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Toward the Crystallization of an Archaeal Dihydrorotase

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Toward the Crystallization of an Archaeal Dihydroorotase

College of Sciences and Health Professions

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Abstract

Dihydroorotase catalyzes the conversion of N-carbamoyl-L-aspartate to L-dihydroorotate in the *de novo* biosynthesis of pyrimidines. *M. jannaschii* is an archaeon that thrives in extreme environments such as the hypothermal vents at the bottom of the oceans in which both temperature and pressure are extremely high. It can serve as a model organism for research purposes. This experiment is a first step toward elucidating the structure of this enzyme in *M. jannaschii*. Our summer research started using a partially purified enzyme preparation from previous experiments. We further purified the enzyme primarily using hydrophobic interaction and hydroxyapatite chromatographies. Twenty-four closely related conditions were tested to determine if a crystal of dihydroorotase could be formed. Several conditions led to whisker-needle clusters and preliminary needle crystals. These findings can be utilized to determine additional steps and other conditions to test. Once a crystal is formed, it can undergo X-ray crystallography to determine its structure. The structure of dihydroorotase in *M. jannaschii* can elucidate what structural characteristics allow this archaeon to survive in extreme heat and pressure. It also provides further understanding of the diversity within the dihydroorotase family of proteins. It can also give further insight into this pathway in humans.