s1.m8.p26 **X-ray structures of Aldose Reductase-inhibitor complexes at 0.9 Å resolution from LN2-cryocooled crystals measured at 10 K.** A. Mitschler[a], S. Ginell[b], T. Petrova[a], I. Hazemann[a], A.Cousido[a], F. Ruiza, M. Van Zandt[c], A. Joachimiak[b] and A. Podjarny[a], [a]*IGBMC, 1 rue Laurent Fries, Illkirch, France.* [b]*Bioscience Division, Structural Biology Center and Midwest Center for Structural Genomics, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439, USA.* [c]*IDD, 23 Business Drive, Branford, Connecticut, USA. E-mail: mitschle@titus.u-strasbg.fr*

Keywords: Protein Crystallography; Helium temperatures; Enzymology

Human Aldose Reductase (AR, MW= 36 kDa, an enzyme in the polyol pathway from the aldo-ketoreductase family, is implied in diabetic complications. Its ternary complexes with the inhibitors IDD-676, IDD-860 and IDD-594 (both hydrogenated and fully deuterated) have been measured at 10 K with an open-flow helium cryosystem at APS-SBC-Argonne National Labs in order to validate within the active site the expected dynamic disorder decrease by lowering the temperature from 100K (Liquid Nitrogen gas stream) down to 10 K (Helium gas stream). The results show a decrease of the temperature factors in the most ordered regions, confirming that the dynamic disorder diminishes with lowering temperature. Furthermore, the protonation states of the catalytic residues are clearly seen and a double conformation of the inhibitor's carboxylate head appears for the case of the complex with IDD-676. This double conformation, which is clearly identified in the 10 K structure while it appears smeared in the 100 K one, has important consequences for the inhibition and enzymatic mechanisms.

s1.m8.p27 **Structure of the PB1 domain of NBR1 and complex formation with the PB1 domain of p62.** S. Müller, I. Kursula and M. Wilmanns, *EMBL, Notkestr. 85, 22603 Hamburg, Germany. E-mail: simone.mueller@embl-hamburg.de*

Keywords: NBR1; PB1 domain; Protein-protein interaction

Specific protein-protein interactions are pivotal in regulation of signal transduction. Various interaction domains like SH2, SH3, PX, WW and PDZ domains have been investigated intensively. The PB1 domain (Phox and Bem1p) is a recently discovered interaction domain [1]. The OPCA motif (according to the OPR, PC and AID motif) within a PB1 domain interacts with its acidic hairpin to the basic back of another PB1 domain [2]. The PB1 domains are accordingly classified into 3 types: A-type (with OPCA motif), B-type (basic back with Lysine) and AB-type (with both OPCA motif and basic back Lysine to form front-to-back interactions) [2]. We have determined the 1.55 Å resolution crystal structure of the OPCA-motif containing PB1 domain of NBR1 (next to breast cancer 1). It shows the typical ubiquitin-like β grasp fold as shown for PB1 domains of Bem1p [3], Cdc24 [4], p40phox and p67phox [5], similar to that found in several Ras-GTP-binding domain families. The scaffold protein p62 is a ligand of NBR1, also presenting a multidomain protein with a similar domain architecture as NBR1. Their N-terminal PB1 domains are responsible for interaction [6]. The complex formation of the PB1 domains of NBR1 with p62 has been investigated and a high affinity in the nM range is proposed. At present, we are in the process to crystallise the PB1 heterodimer of NBR1/p62.

[1] Ito *et al. EMBO J.* **20**, 3938 (2001)
[2] Noda *et al. JBC* **278**, 43516 (2003)
[3] Terasawa *et al. EMBO J.* **20**, 3947 (2001)
[4] Yoshinaga *et al. EMBO J.* **22**, 4888 (2003)
[5] Wilson *et al. Mol. Cell* **12**, 39 (2003)
[6] Lamarck *et al. JBC* **278**, 34568 (2003)