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.

[1] Adachi, M., et al., *Proc. Nati. Acad. Sci. USA* 106, 2009, 4641. [2] Tamada, T., et al., *J. Am. Chem. Soc.* 131, 2009, 11033.

**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

test facility but will be put back into regular user mode operation shortly. BL 14.1 has recently been upgraded with an MD2-microdiffractometer including a kappa-geometry option and an automated sample changer. Additional user facilities include office space adjacent to the beam lines, a sample preparation laboratory, a biology laboratory (safety level 1) and high-end computing resources. On the poster, a summary on the experimental possibilities of the beam lines and the provided ancillary equipment for the user community will be given.

[1] Heinemann U., Büssow K., Mueller, U. & Umbach, P. (2003). *Acc. Chem. Res.* 36, 157-163.

**Keywords: Macromolecular Crystallography, Synchrotron Radiation, Beam Line**

## FA1-MS01-P15

### Crystal Structure of Dichloridobis[5-nitro-1-trimethylsilylmethyl-1H-benzimidazole-κN$^3$] cobalt (II) N,N-dimethylformamide solvate.

Şerife Pınar Yalçın[a*], Mehmet Akkurt[b], Nihat Şireci[c],Hasan Küçükbay[c], M. Nawaz Tahir[d]. [a]*Department of Physics, Faculty of Arts and Sciences, Harran University, 63300, Şanlıurfa, Turkey.* [b]*Department of Physics, Faculty of Arts and Sciences, Erciyes University, 38039,Kayseri, Turkey.* [c]*Department of Chemistry, Faculty of Arts and Sciences, İnönü University, 44280 Malatya, Turkey.* [d]*Department of Physics,University of Sargodha, Sargodha, Pakistan.*
E-mail: serifeyalcin@harran.edu.tr

Benzimidazole compounds are imported because of their metal complexes and their versatile properties such as biological activities and catalytic activities of their metal complexes in many organic syntheses [1]. We were investigate Crystal structure of benzimidazole compounds due to this important feature by single crystal X-ray diffraction.
Benzimidazole is a heterocyclic aromatic organic compound. This bicyclic compound consists of the fusion of benzene and imidazole.
The title compound, $[CoCl_2(C_{11}H_{15}N_3O_2Si)_2].C_3H_7NO$, was synthesized from 5-nitro-1-trimethylsilylmethyl-1H-benzimidazole and cobalt(II) chloride in dimethylformamide. The $Co^{II}$ atom is coordinated in a distorted tetrahedral environment by two Cl atoms and two N atoms. In the crystal structure, there are a number of C—H...Cl and C—H...O hydrogen-bonding interactions between symmetry-related molecules.

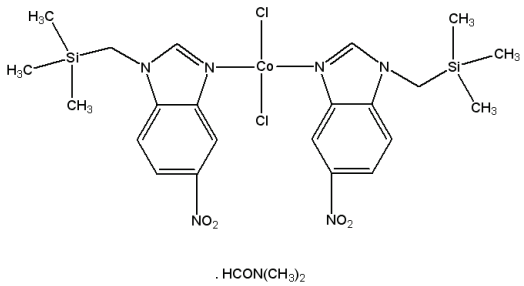


**Fig 1**: The molecular scheme of the title compound.

Using Stoe IPDS II diffractometer system, it was found that crystal system of $[CoCl_2(C_{11}H_{15}N_3O_2Si)_2]$ is triclinic, space group P-1, a = 9.8982(4)Å, b = 11.6936(5)Å, c = 15.9293(6)Å, α = 106.041(2)°, β = 107.408(2)°, γ = 99.040(3)°, Z = 2, D = 1.428 $M.gm^{-3}$ , μ = 0.81 $mm^{-1}$, R = 0.053, $wR(F^2)$ = 0.123, S = 1.02.
Crystal structure were solved by direct methods using Shelxl 97 Sir97. A refinement was carried out by the full–matrix least–squares method using Shelxl 97. For molecular graphics, ORTEP-3 for Windows [2] (Farrugia, 1997) and PLATON program was used.

[1] Çetinkaya, B.; Çetinkaya, E.; Küçükbay, H.; Durmaz, R.,Arzneim.Forsch. Drug Res., (1996), 46, 1154–1158. [2] Farrugia, L. J. J. Appl. Cryst. (1999), 32, 837–838.

**Keywords: Benzimidazole, Single Crystal X-ray Study, N,N-dimethylformamide**

## FA1-MS01-P16

### MAIN 2010: finalizing the structure by validation driven structure improvement

Martin Turk and Dusan Turk *Department of Biochemistry and Molecular and Structural Biology Jožef Stefan Institute, Ljubljana,*
E-mail: Dusan.Turk@ijs.si

At the final stages of crystal structure determination conformations of side chains, peptide bond orientations considering electron density maps as well as hydrogen bonding networks and electrostatic stability and packing of conformations need to considered before the structure can be considered final and ready for deposition in PDB.
In MAIN a procedure has been developed for automated improvements and completion of the structure. The procedure includes side chain and peptide bond density fitting combined with flipping in a combinatorial manner. At first the current state of the model, termed starting model, is validated towards density maps. Dead end elimination, exhausted, rotational search is used to fit atoms into electron density maps followed by the energy minimization. Next side chains of branched residues ILE, VAL, THR and LEU are flipped and adjusted to density by fragment and side chain fitting, each followed by minimization. Each state is validated and compared to the starting model. When local improvement is achieved, the geometry of the modified part replaces that of the starting model. A similar procedure considering peptide bond orientation follows. After an optimal fit to density maps is achieved combinatorial search considering packing of short range (below 4A) electrostatic and vdw interactions as well as hydrogen bond network is considered. To enable this explicit hydrogens are used. Side chain and residue flipping is at this stage applied to electrostatically asymmetric residues HIS, ASN, GLN, and solvent molecules in a combinatorial manner. Each of the states is saved together with their packing energy. The lowest energy state is at the end of procedure transferred to the working model, which is again energetically minimized - as always using real space refinement procedure. The structure can then be refined against crystallographic targets and the cycle repeated unless the structure is considered done.

**Keywords: Macromolecular Crystallography, Structure Refinement, Validation, Automated**