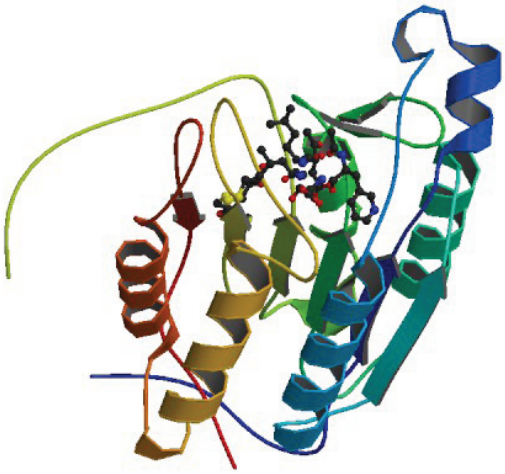

Figure 1. An example of caspase with a ligand (PDB ID: 4PS1) [7]

Keywords: caspases, ligands, electrostatic interactions, UBDB, TAAM

MS5-P39 Crystallization and structural characterization of glyceraldehyde dehydrogenase from *Thermoplasma acidophilum*

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Biotechnological production of chemical compounds is an environmentally more gentle process compared to their fabrication from natural fossil resources. In terms of bio-production, cell-free processes are more effective than microbial production techniques since the enzymes can tolerate higher concentrations of final product than the cells. The glyceraldehyde dehydrogenase from *Thermoplasma acidophilum* (*Ta*AlDH) is a part of an artificial cell-free enzyme cascade for production of isobutanol and ethanol from glucose. *Ta*AlDH catalyzes the oxidation of D-glyceraldehyde to D-glycerate in this synthetic pathway. Various mutants of *Ta*AlDH were constructed by random approach followed by site-directed and saturation mutagenesis in order to improve the enzymes' properties essential for its functioning within the cascade. Further optimization of *Ta*AlDH requires structural information about the enzyme for which crystallization followed by X-ray diffraction analysis was employed.

Different types of *Ta*AlDH wild-type crystals grew within one to two weeks after initial screening in 30 diverse conditions. In order to obtain the best quality crystals, optimization was carried out considering the following parameters: (*a*) already known diffraction quality of crystals; (*b*) size and shape of crystals (big single crystals with sharp edges preferred); (*c*) different crystal forms. Optimization, including variation of pH, protein and precipitant concentrations and ratios, resulted in adequate crystal quality only for condition H6 of the Morpheus screen (Molecular Dimensions Ltd., UK). These crystals diffracted X-rays to 1.95Å resolution and belonged to space group $P2_1$ with 8 molecules per asymmetric unit and unit cell parameters of a = 95.29 Å, b = 152.35 Å, c = 149.90 Å, $\alpha = \gamma = 90.0°$, $\beta = 92.19°$.

The structure of *Ta*AlDHwt was solved by molecular replacement using the coordinates of betaine-aldehyde dehydrogenase from *Pseudoalteromonas atlantica* T6c (sequence identity 38%, PDB ID 3K2W). The final model contains two tetramers in the asymmetric unit that are related by non-crystallographic symmetry with

differences observed in regions participating in crystal contacts. The *Ta*AlDHwt homotetramer consists of two homodimers that display a very tight connection through the formation of an extended beta-sheet between monomers of the dimer. The structure refinement of *Ta*AlDHwt is in progress.

Keywords: glyceraldehyde dehydrogenase, cell-free enzyme cascade, bioproduction

MS5-P40 Structural and biochemical characterization of dipeptidyl peptidase III from *Porphyromonas gingivalis*

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Porphyromonas gingivalis is gram-negative, human pathogenic bacterium. It is found in the oral cavity and it is known to cause periodontal disease by invading human gingival fibroblasts. It also contains an enzyme which belongs to the DPP III family.Dipeptidyl peptidase III (DPP III), also known as enkephalinase B, is an enkephalin-degrading enzyme that cleaves dipeptides sequentially from the N-termini of substrates. All DPPs III described thus far contain the unique zinc binding motif HEXXGH characteristic for metallopeptidase family M49. An important role of DPP III in the mammalian pain modulatory system is supported by several recent findings: low levels of DPP III activity were detected in the cerebrospinal fluid of individuals suffering from acute pain; DPP III exhibits high in vitro affinity towards the important neuropeptides endomorphin-1 and endomorphin-2.The exact function of DPP III from *Porphyromonas gingivalis* is still unknown. It could possibly be involved in pathogenicity. With the human DPP III shares 20,3 % sequence identity. According to homology model, it was possible to obtain two domains: one is DPP III domain also observable in humans and additional alpha-alpha superhelix domain which is not recognized as transmembrane domain.We aim at determining the potential substrates and also to solve the structure of the enzyme in order to get insight into the potential function of the protein. Here we represent the ITC- and SAXS- data as well as crystallization experiments as first important steps towards this goal.

Keywords: DPP III, SAXS, ITC, Crystallization