(DESY) control system, MxCuBE (ESRF) and EDNA (www.edna-site.org).

Keywords: Synchrotron beamlines, instrumentation, microcrystals

FA1-MS01-P06

Structural biology of the nogalamycine biosynthetic pathway. Magnus Claesson, Gunter Schneider. *Mol. Struct. Biol., Karolinska Institutet, Sweden.*
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Anthracyclines are polyketide antibiotics with a high cytostatic potency and are used in the treatment of a wide range of tumor types. The usage is however limited by a cumulative cardiotoxicity, giving rise to severe side effects during and after treatment. There is therefore a need for new polyketide antibiotics, with reduced toxicity and maintained therapeutic effect.
As part of an on-going effort in the laboratory to characterize the biosynthetic pathways of anthracyclines, five genes encoding glycosyltransferases from two *Streptomyces* species have been cloned. One of these was successfully produced in soluble form and purified to homogeneity. Crystallization screening yielded crystals at several conditions and one of these conditions was scaled up, providing one data quality crystal from which data was collected. Crystal reproduction was optimized, however due to size limitations of the crystals no additional data quality crystals could be obtained. Reductive methylation was successfully utilized to improve crystal quality and reproducibility. Methylation appears not to have given rise to any new crystal contacts or changes in crystal packing, and the crystals were obtained in roughly the same conditions. The beneficial, but rather elusive, effect of the methylation seems to be a reduction in protein diversity, where the chemical treatment causes instable protein to aggregate. Furthermore the remaining protein is less likely to aggregate over time and seems less prone to excessive nucleation as compared with the native protein. The structure of this glycosyltransferase has been determined to 2.7Å in complex with a ligand, using molecular replacement.

Keywords: glycosyltransferases, natural products, X-ray macromolecular structure

FA1-MS01-P07

Facilities for Structural Biology and Chemical Crystallography at Diamond. Michael Engel, Elizabeth J. Shotton. *Diamond Light Source Ltd, Harwell Science & Innovation Campus, Didcot, Oxfordshire, OX11 0DE, UK.*
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Diamond Light Source was opened in October 2007. Phase I construction comprised the building, the machine and the first seven experimental stations. As part of Phase I, three macromolecular crystallography beamlines I02, I03 and I04 were built. The three beamlines will be tunable over the wavelength range 0.5 - 2.5 Å, to enable MAD experiments to be carried out with all three beamlines optimised for performance around the Se K-edge. Robotic systems for automated sample handling and crystal centring, and software allowing automated data collection have led to highly efficient and productive beamlines.
Facilities for remote monitoring and beamline operation further enhance the performance, and the implementation of biological containment at category 3 level on beamline I03 will allow pathogens to be studied.
I24, the tunable microfocus macromolecular crystallography beamline, became operational in July 2008 as part of Phase II. With a beamsize of 5-30 microns it enables measurements on small crystals that are not possible on conventional beamlines, due to their small size or mosaicity. With its ability to mount SBS-format plates it also improves the screening of crystals for optimisation of crystallisation conditions.
Two beamlines for structural chemistry have also opened recently. I11 provides high resolution, high throughput powder diffraction and I19 is a small molecule crystallography beamline suitable for studying microcrystals and weakly diffracting samples as well as excited state systems.
Further crystallography beamlines include a fixed wavelength MX station (I04.1) for high throughput experiments (currently under commissioning with 25% user mode) and I23, a long wavelength MX beamline (currently in the design phase).

Keywords: structural chemistry and biology, X-ray crystallography, synchrotron X-ray diffraction

FA1-MS01-P08

Sulfolobus solfataricus Adenine Phosphoribosyl-transferase. Sune F. Husted[a], Kristine S. Jensen[b], Anne Mølgaard[a], Jens-Christian N. Poulsen[b], Anders Kadziola[a], Kaj Frank Jensen[b]. *[a]Department of Chemistry and [b]Department of Biology, University of Copenhagen, Denmark.*
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Phosphoribosyltransferases (PRTases) are a group of enzymes that catalyze the formation of nucleotide 5´-monophosphates as essential precursors in the synthesis DNA or RNA. They all use a common substrate, 5-phosphoribosyl-α-1-pyrophosphate (PRPP), and transfer nucleobases to C1 of the ribose 5´-phosphate moiety of PRPP to form nucleotide 5´-monophosphates under the release pyrophosphate ($P_2O_7^{4-}$). PRTases are involved in both the *de novo* biosynthesis of nucleotides, e.g. orotate PRTase (OPRTase) and in the salvage path-ways, e.g. uracil PRTase (UPRTase), adenine PRTase (APRTase) and hypoxantine-guanine-xanthine PRTases (HGXPRTases; often with mixed specificity). PRTases share a common domain fold (type 1) which defines the specificity for PRPP and a variable domain for recognition of the various nucleobases.
Adenine PRTase structures are known for eukaryotes and bacteria and details of their active sites have been mapped out. APRTase from the thermophile archaean *Sulfolobus solfataricus* is an enzyme with unexpected properties: The substrate binding order is reversed with adenine binding first followed by PRPP. It has a double pH optimum and is potently inhibited by AMP and ADP. Sequentially it does not resemble other APRTases but merely HGXPRTases from eukaryotes, bacteria and archaea. The closest sequence homologues in PDB used for molecular replacement share about 31 % sequence identity for a 143/210 residue stretch of the sequence. The remaining sequence does not resemble any known structure. *Ss*APRTase is to our knowledge the first archaean APRTase to be structurally characterized.

We will present dimeric $P6_1$ structures of "apo" *Ss*APRTase (with SO_4^{2-}) together with the complexes *Ss*APRTase:AMP (product) and *Ss*APRTase:ADP (inhibitor) based on ESRF (Grenoble) synchrotron data to about 2.4 Å resolution. The current work concentrates on obtaining a substrate analog complex with *Ss*APRTase. Large prisms have been obtained by co-crystallization and a $P2_12_12_1$ native data set to 2.0 Å has been recorded at the MAX-lab synchrotron (Lund). The substrate complex is not an easy molecular replacement task.

Keywords: adenine, phosphoribosyltransferase, archaea

FA1-MS01-P09

Crystal Direct : A new system for automatic crystal mounting and diffraction analysis. Florent Cipriani, José A. Márquez. *European Molecular Biology Laboratory, Grenoble Outstation, France.*
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The introduction of automated crystallization and data collection systems has had a significant impact in structural biology both by helping speed up the process of solving structures and enabling very challenging projects. At the same time this has resulted in a significant increase in the number of crystallization experiments performed and the number of crystals tested. However, recovering crystals from crystallization plates and mounting them on the supports used at data collection stations remains still a manual operation. Attempts to automate this step by emulating the manual mounting process with the use micromanipulators have been made, however this still requires significant human intervention. We have recently developed a new concept for protein crystallization and crystal recovery. This system is based on a new crystallization support that can be excised to recover the crystalline material. This system is also compatible with standard cryoprotection and other sample manipulations allowing X-ray diffraction measurements both at RT and under cryocooling conditions. Among the advantages of this new approach is the absence of mechanical stress for the crystals during the mounting process and that it facilitates handling of microcrystals, which can be challenging with standard methods. Moreover this system is designed to enable full automation of the crystal mounting process. The Crystal Direct system will be presented along with examples from a number of samples analyzed so far.

Keywords: Biological-Crystallography, High-througput-Crystallization, Automation, Crystal-mounting, Data-collection

FA1-MS01-P10

Incorporation of Extra-domain B into Fibronectin mediates local dimerization. André Schiefner, Michaela Gebauer, and Arne Skerra, *Department of Biological Chemistry, Technical University Munich, Emil-Erlenmeyer-Forum 5, 85350 Freising-Weihenstephan,Germany*
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Fibronectin (Fn) is an abundant and ubiquitous component of the extracellular matrix (ECM). It mediates a wide variety of cellular interactions, which are essential for cell adhesion, migration, proliferation, and differentiation. Many different cell lines synthesize and secrete Fn as a large glycoprotein consisting of repeating Fn type I, II, and III domains [1]. These domains comprise functional units that mediate interactions with other ECM components such as collagen and heparin, with cell surface receptors such as integrins, and with Fn itself. Fn is encoded by a single gene, which is spliced and expressed in a tissue-specific and developmental stage-dependent manner. The most prominent splice variants are observed in the type III set of domain repeats, termed EDA, EDB, and IIICS. While IIICS and EDA appear to be associated with Fn dimer secretion and cell differentiation, respectively, the function of EDB remains elusive. EDB gets incorporated between the domains Fn7 and Fn8 during embryogenesis, wound healing, and neoplastic vascularization but is absent in normal adult tissue. Thus, its presence serves as a marker for tumorigenesis that can be targeted by antibody fragments, which are currently subject to clinical studies [2]. In order to investigate the role of EDB on Fn structure, we determined the crystal structure of the four-domain Fn fragment Fn7-EDB-Fn8-Fn9 at 2.4 Å resolution. As previously described for Fn7-Fn8-Fn9-Fn10 [3], the individual FnIII domains are assembled in an extended rod-like structure. However, the relative orientations of Fn7-EDB and EDB-Fn8 are significantly different compared to Fn7-Fn8 in Fn7-Fn8-Fn9-Fn10. Unexpectedly and in contrast to the Fn7-Fn8-Fn9-Fn10 structure, Fn7-EDB-Fn8-Fn9 shows an, extended, anti-parallel contact between two Fn molecules in the crystal packing. This interaction is dominated by H-bonds and electrostatics with a total buried surface area of 1800 $Å^2$ for each molecule. Dimerization of Fn7-EDB-Fn8-Fn9 has been confirmed by size exclusion chromatography. Thus, our data suggest that the incorporation of EDB influences the affinity of Fn molecules for each other, which likely affects Fn mediated cell-cell adhesion.

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Keywords: fibronectin, alternative splicing, crystal structure

FA1-MS01-P11

PSPF - A protein sample production facility for structural biologists Anja Schütz[a], Joop van den Heuvel[b], Volker Jäger[b], Konrad Büssow[b], Dirk Heinz[b] and Udo Heinemann[a] [a]*Max Delbrück Center for Molecular Medicine, Berlin,German,* [b]*Helmholtz Centre for Infection Research, Braunschweig, Germany*
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The Protein Sample Production Facility (PSPF) was established in 2007 as the result of an international review of the major bottlenecks and needs for the structural biologists within the Helmholtz Association. The platform is dedicated to develop and provide infrastructure for the production of biomacromolecules for structural biology research. The decentralised facility is shared between the Helmholtz Centre for Infection Research (HZI) in Braunschweig and the Max-Delbrück-Centre (MDC) in Berlin. Since 2010 the PSPF is additionally integrated within the ESFRI project as an