Solution of the crystal structure of thermostable *Bacillus* RAPc8 Nitrile Hydratase

T.L. Tsekoa(1), R.A. Cameron(1), O. Bishop(2), M.F. Sayed(2), B.T. Sewell(3), D.A. Cowan(1)

(1)ARCAM, Department of Biotechnology, University of the Western Cape (2)Department of Biotechnology, University of the Western Cape (3)EMU, University of Cape Town

Microbial nitrile hydratases (NHases) have attracted significant interest from both academic and industrial researchers. These enzymes catalyze the conversion of nitriles to the corresponding amides. The utilization of nitrile hydratase-containing *R. rhodochrous* J1 to produce the commodity chemical acrylamide by Mitsubishi Rayon (Japan) has become a model industrial biocatalysis process.

This lab has previously isolated, cloned and sequenced the nitrile hydratase operon of thermophilic *Bacillus* sp. RAPc8. Here we report our resolution of the crystal structure of RAPc8 NHase. First, it is demonstrated that co-expression in *E. coli* with a small downstream protein (P14K) dramatically stimulated activity of the expressed protein. The strategy for purification of the recombinant protein in three steps is described. The resultant pure, heterotetrameric enzyme was crystallised using the hanging-drop vapour-diffusion method. Crystals produced in the presence of 30% PEG 400, 0.1 M MES pH 6.5 and 0.1 M magnesium chloride were selected for X-ray diffraction studies. A data set complete to 2.5 $\dot{A}$ was collected under cryoconditions at the in-house X-ray source, University of the Western Cape. The space group was determined to be primitive tetragonal (P41212) with unit cell dimensions a=106.61 $\dot{A}$, b=106.61 $\dot{A}$, c=83.23 $\dot{A}$, alpha=beta=gamma=90^0; with one dimer per asymmetric unit. The structure was solved *via* molecular replacement using *Ps. nocardia* NHase as a search model. The solved structure will form the basis of work aimed at engineering NHase for improved activity in industrial biotransformations, with particular emphasis on engineering aromatic specificity into the enzyme.