The toxic extracellular endopeptidase AsaP1 is the causative agent of Aeromonas salmonicida achromogenes and leads to atypical furunculosis, a systematic disease in Atlantic salmon and other farmed fish (1).
AsaP1 is 343 amino acids long zinc-metallopeptidase containing a signal sequence of 22 amino acids and an N-terminal propeptide of 171 amino acids, which is released by autocleavege leading to an active enzyme of 22 kDa.
For biochemical and structural characterisation an *E. coli* expression system was established and the structure elucidation for the two inactive mutants AsaP1_E294Q and AsaP1_E294A was performed by X-Ray crystallography.
The structure was solved by molecular replacement using the search model of the peptidyl-Lys metalloendopeptidase from Grifola frondosa composed of a 172 amino acid protease domain without propeptide (pdb entry 1g12).
Due to inactivity of AsaP1 mutants the propeptide remains still as a part of the protein, but its function, whether it plays a role in protease folding and acts as an intramolecular chaperone or whether it inhibits proteinase activity in the intracellular space, still remains unclear.
The complex build of propeptide domain and protease domain reveals insights for substrate interaction and substrate specificity of AsaP1.

[1] Gudmundsdottir, B. K. 1996. *J. App. Bacteriol.* 80 (1):105-13

Keywords: metalloendopeptidase, aspzincin, propeptide

FA1-MS11-P07

Structure and Biochemistry of the APP E2-domain.
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The Amyloid Precursor Protein (APP) and its sequential cleavage by the proteases beta- and gamma-secretase are generally believed to be of central importance for the development of Alzheimer 's disease (AD) [1]. The resulting neurotoxic peptide Abeta is found in the disease typical senile plaques. Especially the biological function of APP remains mostly unclear until now, not least because of insufficient structural knowledge about the Protein. We will present structural and biochemical data of the E2-domain of APP, which provides new functional insights.

[1] K. Blennow, M. J. de Leon and H. Zetterberg, *Lancet* 368 (2006), p. 387
Keywords: Amyloid Precursor Protein (APP), Alzheimer's Disease (AD), Crystal structure

FA1-MS11-P08

Crystal structure of N-formimino-L-Glutamate Iminohydrolase.
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The members of the aminihydrolase superfamily of protein molecules are found in every organism sequenced to date and are structurally characterized by metal center embedded at the C-terminal end of $(\beta/\alpha)_8$-barrel protein fold.
Here we present the crystal structure of N-formimino-L-Glutamate Iminohydrolase from Pseudomonas aeruginosa – the member of aminodydrolase superfamily. The crystal structure of this enzyme in the presence of the inhibitor, N-formimino-L- aspartate also will be presented.
These crystal structures have provided insight into the mechanism for the deimination reaction and identified conserved residues within the active site that are required for substrate recognition.

Keywords: protein crystal structure, Iminohydrolase, enzyme mechanism

FA1-MS11-P09

Structure of the tetracycline-degrading monooxygenase TetX2.
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The flavin-dependent monooxygenase TetX2 from anaerobic *Bacteroides thetaiotaomicron* confers resistance against tetracyclines on aerobically grown *Escherichia coli* [1]. The enzyme modifies several tetracycline antibiotics including the recently approved 3rd generation antibiotic tigecycline under regioselective hydroxylation of the substrate which leads to non-enzymatical degradation associated with weaker antibiotic properties [2]. In contrast to efflux or ribosomal protection mechanisms, this resistance mechanism is only partly understood. TetX2 has also been found in aerobic *Sphingobacterium* sp. which may be the ancestral source of the *tet*X-genes. The crystal structure was solved in a 3-WL MAD experiment with a SeMet-containing crystal in space group $P2_1$. The native protein crystallized in $P1$ and data were collected to a resolution of 2.5 Å. The self-rotation function of the $P2_1$ data revealed two independent twofold non-crystallographic axes which occur also in the $P1$ data but with a slightly different orientation of the four monomers in the asymmetric unit. TetX2 shares highly conserved homologous domains with other structurally known FAD-binding monooxygenases like PhzS hydroxylase, despite low sequence identity and different substrates. The ADP moiety of the flavin cofactor is bound by a glutathione reductase fold which is comparable to other FAD-binding enzymes. The supposed substrate binding domain contains a seven stranded β-sheet. Two strands connect the FAD-binding domain with the substrate binding domain additional to a helix composed of eight turns. In the absence of a substrate complex molecular modeling studies are now under way to position a tetracycline molecule in the supposed active site of TetX2.

[1] Yang W., Moore I.F., Koteva K.P., Bareich D.C., Hughes D.W., Wright G.D., *JBC,* 2004, 279(50), 52346. [2] Moore I.F., Hughes D.W., Wright G.D., *Biochemistry,* 2005, 44, 11829.

Keywords: flavoenzymes, tetracyclines, monooxygenases

FA1-MS11-P10

Crystal structure of Tpa1 from *Saccharomyces cerevisiae.* Hye Jin Yoon[a], Hyoun Sook Kim[a], Hye Lee Kim[a], Kyoung Hoon Kim[a], Do Jin Kim[a], Sang Jae Lee[a], Ji Young Yoon[a], Se Won Suh[a,b]. [a]*Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151-742, Korea.* [b]*Department of Biophysics and Chemical Biology, College of Natural Sciences, Seoul National University, Seoul 151-742, Korea.*
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Tpa1 (for *t*ermination and *p*oly*a*denylation) from *Saccharomyces cerevisiae* is a component of a messenger ribonucleoprotein complex at the 3' untranslated region of mRNAs. It comprises an N-terminal Fe(II)- and 2-oxoglutarate-dependent dioxygenase domain and a C-terminal domain. The N-terminal dioxygenase domain of a homologous Ofd1 protein from *Schizosaccharomyces pombe* was proposed to serve as an oxygen sensor that regulates the activity of the C-terminal degradation domain. Members of the Tpa1 family are also present in higher eukaryotes including humans. Here we report the crystal structure of *S. cerevisiae* Tpa1 as a representative member of the Tpa1 family. Structures have been determined as a binary complex with Fe(III) and as a ternary complex with Fe(III) and 2-oxoglutarate. The structures reveal that both domains of Tpa1 have the double-stranded β-helix fold and are similar to prolyl 4-hydroxylases. However, the binding of Fe(III) and 2-oxoglutarate is observed in the N-terminal domain only. We also show that Tpa1 binds to poly(rA), suggesting its direct interaction with mRNA in the messenger ribonucleoprotein complex. The structural and functional data reported in this study support a role of the Tpa1 family as a hydroxylase in the messenger ribonucleoprotein complex and as an oxygen sensor [1].

[1] Kim H.S., Kim H.L., Kim K.H., Kim D.J., Lee S.J., Yoon J.Y., Yoon H.J., Lee H.Y., Park S.B., Kim S.-J., Lee J.Y., and Suh S.W., *Nucleic Acids Research*, 2010, 38, 2099.

Keywords: Tpa1, Ofd1, mRNP complex

FA1-MS11-P11

Structure and function of a novel Dienelactone hydrolase Christian Roth[a], Michael Schlömann[b] Norbert Sträter[a]. [a]*Faculty for Chemistry and Mineralogy, Structural Analytics of Biopolymers, University Leipzig, Germany.* [b]*Interdisciplinary Ecological Center, Technical University Bergakademie Freiberg, Germany.*
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Fluoroaromatic compounds are widely used as pesticides or pharmaceuticals and well known environmental pollutants. The bacterium *Cupriavidus necator* is able to degrade such compounds via a modified variant of the β-ketoadipate pathway. One of the key enzymes is the *trans*-dienelactone hydrolase (t-DLH) which degrades (fluoro)dienelactone leading to maleylacetate. The *t*-DLH from C. necator is a monomeric enzyme with a molecular weight of approximately 38 kDa. The enzyme shows no significant similarity to other known dienelactone hydrolases. Furthermore C. necator t-DLH shows activity only in the presence of divalent cations like Mn^{2+} or Mg^{2+}.
The gene of *t*-DLH was cloned expressed in *E.coli*. The protein was purified to at least 90 % homogenity. The wildtype was subjected to crystallization trials using sparse matrix screens. Suitable crystallization conditions could not be identified for this variant. Mutants designed based on the principle of surface entropy reduction were created and purified. Crystals, suitable for diffraction experiments, could be obtained for a double mutant. The phase problem was solved using MIRAS and a model of the enzyme was built and the metal center was assigned. We currently try to get a complex structure to study the reaction mechanism of this new class of dienelactone hydrolases.

[1] Gummow R.J., Liles D.C., *Mat. Res. Bull*, 1993, 28, 1293. [2] Grirrane, A.; Pastor, A.; Galindo, A.; Ienco, A.; Mealli, C. *Chemm. Commun.* 2003, 512.

Keywords: biocrystallography, hydrolase, metallo enzyme X-Ray