Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Enzyme Confined in Silica-based Nanocages for Biocatalysis in Pickering Emulsion

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Experimental Section

Reagents and materials

Amano lipase PS from *Pseudomonas cepacia* (PCL) and lysozyme (LYZ) were purchased from Sigma-Aldrich and Dalian Chenyu Biochemical Reagents Co., respectively. Pluronic copolymer F127 ($EO_{106}PO_{70}EO_{106}$) and (R, S)-1-phenylethanol were acquired from Sigma-Aldrich. Triacetin was commercially available from Alfa Aesar. Tetraethoxysilane (TEOS), hexamethyldisilazane (HMDS), vinyl acetate and other reagents were obtained from Shanghai Chemical Reagent Inc. of Chinese Medicine Group.

Encapsulation of PCL into the nanocages of FDU-12 (PCL-FDU12-HMDS)

FDU-12 was synthesized according to the procedures reported in literature.[1] The PCL solution was prepared by dissolving 0.4 g of PCL powder into 20 mL of phosphate buffer solution (PBS, pH = 8.0, 50 mM). 18 mL of the prepared PCL solution was added into 800 mg of FDU-12 and the suspension was shaken (160 rpm) for 12 h at 4 ^{o}C. The mixture was then centrifuged to remove the supernatant, and dried at ambient temperature (about 20 ^{o}C) under vacuum. The water content of the sample was controlled in virtue of the drying time and estimated according to the mass increment of the sample during this process. Thereafter, the resulting solid was thoroughly ground and dispersed into 4 mL of hexane under vigorous stirring to create a uniform suspension, and then 2.584 g of HMDS (20 mmol $g_{FDU\text{-}12}^{-1}$) was introduced and the reaction mixture was stirred (500 rpm) at 30 ^{o}C for 4 h. After centrifugation, the solid material was washed with 2 mL of PBS several times until the amount of PCL in the washing solutions could not be detected via the Bradford method. After that, the solid was fully dried at ambient temperature under vacuum and the obtained sample was denoted as PCL-FDU12-HMDS. Meanwhile, FDU12-HMDS represents the sample without PCL loading (the exact same method was employed to modify FDU-12 with HMDS but substituting 18 mL of PBS for 18 mL of PCL solution), and PCL-FDU12 means the sample without HMDS tailoring (After separating the

suspension consisting of PCL solution and FDU-12 by centrifugation, the residual solid was directly washed three times with PBS and dried at ambient temperature under vacuum).

Generally, the principal driving forces for enzyme adsorption inside pores are hydrophobic interaction, electrostatic attraction, hydrogen bonding, and van der Waals force.[2-7] As for PCL, due to its special "interfacial activation" effect, hydrophobic interaction is usually the dominant driving force playing a significant role in PCL uptake. However, in our case the support FDH-12 is hydrophilic before silylation thus cannot offer hydrophobic affinity towards PCL. Due to the pI values of FDU-12 and PCL are both lower than the pH value of the buffer solution (pH =8.0), little electrostatic attraction exists between enzyme protein and the pore-wall of FDH-12. Therefore, the hydrogen bond formed between the large amount of Si-OH or $Si-O^-$ groups on the pore surface of FDH-12 and the amino and carboxylic groups on the external surface of PCL, as well as the van der Waals force, are probably the main driving force for PCL uptake into the nanocages of FDU-12 and also the nature of interaction between the immobilized PCL and pore-wall of FDU-12. These two interactions are not sufficiently strong to prevent PCL leaching out of the support, thus physically confining PCL in the nanocages of FDU-12 via a silylation method as reported in our research is very important.

With regard to the encapsulation of LYZ molecules into the nanocages of FDU-12, parallel procedures were performed except that the LYZ solution (using sodium carbonate buffer solution, 10 mM, pH = 10.8) with a concentration of 300 μmol L^{-1} was used instead of the PCL solution, and the amount of LYZ in supernatant and washing solutions could be directly determined by UV spectrophotometer at a wavelength of 280 nm.

Assessment of the catalytic performance of PCL-FDU12-HMDS

As for the PCL-catalyzed hydrolysis reaction of triacetin in aqueous solution, 250 μL of triacetin and 10 mL of PBS were mixed at 30 °C under vigorous stirring for 5 min. Free PCL solution (100 μL) or PCL-loaded solid sample (15 mg) was added and mildly stirred for another 10 min. After that, the mixture was titrated with sodium

hydroxide solution (0.05 M) with phenolphthalein as the indicator. A blank experiment without adding enzyme was carried out following the identical procedure. Based on the consumption amount of sodium hydroxide, the amount of acetic acid produced in the hydrolysis was acquired, and the activity of free PCL, PCL-FDU12 and PCL-FDU12-HMDS could be calculated. In a typical experiment of the PCL-catalyzed kinetic resolution of (R, S)-1-phenylethanol in non-aqueous medium, the (R, S)-1-phenylethanol (0.25 mmol) and vinyl acetate (2.0 mmol) were dispersed in the dry hexane (5 mL) and stirred at 30 °C. The desired amount of PCL-FDU12 or PCL-FDU12-HMDS containing identical protein content was added to the mixture to start the reaction. Aliquots of the samples were periodically withdrawn from the reaction system at fixed time intervals, then centrifuged to remove the precipitate, and analyzed by the gas chromatograph with a chiral column (Agilent HP-19091G-B233, 30 m × 250 μm × 0.25 μm).

Preparation of the Pickering emulsion stabilized by PCL-FDU12-HMDS

200 mg of PCL-FDU12-HMDS was dispersed in a solution containing 1.5 mL of PBS and 1.5 mL of hexane. The mixture was homogenized using an Ultra Turrax T10 homogenizer (8 mm head) at 8000 rpm for 1 min. The emulsion type was determined by measuring the conductivity, and by introducing a water-soluble dye (Methylene Blue trihydrate) into the emulsion system followed by a visual inspection in the optical microscope.

Assessments of the catalytic performance and reusability of the Pickering emulsion stabilized by PCL-FDU12-HMDS

The catalytic performance of the Pickering emulsion stabilized by PCL-FDU12-HMDS was investigated in virtue of the kinetic resolution of (R, S)-1-phenylethanol with vinyl acetate as the acyl donor. 1.5 mL of the substrate solution comprising 400 mM of vinyl acetate in hexane, 1.5 mL of PBS, and 200 mg of PCL-FDU12-HMDS were homogenized using an Ultra Turrax T10 homogenizer (8 mm head) at 8000 rpm for 1 min. Then, 0.375 mmol of (R, S)-1-phenylethanol was

added into the prepared Pickering emulsion to initiate the transesterification reaction. After 10 min, the reaction was stopped by centrifugation at 12000 rpm. The upper phase (oil phase) in the supernatant was collected, diluted with hexane, and analyzed by gas chromatograph to determine the values of conversion and enantiomeric excess (ee %). Following similar procedure, a biphasic PBS/hexane system containing free PCL was used as a benchmark. When performing the recyclable catalytic reaction in Pickering emulsion, the reaction mixture was separated by centrifugation after each batch. The upper phase (oil phase) and lower phase (water phase) of the supernatant was respectively collected for the assessments corresponding to the catalytic efficiency and the leaching of protein. In the meantime, the residual solid sample was washed with hexane and dried at ambient temperature under vacuum prior to the next cycle.

Characterization methods

Nitrogen-sorption experiments were performed at 77 K on a Micromeritics ASAP 2020 system. Prior to the measurements, the sample was degassed at 90 ^{o}C for 6 h. Brunauer-Emmett-Teller (BET) specific surface areas were calculated based on the adsorption isotherms. Pore-size distribution was calculated from the adsorption branch by using the BJH (Barrett-Joyner-Halenda) method. The total pore volume was estimated from the adsorption amount at a relative pressure (P/P_0) of 0.99. FT-IR spectra were recorded on a Thermo Nicolet Nexus 470 Fourier transform infrared (FT-IR) spectrometer by using KBr pellets. UV-Vis spectra were recorded on a SHIMADZU UV-2550 double-beam spectrophotometer by using a 1 cm quartz cell. The transmission electron microscopy (TEM) was performed on FEI Tecnai G^2 Spirit at an acceleration voltage of 120 kV. The scanning electron microscopy (SEM) was undertaken on JEOL JSM-6360 scanning electron microscope operating at an acceleration voltage of 20 kV. The thermogravimetric (TG) analysis was carried out under a flow of air on a Perkin-Elmer Pyris Diamond TG instrument within the temperature range of 20 - 900 ^{o}C with a heating rate of 5 ^{o}C min^{-1}. The water contact angle measurement was conducted using a contact angle measuring system JC 2000A

via the sessile drop technique. The size of the emulsion droplets was examined using an optical microscopy (XSP-3CA) equipped with 10 × magnification lens.

Supplemental Data

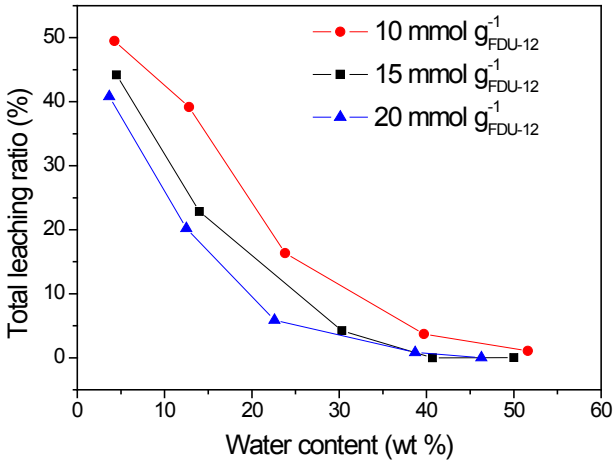

Fig. S1 When encapsulating LYZ into the nanocages of FDU-12, the addition amount of HMDS (10 - 20 mmol g_{FDU-12}^{-1}) and the water content of the LYZ-loaded FDU-12 played significant roles in determining the total enzyme leaching ratio.

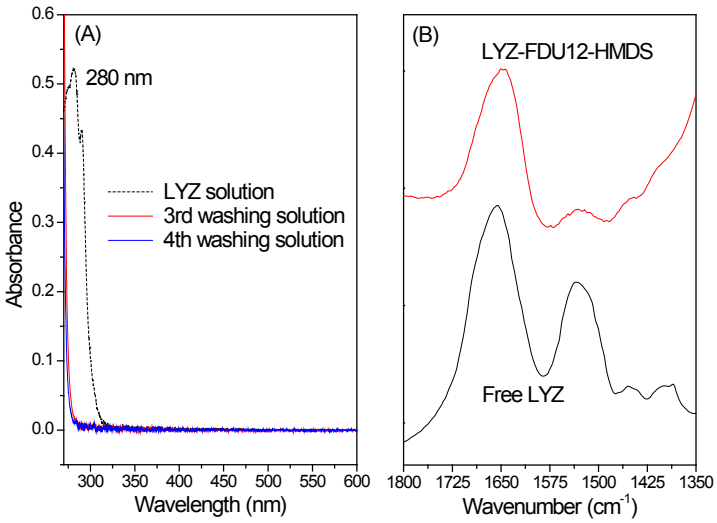

Fig. S2 In the silylation reaction for confining LYZ in the nanocages of FDU-12, the addition amount of HMDS was 20 mmol g_{FDU-12}^{-1} and the water content was controlled in the region of 40 - 50 wt%. After the silylation step, the solid sample was washed several times with the sodium carbonate buffer solution (10 mM, pH = 10.8). Figure (A) shows the UV-Vis spectra of the 3rd and 4th washing solutions and the

LYZ solution with a concentration of 15 μmol L^{-1}. It can be seen that just after washing twice, the LYZ protein can hardly be detected in the washing solutions. Figure (B) presents the FT-IR spectra of the solid sample after thoroughly washing (LYZ-FDU12-HMDS) and also the free LYZ as the benchmark. The result indicates that the silylation procedure can effectively confine a considerable number of LYZ molecules in FDU-12.

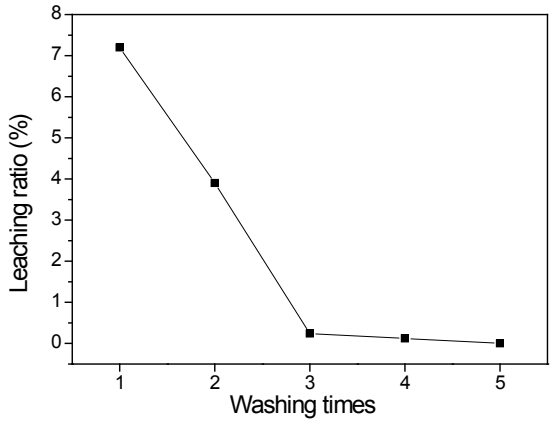

Fig. S3 Following the silylation procedure to confine PCL into the nanocages of FDU-12, the solid sample was washed several times with the phosphate buffer solution (50 mM, pH = 8.0). The leaching ratio corresponding to each washing was derived from the protein assay based on the Bradford method. The leaching ratio distinctly decreased with the washing times and practically no PCL leaching could be detected in the 5th washing.

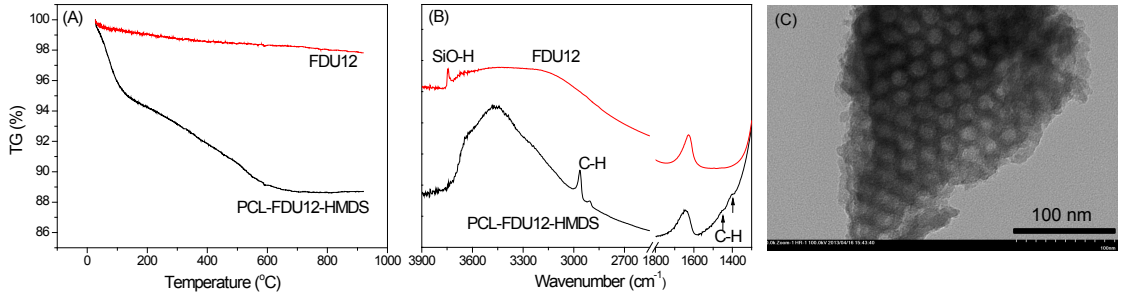

Fig. S4 (A) TG curves of FDU-12 and PCL-FDU12-HMDS. (B) FT-IR spectra of FDU-12 and PCL-FDU12-HMDS. Compared to FDU-12, the FT-IR spectrum of PCL-FDU12-HMDS featured the appearance of several new bands corresponding to the stretching vibration and bending vibration of C-H, as well as the disappearance of

the adsorption peak arising from the stretching vibration of SiO-H. In addition, since the adsorption amount of PCL had to be controlled very low (otherwise the biocatalyst cannot be saturated with substrates in the Pickering emulsion reaction system), the characteristic peaks attributed to PCL protein cannot be clearly observed in the spectrum. (C) TEM image of PCL-FDU12-HMDS.

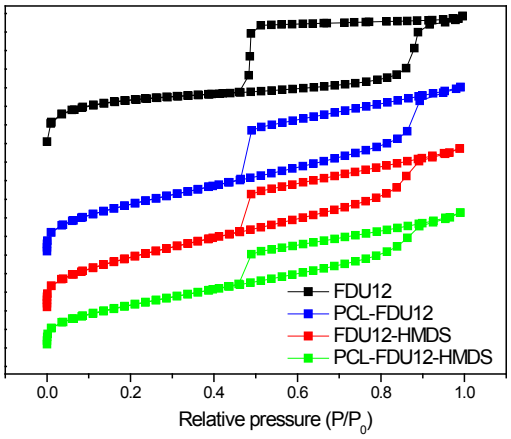

Fig. S5 Nitrogen adsorption-desorption isotherms of FDU-12, PCL-FDU12, FDU12-HMDS and PCL-FDU12-HMDS.

Table S1 Physicochemical properties of FDU-12, PCL-FDU12, FDU12-HMDS and PCL-FDU12-HMDS

Samples	BET surface area (m^2 g^{-1})	Pore volume (cm^3 g^{-1})	Pore size[a] (nm)
FDU-12	605	0.86	17.4
PCL-FDU12	552	0.71	16.5
FDU12-HMDS	548	0.68	13.3
PCL-FDU12-HMDS	437	0.56	13.5

[a] Referring to the cage size

Table S2 Kinetic resolution of (R, S)-1-phenylethanol catalyzed by PCL-FDU12 and PCL-FDU12-HMDS in hexane.

Catalysts	Initial rate ($mM\ min^{-1}$)	Conversion after 3 h (%)	Ee[a] (%)
PCL-FDU12	0.044	12.6	99
PCL-FDU12-HMDS	0.280	49.9	99

[a] Determined in terms of the ester product

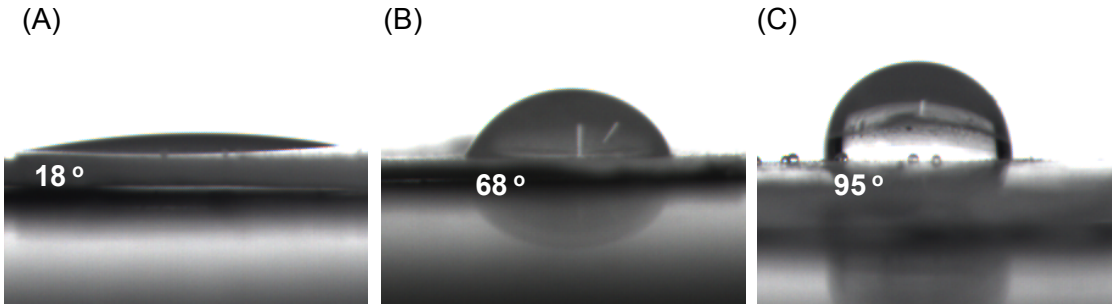

Fig. S6 Water contact angles of (A) FDU-12, (B) FDU12-HMDS and (C) PCL-FDU12-HMDS determined by the sessile drop technique.

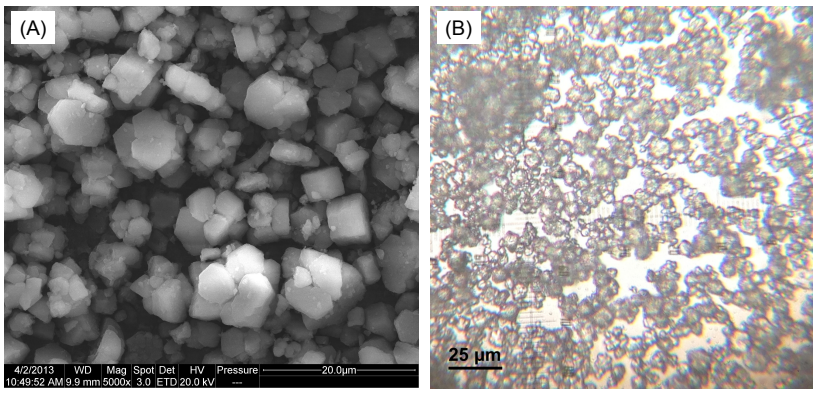

Fig. S7 (A) SEM image and (B) optical micrograph of PCL-FDU12-HMDS.

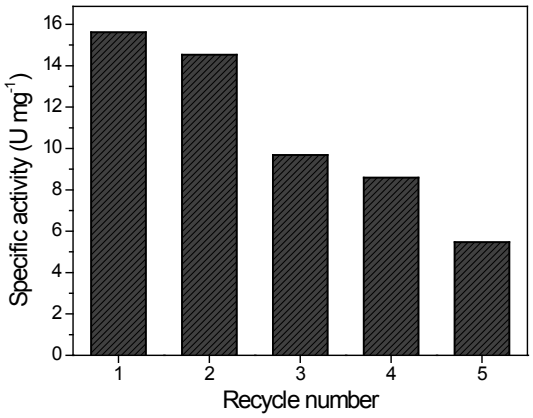

Fig. S8 The reusability of Pickering emulsion stabilized by PCL-FDU12-HMDS in kinetic resolution of (R, S)-1-phenylethanol.

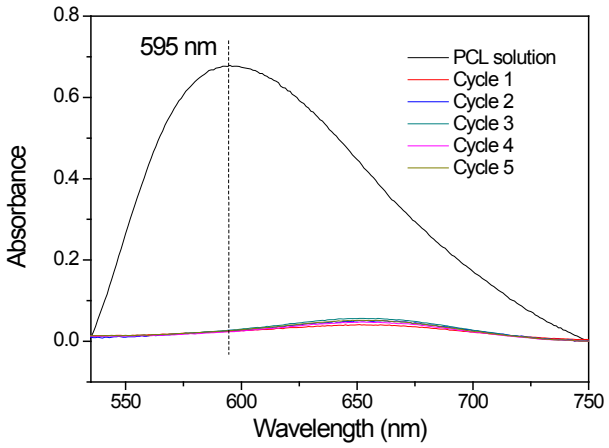

Fig. S9 UV-Vis spectra of the aqueous samples derived from the water phase of the broken emulsion in each cycle of the reusability assay. The UV-Vis spectrum of the PCL solution with a concentration of 189 μg mL^{-1} is also presented as a contrast. Prior to the UV-Vis measurement, all samples were pretreated according to the Bradford method. Since the PCL concentration of each sample from cycle 1 to cycle 5 was lower than the detection limit of 5μg mL^{-1}, it can be estimated that in each cycle the proportion of PCL protein that leached into the water phase during the reaction was less than 3%.

Reference

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