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Time-resolved macromolecular crystallography studies of AmpCEC using synchrotron and XFEL radiation

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The β-lactamase enzymes degrade β-lactam antibiotics, exemplified by penicillin. As such, the families of metallo- and serine β-lactamases are responsible for a major antimicrobial resistance mechanism in many clinically relevant species of Gram-negative bacteria, including Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa. To preserve the antimicrobial activity of β-lactam antibiotics, inhibitors of β-lactamases can be used in combination with a β-lactam antibiotic during treatment of an antimicrobial resistant infection. However, these inhibitors often have a narrow spectrum of activity against β-lactamases, and other bacterial mechanisms of resistance against them. To further development of novel β-lactamase inhibitors, we apply time-resolved serial femtosecond and synchrotron crystallography (tr-SF/SX) techniques to investigate the acylation of the β-lactamase AmpC from Escherichia coli by avibactam, a clinically approved β-lactamase inhibitor. Previously, tr-SF/SX data gathered by using a "drop-on-demand"sample delivery system revealed that covalent binding of avibactam to the AmpC active site occurred in a time frame quicker than 200 ms and as such pre-acylated time-resolved structures could not be obtained using this system. In the interest of capturing earlier time points using this enzymatic system, we have turned our focus to using a "drop-on-chip" fixed target sample delivery system, addressing contamination issues and implementing robust controls in our setup for accurate data collection. Currently, we are employing a drop-on-chip fixed target sample delivery system to access time points >1 ms at XFELs and >10 ms at synchrotrons such as Diamond Light Source.

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