Supporting Information

for

Nanoflowers-Shaped Biocatalyst with Peroxidase Activity Enhances the Reversible Addition-Fragmentation Chain Transfer Polymerization of Methacrylate Monomers

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Experimental

Materials

Copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) was purchased from Aladdin Reagents (Shanghai, China). Hydrogen peroxide (H_2O_2) was purchased from Beijing Chemicals (Beijing, China). Acetylacetone (ACAC) was purchased from Energy Chemical Company (Shanghai, China). Proteins including albumin from bovine serum albumin (BSA) (lyophilized powder) were purchased from Sigma-Aldrich. Phosphate buffered saline (PBS, 1X, pH 7.4) was purchased from Invitrogen. 2-Cyanoprop-2-yl-dithiobenzoate (CPDB) was purchased from J&K Scientific (Beijing, China). *N,N*-Dimethylaminoethyl methacrylate (DMAEMA) and poly(ethylene glycol) methyl ether methacrylate (M_n = 500 g/mol, $PEGMA_{500}$) were purchased from Sigma-Aldrich and passed through a column of basic alumina to remove inhibitors before use.

Characterization

Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Avance III NMR spectrometer (400 MHz) using D_2O as solvent. Chemical shifts (in ppm) were reported downfield using tetramethylsilane as an internal standard. Gel permeation chromatography (GPC) was performed on a Malvern instrument, equipped with a PLgel MIXED guard column followed by a PLgel MIXED guard column (molecular weight range 2.0×10^2–4.0×10^5 g/mol), column thermostated to 60 °C and calibrated by linear polystyrene standards. *N,N*-Dimethylformamide (DMF) was used as the eluent at a flow of 0.8 mL/min at 60 °C. Scanning electron microscopy images were captured on a Hitachi FE-SEM S-4800 instrument with an acceleration voltage of 3 kV. The samples were prepared by depositing sample dispersion onto a freshly cleaved silicon wafer surface.

Preparation of BSA-$Cu_3(PO_4)_2$•$3H_2O$ hybrid nanoflowers

The synthesis of BSA-$Cu_3(PO_4)_2$•$3H_2O$ hybrid nanoflowers were accomplished according to previous report with a minor modification. Typically, 1.33 mL of aqueous $CuSO_4$ solution (120 mM) in molecular biology grade water was added into 20 mL of PBS (pH 7.4) containing 0.1 mg/mL proteins, then incubated at 25 °C for 3 days. After that, blue precipitates were formed at the bottom of mixture and collected by centrifugation and lyophilization. The collected powder was washed by water and centrifuged at 8 000 rpm for 5 min at least 3 times in order to remove all unreacted components.

RAFT polymerization initiated by BSA-$Cu_3(PO_4)_2$•$3H_2O$ hybrid nanoflowers

Poly(DMAEMA) was prepared *via* RAFT polymerization initiated by BSA-$Cu_3(PO_4)_2$•$3H_2O$ hybrid nanoflowers in a mixed solvent (DMF/H_2O = 1/1) with hydrophobic chain transfer agent (CTA), 2-cyanoprop-2-yl-dithiobenzoate (CPDB). In a typical polymerization process, the feed molar ratio of [DMAEMA] : [CPDB] : [H_2O_2] : [ACAC] was maintained at 400 : 1: 0.0028 : 5.57, DMAEMA (2.2 g, 13.99 mmol), CPDB (7.74 mg, 0.035 mmol), BSA-$Cu_3(PO_4)_2$•$3H_2O$ hybrid nanoflowers (4 mg), ACAC (20 μL, 0.1948 mmol), DMF (2 mL) and H_2O (2 mL) were introduced to the branch-necked flask, followed by degassed by three freeze-vacuate-thaw cycles and then heated at 30 °C under nitrogen in a thermostated oil bath. Then, H_2O_2 (10 μL, 9.79×10^{-5} mmol) was injected into the reaction mixture to trigger reaction. After the desired time, the reaction mixture was diluted with water before centrifugation to collect BSA-$Cu_3(PO_4)_2$•$3H_2O$ hybrid nanoflowers. Before lyophilization, the mixture was purified by dialysis against deionized (DI) water at room temperature using a dialysis membrane (molecular weight cutoff: 3500 g/mol). To study the kinetics, samples were periodically withdrawn from the branch-necked flask and filtrated for ^{1}H NMR and GPC analysis.

RAFT polymerization initiated by recycled BSA-$Cu_3(PO_4)_2$•$3H_2O$ hybrid nanoflowers

To evaluate the recyclability of BSA-$Cu_3(PO_4)_2$•$3H_2O$ hybrid nanoflowers, the reaction mixture was centrifuged at 10 000 rpm for at least 20 min and washed 3 times with distilled water after each batch of polymerization. After lyophilization, the nanoflowers were then employed in the next reaction cycle. In the first polymerization cycle, in order to offset inevitable loss of nanoflowers during post-processing, we employed 20 mg nanoflowers. The feed molar ratio of [DMAEMA] : [CPDB] : [H_2O_2] : [ACAC] was maintained at 400 : 1 : 0.0028 : 5.57, and DMAEMA (11 g, 69.95 mmol) was introduced to the branch-necked flask in the first cycle. After degassed by three freeze-vacuate-thaw cycles, heated at 30 °C under nitrogen in a thermostated oil bath, and then triggered by H_2O_2 (50 μL, 4.895×10^{-4} mmol). After polymerized for 6 h, the reaction mixture was diluted with water before centrifugation to collect BSA-$Cu_3(PO_4)_2$•$3H_2O$ hybrid nanoflowers. The nanoflowers were recovered by centrifuging at 10000 rpm for 20 min and washed three times with distilled water. The monomer conversion was determined by ^{1}H NMR spectroscopy, and the molecular weight was analyzed *via* GPC using DMF as the mobile phase with 3.5 mM tetrabutylammonium bromide (TBABr).

Synthesis of polymers with different chains lengths

Poly(DMAEMA) polymers were synthesized with different target degrees of polymerization (DPs), including 50, 100, 200, 400, 700 and 1 000. The concentration of monomer and the polymerization procedures were the same as typical polymerization procedures, while, the amount of nanoflowers (4 mg) and the CPDB (7.74 mg, 0.035 mmol) were kept at a constant value. After each experiment, the monomer conversion and molecular weight were characterized by ^{1}H NMR spectroscopy and GPC.

Chain extension of poly(DMAEMA) *via* RAFT polymerization initiated by nanoflowers

The macro-CTA (poly(DMAEMA)) was synthesized under typical polymerization procedures. The molar ratio of [DMAEMA] : [CPDB] : [H_2O_2] : [ACAC] was maintained at 200 : 1 : 0.0028 : 5.57, and the monomer (1.1 g, 7.0 mmol) was introduced to the branch-necked flask. After 12 h of polymerization, the product was handled by intermediate separation and lyophilization procedures for subsequent chain extension. During the chain extension step, the feed molar ratio of [DMAEMA] : [macro-CTA] : [H_2O_2] : [ACAC] was maintained at 200 : 1 : 0.0028 : 5.57. Followed by reaction for 4 h, the synthesized polymer was sampled for ^{1}H NMR and GPC analyses.

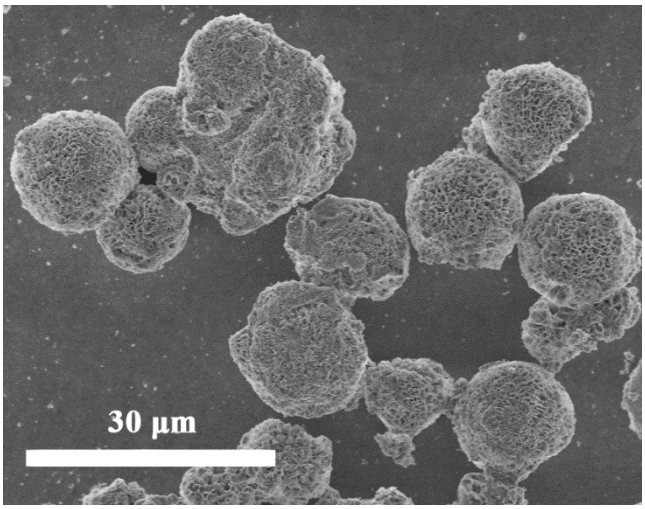

Figure S1. SEM image of $Cu_3(PO_4)_2$•$3H_2O$ hybrid nanoflowers prepared by a simple and facile method of self-assembly.

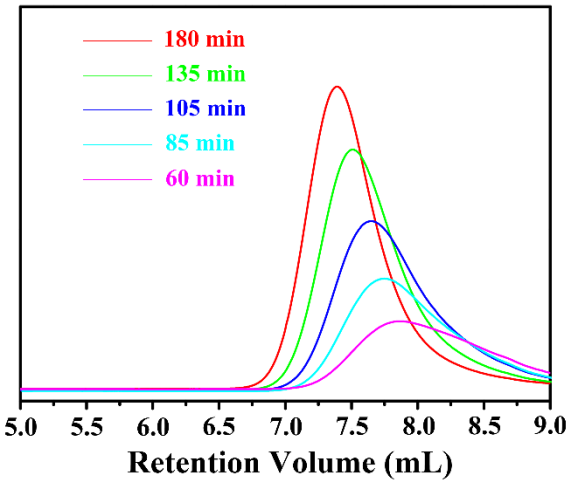

Figure S2. Corresponding GPC traces of poly(DMAEMA) polymers synthesized by RAFT polymerization with 10 µL of ACAC feed volume at 30 °C under the following reaction conditions [DMAEMA] : [CPDB] : [H_2O_2] : [ACAC] = 400 : 1 : 0.0028 : 2.79.

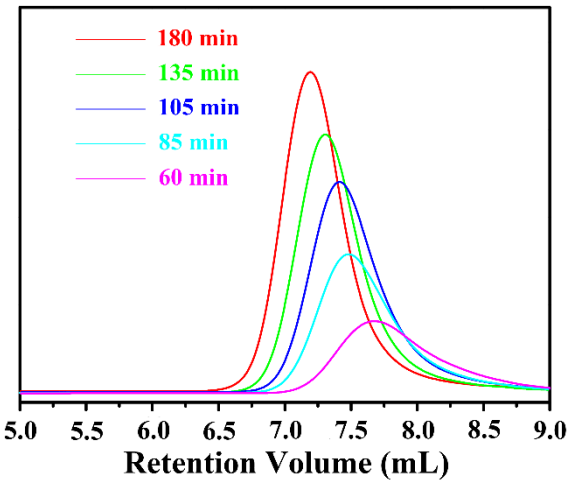

Figure S3. Corresponding GPC traces of poly(DMAEMA) polymers synthesized by RAFT polymerization with 15 µL of ACAC feed volume at 30 °C under the following reaction conditions [DMAEMA] : [CPDB] : [H_2O_2] : [ACAC] = 400 : 1 : 0.0028 : 4.18.