Supporting Information

High activity and convenient ratio control: DNA-directed coimmobilization of multiple enzymes on multifunctionalized magnetic nanoparticles

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Table of Contents

1. Experimental

Table S-1 DNA used in this study

	DNA sequence
P1	CTTAGCTTCATCGAGGTCCAGTCA
C1	TGACTGGACCTCGATGAAGCTAAG
P2	TGCATTAGAGACACGTCCGACTTG
C2	CAAGTCGGACGTGTCTCTAATGCA

C1 and C2 were complementary to P1 and P2, respectively.

2. Transmission electron microscope measurements

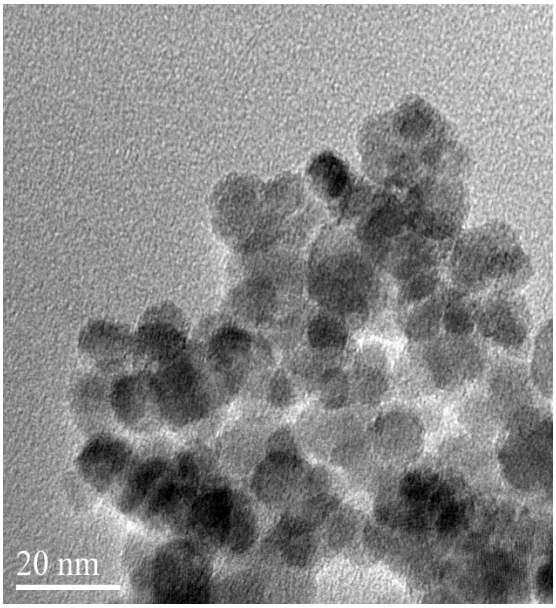

Fig. S-1 TEM image of MPs.

3. Nuclear magnetic resonance (NMR) measurements

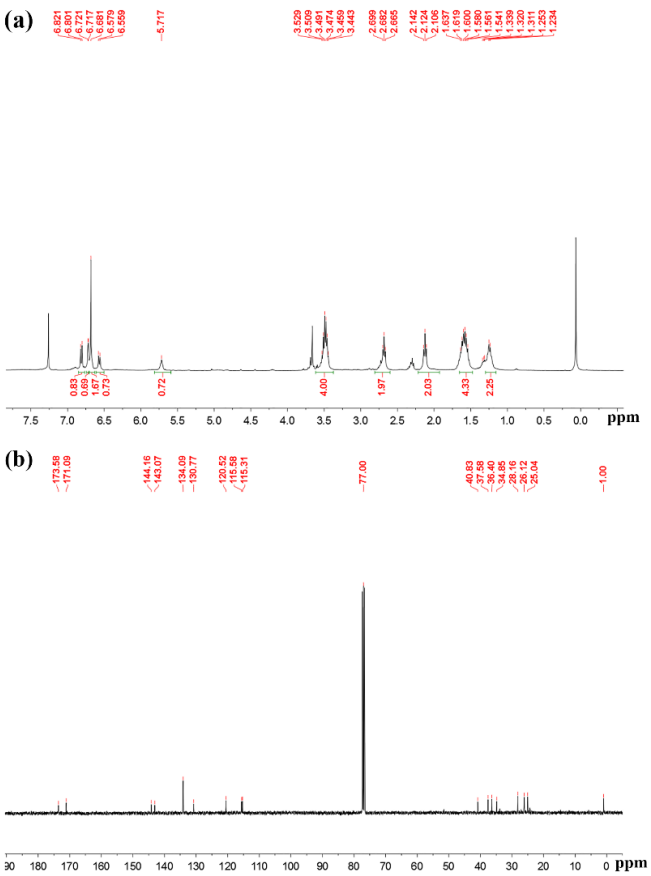

Fig. S-2 (a) ^{1}H NMR spectrum of MA. (b) ^{13}C NMR spectrum of MA.

4. Fourier transform infrared spectroscopy (FTIR) measurements

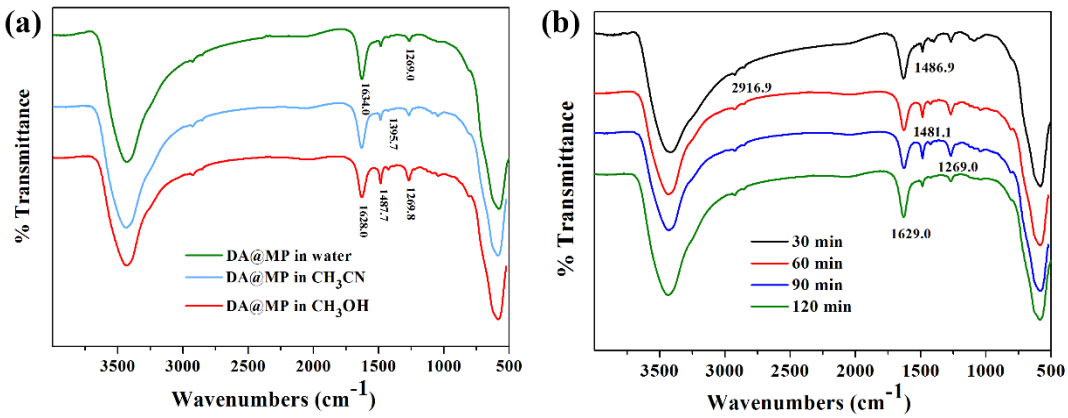

Fig. S-3 FTIR spectra of DA-modified MPs with (a) different solvents and (b) various reaction times.

5. Thermogravimetric analysis (TGA) and vibrating sample magnetometer (VSM) measurements

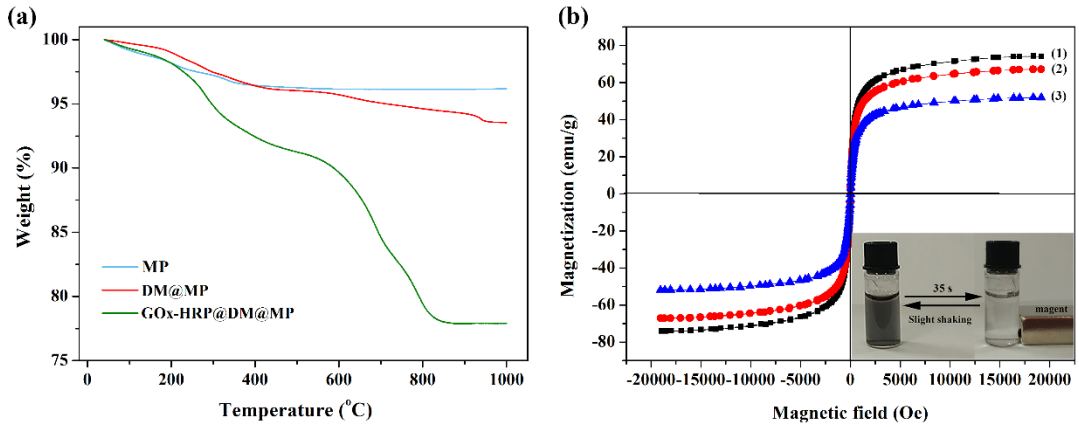

Fig. S-4 (a) TGA curves. (b) Magnetization curves of (1) MPs, (2) DM@MP and (3) GOx-HRP@DM@MP.

6. Enzymatic assays of the bienzymes

Table S-2. Enzymatic kinetics of free GOx&HRP, GOx-HRP@APTES@MP, and GOx-HRP@DM@MP.

	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} mM^{-1})
Free GOx&HRP	2.63	6.64	2.53
GOx-HRP@APTES@MP	1.92	2.99	1.56
GOx-HRP@DM@MP	1.41	7.07	5.02

The overall amount of enzymes in the supernatants after anchoring and washing was determined by Bradford Protein Assay Kit which was purchased from Beyotime Institute of Biotechnology.

Kinetic parameters of enzymes were determined as follows:

$$\frac{1}{v} = \frac{t \cdot \varepsilon \cdot b}{A}$$

ε=5.9×10^4 M^{-1} cm^{-1}; b=1 cm;

$$k_{cat} = \frac{V_{max}}{c_{GOx} + c_{HRP}}$$

GOx MW=160kDa, HRP MW=44kDa;

Table. S-3 The concentrations of GOx and HRP in different states

	GOx (μg mL^{-1})	HRP (μg mL^{-1})
GOx-HRP@DM@MP	1.43	0.18
Free GOx&HRP	1.2	0.15
GOx-HRP@APTES@MP	1.94	0.24