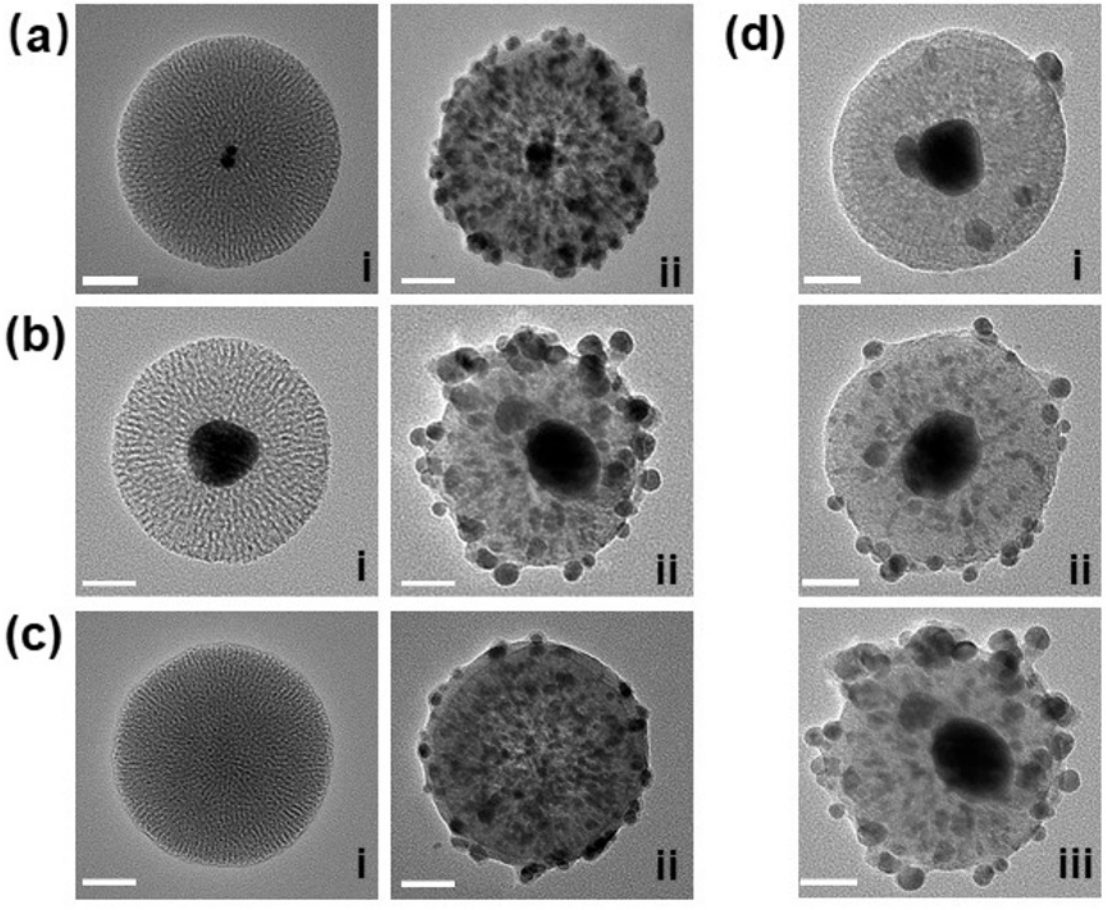

Figure S2 Control and optimization of core-shells nanospheres for LDI MS Analysis: TEM images with (a) 15 nm AuNPs as core and (b) 45 nm AuNPs as core of the (i) Au@MSN, (ii) Au@MSN@Ag. (c) TEM images of (i) MSN and (ii) MSN@Ag. (d) TEM images of Au@MSN@Ag by silver mirror reaction at (i) once, (ii) twice, and (iii) three times. Scale bars, 50 nm.

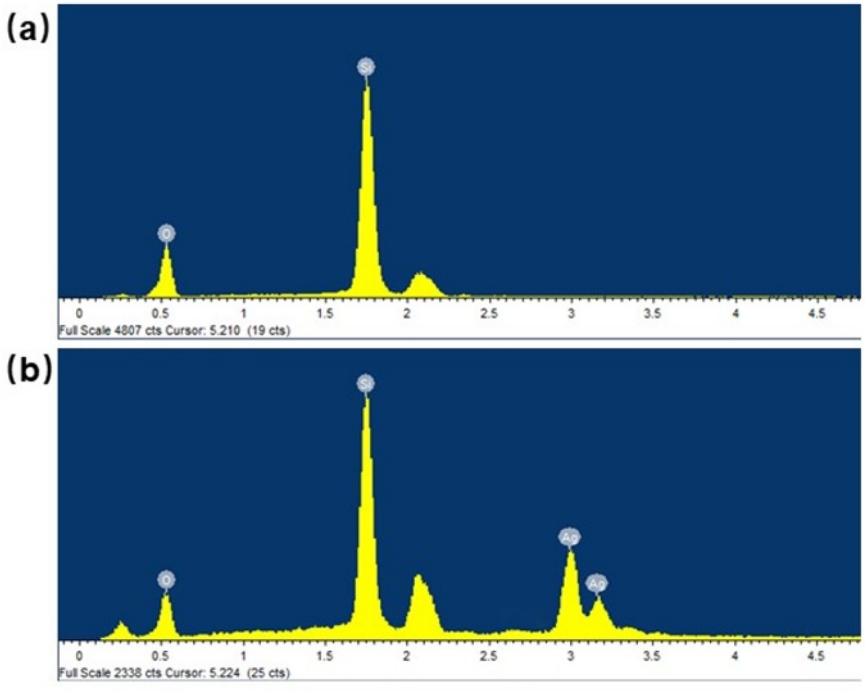

Figure S3 Typical EDX spectra for nanospheres. (a) Au@MSN. (b) Au@MSN@Ag.

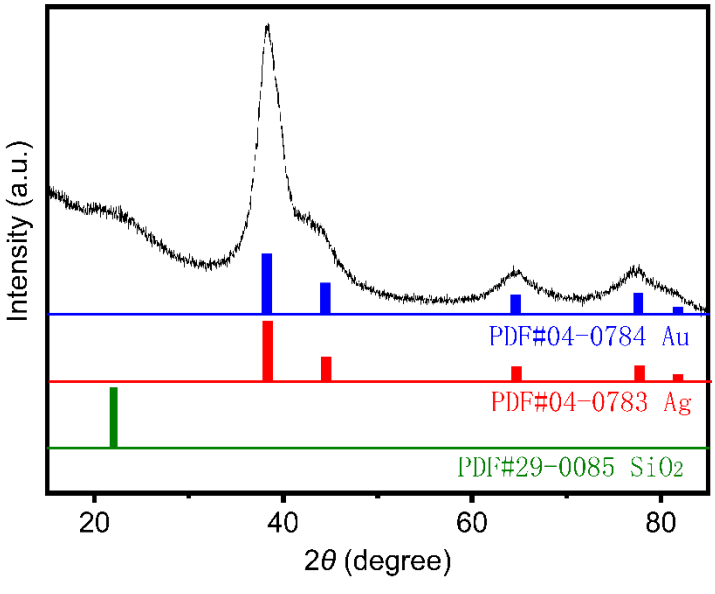

Figure S4 Typical XRD pattern of the sandwich-like Au@MSN@Ag nanospheres.

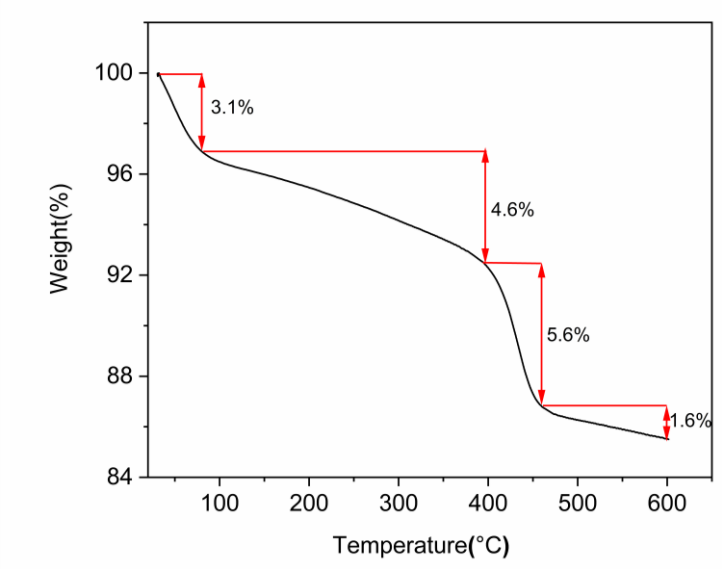

Figure S5 TG A curves of the sandwich-like Au@MSN@Ag nanospheres.

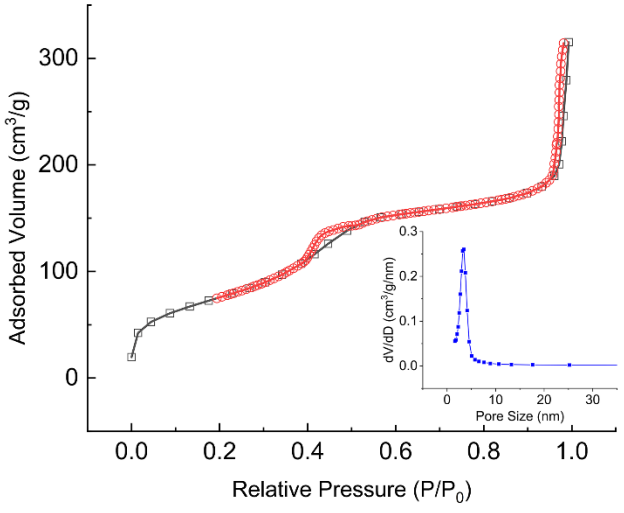

Figure S6 Nitrogen adsorption-desorption isotherms and pore size distribution (inset) of Au@MSN@Ag.

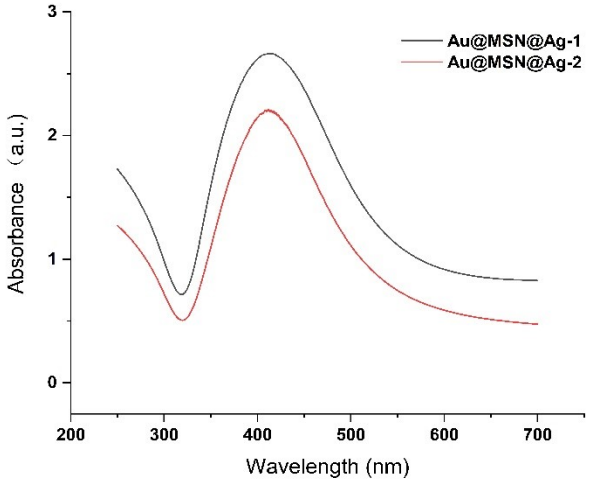

Figure S7 UV-Vis absorption spectra of Au@MSN@Ag-1 with 45 nm AuNPs as core and 55 nm MSN as shell, and Au@MSN@Ag-2 with 15 nm AuNPs as core and 100 nm MSN as shell. The thickness of the silver shell was the same. The nanospheres solutions concentration was 0.02 mg mL^{-1}.

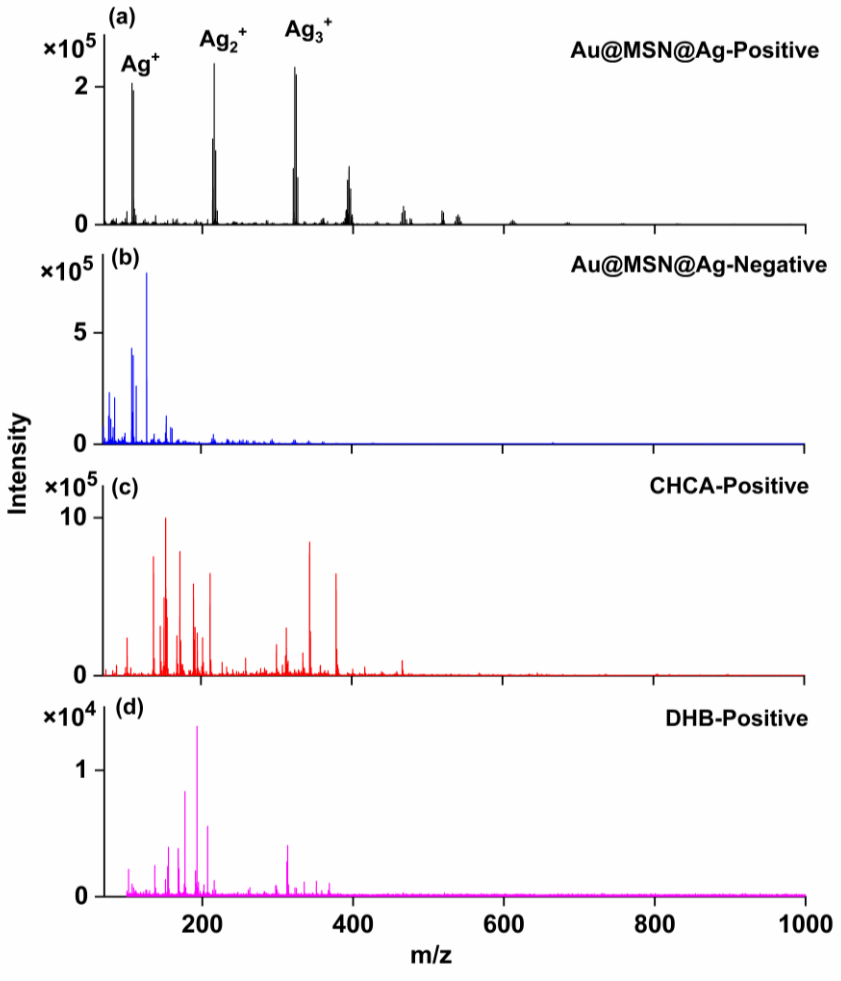

Figure S8 LDI-TOF MS spectra detected in the positive-ion mode only using (a) Au@MSN@Ag, (c) CHCA, (d) DHB, and negative-ion mode only using (b) Au@MSN@Ag as the matrices.

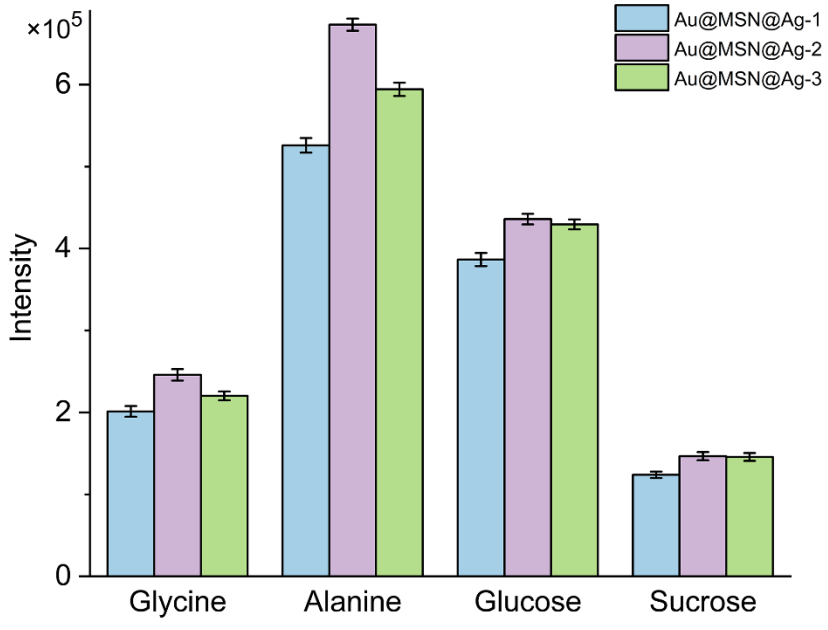

Figure S9 Selection of optimized Au@MSN@Ag. The mean intensities (n=3) of $[M+^{107}Ag]^+$ signals of small molecules obtained using Au@MSN@Ag-1 (with 15 nm AuNPs as core and 100 nm MSN as shell), Au@MSN@Ag-2 (with 45 nm AuNPs as core and 55 nm MSN as shell) and Au@MSN@Ag-3 (with 45 nm AuNPs as core and 70 nm MSN as shell) as matrices.

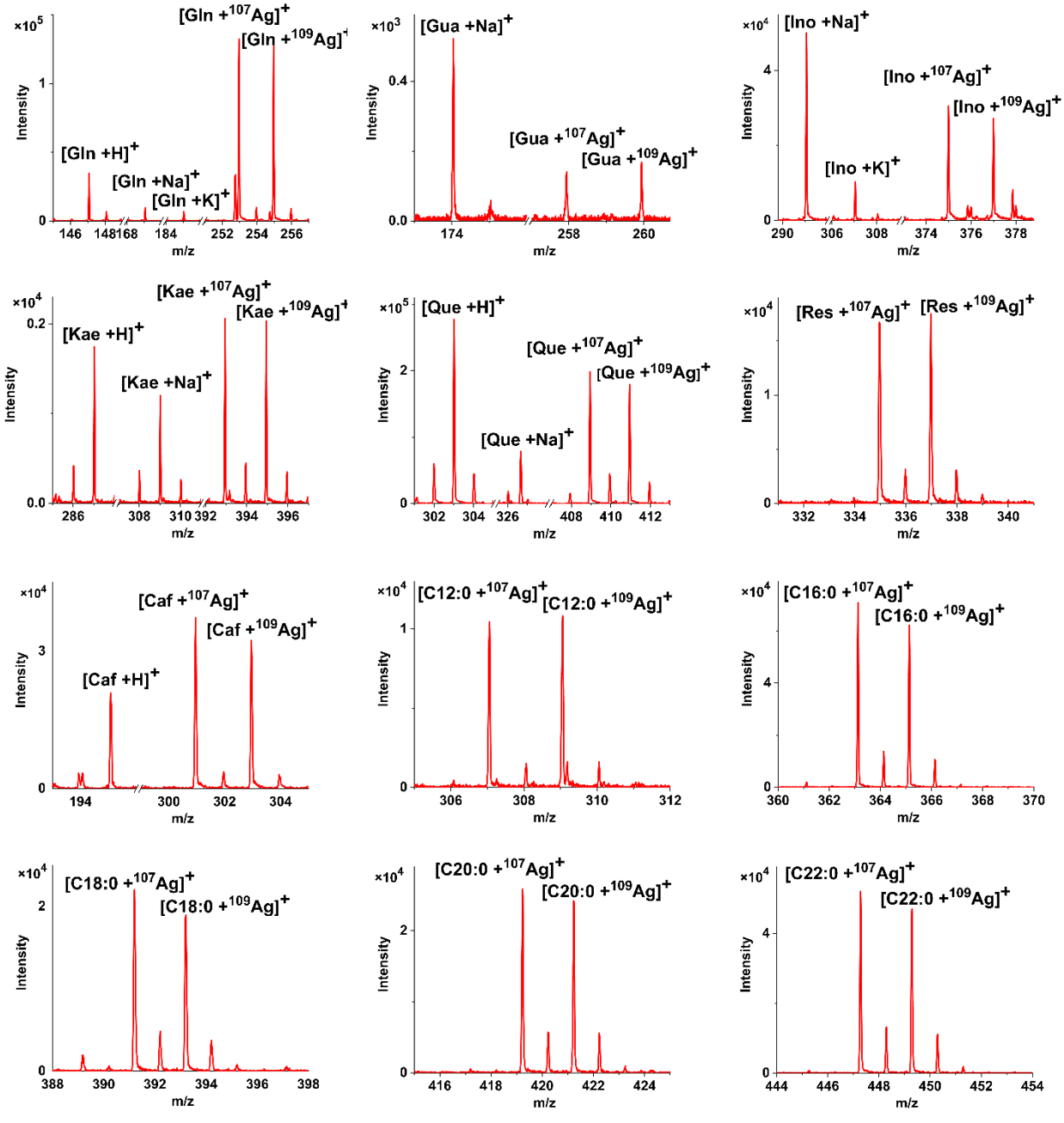

Figure S10 Mass spectra of metabolites (guanine, inosine, and glutamine), flavone (kaempferol and quercetin), non-flavonoid polyphenols (resveratrol), caffeine, and fatty acids (C12:0, C16:0, C18:0, C20:0, C22:0) standards detected in positive ion mode using Au@MSN@Ag as the matrix by LDI MS. The amount of each analyte is 100 μg mL^{-1}.

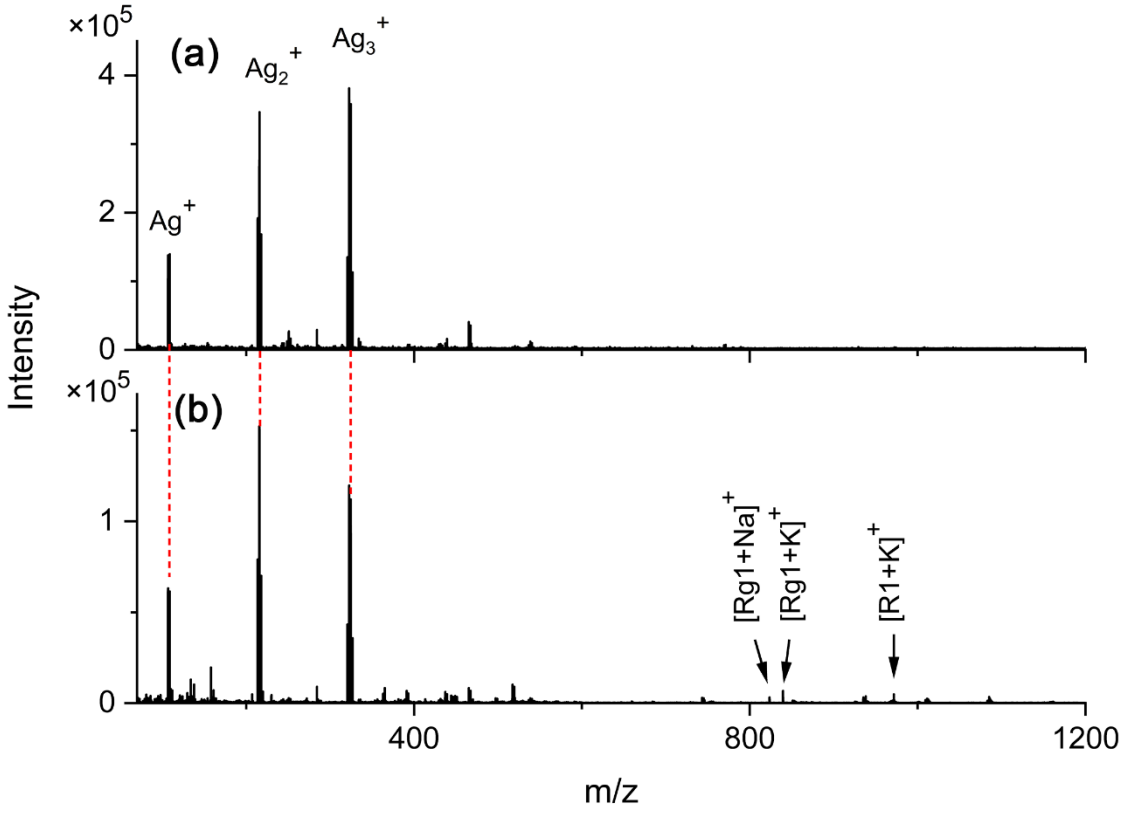

Figure S11 Mass spectra of (a) polypeptide (donkey derived peptide A1, donkey derived peptide A2, horse derived peptide A, and bovine derived peptide A1), and (b) ginsenoside (Rg1, Re, Rb1, and Notoginsenoside R1) standards detected in positive ion mode using Au@MSN@Ag as the matrix by LDI MS. The amount of each analyte is 100 μg mL^{-1}.

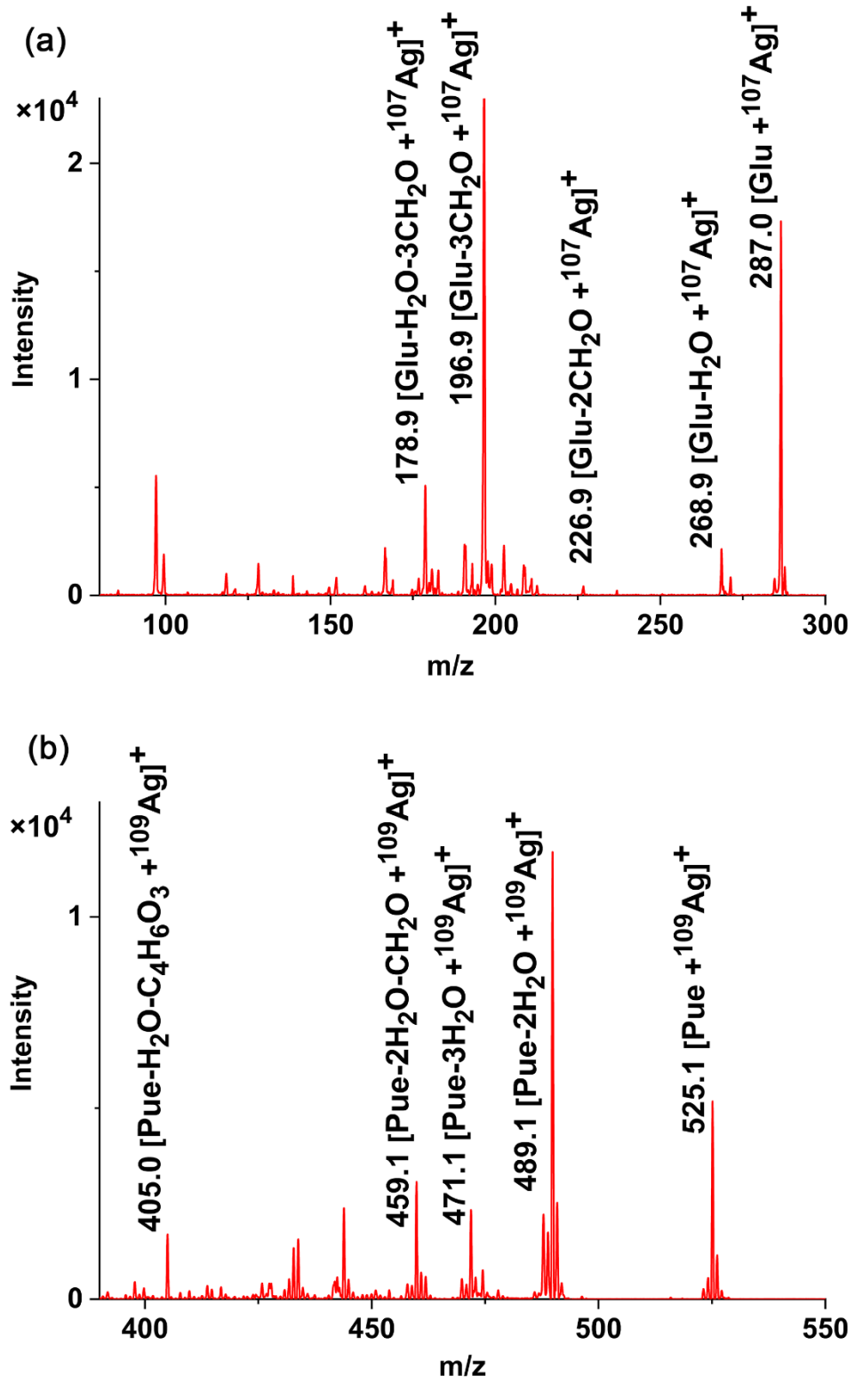

Figure S12 Tandem mass spectra of silver adducted of (a) glucose at m/z of 287.0 for $[M+^{107}Ag]^+$, and (b) puerarin at m/z of 525.1 for $[M+^{109}Ag]^+$.

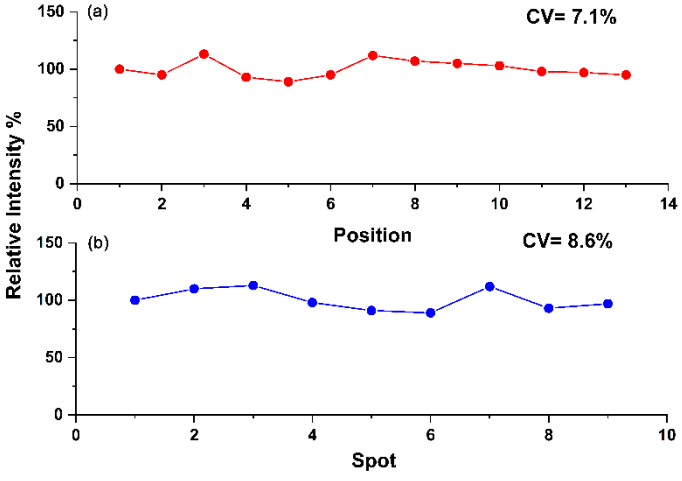

Figure S13 Highly reproducible MS signal intensity by using the Au@MSN@Ag matrix. Analyte: Glucose. (a) 13 different positions in a single spot. (b) 9 different spots in the 3×3 array. The coefficient of variation (CV) values was 7.1% in the position-to-position (a) tests and 8.6% in the spot-to-spot (b) tests.

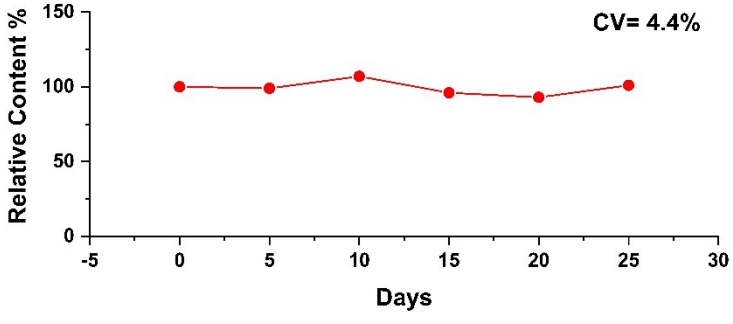

Figure S14 The internal standard quantitative stability of LDI-MS in the 25 days period by using the Au@MSN@Ag matrix. Analyte: Glucose. The coefficient of variation (CV) value was 4.4%.

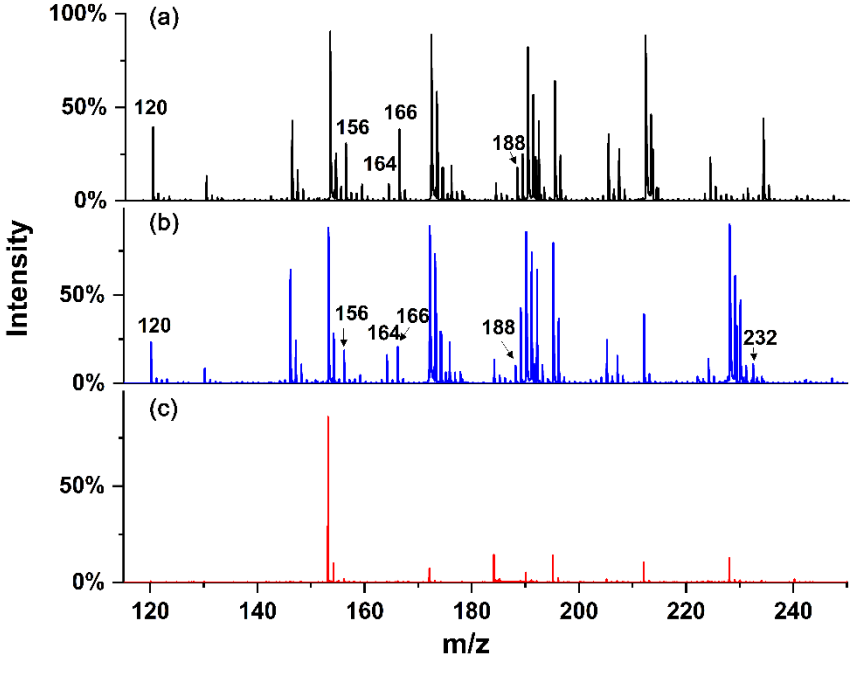

Figure S15 Mass spectra of mixed amino acids solution containing Threonine (m/z = 120.1 $[M+H]^+$, m/z = 164.0 $[M+2Na-H]^+$), Tryptophan (not detect), Histidine (m/z = 156.1 $[M+H]^+$, m/z = 232.3 $[M+2K-H]^+$), and Phenylalanine (m/z = 166.1 $[M+H]^+$, m/z = 188.1 $[M+Na]^+$) obtained using saturated CHCA solution as matrixes. The samples were prepared in 0.5 M NaCl (a), 0.5 M KCl (b), and 5 mg mL^{-1} BSA (c). The amount of each analyte is 500 pmol.

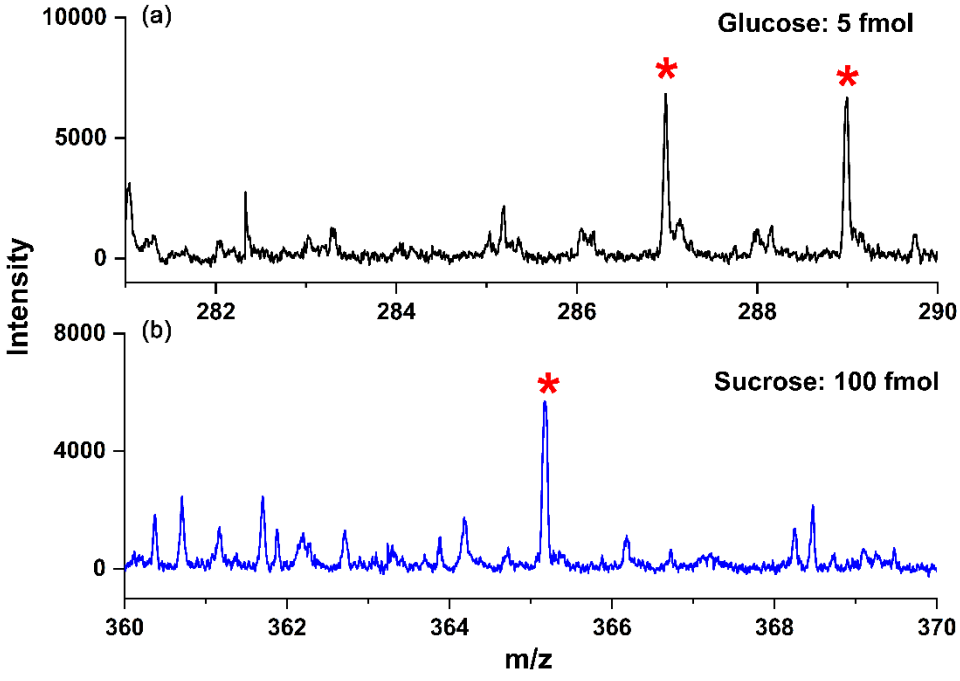

Figure S16 The LODs of glucose (a) and sucrose (b) using the Au@MSN@Ag as matrix.

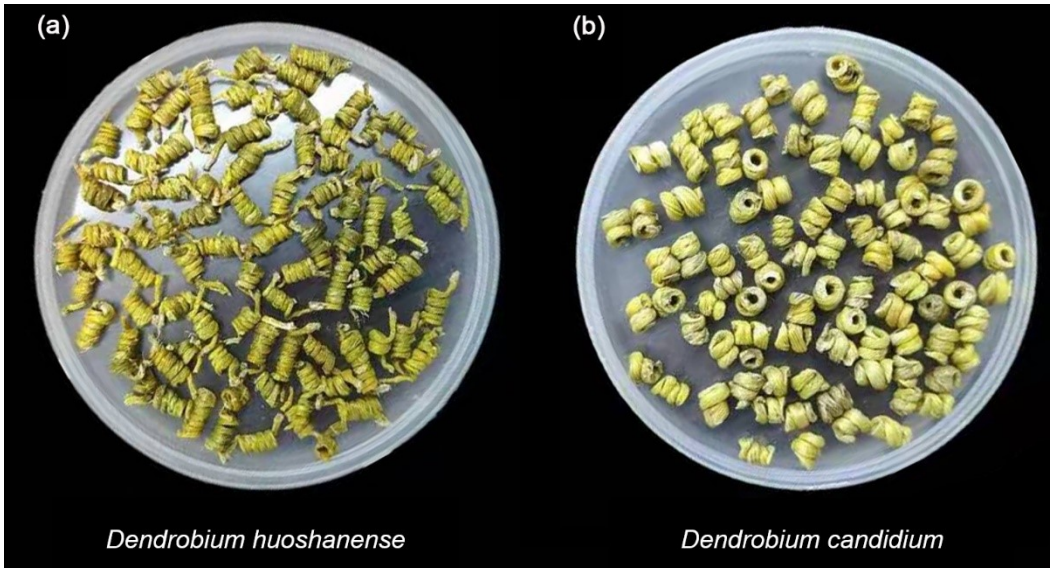

Figure S17 Photos of Medicinal *Dendrobium*. (a) *Dendrobium huoshanense* and (b) *Dendrobium candidum*.

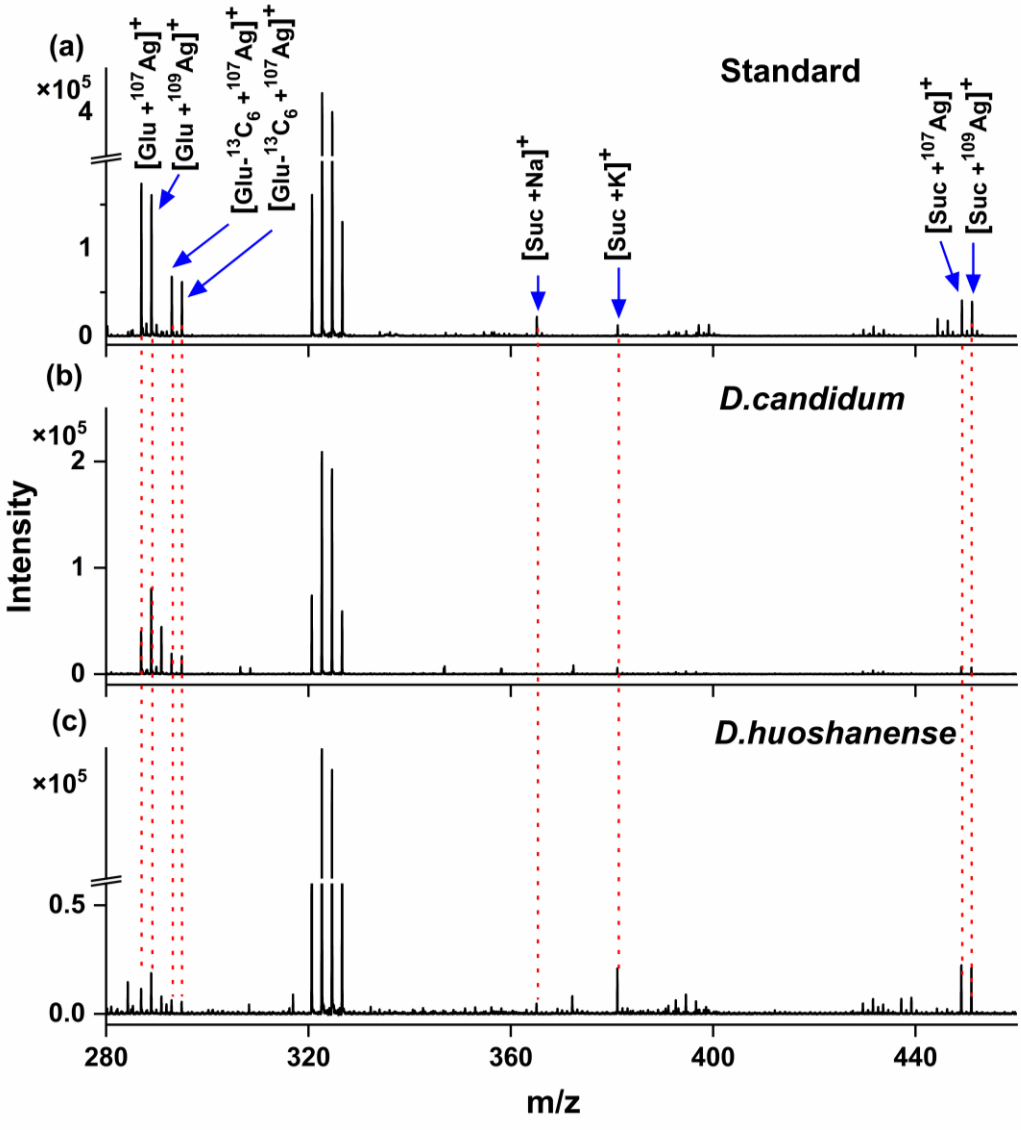

Figure S18 LDI-MS spectra of (a) glucose and sucrose standard solution, (b) *d*ENDROBIUM CANDIDUM extract solution, and (c) *d*ENDROBIUM HUOSHANENSE extract solution with Au@MSN@Ag as matrix.

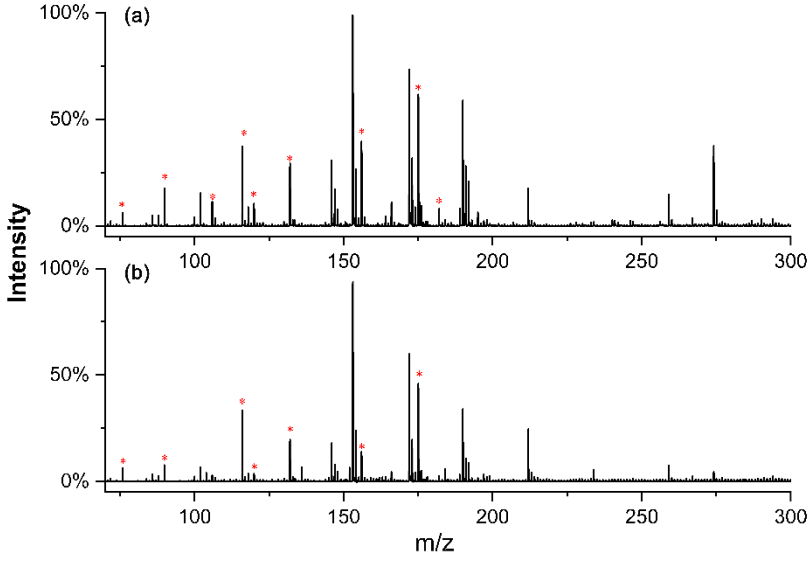

Figure S19 LDI-MS spectra of (a) 16 amino acids standard solution and (b) gelatin extract solution with CHCA as matrixes.

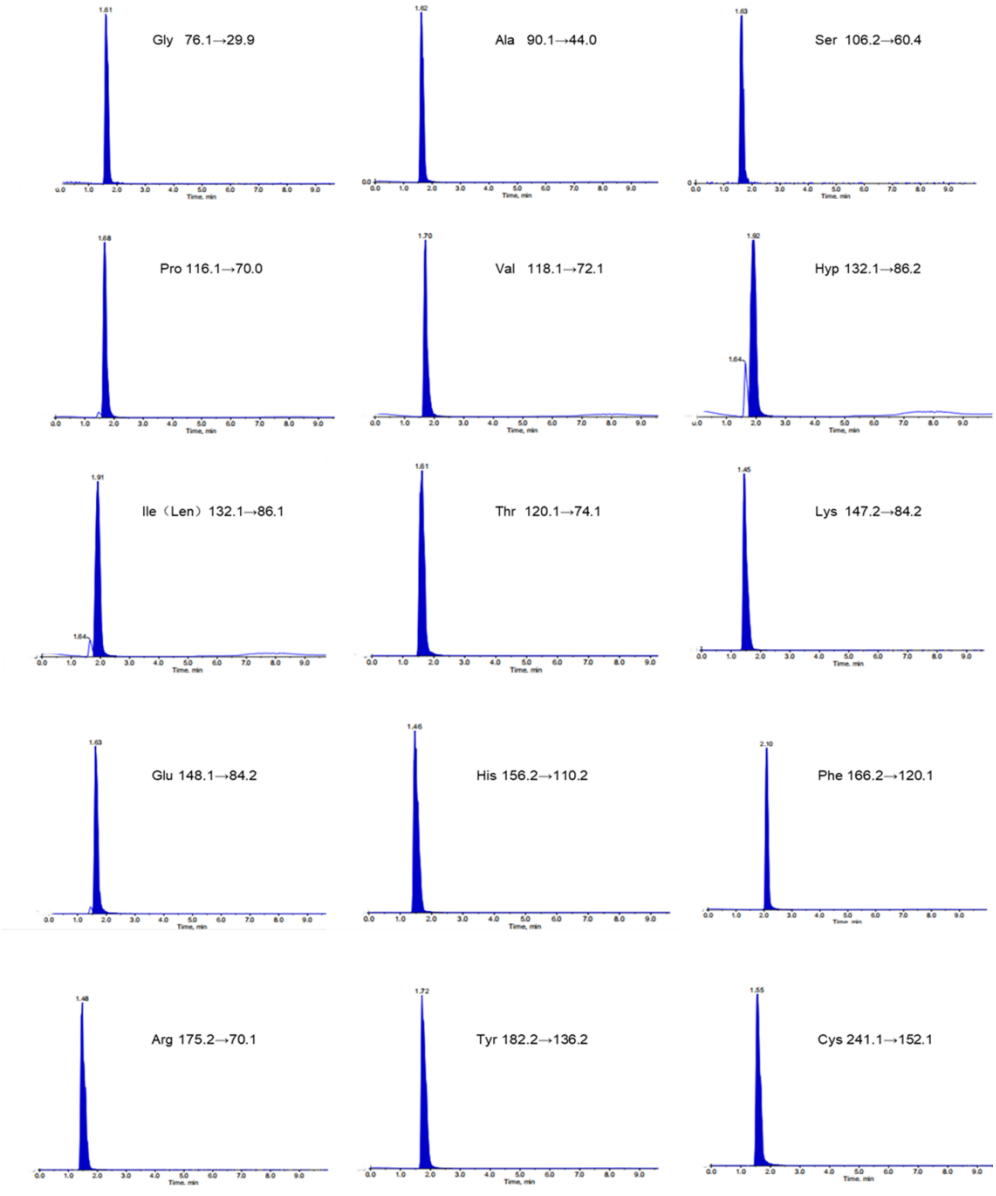

Figure S20 Typical MRM chromatograms of amino acids standard solutions.

Table S1 Structural parameters of nanospheres.

Sample	Average Size (nm)	PDI	Zeta potential (mv)
Au@MSN	155.8±4.43 nm	0.199±0.134	-10.31±1.032
Au@MSN@Ag	186.9±3.53 nm	0.208±0.116	-20.57±1.365

*Three independent synthetic materials, each group of parameters measured three times.

Table S2 Elemental mapping results of nanospheres.

Sample	Weight (%)			Atomic (%)		
	O	Si	Ag	O	Si	Ag
Au@MSN	41.49	58.21	0	55.76	44.24	0
Au@MSN@Ag	24.36	46.86	28.78	44.03	48.25	7.72

*Three independent synthetic materials, each group of parameters measured three times.

Table S3 Small molecules (m/z < 500) detected by LDI MS using Au@MSN@Ag as matrix.

Name	Abbrev.	Molecular Formula	Molecular Weight	$[M+Na]^+$	$[M+K]^+$	$[M+^{107}Ag]^+$	$[M+^{109}Ag]^+$
Glycine	Gly	$C_2H_5NO_2$	75.0	98.0	114.1	181.9	183.9
Alanine	Ala	$C_3H_7NO_2$	89.0	112.0	128.1	196.0	198.0
Serine	Ser	$C_3H_7NO_3$	105.0	128.0	144.1	211.9	213.9
Proline	Pro	$C_5H_9NO_2$	115.1	138.1	154.2	222.0	224.0
Valine	Val	$C_5H_{11}NO_2$	117.1	140.1	156.2	224.0	226.0
Hydroxyproline	Hyp	$C_5H_9NO_3$	131.1	154.0	170.2	238.0	240.0
Isoleucine	Ile	$C_6H_{13}NO_2$	131.1	154.1	170.2	238.0	240.0
Leucine	Leu	$C_6H_{13}NO_2$	131.1	154.1	170.2	238.0	240.0
Threonine	Thr	$C_4H_9NO_3$	119.1	142.0	158.2	226.0	228.0
Lysine	Lys	$C_6H_{14}N_2O_2$	146.1	169.1	185.2	253.0	255.0
Glutamic acid	Glu	$C_5H_9NO_4$	147.1	170.0	186.2	254.0	256.0
Histidine	His	$C_6H_9N_3O_2$	155.1	178.1	194.2	262.0	264.0
Phenylalanine	Phe	$C_9H_{11}NO_2$	165.1	188.1	204.2	272.0	274.0
Arginine	Arg	$C_6H_{14}N_4O_2$	174.1	197.1	213.2	281.0	283.0
Tyrosine	Tyr	$C_9H_{11}NO_3$	181.1	204.1	220.2	288.0	290.0
Cysteine	Cys	$C_6H_{12}N_2O_4S_2$	240.0	263.0	279.1	347.0	349.0
Tryptophan	Try	$C_{11}H_{12}N_2O_2$	204.1	227.1	243.2	311.0	313.0
Glucose	Glu	$C_6H_{12}O_6$	180.1	203.0	219.2	287.0	289.0
Sucrose	Suc	$C_{12}H_{22}O_{11}$	342.1	365.1	381.2	449.0	451.0
Ginkgolide A	GA	$C_{20}H_{24}O_9$	408.1	431.1	447.2	515.0	517.0
Ginkgolide B	GB	$C_{20}H_{24}O_{10}$	424.1	447.1	463.2	531.0	533.0
Ginkgolide C	GC	$C_{20}H_{24}O_{11}$	440.1	463.1	479.2	547.0	549.0
Caffeine	Caf	$C_8H_{10}N_4O_2$	194.1	217.1	233.2	301.0	303.0
Guanine	Gua	$C_5H_5N_5O$	151.0	174.0	190.1	258.0	260.0
Inosine	Ino	$C_{10}H_{12}N_4O_5$	268.1	291.1	307.2	375.0	377.0
Glutamine	Gln	$C_5H_{10}N_2O_3$	146.1	169.1	185.2	253.0	255.0
Kaempferol	Kae	$C_{15}H_{10}O_6$	286.0	309.0	325.1	393.0	395.0
Quercetin	Que	$C_{15}H_{10}O_7$	302.0	325.0	341.1	408.9	410.9
Puerarin	Pue	$C_{21}H_{20}O_9$	416.1	439.1	455.2	523.0	525.0
Lauric acid	C12:0	$C_{12}H_{24}O_2$	200.2	223.2	239.3	307.1	309.1
Palmitic acid	C16:0	$C_{16}H_{32}O_2$	256.2	279.2	295.3	363.1	365.1
Stearic acid	C18:0	$C_{18}H_{36}O_2$	284.3	307.3	323.4	391.2	393.2
Arachidic acid	C20:0	$C_{20}H_{40}O_2$	312.3	335.3	351.4	419.2	421.2
n-Docosanoic acid	C22:0	$C_{22}H_{44}O_2$	340.3	363.3	379.4	447.2	449.2
Resveratrol	Res	$C_{14}H_{12}O_3$	228.1	251.1	267.2	335.0	337.0

Table S4 Large molecules (m/z ≈ 500-1300) detected by LDI MS using Au@MSN@Ag as matrix.

Name	Abbrev.	Molecular Formula	Molecular Weight	$[M+Na]^+$	$[M+K]^+$
Notoginsenoside R1	R1	$C_{47}H_{80}O_{18}$	932.5	955.5	971.6
Ginsenoside Rg1	Rg1	$C_{42}H_{72}O_{14}$	800.5	823.5	839.6
Ginsenoside Re	Re	$C_{48}H_{82}O_{18}$	946.6	969.5	985.6
Ginsenoside Rb1	Rb1	$C_{54}H_{92}O_{23}$	1108.6	1131.6	1147.7
Name	**Amino Acid Sequence**	**Molecular Formula**	**Molecular Weight**	**$[M+Na]^+$**	**$[M+K]^+$**
Donkey derived peptide A1	GPAGPTGPVGK	$C_{41}H_{68}N_{12}O_{13}$	936.5	959.5	975.6
Donkey derived peptide A2	GEAGAAGPAGPAGPR	$C_{51}H_{82}N_{18}O_{18}$	1234.6	1257.6	1273.7
Horse derived peptide A	GASGPAGVR	$C_{31}H_{54}N_{12}O_{11}$	770.4	793.4	809.5
Bovine derived peptide A1	GEAGPSGPAGPTGAR	$C_{52}H_{84}N_{18}O_{20}$	1280.6	1303.6	1319.7

Table S5 The relative content of saccharides in 2 kinds of medicinal *Dendrobium*（n=10,%）

parameter	*Dendrobium huoshanense*	*Dendrobium candidum*
monosaccharides	0.68±0.06[a]	0.97±0.07[b]
disaccharide	2.71±0.16[a]	0.91±0.04[b]
total	3.38±0.22[a]	1.18±0.09[b]
Monosaccharides/ disaccharide	4.01±0.19[a]	0.93±0.06[b]

[a,b] Different letters in the same row indicate significant differences($P < 0.05$) between the means.

Table S6 MS/MS parameters for amino acids analysis by HPLC-MS-MS.

Name	Abbrev.	Q1	Q3	DP (V)	CE (eV)
Glycine	Gly	76.1	29.9	60	15
Alanine	Ala	90.1	44.0	79	10
Serine	Ser	106.2	60.4	67	8
Proline	Pro	116.1	70.0	68	10
Valine	Val	118.1	72.1	54	10
Hydroxyproline	Hyp	132.1	86.2	60	15
Isoleucine	Ile	132.1	86.1	64	10
Leucine	Leu	132.1	86.1	64	10
Threonine	Thr	120.1	74.1	93	20
Lysine	Lys	147.2	84.2	66	14
Glutamic acid	Glu	148.1	84.2	83	14
Histidine	His	156.2	110.2	95	16
Phenylalanine	Phe	166.2	120.1	56	14
Arginine	Arg	175.2	70.1	88	18
Tyrosine	Tyr	182.2	136.2	46	17
Cysteine	Cys	241.1	152.1	60	15

Table S7 Amino acid content of different hide gelatins detected by MALDI-MS and HPLC-MS-MS(n=5, %)[a,c]

Amino Acids	Donkey-hide gelatin		Bovine-hide gelatin		Pig-hide gelatin	
	MALDI-MS	HPLC-MS-MS	MALDI-MS	HPLC-MS-MS	MALDI-MS	HPLC-MS-MS
Gly	23.48±0.47	23.11±0.39	20.54±0.88	21.07±0.93	25.77±0.69	26.18±0.86
Ala	7.79±0.86	7.81±0.23	6.95±0.23	7.18±0.14	7.58±0.37	7.97±0.30
Ser	2.29±0.10	2.30±0.17	2.00±0.08	1.99±0.12	2.60±0.29	2.52±0.28
Pro	12.78±0.43	13.12±0.35	10.74±0.59	10.20±0.83	13.93±0.58	13.40±0.49
Val	2.30±0.13	2.44±0.13	1.99±0.10	2.06±0.11	1.75±0.15	1.85±0.13
Hyp	15.92±0.25[b]	10.30±0.74	13.66±0.43[b]	8.74±0.37	12.10±0.87[b]	7.36±0.67
Leu(Ile)		5.15±0.41		4.92±0.25		4.64±0.27
Thr	1.87±0.12	1.91±0.11	1.73±0.04	1.81±0.07	2.59±0.03	2.72±0.10
Lys	3.21±0.07	3.13±0.06	3.00±0.07	2.78±0.07	3.41±0.12	3.63±0.16
Glu	4.63±0.12	4.72±0.17	3.44±0.12	3.49±0.13	2.49±0.01	2.65±0.06
His	1.19±0.07	1.21±0.10	1.07±0.05	1.11±0.03	1.40±0.10	1.48±0.05
Phe	2.00±0.05	2.14±0.06	1.87±0.09	1.88±0.07	2.39±0.16	2.50±0.14
Arg	3.12±0.13	3.01±0.13	3.00±0.12	3.12±0.12	3.49±0.03	3.63±0.05
Tyr	0.94±0.10	0.98±0.09	0.75±0.04	0.77±0.07	0.84±0.04	0.85±0.06
Total	81.54±0.43	81.33±1.23	70.75±1.14	71.12±1.27	80.37±1.03	81.38±1.33

[a] The result of amino acid content were no significant differences (P > 0.05) between two detect methods.
[b] Total content of Hyp, Ile, and Leu.
[c] The results were calculated as dry samlpes.