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The mechanisms of enzymes of the nitrilase superfamily

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Our goal is to determine the mechanisms of the nitrilase superfamily enzymes. These enzymes have common features such as their fold and conserved residues in their active sites (two glutamates, a lysine and a cysteine) but have a range of different activities. The superfamily derives its name from the nitrilases that convert nitriles to the corresponding carboxylic acids and ammonia, but most of the enzymes in the superfamily are amidases that convert amides to the corresponding carboxylic acid and ammonia. The reaction proceeds via the formation of a thioester intermediate. In our recent work [1] we identified the components of the active site that position the amide substrate for the attack by the cysteine on the carbonyl carbon of the amide. Our approach, which has led to several key insights, involves a combination of structure analysis, site directed mutagenesis, identification of intermediates by mass spectroscopy and quantum mechanical modelling [2]. An example of such an insight, shown in Fig. 1, locates the water molecule that is responsible for the hydrolysis of the thioester intermediate such that its lone pair overlaps with the LUMO of the carbonyl carbon.
Many amidase homo-oligomers, ranging from 2-8 monomers in different instances, have been crystallized, leading to extensive structural knowledge. The nitrilases, on the other hand, form spiral homo-oligomeric structures and, to date, none have crystallized in their complete, active form. The spiral structures are, however, amenable to structure determination by cryoEM [5]. In the case of Nit4 from Arabidopsis thaliana side chains in two adjacent monomers contribute to the active site pocket, playing an important role in substrate specificity. This enables enzymes to be tailored to a wide variety of substrates.

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