Self-Assembly of Amphiphilic Janus Particles into Monolayer Capsules for Enhanced Enzyme Catalysis in Organic Media

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⑤ *Supporting Information*

ABSTRACT: Encapsulation of enzymes during the creation of an emulsion is a simple and efficient route for enhancing enzyme catalysis in organic media. Herein, we report a capsule with a shell comprising a monolayer of silica Janus particles (JPs) (referred to as a monolayer capsule) and a Pickering emulsion for the encapsulation of enzyme molecules for catalysis purposes in organic media using amphiphilic silica JPs as building blocks. We demonstrate that the JP capsules had a monolayer shell consisting of closely packed silica JPs (270 nm). The capsules were on average 5−50 μm in diameter. The stability of the JP capsules (Pickering emulsion) was investigated with the use of homogeneous silica nanoparticles as a control. The results show that the emulsion stabilized via amphiphilic silica JPs presented no obvious changes in physical appearance after 15 days, indicating the high stability of the emulsions and JP capsules. Furthermore, the lipase from *Candida sp.* was chosen as a model enzyme for encapsulation within the JP capsules during their formation. The catalytic performance of lipase was evaluated according to the esterification of 1-hexanol with hexanoic acid. It was found that the specific activity of the encapsulated enzymes (28.7 U mL^{-1}) was more than 5.6 times higher than that of free enzymes in a biphasic system (5.1 U mL^{-1}). The enzyme activity was further increased by varying the volume ratio of water to oil and the JPs loadings. The enzyme-loaded capsule also exhibited high stability during the reaction process and good recyclability. In particular, the jellification of agarose in the JP capsules further enhanced their operating stability. We believe that the monolayer structure of the JP capsules, together with their high stability, rendered the capsules to be ideal enzyme carriers and microreactors for enzyme catalysis in organic media because they created a large interfacial area and had low mass transfer resistance through the monolayer shell.

KEYWORDS: *Janus particle, capsule, Pickering emulsion, self-assembly, enzyme immobilization*
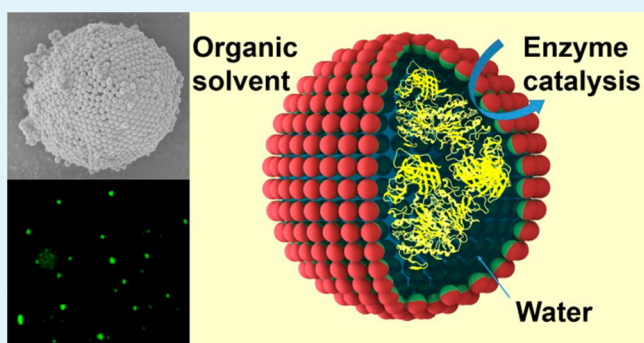
INTRODUCTION

Enzymes catalyze a broad variety of organic reactions with high chemo-, stereo-, and regioselectivity under mild conditions.[1−3] However, the application of free enzymes in organic media is often hampered by the fact that, in many cases, enzymes have low stability and poor recyclability. A simple, gentle, and general way to solve these problems is the encapsulation of enzymes within capsules, which can create a microenvironment to maintain the enzyme activity in biphasic aqueous−organic systems.[3−7] In recent years, such research has advanced in a promising direction toward the use of nanoparticles as building blocks for the construction of such capsules and emulsions (often referred to as Pickering emulsions) as enzyme carriers.[5−9] These nanoparticle-assembled capsules have proven to be very effective for enhancing enzyme stability, facilitating enzyme recycling, and simplifying the separation process in organic media.

In general, an ideal capsule for enzyme encapsulation should have high stability, good recyclability, and efficient substrate/ product transport through the shell, in addition to facile preparation and, in some cases, reversible assembly. To achieve good performance, a simple strategy is the modification of the surface of hydrophilic nanoparticles.[5,10] For example, Wu et al.[5] prepared hydrophobic silica nanoparticles with trimethoxy (octadecyl) silane as the modifier and then used the nanoparticles to stabilize a water-in-oil Pickering emulsion. This was also the first attempt to apply a capsule (or Pickering emulsion) for enzyme immobilization, giving rise to an enhanced specific activity compared to the use of a free enzyme. In addition, Bollhorst et al.[10] demonstrated that oil-

soluble surfactants can also be used to tailor the physicochemical properties of the surface and thus to form submicrometer-sized capsules with tailorable nanopores. Another strategy is to chemically link the nanoparticles at an oil/water interface.[7,11−13] For example, Shi et al.[7] prepared a nanoparticle (TiO$_2$)-stabilized capsule using synergy between the Pickering emulsion and a sol−gel process, in which the sol−gel titania can cross-link the oligodopa-coated TiO$_2$ nanoparticles through catechol-titanium chelation. The enzyme-loaded capsule displays high catalytic activity and stability, as well as superior recyclability. Recently, Zhang et al.[11] reported a silica capsule with different shell structures by chemically linking silica nanoparticles at the oil/water interface using a silica precursor polymer (hyperbranched polyethoxysiloxane). Overall, these capsule systems involve the use of either hydrophilic or hydrophobic nanoparticles, as well as postmodification (e.g., chemical linking) to improve their stability for enzyme encapsulation applications. However, it is still difficult for a single system to satisfy all of the above-mentioned requirements. The design of new and efficient capsules for enzyme encapsulation has attracted extensive attention in recent years. In this study, we employ amphiphilic nanoparticles to form highly stable capsules and encapsulate enzymes for enhanced catalysis in organic media.

Janus particles (JPs) are a special class of colloidal particles that possess two distinct sides in terms of chemistry or polarity, such as one side that is hydrophilic while the other is hydrophobic.[14−17] In recent years, many emulsion-based approaches, such as Pickering emulsion,[18−20] emulsion or miniemulsion polymerization,[15,21] and miniemulsion/solvent evaporation,[22] have been successfully applied to produce large quantities of JPs. The controllable and tunable asymmetric structure of JPs makes them particularly attractive for various applications. For example, the amphiphilic Janus particle exhibits a significantly higher surface activity at an oil/water interface than a uniform particle, showing a remarkable ability to form stable Pickering emulsions.[23−25] Walther et al.[26] used amphiphilic JPs to stabilize emulsions, achieving good performance in the polymerization of styrene and nBuA. When metal catalysts (e.g., Pt, Co−Mo) were deposited one side of the JPs, the resulting JP/catalyst combination was able to stabilize oil/water emulsions while simultaneously catalyzing chemical reactions at the liquid/liquid interface.[27−29] However, to date, no attempt to apply the JP assembled capsule to the encapsulation of active enzymes for enhanced catalysis in organic media has been presented.

Herein, we report a capsule with a shell comprising a monolayer of silica JPs for enzyme encapsulation using amphiphilic silica JPs as building blocks. Specifically, amphiphilic silica JPs were prepared through a classic method based on the Pickering emulsions of wax-in-water, as described previously.[19] The region-selective modification and Janus property of silica JPs were characterized by elemental analysis and the selective adsorption of Au nanoparticles on the amino-modified surface. Then, we used the silica JPs to emulsify an aqueous solution in an organic medium, resulting in the formation of JP capsules and a Pickering emulsion. The structure, diameter distribution, and stability of the JP capsules were further characterized by optical microscopy and scanning electron microscopy using hydrophilic and hydrophobic silica nanoparticles as the control. Moreover, we attempted to use these capsules to encapsulate enzymes for catalysis in organic media. As a model, lipase from *Candida sp.* was chosen to catalyze the esterification of 1-hexanol with hexanoic acid. The encapsulation of enzymes was directly confirmed by laser scanning confocal microscopy using fluorescent dye (FITC) labeled lipase. We further prepared two different capsules, one that was hollow and the other jellified with agarose, to encapsulate lipases and evaluated their catalytic activity, stability, and recyclability in organic media.

■ EXPERIMENTAL SECTION

Materials. Paraffin wax (mp 58−62 °C) and lipase from *Candida sp.* expressed in *Aspergillus niger* was purchased from Sigma-Aldrich. Tetraethyl orthosilicate (TEOS), dodecylethyldimethylammonium bromide, (3-aminopropyl) triethoxysilane (APTES), octadecyl trichlorosilane (ODTS), fluorescein isothiocyanate (FITC), 1-hexanol, hexanoic acid, gold chloride trihydrate (HAuCl$_4$·3H$_2$O), sodium citrate, and sodium borohydride (NaBH$_4$) were obtained from Aladdin Industrial Corp. (Shanghai, China). All other chemicals, such as anhydrous ethanol, aqueous ammonia, chloroform, and methylbenzene, were of analytical grade and were obtained from commercial sources.

Synthesis of Silica Nanoparticles. The silica nanoparticles were synthesized according to the Stöber method.[30] Briefly, 9.38 mL of TEOS and 180 mL of anhydrous ethanol were mixed in a 250 mL flask under vigorous stirring. Then, a mixed solution of 15.4 mL of ammonia and 4.46 mL of deionized water was slowly added. The resulting mixture was incubated at 24 °C and 350 rpm for 12 h. Afterward, the silica nanoparticles were collected by centrifugation (5000 rpm, 10 min) and washed with anhydrous ethanol for six cycles to remove the excess TEOS and ammonia. The cleaned silica nanoparticles were then treated with piranha solution (1:3 mixture of 30% H$_2$O$_2$ and concentrated H$_2$SO$_4$) at 90 °C and 350 rpm for 1 h. (CAUTION: "Piranha" solution reacts violently with organic materials; it must be handled with extreme care.) The treated silica nanoparticles were centrifuged (5000 rpm, 10 min), washed with deionized water (two times), and finally dried at 80 °C under vacuum for subsequent experiments.

Synthesis of Silica Janus Particles. The silica Janus particles were synthesized on the basis of a wax-in-water emulsion proposed by Hong et al.[19] and Perro et al.[20] with some modifications. In a typical experiment, 0.25 g of silica nanoparticles was added to an aqueous solution (10 mL) of dodecylethyldimethylammonium bromide (60 mg L^{-1}) and then heated to 75 °C. Subsequently, 2.5 g of paraffin wax was added to the mixture and incubated at 75 °C for 0.5 h. The resulting mixture was submitted to vigorous stirring in a modular homogenizer at 15 000 rpm for 120 s and then cooled to room temperature, giving rise to a large number of solid wax droplets with trapped silica nanoparticles. Subsequently, the wax droplets were filtered, washed with deionized water to remove the excess and weakly attached silica nanoparticles, and then dried at 25 °C under vacuum for 24 h.

To graft amino groups to one side of the silica nanoparticles, the dried wax droplets were added to a 5 mL methanol solution containing 0.5 mL of APTES and incubated at 25 °C and 50 rpm for 12 h. Then, chloroform was used to dissolve the paraffin wax at 32 °C, thereby releasing the amino-modified silica nanoparticles. The resulting nanoparticles were collected by centrifugation (5000 rpm, 20 min) and washed with chloroform three times. The cleaned nanoparticles were then exposed to two cycles of dispersion in anhydrous ethanol and subsequent centrifugation (5000 rpm, 20 min).

To graft hydrophobic chains to the other side of the silica nanoparticles, the resulting amino-modified silica nanoparticles were dispersed in a toluene solution (5 mL) containing 0.25 mmol of octadecyl-trichlorosilane (ODTS) and then incubated at 25 °C and 150 rpm for 2 h, leading to the formation of amphiphilic silica JPs. Finally, the JPs were collected by centrifugation (5000 rpm, 20 min), washed with ethanol three times, and then dried under vacuum for subsequent experiments.

Surface Modification of Silica JPs with Au Nanoparticles. Citrate-stabilized Au nanoparticles (approximately 5 nm) were prepared by adding a 1 mL aqueous solution of HAuCl$_4$ (1 wt %)

to 100 mL of deionized H$_2$O under vigorous stirring, followed by the addition of 1 mL of aqueous sodium citrate (1 wt %). After an additional 1 min, 1 mL of sodium citrate solution (1 wt %) containing 0.075 wt % NaBH$_4$ was added. The solution was stirred for 5 min and then stored at 4 °C. An ethanol solution (2 mL) containing 10 mg of silica JPs was mixed with a 2 mL aqueous suspension of citrate-stabilized Au nanoparticles, followed by incubation in a shaking bath at 28 °C and 150 rpm for 12 h.

Self-Assembly of Silica JPs into the Monolayer Capsule. In a typical experiment, 20 mg of dried silica JPs was dispersed in 2 mL of heptane under ultrasonication for 30 min. Then, 200 μL of deionized water was added to the JPs solution. The resulting mixture was vigorously stirred in a modular homogenizer at 15 000 rpm for 60 s, giving rise to a uniform emulsion composed of a large number of silica JP capsules.

Enzyme Encapsulation with JPs Capsule. In a typical experiment, a 20 μL aqueous stock solution of lipase (obtained directly from Sigma without any treatment, the specific activity for the first 40 min was measured to be 5.1 U mL^{-1} in the esterification of 1-hexanol with hexanoic acid in a water/heptane system) was diluted to 180 μL of phosphate buffered saline (PBS, 10 mM potassium phosphate, pH 7.4) and mixed under shaking for 3 min. Then, the resulting lipase solution (200 μL) was added to a heptane solution (2 mL) containing well-dispersed silica JPs (20 mg), followed by homogenization in a modular homogenizer at 4 °C and 15 000 rpm for 60 s.

For enzyme encapsulation with a jellified capsule, 7.5 mg of agarose was dissolved in 1 mL of PBS solution (10 mM, pH 7.4) at 50 °C and then cooled down to 25 °C. The resulting solution (180 μL) was mixed with a 20 μL aqueous stock solution of lipase and added to dispersions of silica JPs in 2 mL of heptane, followed by homogenization at 4 °C, as mentioned before.

Assessment of the Catalytic Performance of Lipases. The catalytic performances of the free and encapsulated lipases were determined via the esterification of 1-hexanol with hexanoic acid in a heptane medium. In a typical experiment, a heptane solution (2 mL) containing 400 mmol L^{-1} 1-hexanol and 400 mmol L^{-1} hexanoic acid were added to a Pickering emulsion (2.2 mL) containing lipase prepared as before. For free lipase, except the silica JPs, the other conditions, including substrate concentration, solvent composition, and lipase content, were the same as given before. All of the esterification reactions were carried out on a rotating shaker (80 rpm) at 37 °C for the appropriate amount of time. Aliquots (50 μL) were extracted at different time points and then centrifuged (13 000 rpm, 5 min) for gas chromatography (GC) analysis.

The concentrations of the substrates (1-hexanol, hexanoic acid) and product (hexyl hexanoate) were determined using an Agilent GC system (GC-7090A) equipped with a flame ionization detector (FID) on a column (DB-FFAP column, 30 m × 0.25 mm × 0.25 mm). The temperature program was as follows: start temperature 80 °C, hold for 0.5 min, temperature increase at 20 °C min^{-1} from 80 to 170 °C, and 5 °C min^{-1} increase from 170 °C to the end temperature 200 °C.

One unit of lipase activity (U) was defined as 1 μmol of product produced within 1 min. The specific activities (U mL^{-1}) of free and encapsulated lipase were determined under the same conditions within 40 min. All reactions were repeated at least three times.

Characterization. Optical microscopy imaging was performed on a polarized light microscope (ShunYu XP, China) with an attached charge-coupled device video camera. The diameter distribution of the JP capsules was obtained through the statistical analysis of more than 100 capsules.

The silica nanoparticles, wax droplets, silica JPs, and capsules were imaged using field-emission scanning electron microscopy (FESEM, S-4800, Hitachi Hightechnologies Co., Japan) at an accelerating voltage of 3 kV. All samples were sputter-coated with platinum using an E1045 Pt-coater (Hitachi High-technologies Co., Japan) before SEM observation.

Transmission electron microscopy (TEM) analysis was performed on a field-emission TEM (JEM-2100F, JEOL, Japan) at an accelerating voltage of 120 kV. Scanning transmission electron microscopy

(STEM) and STEM electron energy loss spectroscopy (EELS) were conducted using a JEOL 2100F TEM equipped with a GIF Tridiem EELS spectrometer at 120 kV. The samples were prepared by placing one drop of solution containing silica JPs or Au-labeled silica JPs onto a 300 mesh, carbon film-coated copper grid, and the specimens were then dried in vacuum for 6 h.

Zeta potential measurements were carried out on a Zetasizer Nano-ZS (Malvern Instruments, UK) at 25 °C. The samples were prepared by dispersing 50 mg of bare SiO$_2$, silica JPs, or SiO$_2$-NH$_2$ into 2 mL of deionized water (pH 7.0). The reported values are averages of two measurements recorded for each sample.

To confirm the encapsulation of lipase within a capsule, the lipase was first labeled by reaction with a fluorescent dye (FITC). The FITC-labeled lipase was then encapsulated within silica JP capsules by following the procedure as described before. The resulting lipase-loaded capsules were then directly observed with laser scanning confocal microscopy (LSCM, FV-1000, Olympus, Japan) with an excitation wavelength of 488 nm.

Far-UV circular dichroism (CD) spectra of lipase solutions were measured on a J-810 CD spectropolarimeter (Jasco Inc., Tokyo, Japan) over a wavelength range of 200−280 nm with a resolution of 0.5 nm. A quartz cell with 1 mm path length was used for CD measurements. Spectra were recorded three times using a scanning speed of 50 nm min^{-1} with a bandwidth of 1.5 nm.

■ RESULTS AND DISCUSSION

The synthesis of silica JPs based on a wax-in-water emulsion is illustrated in Scheme 1. First, we prepared the common silica

Scheme 1. Schematic Illustration of the Preparation of Amphiphilic Silica JPs via the Adsorption of Hydroxylated SiO$_2$ Particles onto a Wax/Water Emulsion, Modification with APTES, Separation from the Wax, and Then Modification with Hydrophobic ODTS
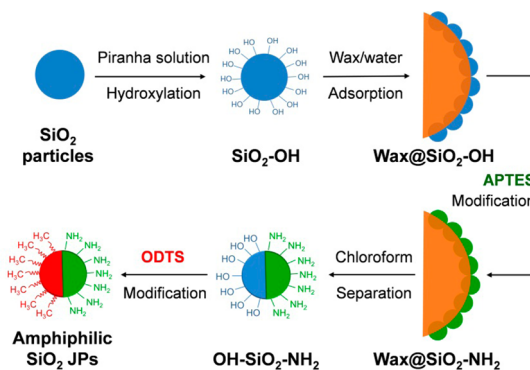
nanoparticles according to the Stöber method. As shown in Figure 1a, uniform silica nanoparticles measuring approximately 270 nm in diameter were obtained in this work. These silica nanoparticles were hydroxylated (SiO$_2$−OH) in piranha solution and then used to form a Pickering emulsion in a wax/water solution at 75 °C. In this case, the silica nanoparticles were adsorbed on the wax-in-water emulsion interface, leading to the formation of silica-covered wax droplets (Wax@SiO$_2$−OH). After cooling to room temperature, the silica colloidosomes became jellified with solid wax. As shown in Figure S1, Supporting Information, the colloidosomes measured 8−35 μm in diameter, which was significantly smaller than similar colloidosomes (20−150 μm) reported in a previous study.[19] On the basis of additional characterization, the silica nanoparticles were locked uniformly on the surfaces of the wax droplets. Subsequently, APTES was used to modify only those faces of the silica nanoparticle that
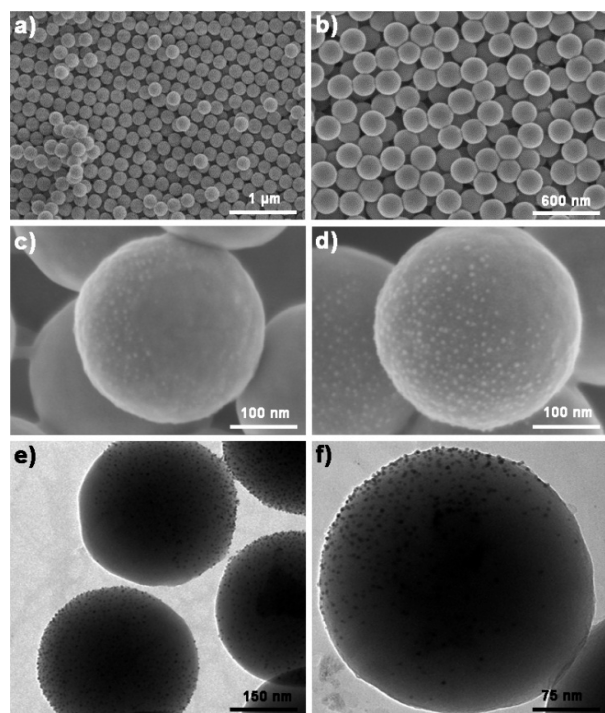
Figure 1. (a,b) SEM images of the silica particles prepared by the Stöber method (a) and silica JPs (b). (c,d) SEM and (e,f) TEM images of silica JPs whose amine grafted area was labeled with Au nanoparticles.

were not buried in wax ($Wax@SiO_2-NH_2$). After the removal of wax, the other side of each silica nanoparticle ($OH-SiO_2-NH_2$) was modified with octadecyltrichlorosilane (ODTS), forming the amphiphilic silica JPs ($C_{18}H_{37}-SiO_2-NH_2$). As shown in Figure 1b, the silica JPs had a uniform diameter of approximately 270 nm, and no aggregation was observed.

To confirm the region-selective modification and Janus characteristic of the obtained silica JPs, we performed elemental analysis using STEM-EELS and STEM-EDX mapping. STEM-based electron energy loss spectroscopy (EELS), as a well-established technique, had been successfully employed to measure the local (even down to atomic dimension) change in electronic structure and composition of many systems. Additionally, the STEM-HAADF images could be simultaneously recorded on a STEM with a HAADF detector. As shown in Figure S2, Supporting Information, the selective area spectra obtained with EELS revealed the presence of the element N, originating from the $-NH_2$ group on one side of the JPs, while almost no N was detected on the other side, which was embedded in and, hence, protected by the wax. As expected, the STEM-EDX maps of silica JPs showed that N was asymmetrically distributed on the surfaces of the JPs (Figure S3, Supporting Information). To precisely characterize silica JPs at the nanoscale, we used the citrate-stabilized Au nanoparticles to label JPs via electrostatic interaction, based on affiliation between the gold atoms and amine groups. As shown in Figures 1c–f and S4, Supporting Information, the Au nanoparticles adsorbed onto a specific zone of the silica JPs. According to SEM/TEM images, the area covered with Au nanoparticles represented a fraction of the total surface area of the JPs, depending on the process of embedment in the wax/water emulsion, as well as the fact that selected particles had been positioned at different angles.

Furthermore, we measured the zeta potential and dispersity of silica particles with different surface characteristics in water/dichloromethane solutions. For this purpose, we also prepared two modified silica nanoparticles, one was an amino-silica (SiO_2-NH_2) nanoparticle using APTES as the modifier, and the other was hydrophobic silica using ODTS as the modifier. As shown in Figure S5, Supporting Information, the zeta potential of the original hydrophilic SiO_2 at pH 7.0 was −16.5 mV, while the values shifted to +11.8 and +38.1 mV for the silica JPs and silica-NH_2 nanoparticles, respectively. The result indicates that the silica JPs have fewer amino groups compared to $SiO_2@NH_2$ particles, which is possibly due to the less area of exposure to the APTES solution during the modification process. Figure S6, Supporting Information, shows photographs of the different silica nanoparticles suspended in water/dichloromethane solutions. The bare silica particles only dispersed in the aqueous phase due to the significant fraction of hydroxyl groups present on their surfaces, while the hydrophobic particles modified with ODTS dispersed well in dichloromethane. In contrast to the bare and hydrophobic silica particles, the silica JPs remained at the interface between water and dichloromethane, suggesting their excellent amphiphilicity. A similar phenomenon was also observed for amphiphilic particles in a previous study.[31]

We further applied the silica JPs to form a Pickering emulsion and then investigated the stability of this emulsion. Specifically, silica JPs were dispersed in heptane under ultrasonication and then were mixed with water. After emulsification (15 000 rpm, 60 s), the resulting emulsion was allowed to stand at room temperature without any disturbance. In the control experiments, bare (hydrophilic) and hydrophobic silica particles were also used to stabilize the emulsions, respectively, according to the same procedure as described before. Any changes in the phases were monitored to evaluate the stability of the emulsions. As shown in Figure 2, for the JP-stabilized emulsions (Case II), no obvious change in physical appearance was observed after 15 days, indicating the high stability of the emulsion. In contrast, the bare silica was unable to stabilize the emulsions due to its high hydrophilicity (Figure 2, case I). Additionally, the use of hydrophobic silica particles as a stabilizer also led to the formation of an emulsion, but it had low stability, as evidenced by the observed phase separation (Figure 2b, case III).

Figure 3a shows a microscopic image of the liquid droplets acquired from the emulsion phase. The droplets were spherical and had a diameter range of 5−50 μm (Figure 3b), similar to that of reported emulsion droplets.[6] Scanning electron microscopy (SEM) imaging clearly revealed that the Pickering emulsion droplets were hollow capsules with a shell comprising a monolayer of silica JPs (Figures 3c–e). The shell of the intact capsule consisted of closely packed silica JPs (Figure 3f), which remained unbroken even in the freeze-drying process. It is worth mentioning that the drying treatment resulted in capsule deformation, leading to the nonspherical shapes shown in Figure 3c. In general, the capsule shells that were prepared with hydrophilic or hydrophobic nanoparticles, as mentioned before, consisted of random and multilayered particles as a result of either chemical linking or aggregation.[5,12] In our case, silica JPs were easily adsorbed to the heptane/water interface and thus self-arranged into a monolayer due to their amphiphilic property. Similarly to surfactant molecules, the hydrophilic side of the JPs adsorbed tightly to the aqueous phase, while the counterpart side adsorbed to the other phase. The irreversible
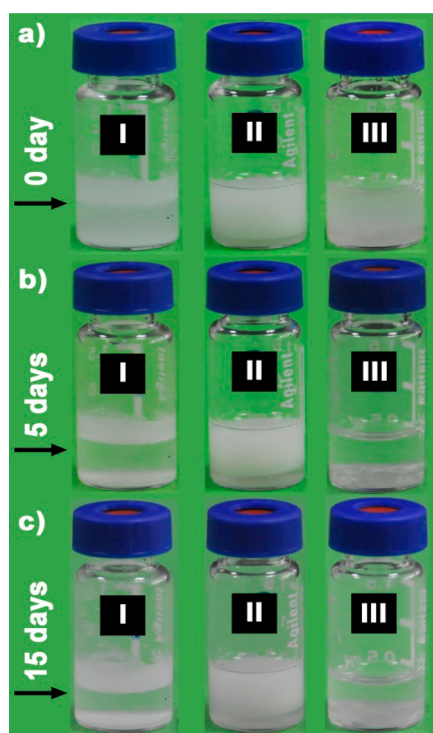
Figure 2. Photos of the emulsions after standing for different times (a: 0 day; b: 5 days; c: 15 days). These emulsions were synthesized in heptane/water (v/v = 10/1) solutions using hydrophilic (I), amphiphilic (II), and hydrophobic (III) silica nanoparticles, respectively.

Figure 3. (a) Optical micrograph of the JPs self-assembled capsules in heptane/water media. (b) The histogram of the diameter distribution of the resulting capsule, derived from the optical micrograph. (c,d) SEM images of monolayer capsules formed by the self-assembly of JPs at the heptane/water interface. (e) SEM image of a cleaved capsule with a hollow structure. (f) SEM image of the surface of a monolayer capsule.

Scheme 2. (a−c) Schematic Illustration of the Self-Assembly of JPs into a Monolayer Capsule and the Simultaneous Encapsulation of Enzymes. (d) Schematic Structure of the Enzyme-Containing Capsule

attachment of JPs at the oil/water interface led to the high stability of the capsules and the emulsions, as well. In addition, the monolayer form had less thickness and significantly more nanopores compared to the multilayer form, which facilitated the transport of substrates or products through the capsule shell. This unique capsule structure, together with its high stability, allowed the capsules to function as potential carriers for enzyme encapsulation and thus improved their catalytic performance in organic media.

To apply the capsule to enzyme immobilization, we used lipase from *Candida sp.* as the target enzyme and the esterification of 1-hexanol with hexanoic acid into hexyl hexanoate as the target reaction. Scheme 2 illustrates the self-assembly of silica JPs into a monolayer capsule with the simultaneous encapsulation of enzymes. In this process, an aqueous solution of lipase was mixed with a heptane solution of silica JPs. The resulting mixture was emulsified at 15 000 rpm for 60 s, yielding a Pickering emulsion that consisted of lipase-loaded capsules. To confirm the encapsulation of lipase within the capsule, the lipase molecules were labeled with fluorescein isothiocyanate (FITC) and then were used for encapsulation by following the same procedure as mentioned before. As shown in the inset image of Figure 4a, a green fluorescent emulsion was generated after homogenization. Confocal laser scanning microscopy (CLSM) imaging clearly revealed that the emulsion was composed of green capsules (Figure 4) and thus indicated that the capsules were loaded with FITC-labeled lipase molecules. After gentle centrifugation, the lipase-loaded emulsion droplets were separated from the water/heptane solution as a fluorescent sediment (Figure S7, Supporting Information).
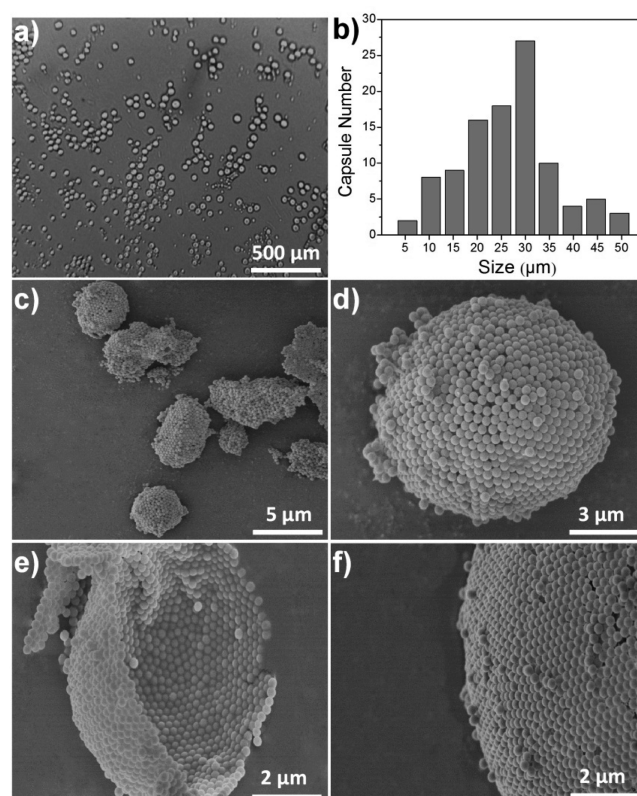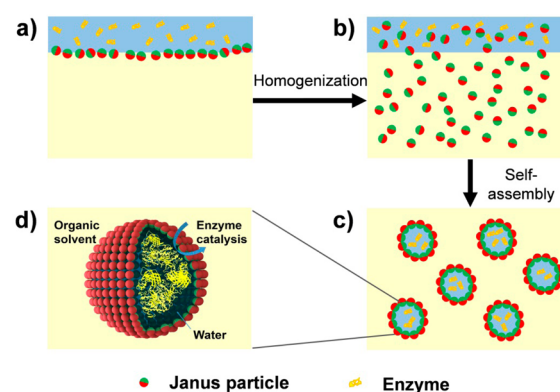
To determine whether the lipase was destroyed during the encapsulation process, we used circular dichroism (CD) to identify the secondary structure of the free lipase before emulsification and after release from the Pickering emulsions via centrifugation and redispersion in buffer solutions. As shown in Figure S8, Supporting Information, the CD spectra showed little change in terms of secondary structure, suggesting
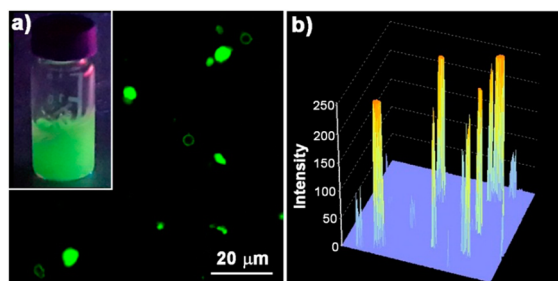
Figure 4. Confocal laser scanning microscopy image (a) and the corresponding fluorescence intensity (b) of the JPs self-assembled capsules containing FITC-labeled lipase. The inset in (a) is a photograph of the emulsions containing lipase-loaded capsules.

that the emulsification process insignificantly impacted the enzymes.

We further investigated the catalytic performance of the encapsulated lipase in the monolayer capsule. For this purpose, we prepared three different capsules for enzyme immobilization, two of which were hollow capsules prepared using 200 μL (named JPs-1) and 300 μL (named as JPs-2) of PBS for emulsification. The third was an agarose-jellified capsule prepared using 200 μL of PBS containing 7.5 mg/mL agarose (named as JPs + agarose) for emulsification. The formed Pickering emulsions are shown in Figure S9B-D, Supporting Information. In addition, a biphasic water/heptane system containing free lipases was used as the control (Figure S9A, Supporting Information). The substrates (1-hexanol, hexanoic acid) were introduced into the heptane phase after homogenization to initiate the enzymatic reaction. Figure 5 shows the
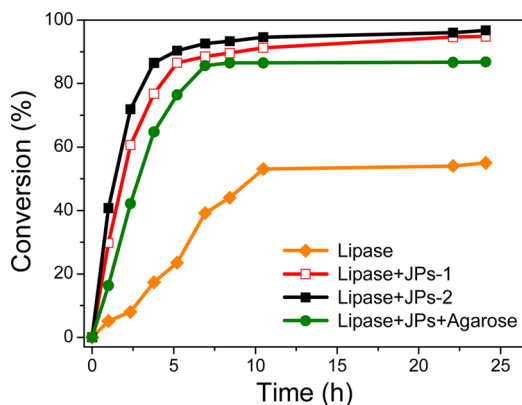

Figure 5. Time courses of the esterification of 1-hexanol with hexanoic acid using free and encapsulated lipase, respectively. The JPs-1, JPs-2, and JPs+agarose represent 200 μL of PBS solution, 300 μL of PBS, and 200 μL of PBS containing 7.5 mg mL^{-1} agarose. These formulations were used to generate the oil/water phase for capsule formation and enzyme encapsulation, respectively. A 20 μL aqueous stock solution of lipase was used in all the experiments.

time course of the bioconversion of 1-hexanol using free and encapsulated lipases. The conversion of 1-hexanol reached 80−92% after 24 h in all three cases using encapsulated lipases, while less than 50% conversion was observed for the free lipases. The higher conversion in the Pickering emulsions can be attributed to the significantly increased interfacial area created by the silica JPs-assembled capsules, which improved mass transfer and the accessibility of the enzymes because the substrates and product were sufficiently soluble in the organic

media (the outside of the capsule) in our cases. In contrast, enzymes typically prefer an aqueous environment (the inside of the capsule). In addition, the long-term exposure of free enzymes in an organic solvent can also lead to reduced enzyme activity, which can achieve low conversion even after a long reaction time (e.g., 24 h).

Furthermore, the specific activities of the free and encapsulated enzymes were calculated according to the product yields obtained within 40 min. As shown in Figure 6a and
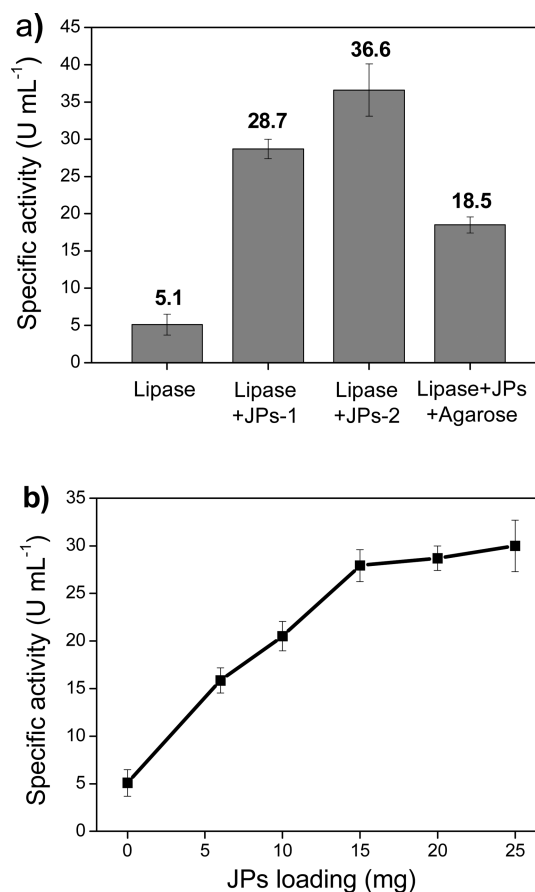

Figure 6. (a) The specific activities of free lipase and encapsulated lipase. The JPs-1, JPs-2, and JPs+agarose represent 200 μL of PBS solution, 300 μL of PBS, and 200 μL of PBS containing 7.5 mg mL^{-1} agarose. These formulations were used to generate the oil/water phase for capsule formation and enzyme encapsulation, respectively. (b) The change in specific activity of encapsulated lipase with increasing JPs loading. The volume of PBS solution was 200 μL; the volume of heptane was 2 mL. A 20 μL aqueous stock solution of lipase was used in all the experiments.

Figure S10, Supporting Information, free lipase dissolved in a biphasic water/heptane system had a specific activity of 5.1 U mL^{-1}, which increased to 28.7 U mL^{-1} after encapsulation in JP capsules. The increased activity suggested that the JPs-assembled capsule significantly increased the interfacial area and created the nanoporous structure of the monolayer, which allowed the substrate and product molecules to diffuse through the capsule more easily. In the case of the bilayer capsules prepared via chemical linking, the catalytic activity of the encapsulated enzymes decreased compared to the native enzymes possibly due to retarded mass transfer through the capsule shell.[11] The chemical linking increased the stability of the capsule, as mentioned before; however, the formation of a

particle bilayer led to limited mass transfer in many cases. In our case, silica JPs self-assembled at the oil/water interface as a result of their amphiphilic property, yielding a stable monolayer (capsule shell) without any postmodification. Our results demonstrate that this monolayer capsule was an ideal enzyme carrier and microreactor for enzyme catalysis in organic media. Actually, some supports such as silica, polymer, etc. can also enhance the activity of lipase due to the increased interfacial area. However, different from these carriers, the JPs capsules could offer an aqueous microenvironment and thus protect them effectively from the organic media.

In general, enlarging the area of the oil/water interface was able to improve the catalytic activity of the enzymes in emulsions.[32,33] In this work, we prepared two Pickering emulsions by varying the volume ratio ($R_{w/o}$) of PBS solution to heptane (JPs-1 was 1:10; JPs-2 was 1.5:10, Figures 5 and 6a). As shown in Figure 6a, with the increase of $R_{w/o}$, the specific activity of the lipase increased from 28.7 to 36.6 U mL^{-1}. The results suggest that the emulsion with a $R_{w/o}$ of 1.5:10 may have had a larger interfacial area, leading to enhanced catalytic performance. To further investigate the effect of interfacial area on enzyme activity, we measured the specific activity of the encapsulated lipases at different JPs loadings. As shown in Figure 6b, when the $R_{w/o}$ value was held constant at 1:10, the specific activity of lipase increased with increasing JPs loading, indicating that the enhancement of enzyme activity should be attributed to the increased interfacial area. In addition, to compare the activity of free and immobilized enzymes inside the capsules, we also determined the enzyme activity in the agarose-jellified capsule. From the data in Figure 6a, it can be seen that the jellification of the agarose core reduced the specific activity of lipase to 18.5 U mL^{-1}, although this value was 3.6 times higher than that of the free lipase in the biphasic system. We found no significant difference in morphology and diameter between the hollow and jellified capsules (data not shown). The decreased enzyme activity should be attributed to the diffusion limitations created by the agarose gel network, in which the enzymes cannot diffuse freely inside the core compared to the case of the hollow capsules. Similar results were also observed in other gel carriers reported previously.[5,34,35]

The stability of the encapsulated enzyme is an important parameter for enzyme catalysis. To evaluate this parameter, the enzyme-loaded Pickering emulsions were incubated with substrates at 37 °C and 80 rpm for 30 h. Then, the capsules were collected via centrifugation and redispersed in fresh heptane solvent for activity assessment. As shown in Figure 7a, the enzyme molecules in hollow capsules retained approximately 87% of their initial activity after continuous reaction for 30 h and centrifugation for one time, demonstrating the high stability of the capsule and its low enzyme leakage during the reaction process. The enzyme stability was higher than that obtained with the hydrophobic silica capsule reported previously (approximately 50% after a 19 h storage).[5] We speculate that the decreased enzyme activity can be attributed to enzyme leakage during centrifugation due to the strong hydraulic shear force, as further confirmed below. It is worth noting that the enzyme encapsulated in jellified capsules had a significantly higher relative activity (95%) after 30 h, suggesting that enhanced stability was obtained as a result of the jellification with agarose gel in the JP capsules.

Finally, the recycling and reuse of the encapsulated enzyme were examined. As shown in Figure 7b, the enzyme fractions in
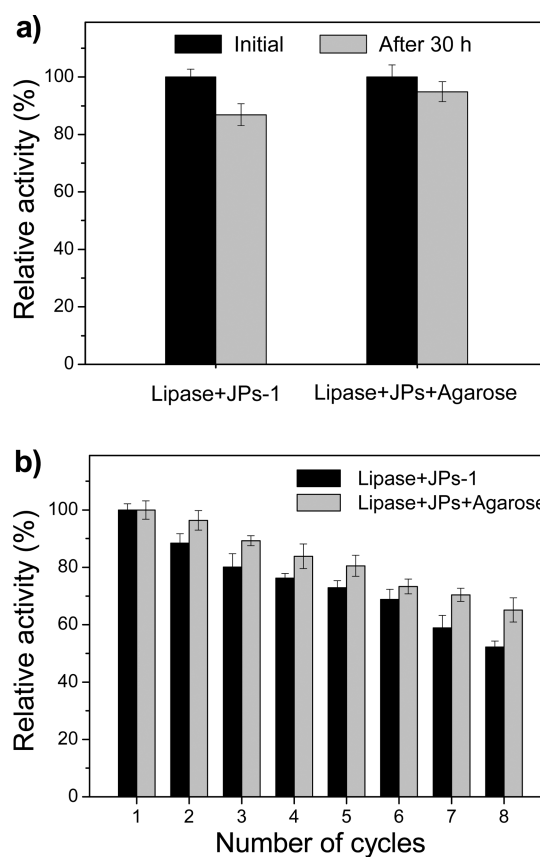

Figure 7. (a) The relative activity of encapsulated lipase after continuous reaction for 30 h. (b) The relative activity of encapsulated lipase during the recycling and reuse process. A 20 μL aqueous stock solution of lipase was used in all the experiments.

the hollow and jellified capsules retained 52% and 65% of their initial specific activity after eight cycles (40 min/cycle), suggesting good stability and recyclability. The recycling performance was close to that obtained with chemically linked silica capsules,[11] in which approximately 70% of the initial activity remained after six consecutive recycling runs. In particular, in the second reaction cycle (total time: 80 min; one time centrifugation), the encapsulated enzyme fractions retained 88% and 96% of their initial activity, which were similar results to those obtained after continuous reaction for 30 h, including centrifugation once as mentioned before. This observation further demonstrated that activity loss might be related to capsule breakage, enzyme leakage, and/or the loss of water during the centrifugation phase, rather than the reaction process.

■ CONCLUSIONS

In summary, we have demonstrated the successful use of JP capsules for the microencapsulation of enzymes for catalysis in organic media. The amphiphilic silica JPs self-assembled into the capsules with a shell comprising a monolayer of silica JPs at the oil/water interface, yielding a Pickering emulsion with high stability. The monolayer capsules did not require post-modification and had significantly higher stability than the hydrophobic silica capsules. On the basis of this concept, a simple and efficient enzyme immobilization method via the direct encapsulation of enzymes (lipase) along with the formation of hollow or agarose-jellified capsules was further

developed. The method displayed a high immobilization efficiency and allowed for reversible encapsulation because the enzymes could be released from disassembled capsules into an aqueous solution. The encapsulated enzyme exhibited a significantly enhanced specific activity and high stability during the reaction process. This excellent catalytic performance was attributed to the large interfacial area, low mass transfer resistance through the non-cross-linked monolayer, and the high stability of the JP capsule. In addition, the encapsulated enzyme also demonstrated good recyclability, although some enzyme loss was experienced during the regeneration process. In view of their enhanced catalytic performance and good recyclability, together with the simplicity of enzyme encapsulation, JP capsules have great potential as enzyme carriers and microreactors for catalysis in organic media.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

SEM images of Wax@SiO$_2$−OH, STEM-EELS spectra of the silica JPs, STEM-HAADF images of the silica JPs, STEM-EDX maps of N, SEM/TEM images of silica JPs labeled by Au nanoparticles, Zeta potential of the silica-based particles, suspension of the silica-based particles in water/dichloromethane solutions, photographs of FITC-lipase loaded emulsion droplets, CD spectra of native lipase and lipase released from JP capsules, photographs of a biphasic system containing lipase, and pickering emulsions before and after homogenization. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes
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