describe the structure of a new class of CBP from the parapoxvirus, Orf virus. The crystals of this protein were challenging to produce and optimized significantly through the use of somewhat surprising additives. Crystals occupy Space Group $P6_522$ with unit cell parameters of a = b = 75.62, c = 282.49 Å, α = 90, β = 90, γ = 120°. The structure was phased using MAD methodologies and currently the 2.1Å structure is undergoing refinement. Early analysis indicates that it is a member of the β-sandwich family but it is quite distinct from other family members when superimposed. Additionally the crystal structure is consistent with a physiologic dimer and displays a very broad β sheet on its surface containing contributions from more than 10 β strands. The dimeric nature of this CBP appears to be a unique property of its class and may be key in explaining how it is able to bind different chemokines from at least two distinct chemokine classes.

Keywords: protein crystallography, chemokine , virology

FA1-MS11-P04

How to minimize X-ray dose used for in-house data collection on protein crystals? Vernon Smith[a], Marianna Biadene[a], Matt Benning[b]. [a]*Bruker AXS GmbH, Karlsruhe, Germany.* [b]*Bruker AXS Inc, Madison, WI, USA.*
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Datasets for *de-novo* structure solution are typically collected at synchrotron beamlines. However, with increasingly bright rotating anode generators, a significant number of datasets are collected in-house. Any exposure to X-ray radiation create free radicals inside the crystal, which can lead to decreased resolution, decreased $\langle I/\sigma(I)\rangle$, increased mosaicity and increased B-factors. It is clear that radiation damage does occur during longer data collections on rotating anode sources, even at 100 K. Unnecessary radiation damage can be avoided by using a system which maximizes detection of diffracted X-ray photons enabling more conservative incident X-ray doses to be inflicted.
The latest generation of microfocus sealed-tube sources deliver an incident X-ray beam of greater intensity than traditional rotating anode generators, but with much better beam properties and stability. Coupling with a high-sensitivity, low-noise detector creates a system (Figure 1) capable of measuring high-quality datasets while minimizing data deterioration through radiation damage.
An example will be presented comparing data from the newly developed solution with data obtained using a 'classical' rotating anode-imaging plate combination. Special attention will be paid on the X-ray dose the investigated sample receives during the data collection.

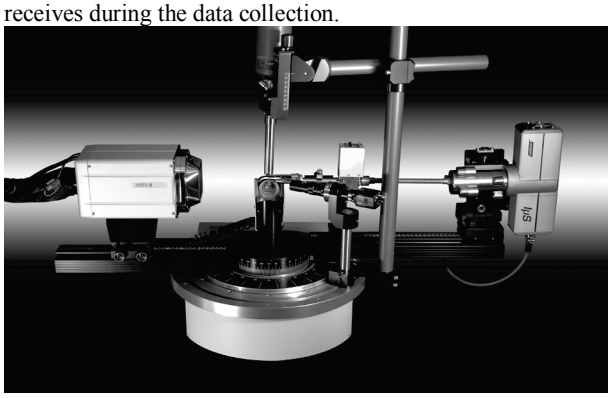

Figure 1: In-house system for X-ray dose minimization: X8 PROSPECTOR

Keywords: X-ray dose, microfocus source, crystal damage

FA1-MS9-P05

Crystal structure of $SppB_{TK}$, a putative signal peptide peptidase from *Thermococcus kodakaraensis.*
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Proteins secreted by the Sec-dependent pathway contain N-terminal signal peptides that are cleaved by signal peptidases following transport. The remnant signal peptides are then degraded by membrane-bound signal peptide peptidases (Spp). The crystal structure of the soluble domain of SppA from *E. coli* ($SppA_{EC}$, 67 kDa) showed that this protein consists of two domains with nearly identical structures, which assemble into a tetrameric ring [1]. *Thermococcus kodakaraensis* is a hyperthermophilic archaeon that possesses a Spp gene ($SppA_{TK}$) with approximately half the size of $SppA_{EC}$ (36 kDa) and is most homologous to the C-terminal half of $SppA_{EC}$ [2]. In addition, it also possesses another putative Spp gene (Tk0130), encoding a protein ($SppB_{TK}$) with 18% homology to $SppA_{TK}$. Biochemical data suggest that this protein functions as a signal peptide peptidase. Here, we present the crystal structure of the soluble domain of $SppB_{TK}$ in the free and substrate-bound forms. $SppB_{TK}$ structure is homologous to ATP-dependent protease ClpP and the C-terminal half of $SppA_{EC}$. It is an oligomeric protease assembled into an octameric ring. The active site of $SppB_{TK}$ consists of Ser_{130}-His_{226}-Asp_{154} triad, different from the Ser-Lys dyad of $SppA_{TK}$ and $SppA_{EC}$. Co-crystallization of S130A-$SppB_{TK}$ with a tetrapeptide substrate revealed the substrate binding mechanism of the protein. Based on these results, we discuss about the possible role of $SppB_{TK}$ in signal peptide degradation in archaea.

[1] Kim A.C., Oliver, D.C., Paetzel, M., *J. Mol. Biol.*, 2007, 376, 352.
[2] Matsumi R., Atomi H., Imanaka T., *J. Bacteriol.* 2005, 187, 7072.

Keywords: signal peptide peptidase, oligomeric proteases, structure-function proteases

FA1-MS11-P06

Crystal structure of AsaP1 metalloendopeptidase in complex with its propeptide. Xenia Bogdanović[a], Rajesh K. Singh[a*], Johanna Hentschke[c], Bjarnheidur K. Gudmundsdóttir[c], Winfried Hinrichs[a]. [a]*Institute for Biochemistry, Ernst-Moritz-Arndt University Greifswald, Germany* [b]*Institute for Experimental Pathology, University of Iceland, Iceland.*
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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.