Electronic Supplementary Information (ESI)

Lipase-embedded Silica Nanoparticles with Oil-filled Core-Shell Structure: Stable and Recyclable Platforms for Biocatalysts

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Experimental

Material

Lipase from *Candida antarctica lipase A* (CALA; E.C. 3.1.1.3; ≥1.0 U/mg), bovine serum albumin-fluorescein isothiocyanate conjugate (BSAf) and 3-aminopropyl triethoxysilane (APTES) were purchased from Sigma-Aldrich and used without any further purification. Tetraethoxyorthosilicate (TEOS) and oleic acid (OA) were purchased from Nacalai Tesque Inc. and used as such. All commercially available organic compounds for catalytic reactions were purified by standard procedures.

Synthesis

Synthesis of CALA@OSN: 60 mL of water including OA (1 mmol) was sonicated for 1 min to achieve emulsion. Into this solution were dispersed 14 mg of CALA. Then a mixture of TEOS (6.7 mmol) and APTES (1 mmol) was added dropwise. The solution was allowed to be stirred for approximately 10 min at room temperature, left to age for 2 h at the same temperature under static conditions and aged for another 24 h at 50 °C to achieve the formation of silica network. The initial molar composition was 1 OA / 1 APTES / 6.7 TEOS / 1600 H_2O and the enzyme loading level was fixed to be 30 mg enzyme/g-SiO_2. The resulting suspension was centrifuged at 20000 *g*, washed twice with distilled water and dried under vacuum overnight to give CALA@OSN. BSAf@OSN with average particle size ~10 μm was obtained as described above but without sonification. The prepared samples were stored in the refrigerator at 4 °C until use for preventing denaturation.

Synthesis of CALA@MCM-41: A MCM-41 mesoporous silica with pore diameter (6.1 nm) approximately comparable to the molecular diameter of CALA, was prepared using cetyltrimethylammonium bromide (CTAB; Wako Pure Chemical Ind., Ltd.) as surfactant and mesitylene and 1,3,5-triisopropylbenzene as swelling agents according to the method developed by Lindlar et al. (B. Lindlar, A. Kogelbauer, P. J. Kooyman and R. Prins, *Microporous Mesoporous Mater.*, 2001, **44**, 89). The immobilization of CALA on MCM-41 was performed by stirring 100 mg of MCM-41 in 2 mL of 50 mM Na-phosphate buffer solution (pH 7.0) containing 20 mg of CALA at 4 °C for 24 h. The resulting products were then filtered, washed with sufficient amount of distilled water, and then stored in a desiccator at room temperature overnight.

Characterization

Powder X-ray diffraction (XRD) patterns were recorded using a Rigaku Ultima IV diffractometer with CuKα radiation (λ=1.54056 Å). Nitrogen adsorption–desorption isotherms were measured at –196 °C using BELSORP-max system (BEL Japan, Inc.). Enzyme-loaded samples were degassed at 80 °C for 12 h to vaporize the physisorbed water, while the calcined samples were outgassed at 350 °C for

3 h prior to the measurements. Specific surface area was calculated by BET (Brunauer–Emmett–Teller) method using nitrogen adsorption data ranging from P/P_0 = 0.05 to 0.35. The pore size distributions were obtained by BJH (Barrett–Joyner–Halenda) method. Thermal analysis was carried out with differential thermal analysis-thermogravimetry (DTA-TG) (MAC Science Co. Ltd., TG-DTA2000S) from room temperature to 1000 °C at a heating rate of 10 °C/min in N_2 flow of 50 cm^3/min using α-Al_2O_3 as standard. Transmission electron microscopy (TEM) micrographs were obtained with a Hitachi Hf-2000 FE-TEM equipped with a Kevex energy-dispersive X-ray detector operated at 200 kV. Fluorescence/Optical microscopy images were taken with an Olympus confocal system (OLYMPUS, BX51WI) equipped with a ×100 objective lens (excitation: 470–490 nm, emission: 510–550 nm).

Catalytic reactions

All liquid-phase reactions were conducted at 40 °C with agitation in a 20 mL vial unless otherwise stated. The typical reaction conditions are given in the Figure captions. For hydrolysis reaction of *n*-butylacetate, the catalyst containing 1.0 mg of CALA was added to the mixture of 1 mL of *n*-butylacetate aqueous solution (50 mM) and 9 mL of 50 mM buffer solution (Na–acetate for pH 3.0–5.0, Na–phosphate for pH 6.0–8.0 and Gly–NaOH for pH 9.0–11.0). The reaction mixture was magnetically stirred at 40 °C for 6 h. A portion of the reaction mixture was taken at appropriate intervals by filtration and then analyzed by GC (Shimadzu GC-14B) with a flame ionization detector equipped with a Porapak Q column. The solid catalysts were recovered by centrifugation (5 min at 20000 *g*) after reaction, washed three times with deionized water and subjected to the next catalytic reaction.

Hydrolysis reaction of triglyceride was also carried out in 10 mL of water-saturated *n*-heptane (10 mL) containing 100 μL of tricaprylin ($C_{27}H_{50}O_6$) previously dissolved in methyl *tert*-butyl ether (50 mg/mL) and a supported biocatalyst containing 4.0 mg of CALA. Transesterification reaction of triglyceride was performed in 10 mL of *n*-heptane containing 100 μL of tricaprylin previously dissolved in methyl *tert*-butyl ether (50 mg/mL), ethanol (7 μL, 120 μmol) and a supported biocatalyst containing 4.0 mg of CALA. In both reactions, the reaction was carried out 40 °C for 24 h with slow magnetic stirring and the conversion of tricaprylin and the amounts of products were monitored using GC with a flame ionization detector equipped with a DB-HT-SIMDIS stainless capillary column. After 24 h of each catalytic run, the solid catalysts were extracted from the reaction solution by filtration and washed three times with *n*-heptane prior to performing a new catalytic run.

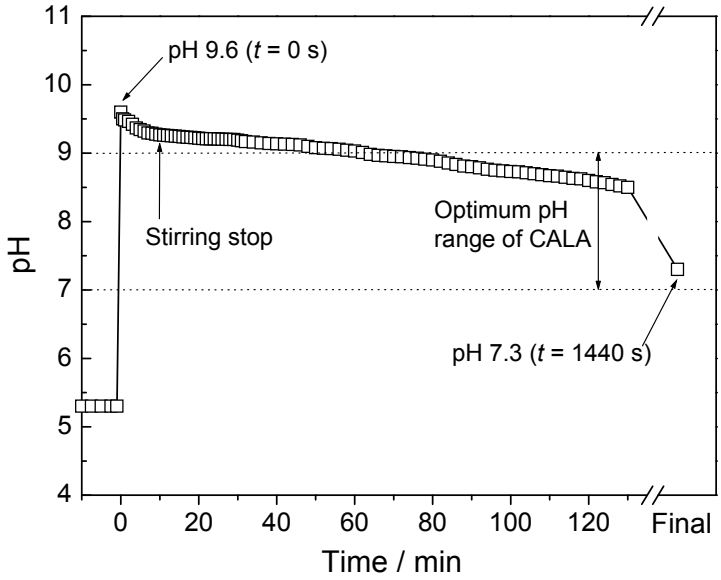

Fig. S1 pH change during the synthesis of CALA@OSN.

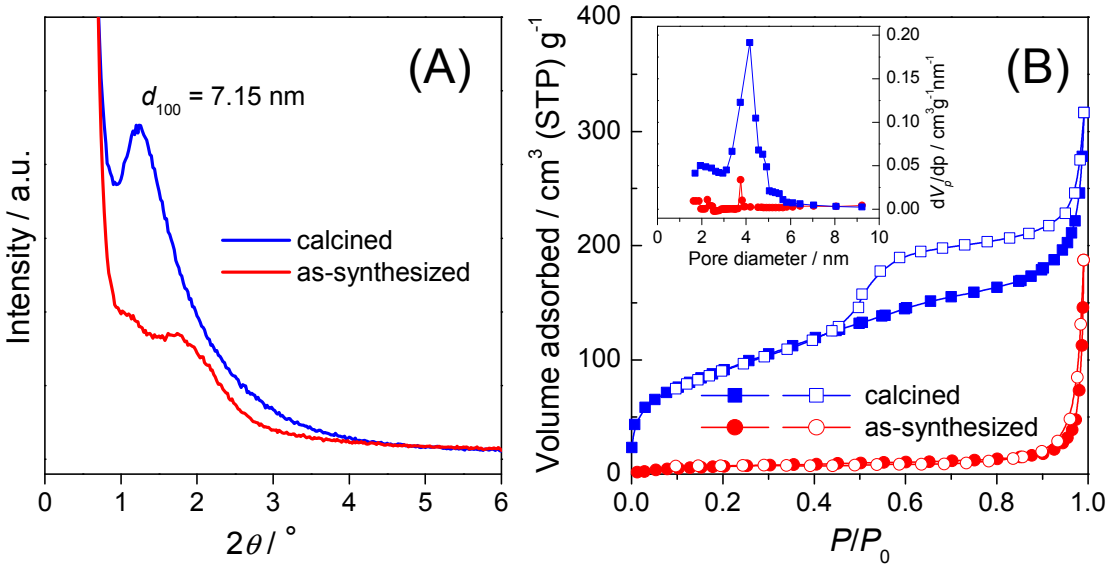

Fig. S2 (A) X-ray diffraction patterns and (B) N_2 adsorption–desorption isotherms of CALA@OSN. Inset in right figure shows BJH pore size distributions. As-synthesized sample was outgassed at 80 °C for 12 h, while calcined sample was degassed at 350 °C for 3 h prior to measurements. The pore size distributions were obtained from desorption branches of the N_2 isotherms.

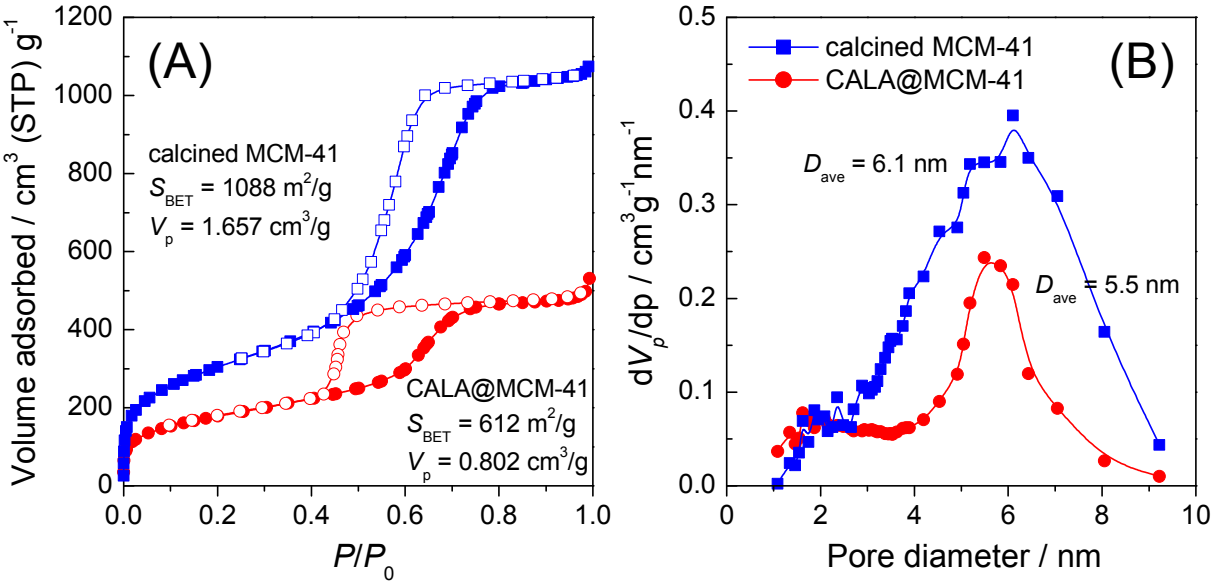

Fig. S3 (A) N_2 adsorption–desorption isotherms and (B) BJH pore size distributions of calcined MCM-41 and CALA@MCM-41. The sample containing CALA was outgassed at 80 °C for 12 h prior to measurements. The pore size distributions were obtained from adsorption branches of the N_2 isotherms.

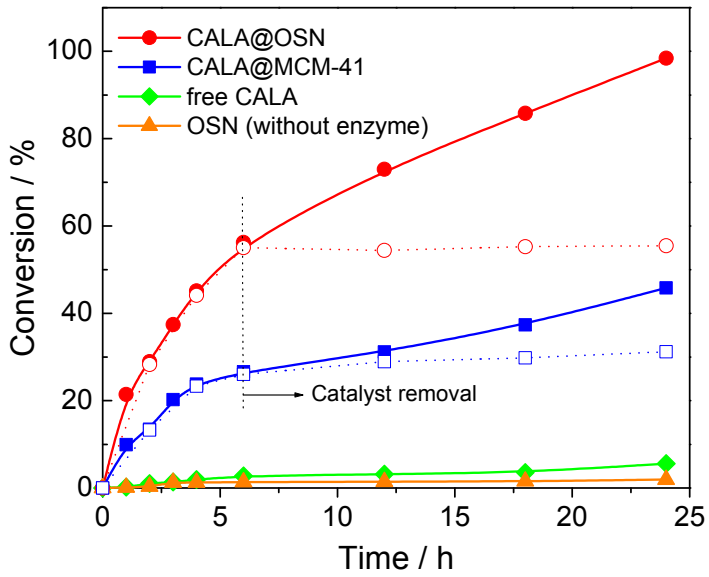

Fig. S4 Hydrolysis of tricaprylin ($C_{27}H_{50}O_6$) in water-saturated *n*-heptane catalyzed by CALA@OSN, CALA@MCM-41, free CALA and OSN (without enzyme). The dotted lines represent time plots after removal of catalysts by filtration of the reaction mixture (The reaction had previously been allowed to proceed for 6 h). Reaction conditions are described in the Experimental Section.

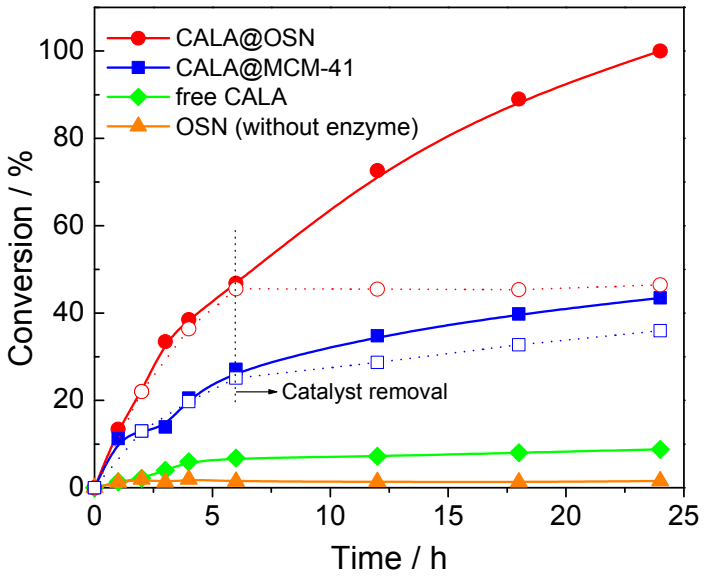

Fig. S5 Transesterification of tricaprylin ($C_{27}H_{50}O_6$) with ethanol in *n*-heptane catalyzed by CALA@OSN, CALA@MCM-41, free CALA and OSN (without enzyme). The dotted lines represent time plots after removal of catalysts by filtration of the reaction mixture (The reaction had previously been allowed to proceed for 6 h). Reaction conditions are described in the Experimental Section.

Table S1 Textural and catalytic properties of free CALA, CALA@OSN and CALA@MCM-41

Biocatalyst	Enzyme loading[a] (mg/g)	N_2 ads-des			Hydrolysis of *n*-butylacetate[e]		Hydrolysis of tricaprylin[f]		Transesterification of tricaprylin[g]	
		S_{BET}[b] (m^2/g)	V_p[c] (cm^3/g)	D_p[d] (nm)	Conv. (%)	enzymatic activity[h] (μmol h^{-1} mg^{-1})	Conv. (%)	enzymatic activity[h] (μmol h^{-1} mg^{-1})	Conv. (%)	enzymatic activity[h] (μmol h^{-1} mg^{-1})
free CALA	-	-	-	-	94.4	15.73	5.59	0.0058	8.78	0.0092
CALA@OSN	18.7	26.2	0.284	4.2	90.8	15.13	98.4	0.1025	>99.9	0.1042
CALA@MCM-41	107	612	0.802	5.5	8.41	1.40	45.8	0.0477	43.5	0.0453

[a] Determinded by thermogravimetric analysis. [b] Specific surface area calculated by BET (Brunauer–Emmett–Teller) method. [c] Total pore volume at P/P_0=0.99. [d] Average pore diameter estimated using BJH (Barrett–Joyner–Halenda) method. [e] Reaction conditions: catalyst containing 1.0 mg of CALA, *n*-butylacetate (50μmol), Na–phosphate buffer solution (10 mL), pH 7.0, 40 °C, 3 h. [f] Reaction conditions: biocatalyst containing 4.0 mg of CALA, Tricaprylin (10 μmol), water-saturated *n*-heptane (10 mL), 40 °C, 24 h. [g] Reaction conditions: biocatalyst containing 4.0 mg of CALA, Tricaprylin (10 μmol), ethanol (7 μL, 120 μmol), triglyceride : ethanol = 1 : 12, *n*-heptane (10 mL), 40 °C, 24 h. [h] Enzymatic activity = [moles of substrate converted]/[reaction time (h)]/[amount of enzyme (mg)].