

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 researchers 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. Smit¹, R. Hibbert^a, J.A. Martejjn^b, B.A. van der Reijden^b, Titia K. Sixma^a. ^a*Division of Biochemistry, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands.* ^b*Division of Hematology, UMC St Radboud Centraal, 6500 HB Nijmegen, The Netherlands.*

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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

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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.*

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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. Natl. 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

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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-12, Macromolecular Crystallography, Albert-Einstein-Str. 15, D-12489 Berlin, Germany.*

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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