Synthesis and Crystallization trials of New Delhi Metallo B-Lactamase

Jesus Valencia[1], Jose L. Olmos, Jr[2], George N. Phillips , Jr.[2]
[1]Department of Chemistry and Biochemistry, St. Mary's University, San Antonio, TX, 78828
[2]Department of BioSciences, Rice University, Houston, TX 77005

` B-Lactam antibiotics are a class of broad spectrum antibiotics that are used to treat illnesses such as urinary track infections and meningitis. Although B-Lactam antibiotics are an effective method of terminating bacteria, bacteria have become resistant due the use of the New Delhi Metallo-B-Lactamase (NDM-1). NDM-1 is a class B B-Lactamase with a binuclear zinc center that renders B-Lactam antibiotics useless via hydrolysis. Since NDM-1 has the ability to hydrolyze nearly all B-Lactam antibiotics, it poses a great threat to the world. In order to fully understand the hydrolysis of B-lactam antibiotics by NDM-1, the protein will be crystallized and its structure will be determined in its static form and in various intermediate states on the path to hydrolysis leading to product release. NDM-1 was successfully cloned in pNIC28-BSA4 by Gibson Assembly. The construct was then transformed in E. Coli (D5-alpha) and the recombinant DNA was then purified and transformed in a protein expression cell line of E.coli (BL21). NDM-1 was expressed and then purified by affinity chromatography using a Ni-column, followed by a TEV protease digest and subtractive immobilized metal affinity chromatography to remove the recombinant 6X-histidine tag and size exclusion chromatography. NDM-1 was set for crystallization trials and is currently under incubation. The crystals generated can then be optimized as microcrystals for use at an X-ray free electron laser to obtain data sets that can be processed to determine protein structures leading to the generation of a molecular movie of NDM-1 catalyzing the hydrolysis of B-lactam antibiotics. The molecular movie would provide an understanding of the NDM-1's relevant conformations and the time frames in which they occur. Ultimately, leading to the design of a new generation of antibiotics.