Supplemental Data

An Amino-Acid Position at the Crossroads of Evolution of Function: Antibiotic-Sensor Domain of the BlaR1 Protein from *Staphylococcus aureus vs*. Class D β-Lactamases

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Running head: Catalytic competence for a mutant BlaR1 protein

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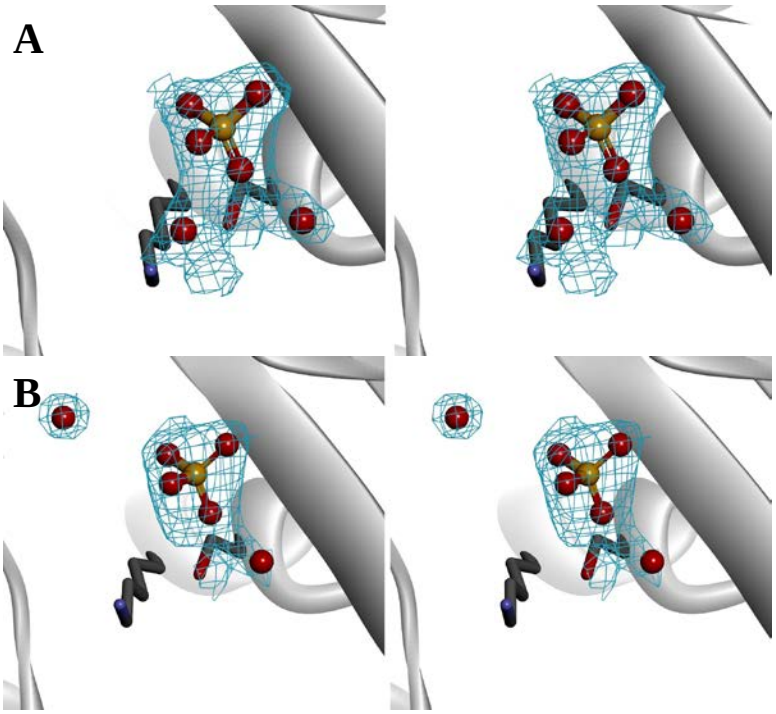

Figure S1. Stereo images of the $2F_o - F_c$ electron density contoured at 1 σ for the sulfate ion and the two water molecules closest to Lys-392 and Ser-389. **A.** The first molecule of the asymmetric unit. **B.** The second molecule of the asymmetric unit.

Imipenem Binding. The imipenem-binding mode (Fig. S1) is significantly different from that of ceftazidime (Fig. 5A). The most important difference is the placement of the 6α-hydroxyethyl moiety of imipenem. It occupies the position of the hydrolytic water molecule seen in the ceftazidime complex (Fig. 5A); thus our observation of the very low probability of favorably positioned hydrolytic water in the acylated imipenem complex. As shown in Fig. S1, Wat1, which serves the role of the hydrolytic water in the normal turnover events, is pushed aside by the hydroxyl moiety of the 6α-hydroxyethyl group. Therefore, the deacylation process is hindered in the case of imipenem binding to the BlaR1 N439V mutant. This very observation was noted for the complex of imipenem with the class A TEM-1 β-lactamase (1). Similarly, we have shown that the acyl-enzyme species that is generated with the acylation of the class D OXA10 β-lactamase with 6α-hydroxyethylpenicillanates is stable to deacylation for the same reason (2).

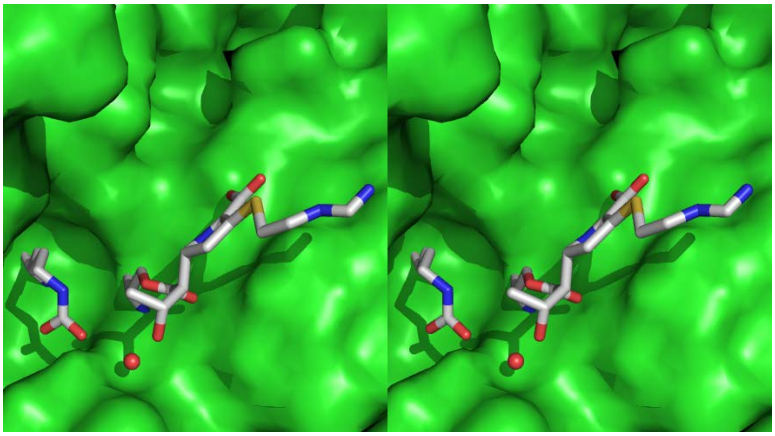

Figure S2. Stereo image of the imipenem bound to the $BlaR^S$ N439V active site of a representative molecular-dynamics snapshot, showing the position of the hydrolytic water molecule as a red sphere. The protein is shown as a Connolly surface in green with important residues depicted as capped sticks with atoms colored by atom types (nitrogen in blue, oxygen in red, carbon in gray; hydrogens not shown).

References

1. Maveyraud, L., Mourey, L., Kotra, L. P., Pedelacq, J. D., Guillet, V., Mobashery, S., and Samama, J. P. (1998) *J. Am. Chem. Soc.* **120**, 9748-9752
2. Golemi, D., Maveyraud, L., Vakulenko, S., Tranier, S., Ishiwata, A., Kotra, L. P., Samama, J. P., and Mobashery, S. (2000) *J. Am. Chem. Soc.* **122**, 6132-6133