Electronic Supplementary Information

Formation of lipase *Candida* sp. 99-125 CLEAs in mesoporous silica: characterization and catalytic properties

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Materials

Polyoxyethylene-polyoxypropylene block copolymer $(EO)_{78}$-$(PO)_{30}$-$(EO)_{78}$ (Pluronic F-68, denoted PF-68, *M.W.*=8350±1000), 1,3,5-Trimethylbenzene(TMB, 98%), and tetraethoxysilane (TEOS) were purchased from Sigma-Aldrich (America). Lipase *Candida* sp. 99-125 was purchased from Beijing CAT New Century Biotechnology Co., Ltd. (China). Hydrochloric acid, glutaraldehyde (GA), 4-nitrophenyl palmitate (pNPP), oleic acid, ethanol, cyclohexane, ethyl oleate and *jatropha* oil were got from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other reagents were of analytical grade.

Enzymatic activity assay

Hydrolytic activities of the native and immobilized lipase were measured by the hydrolysis of 4-Nitrophenyl palmitate (pNPP). 0.1 mL of pNPP (5 mg/mL in ethanol) was added into enzyme solution, and reacted 2 min at room temperature. After the reaction, the reaction mixture was filtered and the absorbance of filtrate was measured at 405 nm. One unit of enzyme activity was defined as the amount of enzyme which liberates 1 μM of pNP per minute under the assay conditions. The specific activity of

immobilized lipase was expressed as U per mg of support, and the specific activity of native lipase was expressed as U per mg of protein. The specific activity value of native lipase is 28.5 U/mg protein.

Determination of adsorption time

100 mg of MPS was added to 5 mL of enzyme solution (30 mg/mL) and incubated at 25 ^{o}C under shaking (200 r/min) for different time, then the mixture was centrifuged and the precipitation was washed with phosphate buffer (100 mM, pH 8.0) twice. The loading amount of lipase on MPS was analyzed by the Bradford protein assay using bovine serum albumin (BSA) as the standard.

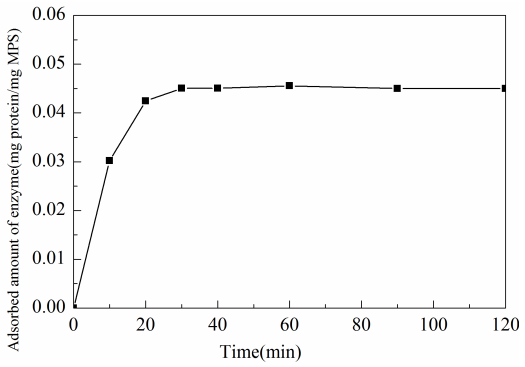

Fig.S1 The adsorbed amount of lipase under different adsorption time

Determination of lipase concentration

100 mg of MPS was added to 5 mL of enzyme solution with different concentrations and incubated at 25 ^{o}C under shaking (200 r/min) for 30 min. The samples were washed with phosphate buffer (100 mM, pH 8.0) twice. Lipase loading amount and the hydrolysis activity were analyzed.

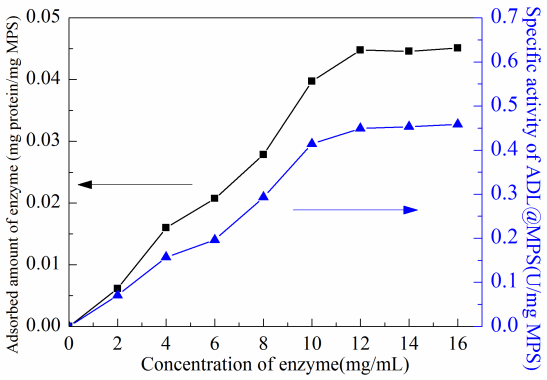

Fig.S2 The lipase loading amount and activities of ADL@MPS with different lipase concentrations

Determination of crosslinking time and crosslinker concentration

For the immobilization of lipase onto MPS particles, 100 mg of MPS was added to 5 mL of enzyme solution (12 mg/mL) and incubated at 25 ^{o}C under shaking (200 r/min) for 30 min. The samples were washed with phosphate buffer (100 mM, pH 8.0) twice, so the immobilized enzyme ADL@MPS was generated. For the preparation of CLL@MPS, 100 mg of ADL@MPS was incubated in 5 mL of GA solution (w/w) under stirring (200 r/min) for a certain time. After the GA treatment, the samples were washed with Tris-HCl buffer (100 mM, pH 8.0) once and phosphate buffer (100 mM, pH 8.0) twice to remove unreacted aldehyde groups, so the immobilized enzyme CLL@MPS was obtained and the hydrolytic activity was analyzed. At the same time, the loading amount of lipase influenced by incubation time at the GA concentration of 0.5% was also tested and the results were shown in Fig.S4.

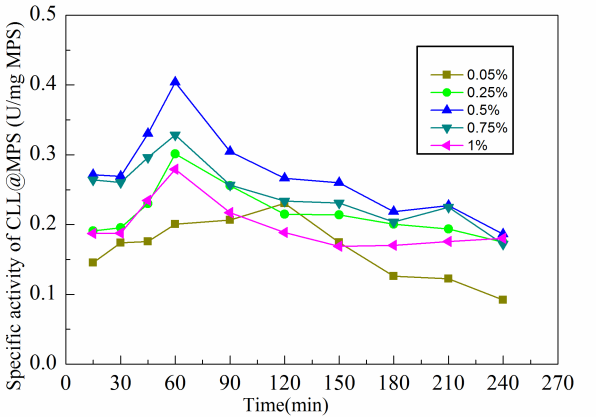

Fig.S3 Effect of crosslinking time and crosslinker concentration on activities of CLL@MPS

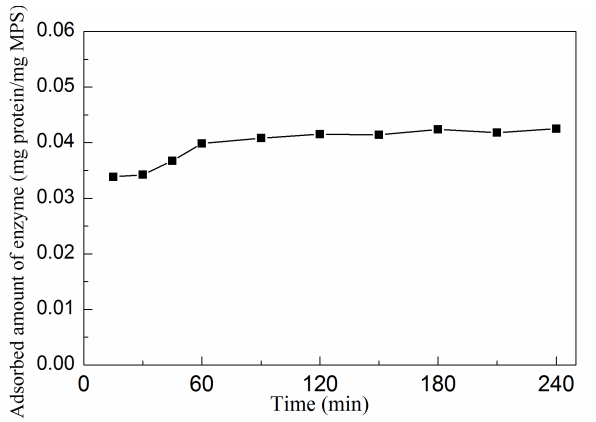

Fig.S4 Effect of crosslinking time on the loading amount of CLL@MPS

Small angle X-ray scattering pattern of MPS

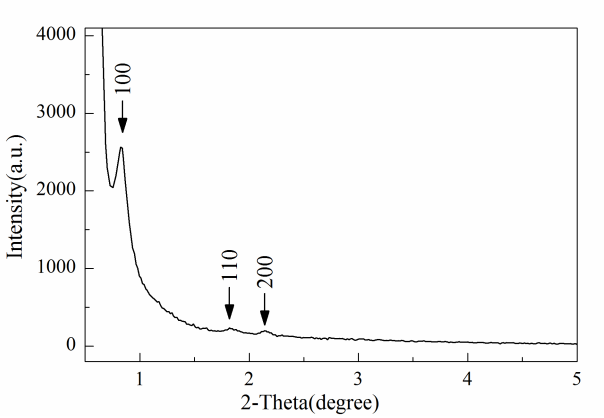

Fig.S5. Small angle X-ray scattering pattern of MPS

Esterification reaction with isooctane as cosolvent at 70 °C

The native lipase, ADL@MPS and CLL@MPS with equal activity were added into the mixture containing 0.42 g of oleic acid, 0.175 mL of ethanol and 3 mL of isooctane. The reaction mixture was magnetically stirred at 70 °C for 6 h. Then lipases were separated from the mixture and washed with isooctane for 3 times, and then the lipases were reused. The yield of ethyl oleate was detected.

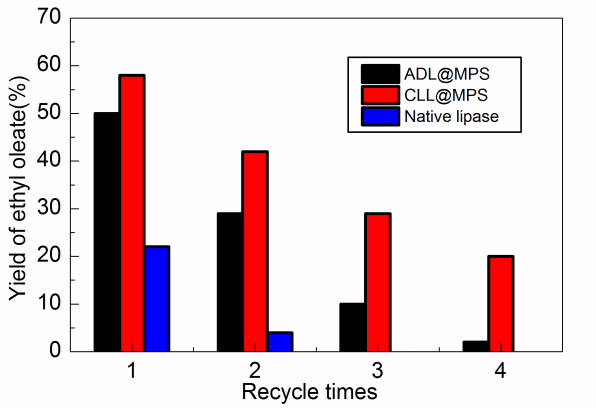

Fig.S6 Reusability of the native and immobilized lipases in esterification reaction at 70 °C

Thermal stability in isooctane-ethanol system

The native lipase, ADL@MPS and CLL@MPS with equal activity were immersed in isooctane-ethanol (17:1, v/v) system at 70 °C and incubated for a certain time. The samples were taken out at each time point and the remaining esterification activities were measured.

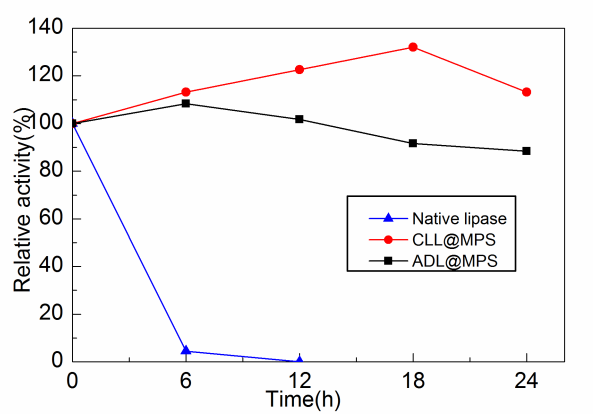

Fig.S7 Thermal stabilities of native and immobilized lipase in isooctane-ethanol at 70°C

Thermal stability in isooctane-oleic acid system

The native lipase, ADL@MPS and CLL@MPS with equal activity were immersed in isooctane-oleic acid (7:1, v/w) system at 70 °C and incubated for a certain time. The samples were taken out at each time point and the remaining esterification activities were measured.

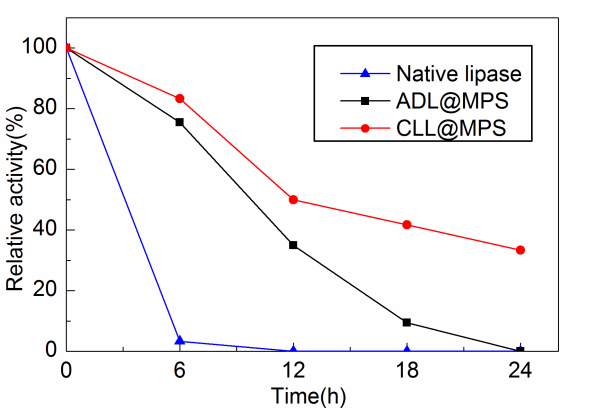

Fig.S8 Thermal stabilities of native and immobilized lipase in isooctane-oleic acid at 70°C