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Electronic Supplementary Information

DNA directed immobilization enzyme on polyamidoamine tethered magnetic composites with high reusability and stability

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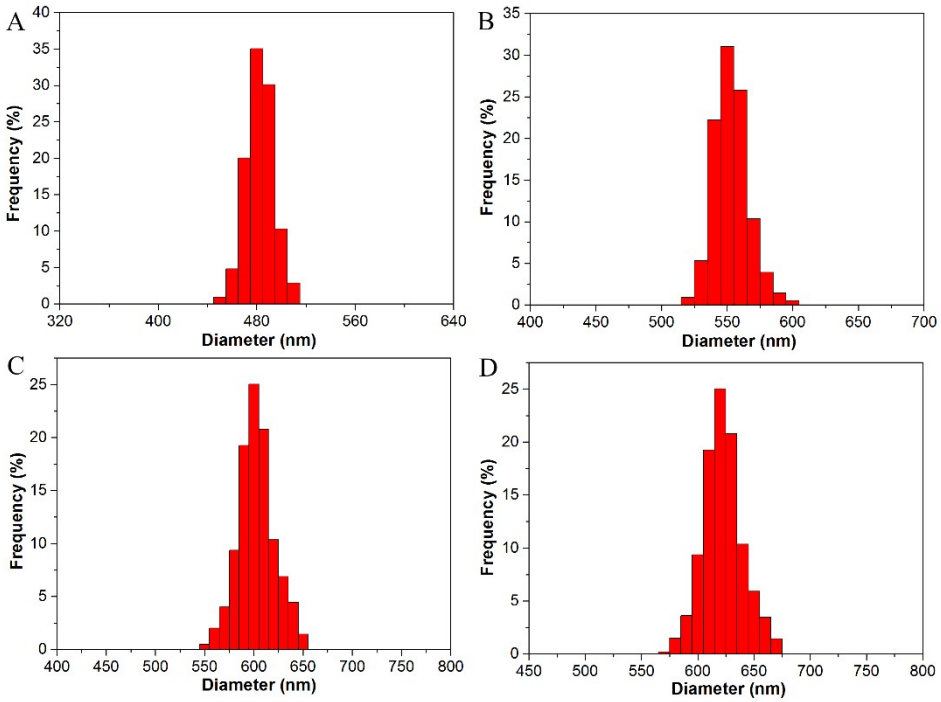

Fig. S1 Particle size distribution measured by DLS for (A) Fe_3O_4, (B) Fe_3O_4@SiO_2, (C) MNPs, (D) MNPs@G3.0PAMAM.

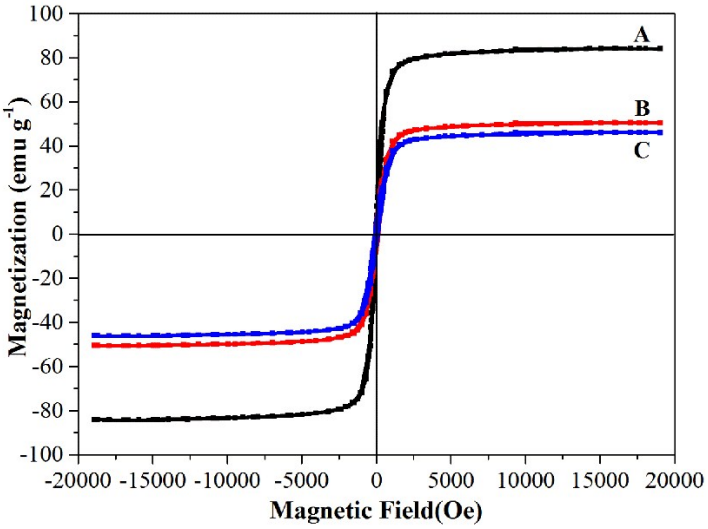

Fig. S2 Magnetization curves of Fe_3O_4 (A), MNPs (B), and MNPs@G3.0PAMAM@DNA-trypsin (C).

Table S1 Sequences of the DNA oligonucleotides used in the experiments

Name		Sequence（from 5′ to 3′）
Capture DNA	C_{14}	5’-NH_2-CTAGCTTGTCGTAA-3’
	C_{24}	5’-NH_2-CTAGCTTGTCGTAATACCAGGGTC-3’
	C_{34}	5’-NH_2- CTAGCTTGTCGTAATACCAGGGTCGTAGTAGTCA-3’
	C_{44}	5’-NH_2-CTAGCTTGTCGTAATACCAGGGTCGTAGTAGTCAG TAGTAGTCA-3’
Target DNA	T_{14}	5’-SH- TTACGACAAGCTAG -3’
	T_{24}	5’-SH- GACCCTGGTATTACGACAAGCTAG -3’
	T_{34}	5’-SH- TGACTACTACGACCCTGGTATTACGACAAGCTAG -3’
	T_{44}	5’-SH- TGACTACTACTGACTACTACGACCCTGGTATTACG ACAAGCTAG -3’

Table S2 Results of elemental analysis for magnetic materials.

Compound	N (%)	C (%)	H (%)
Fe_3O_4	0.01	1.95	0.495
Fe_3O_4@SiO_2	0.11	2.50	0.755
MNPs	0.3	2.97	0.902
MNPs@G3.0PAMAM	1.4	4.2	1.453

For the practical applicability, the enzymatic activity of the immobilized trypsin at optimum temperature, pH, digestion time and the concentration of protein solution have been studied. The temperature and pH are the most important factors affecting the activity of the immobilized enzyme. We investigated the effect of temperature and pH on the activity of the immobilized trypsin and the Cyt-c was chosen as a model protein. As shown in Table S3, the optimum temperature for the immobilized trypsin was 37^{o}C, with the sequence coverage and the number of matched peptides of 78% and 14, respectively. The influence of pH on the activity of the immobilized trypsin was determined in the range of 7.8 to 8.36 (Table S4), maximum activity of the immobilized trypsin was observed at pH value of 8.1. The influence of the digestion time of Cyt-c on activity of the immobilized trypsin is shown in Table S5. The results revealed that the digestion efficiency reached a plateau over 15 min, indicating that 15 min was the optimal digestion time. The optimal concentration of Cyt-C solution for the highest digestion efficiency was 0.25 mg ml^{-1} (Table S6). Further increasing the substrate concentration led to a decrease in the digestion efficiency, most likely because of the saturation of the enzyme binding capacity and digestion ability. As shown in Table S7-S10, similar results were also observed using Myo, HbA1c as model protein substrate and the optimal concentration, digestion time of Myo were 0.25 mg ml^{-1} (Table S7) and 5 min (Table S8), respectively, and those for HbA1c were 1 mg ml^{-1} (Table S9) and 10 min (Table S10), respectively.

Table S3 Effect of the temperature on the activity of the immobilized trypsin toward Cyt-c (n=3).

Temperature (℃)	20	30	37	50	60
Sequence coverage (%)	39	51	78	62	54
Peptides matched	7	8	14	11	9

Table S4 Effect of the pH on the activity of the immobilized trypsin toward Cyt-c (n=3).

pH	7.8	8.0	8.1	8.23	8.36
Sequence coverage (%)	70	74	78	69	62
Peptides matched	12	13	14	12	11

Table S5 Effect of the digestion time on the activity of the immobilized trypsin toward Cyt-c (n=3).

Digestion time (min)	10	15	30	50	70	90
Sequence coverage (%)	55	74	72	59	59	52
Peptides matched	11	13	11	10	10	10

Table S6 Effect of the concentration of Cyt-C solution on the activity of the immobilized trypsin (n=3).

Concentration (mg ml^{-1})	0.0625	0.125	0.25	0.5	1	2	4
Sequence coverage (%)	38	45	78	55	60	60	61
Peptides matched	4	6	14	12	13	9	8

Table S7 Effect of the concentration of Myo solution on the activity of the immobilized trypsin (n=3).

Concentration (mg ml^{-1})	0.125	0.25	0.5	1	2	4
Sequence coverage (%)	95	99	99	99	99	99
Peptides matched	18	20	22	20	20	20

Table S8 Effect of the digestion time on the activity of the immobilized trypsin toward Myo (n=3).

Digestion time (min)	1	5	10	15	30	50
Sequence coverage (%)	90	99	95	99	99	99
Peptides matched	16	20	20	20	21	20

Table S9 Effect of the concentration of HbA1c solution on the activity of the immobilized trypsin (n=3).

Concentration (mg ml^{-1})	0.125	0.25	0.5	1	2	4
Sequence coverage (%)	65	72	79	88	88	88
Peptides matched	8	10	12	16	15	16

Table S10 Effect of the digestion time on the activity of the immobilized trypsin toward HbA1c (n=3).

Digestion time (min)	1	5	10	15	30	50
Sequence coverage (%)	70	79	88	88	88	88
Peptides matched	11	13	16	16	14	15

Table S11 Comparison of the digestion efficiency of immobilized trypsin nanoparticles (MNPs@G3.0PAMAM@DNA-trypsin) and free trypsin for Cyt-c digestion.

Position	Mass	Missed cleavage	Peptide sequence	Immobilizd trypsin	Free trypsin
10 – 26	1992.2317	2	K.IFVQKCAQCHTVEKGGK.H	√	
24 – 39	1676.0644	2	K.GGKHKTGPNLHGLFGR.K	√	√
27 – 39	1433.9101	1	K.HKTGPNLHGLFGR.K	√	√
27 – 40	1562.0248	2	K.HKTGPNLHGLFGRK.T	√	√
29 – 39	1168.7259	0	K.TGPNLHGLFGR.K	√	√
29 – 40	1296.8344	1	K.TGPNLHGLFGRK.T	√	√
40 – 56	1827.0744	2	R.KTGQAPGFSYTDANKNK.G	√	√
41 – 54	1456.8981	0	K.TGQAPGFSYTDANK.N	√	√
41 – 56	1698.9713	1	K.TGQAPGFSYTDANKNK.G	√	√
57 – 73	2010.1405	0	K.GITWGEETLMEYLENPK.K	√	
57 – 74	2138.2537	1	K.GITWGEETLMEYLENPKK.Y	√	
57 – 74	2154.2524	1	K.GITWGEETLMEYLENPKK.Y + Oxidation (M)	√	
57 – 80	2813.6771	2	K.GITWGEETLMEYLENPKKYIPGTK.M+ Oxidation (M)	√	
90 – 100	1306.8282	1	K.GEREDLIAYLK.K	√	√

Table S12 Comparison of the digestion efficiency of immobilized trypsin nanoparticles (MNPs@G3.0PAMAM@DNA-trypsin) and free trypsin for Myo digestion.

Position	Mass	Missed cleavage	Peptide sequence	Immobilizd trypsin	Free trypsin
2 – 17	1816.2641	0	M.GLSDGEWQQVLNVWGK.V	√	√
18 – 32	1607.1203	0	K.VEADIAGHGQEVLIR.L	√	√
33 – 43	1271.8759	0	R.LFTGHPETLEK.F	√	
33 – 46	1662.1329	1	R.LFTGHPETLEKFDK.F	√	
33 – 48	1937.3283	2	R.LFTGHPETLEKFDKFK.H	√	√
49 – 57	1086.7430	1	K.HLKTEAEMK.A	√	
49 – 63	1730.1608	2	K.HLKTEAEMKASEDLK.K	√	
52 – 64	1495.4876	2	K.TEAEMKASEDLKK.H + Oxidation (M)	√	
65 – 78	1379.0849	0	K.HGTVVLTALGGILK.K	√	
65 – 79	1507.1912	1	K.HGTVVLTALGGILKK.K	√	
65 – 80	1635.3010	2	K.HGTVVLTALGGILKKK.G	√	
79 – 97	2110.4975	2	K.KKGHHEAELKPLAQSHATK.H	√	
80 – 97	1982.3908	1	K.KGHHEAELKPLAQSHATK.H	√	
81 – 97	1854.2485	0	K.GHHEAELKPLAQSHATK.H	√	
98 – 103	735.6153	1	K.HKIPIK.Y	√	
104 – 119	1885.3241	0	K.YLEFISDAIIHVLHSK.H+ Oxidation (M)	√	
120 – 134	1502.9182	0	K.HPGDFGADAQGAMTK.A	√	
135 – 140	748.5603	0	K.ALELFR.N	√	√
135 – 146	1360.9728	1	K.ALELFRNDIAAK.Y	√	√
135 – 148	1652.2437	2	K.ALELFRNDIAAKYK.E		√
141 – 154	1554.1082	2	R.NDIAAKYKELGFQG.		√
147 – 154	941.6326	1	K.YKELGFQG	√	√

Table S13 Comparison of the digestion efficiency of immobilized trypsin nanoparticles (MNPs@G3.0PAMAM@DNA-trypsin) and free trypsin for HbA1c digestion.

Position	Mass	Missed cleavage	Peptide sequence	Immobilizd trypsin	Free trypsin
2 – 9	952.7872	0	M.VHLTPEEK.S	√	
2 – 17	1685.4348	2	M.VLSPADKTNVKAAWGK.V		√
2 – 31	3162.4729	2	M.VHLTPEEKSAVTALWGKVNVDEVGGEALGR.L	√	
9 – 32	2485.8790	2	K.TNVKAAWGKVGAHAGEYGAEALER.M		√
10 – 31	2228.7369	1	K.SAVTALWGKVNVDEVGGEALGR.L	√	
13 – 32	2043.5473	1	K.AAWGKVGAHAGEYGAEALER.M		√
18 – 32	1530.1819	0	K.VGAHAGEYGAEALER.M		√
19 – 31	1315.0421	0	K.VNVDEVGGEALGR.L	√	
32 – 41	1275.0745	0	R.LLVVYPWTQR.F	√	
42 – 57	1834.4083	0	K.TYFPHFDLSHGSAQVK.G		√
42 – 60	2059.4833	0	R.FFESFGDLSTPDAVMGNPK.V	√	
42 – 60	2075.4992	0	R.FFESFGDLSTPDAVMGNPK.V + Oxidation (M)	√	
42 – 61	2213.6347	1	K.TYFPHFDLSHGSAQVKGHGK.K		√
42 – 62	2286.7314	1	R.FFESFGDLSTPDAVMGNPKVK.A	√	√
62 – 93	3394.6532	2	K.KVADALTNAVAHVDDMPNALSALSDLHAHKLR.V		√
63 – 83	2191.6835	2	K.AHGKKVLGAFSDGLAHLDNLK.G	√	
63 – 91	2997.2813	0	K.VADALTNAVAHVDDMPNALSALSDLHAHK.L		√
63 – 93	3266.5310	1	K.VADALTNAVAHVDDMPNALSALSDLHAHKLR.V		√
67 – 83	1798.4694	1	K.KVLGAFSDGLAHLDNLK.G	√	
68 – 83	1670.3537	0	K.VLGAFSDGLAHLDNLK.G	√	
84 – 96	1480.2292	0	K.GTFATLSELHCDK.L	√	
84 – 105	2586.8883	1	K.GTFATLSELHCDKLHVDPENFR.L	√	
92 – 100	1087.9656	1	K.LRVDPVNFK.L		√
122 – 133	1379.0851	0	K.EFTPPVQAAYQK.V	√	
134 – 145	1150.0108	0	K.VVAGVANALAHK.Y	√	
134 – 147	1450.1969	1	K.VVAGVANALAHKYH	√	