Supplemental Material

Figure S1. Root mean square deviation (RMSD) for the molecular dynamics simulation of OXA-24/40 (WT) and OXA-160 (OXA-24/40 P227S).

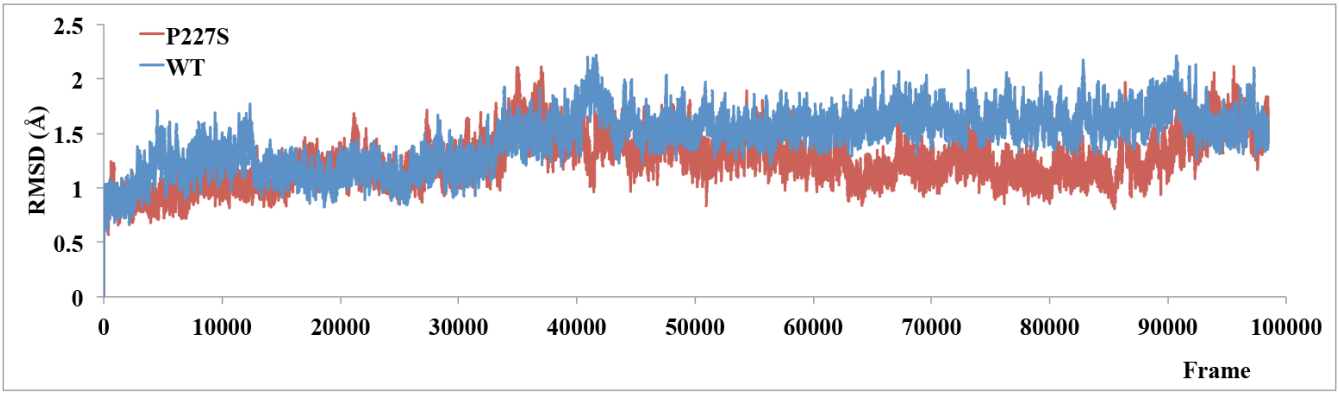


Figure S2. Post-acylation rearrangement of ceftazidime. After ceftazidime forms an acyl intermediate with the nucleophilic serine of the enzyme (left panel), the dihydrothiazine ring rearranges electronically causing the removal of a pyridine group from its R2 side-chain (right panel).

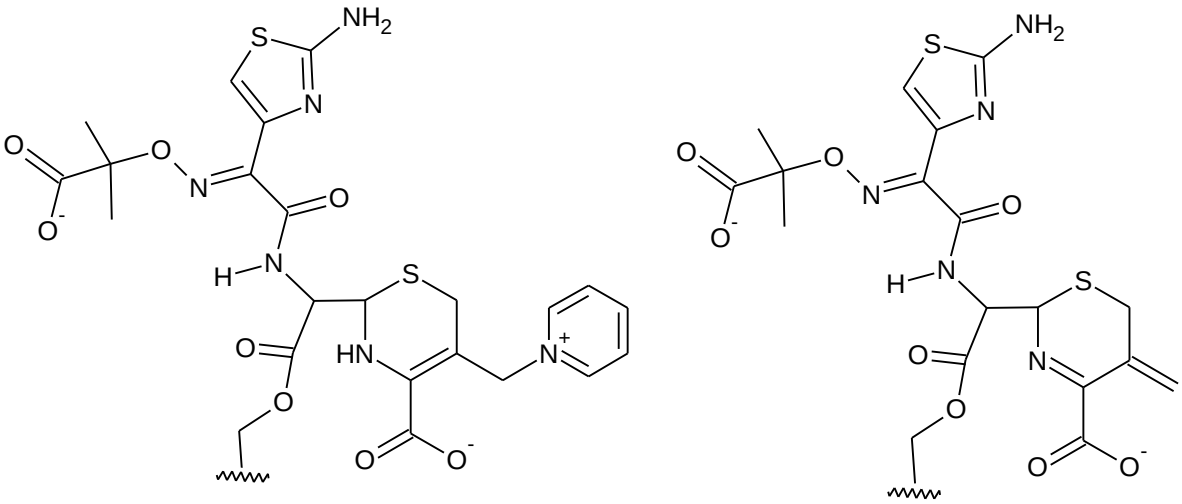
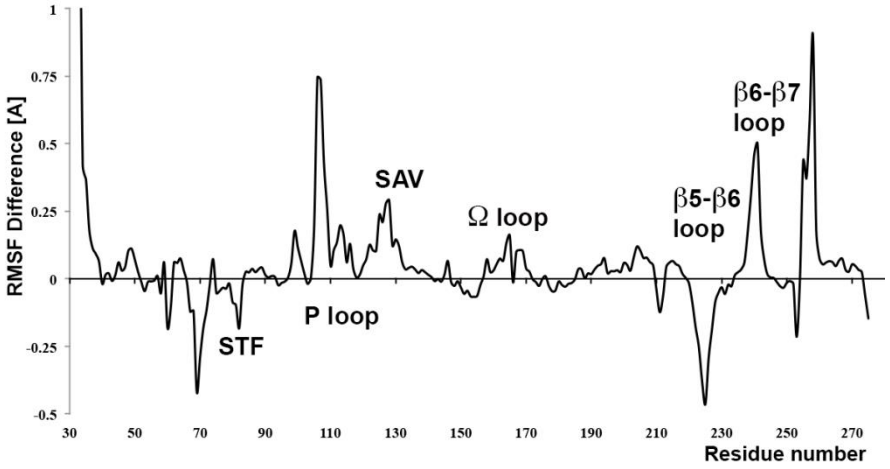
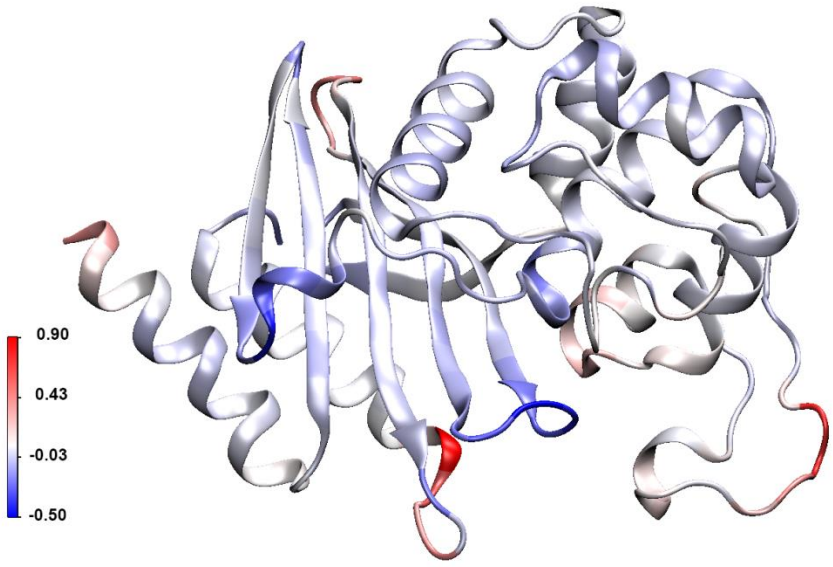


Figure S3. Upper panel: Cα root mean squared fluctuations (RMSF) difference between OXA-24/40 and OXA-160 enzymes. Lower panel: Plot of areas where fluctuations differ most from normal, with blue indicating decrease in fluctuations in OXA-160 (OXA-24/40 P227S) compared to the OXA-24/40 WT. Red segments denote increased fluctuations for the P227S mutant.





Supplementary Data: CHARMM force-field parameters for carboxylated lysine. Carboxylated lysine CHARMM topology and charges (N. Simakov and T. Wymore, manuscript in preparation)

```
PRES   KCX    -1.00 ! Patch for carboxylated lysine, ns
GROUP
ATOM CE   CT2       0.060
ATOM HE1  HA        0.090
ATOM HE2  HA        0.090
ATOM NZ   NECA    -0.810
ATOM HZ1  H         0.300
ATOM CX   CC        0.690
ATOM OQ1  OC      -0.710
ATOM OQ2  OC      -0.710
DELETE ATOM HZ2
DELETE ATOM HZ3
BOND NZ CX
BOND  CX OQ1
BOND CX OQ2
IMPR  CX NZ OQ1 OQ2
```

Carboxylated lysine CHARMM topology and charges (N. Simakov and T. Wymore, manuscript in preparation)

Bond parameters

```
CT2   NECA  380.00      1.4300  ! ECA/KCX, init from NH1  CT1, fq matched, ns
H      NECA  440.00      1.0280  ! ECA/KCX, init from NH1  H, fq matched, ns
CC    NECA  320.00      1.4617  ! ECA/KCX, init from NH2  CC/NH1  C, fq matched, ns
```

Angle parameters

```
CT3  CT2  NECA    67.70     110.93   ! ECA/KCX, init from NH3  CT2  CT3, fq matched, ns
CT2  CT2  NECA    67.70     110.93   ! ECA/KCX, set to CT3  CT2  NECA, ns
HA   CT2  NECA    51.50     107.50   ! ECA/KCX, init from NC2  CT2  HA, fq match ok, ns
CC   NECA CT2      50.00     115.00   ! ECA/KCX, init from CT2  NH1  C, fq matched, ns
CT2  NECA H        60.00     110.00   ! ECA/KCX, init from H    NH1  CT1, fq match ok, ns
CC   NECA H        60.00     110.00   ! ECA/KCX, init from H    NH1  CT2, fq match ok, ns
NECA CC   OC       80.00     110.00   ! ECA/KCX, init from O    C    NH1, fq match ok, ns
```

Torsional parameters

```
HA   CT2  CT3  HA         0.1600   3     0.00   ! ECA/KCX, init from X    CT2  CT3  X , ns
HA   CT3  CT2  NECA     0.0400   3     0.00   ! ECA/KCX, init from HA   CT3  CT2  CA, fq
matched, ns
CC   NECA CT2  CT3      1.1000   1   180.00 ! ECA/KCX, Set from ParamIt:dihfit, ns
CC   NECA CT2  CT3      0.2000   3     0.00   ! ECA/KCX, Set from ParamIt:dihfit, ns
CC   NECA CT2  CT2      1.1000   1   180.00  ! ECA/KCX, Set from ParamIt:dihfit, ns
CC   NECA CT2  CT2      0.2000   3     0.00   ! ECA/KCX, Set from ParamIt:dihfit, ns
```

H NECA CT2 HA 0.0000 1 0.00 ! ECA/KCX, init from HA CT3 NH1 H, ns
CC NECA CT2 HA 0.0000 1 0.00 ! ECA/KCX, init from HA CT2 NH1 C, ns
CT2 NECA CC OC 2.2000 2 180.00 ! ECA/KCX, Set from ParamIt:dihfit, ns
CT3 CT2 NECA H 0.2000 3 0.00 ! ECA/KCX, Set from ParamIt:dihfit, ns
CT2 CT2 NECA H 0.2000 3 0.00 ! ECA/KCX, Set to CT3 CT2 NECA H, ns
H NECA CC OC 1.9250 2 180.00 ! ECA/KCX, Set from ParamIt:dihfit, ns

Improper

CC NECA OC OC 96.0000 0 0.0000 ! ECA/KCX, init from OC X X CC, ns

Non-bonded parameters, Lennard-Jones, epsilon, Rmin/2

NECA 0.000000 -0.200000 1.850000 ! ECA/KCX, N for ECA, ns