# Supporting information for

# Activation of Enzyme Nanogel in Organic Solvents by PEG-substrate Joint Imprinting

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**Materials.** Lipase from *Thermomyces lanuginosus* (Lipozyme TL 100L) was purchased from Novozyme. *N*-acryloxysuccinimide, ammonium persulfate, *N,N,N',N',*-tetramethylethylenediamine, acrylamide,chloramphenicol, vinyl palmitate, polyethyleneglycol, para-nitrophenyl palmitate, fluorescein and palmitoyl chloride were purchased from Sigma-Aldrich. The TSK- Gel G2000 SW XL column and ZORBAX Rx-SIL column (5 μm, 4.6×250 mm) were purchased from TOSOH (Tokyo, Japan) and Agilent, respectively. Other chemicals were all of analytical grade.

**Preparation of imprinted lipase nanogels.** The crude lipase (30 mg/mL) from Novozyme was first dialyzed against acetic buffer (pH 5.0, 50 mM) for 24 h before chemical modification. The first step was the acryloylation, in which 6 mg of *N*-acryloxysuccinimide (NAS) in 0.8 mL of dimethyl sulfoxide (DMSO) was slowly added to 10 mL of acetic buffer (pH 5.0, 50 mM) containing 4 mg/mL of lipase. The mixture was incubated at 30 °C for 6 h. Unreacted reagents were removed by dialysis for 48 h in acetic buffer (pH 5.0, 50 mM). The second step was the *in-situ* polymerization, in which 0.5 g of acrylamide (AM) was added to the acryloylated lipase solution (4 mg/mL). After 30 min $N_2$ purging, *in-situ* polymerization was initiated by adding 30 mg of ammonium persulfate (APS) and 25 μL of *N,N,N',N'*-tetramethylethylenediamine (TEMED). After 10 h reaction, the product solution was subjected to 48 h dialysis against acetic buffer (pH 5.0, 50 mM) at 4 °C to remove

unreacted reagents. The imprinting treatment was conducted during lyophilization. Palmitic acid, or PEG with molecular weight of 200, 1000, 4000, 12000 was added to an acetic buffer (50 mM, pH 5.0) solution containing lipase nanogel or native lipase (1 mg/mL) at the molar ratio of imprinting molecular to lipase of 5:1 under vigorous stirring. The solution was lyophilized to generate lipase nanogel or native lipase in powder form. After washing with toluene for 3 times, the imprinted sample in powder form was subjected to volatilization and then stored at 4°C. The protein concentration of native enzyme and enzyme nanogels was determined by the bicinchoninic acid (BCA) protein assay kit (Beyotime, China ). The hydrolytic activity of different preparations of lipase catalysts were determined using para-nitrophenyl palmitate as the substrate. Para-nitrophenyl palmitate was first dissolved in acetone and then added to phosphate buffer (50 mM, pH 7.0) containing 1.25% (w/v) Triton X-100. The reaction was started by adding 50 μL of enzyme solution to 950 μL of substrate solution and detected at 348 nm. The transesterification activities of different preparations of lipase catalysts with the same protein content (0.5 mg protein) were measured by adding 30 μL of n-butanol to 0.5 mL of n-heptane solution containing 10 mM para-nitrophenyl palmitate, incubating at 40°C at 200 rpm for 2 h. Then 25 μL of the clear supernatant was immediately mixed with 1 mL of 0.1 M NaOH. The para-nitrophenol liberated was extracted by the aqueous alkaline phase, and detected at 410 nm using a UV-visible spectrophotometer (Shimadzu UV2550).

**Transmission electron microscopy (TEM) and scanning electron microscopy (SEM).** The TEM of non-imprinted and imprinted enzyme nanogels were determined with a Hitachi H-7650B high-resolution TEM. The samples were diluted in distilled water to 0.1 mg/mL, and dropped on to the surface of carbon-coated grids. After removing the excess, negative staining was carried out by adding 2% of pH 7.0 sodium phosphotungstate. The sample was then dried at room temperature before visualized under TEM. The powder of lipase nanogel was placed on SEM conductive tapes and directly subjected to SEM measurement.

**Adsorption of FITC-labeled palmitic acid by lipase nanogels.** Palmitoyl chloride (330 mg, 1.20 mmol) and fluorescein (200 mg, 0.6 mmol) was added dropwise to a mixture of pyridine in DMF (10 mL). After 12 h at 40 °C, 15 mL of deionized water was added to the solution to quench the reaction. The solution was then washed with brine (3 × 40 mL), dried with $Na_2SO_4$, and subjected to rotary evaporation. The residue was purified by a column chromatography (hexane/AcOEt 1:1)[1]. 0.05 mg/mL the FITC-labeled palmitic in acetonitrile was added to 0.1 mg imprinted (or non-imprinted) lipase nanogel at 25 °C. The mixture was immediately analyzed under laser scanning confocal microscope (LSCM) (LSM780, Zeiss Company). The relative fluorescence intensity was determined according to the following equation,

$$A = \frac{I_N - I_B}{I_{N0} - I_{B0}},$$

in which $I_{N0}$ and $I_N$ represent the average fluorescence intensity in region of lipase nanogel at 0 s and t s, and $I_{B0}$ and $I_B$ represent the average fluorescence intensity in region of background at 0 s and t s, respectively.

**Transesterification reaction between chloramphenicol and vinyl palmitate.** Transesterification reaction between chloramphenicol and vinyl palmitate catalyzed by different preparations of lipase catalysts was conducted in 2 mL of acetonitrile containing chloramphenicol (10 mg), enzyme catalyst (25 mg) and vinyl palmitate (44 mg). The molar ratio of chloramphenicol to vinyl palmitate was 1: 5. The mixture was shaken at 200 rpm at 20°C. The product was purified by recrystallization in ethanol. The structure of product was determined by $^1$H-NMR and $^{13}$C-NMR[2].$^1$H-NMR ($CDCl_3$, 600.17 MHz):0.87 (t,$^3J_{HH}$=6.9 Hz, 3H), 1.25 (s, 24H), 1.62 (t, $^3J_{HH}$=7.6 Hz, 2H), 2.37 (t, $^3J_{HH}$ =7.9 Hz, 2H), 4.18-4.20 (m, 1H), 4.46-4.48 (m, 2H), 5.05 (d, $^3J_{HH}$=1.5 Hz, 1H), 5.76 (s, 1H), 6.90 (d, $^3J_{HH}$=8.9 Hz, 1H), 7.55 (d, $^3J_{HH}$=8.6 Hz, 2H), 8.19 (d, $^3J_{HH}$=8.6 Hz, 2H); $^{13}$C-NMR ($CDCl_3$, 150 MHz): 14.2 ($CH_3$), 22.8($CH_2$), 24.7($CH_2$), 24.8($CH_2$), 29.3-29.7 (8$CH_2$), 32.0($CH_2$), 33.8($CH_2$), 34.2($CH_2$), 54.2 (CH), 62.4 ($CH_2$), 66.1(CH), 70.6(CH), 123.8 (2CH), 126.8(2CH), 147.1(C), 147.7 (C), 164.5 (C), 174.6 (C).

**Assays.** Size exclusion chromatography was conducted using a TSK-GEL SW4000xL column (TOSHO) with fluorescence detector (RF-10AxL, SHIMADZU) to determine the content of the encapsulated lipase. Samples were eluted with 0.1 M sodium phosphate buffer (pH 6.7) containing 0.1 M $Na_2SO_4$ and 0.05 % $NaN_3$ as the mobile phase at a flow rate of 0.5 mL/min. The elution profiles were recorded by the fluorescence detector with an excitation wave length of 280 nm and an emission wavelength of 330 nm. The protein encapsulation yield was measured in terms of the peak area of the encapsulated lipase over the total area of protein fractions. The yield and purity of chloramphenicol palmitate were determined by high performance liquid chromatography using Agilent ZORBAX Rx-SIL column (5 μm, 4.6×250mm) with a SHIMADSU SPD-10AVP UV-vis detector at 254 nm.[3] In the assay, samples were eluted at 1 mL/min with 4.8 vol % 2-propanol in hexane. For the calculation of the purity and yield, calibration curves were done with standard solution of chloramphenicol and chloramphenicol palmitate.

**References**


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