

# Simultaneous improvement in the thermostability and catalytic activity of epoxidase Lsd18 for the synthesis of Lasalocid A

Ning Liu <sup>1,†</sup>, Hongli Xiao <sup>1,†</sup>, Yongjian Zang <sup>2,3</sup>, Longji Zhou <sup>4</sup>, Jun Mencius <sup>4</sup>, Zhiwei Yang <sup>2</sup>, Shu Quan <sup>4,\*</sup> and Xi Chen <sup>1,\*</sup>

<sup>1</sup> Key Laboratory of Synthetic and Natural Functional Molecule of the Ministry of Education, College of Chemistry and Materials Science, Northwest University, Xi'an 710127, China

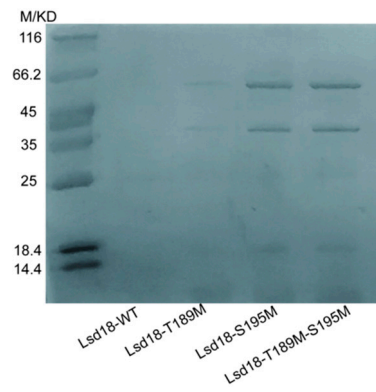
<sup>2</sup> MOE Key Laboratory for Nonequilibrium Synthesis and Modulation of Condensed Matter, School of Physics, Xi'an Jiaotong University, Xi'an 710049, China

<sup>3</sup> Institute of Physics and Electronic Information, Yunnan Normal University, Kunming 650504, China

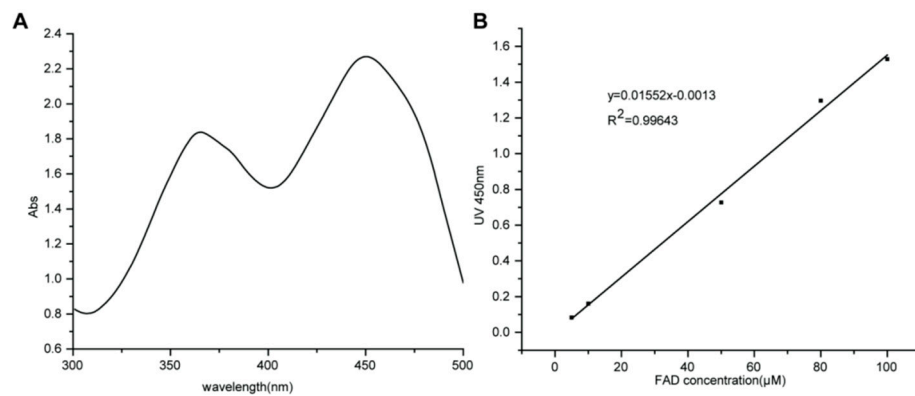
<sup>4</sup> State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China

\* Correspondence: shuquan@ecust.edu.cn (S.Q.); xchen@nwu.edu.cn (X.C.)

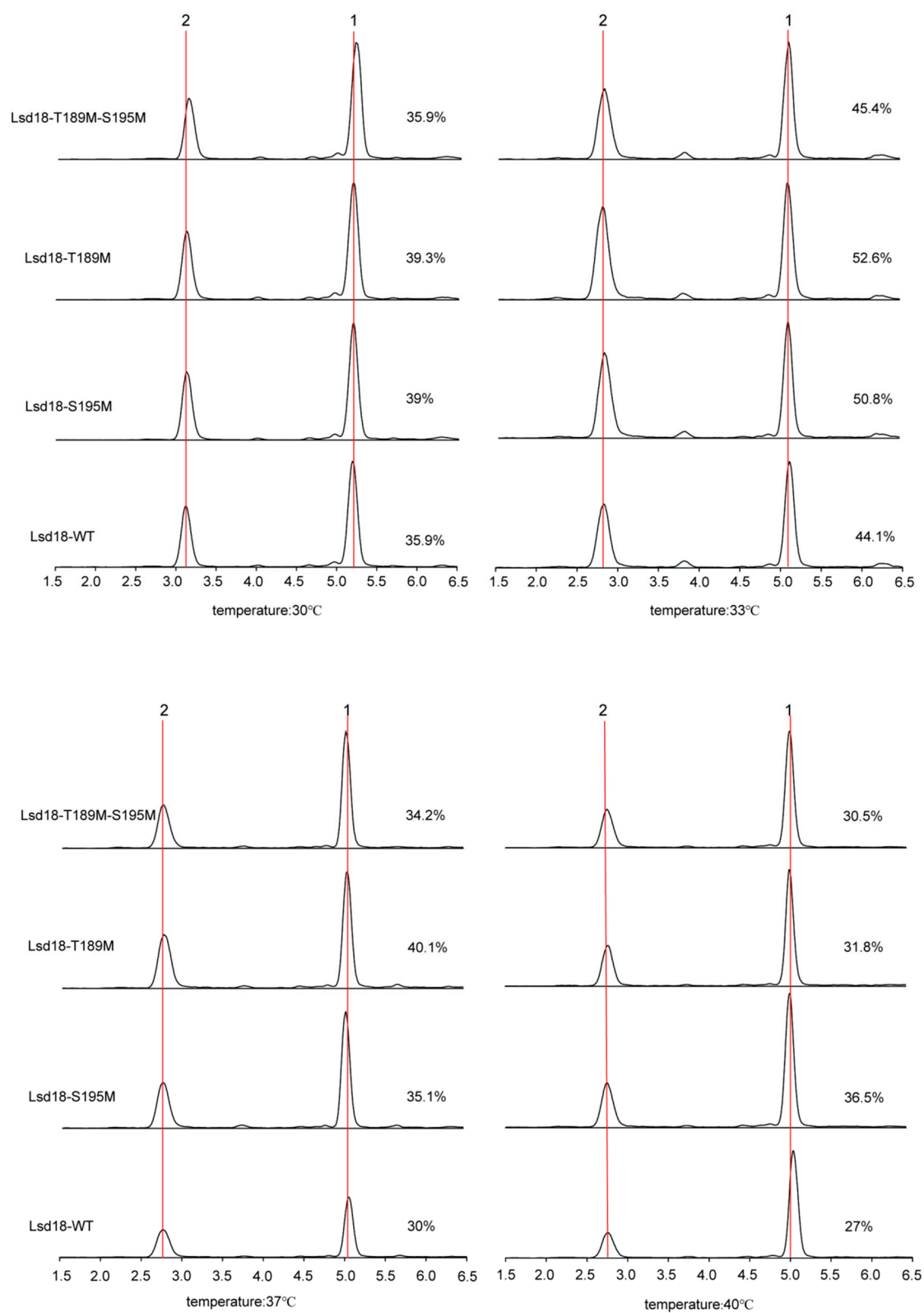
<sup>†</sup> These authors contributed equally to this work.



**Figure S1.** SDS-PAGE of the limited proteolysis of Lsd18 with trypsin.



**Figure S2.** Standard FAD fluorescence spectrum (A) and standard curve of the UV absorbance and the concentration of FAD (B).



**Figure S3.** LC-MS detection of the enzymatic activity of wild-type Lsd18 and the mutants at different temperatures.

**Table S1.** Semi-rational design mutants of Lsd18

Mutant	$\Delta\Delta G$ (kcal/mol)
R51W	-0.69
D52S	-0.40
V64D	-1.12
S108V	-1.79
S237V	-2.31
H239L	-1.14

**Table S2.** Data collection and refinement statistics

	Lsd18-S195M	Lsd18-T189M-S195M
PDB	8WVB	8WVF
<b>Data collection</b>		
Space group	P 1 21 1	P 1 21 1
Cell dimensions		
$a, b, c$ (Å)	62.041, 48.671, 136.049	63.005, 48.116, 135.569
$\alpha, \beta, \gamma$ (°)	90, 91.337, 90	90, 91.559, 90
Resolution (Å)	29.46 - 2.5 (2.589 - 2.5)	19.9- 3.764 (3.897 - 3.764)
$R_{\text{merge}}$	0.370 (0.994)	0.268 (0.389)
$I / \sigma I$	3.9 (2.6)	3.7 (2.2)
Completeness (%)	99.03 (98.52)	87.95 (83.62)
Redundancy	6.7 (6.8)	1.0 (1.0)
<b>Refinement</b>		
Resolution (Å)	2.5	3.764
No. reflections	28322	7506
$R_{\text{work}} / R_{\text{free}}$	0.1955/0.2538	0.2323 /0.3194
No. atoms	7084	6623
Protein	930	936
Ligand/ion	108	106
Solvent	186	0
$B$ -factors	22.42	33.26
Protein	22.54	33.26
Ligand/ion	16.94	33.27
Solvent	21.17	1
R.m.s. deviations		
Bond lengths (Å)	0.003	0.002
Bond angles (°)	0.57	0.58