Supporting information

Incorporating Mobile Nanospheres in the Lumen of Hybrid Microcapsules for Enhanced Enzymatic Activity

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EXPERIMENTAL SECTION

Materials

Catalase (CAT) from *bovine liver*, protamine sulfate from *salmon*, and poly(sodium 4-styrenesulfonate) (PSS, M_w *ca.* 70 kDa) were purchased from Sigma-Aldrich Chemical Company. Tetraethylorthosilicate (TEOS), ethanol, ammonium hydroxide, γ-methacryloxypropyltrimethoxysilane (MPS), 2-methylpropenoic acid (MAA), ethylene glycol dimethylacrylate (EGDMA), azobisisbutyronitrile (AIBN), acetonitrile, sodium silicate were obtained from Guangfu Chemical Company. Water used all through experiments was prepared *via* a Millipore Milli-Q purification system and had a resistivity higher than 18 MΩ·cm. All other reagents were of analytical grade, and used without further purification.

Synthesis of hydrophilic silica nanospheres

The synthesis of hydrophilic silica nanospheres was described as follows.[1] Firstly, pristine silica nanospheres with diameter *ca.* 200 nm were synthesized according to Stöber method:[2] TEOS (10 mL) was added into the mixture of ethanol (200 mL), water (20 mL) and aqueous solution of ammonium hydroxide (7 mL, 25-28%) under vigorous stirring at 30 ^{o}C for 24 h. Excess MPS was then added into the silica mixture with a reaction time of 24 h to functionalize the silica with C=C bonds. The coating of hydrophilic polymer on MPS-functionalized silica spheres was carried out by distillation-precipitation polymerization in acetonitrile. In a dried 100 mL flask attached to a fractionating column, Liebig condenser, and receiver, 200 mg of silica particles was dispersed into 80 mL acetonitrile assisted by sonication. The monomer MAA (0.35 mL, 371 mg), crosslinker EGDMA (0.65 mL, 624 mg), and initiator AIBN (20 mg, 2 *wt*% relative to the co-monomers) were then dissolved into the MPS-modified silica solution. The mixture was heated from ambient temperature till

boiling state and then the solvent was distilled from the reaction system. After 40 mL of acetonitrile was distilled out, the reaction was terminated and the resultant nanospheres were purified by three cycles of ultracentrifugation, decanting, and re-suspension in acetonitrile. The acquired hydrophilic polymer coated silica nanospheres was denoted as hydrophilic silica nanospheres.

Synthesis of nanospheres-encapsulated protamine/silica (NEPS) microcapsules and pristine protamine/silica (PPS) microcapsules

In the synthesis of NEPS microcapsules, nanospheres-doped $CaCO_3$ microparticles were used as sacrificial templates, which were prepared according to the co-precipitation method described in the previous literature.[3] Briefly, hydrophilic silica nanospheres (5 mg) and PSS (30 mg) was completely dissolved in 10 mL of $CaCl_2$ solution (330 mM) in a beaker at room temperature. Then, an equal volume of Na_2CO_3 solution (330 mM) was rapidly added into the beaker under magnetic agitation (800 rpm), and stirred continuously for 20 s. After placed without stirring for 10-15 min, the deposit was centrifuged, and washed three times with water. Finally, nanospheres-doped $CaCO_3$ microparticles with spherical shape of about 5 μm in size were obtained. In a typical layer-by-layer (LbL) assembly of NEPS microcapsules, the nanospheres-doped $CaCO_3$ microparticles as-prepared above were firstly dispersed in protamine aqueous solution (5 mL, 2 mg mL^{-1}) containing NaCl (0.5 M). After shaking for 15 min, $CaCO_3$ microparticles coated with protamine were collected by centrifugation, and washed twice with water to remove the residual protamine. Subsequently, these microparticles were suspended in sodium silicate solution (5 mL, 30 mM), and shaken for 15 min followed by centrifugation. After washed and centrifuged twice with water, nanospheres-doped $CaCO_3$ microparticles coated with one protamine layer and one silica layer were obtained. This procedure could be

repeated until bilayers with desired number were deposited. NEPS microcapsules were obtained after removal of $CaCO_3$ cores through incubating the microparticles coated with $(protamine/silica)_x$ bilayers in EDTA solution (0.015 M).

Additionally, for comparison purpose, PPS microcapsules without nanospheres enclosed in the lumen are prepared through similar procedure. The only difference locates at the non-doping of nanospheres during the preparation of $CaCO_3$ templates.

Synthesis of enzyme-encapsulated NEPS microcapsules and enzyme-encapsulated PPS microcapsules

To encapsulate enzymes in the NEPS microcapsules, CAT was added in the co-precipitation system to prepare nanospheres-doped $CaCO_3$ microparticles. Thereafter, nanospheres-doped $CaCO_3$ microparticles with CAT were used as sacrificial templates to prepare CAT-encapsulated NEPS microcapsules according to the procedure described in the previous section. The activity of free and immobilized CAT was determined by directly measuring the change of H_2O_2 absorbance at 240 nm at room temperature and pH 7.0.

Additionally, for comparison purpose, CAT is also encapsulated in PPS microcapsules. The synthesis procedure is similar as describe above.

Characterizations

The zeta-potentials of $CaCO_3$ microparticles and protamine/silica hybrid microcapsules were measured in water using a Brookhaven zeta-potential analyzer. Five parallel measurements were conducted for every sample, and the average value was calculated. High-resolution SEM images were recorded using a field emission scanning electron microscopy (FESEM, Nanosem 430). FTIR spectra of the prepared microcapsules were obtained on a Nicolet-6700 spectrometer. Thirty two scans were accumulated with a resolution of 4 cm^{-1} for each spectrum.

Mass transfer property

The mass transfer property of enzyme (CAT) and H_2O_2 released from the capsule lumen to bulk solution for NEPS and PPS microcapsules was investigated. Briefly, for the CAT release experiment, 60 μL of concentrated CAT-encapsulted microcapsules solution was immersed in 10 mL of a well-stirred pH 7.0, 30 mM tris-HCl buffer solution. At designed time intervals, the CAT concentration in the bulk solution was determined by a UV/vis spectrophotometer (Hitachi U-3010) as the detector. The fraction of relased CAT in solution was expressed as eq. (1):

$$Fraction\ release\ of\ CAT\ (\%)=(V\times C_{t,\ CAT}/M_{CAT})\times 100 \quad (1)$$

where $C_{t,\ CAT}$ was the CAT concentration in the bulk solution at time t (mg L^{-1}), V was the solution volume (L), and M_{CAT} was the amount of initially encapsulated CAT (mg).

For the H_2O_2 release experiment, 60 μL of concentrated microcapsules solution was immersed in 10 mL of a well-stirred pH 7.0, 30 mM tris-HCl buffer solution containing 19.4 mM H_2O_2 solutions. After saturated adsorption, the as-acquired H_2O_2-encapsulated microcapsules were quickly collected by centrifugation, which were then dispersed into 10 mL of a well-stirred pH 7.0, 30 mM tris-HCl buffer solution. At designed time intervals, the H_2O_2 concentration in the bulk solution was determined by a UV/vis spectrophotometer (Hitachi U-3010) as the detector. The fraction of H_2O_2 in solution was expressed as eq. (2):

$$Fraction\ release\ of\ H_2O_2\ (\%)=(V\times C_{t,\ H2O2}/M_{H2O2})\times 100 \quad (2)$$

where $C_{t,\ H2O2}$ was the H_2O_2 concentrations in the bulk solution at time t (mg L^{-1}), V was the solution volume (L), and M_{H2O2} was the amount of saturatedly adsorbed H_2O_2 (mg). Herein, it should be noted that once the H_2O_2-encapsulated microcapsules was

despersed into the solution, the simultaneously measured concentration of H_2O_2 in solution was normalized into zero.

Assay of enzyme activity and activity recovery of the immobilized enzymes[5]

The immobilization yield (eq. (3)) was used to describe the percentage of total enzyme activity from the free enzyme solution that was immobilized. The "activity that was immobilized" (eq. (4)) was determined by measuring the total residual enzyme activity that remained in the enzyme solution after immobilization and by subtracting this activity from the total starting activity.

Immobilized yield (%)=(immobilized activty/initial activity)×100 (3)

Immobilized activity=initial activity-residual activity (4)

The immobilization efficiency (eq. (5)) was used to describe the percentage of enzyme activity that was observed in the immobilized systems.

Immobilization efficiency (%)=(observed activity/immobilized activity)×100 (5)

Activity recovery (eq. (6)) was used to describe the success of the total immobilization process, which was the immobilization yield multiplied by the immobilization efficiency. With activity recovery, the activity of the immobilized enzymes was compared to that of the total starting activity of the free enzyme.

Activity recovery (%)=(observed activity/initial activity)×100 (6)

The activity of free and immobilized CAT was determined by measuring the decrease in the absorbance of H_2O_2 at 240 nm after 5-min reaction due to the decomposition of H_2O_2. Briefly, the encapsulated enzyme (or free enzyme) was added to the H_2O_2 solution (19.4 mM) with rapid stirring. The decrease in absorbance at 240 nm with time was recorded immediately after the enzyme was mixed into the above solution.

Kinetic Parameters (K_m and V_{max})

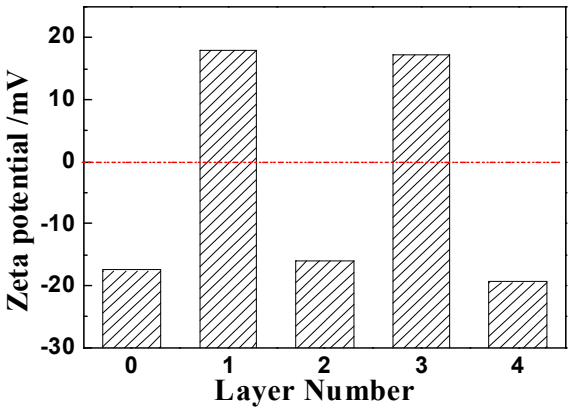
K_m and V_{max} for the free and immobilized enzymes were determined using the Michaelis-Menten model, given by eq.(5)

$$1/V=(K_m/V_{max})\times(1/[S])+(1/V_{max}) \quad (5)$$

where V was the initial reaction rate (mM min^{-1}), $[S]$ was the initial substrate concentration (mM), V_{max} was the maximum reaction rate attained at infinite initial substrate concentration(mM min^{-1}), and K_m was the Michaelis-Menten contant (mM).

Recycling stability

The recycling stability of encapsulated CAT was determined by measuring the residual activity of CAT at room temperature after it was used for several cycles. The microcapsules containing CAT were collected by centrifugation after each reaction batch (25 °C, pH 7.0, reaction time 5 min), thoroughly rinsed with phosphate buffer, and reused in the next reaction cycle. During all the stability experiments, each result was obtained by averaging three individual experiments.



Fgiure S1. Zeta potential as a function of layer number of $CaCO_3$ microparticles coated with protamine/silica layers. The first measurement (layer 0) was the surface potential of nanospheres-doped $CaCO_3$ microparticles.

Figure S1 validated the LbL assembly process by providing the surface zeta-potential of the $CaCO_3$ microparticles after the deposition of each layer. Starting from a nanospheres-doped $CaCO_3$ microparticle with a zeta-potential of -17.56 mV, the zeta-potential alternated between -20 mV and 20 mV during the deposition of 2 protamine/silica bilayers. The obvious switching of zeta-potential indicated alternative deposition of protamine and silica directly on the $CaCO_3$ microparticles. In fact, the pI value of protamine was about 12, which rendered the surface of the protamine layer to be positively charged at pH 7.0. Therefore, the protamine layer could adsorb and concentrate the negatively charged silicates *via* electrostatic attractive interactions. The presence of protamine induces a rapid polycondensation of silicates with the formation of silica particles and eventually a silica layer.[5] Furthermore, because protamine has a strong interaction with silicates, an alternative soaking process can be performed where after one silica layer has been deposited,

protamine can be adsorbed to the surface from solution and another layer of silica can be constructed, which was in accordance with our previous results.[6]

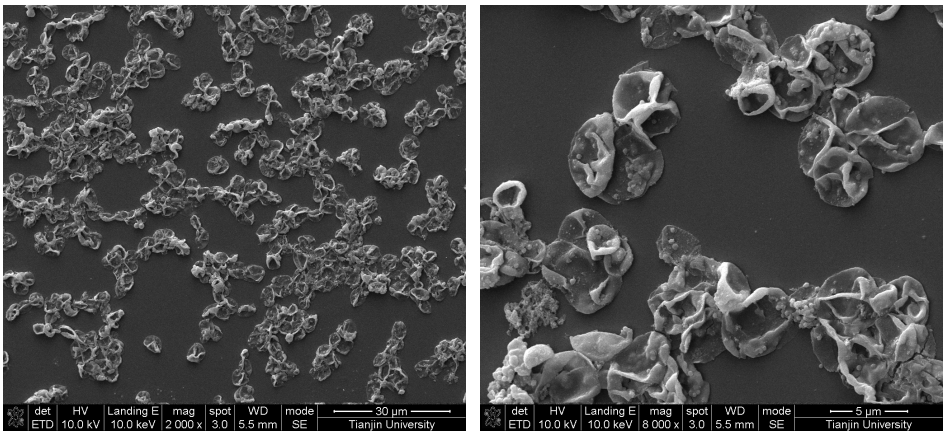


Figure S2. Overview (*SEM images*) of NEPS microcapsules with one protamine/silica bilayer.

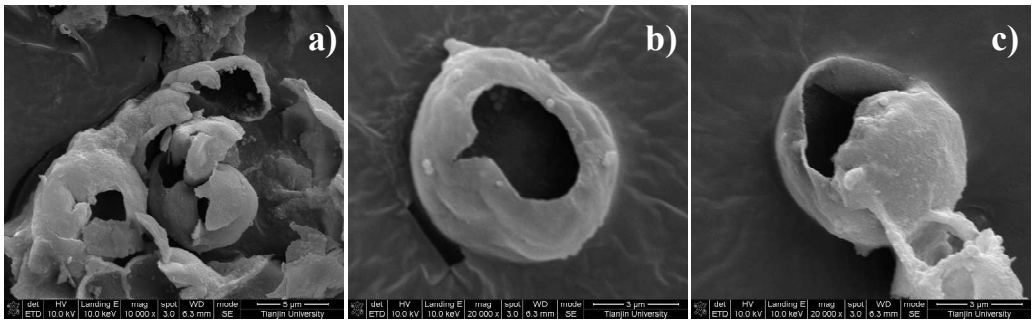


Figure S3 SEM images of NEPS microcapsules, which were frozen dried and manually grounded.

Reference

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