Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Synthesis of zwitterionic hydrophilic magnetic mesoporous silica materials for endogenous glycopeptides analysis in human saliva†

Zidan Wang,[a] Runqing Wu,[a] Hemei Chen,[a] Nianrong Sun [a,*] and Chunhui Deng[a,*]

a. Department of Chemistry, The Fifth People's Hospital of Shanghai, Institutes of Biomedical Sciences, and Collaborative Innovation Center of Genetics and Development, Fudan University, Shanghai 200433, China

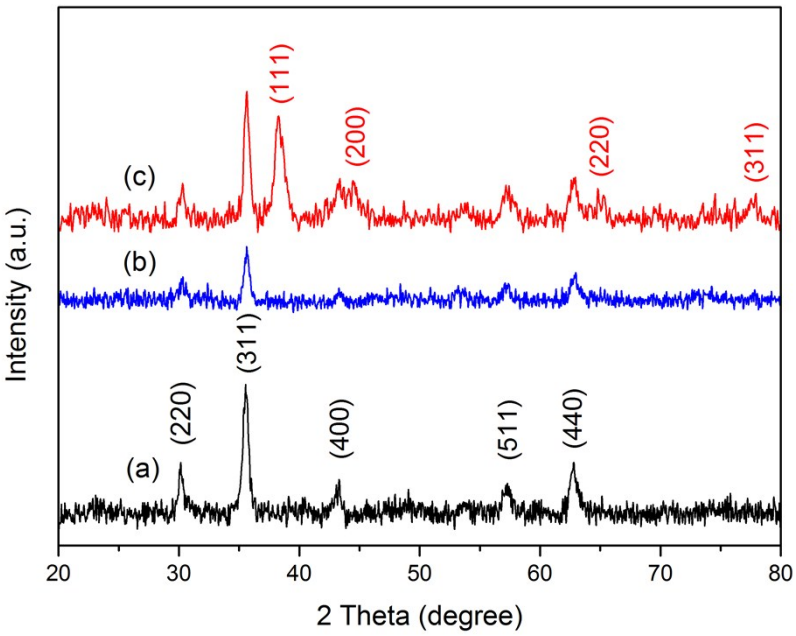

Fig S1. Wide-angle XRD analysis of (a) Fe_3O_4, (b) Fe_3O_4@SiO_2 and (c) FSAu nanoparticles. Black and red numbers marked belong to the characteristic peaks of Fe_3O_4 and Au, respectively.

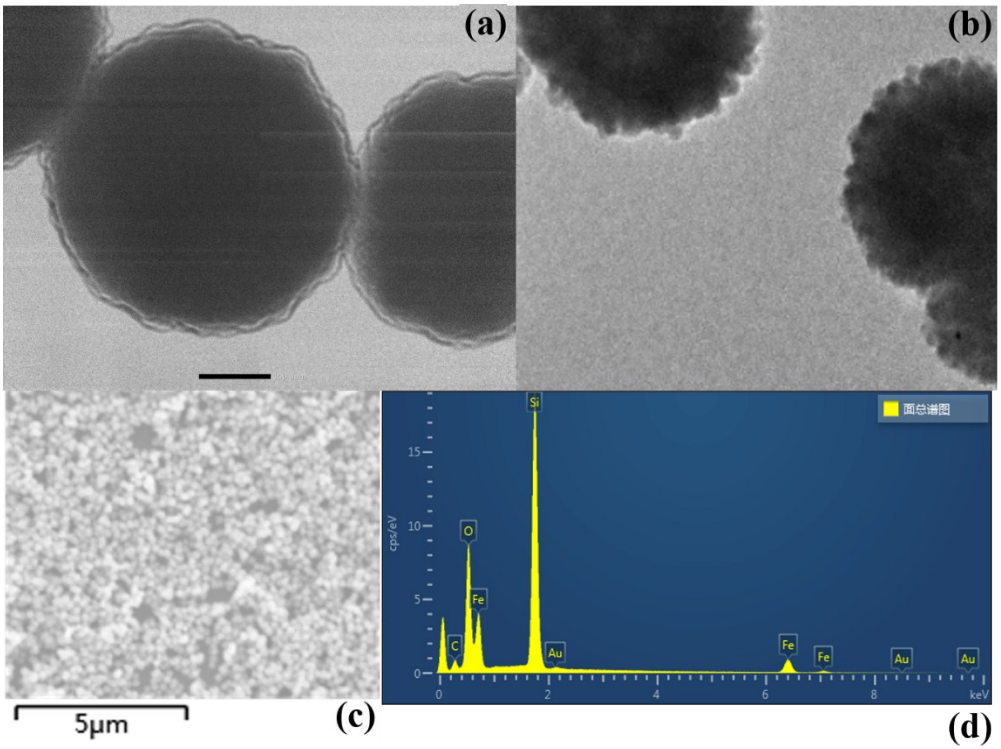

Fig S2. TEM images of (a) Fe_3O_4@SiO_2 and (b) FSAu, SEM image (c) and EDS image (d) of FSAu.

Fig S3. Pictures of FSAu@mSiO$_2$@L-Cys nanoparticles (a) dispersed in distilled water; (b) separated from distilled water by a magnet after 30 seconds.

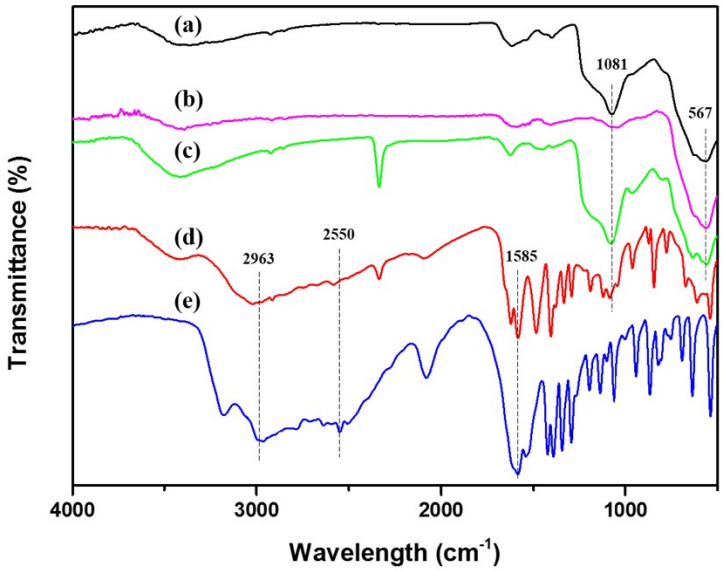

Fig S4. FT-IR spectra of (a) Fe_3O_4@SiO_2, (b) FSAu, (c) FSAu@mSiO_2, (d) FSAu@mSiO_2@L-Cys nanoparticles and (e) L-Cys.

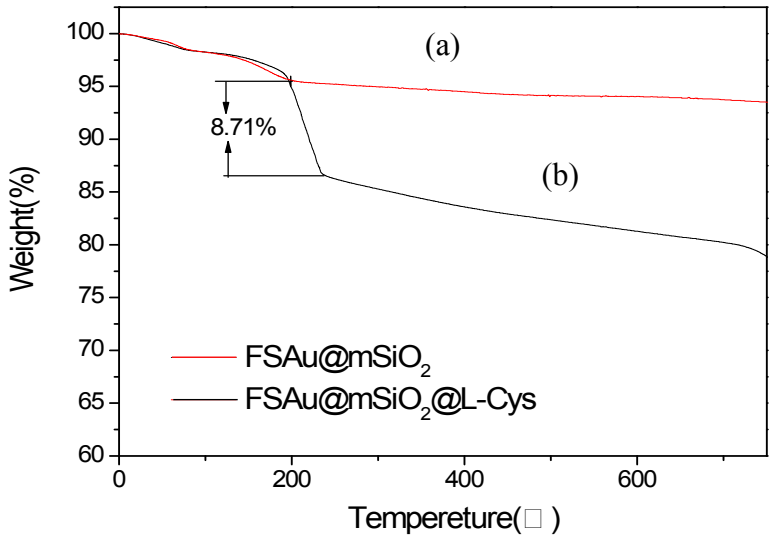

Fig S5. TGA curves of (a) FSAu@$mSiO_2$, (b) FSAu@$mSiO_2$@L-Cys.

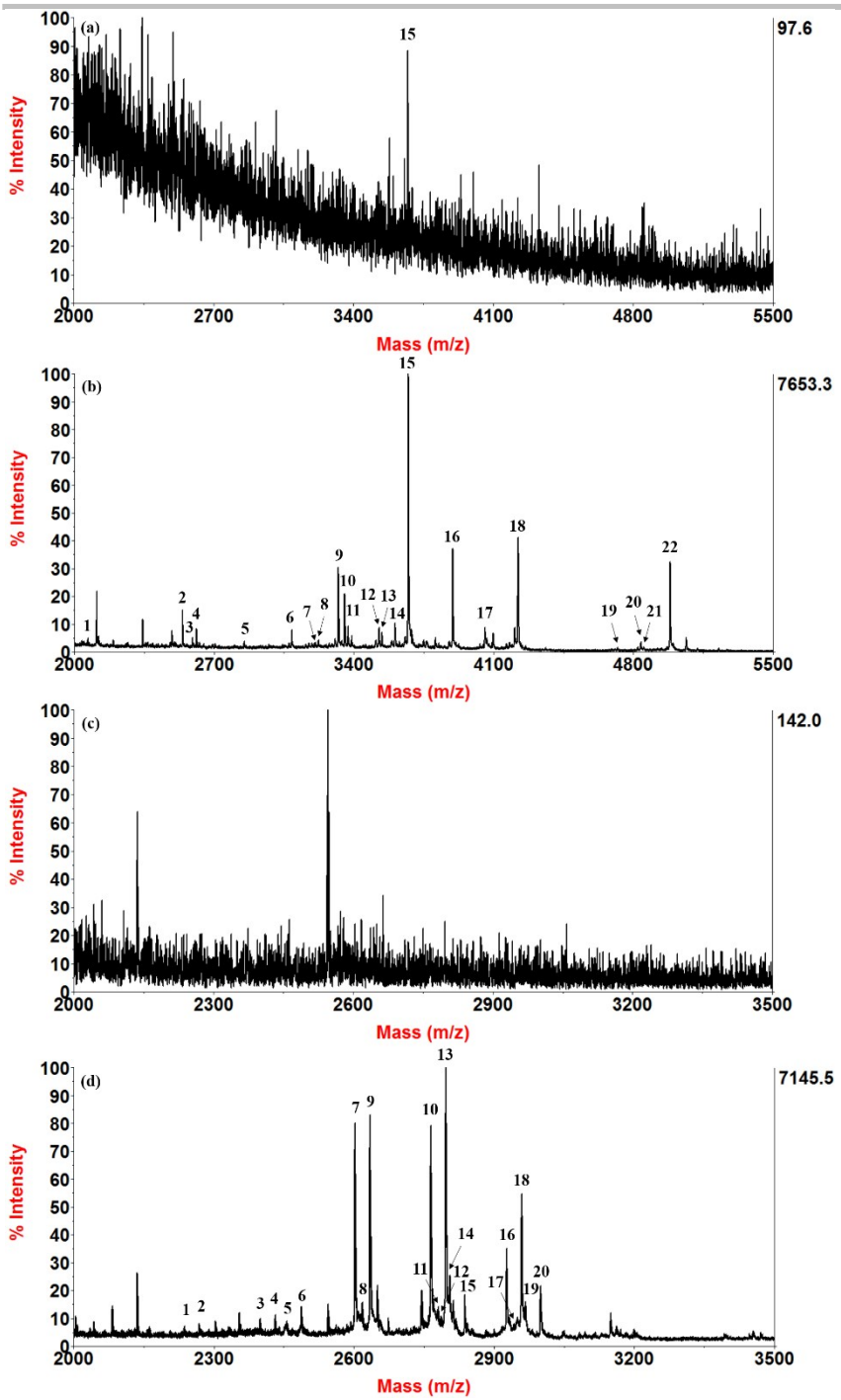

Fig S6. MALDI-TOF MS spectra of 100 fmol/μL HRP tryptic digest (a) direct analysis, (b) after enrichment by FSAu@mSiO$_2$@L-Cys nanoparticles. MALDI-TOF MS spectra of 100 fmol/μL IgG tryptic digest (c) direct analysis, (d) after enrichment by FSAu@mSiO$_2$@L-Cys nanoparticles. Glycopeptides were marked with numbers in the spectra and detailed information was shown in Supplementary Table S3 and S4.

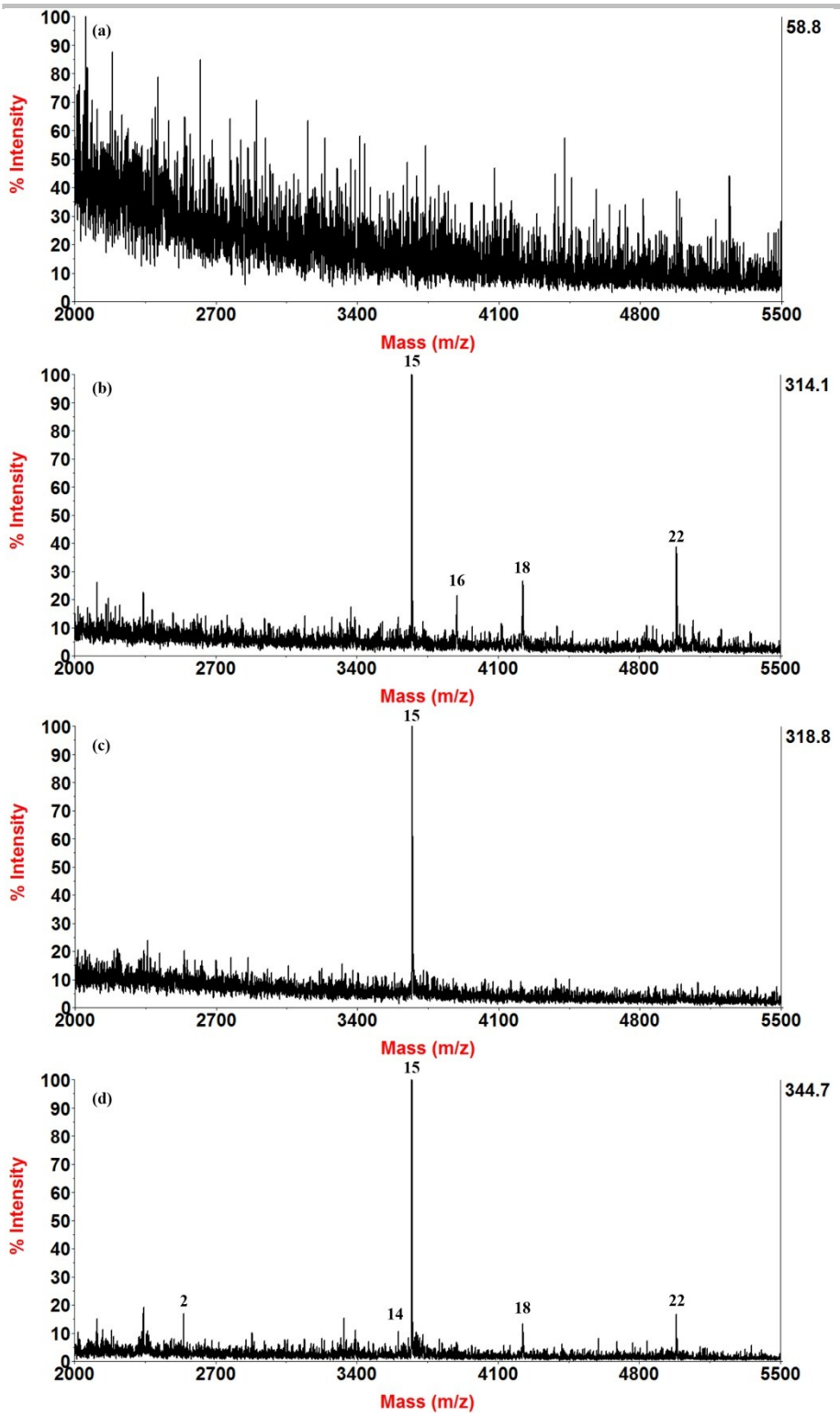

Fig S7. MALDI-TOF-MS spectra of 100 fmol/μL HRP tryptic digest after the enrichment by (a) Fe_3O_4, (b) Fe_3O_4@SiO_2, (c) FSAu and (d) FSAu@mSiO_2 nanoparticles. Glycopeptides were marked with numbers in the spectra and detailed information was shown in Supplementary Table S3.

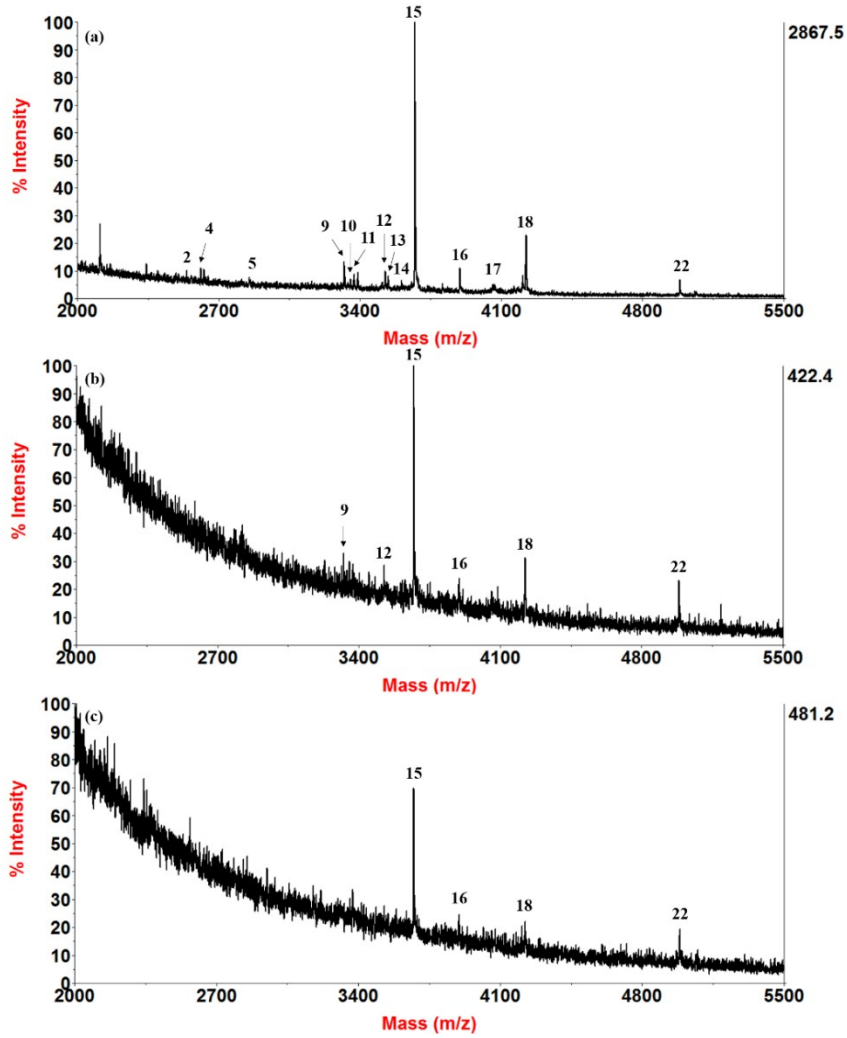

Fig S8. MALDI-TOF MS spectra of (a) 10 fmol/μL, (b) 1 fmol/μL, (c) 0.5 fmol/μL HRP digest enriched by FSAu@mSiO_2@L-Cys nanoparticles. Glycopeptides were marked with numbers in the spectra and detailed information was shown in Supplementary Table S3.

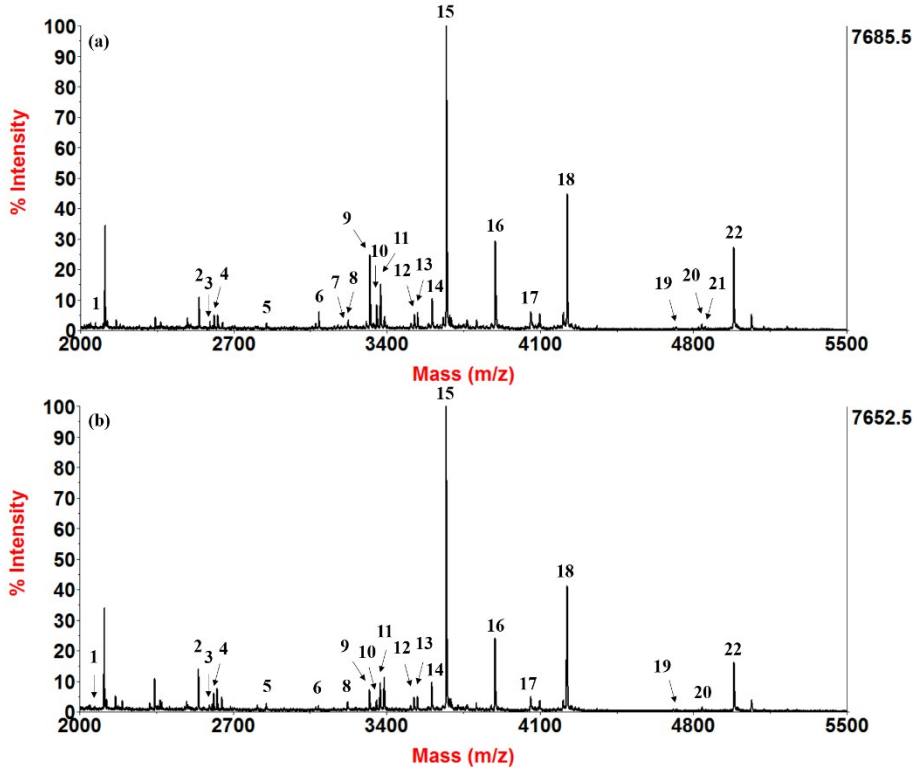

Fig S9. MALDI-TOF-MS spectra of 100 fmol/μL HRP tryptic digest after enrichment by FSAu@mSiO$_2$@L-Cys nanoparticles for (a) the third time and (b) the fifth time. Glycopeptides were marked with numbers in the spectra and detailed information was shown in Supplementary Table S3.

Table S1. Comparison of different techniques and materials in glycoproteomics analysis

Materials	Selectivity (or size-selectivity) (mass ratio)	LOD	LC-MS (min)	Sample/content	Endogenous glycopeptide	glycoprotein	Journal
DEAE-Sepharose microcolumns {PC} [a]	fetuin:$BSA_{(D)}$ 1:5	-	120	Human serum/300ug	-	115	Anal. Bioanal. Chem, 2017, 409.2: 511-518.
Au-Cys modified monolithic column {MC}	-	-	115	Human plasma/5ug	-	122	Anal. Chim. Acta, 2015, 900: 83-89.
Fe_3O_4@PGMA@Au-L-cys {N}	IgG:$BSA_{(D)}$ 1:100	0.0125fmol/ul(IgG)	170	Mouse liver/75ug	-	411	J. Chromatogr. A, 2017, 1482: 23-31.
MIL-101(NH_2)@Au-Cys {N}	IgG:$BSA_{(D)}$ 1:132	12fmol/ul(IgG)	170	Hela cell lysate/30ug	-	614	ACS Appl. Mater. Interfaces, 2017, 9, 19562-19568.
Poly(MBAAm-co-MAA)@L-Cys {N}	IgG:$BSA_{(D)}$ 1:10	33.4fmol/ul(IgG)	110	Human plasma/5ug	-	121	ACS Appl. Mater. Interfaces, 2016, 8(34), 22018-22024.
Fe_3O_4@L-Cys {N}	HRP:$BSA_{(D)}$ 1:100	0.025fmol/ul(HRP)	120	Human serum/2ul	-	118	Anal. Bioanal. Chem, 2017, 1-10.
Fe_3O_4@SiO_2@L-Cys {N}	HRP:$BSA_{(D)}$ 1:50	0.9fmol/ul(HRP)	-	Human serum/2ul	-	88	Talanta, 2016, 160, 461-469.
Fe_3O_4@G6P {N}	HRP:$BSA_{(D)}$ 1:100	0.5fmol/ul(HRP)	120	Human serum/2ul	-	92	Anal. Chem, 2017, 89, 11151-11158.
MIL-101(Cr)-NH_2 {N}	HRP:$BSA_{(D)}$ 1：15	0.2fmol/ul(IgG)	146	Human serum/10ul	-	42	Chem. Commun, 2014, 50, 11504-11506.
Fe_3O_4@SiO_2-APB/PMMA {N}	HRP:$MYO_{\{D\}}$ 1:38	2.23fmol/ul(HRP)	90	Human serum/1ul	-	66	Anal. Chem, 2014, 86, 2057−2064.
Fe_3O_4@mSiO_2-IDA {N}	HRP:$BSA_{\{D\}}$ 1:200	1fmol/ul(HRP)	-	Human serum/2ul	-	140	Anal. Chem, 2017, 89, 1764−1771.
FDU-12-GA{N}	HRP:$BSA_{\{D\}}$ 1:10	0.62fmol/ul(HRP)	-	-	-	-	Anal. Chem, 2009, 81, 503–508.
Chitosan Microspheres{N}	HRP:$BSA_{\{D\}}$ 1:150	0.45fmol/ul(HRP)	100	Human serum/2ul	-	104	Anal. Chem, 2017, 89, 9712-9721
MCM-41-APTES-CPB {N}	HRP:$BSA_{(D)}$ 1:150	5fmol/ul(HRP)	90	Rat serum/1ml	15	-	Anal. Chim. Acta, 2012, 753, 64-72.
This work {N}	HRP:$BSA_{(U)}$ 1:1000	0.5fmol/ul(HRP)	90	Human saliva/10ul	40	-	-

{U} undigested(proteins), {D}: digested(peptides)

{PC} : packed column. {MC}: monolith column. {N}: nanoparticles

Table S2. Data of Zeta potential for prepared materials in deionized water.

Prepared nanoparticles	Zeta potential (mV)
Fe_3O_4	9.7
Fe_3O_4@SiO_2	-16.1
FSAu	-6.77
FSAu@mSiO_2	-19.2
FSAu@mSiO_2@L-Cys	-0.386

Table S3. The detailed information of glycopeptides derived from HRP tryptic digest after enrichment by FSAu@mSiO_2@L-Cys nanoparticles.

No.	m/z	Glycan composition	Amino acid sequence
0	1842.2	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	NVGLn#R
1	2068.7	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	PNVSn#IVR
2	2540.8	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	SSPn#ATDTIPLVR
3	2590.8	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	PTLn#TTYLQTLR
4	2610.8	[Hex]3[HexNAc]2[Xyl]1	MGn#ITPLTGTQGQIR
5	2850	[HexNAc]1[Fuc]1	GLIQSDQELFSSPn#ATDTIPLVR
6	3088.9	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	GLCPLNGn#LSALVDFDLR
7	3207.8	[Hex]3[HexNAc]2[Xyl]1	SFAn#STQTFFNAFVEAMDR
8	3220.9	[Hex]3[HexNAc]2[Fuc]1	SFAn#STQTFFNAFVEAMDR
9	3321.1	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	QLTPTFYDNSCPn#VSNIVR
10	3353	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	SFAn#STQTFFNAFVEAMDR
11	3368.9	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	SFAn#STQTFFNAFVEAM*DR
12	3525.2	[Hex]3[HexNAc]2[Xyl]1	GLIQSDQELFSSPn#ATDTIPLVR
13	3539.3	[Hex]3[HexNAc]2[Fuc]1	GLIQSDQELFSSPn#ATDTIPLVR
14	3605.2	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	NQCRGLCPLNGn#LSALVDFDLR
15	3671.3	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	GLIQSDQELFSSPn#ATDTIPLVR
16	3893.8	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	LHFHDCFVNGCDASILLDn#TTSFR
17	4055.7	[Hex]3[HexNAc]2[Xyl]1	QLTPTFYDNSC(AAVESACPR)Pn#VSNIVR-H2O
18	4221.9	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	QLTPTFYDNSC(AAVESACPR)Pn#VSNIVR
19	4720.2	[Hex]3[HexNAc]2[Fuc]1	LYn#FSNTGLPDPTLn#TTYLQTLR
20	4838.6	[Hex]3[HexNAc]2[Fuc]1[Xyl]1 [Hex]3[HexNAc]2[Xyl]1	LYn#FSNTGLPDPTLn#TTYLQTLR
21	4852.4	[Hex]3[HexNAc]2[Fuc]1[Xyl]1 [Hex]3[HexNAc]2[Fuc]1	LYn#FSNTGLPDPTLn#TTYLQTLR
22	4983.1	[Hex]3[HexNAc]2[Fuc]1[Xyl]1 [Hex]3[HexNAc]2[Fuc]1[Xyl]1	LYn#FSNTGLPDPTLn#TTYLQTLR

HexNAc=N-acetylglucosamine, Hex=mannose, Fuc=fucose, Xyl=xylose. n# denotes the N-linked glycosylation site.

Table S4. The detailed information of glycopeptides derived from IgG tryptic digest after enrichment by FSAu@mSiO$_2$@L-Cys nanoparticles.

No.	m/z	Glycan composition	Amino acid sequence
1	2269.1	[Hex]3[HexNAc]2[Fuc]1	EEQYn#STYR
2	2399.2	[Hex]3[HexNAc]3[Fuc]1	EEQFn#STFR
3	2431.2	[Hex]3[HexNAc]3[Fuc]1	EEQYn#STYR
4	2456.2	[Hex]3[HexNAc]4	EEQFn#STFR
5	2472.2	[Hex]3[HexNAc]4	EEQFn#STYR
6	2488.2	[Hex]3[HexNAc]3[Fuc]1	EEQYn#STYR
7	2601.7	[Hex]3[HexNAc]4[Fuc]1	EEQFn#STFR
8	2618.2	[Hex]4[HexNAc]4	EEQFn#STFR
9	2634.3	[Hex]3[HexNAc]4[Fuc]1	EEQYn#STYR
10	2650.9	[Hex]4[HexNAc]4	EEQYn#STYR
11	2674.7	[Hex]3[HexNAc]5	EEQFn#STYR
12	2764.5	[Hex]4[HexNAc]4[Fuc]1	EEQFn#STFR
13	2780.8	[Hex]5[HexNAc]4	EEQFn#STFR
14	2795.8	[Hex]4[HexNAc]4[Fuc]1	EEQYn#STYR
15	2805.3	[Hex]3[HexNAc]5[Fuc]1	EEQFn#STFR
16	2836.7	[Hex]3[HexNAc]5[Fuc]1	EEQYn#STYR
17	2925.7	[Hex]5[HexNAc]4[Fuc]1	EEQFn#STFR
18	2942.8	[Hex]5[HexNAc]4[Fuc]1	EEQFn#STYR
19	2959.6	[Hex]5[HexNAc]4[Fuc]1	EEQYn#STYR
20	3001.1	[Hex]4[HexNAc]5[Fuc]1,or [Hex]5[HexNAc]5	EEQYn#STYR, or EEQFN#STYR
21	3089.4	[Hex]4[HexNAc]4[Fuc]1[NeuAc]1	EEQYn#STYR
22	3161.7	[Hex]5[HexNAc]5[Fuc]1	EEQYn#STYR

HexNAc=N-acetylglucosamine, Hex=mannose, Fuc=fucose. n# denotes the N-linked glycosylation site.

Table S5. Data of LC/MS run of the N-glycosylation sites in human saliva enriched by FSAu@mSiO$_2$@L-Cys nanoparticles.

No.	Peptide Sequence	MH+ [Da]	Charge	Score	Protein
1	ALLPQn#DTR	1028.54	2	38.53	O43240
2	GGn#KSQGPPPPGKPQ	1446.73	3	20.53	P02812
3	GPPPQGDn#KSQSARSPPGKPQGPPPQGGNQPQ	3188.56	3	13.62	P02812
4	GPPPQGDn#KSR	1153.56	2	42.06	P02812
5	GPPPQGGn#KS	939.45	2	18.48	P02812
6	GPPPQGGn#KSQ	1067.51	2	57.82	P02812
7	GPPPQGGn#KSQGPPPPGKPQ	1922.97	2	95.44	P02812
8	GPPPQGGn#KSQGPPPPGKPQGPPPQ	2399.22	3	48.23	P02812
9	GPPPQGGn#KSQGPPPPGKPQGPPPQGGSKS	2815.41	4	21.5	P02812
10	nKPQGPPPPGKPQGPPPQGDn#KSQSARSPPGKP	3326.68	4	11.29	P02812
11	nKPQGPPPPGKPQGPPPQGDn#KSRSSR	2807.42	3	25.36	P02812
12	PQGPPPQGGn#KSQGPPPPGK	1922.97	3	30.03	P02812
13	PQGPPPQGGn#KSQGPPPPGKP	2020.02	2	21.24	P02812
14	QGGn#KSQGPPPPGKPQ	1574.80	2	26.33	P02812
15	IDDTn#ITRL	1061.55	2	53.89	P05783
16	SESFPHPGFn#MSLLEn#HTR	2201.99	3	46.05	P06870
17	GPPPHPGKPEGPPPQEGn#KSR	2161.08	3	59.03	P10163
18	GPPPHPGKPEGPPPQEGn#KSRSA	2319.15	3	73.94	P10163
19	GPPPHPGKPERPPPQGGn#QSQ	2160.08	3	20.72	P10163
20	GPPPQEGn#KSR	1167.58	2	53.28	P10163
21	GPPPQEGn#KSRSA	1325.64	2	53.72	P10163
22	PGKPQGPPPQGGn#QSQGPPPPPGKPE	2528.25	3	12.05	P10163
23	QSQGPPPPPGKPEGRPPQGGn#QSQ	2423.17	3	11.03	P10163
24	SHRPPPPPGKPERPPPQGGn#QSQ	2443.23	3	40.14	P10163
25	SQGPPPHPGKPEGPPPQEGn#KS	2220.08	4	13.78	P10163
26	SQGPPPHPGKPEGPPPQEGn#KSRSA	2534.23	3	53.72	P10163
27	n#GTLPWLRPDSK	1384.72	2	27.86	P31153
28	GGn#QSQGPPPRPGKPE	1603.78	2	68.34	Q04118
29	GPPPQGGn#QSQGPPPHPGKPE	2060.98	3	38.51	Q04118

30	GPPPQGGn#QSQGPPPRPGKPE	2080.02	2	55.68	Q04118
31	GPPPRPGKPEGQPPQGGn#QSQGPPPRPGKPE	3123.58	4	16.6	Q04118
32	PPQGGn#QSQGPPPRPGKPE	1925.95	2	19.31	Q04118
33	GPPPQGGn#QS	939.42	2	31.74	Q04118; P10163
34	GPPPQGGn#QSQ	1067.48	2	32.95	Q04118; P10163
35	GRPPQGGn#QSQ	1126.52	2	27.63	Q04118; P10163
36	PPPQGGn#QSQ	1010.45	2	13.84	Q04118; P10163
37	LQQTn#FSSAFSSDSK	1647.75	2	56.08	Q86VR7
38	QPPKHPDKn#SS	1235.60	2	30.92	Q8TAX7
39	EASNn#FTEMSQRLE	1656.72	2	54.7	Q9UL52
40	Gn#ASPPAGPSNVPKFGSPK	1809.90	3	16.05	

n# denotes the N-linked glycosylation site.