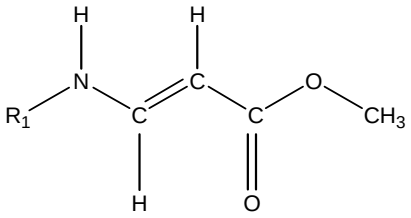
Supplementary Material

Gaussian calculations

In order to improve our understanding of the Raman difference data in Figures 1-3 we undertook calculations based on the Gaussian program - Gaussian 03 ™ (*39*). Preliminary results were reported in Helfand et al. (*21*), where these were based on calculations for the methyl ester of the *trans*-enamine form.



We have extended these calculations by replacing the methyl ester by a derivatized serine moiety, in order to model the active site Ser70 (Supplementary Material, Figure 5S). Moreover, the X-ray coordinates for the acyl-enzyme species, derived from clavulanic acid in the E166A variant provided initial geometries for the serine-enamine calculations (*22*). The complex between serine-clavulanic acid derivative complex shown in Figure 5S represents the smallest of the three intermediates and on this basis it was selected for the calculations. The $-CO_2^-$ group at C_3 was removed, since both the X-ray and the Raman data indicate that the enamine has decarboxylated spontaneously (*22*).

Table 1S summarizes the results for the Raman peaks from a calculation on the compound shown in (Supplementary Material, Figure 5S), where the α, β, γ, δ torsional angles were set to the values reported from the X-ray analysis (*22*). The structure was

allowed to relax whereupon the torsional angles changed slightly, see Table 2S. The results from the calculations have been scaled by the recommended factor of 0.8929 (*39, 41*). The calculations predict that the most intense Raman feature is due to the O=C-C=C-NH-symmetric stretch in clavulanate near 1611 cm^{-1}. This is in good agreement with the experimental band at 1612 cm^{-1}. However, agreement with other putative experimental features is less compelling. The second most intense Raman band (Supplementary Material, Table 1S) is predicted near 1295 cm^{-1}, although there is a weak experimental Raman band here it is possible that the corresponding experimental band is actually within the broad 1250 cm^{-1} profile (Supplementary Material, Figure 2S). Similarly, there is a convincing medium intensity calculated band corresponding to the experimental 1109 cm^{-1} peak (Supplementary Material, Figure 2S). In general, there are two difficulties in making precise assignments. In the absence of suitable isotopomers of the inhibitors, we cannot unambiguously distinguish bands in the difference spectrum arising from the *trans* enamine or from a protein mode that arises from a protein conformational change. Secondly, the calculations show, with the exception of the enamine mode near 1610 cm^{-1}, the other Raman active modes are highly delocalized over most groups of the enamine and include motions of some of the serine atoms.

The calculations indicate that they may be a suite of modes in the 1460-1496 cm^{-1} region due to delocalized motions that include the serine residue. These possibly correspond to the broad feature near 1520 cm^{-1} in the E166A complex (Supplementary Material, Figure 2S). However, at the present level our calculations do not provide a facile explanation for the shift in the broad 1520 cm^{-1} band in the double mutant.

Raman shift cm^{-1}	**Vibrational modes corresponding to the Raman shift values**
1611 cm^{-1}	N-C=C-C=O stretch; NH,CH in plane bending
1496 cm^{-1}	Highly delocalized mode and little contribution from the CH_2 and NH wag
1484 cm^{-1}	Large contribution from the CH_2 and NH wag
1460 cm^{-1}	C_4H and some CH_2 serine wag
1452 cm^{-1}	Serine CH_3
1295 cm^{-1}	NH-C_5=C_6H symmetric stretch, C_5H deformation and delocalized serine motions
1173 cm^{-1}	Highly delocalized chain mode with C-O stretch mode extending into the serine
1035 cm^{-1}	Very similar to the motion at 1173 cm^{-1}

Table 1S: Theoretical Raman shift values for the *trans*-enamine intermediate from clavulanic acid inhibitor linked to a serine residue, determined by Gaussian calculations

Torsional angles	X-ray values	X-ray values allowed to relax
α NH-C=C-C(=O)	-179	-179
β C=C-C-O	167	179
γ HC-C-O-CH_2	169	177
δ C(=O)-O-CH_2-C	94	80

Table 2S: Torsional angles of a *trans* enamine intermediate of an inhibitor linked to a serine residue used for Gaussian calculations

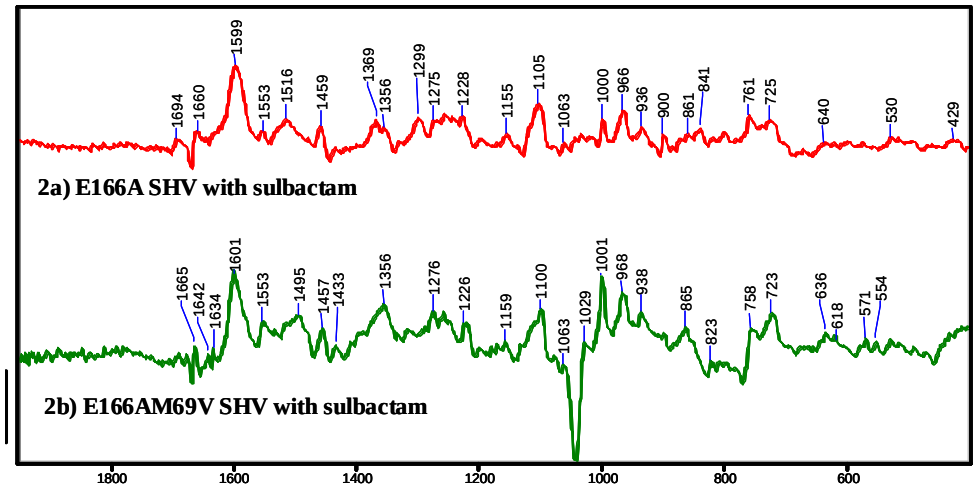


Figure 1S: Raman difference spectra of the E166A and M69V-E166A β-lactamase crystal with sulbactam 5mM in the mother liquor (Hepes 0.1M, pH 7). The vertical bar represents the intensity of a 5 000 photon event.

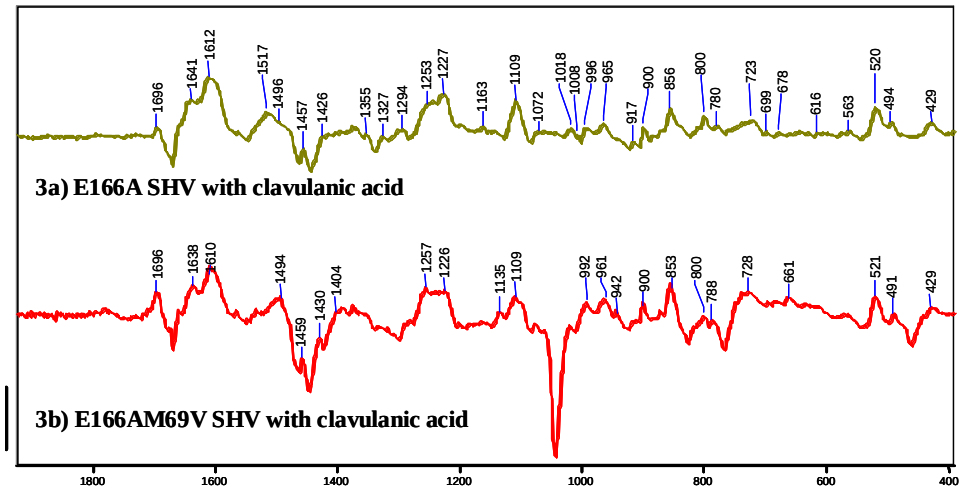


Figure 2S: Raman difference spectra of the E166A and M69V-E166A β-lactamase crystal with clavulanic acid 5mM in the mother liquor (Hepes 0.1M, pH 7). The vertical bar represents the intensity of a 5 000 photon event

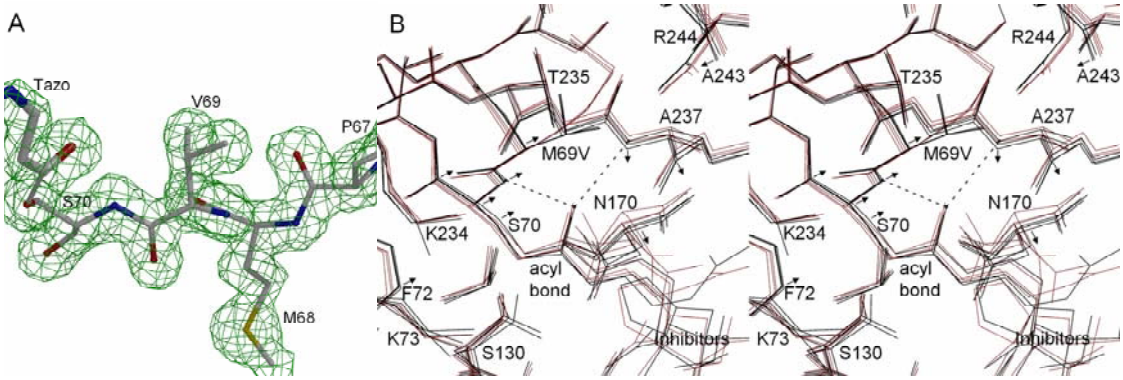


Figure 3S: Conformational changes in the vicinity of the M69V mutation. Panel A: Electron density of the M69V mutation site within the tazobactam bound structure. 2Fo-Fc electron density contoured at 1.0 σ is shown for residues 67-70. Part of the covalently bound tazobactam inhibitor (tazo) is depicted in thick ball-and-stick representation. Panel B: stereo figure of a close-up superposition of the active site of the M69V/E166A and E166A single mutant SHV-1 structures. Shown are the three inhibitor bound E166A inhibitor bound structures (red lines) and the M69V/E166A variant structures (black thicker lines). The site of the inhibitors is highlighted as well as the acyl bond between the S70 and inhibitors. The oxyanion hole interactions between the O8 atom of the inhibitor and the backbone nitrogen atoms of S70 and A237 are shown as dashed lines. Atomic shifts consistently observed in all three double mutant structures with respect to the single mutant structures are highlight by black arrows indicating the direction of the shift.

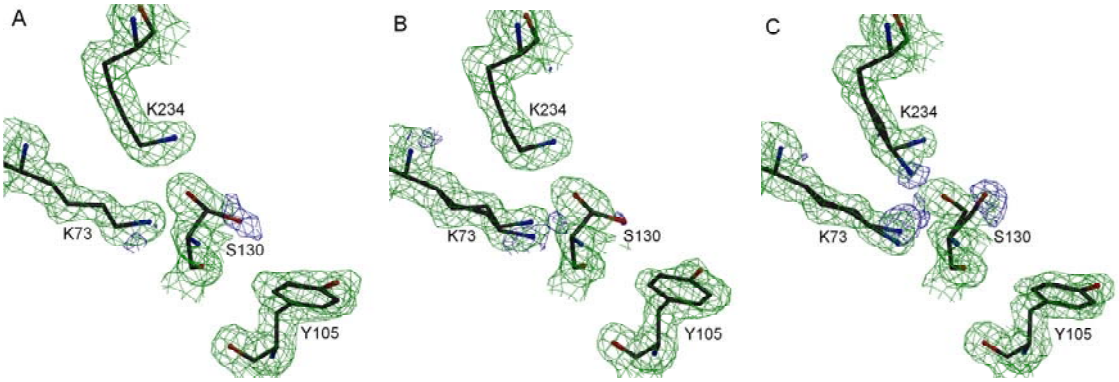


Figure 4S: Alternate conformations of active site residues observed in the E166A/M69V double mutant structures. Depicted are the 2Fo-Fc electron density (green contoured at 1 σ) and positive omit electron density prior to including the alternate conformations (blue lines contoured at 3σ) are depicted for tazobactam (A), sulbactam (B), and clavulanic acid (C).

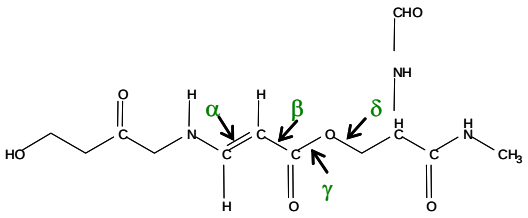


Figure 5S: *Trans* enamine intermediate of clavulanic acid (decarboxylated) linked to a derivatized serine residue. Torsional angles α, β, γ, δ are given in Table 2S.