

Towards the Structure of the *E. coli* dATP-pyrophosphohydrolase

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Beamline(s): X25

Nudix hydrolases, previously known as MutT proteins, are a family of enzymes involved in cleaning the cell from potentially deleterious metabolites and preventing the accumulation of intermediates. These anhydrases are characterized for having a short primary sequence called the Nudix signature sequence and for hydrolyzing a nucleoside diphosphate linkage. To investigate if the enzymes of the family share structural and chemical features, we decided to determine the structure of enzymes that belong to different subfamilies and so catalyse different reactions. The dATP pyro-phosphohydrolase (dATP-ase) hydrolases dATP in AMP and pyrophosphate. Crystal structures of the dATP-ase and other Nudix hydrolases are important for understanding the functional diversity of the family and the conservation of residues.

The dATP-ase crystals grew by seeding in sitting drop experiments using Ammonium sulfate as a precipitant and sodium tungstate. dATP pyrophosphohydrolase crystals appeared in 1 day and took 3 weeks to grow to full size, 0.1x0.1.5x0.03mm. A four wavelength multiple anomalous dispersion data set was collected at the tungstate absorption edge at beam line X25 using frozen crystals. The data was processed with the DENZO/SCALEPACK suite.

The crystals are monoclinic, $a=124\text{\AA}$, $b=42\text{\AA}$, $c=105\text{\AA}$, $\beta=115^\circ$, and diffract to 2.0\AA . The asymmetric unit has two pairs of molecules in the asymmetric unit, which are related by pure translation. The anomalous Patterson map was readily interpretable.

Reference: Bessman, M.J., Frick, D.N., O'Handley, S.F. The MutT Proteins or "Nudix"Hydrolases, a family of versatile, widely distributed, "housecleaning" enzymes. JBC, 271:25059-25062, 1996