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The structure of the C146A variant of the amidase from Pyrococcus horikoshii bound to glutaramide suggests the basis of amide recognition

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The literature suggests that the dinitriles: malononitrile and fumaronitrile are substrates of the nitrilase-like enzyme from Pyrococcus abyssi. We have attempted to verify this and to visualize the bound substrates by X-ray crystallography. No nitriles that we tested are hydrolyzed by the very similar nitrilase-like enzymes from either P. abyssi or P. horikoshii. The enzymes do hydrolyse a variety of amide substrates, with propionamide being the most rapidly hydrolysed of all the substrates tested. Amide substrate docking studies on the wild-type enzyme structures reveal steric hindrance between the active site cysteine sulfhydryl moiety and the incoming amide. The steric hindrance is relieved if the cysteine is replaced by an alanine. The amide then docks in a position in which the amino group of Lys-113 and the backbone amide of Phe-147 are hydrogen bonded to the substrate carbonyl oxygen and the carboxyl oxygen of Glu-42 and the backbone carbonyl oxygen of Asn-171 hydrogen bonded to the amino group of the substrate. This location of the substrate is confirmed experimentally in the case of the well-resolved crystal structure of the C145A mutant of the enzyme from P. horikoshii. Our experiment suggests a different starting position for the hydrolysis reaction sequence than prevailing model in which the amide substrate is positioned with its amino group hydrogen bonded to the two active site glutamate (Glu-42 and Glu-120) carboxyl groups prior to the attack by the cysteine on the substrate carbonyl carbon.

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