The Hpy99I-DNA co-crystal structure provides the first detailed illustration of the e active site in restriction endonucleases and complements structural information on the use of this active site motif in other groups of enzymes such as homing endonucleases (e.g. I-PpoI) and Holliday junction resolvases (e.g. T4 endonuclease VII).

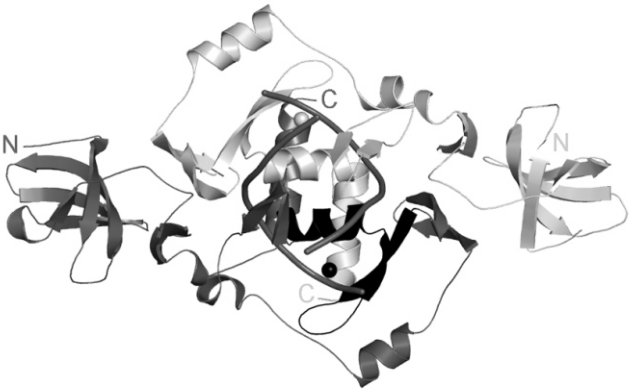

Figure 1. Crystal structure of the -Me type II restriction endonuclease Hpy99I in complex with DNA. One protomer of a dimer is shown in dark grey, the other is presented in light grey. The -Me motif of the first protomer is depicted in black. DNA is shown in dark grey.

Friday Evening Session V - September 25

L16

TURNING THE BAD INTO GOOD: A RATIONAL APPROACH TO CRYSTAL QUALITY IMPROVEMENT

Astrid Rau, Jana Müller, Klaus Korus, Christin Reuter, Thomas Billert and Mathias Gruen

Jena Bioscience GmbH, Loebstedter Strasse 80, 07749 Jena, Germany

mathias.gruen@jenabioscience.com

Structural genomics projects demonstrate that about half of all crystallized proteins cannot be optimized to form suitable crystals for structure determination - which translates into roughly 15% of all proteins [1,2]. While a thorough optimization of initial crystals of a particular protein is antagonistic to the high throughput idea of structural genomics projects the situation is different in most academic or industrial protein crystallization labs. After obtaining initial crystals usually great effort is invested into optimizing these "hits" into well-diffracting crystals. However, this optimization very often focuses mainly on fine-tuning of the chemical composition of the crystallization buffer (i.e. grid-screening of pH, precipitants, additives) or simply on using a large number of commercially available crystal screens. While this yields satisfactory results in some cases it does not in many others.

For optimization of initial hits one may in principle deal with either the crystallization experiment itself or - very often neglected - with the input-protein sample. In the crystallization experiment it is - alongside fine-tuning the chemistry - mainly optimization of physical/thermodynamic parameters (method/instrumentation for crystallization, temperature). In the input-protein sample it is either dealing with an already existing protein preparation (provided there is sufficient material available) or making new (better) protein by optimizing its sequence or changing the method of protein production.

In this year's HEC-Meeting we present three approaches that deal with the crystallization experiment as well as three strategies that aim at improving the input-protein sample. We show the pH-2D screen [3] that allows fine-tuning of pH from 4.0…10.0 with only one buffer system and therefore, eliminates undesired matrix effects inevitably occurring when conventional buffers are used. We discuss Hofmeister's original concept of kosmotropic and chaotropic small molecules in the context of protein crystallization [4,5] and we present a microfluidic Crystal Former^{TM} device with unique equilibration kinetics.

Regarding the input-protein, we elucidate the ready-to-use Choppy-Floppy Kit for in-situ proteolysis targeting proteins containing floppy regions or tags [2]. For cases in which the existing protein preparation does not yield sufficient material for manipulation we discuss strategies for creation of protein variants by random mutagenesis that may be more prone to crystallization [6, and references there]. Finally, we highlight the protein production platform LEXSY which is a eukaryotic protein expression system that is handled similar to *E. coli* [7,8]. By using the example of glycosylation we demonstrate that LEXSY yields highly homogenous protein preparations which is a prerequisite for successful crystallization. We discuss two structures that were determined from LEXSY-derived proteins – one by the means of X-ray crystallography and the other by NMR [9,10]. Most recently a version of LEXSY has been developed that allows cell-free *in vitro* production of up to several mg of any protein within hours [11] which will be available to the research community in late 2009.

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L17

REFOLDING OF ECTO-NUCLEOTIDASES

K. Yates, N. Sträter

Biotechnologisch-Biomedizinisches Zentrum, Fakultät für Chemie und Mineralogie, Universität Leipzig, Germany, karen.yates@bbz.uni-leipzig.de

In addition to its important cellular functions as an energy carrier, ATP also serves as an extracellular signalling substance. It acts on P2X and P2Y receptors. These signalling pathways via ATP and other nucleotides are termed purinergic signalling. Extracellular nucleotides influence a wide variety of physiological processes, including exocrine and endocrine secretion, immune responses, inflammation, platelet aggregation, endothelial-mediated vasodilatation as well as cell proliferation, differentiation, migration and death in development, regeneration and cancer. Extracellular nucleotidases are involved in the hydrolysis of the nucleotides, including the NTPDases which dephosphorylate ATP via ADP to AMP and the 5'-nucleotidases, which catalyze the hydrolysis of AMP to adenosine (Fig. 1).

The aim of our work is to study the structure and function of ecto-nucleotidases in purinergic signalling. Most of these extracellular enzymes contain disulfide bridges and are expressed as inclusion bodies in *E.coli*. The resolubilised enzymes are purified via Ni-NTA affinity chromatography and optimal refolding conditions are determined by sparse matrix screens and systematic screens. A major problem is the separation of the correctly refolded enzyme from misfolded species via different chromatography techniques, such that a homogenous protein solution is obtained for crystallization. We also report on the use of the influence of different variants of the proteins on the refolding yield.

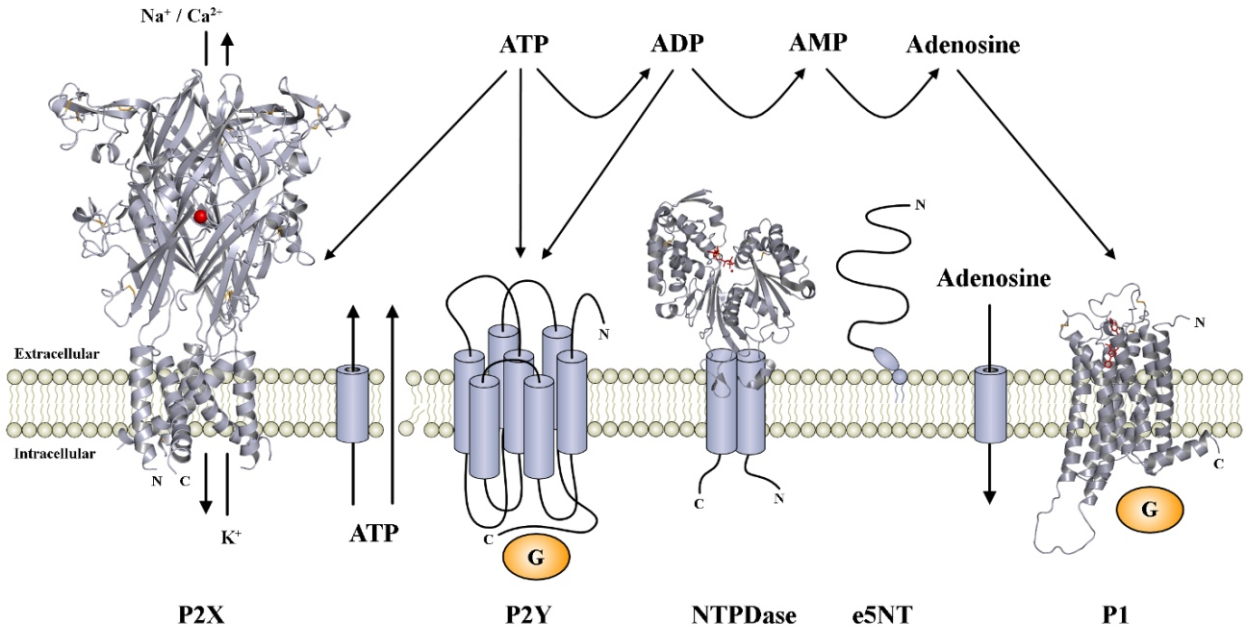

Figure 1. Overview of Purinergic Signalling. Extracellular ATP and adenosine act as signaling molecules binding to the P2 and P1 receptors respectively. The NTPDases (ecto-nucleoside triphosphate diphosphohydrolases) dephosphorylate ATP via ADP to AMP and the ecto-5'-nucleotidase (e5NT) catalyses the hydrolysis of AMP to adenosine.

L18

STRUCTURAL CHANGES OF PATHWAYS IN MUTATED DHAA PROTEINS FROM *Rhodococcus rhodochrous*

A. Stsiapanava[1], J. Dohnalek[3], M. Kuty[1,2], J. A. Gavira[4], T. Koudelakova[5], J. Damborsky[5] and I. Kuta Smatanova[1,2]

[1]*Institute of Physical Biology University of South Bohemia Ceske Budejovice, Zamek 136, 373 33 Nove Hrady, Czech Republic*
[2]*Institute of Systems Biology and Ecology Academy of Science of the Czech Republic, Zamek 136, 373 33 Nove Hrady, Czech Republic*
[3]*Institute of Macromolecular Chemistry AS CR, Heyrovskeho nam.2, 162 00, Prague 6, Czech Republic*
[4]*Laboratorio de Estudios Cristalografico, Edificio BIC-Granada, Avda. de la Innovacion 1, P.T. Ciencias de la Salud, 18100-Armilla, Granada, Spain*
[5]*Loschmidt Laboratories, Faculty of Science, Masaryk University, Kamenice 5/A4, 62500 Brno, Czech Republic*
stepanova@greentech.cz

Haloalkane dehalogenases (EC 3.8.1.5) are members of the -hydrolase fold family and catalyze hydrolytic conversion of a broad spectrum of hydrocarbons to the corresponding alcohols [1]. Besides a wide range of haloalkanes, DhaA can slowly convert serious industrial pollutant 1,2,3-trichloropropane (TCP) [2]. Three mutants marked as DhaA04, DhaA14 and DhaA15 were designed and constructed to study the relevance of the tunnels connecting the buried active site with the surrounding solvent for the enzymatic activity.

The three mutants of DhaA were crystallized using the sitting-drop vapor-diffusion technique [3]. Crystal growth conditions were optimized [4] and crystals were used for synchrotron diffraction measurements at the beamline X11 of the DORIS storage ring at the EMBL Hamburg Outstation. X-ray intensities data for DhaA04, DhaA14 and DhaA15 mutants were collected to a resolutions limit of 1.23 Å, 0.95 Å and 1.22 Å, respectively. Crystals of DhaA04 belong to the orthorhombic space group $P2_12_12_1$ while crystals of DhaA14 and DhaA15 mutants belong to the triclinic space group $P1$. The known structure of the haloalkane dehalogenase from *Rhodococcus species* (PDB code 1bn6) [5] was used as a template for the molecular replacement. In all three cases a solution was found comprising one molecule per asymmetric unit as expected from the Matthews number estimation.

Analyses of crystal structures of mutants allow determine of electron densities observed for the ligands. In the case of DhaA04 the ligand is benzoic acid. DhaA14 and DhaA15 proteins contain isopropanol in the active site cavity. Mutations in Dha04 and DhaA15 partially block the main tunnel and almost completely block small slot in DhaA14 and DhaA15 enzymes. These results suggest that key role in the substrate access and product egress in DhaA protein has main tunnel. The functions of blocked slot could perform main tunnel or other secondary tunnels of DhaA.

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L19

STRUCTURAL INSIGHTS INTO THE GIMAP FAMILY

D. Schwefel, C.Fröhlich, K. Köchert, S. Mathas, J. Behlke, O. Daumke

Max-Delbrück-Centrum for Molecular Medicine, Berlin-Buch
david.schwefel@mdc-berlin.de

Recently, a family of GTP-binding proteins with unique properties was described, termed GTPases of immunity associated proteins (GIMAP), which appear only in vertebrates and plants. There are 7 GIMAP family members in humans, consisting of an N-terminal GTP-binding domain, followed by predicted coiled-coil regions. Some contain a predicted C-terminal transmembrane helix. GIMAPs are predominantly expressed in tissues of the immune system, restricted to T and B cells. In several independent studies, GIMAPs have been shown to be differentially regulated during T cell selection events in the thymus. Functional investigations proved their involvement in T cell apoptosis regulation. A rat strain which is deficient in GIMAP5 due to a frameshift mutation shows severe loss of peripheral T cells (lymphopenia), concomitant with an increased number of apoptotic T cells. Most of these rats develop spontaneous autoimmune diabetes. GIMAP5 knock-out mice have a similar phenotype. GIMAP dysregulation was also detected in certain cancer cells. The scope of the project is to investigate the structure and cellular function of GIMAPs. Three family members (GIMAP2, 5 and 7) were succesfully expressed in bacteria, purified and biochemically characterized. The crystal structures of different GIMAP2 constructs were solved in the nucleotide-free, GDP- and GTP-bound state. The crystal structure of GDP-bound GIMAP5 was solved. The proteins show similarities to Ras-like small GTPases but exhibit a unique C-terminal alpha-helical amphipatic extension. There are also striking differences in the amino acid composition of the nucleotide-binding pocket when compared to Ras-like GTPases. The switch I and II regions show significant structural differences in different nucleotide loading states. At the moment, functional studies on GIMAPs are in progress. Thus we expect to gain new insights how GIMAPs are integrated in programmed cell death processes during lymphocyte development.

L20

X-RAY CRYSTALLOGRAPHIC SNAPSHOTS OF INTERMEDIATES IN TRANSALDOLASE

A. Lehwess-Litzmann[1], P. Neumann[2], C. Parthier[2], M.T. Stubbs[2], K. Tittmann[1]

[1]Department of Biology and Göttingen Center for Molecular Biosciences, Georg-August University Göttingen, Justus-von-Liebig-Weg11, 37077 Göttingen, Germany
[2]Institute of Biochemistry and Biotechnology, Martin Luther University Halle-Wittenberg, Kurt-Mothes Strasse 3, D-06120 Halle/Saale, Germany
alehwes@gwdg.de

Transaldolase is a key enzyme of the non-oxidative branch of the pentose phosphate pathway. As a member of the aldolase class I- family it utilises a Schiff-base forming lysine to transfer a dihydroxyacetone moiety from a ketose donor to an appropriate aldose acceptor. Transaldolase from *Thermplasma acidophilum (T.ac.)* belongs structurally to the group of the shorter transaldolase-like proteins such as fructose 6-phosphate aldolase (FSA) from *E. coli* [1]. In contrast to the latter, the *T. ac.* enzyme exhibits transaldolase activity. Transaldolase from *T. ac.* has been crystallised in two crystal forms containing either a decamer (akin to FSA) or a pentamer in the crystallographic asymmetric unit. We have co-crystallised transaldolase with different substrates and were able to trap different intermediates along the pathway including the Schiff-base intermediate of donor substrate fructose-6-phosphate covalently attached to the catalytic lysine, and the acceptor sugar erythrose-4-phosphate non-covalently bound in the substrate channel. In addition to residues previously suggested as being important for catalysis in *E. coli* transaldolase [2] we could identify an additional residue that seems to be important for conferring substrate specificity by forming a hydrogen bond with the C1-hydroxyl group of the donor sugar fructose-6-phosphate.

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