E0012

Mechanisms of Antibiotic Resistance Studied by Raman and Protein Crystallography. Padayatti, P., Totir, M. Helfand, M., Carey, P., Bonomo, R. van den Akker, F., Dept. of Biochemistry, Case Western Reserve Univ., Cleveland, OH 44106.

β-Lactamases are the major mechanism through which life threatening bacterial infections become antibiotic resistant. There are currently three clinical β-lactamase inhibitors: tazobactam, sulbactam, and clavulanic acid. Mutations in β-lactamases severely threaten the effectiveness of these inhibitors. These inhibitors act as slow substrates and undergo several covalent molecular rearrangements which make it difficult to track using protein crystallography. A novel approach entails Raman crystallography which can identify and track the reaction intermediates prior to protein crystallographic analysis. This allowed us to successfully obtain protein crystallographic snapshots of the trans-enamine intermediate in SHV-1 β-lactamase of all three of these inhibitors. Besides these long awaited snapshots, our studies showed a remarkable agreement between Raman peak height, peak width (indicating local flexibility near the enamine bond), intermediate half-life, and the resulting electron density distribution along the linearized inhibitors, all of which can possibly be explained on the basis of the observed inhibitor:protein interactions. We recently have also applied this novel approach to study the inhibitor resistant β-lactamase variant M69V.