Supporting Information for:

Structural and chemical aspects of resistance to the antibiotic, fosfomycin, conferred by FosB from *Bacillus cereus*

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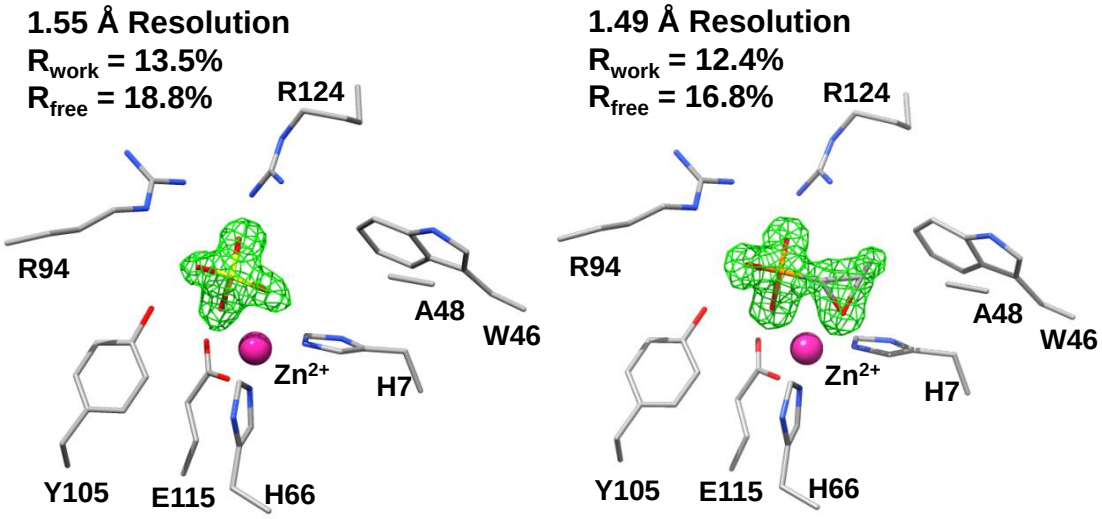

Figure S1. (Left) The active site residues that define metal-ion and sulfate binding sites from x-ray crystal structure of the FosB protein from *Bacillus cereus* in complex with Zn^{2+} and sulfate at 1.55 Å resolution (PDB 4JH1). Final refinement had an R_{work} = 13.5% and R_{free} = 18.8%. **(Right)** The active site residues that define metal-ion and fosfomycin binding sites from the second crystal structure of $FosB^{Bc}$ in complex with Zn^{2+} and fosfomycin at 1.49 Å resolution (PDB 4JH3). Final refinement had an R_{work} = 12.4% and R_{free} = 16.8%. The difference electron density shown for phosphate and fosfomycin is contoured 4σ, emphasizing the quality of the data. Both structures were determined with experimental phases from a SAD experiment at 1.28 Å and the anomalous signal from the two Zn^{2+} sites in the dimer.

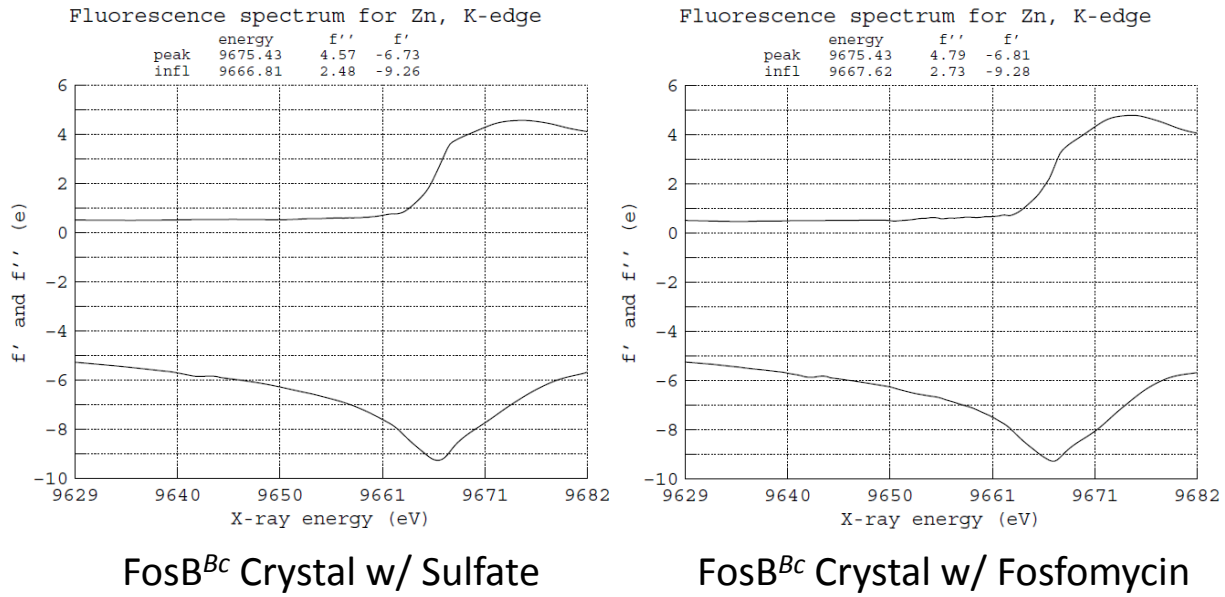

Figure S2. X-ray fluorescence scans of the $FosB^{Bc}$•Zn^{2+} crystals. **(Left)** $FosB^{Bc}$ crystal with Zn^{2+} and sulfate in the active site. **(Right)** $FosB^{Bc}$ crystal with Zn^{2+} and fosfomycin in the active site.

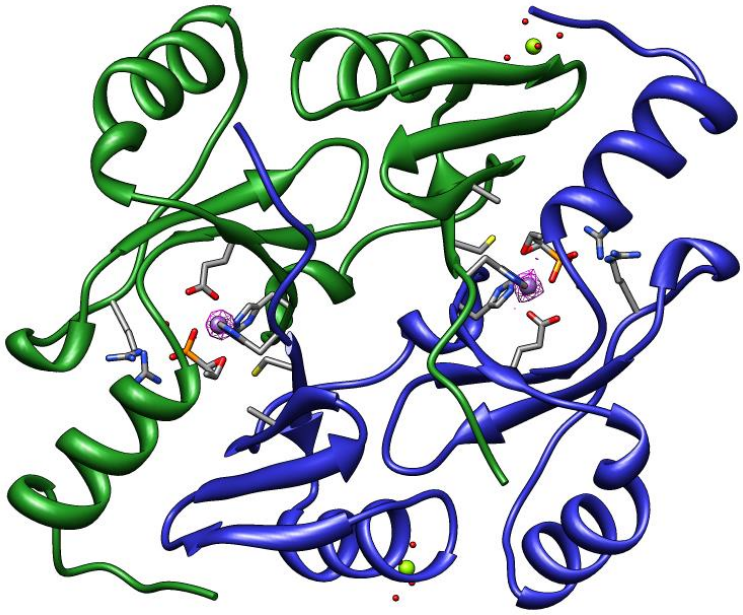

Figure S3. Overall x-ray crystal structure of $FosB^{Bc}$ in complex with Zn^{2+} and fosfomycin at 1.50 Å resolution (PDB 4JH3). The anomalous density map from the SAD experiment shown around the Zn^{2+} metal ions is contoured at 5 σ. Final refinement had an R_{work} = 12.36% and R_{free} = 16.83%.

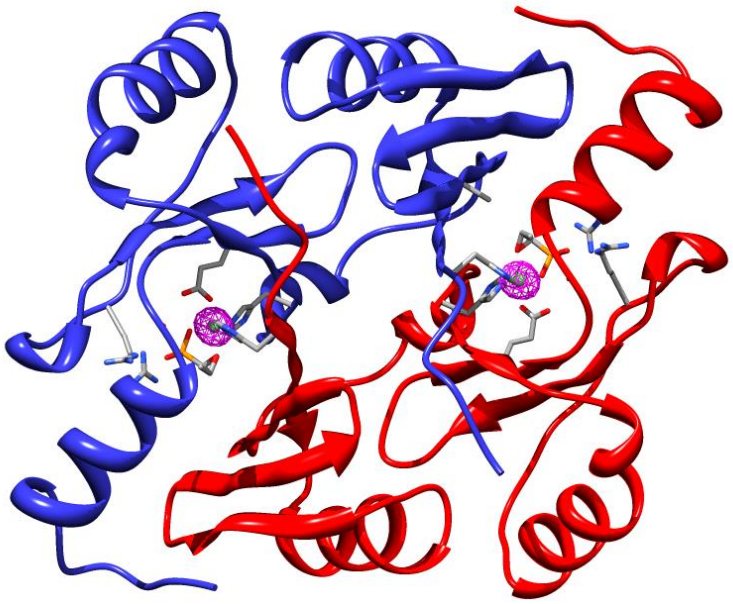

Figure S4. Overall x-ray crystal structure of FosB^{Bc} in complex with Ni^{2+} and fosfomycin at 1.89 Å resolution (PDB 4JH4). The anomalous density map from the SAD experiment shown around the Ni^{2+} metal ions is contoured at 5 σ. Final refinement had an R_{work} = 17.22% and R_{free} = 21.22%.

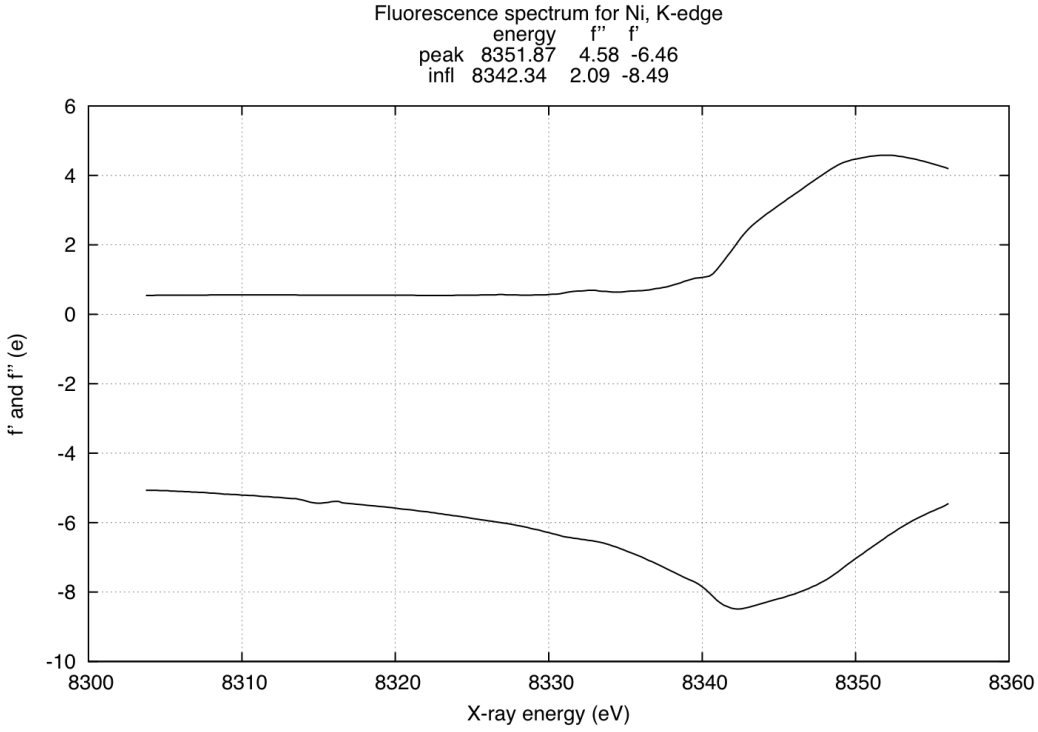

Figure S5. X-ray fluorescence scan of the FosB^{Bc}•Ni^{2+}-Fosfomycin crystal.

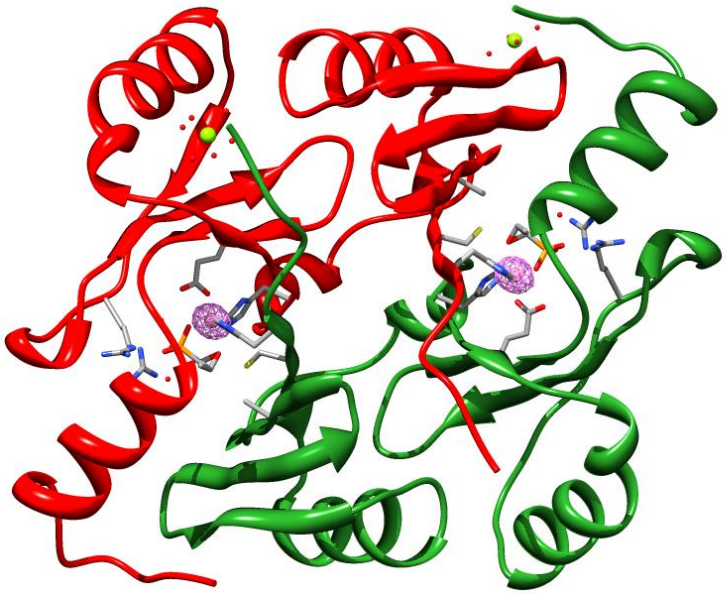

Figure S6. Overall x-ray crystal structure of FosB^{Bc} in complex with Co^{2+} and fosfomycin at 1.77 Å resolution (PDB 4JH5). The anomalous density map from the SAD experiment shown around the Co^{2+} metal ions is contoured at 5 σ. Final refinement had an R_{work} = 16.94% and R_{free} = 20.93%.

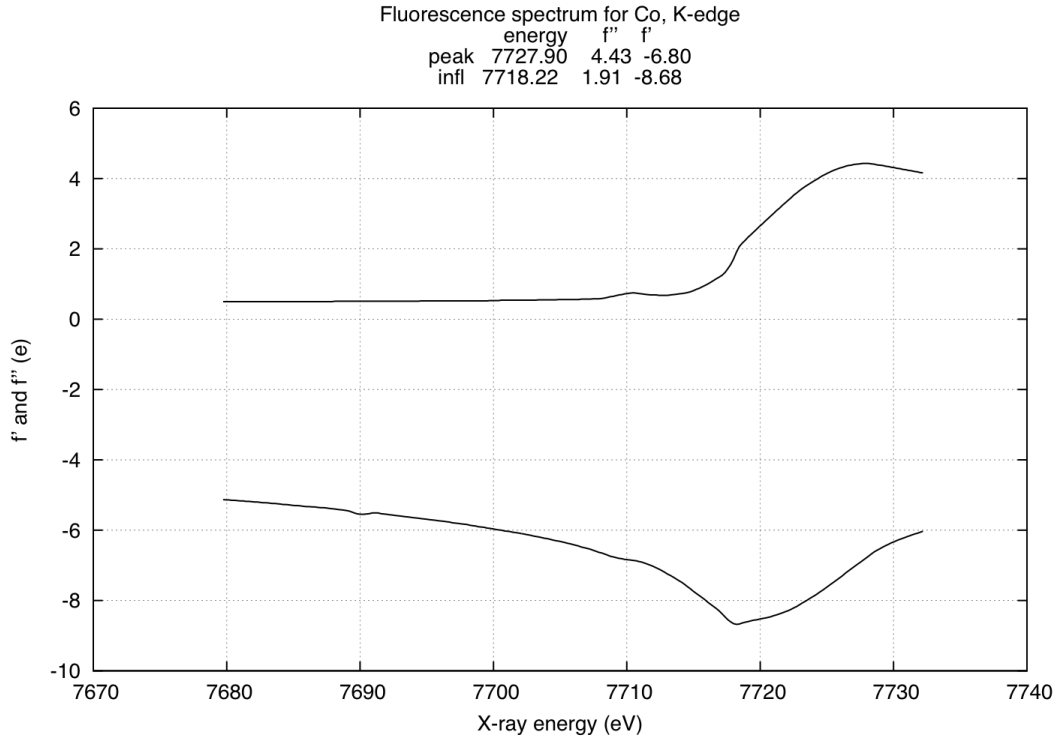

Figure S7. X-ray fluorescence scan of the FosB^{Bc}•Co^{2+}-Fosfomycin crystal.

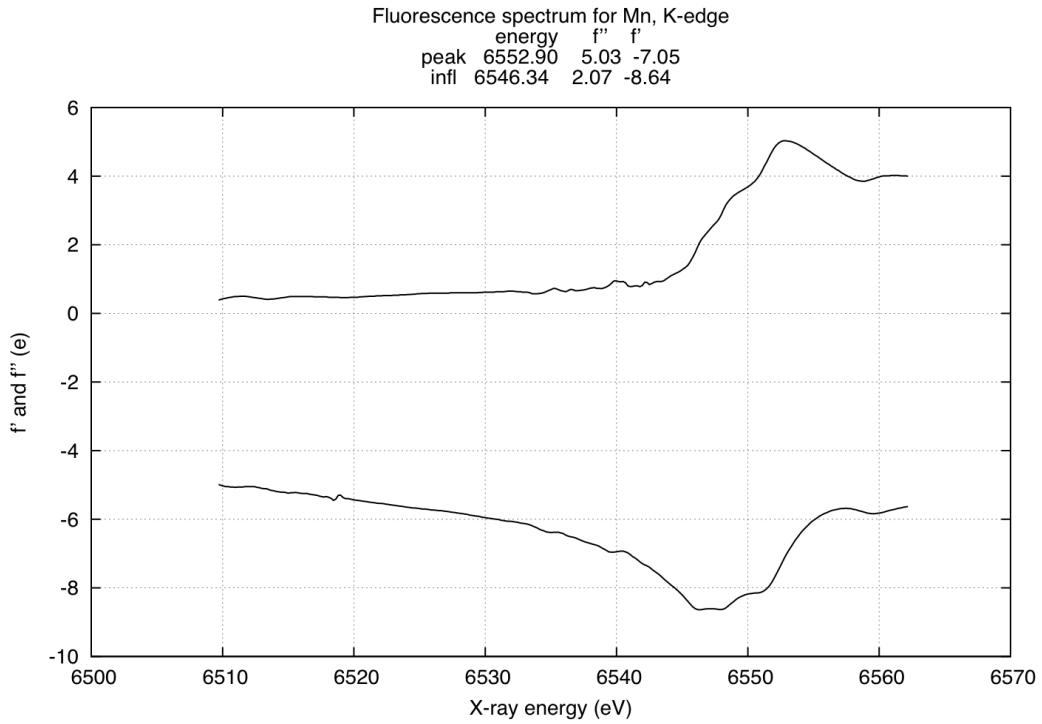

Figure S8. X-ray fluorescence scan of the FosB^{Bc}•Mn^{2+}-Fosfomycin crystal.

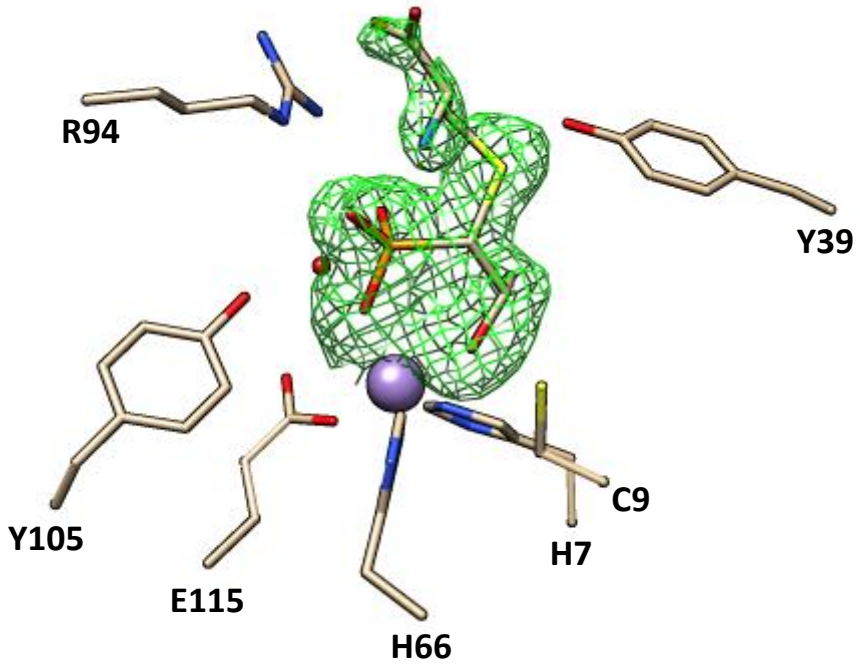

Figure S9. The active site of FosB^{Bc} in complex with Mn^{2+} in the presence of **3** at 1.77 Å resolution (PDB 4JH9). The model was refined with **2** since the complete density for **3** is missing. Final refinement had an R_{work} = 18.30% and R_{free} = 23.55%. The difference electron density shown for product is contoured 3σ. The unit cell dimensions for this crystal are different than the others reported (Table 1) indicating a significant difference in crystal packing. That product has been formed is evidenced by opening of the epoxide ring of the antibiotic and a C1-S bond length of 1.85 Å. Unlike what is observed in the FosB^{Bc}•Mn^{2+}•**2** structure, the electron density in this structure weakens near the C_α end of the L-cysteinyl domain. Thus, what is observed in this structure is the phosphonate end of **3** tethered to the Mn^{2+} metal within the enzyme while the glucosamine-malate moiety of **3** solvent exposed and disordered on the surface of the enzyme within the crystal.

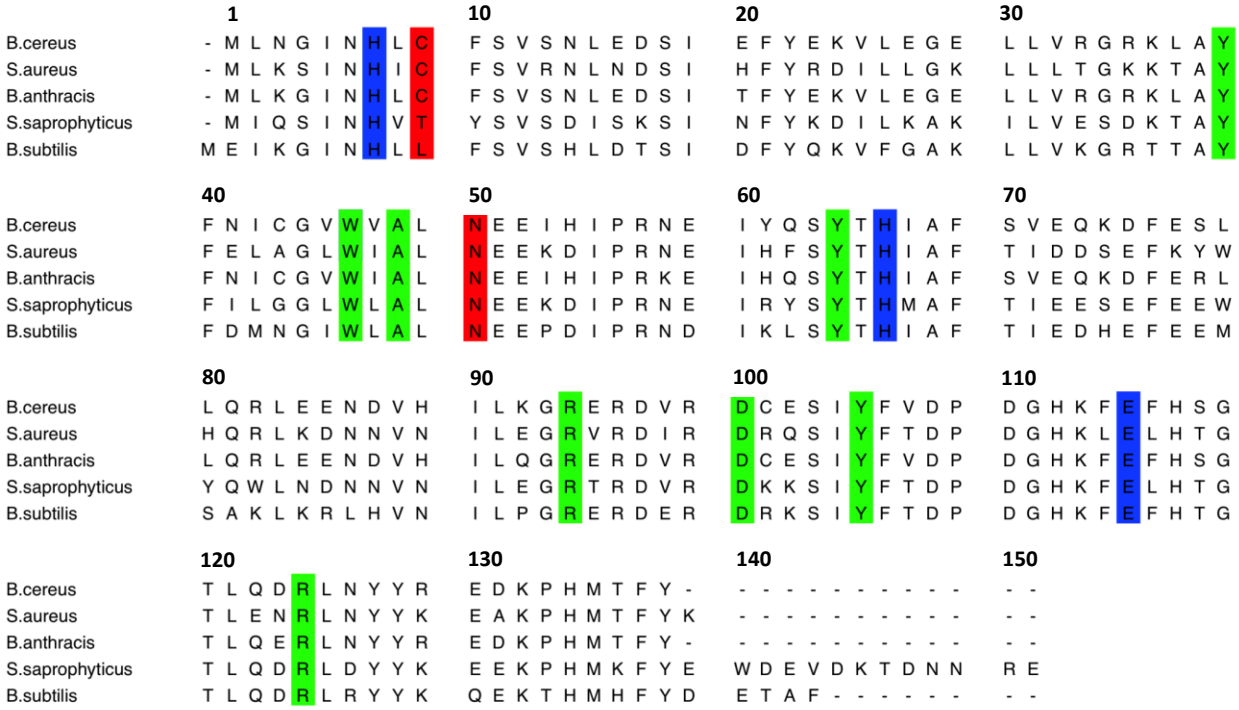

Figure S10. Sequence alignment of current FosB enzymes. Residues highlighted in blue are the metal coordinating amino acids. Residues highlighted in green form the fosfomycin cage. Residues highlighted in red are involved in activation.

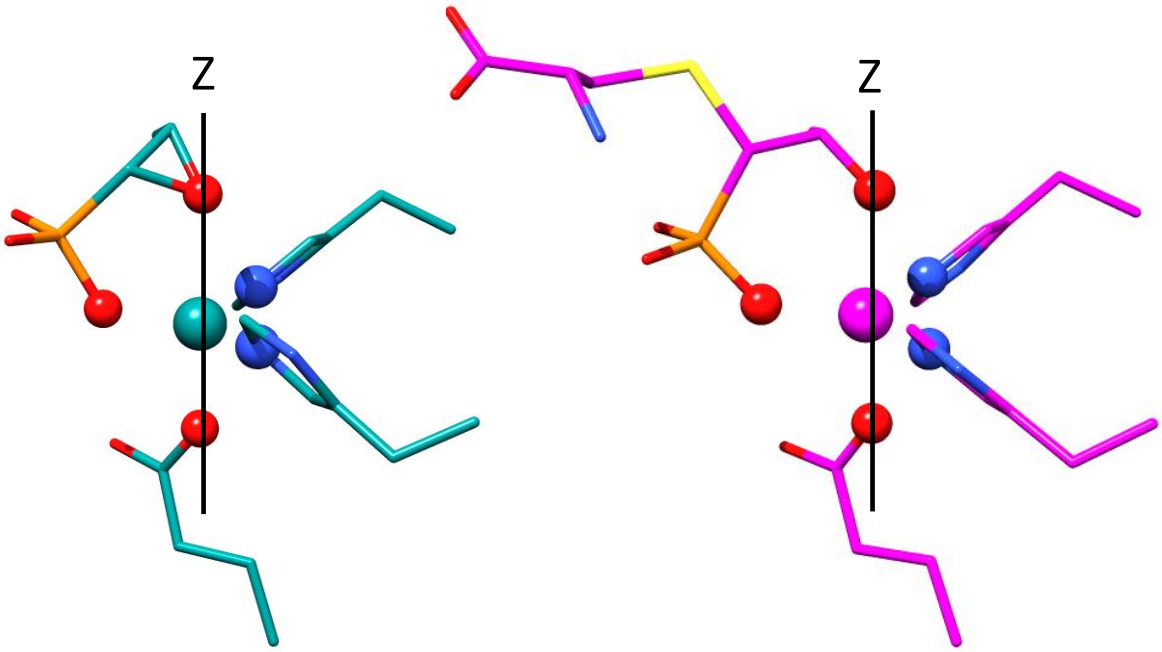

Figure S11. Comparison of the FosB^{Bc}•Mn^{2+}•**1** (PDB 4JH6) and FosB^{Bc}•Mn^{2+}•**2** (PDB 4JH7) structures. If the Z-axis is defined along $E115O_{\varepsilon}$–Mn^{2+}–$FosfomycinO_{(Oxirane)}$, there is a distorted trigonal bipyramidal geometry about the Mn^{2+} center similar to that observed in FosA. When the product is formed, the equatorial distances expand, and the metal-$oxygen_{(oxirane)}$ bond shortens, effectively creating a more symmetric trigonal bipyramidal geometry around Mn^{2+} and relieving strain on both the atomic orbitals of the metal and the epoxide ring of the antibiotic (see Table 2).