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.*
E-mail: marquez@embl.fr

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*
E-mail: schiefner@wzw.tum.de

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*
E-mail: anja.schuetz@mdc-berlin.de
Joop.vandenHeuvel@helmholtz-hzi.de

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

INSTRUCT associate centre in protein production, providing infrastructure and expertise in the field of protein sample production for the whole European structural biology community.
The PSPF dedicates fifty percent of its resources to cooperation and offers services for researchers at academic institutions to produce proteins for subsequent structural analysis by X-ray crystallography, NMR spectroscopy and electron microscopy. The PSPF also trains external researches in its laboratories.
Core activity of the PSPF at the MDC is high-throughput protein production in *E. coli* including construct design, cloning, expression/solubility testing, protein purification, crystallisation, and structure determination. At the HZI, large scale protein sample production in eukaryotic expression systems such as yeast, baculovirus/insect cells and mammalian cells is performed, including labelled proteins. Furthermore, a junior research group is focussing on the development of new and fast strategies for creating stable mammalian expression cell lines.
The PSPF currently accepts the submission of research proposals (http://www.pspf.de). All projects will be reviewed by a scientific committee prior acceptance.

Keywords: facility, protein production, structure determination

FA1-MS01-P12

Mechanistic studies on ubiquitin chain formation by Triad1. Judith J. Smit[1], R. Hibbert[a], J.A. Marteijn[b], B.A. van der Reijden[b], Titia K. Sixma[a]. [a]*Division of Biochemistry, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands.m*[b]*Division of Hematology, UMC St Radboud Centraal, 6500 HB Nijmegen, The Netherlands.*
Email: j.smit@nki.nl

Ubiquitination is a posttranslational modification that targets proteins for degradation or translocation in the cell. The ubiquitin pathway consists of a cascade of an E1, E2 and E3 enzyme that mediate the transfer of ubiquitin towards a target. E3 enzymes form the bridge between the E2-ubiquitin and the target protein. RING domain containing E3s mediate the transfer of the ubiquitin to the target by bringing the target and the E2-ubiquitin in close proximity. We study the E3 ligase Triad1, which is a homologue of the E3 ligase Parkin. These proteins belong to the TRIAD family of E3 ligases, containing two RING fingers and an In-between RING (IBR) domain that are responsible for the E3 function. The RING domains interact with different E2 enzymes, resulting in the formation of different types of ubiquitin chains which mediate different functions. The present study aims to unravel how the TRIAD domain forms a functional unit for ubiquitin chain formation.
We use crystallography and biophysical methods to study the structural and functional properties of Triad1, which has its different possible catalytic domains spread over the two RING domains and the IBR.
Triad1 was expressed in the baculovirus expression system. The purified E3 ligase is active *in vitro* with a subset of the E2 enzymes. We can follow the *in vitro* formation of free ubiquitin chains and, in addition, the mono-ubiquitination of P53, which is most likely an *in vivo* target for Triad1. Interestingly, the two separate activities have different E2 specificity. Surface Plasmon Resonance and analytical gel-filtration assays have shown that Triad1 interacts strongly with the E2 Ubc13 and with lower affinities to the other active E2s. The purified protein from insect cells crystallizes into 200μm crystals. Nevertheless, these crystals show poor resolution diffraction that needs to be optimized for data collection. We will discuss our investigations of the contributions of the different domains of Triad1 to its ubiquitin ligase activity, and its specificity for subsets of E2 enzymes.

Keywords: ubiquitin system, protein biochemistry, protein crystallography

FA1-MS01-P13

Recent activity of neutron diffractometers for biological crystallography at Japan Atomic Energy Agency. Taro Tamada, Kazuo Kurihara, Takashi Ohhara, Nobuo Okazaki, Ryota Kuroki. *Quantum Beam Science Directorate, Japan Atomic Energy Agency, Japan.*
E-mail: tamada.taro@jaea.go.jp

Neutron crystallography, which is a powerful technique for locating hydrogen atoms, enables us to obtain accurate atomic positions within proteins. Neutron diffraction data can provide information of the location of hydrogen atoms to the structural information determined by X-ray crystallography. There are two neutron diffractometers for biological crystallography (BIX-3 and BIX-4) installed at research reactor (JRR-3) in Japan Atomic Energy Agency (JAEA), which have been contributed to 15 structure analyses of biological macromolecules. Here, we report the recent activities for developments of the neutron diffractometers and the structure analysis of drug-target proteins.

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Keywords: Neutron diffraction, hydrogen bonds, X-ray neutron structure

FA1-MS01-P14

Facilities for Macromolecular Crystallography at BESSY II. Manfred. W. Weiss, Martin Bommer, Nora Darowski, Ronald Förster, Michael Hellmig, Michael Krug, Karthik Paithankar, Sandra Pühringer, Uwe Mueller. *Helmholtz-Zentrum Berlin für Materialien und Energie, Institute F-I2, Macromolecular Crystallography, Albert-Einstein-Str. 15, D-12489 Berlin, Germany.*
E-mail: msweiss@helmholtz-berlin.de

The Macromolecular Crystallography (MX) group at the Helmholtz-Zentrum Berlin (HZB) has been in operation since 2003. Since then, three state-of-the-art synchrotron beam lines (BL14.1-3) for MX have been built up on a 7T-wavelength shifter source [1]. Currently, the three beam lines represent the most productive MX-stations in Germany. BL14.1 and 14.2 are energy tunable in the range 5.5-15.5 keV, while BL14.3 is a fixed-energy side station (13.8 keV). All three beam lines are equipped with CCD-detectors. Beam lines BL 14.1 and BL 14.2 are in regular user operation providing about 200 beam days per year and about 600 user shifts to approximately 50 research groups across Europe. BL14.3 is currently used as a