Supporting Information for

Silver cluster-assembled material as matrix for enzyme immobilization towards highly efficient biocatalyst

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Experimental details

Materials

Unless otherwise indicated, all reagents and solvents were acquired from commercial sources and used as received. *tert*-butyl mercaptan was purchased from Tokyo Chemical Industry Co., Ltd. Silver trifluoroacetate (CF_3COOAg) was purchased from FUJIFILM Wako Pure Chemical Corporation. Silver nitrate ($AgNO_3$), toluene, tetrahydrofuran, ethyl acetate, acetone, *n*-hexane, isopropanol, *N,N*-Dimethylacetamide (DMAc) and methanol (MeOH) were purchased from Kanto Chemical Co., Inc. Amano lipase PS (from *Burkholderia cepacia*) was purchased from Sigma-Aldrich. Protein Assay BCA Reagent A, Protein Assay BCA Reagent B, phosphate buffer solution (pH 7.0), vinyl acetate, 1-phenylethanol, mesitylene and acetonitrile (MeCN) were purchased from FUJIFILM Wako Pure Chemical Corporation.

Instrumentation

Regarding the single-crystal X-ray diffraction (SCXRD) data collection, the single crystal was immersed in the cryoprotectant Parabar 10312 (Hampton Research, 34 Journey, Aliso Viejo, CA 92656-3317 USA) and mounted on a Dual-Thickness MicroMounts™ (MiTeGen, LLC, Ithaca, NY, USA). SCXRD was carried out on a Bruker D8 QUEST diffractometer equipped with monochromated Mo Kα radiation (λ = 0.71073 Å). The crystal structure was solved using Apex3 Bruker software package.[1] Powder X-ray diffraction (PXRD) patterns were obtained on a Rigaku X-ray diffractometer with a Cu Kα source (λ = 1.5418 Å) at 40 kV and 40 mA. The data was collected from 2θ = 5-40º with a step size of 0.02º and scan speed of 2.0º per min. The nitrogen physisorption isotherms were recorded on a Quantachrome's Quadrasorb evo gas sorption analyzer at 77 K using sample degassed at 50 ºC for 8 h. The Brunauer−Emmett−Teller (BET) theory was used to calculate the specific surface area based on the adsorption data in the relative pressure (P/P_0) range from 0.001 to 0.05. The pore size distribution was estimated from the adsorption data using the nonlocal density functional theory (NLDFT). X-ray photoelectron spectroscopy (XPS) spectra were acquired on a JPS-9-1-MC electron spectrometer (JEOL, Tokyo, Japan) using Mg K_α radiation (1253.6 eV) as the excitation source. All the binding energies were referenced to the neutral C 1s peak at 283.3 eV. The optical microscope images were acquired with an

Olympus SZX7 stereo microscope. Scanning electron microscopy/energy-dispersive X-ray spectroscopy (SEM-EDX) was conducted on a JEOL JSM-IT800SHL field emission scanning electron microscope. The thermal stability of the SCAM was probed by thermogravimetric analysis (TGA) using a Bruker TG-DTA2010SA instrument from room temperature to 800 ºC under nitrogen flow at 50 mL min^{-1}, ramping at 10 ºC min^{-1}. UV-Vis absorption spectra were recorded with a JASCO V-770 spectrophotometer. The water contact-angle was measured with a DropMaster 300, Kyowa Interface Science Co., Ltd. (Tokyo, Japan) contact angle meter. For high-performance liquid chromatography (HPLC), the Shimadzu Prominence UFLC system was used.

Synthesis methods

Synthesis of silver *tert*-butylthiolate (AgS^{t}Bu). AgS^{t}Bu was synthesized according to the method described previously.[2,3]

Synthesis of TUS 5

First, AgS^{t}Bu (24 mg, 0.12 mmol) was dispersed under continuous stirring in 5 mL of DMAc solvent in a glass vial. Afterwards, CF_3COOAg (24 mg, 0.11 mmol) was added to the solution and stirred until a clear solution was obtained. In a separate glass vial, THIT (16.75 mg, 0.06 mmol) linker was dissolved in 5 mL of MeOH/MeCN (1:1, v/v) solution to obtain a clear solution. The linker solution was then slowly added to the former solution. The resulting mixture was kept for crystallization at room temperature for 1 day under dark, following which colorless hexagonal-shaped crystals of TUS 5 (17.97 mg, 64.73% yield based on Ag metal) were obtained from the bottom of the vial.

Loading of amano lipase PS on TUS 5

First, 120 mg of lipase was dissolved in 5 mL of phosphate buffer solution (pH 7.0). Let the resultant solution be denoted as **solution 1**. Then 50 mg of TUS 5 was added to 5 mL of DI water and sonicated for 15 min. Let the resultant mixture be **solution 2**. The **solution 1** was transferred into the **solution 2** and the mixture was stirred for 5 h. The lipase@TUS 5 was isolated by centrifugation.

Evaluation of the loading capacity of amano lipase PS by TUS 5 from UV-Vis spectroscopy[4,5]

(1) The bicinchoninic acid (BCA) working reagent was prepared by mixing 50 vol of Protein Assay BCA Reagent A with 1 vol of Protein Assay BCA Reagent B (Cu^{2+} solution).

(2) 60 μL of each standard or unknown sample were pipetted into separate test tubes. After this, 1.2 mL of the working reagent was added to each tube and mixed thoroughly. The tubes were covered and incubated at 50 ºC for 15 min, followed by cooling the samples to room temperature for 30 min. Next, the absorbances were measured by UV-Vis spectroscopy at 561 nm. A standard curve was prepared (Fig. S12a). The standard curve was employed to estimate the lipase concentration of each unknown sample (Fig. S12b).

(3) UV-Vis absorbance of the supernatant was obtained as follows:

Before loading: 0.779334

After 5 h loading: 0.662647

From the standard curve,

$y = 3.578\times10^{-5}x + 0.00651$, $R^2 = 0.9999$

where y = absorbance, x = concentration of lipase (ppm)

Concentration (Calculation from curve)

Before loading (C_1): 21598.66 ppm

After 5 h loading (C_2): 18337.51 ppm

Loading weight of lipase (mg)

$$= \frac{(C_1 - C_2) \times Amount\ of\ solutions\ 1\ and\ 2\ (mL)}{1000}$$

$$= \frac{(21598.66 - 18337.51) \times 10}{1000}$$

= 32.61 mg

Lipase uptake capacity of TUS 5

= 32.61 mg/50 mg

= 0.65 mg mg^{-1}

Enzymatic activity of lipase@TUS 5

For the kinetic resolution of (*R*,*S*)-1-phenylethanol using vinyl acetate as the acyl donor and *n*-hexane as the reaction solvent, the reactions were performed in a 10 mL vial with magnetic stirrer. 1-phenylethanol (30.3 μL, 0.25 mmol), vinyl acetate (60.4 μL, 0.65 mmol), *n*-hexane (1.2 mL) and lipase@TUS 5 (2.5 mg) were inserted into the vial. After sealing, the vial was placed in an oil bath heated at 50 ºC and stirred for an appropriate time. At the end of the reaction time, the catalyst was taken out from the system by centrifugation and the supernatant was analyzed by HPLC to determine the conversion (%) from the peak areas after reaction. Regarding the recycling test of the catalyst, the catalyst was isolated via centrifugation, washed three times with *n*-hexane (1.5 mL for each time), and then utilized directly for the next catalytic run without further drying.

Table S1. Conditions of the HPLC analysis.

Column	CHIRAL ART Cellulose-SB
Mobile phase	*n*-hexane:isopropanol (95:5, v/v)
Flow rate	1.0 mL/min
Injection volume	20 μL
Column temperature	25 ºC
UV detector (wavelength)	254 nm
Analysis duration	15-20 min

Table S2. Crystal data and structure refinement parameters of TUS 5.

Identification code	TUS 5
Empirical formula	$C_{60}H_{72}Ag_{12}F_{18}N_{18}O_{12}S_6$
CCDC number	2352291
Formula weight	3066.15
Temperature/K	273.15
Crystal system	Trigonal
Space group	*P*3
a/Å	30.4190(6)
b/Å	30.4190(6)
c/Å	11.1141(4)
α/°	90
β/°	90
γ/°	120
Volume/Å^3	8906.2(5)
Z	3
ρ_{calc}/g cm^{-3}	1.715
μ/mm^{-1}	2.113
F(000)	4428
Crystal size/mm^3	0.18 × 0.16 × 0.15
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	1.989 to 24.706°
Index ranges	-35 ≤ h ≤ 35, -35 ≤ k ≤ 35, -13 ≤ l ≤ 13
Reflections collected	93448
Independent reflections	20162 [R_{int} = 0.0970]
Data/restraints/parameters	20162/1552/1062
Goodness-of-fit on F^2	1.020
Final R indexes [I>=2σ (I)]	R_1 = 0.0712, wR_2 = 0.1880
Final R indexes [all data]	R_1 = 0.1013, wR_2 = 0.2137
Largest diff. peak/hole / e Å^{-3}	5.039/-1.914

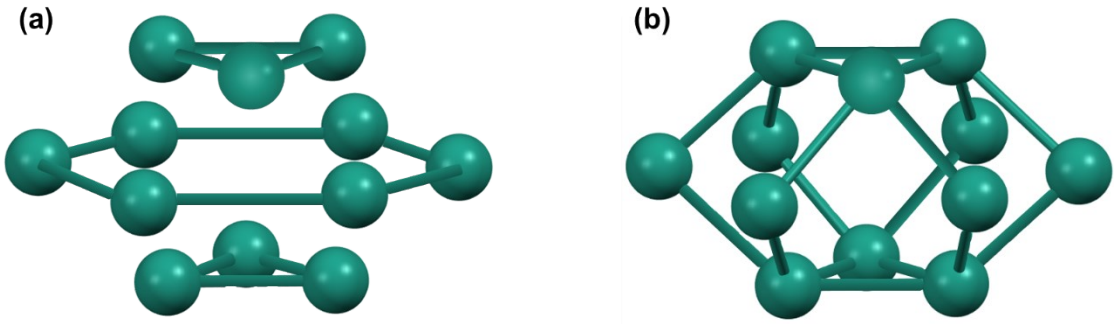

Fig. S1 (a) Three distinct layers of silver atoms building the Ag_{12} core, (b) empty cuboctahedron structural representation of Ag_{12} when considering the connections among the three layers.

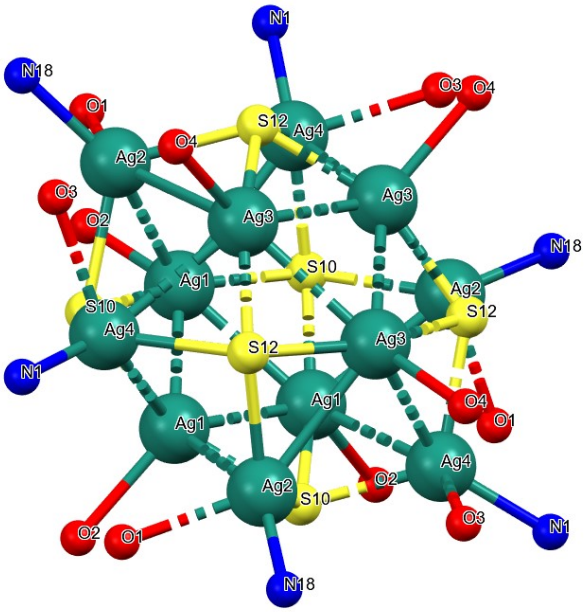

Fig. S2 Labeled Ag_{12} core co-protected by six S^tBu^- and six CF_3COO^- ligands, and coordinated to six linker molecules.

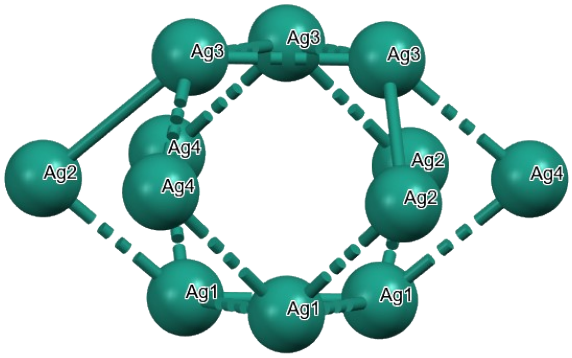

Fig. S3 Empty cuboctahedron-like geometry of labeled Ag_{12}.

Table S3. Ag−Ag bond lengths for the Ag_{12} empty cuboctahedron structure shown in Fig. S3

Atom 1	Atom 2	Bond length/Å		Bond length/Å
Ag1	Ag1	3.017	**Maximum**	3.207
Ag1	Ag2	3.031	**Minimum**	3.017
Ag1	Ag4	3.207	**Average**	3.088
Ag2	Ag3	3.170	**S.D.**	0.080
Ag3	Ag3	3.055		
Ag3	Ag4	3.049		

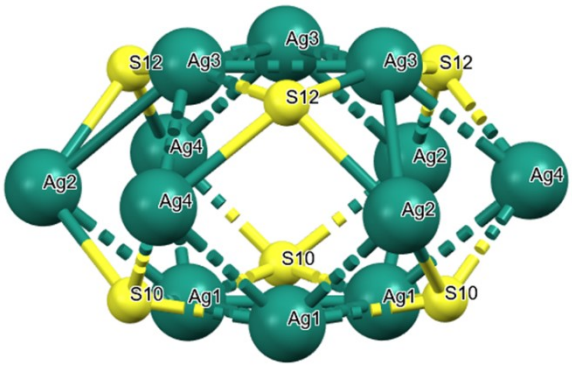

Fig. S4 Attachment of six thiolates on the Ag_{12} cluster node.

Table S4. Ag−S bond lengths corresponding to Fig. S4

Atom 1	Atom 2	Bond length/Å		Bond length/Å
Ag1	S10	2.478	**Maximum**	2.586
Ag1	S10	2.501	**Minimum**	2.448
Ag2	S10	2.586	**Average**	2.506
Ag4	S10	2.485	**S.D.**	0.050
Ag2	S12	2.510		
Ag3	S12	2.448		
Ag3	S12	2.467		
Ag4	S12	2.576		

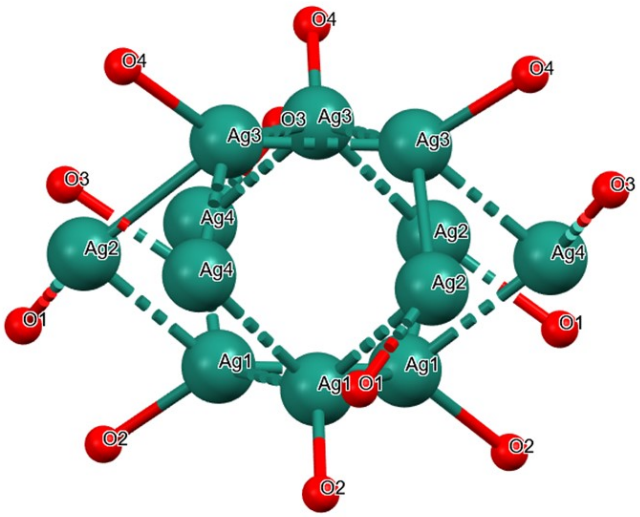

Fig. S5 Attachment of six trifluoroacetates on the Ag_{12} cluster node.

Table S5. Ag–O bond lengths corresponding to Fig. S5

Atom 1	Atom 2	Bond length/Å		Bond length/Å
Ag1	O2	2.514	**Maximum**	2.622
Ag2	O1	2.536	**Minimum**	2.445
Ag3	O4	2.445	**Average**	2.529
Ag4	O3	2.622	**S.D.**	0.073

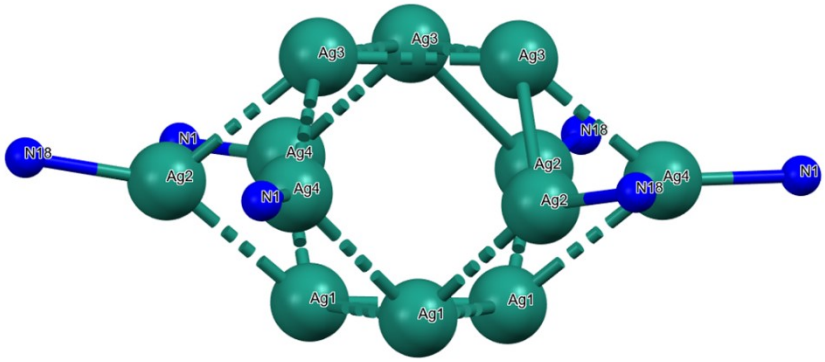

Fig. S6 Attachment of six linker molecules on the Ag_{12} cluster node.

Table S6. Ag–N bond lengths corresponding to Fig. S6

Atom 1	Atom 2	Bond length/Å		Bond length/Å
Ag2	N18	2.229	**Maximum**	2.229
Ag4	N1	2.208	**Minimum**	2.208
			Average	2.219
			S.D.	0.015

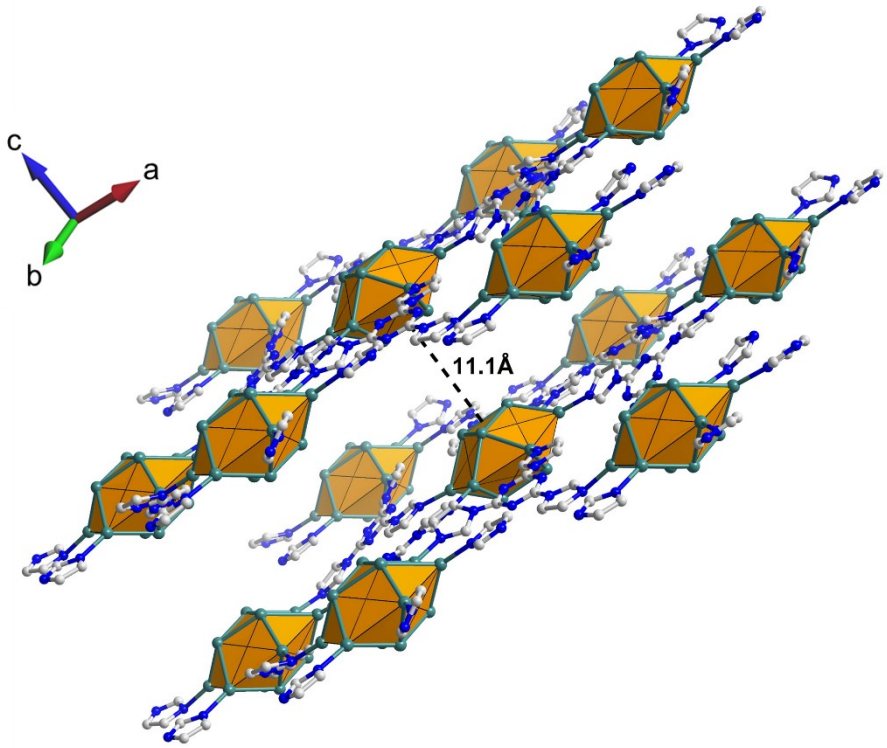

Fig. S7 Interlayer spacing between the adjacent 2D layers in TUS 5. Color code: Ag, dark green; N, blue C, gray. H atoms, thiolate ligands, and trifluoroacetate ligands are removed for clarity.

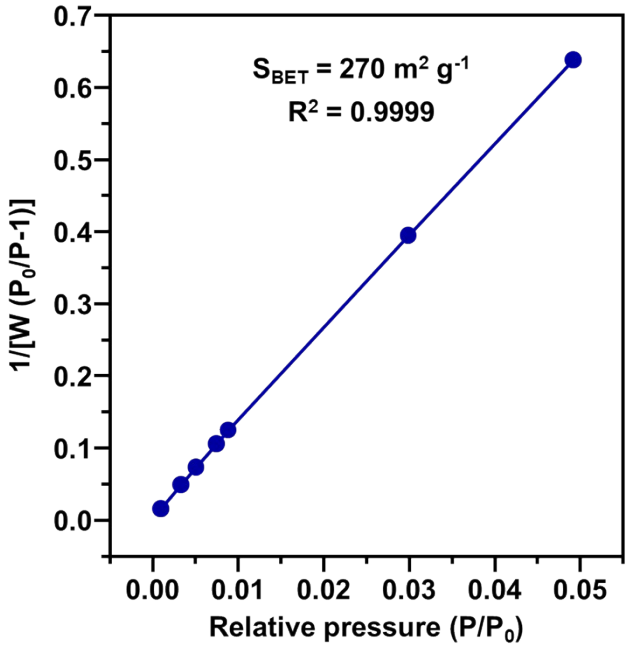

Fig. S8 BET plot for TUS 5 calculated from the N_2 adsorption isotherms at 77 K.

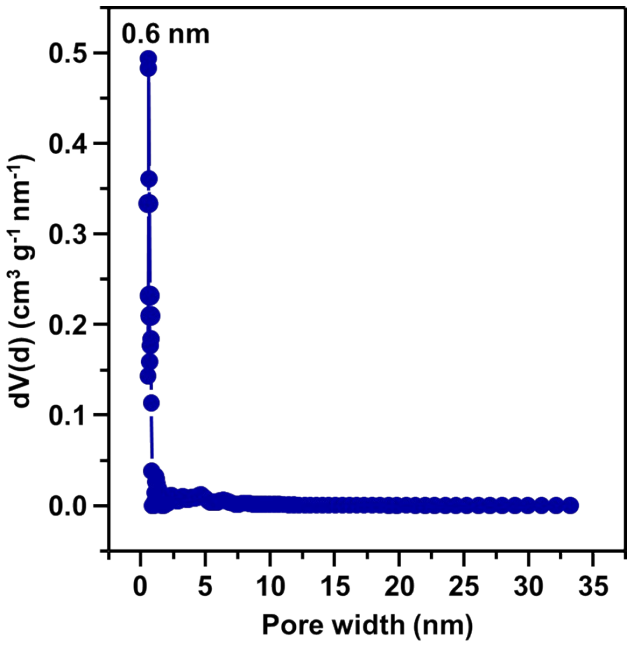

Fig. S9 Pore-size distribution for TUS 5.

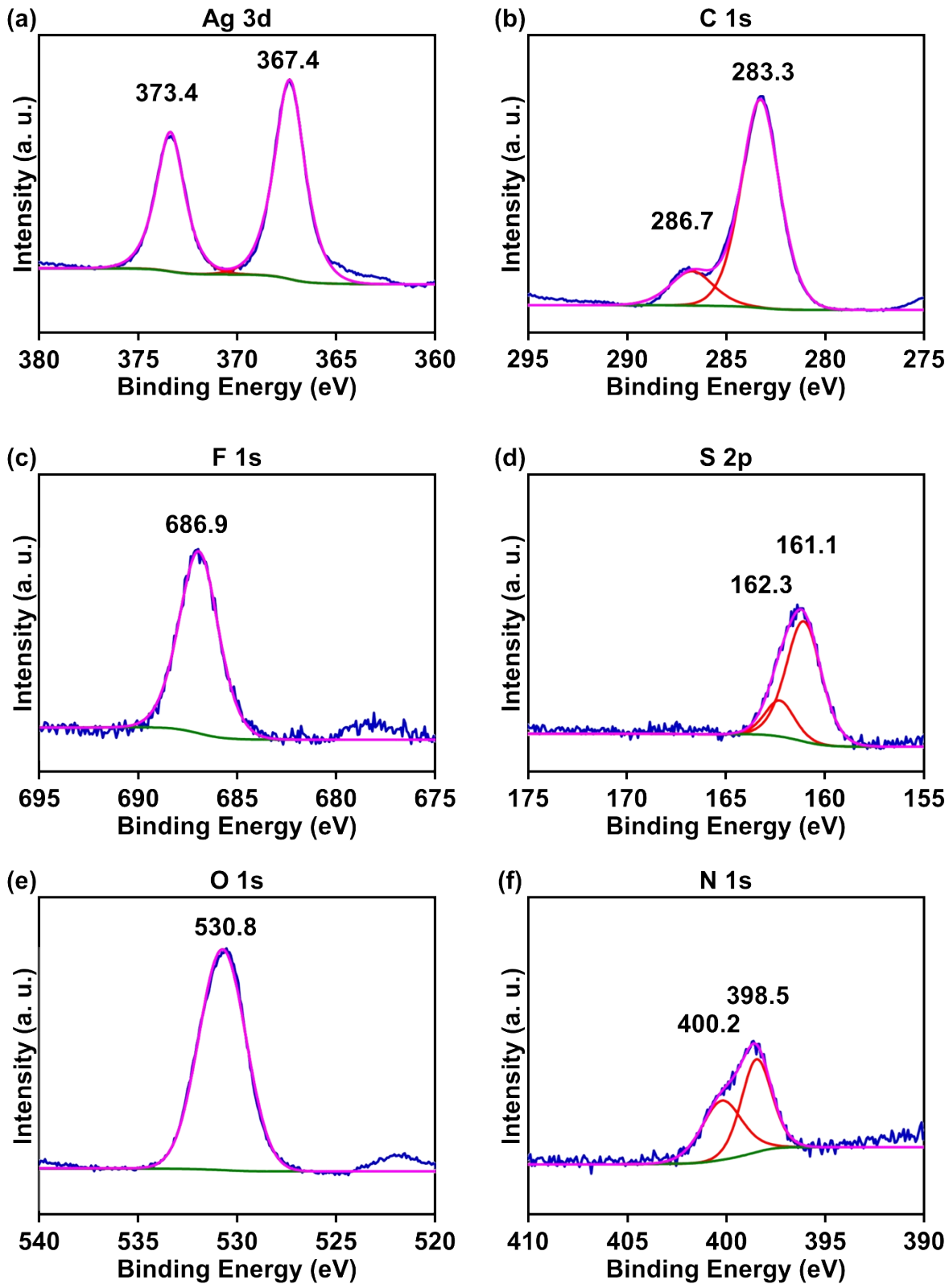

Fig. S10 High-resolution binding energy plot of each element obtained from the XPS measurement of TUS 5.

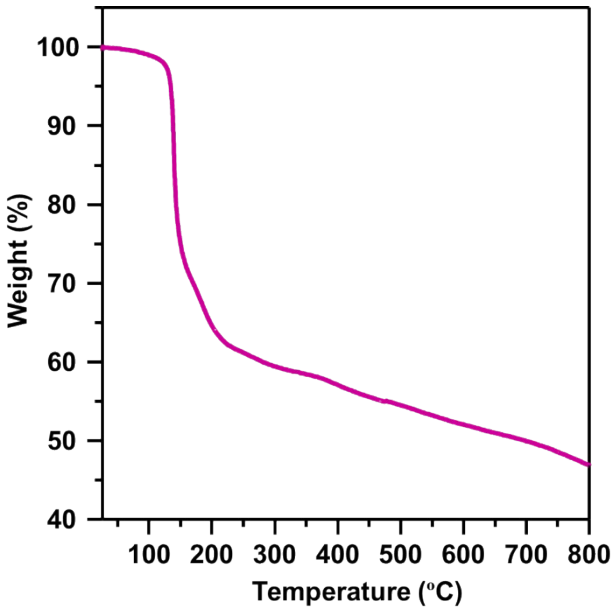

Fig. S11 TGA trace of TUS 5 under N_2 atmosphere.

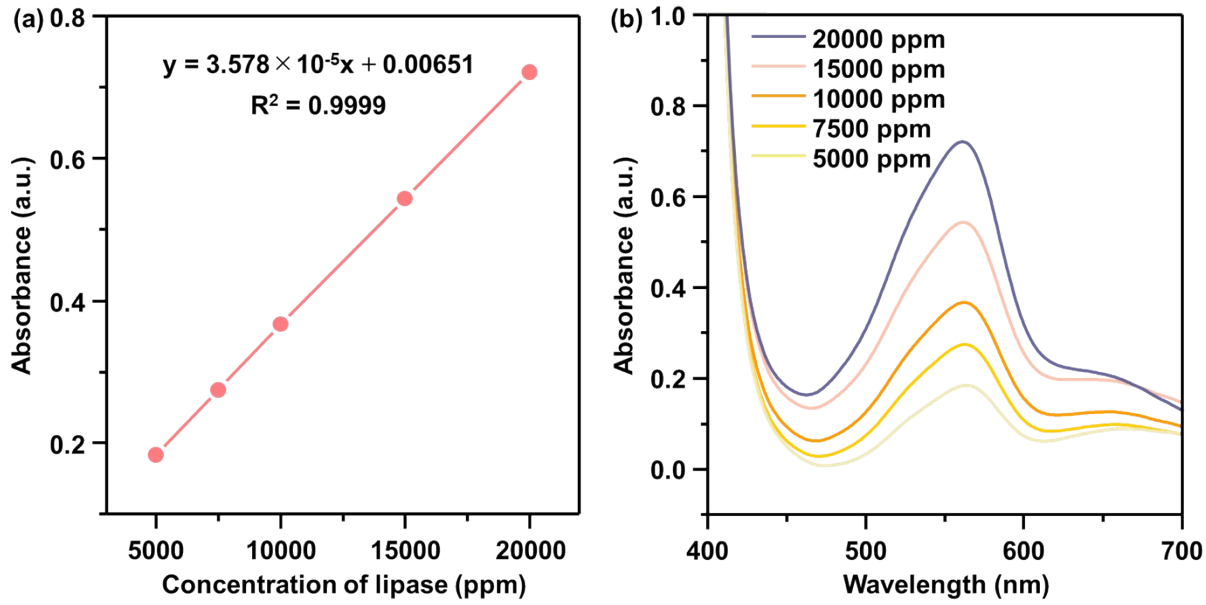

Fig. S12 (a) Standard curve and (b) UV-Vis spectra displaying the absorbance of different concentrations of lipase using BCA assays.

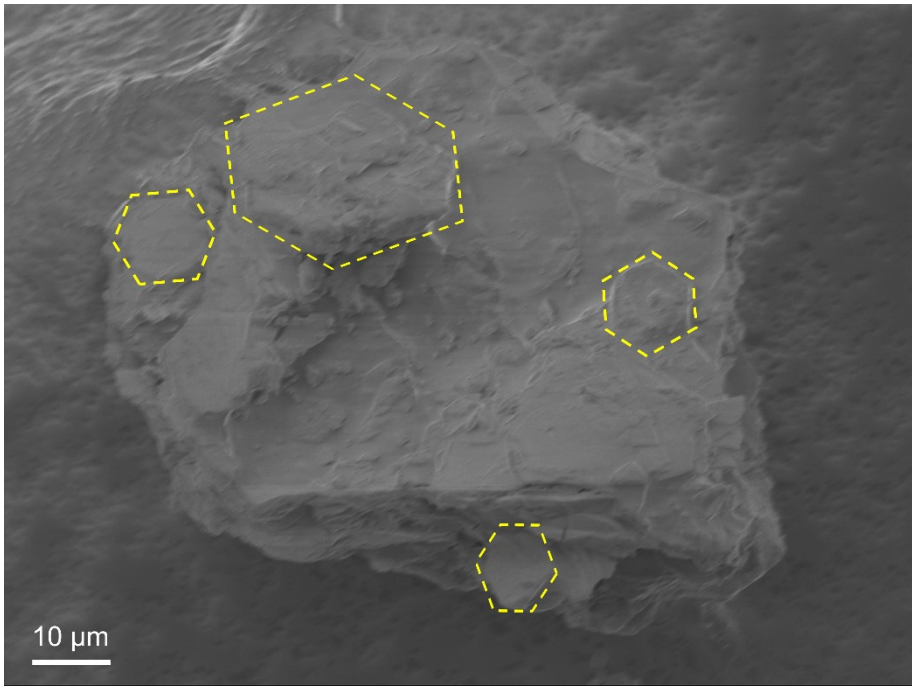

Fig. S13 SEM image of lipase@TUS 5.

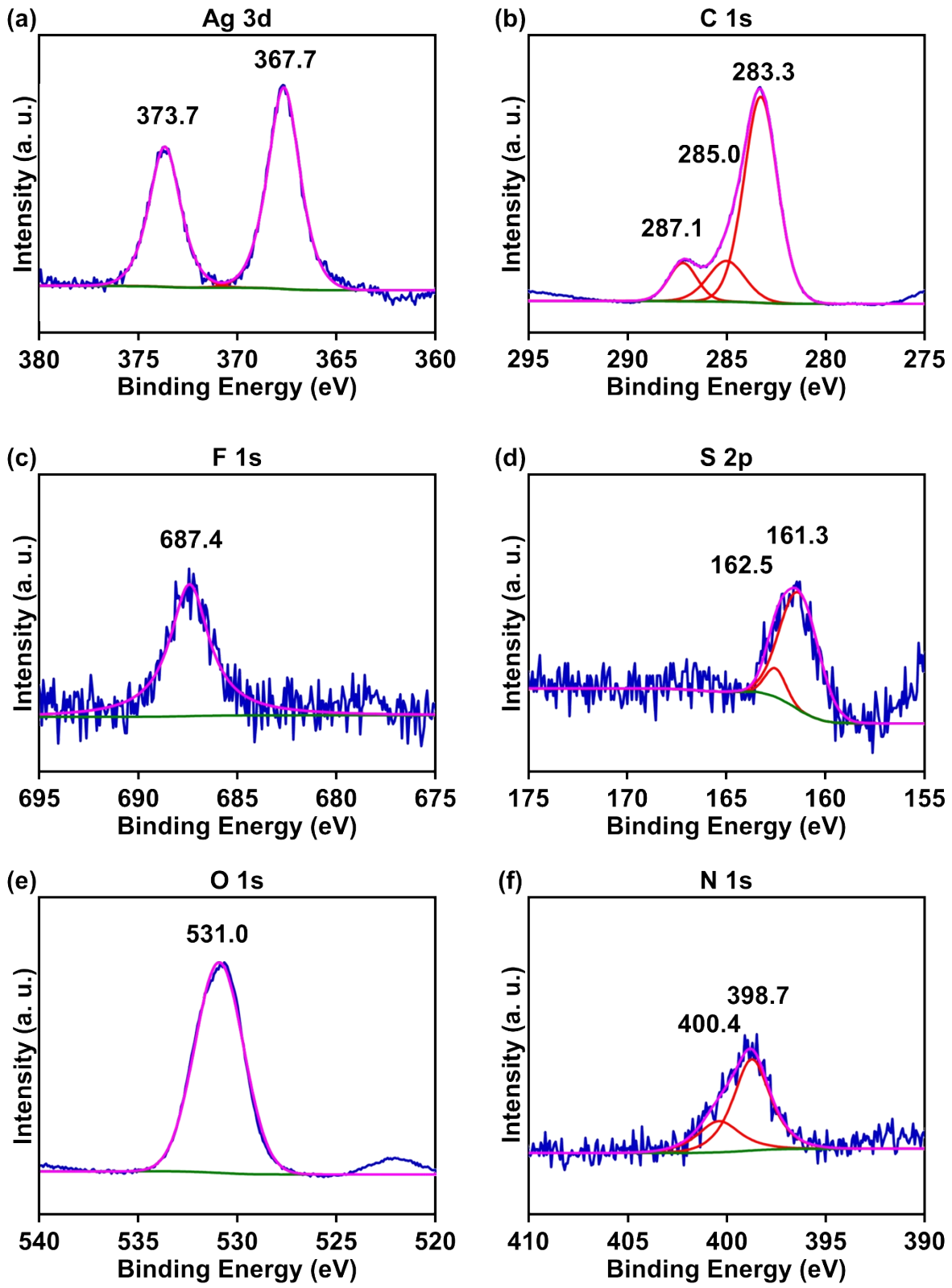

Fig. S14 High-resolution binding energy plot of each element obtained from the XPS measurement of lipase@TUS 5.

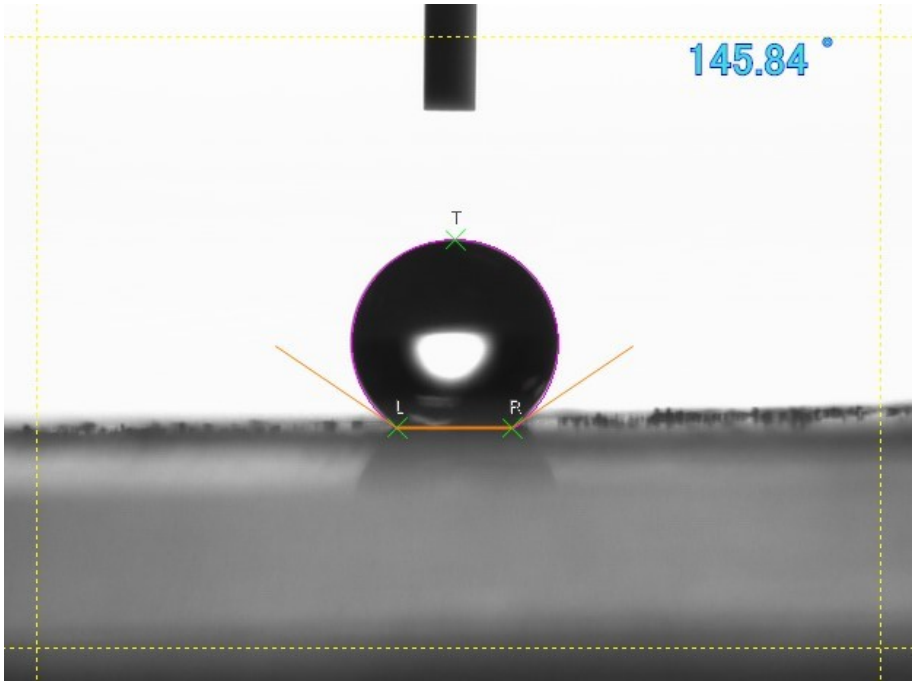

Fig. S15 Water contact-angle test for TUS 5.

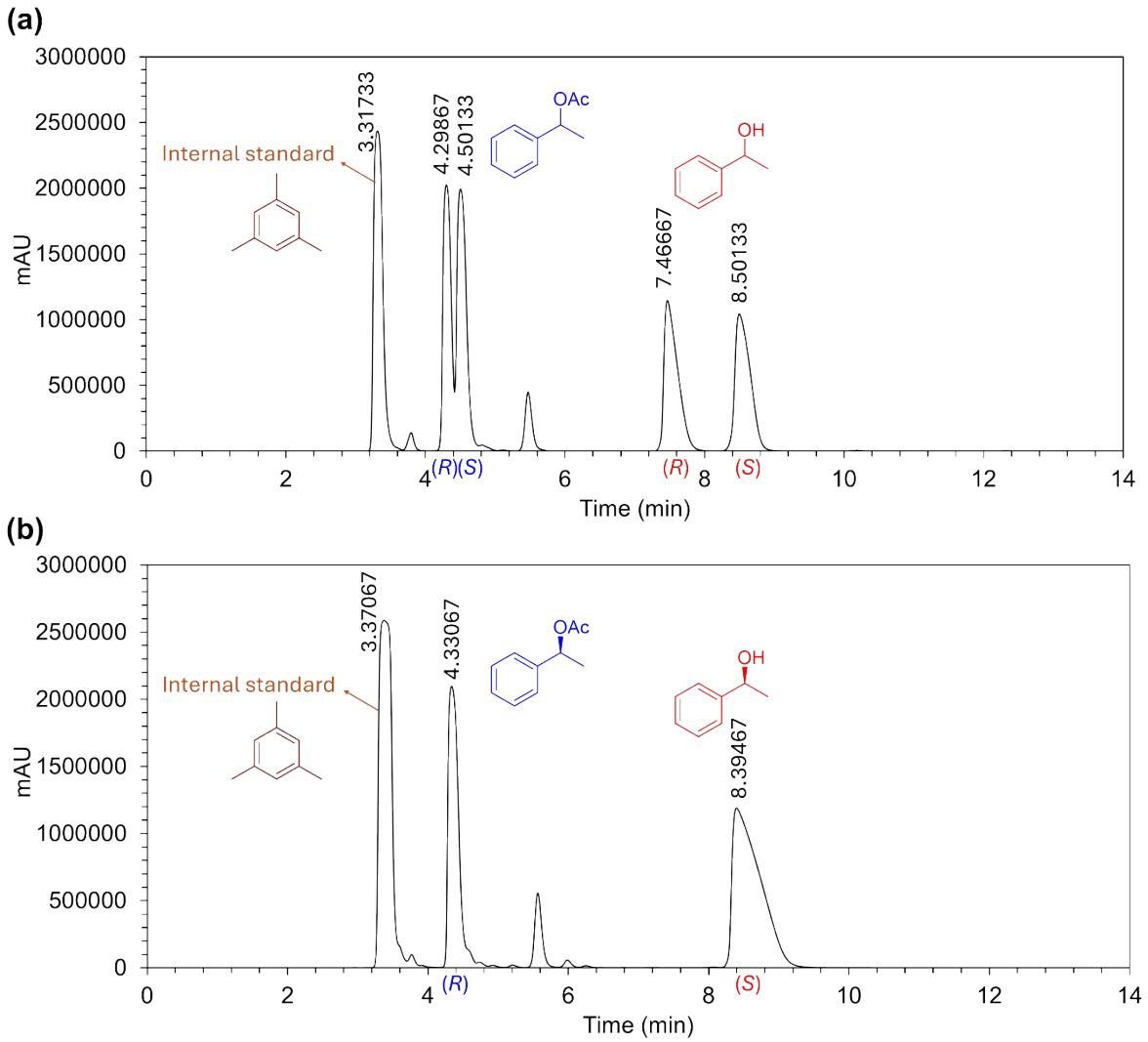

Fig. S16 HPLC spectra of (a) (*R*,*S*)-1-phenylethyl acetate and (*R*,*S*)-1-phenylethanol, and (b) the solution obtained after kinetic resolution of (*R*,*S*)-1-phenylethanol using vinyl acetate as the acyl donor, *n*-hexane as the reaction solvent and catalyzed by lipase@TUS 5.

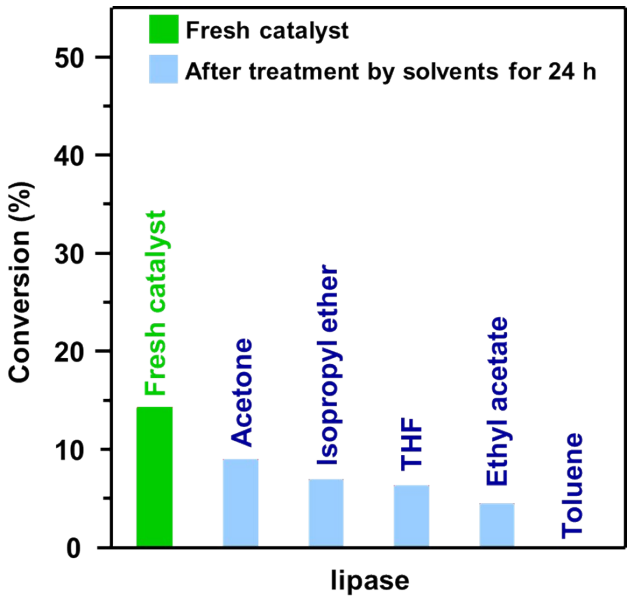

Fig. S17 Retention of catalytic activities of free lipase upon treatment with different organic solvents for 24 h.

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