

Supporting Information

Open-mouthed Hybrid Microcapsules with Elevated Enzyme Loading and Enhanced Catalytic Activity

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Experimental

Materials:

Poly (allylamine hydrochloride) (PAH, $M_w \approx 70$ kDa), Ti(IV)-bis-(ammonium lactate) dihydroxide (Ti-BALDH, 50 wt.% aqueous solution), tris(hydroxymethyl)-amionmethane (tris) and fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich Chemical Co. Calcium chloride (CaCl_2), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), hydrochloric acid (HCl), ethylenediaminetetraacetic acid (EDTA), sodium dihydrogen phosphate and disodium hydrogen phosphate were obtained from Guangfu Reagent Chemicals Co. (Tianjin, China). Water used all through experiments was prepared *via* a Millipore Milli-Q purification system and had a resistivity higher than $18 \text{ M}\Omega \cdot \text{cm}$. All other reagents were analytical grade and used without further purification.

Fluorescent-labeled enzymes were prepared using overnight incubation at room temperature of their mixtures with FITC phosphate buffer solution (PBS, 50 mM, pH 8.0, protein concentration 1 mg mL^{-1} , $[\text{dye}]/[\text{protein}]=5$) followed by exhaustive dialysis (MW cutoff 14 kDa) against PBS (50 mM, pH 7.0) for 72 h and deionized water for 48 h.

Preparation of the open-mouthed hybrid microcapsules (HMCs):

PAH-segregated CaCO_3 (PCa) microspheres with narrow size distribution (average diameter around 3-5 μm) were used as sacrificial templates, which were prepared according to the coprecipitation method previously described in the literature.¹⁻³ Briefly, a certain amount of PAH (2 mg) was dissolved in 1 mL of 330 mM CaCl_2 solution in a beaker under magnetic agitation ($\sim 800 \text{ rpm}$), into which an equal volume of 330 mM Na_2CO_3 solution was rapidly poured at room temperature ($\sim 20^\circ\text{C}$). The mixture was vigorously stirred for 30 s. After gently stirring for 10 min followed by 3 min-standing, PCa microspheres were centrifuged and washed for 3 times with deionized water to get rid of the free PAH.

Then, PCa microspheres were dispersed in 1 mL of Ti-BALDH aqueous solution with a certain concentration (50 mM, pH 7.0). After the mixture was kept in a vessel under mild agitation for 30 min, centrifugation and washing with deionized water were conducted several times until the excess Ti-BALDH was washed away. The resultant particles were denoted as TPCa microspheres.

Next, TPCa microspheres were dispersed in 1.25 mL of pre-polymerized dopa

aqueous solution (oligodopa, pH 6.8±0.2). The initial dopa concentrations were 2 mg mL⁻¹. After the mixture was kept in a vessel under mild agitation for 30 min, centrifugation and washing with deionized water were conducted several times until the excess oligodopa was washed away. The resultant particles were denoted as DTPCa microspheres.

Finally, DTPCa microspheres were incubated in 1 mL, 50 mM EDTA solution for 5 min under shaking to obtain the open-mouthed hybrid microcapsules (HMCs), which were further washed with water and fresh EDTA solution, each for 3 times, with the assistance of centrifugation operation (3000 rpm, 3 min).

Enzyme immobilization on the open-mouthed HMCs:

A certain amount (2.0 mg) of the open-mouthed HMCs were transferred into 1.0 mL of 0.5 mg mL⁻¹ CAT solution (50 mM tris-HCl buffer solution, pH 7.0), and then the immobilization process continued for 8 h under ambient conditions. After centrifugation, the obtained enzyme-adsorbed open-mouthed HMCs were washed more than three times with tris-HCl buffer solution.

The enzyme immobilization yield (%; eq. (1)) was used to describe the percentage of total enzyme amount from the free enzyme solution that was immobilized.

$$\text{Immobilized yield (\%)} = M_{\text{enzyme, immobilized}} / M_{\text{enzyme, added}} \times 100\% \quad (1)$$

The enzyme loading capability (mg g⁻¹ (carrier), eq. (2)) was used to describe the amount of the immobilized enzyme per g of the carrier (*microcapsules*).

$$\text{Enzyme loading capability (mg g}^{-1} \text{ (carrier))} = M_{\text{enzyme, immobilized}} / M_{\text{carrier}} \quad (2)$$

where $M_{\text{enzyme, immobilized}}$ was the amount of the enzymes immobilized on the carrier, mg; $M_{\text{enzyme, added}}$ was the amount of the enzymes added in the solution initially, mg; M_{carrier} was the amount of the carrier, g.

Preparation of the enclosed HMCs:

The preparation procedure of the enclosed HMCs was quite similar to that of the open-mouthed HMCs. The difference was from the synthesis process of PCa microspheres. Specifically, a certain amount of PAH (2 mg) was dissolved in 1 mL of 330 mM CaCl₂ solution in a beaker under magnetic agitation (~800 rpm), into which an equal volume of 330 mM Na₂CO₃ solution was rapidly poured at specific temperature. The mixture was vigorously stirred for 30 s. After gently stirring for <1 min instead of 10 min, PCa microspheres were centrifuged and washed for 3 times with deionized water to get rid of the free PAH.

Enzyme immobilization on the enclosed HMCs:

Enzyme encapsulation:

A certain amount of CAT was dissolved in 5 mL of tris-HCl buffer solution (50 mM, pH 7.0). The enzyme solution was added into the 5 mL CaCl₂ solution (final concentration, 0.33 M). The enzyme-encapsulated enclosed HMCs were prepared following the same procedure as described above. The enzyme loading capability can be tailored through changing the amount of CAT.

Enzyme adsorption:

A certain amount of the enclosed HMCs were transferred into 1.0 mL of 0.5 mg mL⁻¹ CAT solution (50 mM tris-HCl buffer solution, pH 7.0), and then the immobilization process continued for 8 h under ambient conditions. After centrifugation, the obtained enzyme-adsorbed enclosed HMCs were washed more than three times with tris-HCl buffer solution. Similar to the adsorption process enzyme on the open-mouthed HMCs, the enzyme adsorption capability of the enclosed HMCs can be tailored through changing the amount of the enclosed HMCs.

Assay of the immobilized enzymes:

The activity of immobilized CAT or free CAT was determined by direct measurement of the decrease in the absorbance of H₂O₂ at 240 nm due to enzymatic decomposition. Briefly, 30 μ L of the immobilized CAT (or free CAT) (*approximately 0.0463 mg of enzyme*) was dispersed in 19.4 mM H₂O₂ in 10 mL of phosphate buffer solution (PBS, 50 mM, pH 7.0) at 25 °C. After 5 min, the decrease in absorbance at 240 nm was recorded, and the activity was calculated.

Characterizations:

High-resolution SEM images were recorded using a field emission scanning electron microscope (FESEM, Nanosem 430). Elemental analysis was accomplished by energy dispersive spectroscopy (EDS) attached to FESEM. FTIR spectra of the microcapsules were obtained on a Nicolet-6700 spectrometer. Thirty-two scans were accumulated with a resolution of 4 cm⁻¹ for each spectrum. Confocal laser scanning microscopy (CLSM) images were taken with a LSM 710 Confocal Microscope. The excitation wavelength was chosen as 488 nm according to the FITC-labeled enzymes. The pore-size distribution of the microcapsules was determined by nitrogen adsorption-desorption isotherm measurements performed at 77 K on a Tristar 3000 gas adsorption analyzer. Pore-size distribution curves were calculated on the basis of

the adsorption branch of nitrogen isotherms using the Barrett-Joyner-Halenda (BJH) method.

Recycling stability

The recycling stability of encapsulated CAT was determined by measuring the residual activity of CAT at 25 °C after it was used for several cycles. The CAT-adsorbed microcapsules were collected by centrifugation after each reaction batch (25 °C, pH 7.0, reaction time 60 min), thoroughly rinsed with PBS, and reused in the next reaction cycle. During all the stability experiments, each result was obtained by averaging three individual experiments.

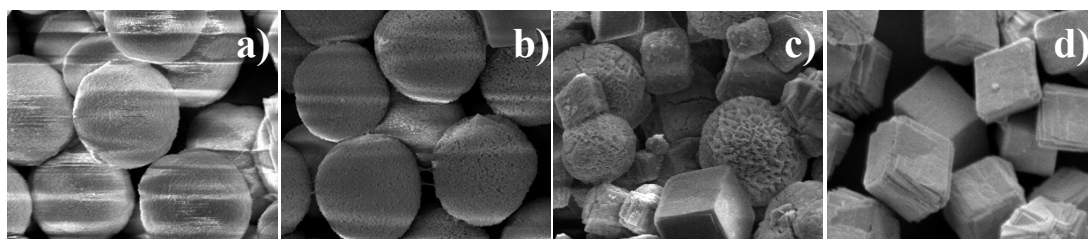


Figure S1. SEM images of PAH-segregated CaCO_3 synthesized through mixing PAH-containing CaCl_2 and NaCO_3 followed by stirring for a) 1, b) 10, c) 30, and d) 60 min.

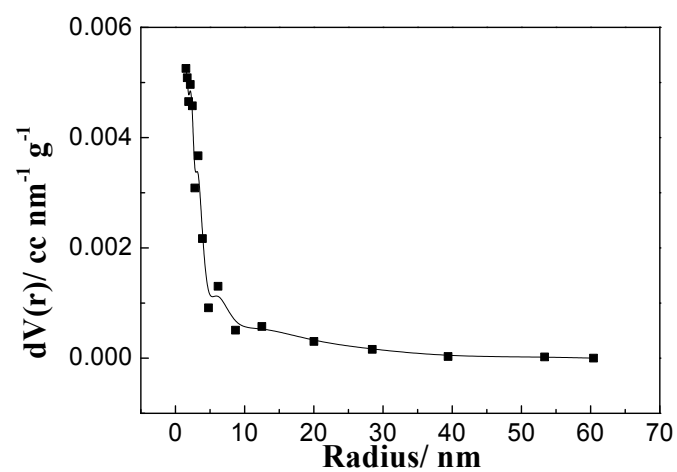


Figure S2. The calculated pore size distribution (Barrett-Joyner-Halenda method) of the open-mouthed HMCs.

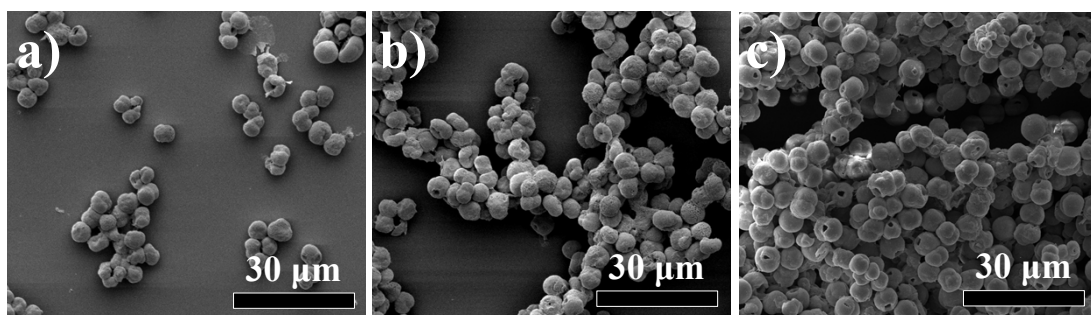


Figure S3. SEM images of the enclosed HMCs utilized for a) encapsulating enzymes and b) adsorbing enzymes, and the open-mouthed HMCs utilized for c) adsorbing enzymes.

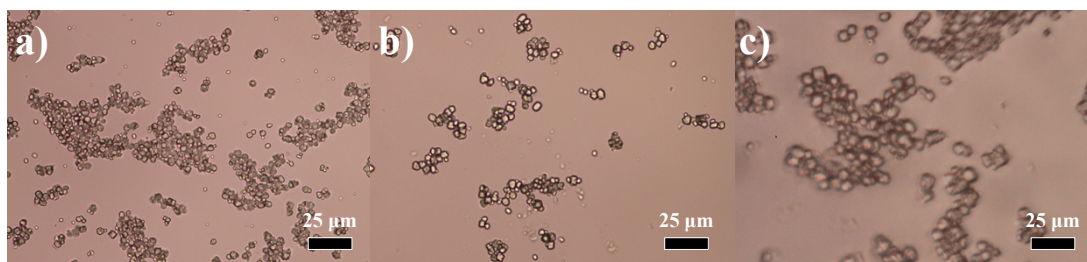


Figure S4. Optical microscopy images of the PAH-segregated CaCO_3 sacrificial templates synthesized from different stirring rate during the mixing of PAH-contained CaCl_2 and Na_2CO_3 aqueous solutions: a) 1200, b) 800, and c) 500 rpm.

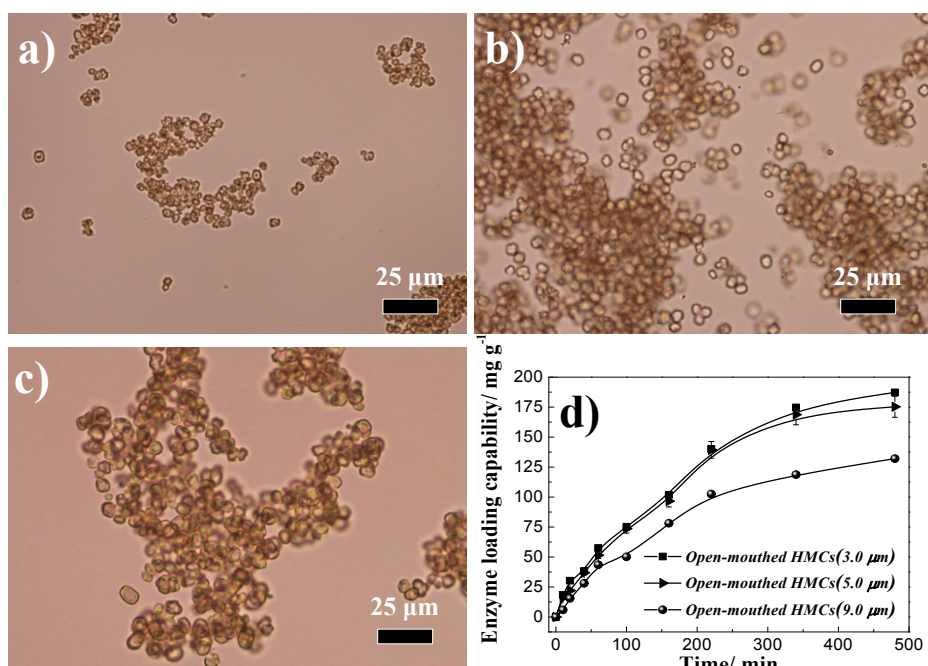


Figure S5. a-c) Optical microscopy images of the open-mouthed HMCs synthesized by using different-sized sacrificial templates: a) 3.0 μm , b) 5.0 μm , and c) 8.0 μm ; and d) the enzyme loading capability as a function of adsorption time for the three kinds of HMCs.

Table 1. The initial reaction rate and equilibrium conversion ratio of H₂O₂ for the enzyme-adsorbed open-mouthed HMCs with different sizes.

Types of enzyme systems	1#	2#	3#
Average capsule size/ μm	3.0	5.0	8.0
Initial reaction rate ^a / % s ⁻¹	0.48	0.40	0.35
Equilibrium conversion ratio ^b / %	98.6	99.0	97.9

^a the initial reaction rate was calculated based upon the initial reaction time of 15 s.

^b the equilibrium conversion ratio was calculated based upon a reaction time of 600 s.

References:

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2. Z. Wang, H. Möhwald and C. Gao, *Langmuir*, 2010, **2**, 1286-1291.
3. J. Shi, W. Zhang, X. Wang, Z. Jiang, S Zhang, X. Zhang, C. Zhang, X. Song and Q. Ai, *ACS Appl. Mater. Interfaces*, 2013, **5**, 5174-5185.