A Structural Approach to a Global Concern, New Delhi Metallo β-Lactamase(NDM-1)

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NDM-1 is a class B β-lactamase that grants bacterial pathogens a resistance to a broad spectrum of currently available β-lactams. This enzyme hydrolyses β-lactams through two zinc cofactors that come from two identical tertiary structures rotated 180 degrees that make up this dimer protein. In this study we will attempt to determine optimal purification protocols for this enzyme as well as optimal crystallization conditions after which we will obtain a structure using X-ray crystallography methods. The protein was produced by inserting a plasmid containing a pET28a vector and the NDM-1 insert from G29 to R270 excluding 28 residues. This process was done using the Gibson Assembly protocol for vector and insert combination. After creating the plasmid DH5α cells were elected first for a successful cloning transformation through effective plasmid retention. A second transformation into BL21 cells was performed for expression purposes. A 50 mL starter culture containing transformed BL21 cells with our plasmid was grown overnight at 37° Celsius for 18 hours. The cells were then transferred into flasks containing 1 L LB media that reached an absorption of ~0.5 at a wavelength of 600 nm before being induced with IPTG for a final concentration of 0.5mM. Cells were then lysed through sonication and centrifuged at 4500 RPM to separate organelles and have the protein relatively isolated for purification. The first process of the purification was performed using a nickel 2+ affinity column and then concentrated after which a size exclusion column was run using fast protein liquid chromatography(FLPC) for further purification. The fractions of the desired peaks were concentrated and stored. The nickel column showed that the protein was purified successfully but without a native gel, size exclusion was elected to continue through the purification process. The graph data for size exclusion using FPLC showed two peaks for the protein that based on standards were determined to be the protein in both a monomer and dimer form in liquid solution. This process proved successful and allowed for the setup of crystal screens. This was done by setting up crystal screens with varying conditions in a vapor diffusion process that was evaluated over time. Moving forward, crystallization conditions will be optimized and a structure determined based on the diffraction patterns obtained.

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