

Supporting Information

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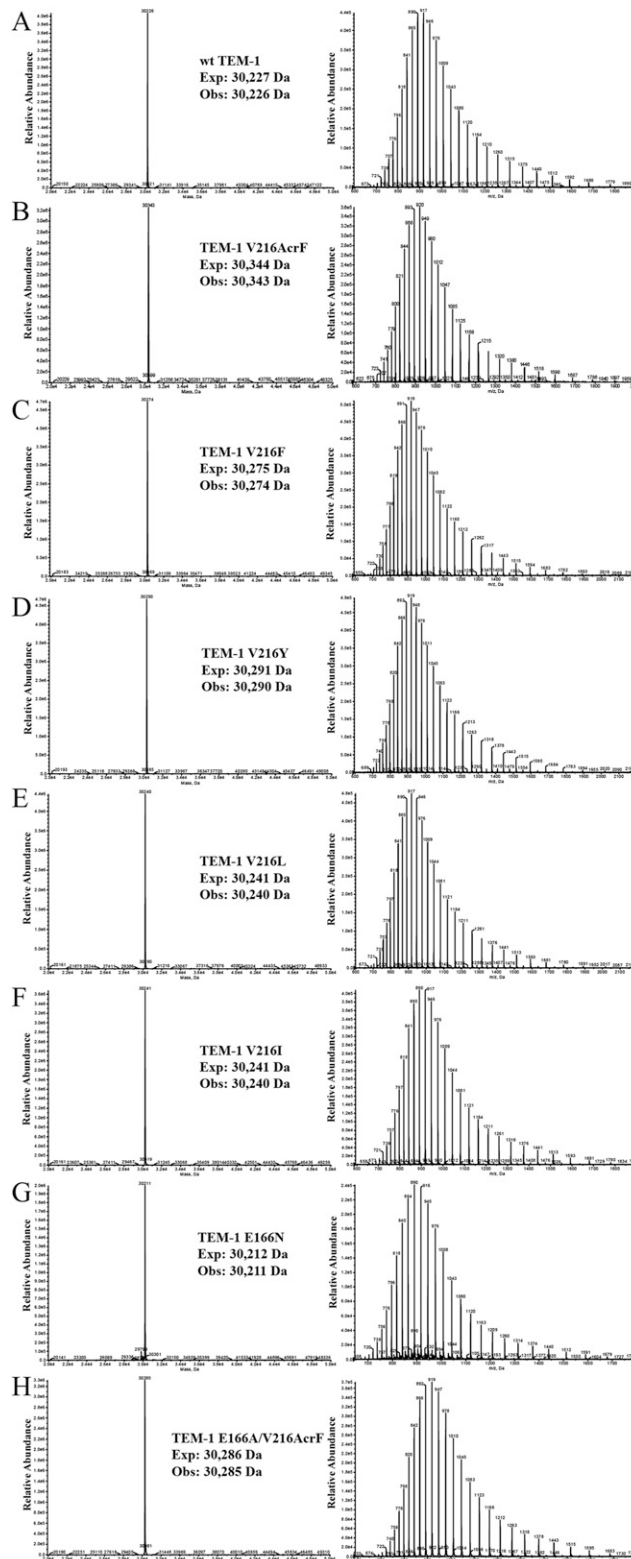


Fig. S1. ESI-MS spectra of wild-type and mutant β -lactamases.

Table S1. MICs of ceftazidime for D179X mutant enzymes

Amino acid 179	MIC, $\mu\text{g}\cdot\text{mL}^{-1}$
AzMeF	14
AcF	4
BrF	8
Asp	0.25
Tyr	4
Phe	4
Cys	2
Glu	0.25
Ala	1
Ser	2
His	1
Gln	1
Pro	0.5
TAG (no ncAA)	0.125
O-allyl-Y	4
AzF	4
Met	4
Gly	4
Trp	2
Leu	1
Ile	0.5
Val	0.25
Thr	0.25
Arg	0.5
Lys	0.25
Asn	8

Table S2. MICs of cephalexin for wild-type and Val-216-AcrF mutant β -lactamases in the presence of various ncAAs

ncAA	Enzyme MIC, $\mu\text{g}\cdot\text{mL}^{-1}$	
	Wild-type	V216TAG
no ncAA	10	<10
AcrF	10	90
IF	20	10
BrF	30	20
AcF	10	10
OMeY	20	10
AzF	20	10
O-allyl-Y	10	10
AzMeF	30	30
PheF	20	20
OtBuY	20	10

OMeY, O-methyl-tyrosine; OtBuY, O-tert-butyl-tyrosine; PheF, biphenylalanine.

Table S3. DNA oligomers used to prepare the library

Oligonucleotide	Nucleotide sequence (5'–3')	Oligonucleotide	Nucleotide sequence (5'–3')	Oligonucleotide	Nucleotide sequence (5'–3')
37tagFwd	gaagatTAGttgggtgcaacaggtgggttacatcgaac	121tagFwd	gaatattAGagtgctgccataaacacatgagtgataaac	200tagFwd	ctctaTAGtccccggcaacaataatagactggatg
37tagRev	cacccactAatcttcagcatcttttactttccaccgc	121tagRev	gcaactATAatcttcactgtcatgccatccg	200tagRev	cgggaCTAagagtagtagtccgcagttaatag
38tagFwd	gatacagTAGgggtcagaggtgggtttacatcgaactgg	122tagFwd	ttatgctTAGgctgccataaacatgagtgataaacactg	201tagFwd	ctagctTAGcggcaacaataatagactggatgg
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Table S3. Cont.

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Table S4. DNA oligomers used in this study

Oligonucleotide	Nucleotide sequence (5'-3')
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HX0976	CTGCAGTTTCAAACGCTAAATTGCCTG
HX0977	GCCTGTCCCGCTTATAAGGACGAAAGGGCCCTCGTGATACGCC
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HX1057	CGGCAATAGTTAATAGACTGGATGGAGGCGGATAAAG
HX1058	CTATTAACCTATTGCCGGGAAGCTAGAGTAAGTAGTTC
HX1065	CGGATAAAATCGCAGGACCACTTCTGCGCTCGGCCCTTCCG
HX1066	GGTCCTGCGAATTTATCCGCCTCCATCCAGTCTATTAATTGTTG
HX1063	CGGATAAAATACGACGACCACTTCTGCGCTCGGCCCTTCCG
HX1064	GGTCCTGCGTATTTATCCGCCTCCATCCAGTCTATTAATTGTTG
HX1067	CGGATAAAATCGCAGGACCACTTCTGCGCTCGGCCCTTCCG
HX1068	GGTCCTGCGAGTTTATCCGCCTCCATCCAGTCTATTAATTGTTG
HX1069	CGGATAAAATCGCAGGACCACTTCTGCGCTCGGCCCTTCCG
HX1070	GGTCCTGCGAATTTATCCGCCTCCATCCAGTCTATTAATTGTTG

Table S5. Data collection and structure refinement statistics

	V216-AcrF	E166N + Cephalexin	E166A/V216-AcrF + Cephalexin
Data collection			
X-ray source	Rigaku FR-E SuperBright	SSRL-11-1	SSRL-7-1
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
Unit cells parameters, Å	<i>a</i> = 45.47 <i>b</i> = 7.24 <i>c</i> = 128.33	<i>a</i> = 46.46 <i>b</i> = 47.04 <i>c</i> = 127.58	<i>a</i> = 46.29 <i>b</i> = 47.03 <i>c</i> = 128.31
No. of reflections measured	306,191	217,768	225,670
No. of unique reflections	41,549	26,744	31,711
Resolution, Å	26.54–1.54 (1.63–1.54)	37.86–1.80 (1.90–1.80)	37.93–1.70 (1.79–1.70)
<i>R</i> _{merge}	0.064 (0.188)	0.097 (0.604)	0.112 (0.812)
Mean <i>I</i> /σ (<i>I</i>)	16.3 (6.5)	15.0 (3.0)	15.2 (2.5)
Completeness, %	100 (100)	100 (100)	100 (99.9)
Redundancy	7.4 (7.1)	8.1 (8.4)	7.1 (7.2)
Refinement			
Resolution, Å	25.00–1.54	25.00–1.80	25.00–1.70
No. of reflections (test set)	39,324 (2,082)	25,336 (1,343)	30,049 (1,593)
<i>R</i> _{work} / <i>R</i> _{free}	0.140/0.170	0.166/0.210	0.154/0.188
No. of atoms			
Protein	2,210	2,110	2,172
Other	505	214	311
Mean overall B value, Å ²	16.4	23.7	19.7
rms bonds, Å ²	0.016	0.015	0.017
rms angles, °	1.85	1.82	1.92
Ramachandran,* %			
Favored regions	99.3	98.5	98.9
Allowed regions	0.7	1.5	1.1
Disallowed regions	0	0	0

SSRL, Stanford Synchrotron Radiation Lightsources. $R_{\text{sym}} = \frac{\sum h \sum j |I(h) - I(h)j|}{\sum h \sum j I(h)}$ where $I(h)$ is the mean intensity of symmetry-related reflections. $R_{\text{work}} = \frac{\sum ||F_{\text{obs}}| - |F_{\text{calc}}||}{\sum |F_{\text{obs}}|}$. *R*_{free} for 5% of reflections was excluded from refinement.

*As defined in Molprobity.