Supplementary material

Significantly increased catalytic activity of *Candida antarctica* Lipase B for the resolution of *cis*-(±)-dimethyl 1-acetylpiperidine-2,3-dicarboxylate

Jiang-Wei Shen, Jia-Mei Qi, Xiao-Jian Zhang, Zhi-Qiang Liu*, Yu-Guo Zheng

Key Laboratory of Bioorganic Synthesis of Zhejiang Province, College of Biotechnology and Bioengineering, Zhejiang University of Technology, Hangzhou 310014, China

***Corresponding author:**

Zhi-Qiang Liu, Email: microliu@zjut.edu.cn; Tel: +86-571-88320614; Fax: +86-571-88320630

Table S1 Primers for the combinatorial active-site saturation test and site-saturation mutagenesis

Library	Oligonucleotide sequences
A-f:	CTTATGGCCTTTGCTCCT**NDT**TACAAAGGT**NDT**GTGTTGGCTGGTCCA
B-f:	CCGTGTTGGCTGGTCCA**NDT**GACGCCTTGGCA**NDT**TCCGCTCCTTCCGTC
C-f:	CAGTGTCCGCTCCTTCC**NDT**TGGCAA**NDT**ACCACGGGTTCAGCT
D-f:	TACTCAGCTACAGACGAA**NDTNDT**CAGCCTCAAGTTAGTA
A-D-r:	GAGAACTGTGATGTCAAAGATCCTGCATGATCGATAAC
E-f:	GCAGCCCTGCTGGCTCCA**NDTNDT**GCCGCTATCGTTGCTG
E-r:	GTTCTAGATCAATGATGATGATGGTGGTGAGGAGTAA
L278-f:	AAGGTCGCTGCCGCAGCCCTG**NNK**GCTCCAGCAGCTGCCGCTATCG
L278-r:	CGATAGCGGCAGCTGCTGGAGC**MNN**CAGGGCTGCGGCAGCGACCTT
I285-f:	CTGGCTCCAGCAGCTGCCGCT**NNK**GTTGCTGGTCCAAAACAAAATT
I285-r:	AATTTTGTTTTGGACCAGCAAC**MNN**AGCGGCAGCTGCTGGAGCCAG

Table S2 Comparison of mut-I189K with other reported counterparts for resolution of *cis*-(±)-**1**.

Enzyme	Substrate conc. (g L^{-1})	Catalyst load (g L^{-1})	Time (h)	Conv (%)	*E*	S/C (g/g)	Reference
Immobilized CALB	80	40[a]	140	>49.9%	>2000	2	Riccardo et al., 2014
Addzyme CALB	80	40[b]	16	>49.9%	>80	2	Ramesh et al., 2014
mut-I189K	243.26	0.1[c]	5	>49.9%	>200	2432.6	This study
mut-I189K	486.52	0.8[c]	8	>49.9%	>200	608.15	This study

[a] The Immobilized CALB used as catalyst.
[b] The CALB enzyme solution (activity 5000 TBU, obtained from Advanced Enzyme Technologies Ltd.,) used as catalyst.
[c] The purified enzyme used as catalyst.

Reference

M. Riccardo, A. Giancarlo, B. Elisabetta, C. Andrea, F. Stefano and G. Marco, US Pat., 8680276B2, 2014.

P. Ramesh, T. Harini and N. W. Fadnavis, *Org Process Res & Dev*., 2014, **19**, 296-301.

Fig. S1. CALB mediated resolution of *cis*-(±)-dimethyl 1-acetylpiperidine-2,3-dicarboxylate.

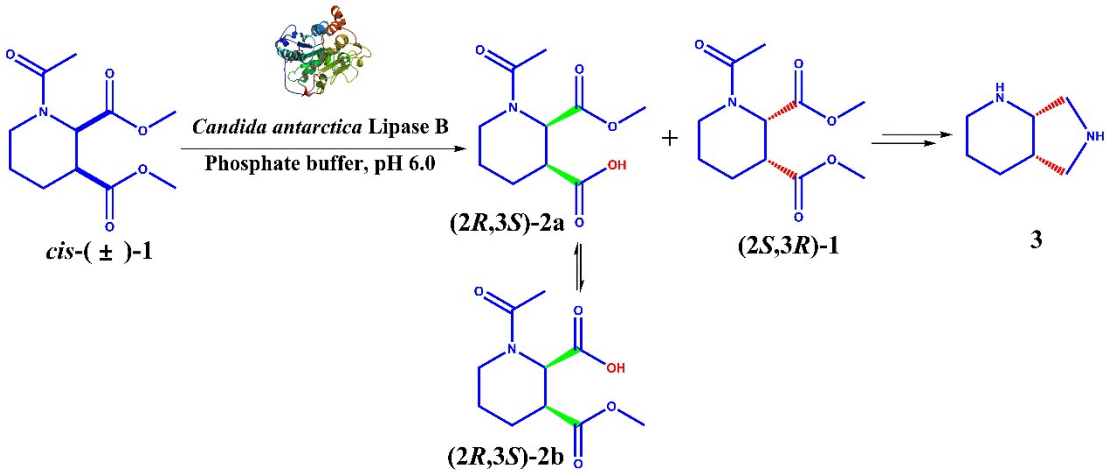
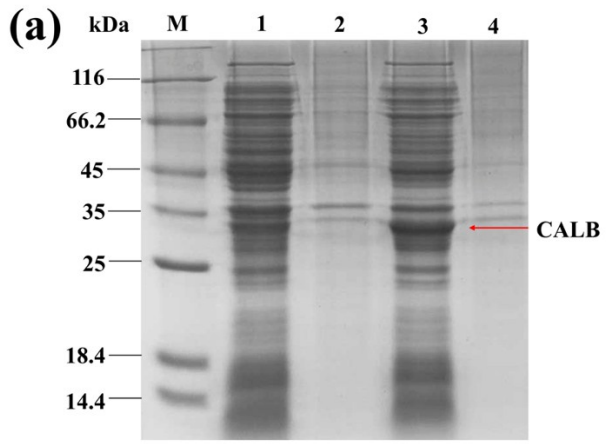
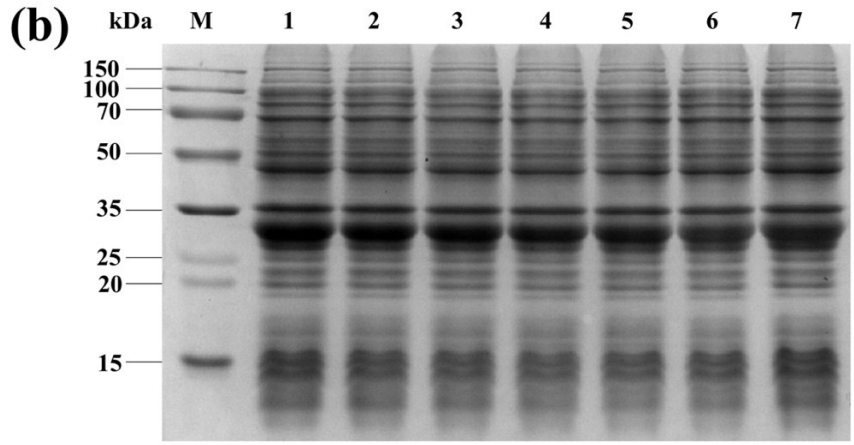
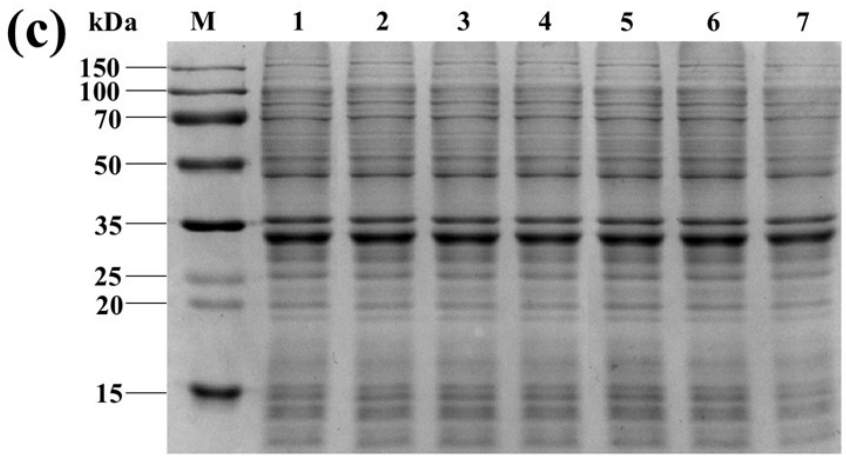


Fig. S2. SDS-PAGE analysis of expressed and purified proteins. (a) lane M: protein marker; lane 1, 2: supernatant extract and insoluble fraction of *E. coli* Rosetta (DE3)/ pET22b-CALB without induced; lane 3, 4: supernatant extract and insoluble fraction of *E. coli* Rosetta (DE3)/ pET22b-CALB induced by 0.1 mM IPTG. (b) lane M: protein marker; lane 1-7: the whole cell of wild-type CALB, mut-I189K, mut-I189R, mut-I189A, mut-I189N/V190L, mut-I189Y/V190L and mut-I189H/V190L. (c) lane M: protein marker; lane1: the whole cell of wild-type CALB; lane 2-7: the whole cell of activity-unchanged variants, (d) purified mut-I189K.






(c)
kDa
M
1
2
3
4
5
6
7
150
100
70
50
35
25
20
15

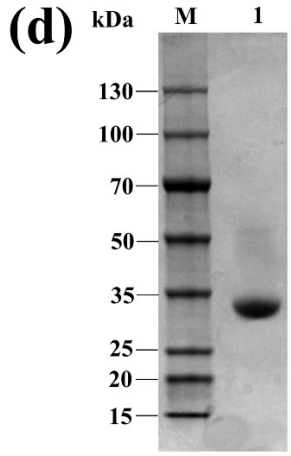

(d)
kDa
M
1
130
100
70
50
35
25
20
15

Fig. S3. The colorimetric high-throughput method for the screening of positive mutants. The 96-well plate with different colors in the wells containing different mutants, the wells of A1-A3 which containing wild-type CALB was used as the control. The wells with the color closer to yellow indicate the corresponding mutants with higher hydrolytic activity.

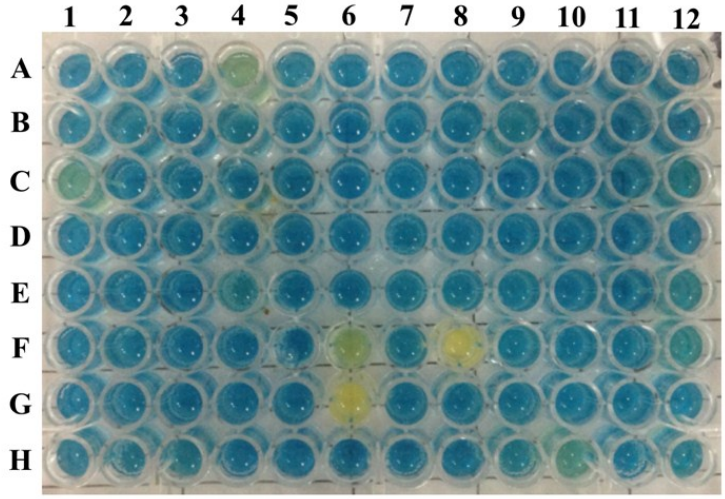


Fig. S4. Effects of temperatures on the thermostabilities of wild-type CALB and mut-I189K.

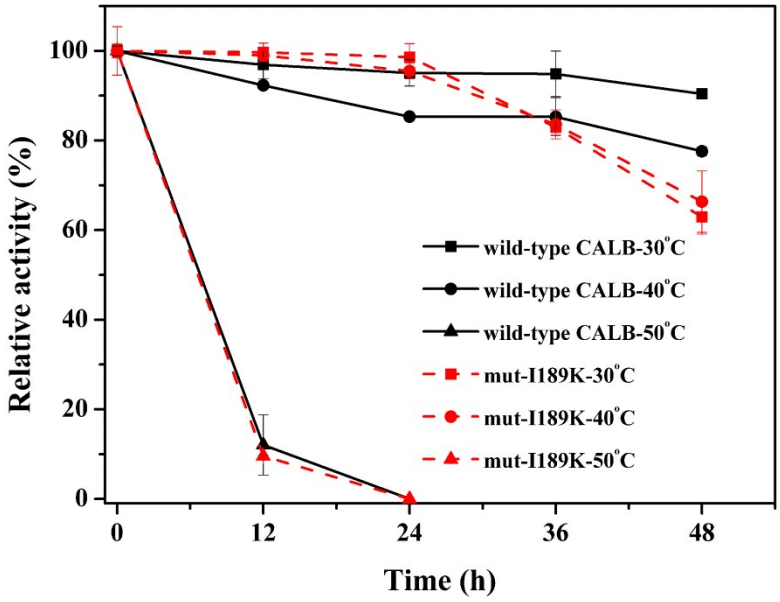


Fig. S5. Enzymatic resolution of *cis*-(±)-**1** at 2M, employing mut-I189K (0.8 g L^{-1}). The reaction was conducted at 35 °C, and the pH was maintained at 6.0 by automatically titrating 4 M NaOH.

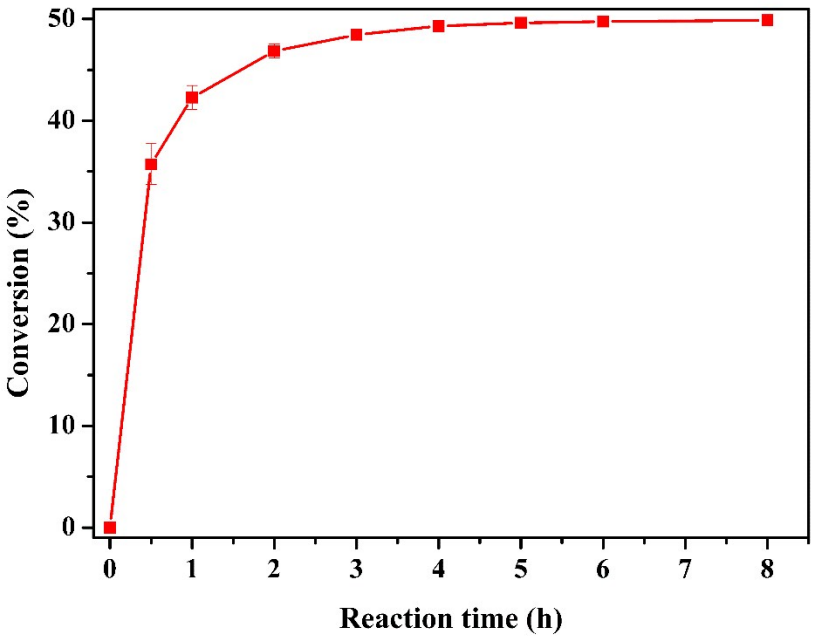


Fig. S6. Enzymatic resolution of *cis*-(±)-**1** at 1 M, adding product 0 M (black squares), 0.5 M (red dots) and 1.0 M (blue triangles) at a catalyst load of 0.1 g L^{-1} of purified mut-I189K. The reaction was conducted at 35 °C, and the pH was maintained at 6.0 by automatically titrating 4 M NaOH.

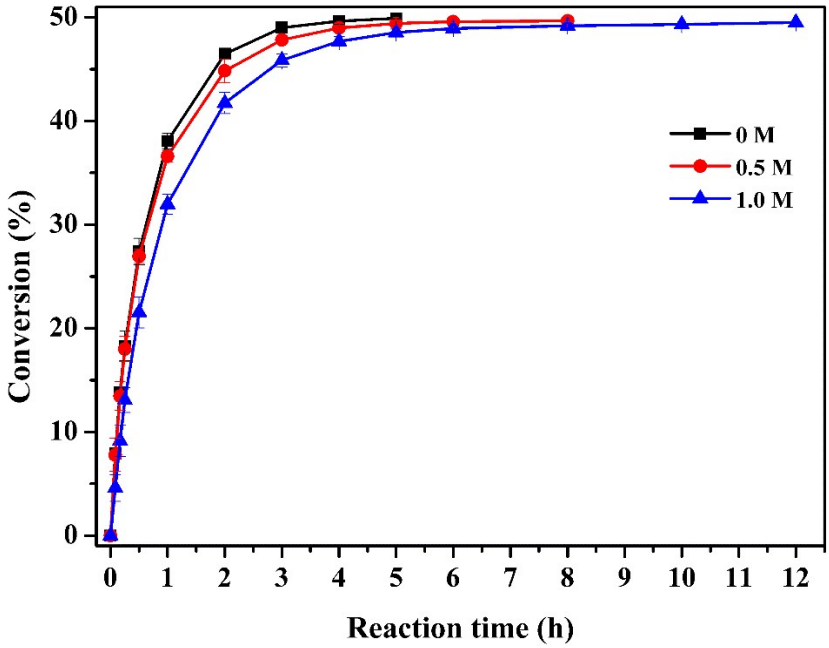
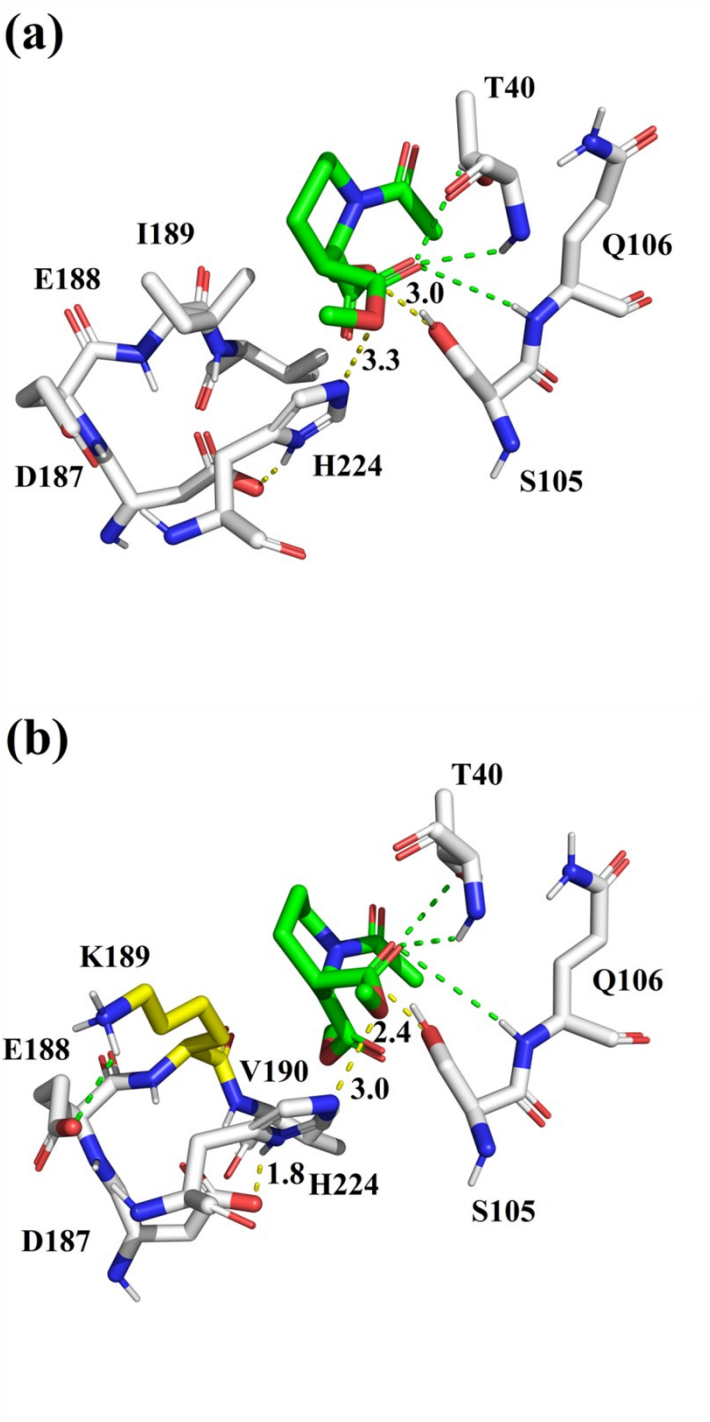
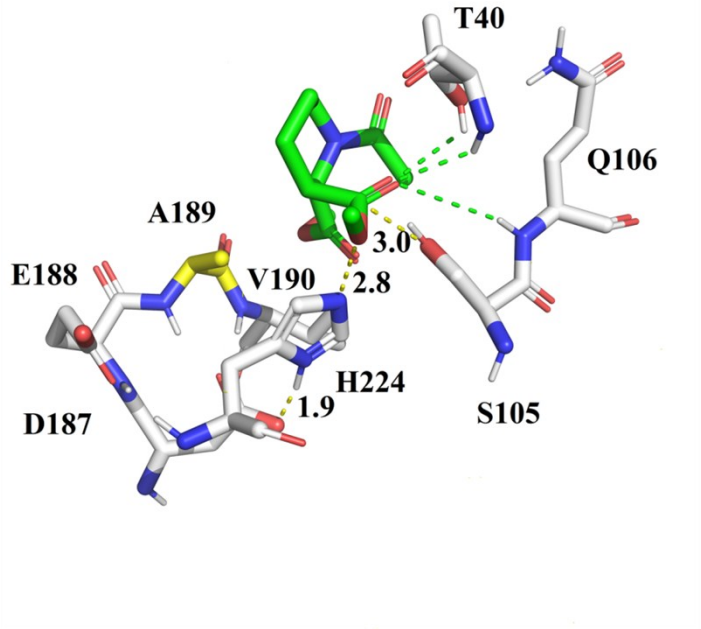


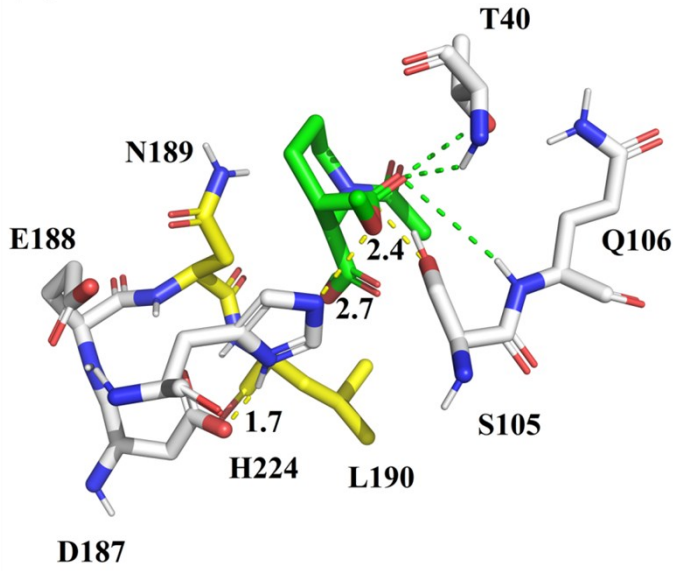
Fig. S7. (2*R*,3*S*)-**1** in the active site of wild-type CALB (a) and mutants mut-I189K, mut-I189A, mut-I189N/V190L (b, c, d), as predicted from the docking analysis. H bonds for interaction and the stabilization of the oxyanion indicated by green dotted lines. The distances for effective nucleophilic attack of serine in the catalytic triad at the carbonyl function of (2*R*,3*S*)-**1** are labeled as yellow dotted lines.



(c)


T40
Q106
A189
3.0
E188
V190
2.8
H224
1.9
D187
S105

(d)


T40
N189
E188
2.4
Q106
2.7
1.7
S105
H224
L190
D187