Dramatically improved performance of an esterase for Cilastatin synthesis by cap domain engineering

Zheng-Jiao Luan[#[a]], Hui-Lei Yu[#[a]], Bao-Di Ma[[b]], Yi-Ke Qi [[a]], Qi Chen[[a]] and Jian-He Xu[*[a]]

[a] State Key Laboratory of Bioreactor Engineering and Shanghai Collaborative Innovation Centre for Biomanufacturing, East China University of Science and Technology, Shanghai 200237, China.

[b] School of Chemical and Environmental Engineering, Shanghai Institute of Technology, 100 Haiquan Road, Shanghai 201418, China.

[#] Zheng-Jiao Luan and Hui-Lei Yu contributed equally to this paper.

[*] Corresponding author. E-mail: jianhexu@ecust.edu.cn; Fax: +86-21-6425-0840.

Supporting Information

Contents

Table S1 Primers for directed evolution of the cap domain

Primers	Oligonucleotide sequences (5'-3')
F1	GCGATCACCAGCATCTATCCGCAGGGTGTCCCGCT
R1	AGCGGGACACCCTGCGGATAGATGCTGGTGATCGC
F2	CCGGAATTCATGTCTATTCGTGAAGCCGT
R2	CCAGGTTGCGAAGCAACTCGTCGTGACCGA
F3	TCGGTCACGACGAGTTGCTTCGCAACCTGG
R3	CCCAAGCTTTTAACCGAGGCTCGAGATGAAG

The underlined bases are the restriction sites

Table S2 Primers for site-directed evolution of the cap domain

Primers	Oligonucleotide sequences (5'-3')
S142-F	TGGCAAGGTCGGCNNKGCGATGCGTAGCAT
S142-R	ATGCTACGCATCGCMNNGCCGACCTTGCCA
A143-F	TGGCAAGGTCGGCTCGNNKATGCGTAGCAT
A143-R	ATGCTACGCATMNNCGAGCCGACCTTGCCA
M144-F	TGGCAAGGTCGGCTCGGCGNNKCGTAGCGCAGTTCCCGGC
M144-R	GCCGGGAACTGCGCTACGMNNCGCCGAGCCGACCTTGCCA
R145-F	GGCTCGGCGATGNNKAGCATTTTTCCCGGC
R145-R	GCCGGGAAAAATGCTMNNCATCGCCGAGCC
S146-F	GGCTCGGCGATGCGTNNKATTTTTCCCGGC
S146-R	GCCGGGAAAAATMNNACGCATCGCCGAGCC
P149-F	TGCGTAGCATTTTTNNKGGCGCGATGTCCG
P149-R	CGGACATCGCGCCMNNAAAAATGCTACGCA
G150-F	TGCGTAGCATTTTTCCCNNKGCGATGTCCG
G150-R	CGGACATCGCMNNGGGAAAAATGCTACGCA
A151-F	TTTTCCCGGCNNKATGTCCGAAGATCCCCG
A151-R	CGGGGATCTTCGGACATMNNGCCGGGAAAA
M152-F	TTTTCCCGGCGCGNNKTCCGAAGATCCCCG
M152-R	CGGGGATCTTCGGAMNNCGCGCCGGGAAAA

The underlined bases are the mutation sites.

Table S3 Enzyme immobilization on resins with different functional groups

Style	Name	hydrophobic property	Size (μm)	Specific activity (U/g) [a]	Activity recovery (%) [b]
Epoxy group	ES-1	hydrophilic	150 – 300	0.29 ± 0.05	3
	ES-101	hydrophobic	150 – 300	1.1 ± 0.2	12
	ES-103	hydrophobic	100 – 250	1.4 ± 0.1	16
Amine group	ESR-1	hydrophilic	100 – 300	4.2 ± 0.8	48
	ESR-2	hydrophobic	100 – 300	6.0 ± 0.2	70
	ESR-3	hydrophobic	100 – 300	4.5 ± 0.7	51

[a] Specific activity of immobilized enzyme towards (*RS*)-DmCpCe.

[b] Activity recovery was calculated as the rate of immobilized enzyme activity and activity of the enzyme bound to the resins.

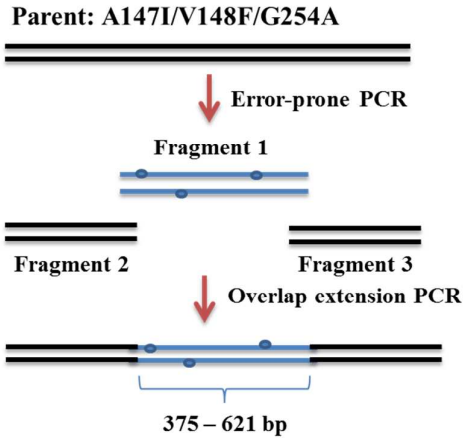
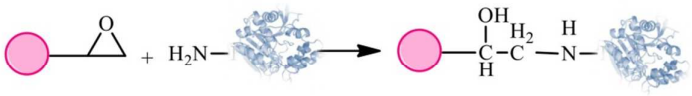
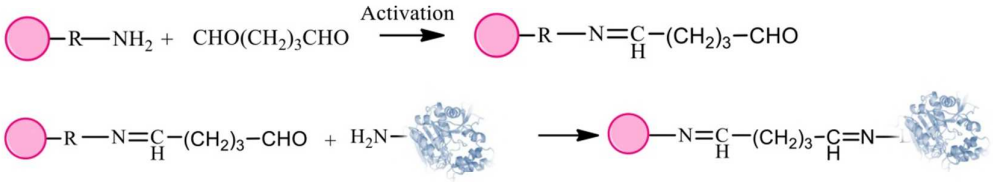


Fig. S1 Scheme presentation for the cap-domain error-prone PCR.



Enzyme immobilization to resins with epoxy group



Enzyme immobilization to glutaraldehyde activated resins with amino group

Fig. S2 Covalent immobilization of enzyme on resins with epoxy group or amino group.

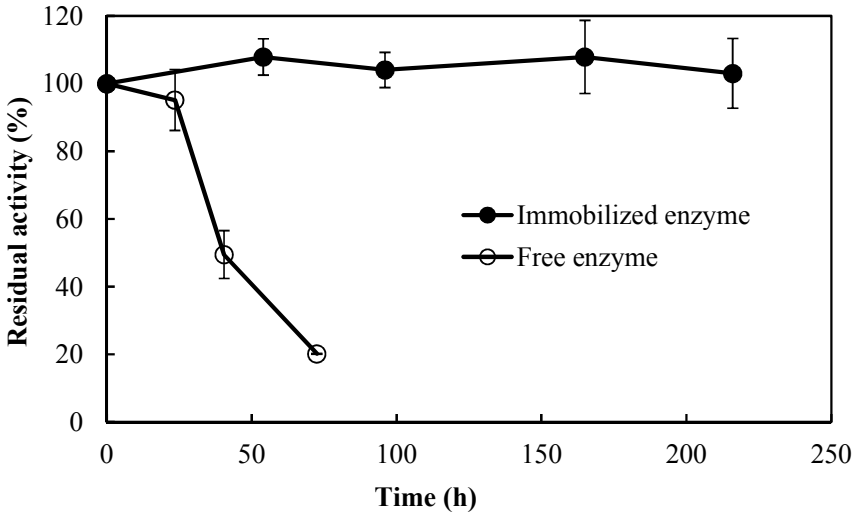


Fig. S3 Thermostabilities of the free enzyme and immobilized enzyme at 30°C.