Supporting Information

Laboratory evolution of enantiocomplementary *Candida antarctica* lipase B mutants with broad substrate scope

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Experimental Section

General

All chiral *p*-nitrophenyl esters (**1, 4-14**) were synthesized in our laboratory by standard procedures.

Library Generation

Saturation mutagenesis libraries were created at sites A (W104 and S105), B (L144 and V149), C (I189 and V190), D (A281 and A282) and E (V154 and Q157) using forward primers and a silent reverse primer (see Table S9) according to an improved PCR-based method for creating saturation mutagenesis libraries.[1] PCR reactions were performed with KOD Hot Start polymerase (Novagen, USA) using these primers and WT-CALB plasmid (pETM11-CALB) as the template DNA. The reaction (100 μL final volume) contained: 10 × KOD buffer (10 μL), $MgCl_2$ (4 μL, 25 mM), dNTP (10 μL, 2 mM each), forward primers (4 μL, 2.5 μM each), silent reverse primer (4 μL, 2.5 μM), template plasmid (1 μL, 100 ng/μL) and 1 μL of KOD polymerase. PCR conditions used were 95 °C, 3 min; five cycles of (98 °C, 1 min; 65 °C, 1 min; 72 °C, 5 min) for the generation of megaprimer; 20 cycles of (98 °C, 1 min; 72 °C, 8 min); and final extension at 72 °C, 10 min. To ensure elimination of the circular polymethylated template plasmid, 20 μL of PCR reaction mixture were mixed with 1 μL DpnI (10 U/μL) and incubated overnight at 37°C, followed by an additional 1 μL of DpnI for 3 h. Upon purification of the DpnI-digested product with a QIAquick PCR purification spin column, an aliquot of 5 μL was used to transform 100 μL of *E. coli* Origami2 cells (containing chaperone plasmid pGro7, Takara, Japan) electrocompetent cells. The transformation mixture was incubated with 1 mL of LB medium at 37°C with shaking and spread on LB-agar plates containing Kanamycin (34 μg/mL) and Chloramphenicol (34 μg/mL).

Expression and Screening of Libraries

Colonies appeared in 24–36 h and were picked by a colony picker QPIX (Genetix, New Milton, UK), and were transferred into 96-deep-well plates (capacity, 2.2 mL, Thermo Scientific, UK) containing 800 μL of TB media with Kanamycin (34 μg/mL) and Chloramphenicol (34 μg/mL), and incubated at 37 °C under shaking of 800 rpm overnight. 100 μL preculture and 70% glycerol were mixed in 1:1 volume to make 200 μL in a 96-round-well glycerol stock storage plate (NUNC; Thermo-Fisher Scientific, Denmark) and stored at −80 °C. A fresh 700 μL of TB media supplemented with 1 mg/mL L-arabinose as the inducer for expression of chaperone pGro7, Kanamycin (34 μg/mL) and Chloramphenicol (34 μg/mL), was inoculated from 100 μL preculture by replication program of liquid handling robotics (TECAN, Switzerland). The duplicate plates were allowed to grow at 37 °C for 4 h, and then cooled in the 4°C fridge for 1h. Then 1 mM isopropyl β-thiogalactopyranoside (IPTG) was added to induce CALB expression. The cultures were allowed to express lipase for 24 h at 18°C. The plates were centrifuged at 4000 rpm and 4 °C for 25 min, and then the supernatants were discarded.

The cell pellet of each well in the original plates was resuspended in 600 μL of 50 mM Tris-HCl (pH 8.0) containing 1 mg/mL lysozyme and 2 units/mL Dnase I. Lysis were performed at 37 °C and 800 rpm for 1 h. Cell debris was precipitated by centrifugation at 4000 rpm and 4 °C for 25 min. 60 μL of each cleared supernatant was trans-

ferred to a 200 μL 96-well microtiter plate. Screening buffer (14 mL, 50 mM potassium phosphate, 0.1% Triton X-100, pH 8.0) and *S*-**1** or *R*-**1** (140 μL, 10 mg/mL in acetonitrile) were premixed before dispersion, and then the mixed solution (60 μL) were dispensed into the microtiter plate containing CALB libraries supernatant. The hydrolytic reaction was followed at 405 nm for 10 min by using a Spectramax Plus384 from Molecular Devices (Sunnyvale, USA). Positive hits were selected for a kinetic resolution experiment to determine the enantioselectivity (*E*) and sequenced by Eurofins Medigenomix GmbH, Germany.

Enzyme Purification and Kinetic Measurements

An overnight culture of the appropriate overexpression strain grown in LB media was diluted 1:100 into 200 mL of TB media with 1mg/mL L-arabinose as inducer and 34 μg/mL Kanamycin and 34 μg/mL Chloramphenicol in 1-L flask. The culture was shaken at 37 °C until the optical density at 600 nm reached 0.6, then IPTG was added to a final concentration of 1 mM and the culture was shaken for additional 24 h at 18 °C. Cells were harvested by centrifugation at 4000 rpm for 25 min at 4 °C. The cell pellets were resuspended in 5 mL 50 mM Tris-HCl buffer (pH 8.0) and lysed by sonification (Bandelin, 15×10 sec with 10 sec interval, 40% pulse, on water-ice bath). The cell debris was removed by centrifugation at 10,000 rpm for 30 min at 4 °C. The supernatant was filtered and loaded on a GE Healthcare HisTrap FF Crude column (5 mL) pre-equilibrated with 50 mM Tris-HCl buffer containing 0.5 M NaCl and 5 mM imidazol. Impurity was removed by imidazol at the concentration of about 40 mM and the enzyme was eluted by 50 mM Tris-HCl buffer with 0.5 M NaCl and 200 mM imidazol. The enzyme fraction was desalted and concentrated by using an ultrafiltration centrifugal filter (10 kD cut-off membrane, Amicon), then another ultrafiltration centrifugal filter with 50 kD cut-off membrane was used to cut off the small amount of chaperone pGro7 that remained in the enzyme fraction. The purified enzymes were dissolved by 50 mM Tris-HCl buffer (pH 8.0) and stored at -80 °C. The purity of the enzyme was tested by SDS–PAGE (Figure S1), and the concentration of the purified enzyme was estimated by the Bradford method (Bio-Rad protein assay kit).

The hydrolytic activities of the purified enzymes were performed at 25 °C in potassium phosphate buffer (50 mM, pH 7.5, 1.0% Triton X-100) by using both enantiomers of *p*-nitrophenyl esters of **1, 4, 6**, and **11** separately at various concentration ranges. The kinetics measurements were performed on a Molecular Devices Spectramax (Molecular Devices GmbH, Germany). The obtained data were fitted to the Michaelis-Menten equation by nonlinear regression analysis.

Kinetic Resolution of Racemic Substrates 1, 4–14

The reactions of substrate **1, 4–13** were performed as follows: Potassium phosphate buffer (420 μL, 50 mM, pH 7.5, 1.0% Triton X100) and substrates *rac*-**1, 4–13** in acetonitrile (30 μL, 10 mg/mL) were added to the enzymes solution (150 μL). The reaction mixture was shaken at 30 °C until the reaction had reached 30-50% conversion. The process was monitored by a Spectramax Plus384 from Molecular Devices, and the A_{405nm} was controlled in the range of 0.2-0.35. Then the reaction solution was acidified by adding 10% HCl (20 μL). The acidified solution was extracted with 600 μL dichloromethane and diethyl ether, respectively. The extracted organic phase was evaporated by using Speed-Vac alpha RVC (Christ, Germany). 400 μL MTBE containing $C_{12}H_{26}$ internal standard were added to the tubes. The conversion and the enantiomeric excess of *rac* **1, 4, 5, 6, 11, 12, 13** were determined by chiral GC. And the other substrates of *rac* **7-10** were analyzed by chiral HPLC. GC analysis was performed as described in the supporting information (Table S10). The HPLC analysis were performed by using a Chiralpak AD-3 chiral column, 150 mm × 4.6 mm i.d. Conditions: mobile phase, n-heptane/2-propanole/TFA = 98:2:0.1; flow, 1 mL/min; temperature profile, 298 K; detection, UV at 254 nm. Absolute stereochemistry of rac-**1, 4, 6, 11** were determined by comparison with S-standards, while the other substrates with literature values.

The reactions of substrate **14** were performed as follows: Potassium phosphate buffer (420 μL, 50 mM, pH 7.5, 1.0% Triton X100) and substrates *rac*-**14** in acetonitrile (30 μL, 10 mg/mL) were added to the enzymes solution (150 μL). The reaction mixture was stopped at different reaction time. After extraction for twice, the conversion and selectivity were determined by chiral GC. Absolute stereochemistry was determined by comparison with S-standards.

Scale-up of Kinetic Resolution of Substrate *rac*-1

Only one experiment was performed without optimizing the reaction conditions or purification. The cell pellets of mutant SG301 was prepared as described in the section “Expression and Screening of Libraries”. Approximately 1.5 g (wet cell weight) of fresh cell pellet which was harvested from ca. 150 ml of culture were utilized for the scale-up reaction. The cell pellets were resuspended in 10 mL 50 mM Tris-HCl buffer (pH 8.0) and lysed by sonification (Bandelin, 15×10 sec with 10 sec interval, 40% pulse, on water-ice bath). The cell debris was removed by centrifugation at 10,000 rpm for 30 min at 4 °C. The scale-up kinetic resolution of *rac*-**1** was performed as follows: Enzyme solution (2 mL) and substrate *rac*-**1** (40 mg) in acetonitrile (200 μL, 200 mg/mL) were added to potassium

phosphate buffer (2 mL, 50 mM, pH 7.5, 1.0% Triton X100). The reaction mixture was shaken at 30 °C until the reaction had reached 30-50% conversion. The process was monitored by a Shimadu UV-2550 UV-Vis Spectrophotometer at 405 nm wavelength. Then the reaction solution was acidified by adding 10% HCl (200 μL). The acidified solution was extracted three times with 4 mL dichloromethane and diethyl ether, respectively. The combined organic phases were evaporated under vacuum. The obtained crude product was further separated and purified using flash column chromatography, providing 9 mg of *S*-**2** (87% ee) and 16 mg of *R*-**1** (95% ee).

Determination of Thermostability

For accessing the thermostability of WT and the mutants, 50 μL of enzyme supernatants from the same deep-well plate was transferred to 200-μL 96-well PCR plates that were then heated for 45 min at a defined temperature from 37°C to 77°C; after cooling to 4°C for 5 min (stop the heat shock), the samples were kept at room temperature for 15 min. 100 μL distilled water was added to the plate and then the plate was centrifuged at 4000 rpm and 4 °C for 25 min. A volume of 60 μL supernatants was transferred from each well to a microtiter plate, and 60 μL solution of *p*-phenyl caprate (1mM) diluted from stock solution (20mM in acetonitrile) with phosphate buffer (50 mM potassium phosphate, 0.1% Triton X-100, pH 8.0) was dispensed into the microtiter plate. The hydrolytic reaction was followed at 405 nm for 10 min by using a Spectramax Plus384 from Molecular Devices (Sunnyvale, USA). The obtained data were transferred into the relative activity and fitted to obtain T_{50}^{45} values.

Molecular Modeling and MD Simulation

The molecular dynamic simulations for WT-CALB, and the evolved mutants SG303, RG401 were performed using the GROMACS 4.0 software (http://www.gromacs.org).[2] The X-ray crystal structure of CALB (PDB code 1TCA.pdb)[3] was used to construct the initial models for our MD simulations. Amino acid mutations were introduced into WT-CALB using Pymol 0.99rc6 programme to construct the input PDB files of SG303 and RG401.[4]

The protein was solvated with the default simple point charge (SPC) water, in a cubic box with a space of 10 Å around the solute. Periodic boundary condition was applied. To neutralize the redundant charges, one sodium counter ion was added to the system. Then, the system was subjected to a 1000 step energy minimization with the steepest descents method to remove bad van der Waals contacts. To maintain the simulated systems at a constant temperature and pressure, the Berendsen temperature coupling and Berendsen pressure coupling (the coupling constants were both set to 0.1) were used to keep the system in a stable environment. The reference temperature was fixed at 300 K. During the simulation, all bonds in the systems were constrained by the LINear Constraint Solver (LINCS) algorithm.[5] The particle mesh Ewald (PME)[6] method was used for computing long-range electrostatics. In all these simulations, a 30 ps position-restrained simulation was performed by keeping the protein coordinates fixed, and making the water molecules soak into the macromolecule. Finally, a full molecular dynamic simulation for 4 ns was submitted at 300 K temperature and 1 bar pressure. The time step used was 2 fs, and the coordinates were saved every 1 ps. The dynamics trajectories were analyzed by using VMD software,[7] OriginPro 7.5 and Microsoft Excel. Equilibrated conformers of WT-CAL B, SG303 and RG401 after MD simulation were shown in Figure 4 and Figure S2.

The complexes of WT or mutated CALB with *R*-**1** or *S*-**1** substrates were derived by docking *R*-**1** or *S*-**1** to the equilibrated conformation of WT, SG303 and RG401 extracted from their dynamics trajectories using VMD software. The docking process was performed by using Autodock 4.0.[8] For the ligands, Gasteiger charges were calculated using AutoDock Tools.[9] The receptor model was prepared with AutoDock Tools adding polar hydrogens and loading Kollman United Atom charges. With the use of AutoGrid, a searching grid box was set in appropriate size prior to docking. The box center was set exactly at the OG atom of the catalytic serine (Ser 105). The spaced box size was set 50, 50 and 50 Å (x, y, and z). After running AutoGrid and AutoDock, 100 possible docking conformations were obtained by Lamakian genetic algorithm under other default docking parameters. The docking conformations of each ligand were clustered on the basis of rootmean-square deviation (RMSD) and ranked on the basis of free energy of binding. The docking free energy (ΔGdocking) was the sum of vdw, electrostatic, hydrogen bond, desolvation and torsion items. The energetically favorable poses of the substrates *R*-**1** and *S*-**1** binding to the targeted binding site of WT-CALB, SG303, and RG401 mutants were extracted. Optimized poses of substrate *R*-**1** and *S*-**1** in *S*-selective mutant SG303 and *R*-selective mutant RG401 respectively were shown in Figure 5 and Figure S3.

Figures and Tables in Supporting Information

Table S1. All Results of the Hydrolytic Kinetic Resolution of rac-**4** with Different (R) and (S)-Selective CALB Mutants.

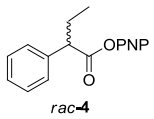
Entry	Substrate	Enzyme	Time(h)	Conv. (%)	ee_p (S)	*E* (S)	*E* (R)
1		SG301	10	43%	88%	31±2	
2		SG201	10	32%	85%	18±1	
3		SG204	6,5	44%	94%	73±20	
4		WT	16,5	11%	4%	1±1	
5		RG401	6,5	41%	95%		69±12
6		RG302	6,5	42%	92%		46±4
7	*rac*-**4**	RG303	4,5	49%	93%		87±15
8		RG304	6,5	46%	92%		66±18
9		RG305	4,5	48%	94%		82±2
10		RG308	6,5	44%	93%		61±5

Table S2. All Results of the Hydrolytic Kinetic Resolution of rac-**5** with Different (R) and (S)-Selective CALB Mutants.

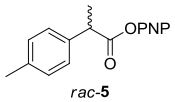
Entry	Substrate	Enzyme	Time(h)	Conv. (%)	ee_p (S)	*E* (S)	*E* (R)
1		SG301	4,5	50%	79%	20±2	
2		SG302	4,5	52%	75%	17±1	
3		SG303	4,5	54%	83%	46±8	
4		SG201	4,5	47%	79%	18±1	
5		SG204	1	42%	85%	23±4	
6		WT	16,5	39%	18%		2±1
7	*rac*-**5**	RG401	6,5	45%	85%		26±3
8		RG303	4,5	44%	84%		23±3
9		RG305	4,5	48%	82%		24±2
10		RG309	4,5	51%	84%		33±3
11		RG310	6,5	50%	83%		30±4
12		RG311	6,5	54%	83%		43±10

Table S3. All Results of the Hydrolytic Kinetic Resolution of rac-**6** with Different (R) and (S)-Selective CALB Mutants.

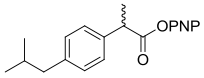
Entry	Substrate	Enzyme	Time(h)	Conv. (%)	ee_p (S)	*E* (S)	*E* (R)
1		SG301	6,5	43%	65%	8±2	
2		SG302	4,5	40%	68%	8±1	
3		SG303	6,5	42%	75%	12±1	
4		SG201	6,5	44%	69%	9±2	
5		SG204	4,5	42%	70%	9±1	
6	*rac*-**6**	WT	16,5	11%	8%		1±1
7		RG401	16,5	39%	77%		13±2
8		RG305	10	42%	75%		12±1
9		RG308	10	35%	73%		9±1

Table S4. All Results of the Hydrolytic Kinetic Resolution of rac-**11** with Different (R) and (S)-Selective CALB Mutants.

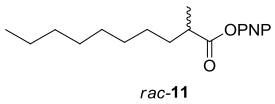
Entry	Substrate	Enzyme	Time(h)	Conv. (%)	ee_p (S)	*E* (S)	*E* (R)
1	 *rac*-**11**	SG301	0,5	47%	84%	26±8	
2		SG302	0,5	46%	85%	27±8	
3		SG303	0,5	43%	87%	29±1	
4		SG201	0,5	45%	81%	19±4	
5		SG203	1,5	50%	92%	75±8	
6		SG204	0,5	51%	63%	8±2	
7		SG205	1,5	50%	94%	115±1	
8		wt	3,5	68%	24%	3±1	
9		RG401	23	17%	66%		6±1
10		RG301	23	23%	66%		6±1
11		RG311	23	24%	69%		7±1

Table S5. All Results of the Hydrolytic Kinetic Resolution of rac-**12** with Different (R) and (S)-Selective CALB Mutants.

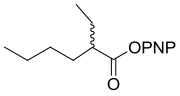
Entry	Substrate	Enzyme	Time(h)	Conv. (%)	ee_p (S)	*E* (S)	*E* (R)
1	 *rac*-**12**	SG301	1,5	50%	>99%	910±65	
2		SG302	1,5	51%	>99%	//	
3		SG303	1,5	49%	>99%	768±44	
4		SG201	1,5	47%	>99%	568±40	
5		SG202	1,5	51%	>99%	//	
6		SG204	1,5	49%	>99%	820±46	
7		WT	23	14%	90%	21±2	
8		RG401	23	23%	87%		19±2
9		RG101	23	28%	93%		41±6

Table S6. All Results of the Hydrolytic Kinetic Resolution of rac-**7-10, 13** with Different (R) and (S)-Selective CALB Mutants.

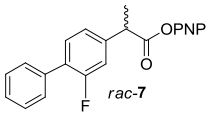
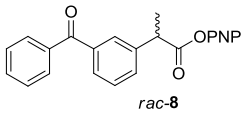
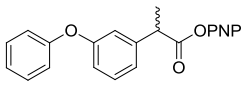
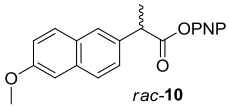
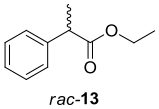
Substrate	Enzyme	Time (h)	ee_p (%)	ee_s (%)	Conv. (%)	*E* (S)	*E* (R)
 *rac*-**7**	SG301	3	74	31	23	9±1	
	SG303	5	71	25	21	7±1	
	SG201	5	73	31	24	8±1	
	SG204	6,5	63	86	62	8±2	
	wt	15	8	1	13		1±1
	RG401	20	59	8	12		4±1
	RG311	15	68	22	22		7±1
 *rac*-**8**	SG301	5	42	99	79	11±2	
	SG303	6,5	54	27	31	4±1	
	SG201	6,5	48	24	32	3±1	
	SG204	6,5	23	11	33	2±1	
	SG101	3	2	2	43	1±1	
	wt	4	21	11	31	2±1	
	RG401	20	33	1	6		2±1
	RG305	15	43	22	37		3±1
	RG311	4	58	33	34		5±1
 *rac*-**9**	SG301	0,5	84	36	26	15±2	
	SG303	4	81	40	10	11±1	
	SG201	4	63	34	32	6±1	
	SG204	0,5	81	41	29	13±3	
	SG101	0,5	25	17	41	2±1	
	wt	4	34	9	25	2±1	
	RG401	20	41	15	16		3±1
	RG305	15	52	36	30		5±1
	RG311	4	16	15	30		2±1
 *rac*-**10**	SG301	3	67	39	32	7±1	
	SG303	4	75	39	30	9±2	
	SG201	6,5	71	39	31	9±1	
	SG204	2	69	38	31	8±1	
	wt	20	9	1	9	1±1	
	RG401	20	81	24	19		12±1
	RG305	6,5	67	27	25		6±1
	RG311	15	78	37	28		11±1
 *rac*-**13**	SG301	3	16	-	41	2±1	
	SG303	3	13	-	47	1±1	
	SG204	3	17	-	46	2±1	
	wt	3	13		48	1±1	
	RG401	6,5	93	-	17		35±4
	RG305	6,5	95	-	23		50±6
	RG311	6,5	91	-	43		46±6

Table S7. Results of the Hydrolytic Kinetic Resolution of rac-**14** with (R) and (S)-Selective CALB Mutants.

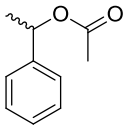
Entry	Substrate	Enzyme	Time (h)	Conv.(%)	ee_p (%)	*E* (R)	*E* (S)	Mean *E*
1		WT	2	48%	99%	789		919±184
2		WT	12	50%	99%	1050		
3		SG303	2	32%	99%	218		332±161
4		SG303	12	47%	99%	446		
5		SG204	2	14%	96%	65		96±43
6		SG204	12	30%	98%	126		
7		W104G	6	47%	99%	510		471±55
8		W104G	11	49%	98%	432		
9		W104L	6	38%	88%	26		33±10
10		W104L	11	50%	87%	40		
11	*rac*-**14**	W104M	6	40%	62%	6		7±1
12		W104M	11	44%	56%	7		
13		W104C	6	36%	23%		2	2±1
14		W104C	11	44%	21%		2	
15		RG309	6	39%	83%		18	20±4
16		RG309	11	50%	81%		23	
17		RG310	6	36%	83%		17	20±4
18		RG310	11	47%	82%		22	

Table S8. Kinetic Data of WT CALB and Mutants with Different Selectivity as Catalyst in the Hydrolysis of Racemic Substrates **5, 7, 8, 10, 12**

Enzyme	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1}s^{-1}$)
		rac-**5**	
RG401	0,26	0,80	3077
WT	2,60	0,18	69
SG303	0,58	0,10	172
		rac-**7**	
RG401	0,19	0,60	3150
WT	1,84	0,09	49
SG303	2,18	0,24	110
		rac-**8**	
RG401	0,44	1,66	3770
WT	0,50	0,11	215
SG303	0,53	0,09	170
		rac-**10**	
RG401	0,20	0,82	4050
WT	0,64	0,05	78
SG303	1,87	0,20	107
		rac-**12**	
RG401	0,24	4,57	19400
WT	0,81	0,05	60

SG303	0,11	0,01	100

Table S9. List of Forward and Reverse Primers

	Library	Primer	Sequence
(S)-selective libraries	D	For A281A282 NDT	CCTGGCGCCG**NDTNDT**GCAGCCATCGTGGCGGGTCCAAAGC
	C	For I189V190 NDT	GGCGACCGACGAG**NDTNDT**CAGCCTCAGGTGTCC
	B	For L144V149NDT	CGCCGGCCCT**NDT**GATGCACTCGCG**NDT**AGTGCACCC
(R)-selective libraries	A	For W104S105 NDT	GCTTCCCGTGCTCACC**NDTNDT**CAGGGTGGTCTGG
	D	For A281A282 NDT	CCTGGCGCCG**NDTNDT**GCAGCCATCGTGGCGGGTCCAAAGC
	B	For L144V149NDT	CGCCGGCCCT**NDT**GATGCACTCGCG**NDT**AGTGCACCC
	E	For V154Q157 NDT	GTGCACCCTCC**NDT**TGGCAG**NDT**ACCACCGGTTCGG
		silent reverse primer	GATGCCGGGAGCAGACAAGCCCGTCAGGGCGC

Table S10. Chiral GC Separation Conditions

Comp.	Conditions	Acid		Ester	
		t_R/min	t_R/min	t_R/min	t_R/min
1, 13	128°C; 1.5°C /min, 152°C; 40°C /min, 180°C (5 min). Carrier: H2, 70kpa. Column: 25 m Ivadex 1/OV 1701 G/294	12.57 (S)	13.01 (R)	ND.	ND.
4	50°C; 2°C /min, 164°C; 10°C /min, 200°C. Carrier: H2, 70kpa. Column: 25 m Ivadex 1/OV 1701 G/294	49.69 (S)	49.97 (R)	28.31 (S)	28.46 (R)
5	125°C (15min); 2.5°C /min, 153°C (3min); 20°C /min, 190°C (5min). Carrier: H2, 70kpa. Column: 25 m Ivadex 1/OV 1701 G/294	25.65 (S)	26.99 (R)	ND.	ND.
6	125°C (15min); 2.5°C /min, 153°C (3min); 20°C /min, 190°C (5min). Carrier: H2, 70kpa. Column: 25 m Ivadex 1/OV 1701 G/294	33.85 (S)	34.25 (R)	15.22 (S)	15.69 (R)
11	105°C (2min); 0.85°C /min, 124°C (1min); 20°C /min, 190°C (5min). Carrier: H2, 70kpa. Column: 25 m Ivadex 1/OV 1701 G/294	28.20 (S)	28.38 (R)	ND.	ND.
12	50°C; 2°C /min, 164°C; 10°C /min, 200°C. Carrier: H2, 70kpa. Column: 25 m Ivadex 1/OV 1701 G/294	32.51 (S)	32.87 (R)	ND.	ND.
		Ester		Alcohol (**15**)	
14	90°C (2min); 4°C /min, 110°C (3min); 60°C /min, 130°C. Carrier: H2, 70kpa. Column: 25m Hydrodex-β 0.25 i. D. G/533	7.1 (S)	8.8 (R)	9.1 (R)	9.7 (S)

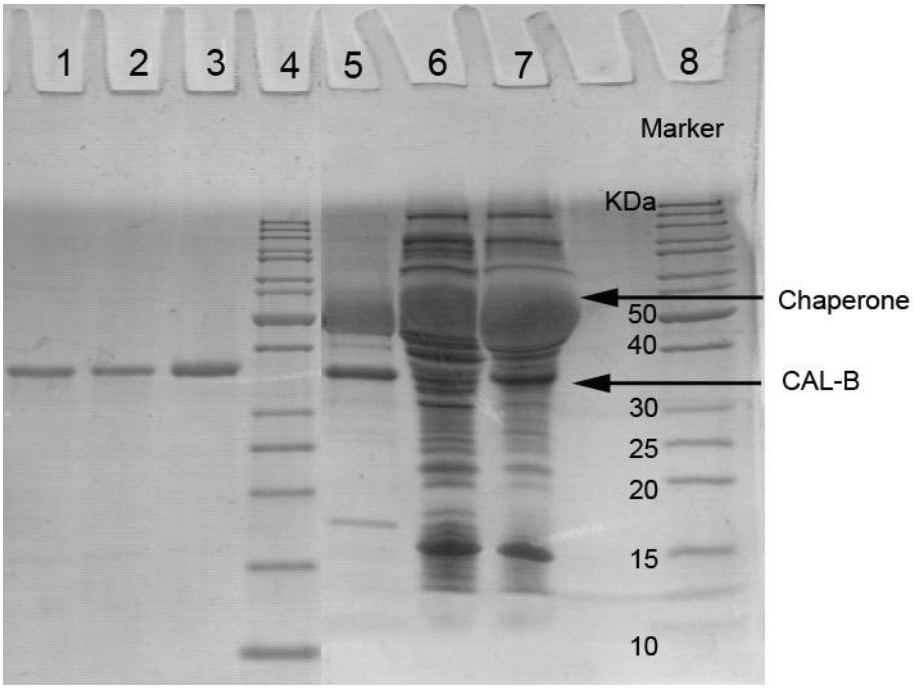


Figure S1. SDS-PAGE of pure WT-CALB (Lane 1), RG 401 (Lane 2), and SG 303 (Lane 3). Lane 4 and 8 are protein markers. Lane 5 is the protein mixture of CALB (33 KDa) and Chaperone pGro7 (60 KDa), Lane 6 and 7 are the unpurified supernatant.

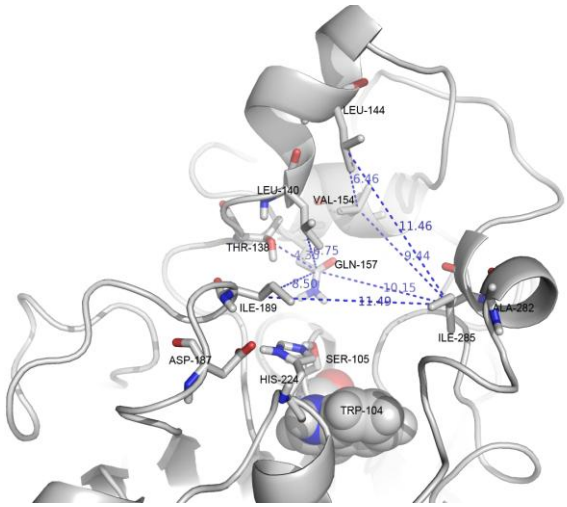


Figure S2. Equilibrated conformer of WT-CALB, some important residues surrounding the acyl binding sites and catalytic triad (S105/H224/D187) were shown as sticks. Distances defining the dimension of cavity A and crevice B were labeled on the blue dotted lines.

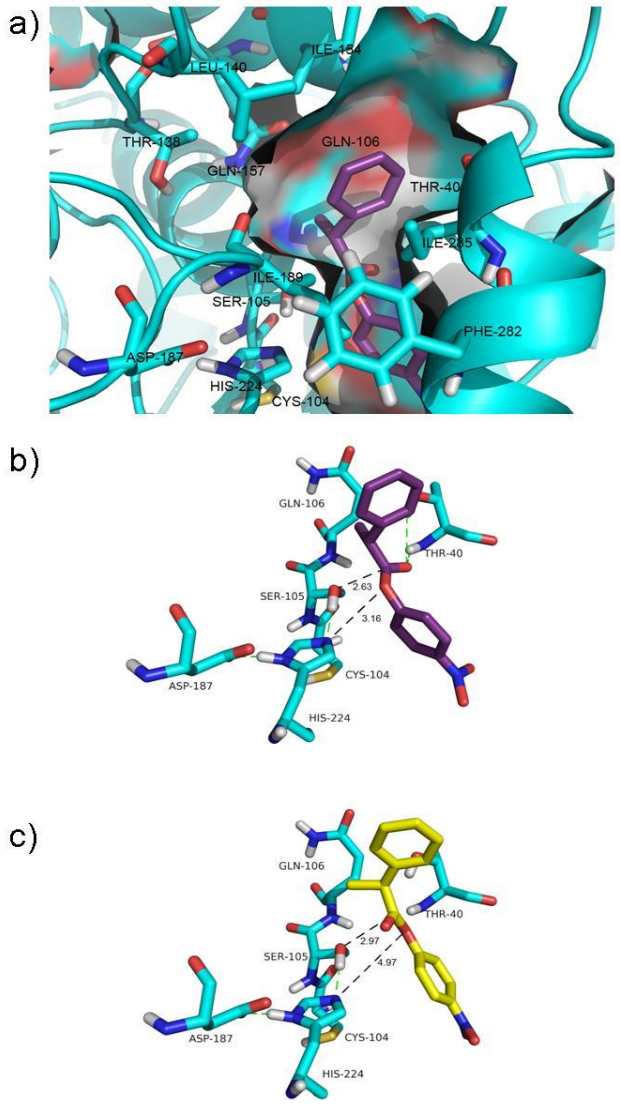


Figure S3. a) Optimized poses of substrate R-**1** in R-selective mutant RG401. R-**1** is shown as purple sticks. S105, H224 and D187 are catalytic triad. b) Necessary H-bonds for catalysis and the stabilization of the oxyanion indicated by green dotted lines occur in the complex of RG401 and R-**1**, the distances for effective nucleophilic attack of catalytic triad toward the ester bond of R-**1** were labeled by the black dotted lines. c) Oxyanion-stabilizing H-bonds in the nonproductive complex of RG401 and S-**1** are lost, and the distances for nucleophilic attack of catalytic triads toward the ester bond of S-**1** were beyond the suitable range.

References

(1) Sanchis, J.; Fernández, L.; Carballeira, J. D.; Drone, J.; Gumulya, Y.; Höbenreich, H.; Kahakeaw, D.; Kille, S.; Lohmer, R.; Peyralans, J. J.-P.; Podtetenieff, J.; Prasad, S.; Soni, P.; Taglieber, A.; Wu, S.; Zilly, F. E.; Reetz, M. T. *Appl. Microbiol. Biotechnol.* **2008**, *81*, 387–397.

(2) (a) Berendsen, H. J. C.; van der Spoel, D.; van Drunen, R. *Comput. Phys. Commun.* **1995**, *91*, 43-56. (b) van der Spoel, D.; Lindahl, E.; Hess, B.; Groenhof, G.; Mark, A. E.; Berendsen, H. J. C. *J. Comput. Chem.* **2005**, *26*, 1701-1718. (c) Hess, B.; Kutzner, C.; van der Spoel, D.; Lindahl, E. *J. Chem. Theory Comput.* **2008**, *4*, 435-447

(3) (a) Uppenberg, J.; Hansen, M. T.; Patkar, S.; Jones, T. A. *Structure* **1994**, *2*, 293-308. (b) Uppenberg, J.; Oehrner, N.; Norin, M.; Hult, K.; Kleywegt, G. J.; Patkar, S.; Waagen, V.; Anthonsen, T.; Jones, T. A. *Biochemistry* **1995**, 34, 16838-16851. (c) Otto, R. T.; Scheib, H.; Bornscheuer, U. T.; Pleiss, J.; Syldatk, C.; Schmid, R. D. *J. Mol. Catal. B: Enzym.* **2000**, *8*, 201-211.

(4) DeLano, W. L. The PyMOL Molecular Graphics System; DeLano Scientific: San Carlos, CA, USA, 2002 (http://www.pymol.org).

(5) Hess, B.; Bekker, H.; Berendsen, H. J. C.; Fraaije, J. G. E. M. *J. Comp. Chem.* **1997**, *18*, 1463-1472.

(6) Essman, U.; Perela, L.; Berkowitz, M.L.; Darden, T.; Lee, H.; Pedersen, L.G. *J. Chem. Phys.* **1995**, *103*, 8577-8592.
(7) van der Werf, M. J.; de Bont, J. A. M.; Swarts, H. J. *Tetrahedron: Asymmetry.* **1999**, *10*, 4225-4230.
(8) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639-1662.
(9) Sanner, M. F. *J Mol Graph Model*. **1999**, *17*, 57-61.