

WHY DO NITRILASES NEED TO FORM HELICES TO BE ACTIVE?

Trevor Sewell^a, Serah Kimani^b and Muhammed Sayed^c

^a*Electron Microscope Unit;* ^b*Department of Molecular and Cell Biology, University of Cape Town;* ^c*Department of Biotechnology, University of the Western Cape, South Africa.*

We have recently solved the crystal structure of the amidase from *Geobacillus pallidus* RAPc8. The structure of this enzyme, which has approximately 20% identity to the fibre forming, cyanide hydratases, cyanide dihydratases and nitrilases gives a series of previously inaccessible insights. Firstly the extended C-terminus forms an interlock on at the “A surface” which we have described previously - giving the reason for the stability of this interface. Furthermore a hydrogen bond across this interface stabilises the position of the 3₁₀ helix on which the catalytic cysteine is located. The most interesting observation arises from the very small active site of the amidase. Because of this it can be seen that the oxygen of the acyl intermediate restricts access to glu59 thought to be the general base catalyst responsible for assisting the hydrolysis of the acyl intermediate. An alternative choice for general base catalyst is glu142 which is located in a loop which we have previously identified as being part of the “C surface” which together with the “A surface” form the interactions along the one-start left handed helix. We postulate, based on a series of cryo and negative stain structures, that the formation of the “C surface” moves glu142 into position so that it can perform a catalytic role in the nitrilases.