Chapter 1

Appendix A: Supplementary Material on "Antagonism between substitutions in β-lactamase explains a path not taken in the evolution of bacterial drug resistance"

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1.1 Supplementary Data

Table 1.1: Table S1. X-ray crystallography data collection and refinement statistics for CTX-M-14 mutant enzymes.

	P167S/D240G	E166A/D240G	E166A/P167S/ D240G	E166A/D240G-CTX	E166A/P167S/ D240G-CTX-1	E166A/P167S/ D240G-CTX-2
PDB ID	6V5E	6V6P	6V6G	6V7T	6V83	6V8V
Data collection						
Space group	P 41 21 2	P 41 21 2	P 32 2 1	P 21	P 41 21 2	P 32 2 1
a, b, c ()	42.2, 42,2, 261.6	41.9, 41.9, 259.2	41.4, 41.4, 231.1	45.1, 107.3, 47.8	42.4, 42.4, 262.7	41.3, 41.3, 231.6
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 120	90, 99.9, 90	90, 90, 90	90, 90, 120
Resolution Range (Å)	41.70 - 2.30	39.89 - 1.55	35.88 - 1.50	35.48 - 1.34	41.82 - 1.80	32.44 - 1.80
	(2.38 - 2.30)	(1.61 - 1.55)	(1.56 - 1.50)	(1.39 - 1.34)	(1.84 - 1.80)	(1.87 - 1.80)
R-merge (%)	8.4 (12.1)	9.7 (18.7)	5.6 (46.5)	4.7 (11.8)	9.4 (60.6)	8.7 (11.8)
I/sigma	17.4 (9.6)	16.6 (10.3)	30.8 (5.1)	17.1 (8.5)	31.3 (5.4)	11.7 (4.8)
Multiplicity	7.0 (6.4)	11.8 (14.0)	8.4 (10.6)	3.7 (3.7)	16.7 (19.9)	4.8 (2.0)
Completeness (%)	88.3	85.5	98.8	99.4	100	97.9
Wilson B-factor ($Å^2$)	29.4	10.3	12.1	9.1	18.2	14.1
Refinement						
Molecules per asymmetric unit	1	1	1	2	1	1
No. of unique reflections	10104 (880)	30051 (2829)	37869 (3494)	99523 (9924)	23526 (2299)	21924 (1871)
R-work/R-free (%)	18.5 / 25.1	16.8 / 19.4	18.3 / 21.7	14.3 / 16.1	17.1 / 20.8	14.9 / 18.9
No. of protein residues	263	263	260	526	261	260
Ramachandran						
Favored (%)	98.5	98.1	97.7	98.1	98.5	98.1
Outliers (%)	0	0	0.39	0.38	0	0.39
Average B-factor ($Å^2$)	31	14.2	21.2	14.1	23.3	16.6
Protein	30.8	12.4	19.6	11.7	21.1	14.4
Ligand	-	23.4	37.3	18.6	62.2	28.7
Solvent	34.7	29.1	32.1	26.7	36.3	30.2
RMS deviations						
Bond length ($Å$)	0.003	0.011	0.007	0.006	0.006	0.011
Bond angles (°)	0.685	1.16	0.92	0.93	0.96	1.204

Table 1.2: *Values in parentheses represent the highest-resolution bin.

1.2 Supplementary Figures

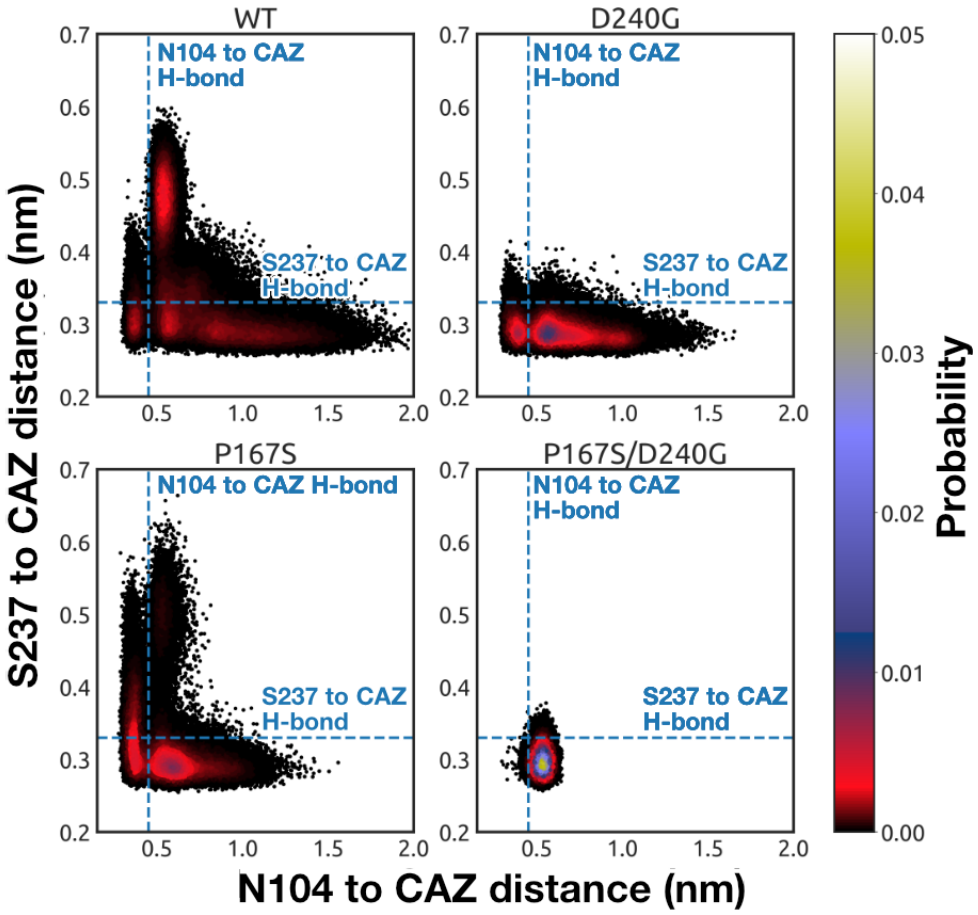

Figure 1.1: The β3 loop and Asn104 contact CAZ in the single mutants. Joint distributions of two hydrogen-bonding distances that capture the contacts between CTX-M and ceftazidime (CAZ) in the acyl-enzyme complex: i) Asn104 to the imino group of ceftazidime and ii) the backbone nitrogen of S237 on the β3 loop to the β-Lactam carbonyl oxygen of ceftazidime. Distance cutoffs for hydrogen-bonding interactions are marked (dashed lines) to indicate whether or not an interaction occurs. Distributions are shown for wild type (top left), D240G (top right), P167S (bottom left), and P167S/D240G (bottom right). Each point represents a snapshot from the molecular dynamics simulations colored according to its probability based on a 2D histogram.

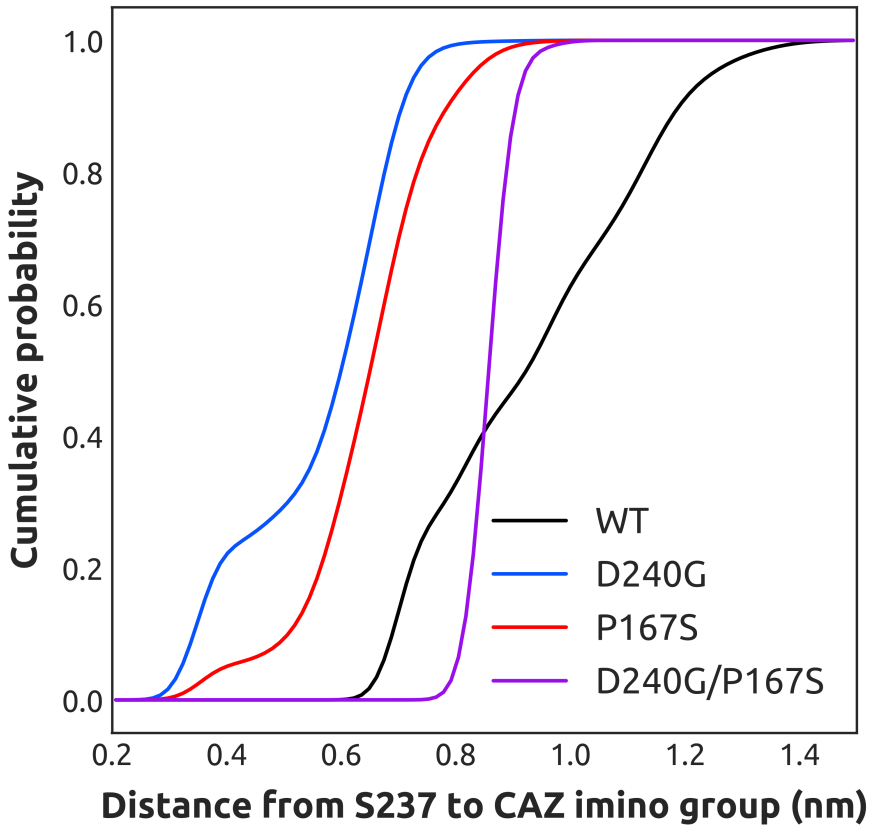

Figure 1.2: Ser237 makes contacts with the imino group of ceftazidime. Cumulative distance distribution of the sidechain oxygen of Ser237 to the carboxylate of the imino group of ceftazdime in the acyl-enzyme complex. Distributions are shown for wild type (black), D240G (blue), P167S (red), and P167S/D240G (purple).

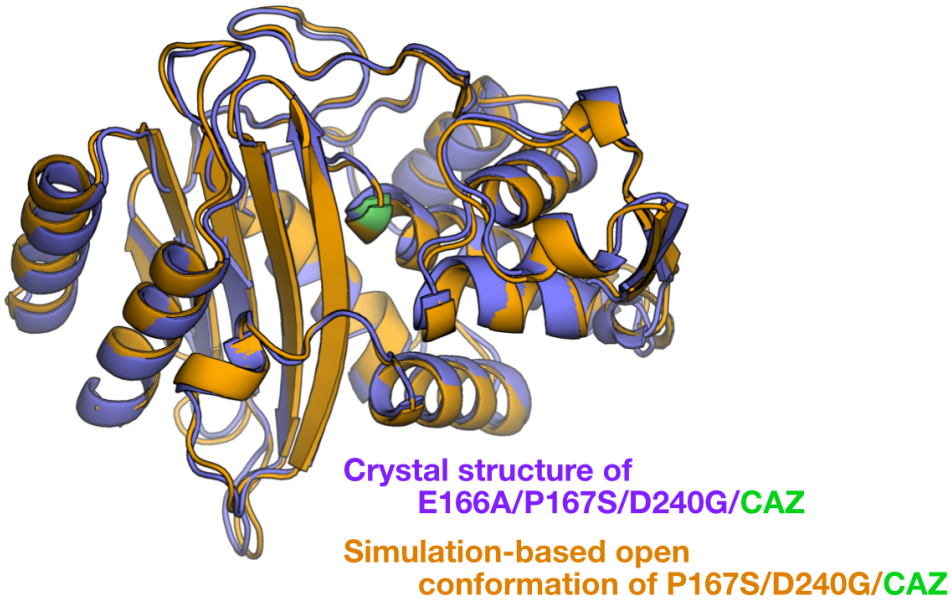

Figure 1.3: MD simulations of the closed conformation of P167S/D240G capture an open conformation of the Ω-loop. Overlay of the crystal structure of E166A/P167S/D240G/CAZ (purple, Ser70 colored in green) with a representative conformation from simulation of the open conformation of the Ω-loop (orange, Ser70 colored in green) sampled from simulations of the P167S/D240G variant starting from the closed conformation. In both constructs the catalytic serine that forms the acyl-enzyme complex with the serine that binds ceftazidime (labelled CAZ) is colored green. The ceftazidime molecule is not shown for clarity.