Electronic Supplementary Information for

A self-enhanced and recyclable catalytic system constructed by magnetic bi-nano-bionic enzymes for real-time control RAFT polymerization†

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1. Experimental Details

1.1 Materials

All chemical reagents used in the experiments were of analytical grade. Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, AR), ethanolamine (ETA, AR), potassium carbonate (K_2CO_3, AR), ethanol, formaldehyde (HCHO, AR), ethylene glycol (EG, AR), sodium acetate anhydrous (NaAc, AR), trisoydium citrate dehydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$, AR), were provided by Sinopharm Chemical Reagent Co, Ltd (Shanghai, China). 3-Aminopropyl-triethoxysilane (APTES, AR), sodium boro-hydride ($NaBH_4$, 98%), polyethylene glycol 2000 (PEG-2000, AR) and 3, 3', 5, 5'-tetramethylbenzidine (TMB, AR) were obtained from Aladin. Acrylic acid (AA, AR), sodium p-styrene sulfonate (SSS, AR) and styrene (AR) were bought from Shandong Laiyang Economic and Technological Development Zone Chemical Plant (Laiyang, China). RAFT agent, 4-cyano-4-ethyl-trithiopentanoic acid (RAFT-a) was synthesized using a well-defined procedure by Liu etal.[1] S, S'-bis (α, α'-dimethyl-α''-acetic acid) trithiocarbonate (RAFT-b) was prepared on the basis of the method previously reported.[2] Deionized water was used to prepare all the solutions in this study.

1.2 Instruments

The morphologies and sizes of Au nanoparticles (NPs), flower-like Fe_3O_4 NPs and Fe_3O_4@Au NPs were characterized using transmission electron microscope (TEM) measurements which were carried out on a JEM-1200EX transmission electron microscopy operated at an accelerating voltage of 100 kV. Scanning electron microscopy (SEM) images were carried out using an FEI Quanta 200 F scanning electron microscope (Merlin, Zeiss Co., Germany). X-ray powder diffraction (XRD) patterns were tested using a Rigaku DMax-2600 PC diffraction meter, employing monochromatic Cu K radiation. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a JNM-ECP 600 (600 MHz) spectrometer. Gel permeation chromatography (GPC) (Shimadzu modular system) was used to figure out the molecular weight in number (Mn) and polydispersity index (Đ) of polymers. Ultraviolet spectrophotometer (UV) spectra were obtained on a UV/V-16/18 UV spectrophotometer (Shanghai, Mapada) at room temperature. Infrared spectroscopy was carried out on a PerkinElmer Spectrum One Fourier transform infrared (FTIR) spectra. X-ray photoelectron spectroscopy (XPS) spectra were recorded on an Axis Ultra DLD (SHIMADZU, Japan). The magnetic properties of the samples were measured using a vibrating sample magnetometer (VSM, Lake-shore 7410) at room temperature.

1.3 Preparation of flower-like Fe_3O_4 NPs

The monodisperse Fe_3O_4 NPs were prepared on the basis of the reported method with slight modifications.[3] In detail, $FeCl_3 \cdot 6H_2O$ (3.0 g, 11.0 mmol) was dissolved in 80 mL of solvent containing ETA (20 mL) and EG (60 mL) to obtain a stable solution. Then, PEG-2000 (2.0 g) and NaAc (8.0 g, 100 mmol) were added into the obtained solution under continuous magnetic stirring. The afforded solution was then moved to a Teflon-lined stainless-steel autoclave (100 mL), sealed and then heated at 200 ^oC for 10 h. After the heat treatment, the autoclave was cooled to room temperature. The black solid products were then collected via a magnet, and then washed using ethanol and deionized water for several times, respectively. The obtained product was dried at 70 ^oC under vacuum for 12 h.

1.4 Preparation of APTES-modified flower-like Fe_3O_4 NPs

100 mg of flower-like Fe_3O_4 NPs were dispersed into 80 mL deionized water, and then the suspension was ultrasonicated for 30 min to afford a uniform dispersion. Then, APTES (2 mL) was added into the suspension under vigorous mechanical stirring for 4 h at ambient temperature. Next, the APTES-modified flower-like Fe_3O_4

NPs were obtained using a magnet, after repeated washing with deionized water and ethanol, respectively, and finally redispersed in deionized water (100 mL) to obtain a homogeneous suspension for the further applications.

1.5 Synthesis of flower-like Fe_3O_4@Au NPs

The synthesis of flower-like Fe_3O_4@Au NPs contains two steps as shown in Fig. 1A. The detailed preparation processes are shown as follows.

1.5.1 Synthesis of flower-like Fe_3O_4@Au nanoseeds

The gold nanoseeds were synthesized using $NaBH_4$ as the reductant. In details, aqueous solution (40 mL) containing 6.25×10^{-4} M trisoydium citrate dehydrate and 6.25×10^{-4} M $HAuCl_4$ was prepared in a 100 mL conical flask. Next, ice-cold $NaBH_4$ solution (0.5 mL, 0.1 M) was added to the obtained solution above while stirring. It could be observed that the colour of solution could turn into claret after the addition of $NaBH_4$, confirming the generation of Au NPs. For the synthesis of Fe_3O_4@Au NPs, aqueous dispersion of APTES-modified Fe_3O_4 NPs (80 mL) was mixed with Au NPs suspension (40 mL) by ultra-sonication in a deionized water bath. The mixture was treated under mechanical stirring for 3 h to afford the Fe_3O_4@Au NPs, which were collected employing a magnet, then washed with deionized water and ethanol 3 times, respectively and redispersed in deionized water (5 mL) to obtain a homogeneous suspension.

1.5.2 Growth process of Au NPs on Fe_3O_4@Au NPs platform

$HAuCl_4$ (1.5ml, 5.0×10^{-3} M) was added to K_2CO_3 solution (100 mL, 2 mM), under continuous stirring for 25 min. Then, the obtained solution was stored in refrigerator at 4 ^oC for 12 h to form $Au(OH)^-_4$. In the Au growth step, firstly, Fe_3O_4@Au nanoseeds suspension (5 mL) was added into 100 mL $Au(OH)^-_4$ solution. And then formaldehyde solution (38.5%, 0.1 mL) was added to the obtained suspension above under continuous mechanical stirring for 3 h. Finally, the flower-like Fe_3O_4@Au NPs were collected using a magnet, then washed with ethanol and deionized water, respectively and dried for 12 h in a vacuum at 50 ^oC.

1.6 The assay for catalytic activity of the constructed bi-nano-bionic enzymes (Fe_3O_4@Au NPs)

TMB reagent was used in this study to detect the hydroxyl radicals (which could oxidize TMB to produce the blue product, ox-TMB) as shown in Fig. 1B.[4] Measurements were carried out in a 2.7 mL aqueous suspension (Fig. 1B(a)), containing H_2O_2 (0.5 mL, 50 mM), TMB (2 mL, 500 μM) and flower-like Fe_3O_4 NPs (0.2 mL, 0.1 mg/mL) or flower-like Fe_3O_4@Au NPs (0.2 mL, 0.1 mg/mL) at room temperature. In addition, absorbance spectra of flower-like Fe_3O_4@Au NPs solutions with different concentrations (0.1 mg/mL-1.2 mg/mL) was detected using a UV-vis spectrometer at λ_{max} = 652 nm.

1.7 Synthesis of polymers using the bi-nano-bionic enzymes/H_2O_2 system

The detailed preparation processes of polymers via free radical polymerization and RAFT polymerization, respectively, using the bi-nano-bionic enzymes/H_2O_2 as initiating system are shown as follows.

1.7.1 Synthesis of polyacrylic acid (PAA) via free radical polymerization using the bi-nano-bionic enzymes/H_2O_2 system without RAFT agents

In a 10 mL vial glass, AA (0.72 mL, 10 mmol), flower-like Fe_3O_4@Au NPs (1 mg/mL) were dissolved in 6 mL deionized water. Then, the resulting mixture was deoxygenated for 30 min using highly pure nitrogen, then injected H_2O_2 solution (0.01 mL, 0.1 mmol) using a injector followed by the stirring at ambient temperature for 24 h. Fe_3O_4@Au NPs were separated from the suspension employing a magnet, then the obtained PAA was purified by rotary evaporation before vacuum drying. The as-prepared product was characterized by ^1H NMR. ^1H NMR (D_2O, 298 K, 600 MHz), δ (ppm from TMS): 3.71 (t, 2H, b), 3.10-2.51 (t, 1H, c), 1.84 (t, 3H, a), 1.17 (t, 3H, d).

1.7.2 Synthesis of PAA via RAFT polymerization using the bi-nano-bionic enzymes/H_2O_2 system with RAFT-a

To a 10 mL vial glass with 6 mL deionized water, AA (0.72 mL, 0.1 mmol), RAFT-a (15.68 mg, 0.05 mmol) and flower-like Fe_3O_4@Au NPs (1 mg/mL) were added. The obtained mixture was deoxygenated for 20 min employing highly pure nitrogen, then injected H_2O_2 solution (0.01 mL, 0.1 mmol) using a injector followed by the vigorous stirring at ambient temperature for 24 h. Fe_3O_4@Au NPs were separated from the suspension using a magnet, then PAA was obtained by rotary evaporation before vacuum drying. The product was analyzed by ^{1}H NMR. ^{1}H NMR (D_2O, 298 K, 600 MHz), δ (ppm from TMS): 3.14 (t, 2H, f), 2.71 (t, 1H, g), 2.60 (t, 2H, a), 2.53 (t, 2H, b), 1.92 (t, 3H, c), 1.72 (t, 2H, d), 1.61 (t, 3H, e). A final PAA conversion of 82.4 % was determined by ^{1}H NMR analysis.

1.7.3 Synthesis of PAA-*b*-poly (sodium-p-styrenesulfonate) (PAA-*b*-PSS) via RAFT polymerization using the bi-nano-bionic enzymes/H_2O_2 system with RAFT-a

As a typical example, PAA-*b*-PSS was synthesized as follows. To a 10 mL vial glass with 6 mL deionized water, purified PAA (0.36 mg, 5 mmol), flower-like Fe_3O_4@Au NPs (1 mg/mL) were added. The resulting mixture was deoxygenated for 20 min employing highly pure nitrogen, then injected H_2O_2 solution (0.01 mL, 0.1 mmol) using a injector followed by the stirring at room temperature for 24 h. Fe_3O_4@Au NPs were separated from the suspension with a magnet, then PAA-*b*-PSS was obtained by rotary evaporation before vacuum drying. The product was determined by ^{1}H NMR. ^{1}H NMR (D_2O, 298 K, 600 MHz), δ (ppm from TMS): 7.80 (t, 1H, j, k), 3.91 (t, 2H, h), 3.82 (t, 1H, i). As a typical example, PAA-*b*-PSS was synthesized as follows. To a 10 mL vial glass with 6 mL deionized water, purified PAA (0.36 mg, 5 mmol), flower-like Fe_3O_4@Au NPs (1 mg) were added. The resulting mixture was deoxygenated for 20 min employing highly pure nitrogen, then injected H_2O_2 solution (0.01 mL, 0.1 mmol) using a injector followed by the stirring at room temperature for 24 h. Fe_3O_4@Au NPs were separated from the suspension with a magnet, then PAA-*b*-PSS was obtained by rotary evaporation before vacuum drying. The product was determined by ^{1}H NMR. ^{1}H NMR (D_2O, 298 K, 600 MHz), δ (ppm from TMS): 7.80 (t, 1H, j, k), 3.91 (t, 2H, h), 3.82 (t, 1H, i).

1.7.4 Synthesis of PAA using the bi-nano-bionic enzymes/H_2O_2 system with RAFT-b

In a 10 mL vial glass, AA (0.72 mL, 10 mmol), RAFT-b (14.4 mg, 0.05 mmol) and flower-like Fe_3O_4@Au NPs (1 mg/mL) were dissolved in 6 mL deionized water. The resulting mixture was deoxygenated for 30 min using highly pure nitrogen, then injected H_2O_2 solution (0.01 mL, 0.1 mmol) using a injector, followed by the stirring at room temperature for 24 h. Fe_3O_4@Au NPs were separated from the dispersion using a magnet, then the obtained PAA was purified by rotary evaporation for 1 h before vacuum drying. A final PAA conversion of 82.3% was determined by ^{1}H NMR analysis.

1.7.5 Synthesis of polystyrene (PS) via RAFT polymerization using the bi-nano-bionic enzymes/H_2O_2 system with RAFT-b

In a 10 mL vial glass, styrene (1.04 mL, 10 mmol), RAFT-b (14.4 mg, 0.05 mmol) and flower-like Fe_3O_4@Au NPs (1 mg/mL) were dissolved in 6 mL DMSO. The resulting mixture was deoxygenated for 30 min using highly pure nitrogen, then injected H_2O_2 solution (0.01 mL, 0.1 mmol) using a injector, followed by the stirring at room temperature for 24 h. Fe_3O_4@Au NPs were separated from the solution using a magnet to obtain PAA which was purified by rotary evaporation for 1 h before vacuum drying.

2. Results and Discussion

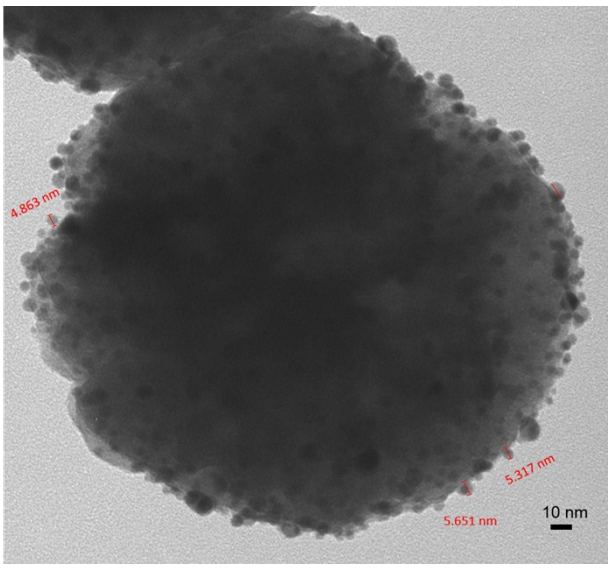

Fig. S1 Characterization of Au NPs. TEM images of flower-like Fe_3O_4@Au NPs.

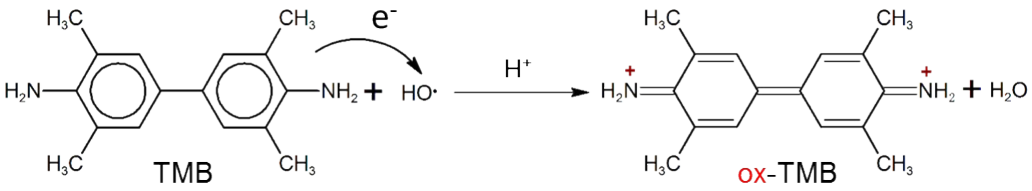

Fig. S2 TMB could be oxidized to be ox-TMB by the produced •OH.

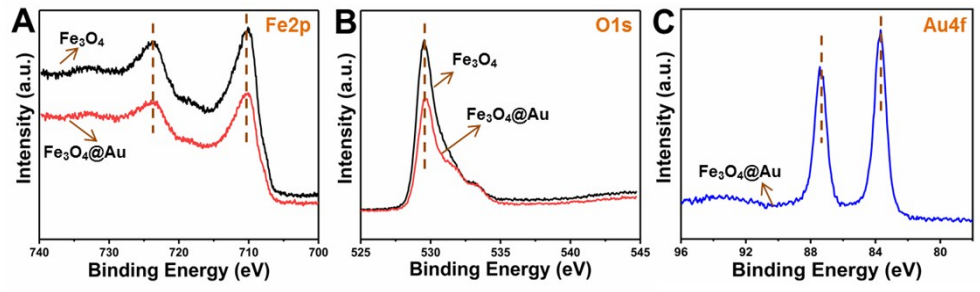

Fig. S3 XPS spectra of flower-like Fe_3O_4 and Fe_3O_4@Au NPs. (A) Fe2p, (B) O1s, (C) Au4f.

The surface electron structures of flower-like Fe_3O_4 and Fe_3O_4@Au NPs were determined from XPS patterns. For the flower-like Fe_3O_4 NPs, the signals at 724.35 eV and 710.25 should correspond to $Fe2p_{1/2}$ and $Fe2p_{3/2}$ levels while the signal at 529.55 eV correlates to the binding energy of O_{1s}, which is consistent with the Fe_3O_4 data reported in the literature.[5] After deposition of Au NPs on the surface of flower-like Fe_3O_4 NPs, the $Fe2p_{1/2}$,$Fe2p_{3/2}$ and O_{1s} binding energies increase to 724.99, 710.99 and 531.23 eV, respectively (Fig.S3 (A) and (B)). All of these results indicate the presence of strong electron interaction between Fe_3O_4 and Au NPs. As shown in Fig. S3(C), the binding energy of metallic $Au4f_{5/2}$ is 87.43 eV and the $Au4f_{7/2}$ binding energy is 83.68 eV which is lower than the reported value.[6] To sum up, the $Fe2p_{1/2}$, $Fe2p_{3/2}$ and O_{1s} binding energies of Fe_3O_4@Au NPs are higher than that of pure Fe_3O_4 which might result from the electron loss from Fe_3O_4 NPs owning to the deposition of Au NPs.

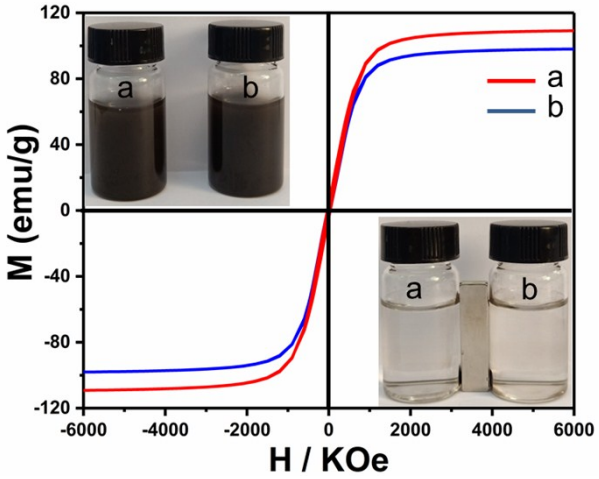

Fig. S4 Magnetization curves of the (a) flower-like Fe_3O_4 NPs and (b) Fe_3O_4@Au NPs. The insets show their suspensions before and after magnetic separation by an external magnet.

In our experiments, both freshly prepared flower-like Fe_3O_4 NPs and Fe_3O_4@Au NPs showed their strong magnetic properties and could be easily collected using a permanent magnet (inset of Fig. S4). In order to quantify the magnetism and make sure whether the presence of Au NPs on the Fe_3O_4 NPs could decrease the magnetism of Fe_3O_4@Au NPs , the magnetic hysteresis loops of Fe_3O_4 NPs and Fe_3O_4@Au NPs were measured using a vibrating sample magnetometer. As illustrated in Fig. S4, the Fe_3O_4 NPs and Fe_3O_4@Au NPs were superparamagnetic and both the remanent magnetizations and coercivities were close to zero. The magnetization saturation value of the flower-like Fe_3O_4 NPs was 109.13 (emu/g). After modification using Au NPs, the magnetization saturation value of the as-prepared Fe_3O_4@Au NPs was 98.57 (emu/g). As a result, the decoration of Au NPs on the surface of Fe_3O_4 NPs could weaken the magnetic saturation value of the Fe_3O_4 NPs. However, the magnetic saturation value of flower-like Fe_3O_4@Au NPs still remained at a high level (90.32%), and the Fe_3O_4@Au NPs could still be easily collected using a using a permanent magnet.

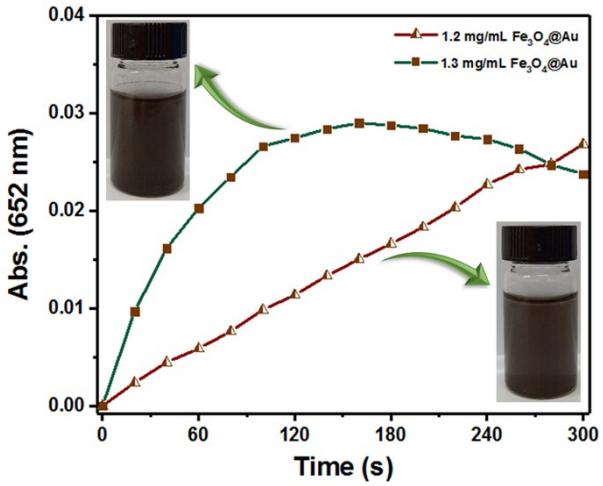

Fig. S5 Catalytic activities of flower-like Fe_3O_4@Au NPs tested using TMB as the substrate [50 mM H_2O_2, 500 μM TMB (pH 7.0) and 1.2 mg/mL flower-like Fe_3O_4 NPs or 1.3 mg/mL flower-like Fe_3O_4@Au NPs, respectively]. The oxidation of TMB was monitored by the absorption increase at λ_{max} = 652 nm at room temperature.

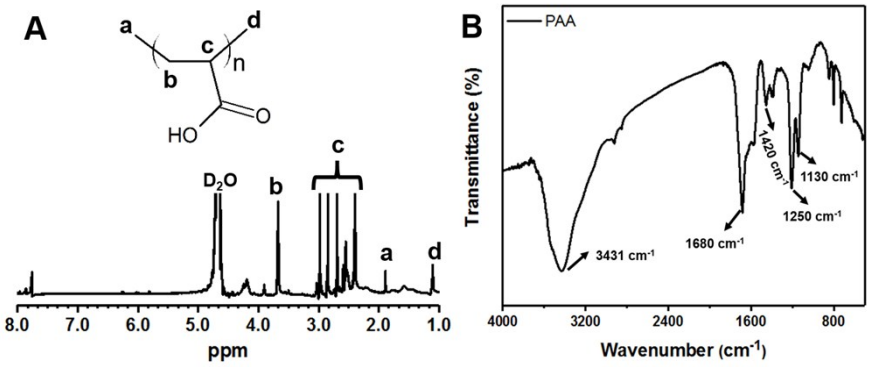

Fig. S6 (A) ^{1}H NMR spectrum of PAA using DMSO as solvent and (B) FTIR spectra of PAA.

The successful synthesis of PAA via free radical polymerization was supported by the ^{1}H NMR spectra. As shown in Fig. S3 (A), the peaks at 1.17 and 1.84 ppm represent the two methyl groups and the peaks range from 2.51 to 3.10 represent methyne groups, and the peaks at 3.71 ppm represent methylene groups, indicating the successful preparation of PAA via free radical polymerization. Further analysis of PAA was also performed using FTIR and the results were shown in Fig. S3 (B). Characteristic peak in FTIR spectrum of PAA at 1130 cm^{-1} corresponded to the methyl groups, and absorption peaks at 1250 cm^{-1} and 1420 cm^{-1} corresponded to methyne and methylene groups, respectively. Moreover, the absorption peaks at 1680 cm^{-1} corresponded to the C=O stretching, and the peak at 3431 cm^{-1} was attributed to the intramolecular hydrogen bond stretch which were formed by vast carboxyl groups.

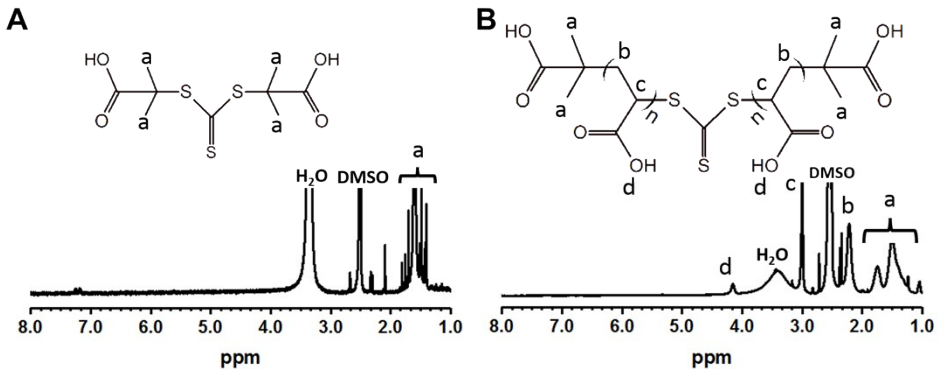

Fig. S7 ^{1}H NMR spectrum of (A) RAFT-b and (B) PAA using DMSO as solvent.

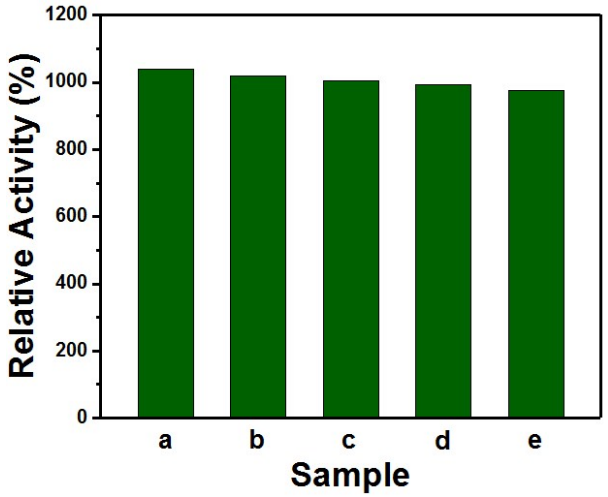

Fig. S8 Relative catalytical activity of the reclaimed bi-nano-bionic enzymes after RAFT polymerization tested using TMB as the substrate [50 mM H_2O_2, 500 μM TMB (pH 7.0) and 1.2 mg/mL flower-like Fe_3O_4@Au NPs]. The oxidation of TMB was monitored by absorption increase at 652 nm at room temperature. (Tips: a represents the freshly-prepared Fe_3O_4@Au NPs, while b-e represents the reclaimed Fe_3O_4@Au NPs for RAFT polymerization for once, twice, three times and four times respectively.)

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