Supporting Information for

Microgels Coating Magnetic Nanoparticles via Bienzyme-mediated Free-radical Polymerization for Colorimetric Detection of Glucose

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1. Materials

Glucose oxidase from Aspergillus niger (GOx, MW = 160 kDa, EC 1.1.3.4), Horseradish Peroxidase (HRP, MW = 44 kDa, EC.1.11.1.7), Poly (ethylene glycol) dimethacrylate (PEGMA, mw=360) and Poly (ethylene glycol) diacrylate (PEGDA, mw=250) were purchased from Sigma-Aldrich. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Energy Chemical, PEG (Succinimidyl Carboxymethyl Ester)2 (SCM-PEG_{1000}-SCM) was purchased from JenKem Technology Co., Ltd. β-D-glucose, maltose, α-lactose, D-fructose, o-phenylenediamine (OPD) and Acetylacetone (ACAC) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All materials were used without further purification.

2. Instruments

UV-vis spectra were obtained by UV-2700 (Shimadzu Corporation). Dynamic light scattering (DLS) studies of the microgels were conducted on Zetasizer Nano instrument (Malvern Instruments Ltd., United Kingdom). Scanning electron microscope (SEM) images were obtained on Hitachi S-4800 with 3 kV accelerating voltages. TEM images were taken with a JEOL 2100 microscope (Japan) operated at 200 kV.

3. The detailed preparation of the microgels

The magnetic Fe_3O_4 nanoparticles(MNPs) were synthesized according to the literature.[1] Typically, the magnetite particles were synthesized by a modified solvothermal reaction at 200 ˚C by reduction of $FeCl_3$ with EG in the presence of sodium acetate and trisodium citrate (Na_3Cit). In detail, $FeCl_3$ (0.65 g, 4.0 mmol) and trisodium citrate (0.26 g, 0.88 mmol) were first dissolved in ethylene glycol (20 mL), afterward, NaAc (1.20 g) was added with stirring. The mixture was

stirred vigorously for 30 min and then sealed in a Teflon-lined stainless-steel autoclave (50 mL capacity). The autoclave was heated at 200 ˚C and maintained for 10 h, and then cooled to room temperature. The black products were then washed with ethanol and deionized water for several times and then were resuspended in deionized water for further use.

Carboxyl-functionalized MNPs (2 ml, 15 mg/mL) were redispersed in 20 mL phosphate buffer solution at pH 5.8 by sonication, with EDC (200 mg), NHS (200 mg), and stirred for 2h. The nanoparticles were then separated by magnetic decantation, washed, and resuspended in 20 mL glucose oxidase solution (1 mg/mL, pH 5.5), a solution prepared by dissolving 20 mg enzyme in 20 mL phosphate buffer solution at pH 5.5. The reaction mixture was stirred for 2h. The residual unreacted glucose oxidase were separated from GOx-MNPs nanoparticles by magnetic decantation and then washed by deionized water for three times. The supernatant and the last wash solution were kept for determination of enzyme concentration by Bradford method. The GOx-MNPs nanoparticles were then resuspended in 40 mL phosphate buffer solution at pH 7.0 with 40 mg SCM-PEG_{1000}-SCM, and stirred for 2h. The MNPs-GOx-SCM nanoparticles were separated by magnetic decantation and then washed with deionized water for three times. The obtained nanoparticles were resuspended in 40 mL HRP solution (0.5 mg/mL, pH 5.5), and stirred for 2h. The residual unreacted HRP were separated from the magnetite nanoparticles by magnetic decantation and then washed with deionized water for three times. The supernatant and the washed solution were kept for determination of enzyme concentration by UV-vis absorption spectroscopy (absorption at 403 nm). The obtained MNPs-GOx-HRP nanoparticles were redispersed in 20 mL deionized water at approx. 4 ℃ for use.

The shell synthesis was achieved by the polymerization of a comonomer mixture in the presence of the MNPs-GOx-HRP as seeds. Formation of the shell was manipulated by decanting the coating solution (10 mL) into the obtained MNPs-GOx-HRP nanoparticles suspension (200 μL) for interfacial polymerization. The aqueous coating solution was constituted of $PEGMA_{360}$ (10%, v/v), $PEGDA_{250}$ (1%, v/v), glucose (0.1 M), ACAC (1%, v/v) and deionized water. The following interfacial polymerization was initiated by the bienzyme (GOx and HRP), for the appropriate stirring time. The supernatant was then removed by magnetic separation, and the obtained nanoparticles were resuspended in deionized water for characterization.

4. Test of magnetization saturation value (Ms)

The magnetic properties of the obtained magnetic nanoparticles and magnetic core-shell microgels were investigated with a vibrating sample magnetometer (VSM). The samples of magnetic nanoparticles and magnetic core-shell microgels were freeze-dried before this test. The Fe_3O_4 and microgels have the magnetization saturation values (Ms) of 103.90 and 93.97 emu/g,

indicating that the core-shell microgels retained strong magnetization of the magnetic nanoparticles, thus providing an extremely efficient way for separating these enzymes. (Fig. S1)

5. Test of Electron Paramagnetic Resonance (EPR)

The EPR spectra were recorded on a Bruker EMX-8/2.7 Spectrometer operating at 9.873 GHz (microwave power: 20 mW; modulation frequency: 100 kHz; modulation amplitude: 0.5 G; receiver gain: 4×105). As for the signal of initiation radical, the mixture of GOx-HRP/ACAC /glucose initiation system and the spin trap (POBN) in deionized water was rapidly transferred to a standard quartz capillary (1 mm in diameter) and placed into the EPR spectrometer. The spectrum recorded after 5 min reaction is shown in Fig. S2b, indicating the successful generation of ACAC radicals. Fig. S2a shows the mechanism of the generation of ACAC radicals for initiating the polymerization of hydrogels.

6. Test of catalytic activity and reusability

We used glucose and o-phenylenediamine (OPD) as the substrates to assess the catalytic activity of the bound and native enzymes by UV-vis spectroscopy at 450 nm. In the participation of glucose and OPD, an enzymatic cascade reaction will take place catalyzed by GOx and HRP, which was summarized in Scheme S1. Spectroscopic properties of HRP and GOx were used to quantify the coverage on the magnetic nanoparticles. we employed Bradford methods to quantify GOx concentration. As for HRP, the Soret peak at 403 nm was used to quantify its concentration, since the HRP heme has a maximal peak at 403 nm.

Kinetic measurements were performed as follows: The mixture of the OPD (20 mM) and glucose in 2 mL buffer was catalyzed by 3 μg/mL GOx and 5 μg/mL HRP in different states (native or co-immobilized). During the reaction course, the reaction mixture was incubated at 25˚C for 5 min. During the incubation, absorbance at 450 nm was collected continuously with a UV-vis spectrometer. The concentrations of the product in buffer were corrected according to the molar extinction coefficients in aqueous buffer. The increase of the absorbance at 450 nm in the first minute was measured by the two types of GOx and HRP. Then the initial reaction rate was obtained by linear fitting the product concentration with time. By changing the glucose concentration from 10 mM to 1 mM with a fixed OPD concentration at 10 mM, we obtained a series of initial reaction rates for constructing the Lineweaver-Burk plot to estimate the kinetic constant values.

To test the reusability of the microgels-immobilized enzymes, we used fresh and recovered enzymes in core-shell microgels (400 μL) to catalyze the oxidation of OPD (10 mM) in 2 ml buffer. The total amount of phenazine-2, 3-diamine was measured by UV-vis spectrometer after

15 minutes reaction. The recovered co-confined enzymes were separated from the reaction mixture by a magnet, then washed with buffer 3 times to remove the product. The recycled co-confined enzymes were then mixed with a fresh reaction mixture again to measure the 15 minutes total amount of phenazine-2, 3-diamine.

7. Test of the glucose detection

Glucose detection was performed as follows: 800μL glucose with various concentrations was added to 1.2 mL phosphate buffer (20 mM, pH 5.5) containing the core-shell microgels (200 μL) and OPD (1mL, 20 mM). The obtained mixture was incubated for 25 min at 35 ˚C. The magnetic nanoparticles were then removed from the reaction solution by magnet, and the remaining solution was used to carry out the adsorption spectroscopy measurement. As for the control experiments, 5 mM fructose, 5 mM lactose and 5 mM maltose were used instead of glucose for the experiments.

8. Figures

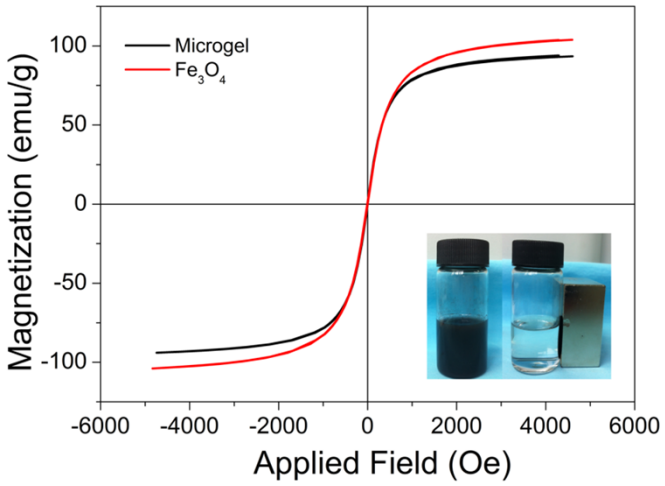


Fig. S1 Magnetization curve of Fe_3O_4 and microgels. Inset: image of solution before and after magnetic separation of microgels.

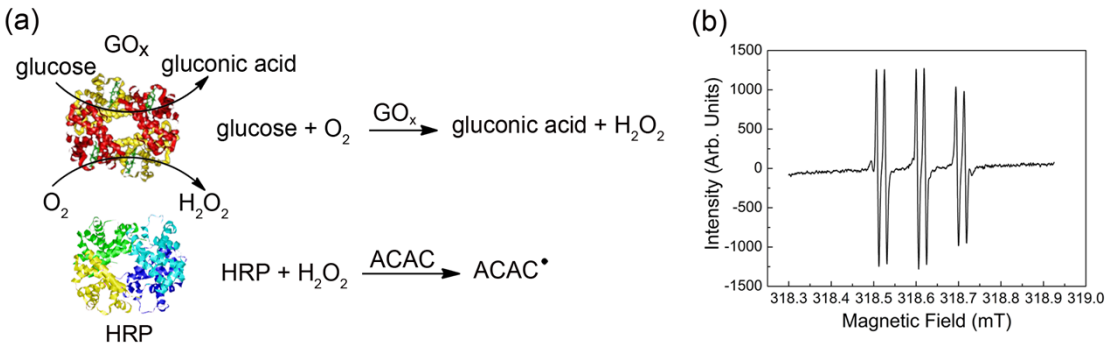
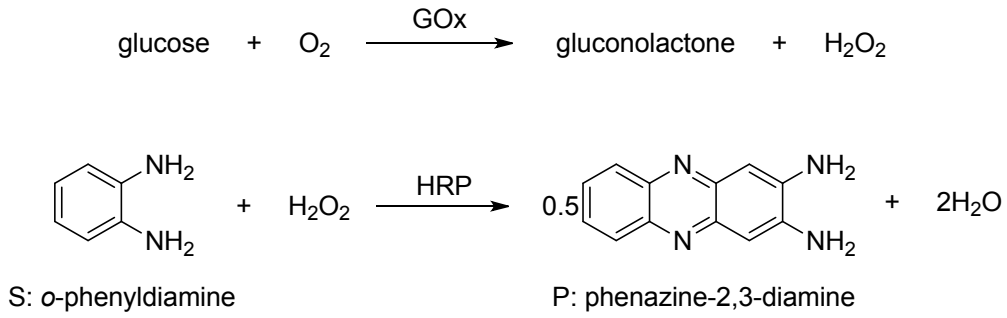


Fig. S2 (a) The mechanism of the bienzyme-mediated free-radical polymerization of the core-shell microgels; (b) EPR spectra of the POBN radical adducts formed in the GOx/HRP-mediated ternary initiation system without monomer PEGMA.



Scheme S1. Schematic illustration of oxidation color reaction of OPD by two enzymes system.

Table S1. Comparison of glucose detection using colorimetric method of the previously reported colorimetric glucose sensors.

Materials	Substances	LOD for glucose	Reference
Graphene Oxide	TMB	1 μM	44
Fe_3O_4 NPs	ABTS	30 μM	49
GO-Fe_3O_4	TMB	0.74 μM	51
Co_3O_4 NPs	TMB	5 μM	56
$ZnFe_2O_4$ NPs	TMB	0.3 μM	57
AuNPs	TMB	4 μM	58
GOx&HRP-$Cu_3(PO_4)_2$ •$3H_2O$ nanoflowers	TMB	0.2 μM	59

References

1. J. Liu, Z. K. Sun, Y. H. Deng, Y. Zou, C. Y. Li, X. H. Guo, L. Q. Xiong, Y. Gao, F. Y. Li and D. Y. Zhao, *Angew. Chem. Int. Ed.*, 2009, **48**, 5875-5879.