before [2]. Here, another c-diGMP binding mode is observed that cross-links the two DGC domains of the dimer. This new mode of domain immobilization may be of general importance for a large sub-class of DGC proteins.

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MS03 P18

Structural characterization of threonine aldolases

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Threonine aldolases (TAs) are PLP dependent enzymes which catalyze the reversible conversion of β-hydroxy-α-aminoacids into glycine and the corresponding aldehyde. In general it's possible to distinguish between two main types, L-selective and D-selective threonine aldolases, depending on their enantioselectivity with respect of the α-carbon of the amino acid. Threonine aldolases possess a high potential as biocatalysts for the synthesis of β-hydroxy-α-amino acids which are important chiral pharmaceutical intermediates.
We are specifically working with two bacterial enzymes, an LTA and a DTA, which share only very low sequence similarity.
For LTA, a microbatch screen was performed, using solutions of the Index Screen (Hampton Research) which yielded first conditions. After optimization it was possible to collect a dataset to a maximum resolution of 3.5 Å at our home x-ray source. The thus obtained LTA crystals are monoclinic (spacegroup $P2_1$) with unit cell dimensions a=106.5 Å; b=116.1 Å; c=106.8 Å and β=93.0°. The asymmetric unit consists of one homotetramer of the enzyme and contains about 70% solvent. First steps towards a structure solution by molecular replacement have been performed using this dataset and a previously generated homology model of LTA.
For DTA we had to employ reductive methylation of lysine residues (using formaldehyde and $NaBH_4$) in order to obtain diffraction quality crystals. With the thus modified DTA a standard crystallization screen was carried out. At our home x-ray source, first crystals yielded a diffraction pattern up to a maximum resolution in the range of 3.5 Å. They belong to spacegroup $P2_1$ with unit cell dimensions of a=62.4 Å; b=83.8 Å; c=73.3 Å and β=112.1°.
Further optimization of the crystallization conditions in order to improve the resolution of the diffraction data, as well as soaking experiments with substrates and inhibitors are underway.

MS03 P19

Crystallographic studies of an active-site mutant of plant-type L-asparaginase

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Keywords: plant-type asparaginase, isoaspartyl peptidase, Ntn-hydrolase, autoactivation

Plant asparaginases hydrolyze the side-chain amide bond of asparagine or of isoaspartyl dipeptides. Close homologs have been found not only in plants, but also in bacteria, with the *E. coli* protein, EcAIII, being particularly well studied. The enzymes from both sources belong to the family of Ntn-hydrolases with a catalytic Thr nucleophile located at the free N-terminus of subunit β, liberated during an autoproteolytic maturation event which generates two separate subunits (α and β) from a single-chain precursor. It is believed that the maturation mechanism involves the very same Thr nucleophile that is liberated in its consequence. However, the details of the maturation reaction are obscure, as the free amino group, supposed to activate the nucleophilic hydroxyl, is not present in the immature protein. To investigate the structural basis of EcAIII maturation, we have generated and crystallized an active-site mutant with the catalytic Thr residue substituted by alanine (T179A). The structure has been solved in two crystal forms, one of which corresponds to that of the wild-type enzyme. The protein exists as a dimer resembling the $(\alpha\beta)_2$ oligomeric form of the mature enzyme. The linker connecting the α and β portions of the precursor can be traced nearly in its entirety, allowing a discussion of a nucleophilic attack launched by residue 179 on the preceding peptide bond. In variance with the wild-type structure, a fragment of the linker chain is seen in the active-site cavity. A tightly coordinated sodium cation, which in the mature protein shapes a loop supporting the active site elements, is found in an analogous structure of the T179A mutant. This suggests that the metal cation is coordinated at an early stage during the protein folding process and that its presence is essential for both the maturation reaction and the final L-asparaginase activity.

MS03 P20

Structural Properties and Electrostatics of Cold-active beta-Galactosidase

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Arthrobacter sp. C2-2, a soil bacteria found on an island near Antarctica, belongs to psychrotrophic, i.e. cold tolerant, microorganisms. Structure of its beta-galactosidase (hydrolase cleaving lactose into galactose and glucose), iso-enzyme C-2-2-1, was solved up to 1.9 Å resolution.
The beta-galactosidase belongs to glycosyl hydrolase structural family 2 and has 30% sequence identity with *Escherichia coli* beta-galactosidase. In spite of the chain similarity, both enzymes differ in their oligomerization states.