Supporting Information

Table S1. Omega angles for CTX-M-14 structures with Asn106 versus Ser106.

	residue position			
CTX-M-14 β-lactamase with Asn106	103	104	105	106
1YLT wt CTX-M-14-chain A[a]	166.9	175.7	168.1	171.2
4PM6 S70G -chain A[b]	165.0	176.7	165.3	171.7
4PM5 S70G-CTX chain A[b]	168.6	177.9	166.7	169.6
4PM8 S70G:S237A chain A[b]	166.1	176.9	165.7	170.9
4PM7 S70G:S237A:CTX chain A[b]	168.7	177.7	166.0	169.5
4PMA S70G:S237A:R276A chain A[b]	168.1	176.9	164.4	170.7
4PM9 S70G:S237A:R276A:CTX chain A[b]	169.6	177.4	167.4	170.9
average	167.6	177.0	166.2	170.6
std. dev.	1.63	0.74	1.27	0.81
CTX-M-14 β-lactamase with Ser106	103	104	105	106
6CYK N106S chain A	172.8	178.0	174.1	173.0
6CYK N106S chain B	175.1	176.3	172.4	173.4
6CYN N106S:D240G chain A	177.9	179.1	177.8	172.3
6CYN N106S:D240G chain B	179.8	178.9	178.4	172.0
6CYN N106S:D240G chain C	179.2	176.8	179.3	172.4
6CYN N106S:D240G chain D	179.1	177.7	180	170.8
6CYN N106S:D240G chain E	178.1	179.4	177.6	172.3
6CYN N106S:D240G chain F	179.6	177.7	178.3	172.4
6CYN N106S:D240G chain G	179.1	176.3	178.4	171.8
6CYN N106S:D240G chain H	179.9	177.7	178.3	171.9
6CYQ S70G:N106S:CTX chain A	177.2	178.7	178.8	170.0
6CYQ S70G:N106S:CTX chain B	178.0	176.4	179.2	170.2
6CYQ S70G:N106S:CTX chain C	175.0	168.1	171.1	169.7
6CYQ S70G:N106S:CTX chain D	175.7	167.0	171.4	170.7
6CYQ S70G:N106S:CTX chain E	174.8	176.5	174.6	171.1
6CYQ S70G:N106S:CTX chain F	175.6	176.3	175.2	171.3
6CYQ S70G:N106S:CTX chain G	179.3	176.2	179.5	170.6
6CYQ S70G:N106S:CTX chain H	179.6	175.4	179.8	169.3
6CYU S70G:N106S:D240G:CTX chain A	176.3	178.4	179.7	172.9
average	177.5	176.4	177.0	171.5
std. dev.	2.13	3.32	2.95	1.19

[a] Chen et al. (2005). J. Mol. Biol. 348:349-362.

[b] Adamski et al. (2015). Biochemistry 54:447-457.

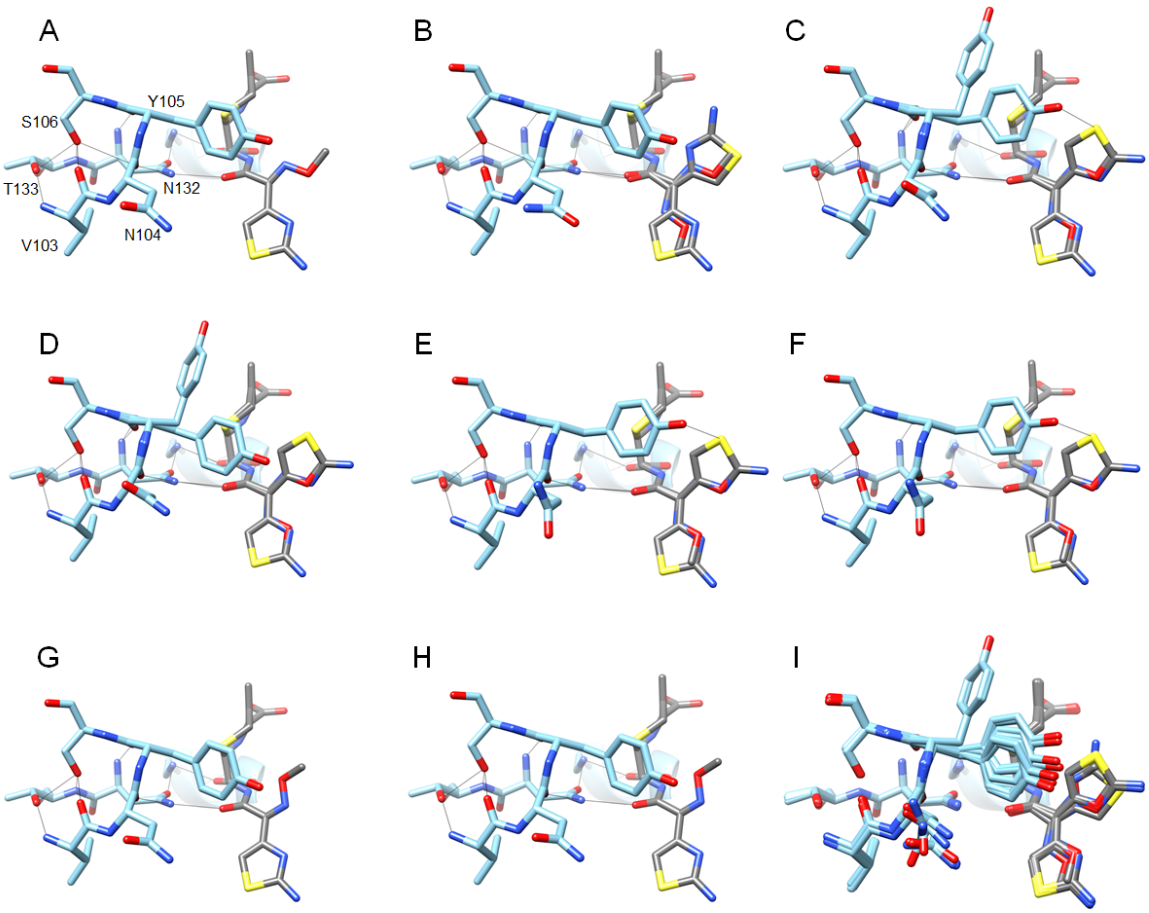

Figure S1. Schematic illustration of CTX-M-14 S70G/N106S structure in complex with cefotaxime. Residues 103-106, 132-133, and 70, 73 as well as bound cefotaxime are shown for each of the eight molecules in the asymmetric unit. **A-H**. Structures of chains A-H from the asymmetric unit, respectively. Residues are labeled in panel A and cefotaxime is shown in dark gray. Note that the side chain of cefotaxime is found in two conformations for chains B, C, D, E, and F. Also note the correlated conformations of Asn104 and Tyr105 for chains A, B, G, H versus chains C, D versus chains E, F. **I**. Structural alignment of chains A-H. In all panels, oxygen and nitrogen are colored red and blue, respectively.

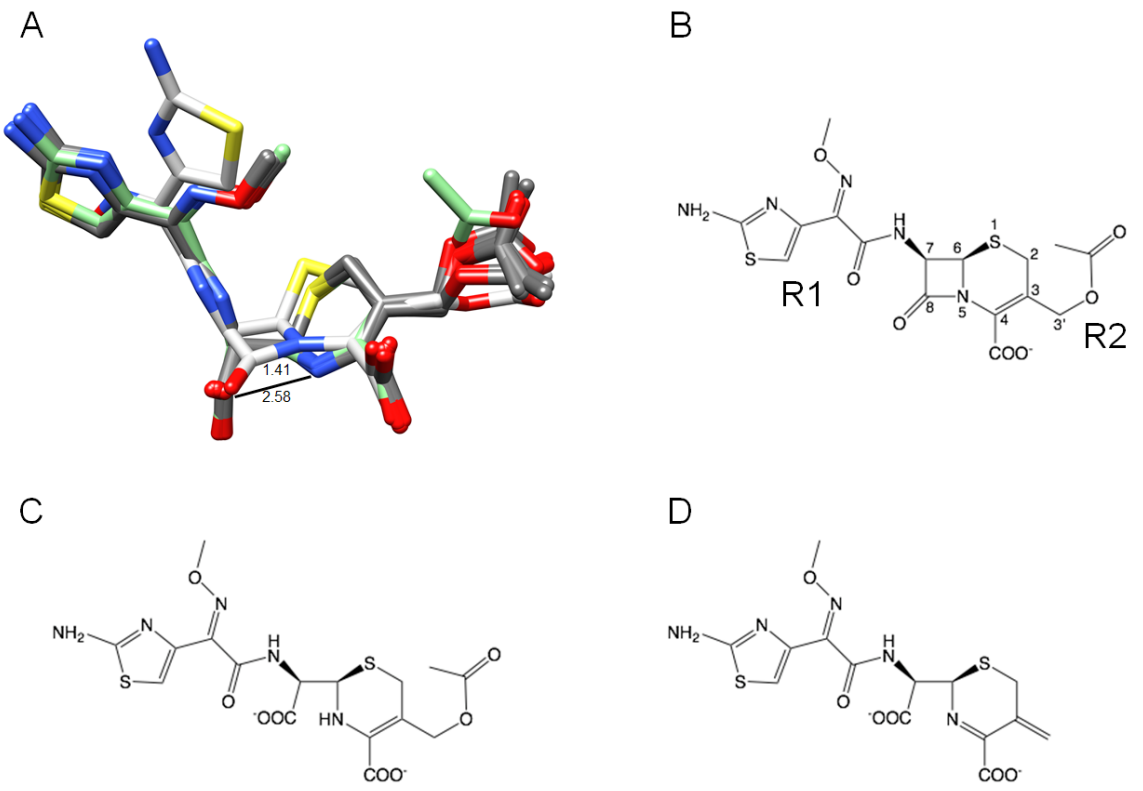

Figure S2. Cefotaxime structures and hydrolytic products. **A.** Alignment of cefotaxime molecules based on the structures of CTX-M-14 S70G/CTS (white)(PDB ID: 4PM5), S70G/N106S/CTX (dark gray) and S70G/N106S/D240G/CTX (light green). All eight molecules from the asymmetric unit of the S70G/N106S/CTX structure are shown. The distances in angstroms from C-8 to N-5 for the intact cefotaxime in the S70G/CTX (1.41 Å) and S70G/N106S (2.53 Å) structures with hydrolyzed cefotaxime are indicated. **B.** Schematic illustration of intact cefotaxime. Relevant atoms are numbered. The R1 (attached to C-7) and R2 (attached to C-3) groups are labeled. **C.** Schematic illustration of hydrolyzed cefotaxime with the N-5 position protonated. **D.** Hydrolyzed cefotaxime with tautomerization to create double bonds at N5-C4 and C3-C3' with the elimination of the R-2 group.

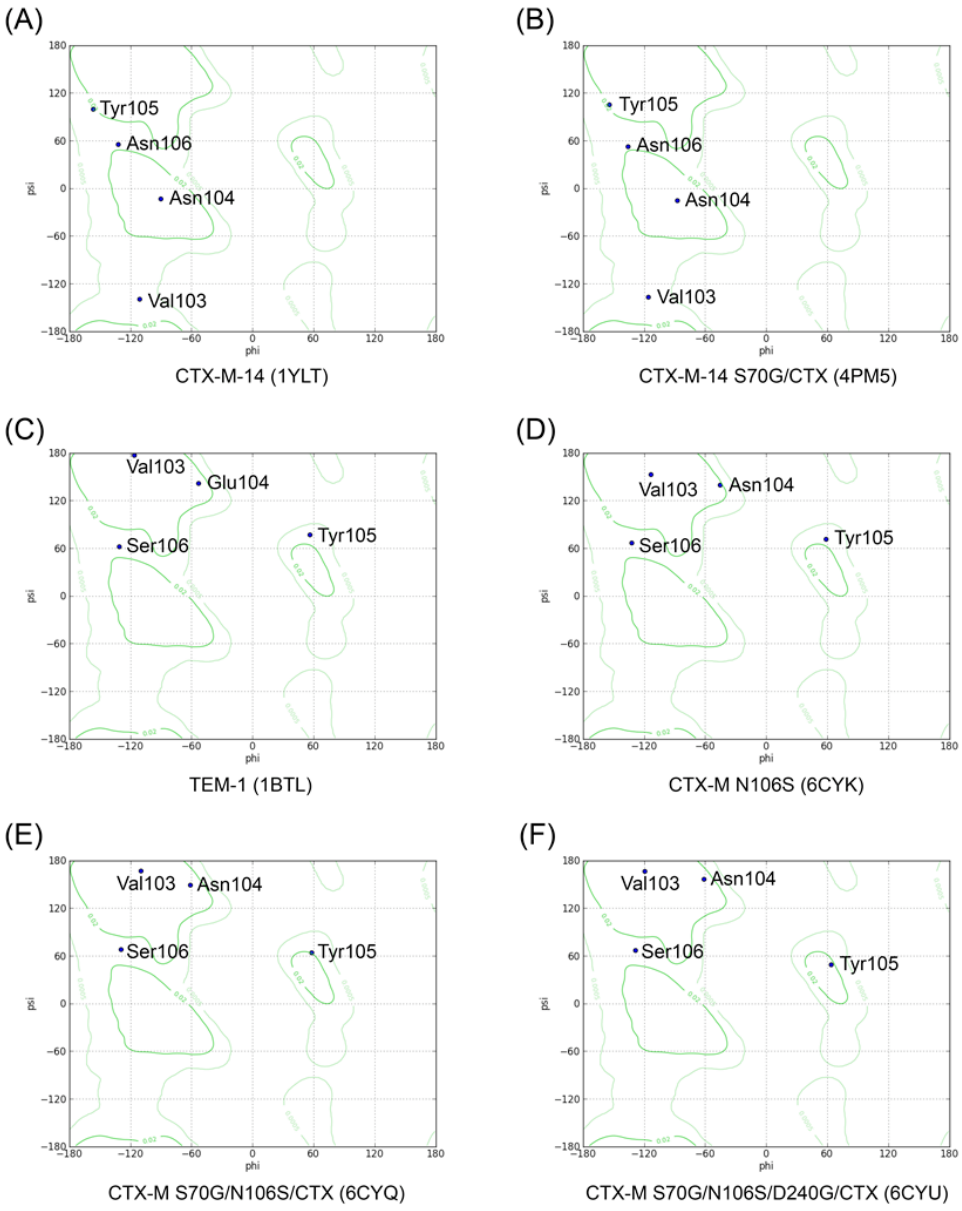

Figure S3. Ramachandran plots showing the phi (x-axis) and psi (y-axis) angles of β-lactamases in the residue 103-106 loop. (**A**) Wild-type CTX-M-14 (PDB ID: 1YLT) (1) and (**B**) S70G/CTX (4PM5) (2) contain Asn106. (**C**) TEM-1 β-lactamase (1BLT) (3) contains Ser106. (**D-F**) CTX-M mutants containing the N106S mutation. Dark green lines encircle favored regions while light green lines encircle allowed regions of the plot. Note that the N106S substitution results in a large shift in the phi-psi angles for residues 103-105 in the CTX-M mutants (**D-F**) versus CTX-M containing Asn106 (**A-B**) while the angles for residue 106 are similar for all structures. Also, note the phi-psi angles for residues 103-106 of the CTX-M-mutants (**D-F**) are closely similar to those for TEM-1 (**C**). Further, note the angles for Val103 move from allowed regions in wild-type CTX-M-14 and S70G/CTX (**A-B**) to favorable regions in the CTX-M N106S-containing mutants (**D-F**).

Supporting Information References

1. Chen, Y., Delmas, J., Sirot, J., Shoichet, B. K., and Bonnet, R. (2005) Atomic resolution structures of CTX-M beta-lactamases: extended spectrum activities from increased mobility and decreased stability. *J. Mol. Biol.* **348**, 349-362
2. Adamski, C. J., Cardenas, A. M., Brown, N. G., Horton, L. B., Sankaran, B., Prasad, B. V., Gilbert, H. F., and Palzkill, T. (2015) Molecular basis for the catalytic specificity of the CTX-M extended spectrum β-lactamases. *Biochemistry* **54**, 447-457
3. Jelsch, C., Mourey, L., Masson, J. M., and Samama, J. P. (1993) Crystal structure of Escherichia coli TEM-1 beta-lactamase at 1.8 A resolution. *Proteins* **16**, 364-383