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Electronic supplementary information

Multifunctional magnetic particles for effective suppression of non-specific adsorption and coimmobilization of multiple enzymes by DNA directed immobilization

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Table S1 Sequences of the DNA oligonucleotides used in the experiments.

Name	Sequence（from 5′ to 3′）
A	NH_2-GCTACCAGTACACATCCGCAGTCATGACCT
A*	SH-AGGTCATGACTGCGGATGTGTACTGGTAGC
B	NH_2-TAGCTTGTCGTAATACCAGGGTCGTAGTAG
B*	SH-CTACTACGACCCTGGTATTACGACAAGCTA

Table S2 Synthesis of multi-functionalized magnetic particles with varying ratios of THPMP, DETAPTMS, and MPTMS, and the influences of different ratios of THPMP, DETAPTMS, and MPTMS on relative enzymatic activity, enzyme loading amount, and the zeta potential (n=3).

Entry	DETAPTMS (μl)	THPMP (μl)	MPTMS (μl)	Relative activity of Cht (%)	Enzyme loading amount (mg/g)	Zeta Potential [mV]
1	0	0	0	100	15.35	-30.5
2	0	5	0	66.9	8.23	-33.2
3	0	10	0	34.6	4.86	-35.2
4	0	20	0	13.1	2.23	-41.8
5	*0*	*40*	*0*	*12.5*	*1.83*	*-44.6*
6	0	80	0	12.3	1.85	-47.3
7	0	160	0	25.4	4.60	-45.0
8	5	40	0	4.7	1.20	19.0
9	10	40	0	4.1	0.86	3.48
10	20	40	0	3.5	0.74	28.4
11	*40*	*40*	*0*	*1.8*	*0.30*	*38.8*
12	80	40	0	3.5	0.65	23.2
13	160	40	0	3.7	0.81	18.6
14	320	40	0	0.9	0.23	14.2
15	40	40	5	0.7	0.22	34.7
16	*40*	*40*	*10*	*0.8*	*0.13*	*36.5*
17	40	40	20	1.1	0.32	29.3
18	40	40	40	Minor precipitated		
19	40	40	80	More precipitated		
20	40	40	160	Large precipitated		
21	40	40	320	Precipitated		

Table S3 The influence of before and after modification of MNPs-2 with THPMP, DETAPTMS, and MPTMS (4:4:1) on relative enzymatic activity and enzyme loading amount of different enzymes (n=3).

Enzyme	Relative activity of enzymes before modification (%)	Relative activity of enzymes after modification (%)	Enzyme loading amount before modification (mg/g)	Enzyme loading amount after modification (mg/g)
Trypsin	100	0.014	12.35	Not detected
HRP	100	0.078	10.45	Not detected
GOx	100	0.021	11.36	Not detected

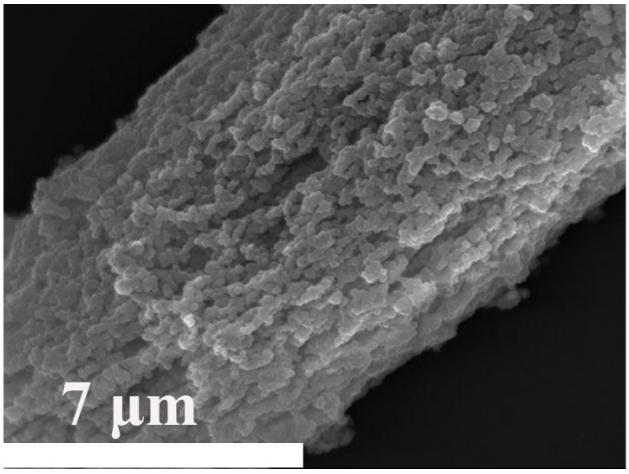

Fig. S1 SEM image of MNPs-4 for EDX elemental mapping.

Table S4 Elemental analysis of N, C, H, and S for MNPs-1, MNPs-2, and MNPs-4.

	N [%]	C [%]	H [%]	S [%]
MNPs-1	0.14	2.04	0.611	0.02
MNPs-2	0.31	3.37	0.827	0.96
MNPs-4	0.58	4.56	1.996	1.23

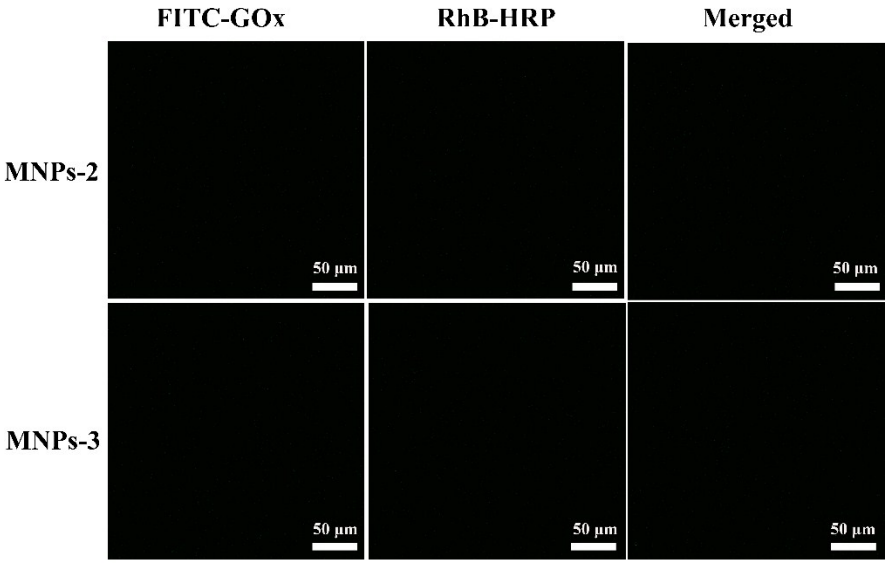

Fig. S2 CLSM images of FITC-GOx-A* conjugates and RhB-HRP-B* conjugates were co-immobilized on MNPs-2 or FITC-GOx and RhB-HRP were immobilized on MNPs-3.

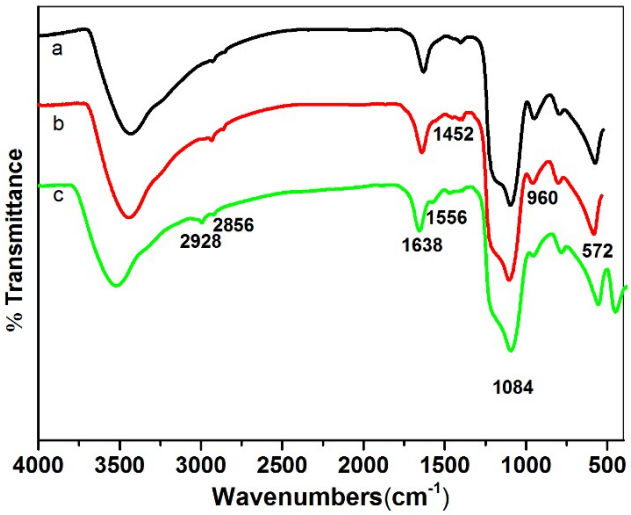

Fig. S3 FT-IR spectra of (a) MNPs-1, (b) MNPs-2, and (c) and MNPs-4.

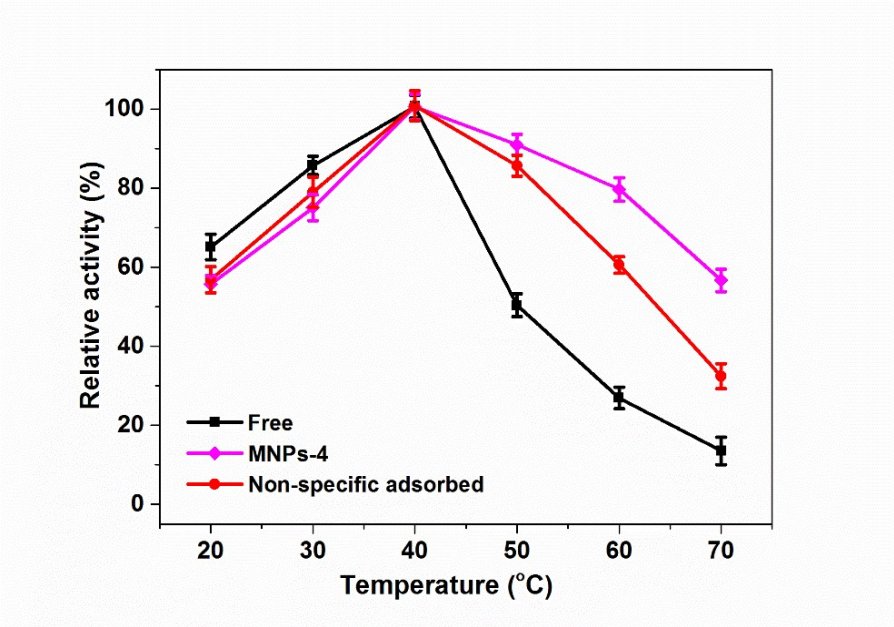

Fig. S4 Effect of temperature on the performance of free and immobilized GOx/HRP.

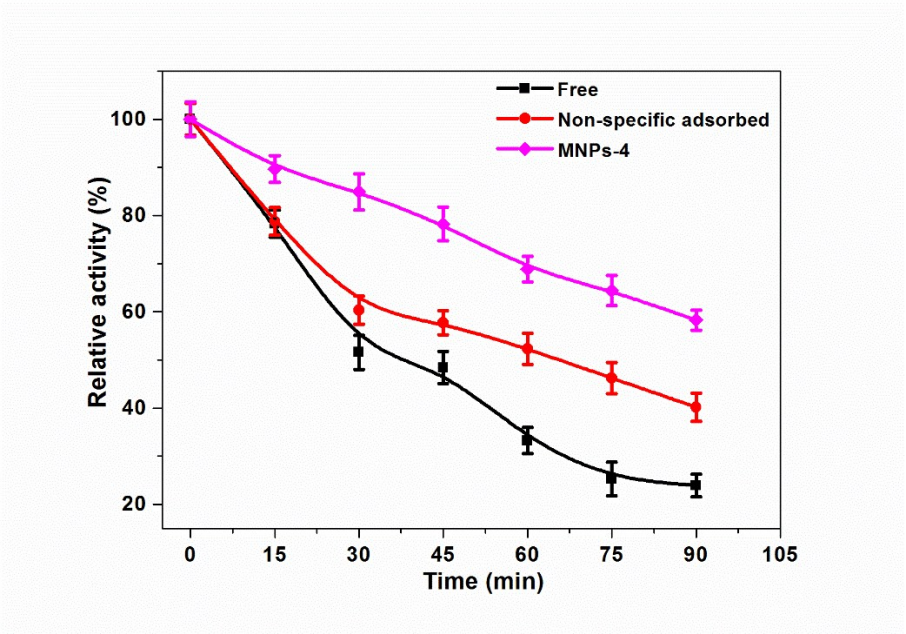

Fig. S5 Stability of the MNPs-4 compared with the equivalent free enzymes and non-specific adsorbed enzymes at 60°C.

Table S5 Determination of the denaturation constant (k_d) and $t_{1/2}$ values for the free, non-specific adsorbed, and MNPs-4.

Temperature (°C)	MNPs-4		Non-specific adsorbed		Free enzyme	
	k_d (h^{-1})	$t_{1/2}$ (h)	k_d (h^{-1})	$t_{1/2}$ (h)	k_d (h^{-1})	$t_{1/2}$ (h)
50	0.2492	2.78	0.4710	1.47	0.7083	0.98
60	0.3562	1.95	0.5635	1.22	0.9989	0.69

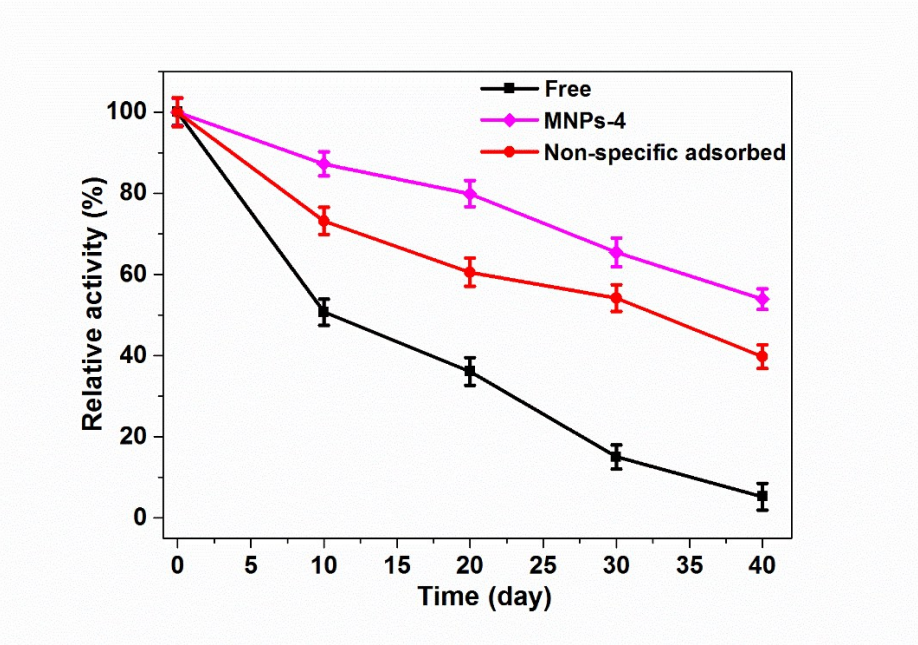

Fig. S6 Long-term storage stability of the MNPs-4 compared with the equivalent free enzymes and non-specific adsorbed enzymes at room temperature.

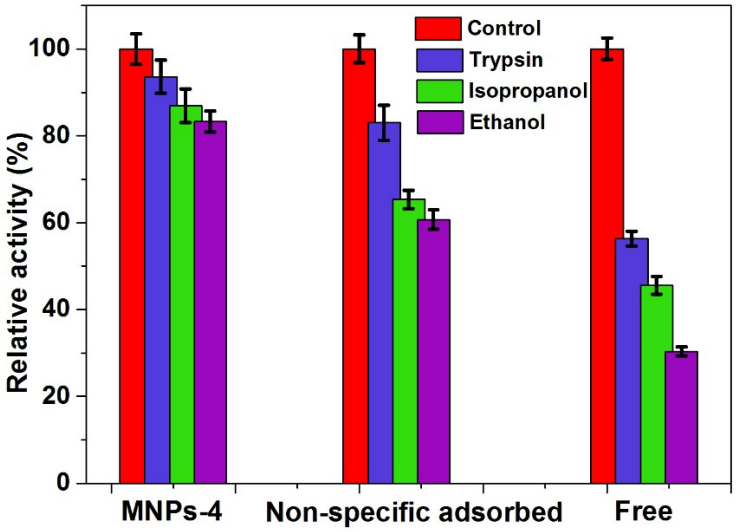

Fig. S7 Stability of the MNPs-4 and non-specific adsorbed enzymes compared with the equivalent free enzymes in the presence of 1 mg mL^{-1} trypsin or 50 wt% ethanol or isopropanol in pH 7.4 buffer.