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Combining Raman Spectroscopy and Protein Crystallography to Analyse Reaction Intermediates in beta-Lactamase Crystals. Pius S. Padayatti[§], M.S. Helfand[|], M.A. Totir[^], M.P. Carey[§], A.M. Hujer [|], R.A. Bonomo[|], P.R. Carey[§], Focco Van Den Akker[§]. [§]Dept. of Biochemistry, [^]Dept. of Chemistry, Case Western Reserve Univ., Cleveland, OH, Research Div., [|]Louis Stokes Cleveland Veterans Affairs Medical Center, Cleveland, OH.

Protein crystallography is a powerful method to gain mechanistic insights into reaction intermediates inside protein crystals. However, identifying a particular reaction intermediate inside protein crystal and timing the best moment to trap it upon freezing the crystal prior to crystallographic data collection is often a difficult task. We therefore used Raman crystallography to determine the time point at which best to stop the soaking experiment by freezing the crystal. We used the E166A deacylation deficient mutant of beta-lactamase for our experiments. Beta-lactamases are in part responsible for the recent surge in antibiotic resistance and delineating their precise reaction mechanism is therefore of great importance. The reaction of the tazobactam antibiotic within the beta-lactamase mutant crystal was followed using a Raman microscope. The trans-enamine reaction intermediate was detected as evidenced by a characteristic band near 1595 cm-1 with a maximum intensity at 20-30 minutes. We therefore collected a 1.63 Å data set for a beta-lactamase crystal soaked for 20 minutes with 5mM tazobactam. Our crystallographic analysis showed the presence of a fully occupied enamine intermediate of tazobactam in the trans configuration. The interpretation of the electron density was further aided by an anomalous difference fourier electron density map highlighting the position of the sulfone group of tazobactam. We conclude that Raman crystallography and protein crystallography are a powerful combination to track and trap reaction intermediates inside protein crystals.