

Supplementary Information

Recyclable

trypsin immobilized magnetic nanoparticle based on hydrophilic
polyethylenimine modification and its proteolytic characteristics

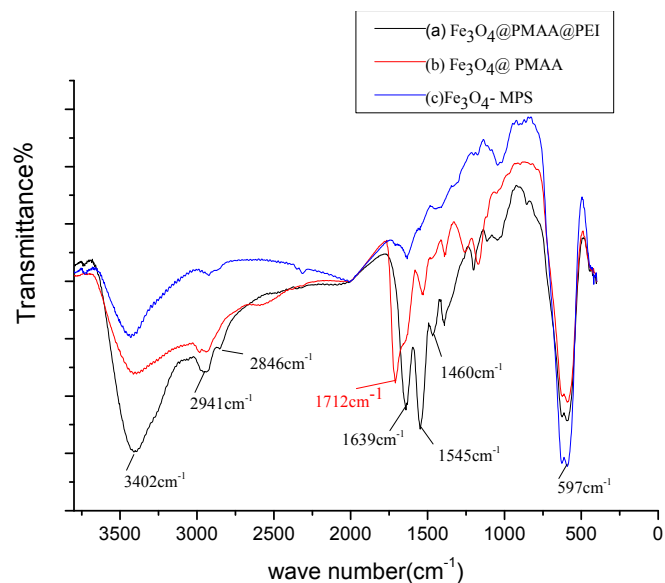
Lingyi Zhang ^{*1}, Bingbing Wang¹, Shulei Wang, Weibing Zhang

Shanghai Key Laboratory of Functional Materials Chemistry, School of Chemistry &
Molecular Engineering, East China University of Science and Technology, Shanghai
200237, P. R. China

*Corresponding author: E-mail: zhanglingyi@ecust.edu.cn; Fax: +86-021-64233161;

Tel: +86-021-64253977

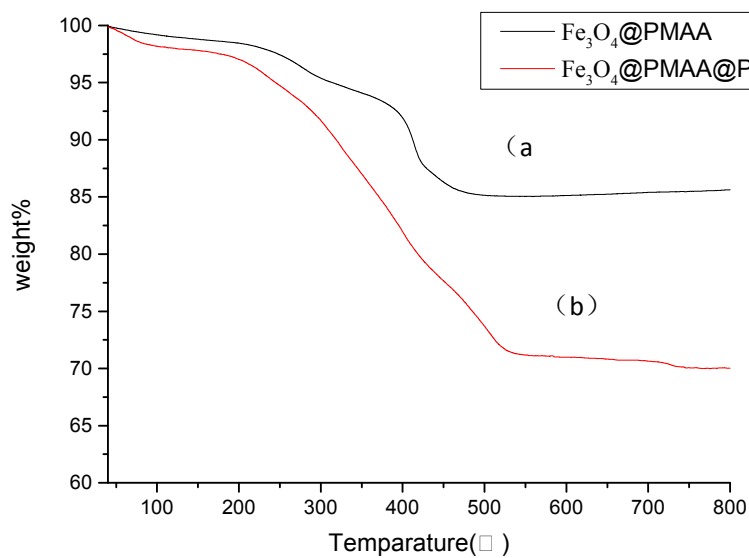
¹ These two authors contributed equally



FigureS-1. Representative FT-IR spectra of $\text{Fe}_3\text{O}_4@\text{PMAA}@\text{PEI}$ (a), $\text{Fe}_3\text{O}_4@\text{PMAA}$ (b), Fe_3O_4 -MPS (c)

Table S-1. Elemental composition of $\text{Fe}_3\text{O}_4@\text{PMAA}@\text{PEI}$ and $\text{Fe}_3\text{O}_4@\text{PMAA}$ obtained from elemental analysis

nanoparticles	Weight/mg	N(%)	C(%)	H(%)
$\text{Fe}_3\text{O}_4@\text{PMAA}$	2.231	1.35	15.77	2.28
	2.537	1.27	15.69	2.24
$\text{Fe}_3\text{O}_4@\text{PMAA}@\text{PEI}$	2.293	9.90	23.21	5.58
	2.243	9.73	23.01	5.70



FigureS-2: TGA curves of $\text{Fe}_3\text{O}_4@\text{PMAA}$ (a), $\text{Fe}_3\text{O}_4@\text{PMAA}@\text{PEI}$ (b)

Measurement of amount of trypsin immobilized in supports

Protein standard solution was obtained by diluting 8mg/mL standard protein into 32.5, 62.5, 125, 250, 500 $\mu\text{g/mL}$. A volume of 100 μL supernatant solution collected after immobilization and standard protein solution were mixed with 2mL working reagent and the mixture was kept at 37°C for 30 minutes. The absorbance was measured at 562nm. Standard curve was shown in Figure S-3. By comparing amounts of trypsin before and after immobilization, the results indicate that the concentration immobilized on the surface of $\text{Fe}_3\text{O}_4@\text{PMAA}$ and $\text{Fe}_3\text{O}_4@\text{PMAA}@\text{PEI}$ are about 0.080mg/mg and 0.170mg/mg.

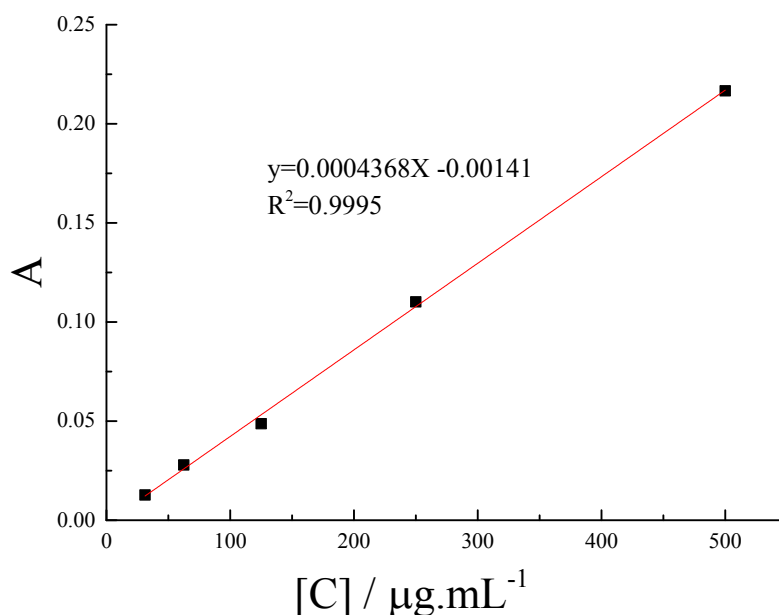


Figure S-3. Standard curve of standard protein by BCA protein assay

Table S-2. The identified peptide mass values of BSA, myoglobin and Cyt-C.

protein	Digestion method	position	Observed Peptide mass	Amino acid sequence
BSA	$\text{Fe}_3\text{O}_4@\text{PMAA}$	25–34	1193.6609	DTHKSEIAHR
		35–44	1249.6703	FKDLGEEHFK
		66–75	1163.6603	LVNELTEFAK
		76–88	1462.8369	TCVADESHAGCEK
		89–100	1419.7465	SLHTLFGDELCK
		139–155	2020.0645	LKPDPNTLCDEFKADEK
		161–167	927.5341	YLYEIAR
		161–168	1083.6326	YLYEIARR
		168–183	2045.1194	RHPYFYAPELLEYANK

@PEI-tryisin

		184-197	1747.8041	YNGVFQECCQAEDK
		223-232	1195.6573	CASIQKFGER
		264-280	2113.9900	VHKECCHGDLLECADDR
		267-280	1749.7765	ECCHGDLLECADDR
		267-285	2248.0652	ECCHGDLLECADDRADLAK
		286-297	1443.7657	YICDNQDTISSK
		298-309	1532.8416	LKECCDKP LLEK
		347-359	1567.8060	DAFLGSFLYEYSR
		360-371	1439.8733	RHPEYAVSVLLR
		361-371	1283.7683	HPEYAVSVLLR
		375-386	1501.8220	EYEATLEECCA K
		387-401	1795.8978	DDPHACYSTVFDK LK
		402-412	1305.7659	HLVDEPQNLIK
		413-420	1068.4735	QNCDQFEK
		413-433	2529.3438	QNCDQFEKLG EYGFQNALIVR
		421-433	1479.8612	LGEYGFQNALIVR
		437-451	1640.0172	KVPQVSTPTLVEVSR
		460-468	1166.5527	CCTKPESER
		460-482	1724.9095	CCTKPESERMPCTEDYLSLILNR
		469-482	1724.9095	MPCTEDYLSLILNR
		483-495	1539.8702	LCVLHEKTPVSEK
		499-507	1138.5521	CCTESLVNR
		508-523	1881.0074	RPCFSALTPDETYVPK
		524-544	2498.3250	AFDEKLFTHADICTLPDTEK
		529-544	1908.0061	LFTFHADICTLPDTEK
		549-561	1504.8132	QTALVELLKHKPK
		569-580	1399.7452	TVMENFVAFVDK
		581-597	1927.8894	CCAADDKEACFAVEGPK
		2-17	1816.0238	GLSDGEWQQVLNVWGK
		2-32	3403.9944	GLSDGEWQQVLNVWGKVEADIAGHGQEV LIR
		18-32	1606.9832	VEADIAGHGQEV LIR
		33-43	1271.7356	LFTGHPETLEK
		33-46	1661.9453	LFTGHPETLEKFDK
		65-78	1378.9218	HGTVVLTALGGILK
		65-79	1507.0221	HGTVVLTALGGILKK
		80-97	1982.1962	KGHHEAELKPLAQSHATK
		81-97	1854.0931	GHHEAELKPLAQSHATK
		104-119	1885.1412	YLEFISDAIHVLH SK
myoglobin	Fe ₃ O ₄ @PMAA	120-134	1502.7552	HPGDFGADAQGAMTK
	@PEI-tryisin	120-140	2248.3535	HPGDFGADAQGAMTKALELFR
		135-146	1360.8427	ALELFRNDIAAK

		147-154	941.5123	YKELGFQG
		47-57	1361.8368	FKHLKTEAEMK
		58-79	2278.3574	ASEDLKKHGTVVLTALGGILKK
		79-97	2110.2898	KKGHHEAELKPLAQSHATK
		135-154	2283.3765	ALELFRNDIAAKYKELGFQG
Cyt-c	Fe ₃ O ₄ @PMAA @PEI-trypsin	1-14	1606.8995	MGDVEKGKKIFVQK
		2-9	861.0699	GDVEKGKK
		9-23	1875.7871	KIFVQKCAQCHTVEK
		27-39	1433.8022	HKTGPNLHGLFGR
		29-39	1168.6924	TGPNLHGLFGR
		29-40	1296.7305	TGPNLHGLFGRK
		40-54	1584.8295	KTGQAPGFSYTDANK
		41-54	1456.7212	TGQAPGFSYTDANK
		41-56	1698.8240	TGQAPGFSYTDANKNK
		55-73	2252.0642	NKGITWGEETLMEYLENPK
		57-73	2009.9917	GITWGEETLMEYLENPK
		57-80	2797.5027	GITWGEETLMEYLENPKKYIPGTK
		75-87	1438.8290	YIPGTKMIFAGIK
		89-101	1562.9208	KGEREDLIAYLKK
		90-100	1306.7051	GEREDLIAYLK
90-101	1434.8154	GEREDLIAYLKK		
90-105	1850.0038	GEREDLIAYLKKATNE		
93-100	964.5173	EDLIAYLK		

Protein	Digestion method	position	Observed peptide mass	Amino acid sequence
BSA	In-solution based digestion	25-34	1193.6558	DTHKSEIAHR
		35-44	1249.6768	FKDLGEEHFK
		66-75	1163.6729	LVNELTEFAK
		76-88	1463.6536	TCVADESHAGCEK
		89-100	1419.7627	SLHTLFGDELCK
		118-138	2541.3767	QEPERNECFLSHKDDSPDLPK
		161-167	927.5619	YLYEIAR
		161-168	1083.6418	YLYEIARR
		198-211	1662.0239	GACLLPKIETMREK
		205-211	906.5392	IETMREK
		264-280	2114.0164	VHKECCHGDLLECADDR
		264-285	2612.3508	VHKECCHGDLLECADDRADLAK
		347-359	1567.8337	DAFLGSFLYEYSR
		360-371	1439.8810	RHPEYAVSVLLR
		375-386	1501.8529	EYEATLEECCA

		413-433	2529.3928	QNCDQFEKLG EYGFQNALIVR
		437-451	1479.8773	LGEYGFQNALIVR
		469-482	1724.9436	MPCTEDYLSLILNR
		483-498	1868.1522	LCVLHEKTPVSEKVTK
		499-507	1138.5936	CCTESLVNR
		508-523	1881.0475	RPCFSALTPDETYVPK
Myoglobin	In-solution based digestion	18-32	1607.5985	VEADIAGHGQEVLR
		33-43	1271.7577	LFTGHPETLEK
		33-46	1661.9861	LFTGHPETLEKFDK
		47-57	1361.8596	FKHLKTEAEMK
		65-78	1378.9500	HGTVVLTALGGILK
		65-79	1507.0597	HGTVVLTALGGILKK
		79-97	2110.3472	KKGHHEAELKPLAQSHATK
		80-97	1982.2474	KGHHEAELKPLAQSHATK
		81-97	1854.1326	GHHEAELKPLAQSHATK
		104-119	1885.1884	YLEFISDAIIHVLHSK
		120-134	1502.7954	HPGDFGADAQGAMTK
		120-140	2248.3784	HPGDFGADAQGAMTKALELFR.
		135-154	2283.4250	ALELFRNDIAAKYKELGFQG
		147-154	941.5297	YKELGFQG
Cyt-c	In-solution based digestion	27-39	1433.8340	HKTGPNLHGLFGR
		27-40	1561.9412	HKTGPNLHGLFGRK
		29-39	1168.6919	TGPNLHGLFGR
		40-54	1584.8112	KTGQAPGFSYTDANK
		41-54	1456.7118	TGQAPGFSYTDANK
		57-73	2010.0289	GITWGEETLMEYLENPK
		57-74	2138.1426	GITWGEETLMEYLENPKK
		89-101	1562.9460	KGEREDLIAYLKK
		90-100	1306.7406	K.GEREDLIAYLK.K
		90-101	1434.8470	K.GEREDLIAYLKK.A
		29-40	1296.7582	TGPNLHGLFGRK
Protein	Digestion method	position	Observed peptide mass	Amino acid sequence
		24-34	1349.7852	RDTHKSEIAHR
		25-34	1193.7000	DTHKSEIAHR
		35-44	1249.7152	FKDLGEEHFK
		65-75	1163.7159	LVNELTEFAK
		76-88	1463.7452	TCVADESHAGCEK
		89-100	1419.8082	SLHTLFGDELCK
		118-138	2541.3923	QEPERNECFLSHKDDSPDLPK
		139-155	2020.1472	LKPDNTLCDEFKADEK
		161-167	927.5651	YLYEIAIR

		168-183	2045.1848	RHPYFYAPELLYYANK
		184-197	1746.9583	YNGVFQECCQAEDK
		198-211	1662.0653	GACLLPKIETMREK
		205-211	906.5421	IETMREK
		205-218	1590.8683	IETMREKVLASSAR
		242-248	847.5549	LSQKFPK
		264-280	2114.0737	VHKECCHGDLLECADDR
		264-285	2612.3999	VHKECCHGDLLECADDRADLAK
		267-280	1749.8380	ECCHGDLLECADDR
		267-285	2248.1396	ECCHGDLLECADDRADLAK
		286-297	1442.8533	YICDNQDTISSK
		298-309	1532.9083	LKECCDKPLLEK
BSA		347-359	1567.8718	DAFLGSFLYEYSR
		360-371	1439.9355	RHPEYAVSVLLR
		361-371	1283.8180	HPEYAVSVLLR
		375-386	1501.8907	EYEATLEECCA
	regenerative	387-399	1554.8951	DDPHACYSTVFDK
	Fe ₃ O ₄ @PMAA	402-412	1305.8090	HLVDEPQNLK
	@PEI-trypsin			
		421-433	1479.9226	LGEYGFQNALIVR
		437-451	1640.0813	KVPQVSTPTLVEVSR
		460-468	1166.5879	CCTKPESER
		469-482	1724.9847	MPCTEDYLSLILNR
		483-489	898.5428	LCVLHEK
		496-507	1466.7468	VTKCCTESLVNR
		499-507	1138.5889	CCTESLVNR
		508-523	1881.0902	RPCFSALTPDETYVPK
		529-544	1908.0991	LFTFHADICTLPDTEK
		549-561	1504.8798	QTALVELLKHKPK
		581-597	1927.9650	CCAADDKEACFAVEGPK

Protein	Digestion method	position	Observed mass	Amino acid sequence
		1-24	2877.6711	MKWVTFISLLLLFSSAYSRGV FRR
		25-34	1193.7222	DTHKSEIAHR
		35-44	1249.7229	FKDLGEEHFK
		76-88	1462.9528	TCVADESHAGCEK
		89-100	1419.8207	SLHTLFGDELCK
		89-105	1946.2227	SLHTLFGDELCKVASLR
		161-167	927.5721	YLYEIAR
		161-168	1083.7090	YLYEIARR
BSA	Fe ₃ O ₄ @PMAA	205-220	1891.1145	IETMREKVLASSARQR

-trypsin

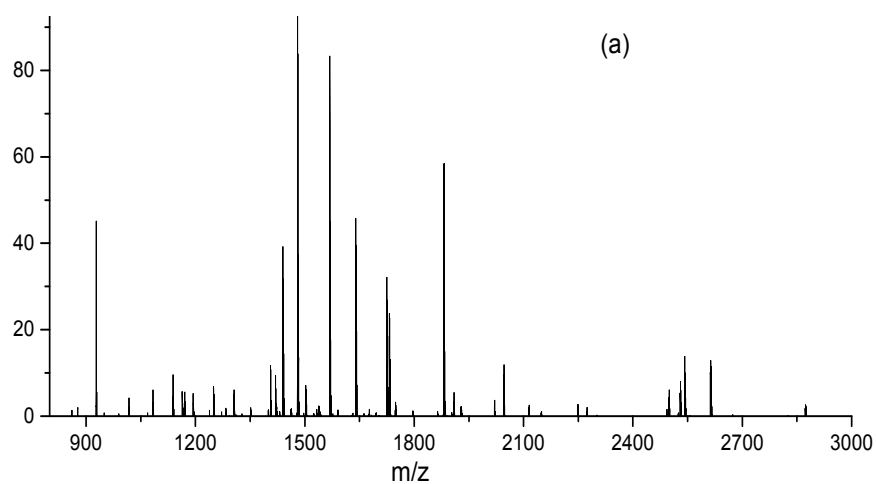
		264-285	2612.4692	VHKECCHGDLLECADDRADLAK
		286-297	1443.9121	YICDNQDTISSK
		341-359	2301.3511	NYQEAKDAFLGSFLYEYSR
		347-359	1567.9141	DAFLGSFLYEYSR
		360-371	1439.9565	RHPEYAVSVLLR
		361-371	1283.8307	HPEYAVSVLLR
		372-401	3592.0681	LAKEYEATLEECCA KDDPHACYSTVFDKL K
		375-386	1501.9216	EYEATLEECCA K
		402-412	1305.8215	HLVDEPQNLIK
		402-433	3816.3491	HLVDEPQNLIKQNC DQFEKLGEYGFQNALI VR
		413-433	2529.5029	QNC DQFEKLGEYGFQNALIVR
		421-433	1479.9423	LGEYGFQNALIVR
		437-451	1640.1062	KVPQVSTPTLVEVSR
		460-482	2872.6367	CCTKPESERMPCTEDYLSLILNR
		469-482	1725.018	MPCTEDYLSLILNR
		483-495	1539.9642	LCVLHEKTPVSEK
		499-507	1138.6078	CCTESLVNR
		508-523	1881.1206	RPCFSALTPDETYVPK
		508-528	2471.4578	RPCFSALTPDETYVPKAFDEK
		529-544	1908.1150	LFTFHADICTLPDTEK
		18-32	1606.9572	VEADIAGHGQEVLR
		33-43	1271.7151	LFTGHPETLEK
		65-78	1378.9167	HGTVVLTALGGILK
		79-97	2110.2502	KKGHHEAELKPLAQSHATK
		80-97	1982.1681	KGHHEAELKPLAQSHATK
		104-119	1885.1340	YLEFISDAIIHVLHSK
myoglobin	Fe ₃ O ₄ @PMAA	120-134	1502.7461	HPGDFGADAQGAMTK
	-trypsin	120-140	2248.3276	HPGDFGADAQGAMTKALELFR
		135-154	2283.349	ALELFRNDIAAKYKELGFQG
		147-154	941.5030	YKELGFQG
		1-14	1606.8558	MGDVEKGKKIFVQK
		2-9	861.1061	GDVEKGKK
		27-39	1433.8314	HKTGPNLHGLFGR
		27-40	1561.9294	HKTGPNLHGLFGRK
		29-39	1168.6830	TGPNLHGLFGR
		29-40	1296.7589	TGPNLHGLFGRK
Cyt-c	Fe ₃ O ₄ @PMAA	40-54	1584.8254	KTGQAPGFSYTDANK
	-trypsin	41-54	1456.7263	TGQAPGFSYTDANK
		57-73	2010.0515	GITWGEETLMEYLENPK

57-74	2138.1589	GITWGEETLMEYLENPKK
89-101	1562.9392	KGEREDLIAYLKK
90-100	1306.7384	GEREDLIAYLK
90-101	1434.8387	GEREDLIAYLKK
93-100	964.5387	EDLIAYLK

Digestion condition: amount of $\text{Fe}_3\text{O}_4@\text{PMAA}@\text{PEI}$ and $\text{Fe}_3\text{O}_4@\text{PMAA}$: 0.8mg; sample, 0.3mg/mL standard protein ; digestion temperature: 37°C , 10minutes ; In-solution digestion, enzyme/protein=1/50 (w/w), 24 h, 37°C



Figure. S-4. Photographic images of $\text{Fe}_3\text{O}_4@\text{PMAA}@\text{PEI}$ dispersing in NH_4HCO_3 buffer solution (pH=8.0)



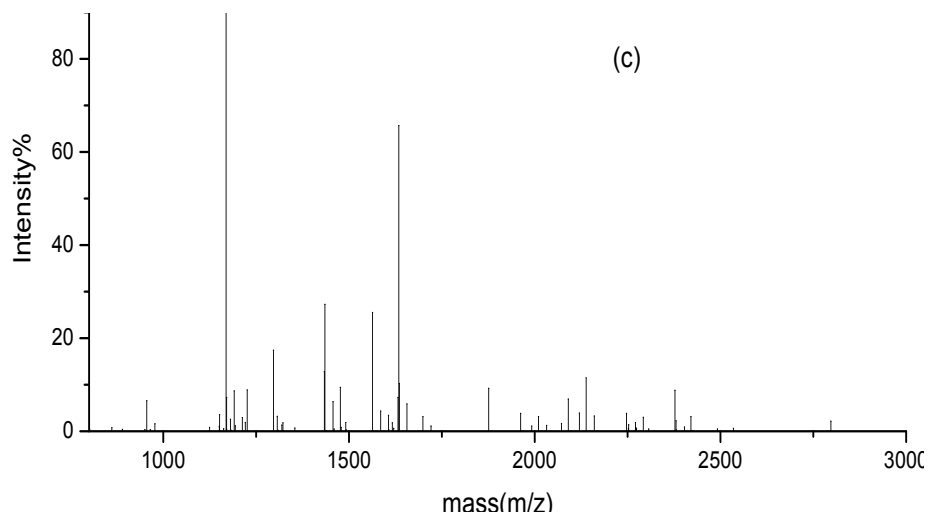
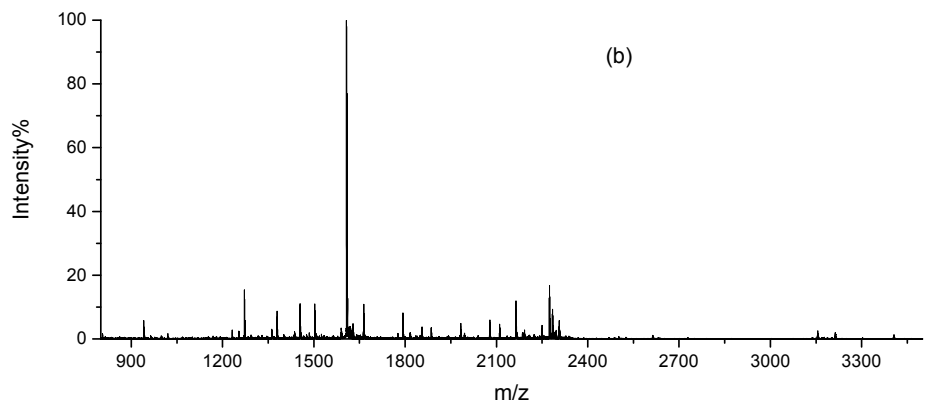


Figure S-5. MALDI-TOF/TOF mass spectra of the digests of (a) BSA (0.3mg/mL), (b) myoglobin (0.3mg/mL) and (c) Cyt-*c* (0.3mg/mL) obtained by using Fe₃O₄@PMAA@PEI-trypsin.