Student abstracts

P1

CRYSTALLIZATION AND INVESTIGATION OF THE STRUCTURE OF THE COMPLEXES OF ARCHAEAL TRANSLATION INITIATION FACTOR 2 WITH RNA

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In Eukarya and Archaea heterotrimeric translation initiation factor 2 (e/aIF2) plays a key role in the initiation of protein synthesis on the ribosome. e/aIF2 complexed with GTP delivers charged initiator methionyl-tRNA into the P site of the small ribosomal subunit; in eukaryotic cells this factor regulates translation initiation by its specific phosphorylation. We study the structure and functions of archaeal translation initiation factor 2 from *Sulfolobus solfataricus* (Sso-aIF2). These investigations are necessary for understanding mechanism of translation initiation both in Eukarya and Archaea.

Recently in our laboratory the crystals of full-sized heterotrimeric aIF2 was obtained and its spatial structure was determined, making it possible to reveal high conformational flexibility in the - and -subunits. At present the main goal of our investigation is to determine and analyze the structure of the ternary complex Met-$tRNA_i$*aIF2*GTP. We crystallized the complex Met-$tRNA_f$*SsoIF2 $_{\text{domain III}}$ *GDPNP. Currently determination of this complex structure is leaded. Also we continue crystallization of other variants of the ternary complex using -dimer and heterotrimeric aIF2.

Besides the delivering of $tRNA_i$ to the ribosome the archaeal translation initiation factor 2 binds to the 5'-triphosphate end of mRNA and protects its 5'-part from degradation. Using our tentative experimental data, we marked out the supposed area on the surface of the -subunit of aIF2 that binds 5'-end of mRNA. In order to check up these data and determine the area of binding mRNA on the protein, we carry out site-directed mutagenesis of the supposed area on the aIF2 . Also we try to get crystals of the complex of aIF2 with mRNA. We have already obtained microcrystals of this complex. And now we are searching the better conditions of its crystallization.

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P2

INITIAL STEPS IN ANTIGEN PRESENTATION OF IMMUNIGENIC PROTEINS

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Endosomal antigen degradation is one of the key subsystems of the adaptive immunity. Proteases residing within the endocytic compartments of professional antigen presenting cells (APCs) play essential role in two key steps: (i) they activate MHC class II molecules by proteolytic degradation chaperone called invariant chain and (ii) they degrade endocytosed proteins to short antigenic peptides that are bound to activated MHC class II molecules presented on the surface of professional APCs [1, 2]. It was suggested that a number of different lysosomal cysteine proteases are involved in these processes, however, their precise role still remain elusive [3].Our ultimate goal is to unravel the complexity of these processes at molecular level by in vitro reconstruction of conditions inside endosomes (proteolytic activity, redox potential, inhibitors), apply it to antigenic proteins and evaluate the process by testing the binding capability of generated antigenic peptides to MHC class II molecules. The starting steps in this process are (1) establishment of infrastructural pipeline for production and structural characterization of endosomal hydrolyses and immunogenic proteins from different pathogens and (2) development of assay for monitoring their proteolytic processing.Here we report the recently determined crystal structure of Autolysin E (AtlE), a cell wall degrading enzyme from Staphyloccocus aureus Mu50. Crystal structure reveals a heart-like globular fold with several distinct -helices surrounding the central lysozyme-like fold. The protein exhibits a high degree amino acid sequence homology to glucosaminidase domain of *S. aureus* major autolysin.

1. Honey K, Rudensky AY. "Lysosomal cysteine proteases regulate antigen presentation. " *Nat Rev. Immunol* **2003**, 3:472–82.
2. Chapman HA. Endosomal proteases in antigen presentation. *Curr. Opin. Immunol*., **2006**, 18: 78–84.
3. Turk B. et al. " Cysteine cathepsins in immune response". *Tissue antigens*, **2006,** Review article.

P3

CRYSTALLOGRAPHIC STUDIES OF THE STAS DOMAIN OF SULP ANION TRANSPORTERS

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The SulP (Sulfate Permease) family is a large and ubiquitous family of anion transporters, whose members are found in eubacteria, plants, fungi and mammals.

The human members of this family form the SLC26 family; these transporters play important roles in normal human physiology in different tissues and many of them are involved in genetic diseases.

The SulP proteins show a similar structural organisation: a highly conserved transmembrane central core and a less conserved cytoplasmic C-terminal portion, comprising a STAS domain. The name STAS (Sulfate Transporter and Anti-Sigma Factor antagonist) is due to a remote but significant sequence similarity with bacterial ASA (Anti-Sigma factor Antagonist) proteins [1].

The bacterial ASA proteins are functionally and structurally well characterised in their 3D structure both by NMR spectroscopy and X-ray crystallography. Unlike these proteins, the STAS domains present in anion transporters are poorly characterised in terms of both their function and structure. Despite the fact that their precise role is unclear, the STAS domains play a fundamental role in the function/regulation of SulP anion transporters.

The final aim of my research project is the structural characterisation of the STAS domains of SulP transporters; the structural information is then integrated with functional data in order to understand the role and the function of this domain.

Recently, in my group, a variant of the STAS domain of the mammal transporters SLC26A5 (prestin) was crystallised and its crystal structure was determined [2]. Prestin is the motor protein responsible for the somatic electromotility of cochlear outer hair cells and it is essential for normal hearing sensitivity and frequency selectivity of mammals [3]. Prestin STAS significantly deviates from the related bacterial ASA proteins, especially in the N-terminal region. A structure-function analysis suggests that prestin STAS model can supports the notion that STAS domains are involved in functionally important intramolecular and intermolecular interactions.

Now I am trying to crystallise other variants of the STAS domains.

1. L. Aravind, Eugene V. Koonin (2000) *The STAS domain- a link between anion transporters and antisigma-factor antagonists*. Current Biology Vol 10 n. 2.
2. E. Pasqualetto, R. Aiello, L. Gesiot, G. Bonetto, M. Bellanda, R. Battistutta (2010) *Structure of the cytosolic portion of the motor protein prestin and functional role of the STAS domain in SLC26/SulP anion transporters*. J. Mol. Biol.
3. P. Dallos and B. Fakler (2002) *Prestin, a new type of motor protein*. Nature Reviews Molecular Cell Biology Vol 3.

P4

INVESTIGATION INTO ORDERING PROPENSITIES OF INTRINSICALLY DISORDERED TAU PROTEIN BY CO-CRYSTALLIZATION WITH MONOCLONAL ANTIBODY

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Alzheimer's disease associated protein tau is an intrinsically disordered protein (IDP) with no preferred secondary and/or tertiary structure under native conditions in solution. It is difficult to obtain direct insight into atomic structure of IDP tau physiological conformations and/or its assembled form in Alzheimer's paired helical filaments as neither of them can be prepared in the form amenable to X-ray crystallography; whereas NMR technique could confer prevalently time-averaged structural data. It has been hypothesized that tau adopts a stable structures upon interaction with its cellular partners. Using this IDP tau property, we are proposing monoclonal antibodies as surrogate binding partners for protein tau to form complexes and crystals for structure solution by X-ray technique [1,2].

We succeeded in preparation and crystallization of such complexes of monoclonal antibody Fab fragments with tau peptides of various lengths derived from their antigenic structures. We ascertained the presence of tau peptides in crystals by mass spectrometry. Structure of complex could be solved by molecular replacement in most cases.

This work was supported by the Slovak Research and Development Agency under the contracts Nos. APVV-0471 -06, LPP-0038-09 and by the Slovak Grant Agency VEGA grants Nos. 2/0162/10, 2/0217/10.

1. Sevcik J., Skrabana R., Kontsekova E., Novak M. (2009) *Prot. Pept. Lett.* 16, 61-64.
2. Skrabana R., Dvorsky R., Sevcik J., Novak M., (2010) *J. Struct. Biol.* in press, doi:10.1016/j.jsb.2010.02.016.1.

P5

IMMUNE RESPONSE MODULATING PROTEINS FROM *Onchocerca volvulus*

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The most complex organisms to invade the human body are the multicellular helminths. The filarial parasite *O. volvulus* is the causative agent of human onchocerciasis or river blindness, a disease characterized by chronic skin and eye lesions. It is the world`s second leading infectious cause of blindness. Pathogenesis of filarial disease is caused mainly by host inflammatory and immune responses to the parasite and to microfilarial and adult worm antigens. In order to avoid an inflammatory environment which is most favorable to parasite survival, *O. volvulus* has generated a variety of strategies to evade and down-modulate the host´s immune system (Allen *et al.*, 2008), thereby also compromising its capability to deal with other infections.

Helminths are not equipped to outpace the immune system by rapid antigen variation, faster cell division or sequestration in specialized niches. Instead they rely on influencing and regulating the immune responses away from the mode most damaging to them (Maizels and Yazdanbakhsh, 2003; Maizels *et al.*, 2004). Here are several proteins are involved and include also homologs of mammalian immune system genes (Maizels *et al*., 2004). They are called excretory-secretory proteins (ESP's). However, proteases are common constituents of endolysosomal compartments and they have important roles in the immune response. Conversely many parasites use similar proteases to invade the host and evade, suppress or subvert its immune response. With regard to protease inhibitors expressed by pathogens, like onchocystatin (OV7) from *Onchocerca volvulus*, a wide range of immunoregulatory properties have been attributed to these molecules include the inhibition of antigen presentation, induction of IL-10 expression and macrophage stimulation, similar to the functions of host cystatins (Bird *et al.*, 2009). Previous research efforts coordinated by the "Oncho Task Force" identified a number of candidate proteins that have not been fully characterized, and it was postulated that a vaccine should contain proteins found in the ESPs or on the surface of larval stages (Lustigman *et al.*, 2002).

1. Allen JE, Adjei O, Bain O, Hoerauf A, Hoffmann WH, Makepeace BL, Schulz-Key H, Tanya VN, Trees, AJ, Wanji S, Taylor DW. (2008) PLoS Negl Trop Dis. 2:e217.
2. Bird PI, Trapani JA, Villadangos JA (2009) Nat Rev Immunol. 9:871-82.
3. Lustigman S, James ER, Tawe W, Abraham D. (2002) Trends Parasitol. 18:135-41.
4. Maizels RM, Yazdanbakhsh M. (2003) Nat Rev Immunol. 3:733-44.
5. Maizels RM, Balic A, Gomez-Escorbar N, Nair M, Taylor MD, Allen JE. (2004) Immunol Rev. 201:89- 116.

P6

DECONSTRUCTING HONEYBEE VITELLOGENIN: NOVEL 40 KDA FRAGMENT ASSIGNED TO ITS N-TERMINUS

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Vitellogenin, an egg-yolk protein precursor common to oviparous animals, is found abundantly in honeybee workers a caste of helpers that do not usually lay eggs. Instead, honeybee vitellogenin (180 kDa) participates in processes other than reproduction: it influences hormone signaling, food-related behavior, immunity, stress resistance and longevity. The molecular basis of these functions is largely unknown. We have tested properties of vitellogenin purified from two honeybee body compartments: abdominal fat body (the main site for vitellogenin synthesis and storage), and hemolymph (blood). We have examined the protein's fragmentation behavior, phosphorylation and glycosylation status. Our results reveal a novel 40 kDa vitellogenin fragment in abdominal fat body tissue. Using mass-spectroscopy, the 40 kDa fragment was assigned to the N-terminus of vitellogenin, while a previously observed 150 kDa fragment corresponded to the remainder of the protein. We found that both protein units were N-glycosylated and phosphorylated. Focusing on the novel 40 kDa fragment, we present a homology model of the N-terminal domain that visualizes a conserved -barrel-like shape, with two insect-specific loops and a lipophilic cavity in the interior. We suggest that this 40 kDa domain is required for vitellogenin to be secreted from the fat body, since only the intact 180 kDa protein form is detected in hemolymph.

P7

SEARCH FOR NEW ANTIVIRAL COMPOUNDS AGAINST HUMAN ENTEROVIRUSES USING FRAGMENT SCREENING METHODOLOGY

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Picornaviridae are among the most diverse and oldest "known" viral families that include many important pathogens of humans and animals. They are small, icosahedral ss+RNA viruses, causing a variety of diseases. Vaccines are available for PV, HAV and FMDV, but no effective prophylaxis is implemented for other picornaviruses. So far, anti-viral research has focused on the capsid, whereas inhibitors targeting non-structural proteins (i.e. proteases, helicases, polymerases) have remained largely unaddressed.

We are developing project focused on searching for novel antiviral compounds against human enteroviruses (HEV) via fragment screening methodology based on STD-NMR. The protein targets are picornaviridae RNA-dependent RNA polymerases, helicases and proteases.

Co-crystallization/soaking of the most successful STD hits with their protein target will be carried out to obtain their 3D structures by X-ray crystallography. The data provided by NMR and crystallography techniques will identify the close contacts between a fragment hit and a protein. It can help to infer the requirements underlying the association and suggest novel ligands by both fragment-growth and fragment-linking strategies. Consequently, new binders could be obtained that eventually will become leads for further development.

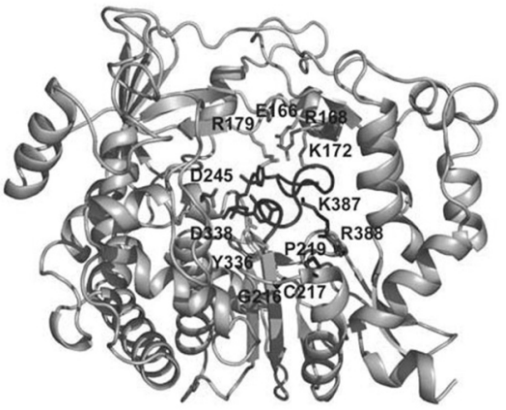

Figure 1: Structure of the FMDV polymerase bound to VPg [1].

1. Ferrer-Orta *et al. EMBO J.* 25 (2006) *880-888.*

P8

CRYSTALLIZATION AND MOLECULAR DYNAMIC STUDY OF HALOALKANE DEHALOGENASES

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Organohalides are integral to a variety of industrial applications, including use as solvents, pharmaceuticals, hydraulic and intermediates for chemical synthesis [1]. Distribution of halogenated organic compounds in the nature makes them one of the largest groups of the environmental pollutants. Haloalkane dehalogenases are bacterial enzymes enable to decontaminate these kind of pollutants [2]. These enzymes with the a/ß-hydrolase fold catalysing hydrolysis a broad range of chlorinated, brominated and iodinated hydrocarbons. The enzymes catalyze the hydrolysis of a carbon–halogen bond in organohalide substrates, producing a corresponding alcohol, a halide ion and a proton [3]. In order to design novel haloalkane dehalogenases that can catalyze the hydrolysis of recalcitrant environmental pollutants, it is necessary to find out the 3D-structure of these enzymes. To date, the crystal structures of different type of haloalkane dehalogenases like DhlA from *Xanthobacter autorophicu*s GJ10 [4], DhaA from *Rhodococcus rhodochrous* NCIMB 13064 [5] and LinB from *Sphingobium japonicum* UT26 [6] have been determined. To make the efficiency of enzymes higher experimentalist try to define new medium for enzymes [7]. It has been shawn that different organic solvents has different effect on dynamic and activity of the enzymes. To find out the behavior of proteins at the molecular level several molecular dynamics simulation was done. These enzymes are structural and functional homologue with a primary sequence identity of average 50%. Despite this fact, the results of molecular dynamic simulations show that different haloalkane dehalogenases exhibit a very different behavior in various solvents. Therefore a detailed analysis of the molecular interactions on the protein surface and in the solvent shell around the protein leads to a fundamental understanding and a generalization of the organic solvent effects on protein structure-dynamics-function. To confirm these results on a basis of the three- dimensional structure of DhaA, LINb and DbjA, we initiated crystal structure analysis of the enzymes in new situation.

1. Janssen, D. B., Pries, F. & van der Ploeg, J. R. (1994). *Annu. Rev. Microbiol.***48**, 163–191.
2 Yukari Sato,ab Ryo Natsume,b Masataka Tsuda,a Jiri Damborsky,c Yuji Nagata,a and Toshiya Senda, *Acta Crystallogr* Sect F Struct Biol Cryst Commun. 2007 April 1; 63(Pt 4): 294–296.
3 Kulakova, A. N., Larkin, M. J., Kulakov, L. A. (1997) *Microbiology* **143**, 109-115.
4. Verschueren, K. H., Seljee, F., Rozeboom, H. J., Kalk, K. H. & Dijkstra, B. W. (1993). *Nature (London)*, **363**, 693–698.
5. Newman, J., Peat, T. S., Richard, R., Kan, L., Swanson, P. E., Affholter, J. A., Holmes, I. H., Schindler, J. F., Unkefer, C. J. & Terwilliger T. C. (1999). *Biochemistry*, **38**, 16105–16114.
6. Marek, J., Vevodova, J., Smatanova, I. K., Nagata, Y., Svensson, L. A., Newman, J., Takagi, M. & Damborsky, J. (2000). *Biochemistry*, **39**, 14082–14086.
7. Asakura, T., Adachi, K. and Schwartz, E., Stabilizing effect of various organic solvents on protein. *J. Biol. Chem.* 1978 **253**: 6423-6425.

P9

STRUCTURAL STUDIES ON PHOSPHOFRUCTOKINASE OF *PICHIA PASTORIS*

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The enzyme 6-phosphofructokinase (Pfk) catalyses the formation of fructose 1,6-bisphosphate from fructose 6-phosphate and MgATP and contributes to the control of glycolysis in prokaryotic and eukaryotic cells. Mutations in the gene encoding Pfk are responsible for genetic disorders (e.g. Tarui disease) in some ethnic groups. The catalytic activity is tightly regulated in a wide variety of organisms by diverse positive (e.g. fructose-2,6-bisphosphate, AMP) and negative (e.g. ATP, citrate) effectors. Eukaryotic phosphofructokinase has evolved by a process of tandem gene duplication and fusion to yield a protein that has a multiple size of prokaryotic Pfks. The N-terminal half of a Pfk subunit obviously retained the catalytic function, whereas in the C-terminal half allosteric ligand binding

sites have evolved from former catalytic and regulatory sites. *Pichia pastoris* Pfk (PpPFK) is a heterododecamer (4 4 4) of molecular weight approximately 1 MDa. Accordingly, it is the largest and most complex Pfk identified yet [1]. In comparison with the enzymes from *Saccharomyces cerevisiae* and *Kluyveromyces lactis*, the activation ratio of PpPFK by AMP is several times higher, the ATP inhibition is stronger and the apparent affinity to fructose 6-phosphate is significantly lower [2]. The -subunit of PpPFK is not required for enzymatic activity although a mutant strain which is deficient for the -subunit displays decreased growth upon nutrient limitation and reduced cell flocculation when compared with wild-type strain. The sequence of the -subunit shows no similarity to classic Pfk subunits or to other proteins. Only a structure alignment reveals the relation to catechol-O-methyltransferase. But the evolutionary origin of the -subunit is still unknown. The objective of our work is to study structure and function of eukaryotic PFKs and the evolutionary origin of the unique -subunit in PpPFK. Mutations in the gene encoding Pfk are responsible for genetic disorders (e.g. Tarui disease) in some ethnic groups.

1. Tanneberger K. et al., A Novel Form of 6-Phosphofructokinase: Identification and functional relevance of a third type of subunit in pichia pastoris; *JBC* 2007; 282; 23687-23697.
2. Kirchberger J. et al.; 6-phosphofructokinase from Pichia pastoris: purification, kinetic and molecular characterization of the enzyme; *Yeast* 2002; 19; 933–947.

P10

CRYSTALLIZATION AND CRYSTAL STRUCTURE OF TWO PEPTIDASE INHIBITORS FROM TICK SALIVA

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The saliva of blood-feeding parasites is a rich source of peptidase inhibitors that help overcome the host's defense during host-parasite interactions. An immunomodulatory protein OmC2 belonging to the cystatin superfamily is from the saliva of the soft tick *Ornithodoros moubata*, an important disease-vector transmitting African swine fever virus and the spirochaete Borrelia duttoni. IRS-2 is an anti-inflammatory protein from serpin superfamily found in the saliva of *Ixodes ricinus*, the vector of Lyme disease and tick-borne encephalitis. Screening for crystallization conditions for both inhibitors was performed using the hanging drop vapor diffusion technique. X-ray diffraction data were collected using synchrotron radiation. The crystal structures of IRS-2 and OmC2 were determined by molecular replacement and refined using data to 1.8 Å and 2.45 Å resolution for IRS-2 and OmC2 respectively.

P11

STRUCTURAL CHARACTERIZATION OF RAC AND ITS RIBOSOMAL INTERACTION

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Nascent proteins need to adopt their native conformations to become biologically active. They are assisted in this process already cotranslationally by molecular chaperones and chaperonins. From eubacteria to humans the first chaperones to interact with a nascent protein chain are ribosome associated chaperones like the *Eschericha coli* trigger factor or Ssb1/2p and RAC in *Saccharomyces cerevisiae*. Yeast RAC is a stable, heterodimeric complex of the Hsp70 homolog Ssz1p and the Hsp40 homolog zuotin that mediates the interaction with the ribosome. The functional complex serves as the J-protein partner for the nearly identical Hsp70 homologs Ssb1p and Ssb2p. We show a SAXS model of the complete RAC complex and a cryo-EM map that reveals the overall arrangement and details on its interaction with the translating ribosome. Also, we have identified the first 50 residues of zuotin to be sufficient to form a stable complex with Ssz1p and are currently conducting protein crystallographic experiments to obtain details on the unique interaction between the two chaperones.

P12

EXPRESSION AND PURIFICATION OF TRUNCATED CONSTRUCTS OF THE CTP:PHOSPHOCHOLINE CYTIDYLTRANSFERASE ENZYME FROM *Plasmodium falciparum*

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The enzyme CTP:phosphocholine cytidylyltransferase (CCT) catalyzes a rate-limiting step of phospholipid biosynthesis [1]. The enzyme from *Plasmodium falciparum* has not yet been characterized at high-resolution structural details. An in-depth analysis of structure-function relationships is indispensable to understand the mechanism of action of this important enzyme. Our aim is to provide a high-resolution three-dimensional structure by X-ray crystallographic studies on this protein and its liganded complexes.

To this end, several truncated constructs of the full length *P. falciparum* CCT enzyme were created. As not much is known of the 896 residue long plasmodial protein, the core of the constructs is the highly conserved folded catalytic domain of the enzyme that constitutes a Rossman fold for accommodation of CTP. We wish to investigate the effect of the N- and C- terminal extensions on protein folding, stability and activity. Presently we are making efforts to achieve soluble overexpression, purification and characterization of these constructs.

Supported by ANR-NKTH AddMal grant

1. Déchamps et al, 2010, Mol Biochem Parasitol. doi:10.1016/j.molbiopara.2010.05.006

P13

PRELIMINARY CRYSTALLOGRAPHIC ANALYSIS OF THE *M. magneticum* AND *M. gryphiswaldense* STRAIN MAGNETOSOME ASSOCIATED PROTEIN, MAMA

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Tetra- tricopeptide repeat (TPR) is a structural motif found as such or forming part of a bigger fold in a wide range of proteins. It serves as a template for protein-protein interactions and mediates multiprotein complexes . MamA is a unique, highly abundant, Magnetosome associated protein and predicted to contain 5 TPR motifs as well as predicted putative one. Magnetosome is a subcellular organelle that consists of a linear-chain assembly of lipid vesicles each able to biomineralize and enclose a ~50-nm crystal of magnetite or greigite. Magnetosome allows magnetotactic bacteria, a diverse group of aquatic microorganisms, to orientate themselves along geomagnetic fields in search of suitable environments *(2)*. MamA is one of the most characterized magnetosome-associated proteins *in vivo* and yet, its function is not clear *(3-5)*. Here, we report on the crystallization and preliminary X-ray analysis of recombinant *M. magneticum* (AMB-1) and *M. gryphiswaldense* (MSR-1) MamA deletion mutants. Crystals diffracted to a resolution of 2.0 Å and 1.95 Å respectively, to yield a reasonable data sets which are currently used for molecular replacment.

1. D'Andrea, L. D., and Regan, L. (2003) TPR proteins: the versatile helix, *Trends Biochem Sci 28*, 655-662.
2. Faivre, D. & Schuler, D. Magnetotactic Bacteria and Magnetosomes, Chem Rev 108, 4875-4898 (2008).
3. Komeili, A., Vali, H., Beveridge, T. J. & Newman, D. K. Magnetosome vesicles are present before magnetite formation, and MamA is required for their activation, Proc Natl Acad Sci USA 101, 3839-3844 (2004).
4. Okuda, Y. & Fukumori, Y. Expression and characterization of a magnetosome-associated protein, TPR-containing MAM22, in Escherichia coli, FEBS Lett 491, 169-173 (2001).
5. Taoka, A., Asada, R., Sasaki, H., Anzawa, K., Wu, L. F. & Fukumori, Y. Spatial localizations of Mam22 and Mam12 in the magnetosomes of Magnetospirillum magnetotacticum, J Bacteriol 188, 3805-3812 (2006).

P14

STUDY OF THE EFFECT OF TEMPERATURE ON PROTEIN CRYSTALLIZATION USING TG40

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Successful crystallization depends on specific solution conditions and temperature. While the effects of precipitant type and concentration, buffer type and pH and protein concentration on protein crystallization are investigated, the temperature remains the least tested parameter. Solubility of as many as 86% of proteins depends on temperature [1]. An extensive study on 10 different proteins has shown that determining the most suitable temperature can increase the number of hits by 65.1% [2]. Proteins can crystallize at temperatures ranging from less than 0°C to around 60°C [3], although the solubility is usually assessed at 20°C and 4°C, limiting factors being both the amount of available protein and temperature control.

To obtain highly accurate temperature control, the Temperature- Controlled Microplate for protein crystallization (TG40) was used. The device allows for screening at 5 different temperatures (from 4° to 60°C) simultaneously. TG40 System is portable thus eliminating temperature fluctuations during observation and crystal manipulations.

It is also possible to dispense very small drop volumes using Oryx, reducing the amount of protein required.

As crystallization of Lysozyme is highly dependent on temperature, we are investigating the effect of temperature on the crystallization of Lysozyme and other proteins currently under study in the Division using TG40, focusing on constant temperature and temperature cycling programmes and exploring wide range of temperatures and their effect

on crystal growth, yield, morphology and diffraction quality.

1. Christopher, G.K; Phipps, A.G; Gary, R.J. Temperature-dependent solubility of selected proteins. *J. Cryst. Growth* 1998, **191**, 820- 826.
2. Lin, Y.; Zhu, D.; Wang, T.; Song, J.; Zou, Y.; Zhang, Y.; Lin, S. An Extensive Study of Protein Phase Diagram Modification: Increasing Macromolecular Crystallizability by Temperature Screening. *Crystal Growth & Design* **8** (12), 2008.
3. L. Lloyd Haire, in T.M. Bergfors (ed) Protein Crystallization, I.U.L. 1999 pp. 65- 68.

P15

TOWARDS THE STRUCTURAL CHARACTERIZATION OF THE N-TERMINAL RELAXASE DOMAIN OF MOBM IN COMPLEX WITH ITS COGNATE DNA. INSIGHTS INTO THE DNA TRANSFER MECHANISM OF GRAM-POSITIVE BACTERIA

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Bacterial conjugation is a major mechanism for genetic exchange in bacteria and thus an important component of bacterial evolution. It provides a route for the rapid acquisition of new genetic information and contributes to the spread of antibiotic resistance. It is also a paradigmatic example of horizontal gene transfer and the best-studied system for cell-to-cell DNA translocation.

Mobilizable DNA transfer in Gram-positive bacteria is poorly understood at the molecular level. Well studied plasmid in Gram-positive bacteria is pMV158, which encodes for MobM, a protein of the relaxase family that initiates DNA replication and transfer. The full length MobM is a 57.9 kDa protein which dimerizes in solution (the C-terminal part harbors a Leu-zipper motif). The N-terminal domain bears the relaxase activity of the protein, similarly as conjugative relaxases from Gram-nagative bacteria TrwC and TraI. MobM and other super-family pMV158 relaxases specifically bind a 6-10 bases long loop formed

by an inverted repeat sequence (*oriT*) and cut at the nic site downstream of the loop.

We are investigating the full length MobM from *Streptococcus agalactiae*, N-terminal relaxase domain constructs and a longer construct that contain a domain believed to be responsible for the protein dimerization. Co-crystals of the complex between different MobM variants and oligonucleotides that mimic the *oriT* sequence just upstream the *nic* site are being prepared.

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STRUCTURAL STUDIES OF THE PROTEIN KINASE CK2: INHIBITION MECHANISM AND STRUCTURE-ACTIVITY RELATIONSHIP

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A significant part of the genome consists of genes that encode proteins with the ability to catalyze the transfer of phosphate to protein substrates thereby altering their biological properties and function; these proteins are called Protein Kinase [1].

Protein kinase CK2 (casein kinase 2) is a Ser/Thr kinase and it is one of the most highly conserved molecules, is present in every eukaryotic cell at a strictly regulated level and it is one of the most unspecific eukaryotic protein kinases; to date the repertoire of CK2 substrates includes 307 proteins (which are proteins implicated in signal transduction, gene expression and other nuclear functions) [2]. Furthermore CK2 shows the rare ability to use either ATP or GTP as phosphoryl donor (dual-co-substrate specificity) [3] and the capability of CK2 to phosphorylate tyrosine has been reported repeatedly in recent years [4].

CK2 is involved in many fundamental aspects of normal cell life, but it is also able to establish favourable conditions for tumorigenesis: high level of CK2 have been found in various cancer cells, CK2 can act as a apoptosis suppressor and it strongly promotes the multi-drug-resistant phenotype of the cells. For these reasons CK2 can be considered a valuable drug target for cancer therapy [5].

CK2 exists in vivo mainly as a holoenzyme composed by two catalytic subunits (CK2) and two regulatory subunits (CK2). Different crystal structures of CK2 have been published: many of them are about catalytic and regulatory subunits with one 3D structure about the holoenzyme.

Given the pharmacological interest of CK2, many inhibitors have been crystallized in complex with the maize and the human enzymes, especially in my laboratory. I am continuing this research line, performing structural studies on protein kinase CK2.

1. Ahmed K., Preface, *Molecular and Cellular Biochemistry* 2008, **316** 1-3.
2. Lorenzo A. Pinna, Protein kinase CK2: a challenge to canons, *Journal of Cell Science* 2002, **115** 3873-3878.
3. Niefind,K., Putter,M., Guerra,B., Issinger,O.-G. and Schomburg,D. *GTP plus water mimics ATP in the active site of protein kinase CK2*, *Nature Struct. Biol.* 1999, **6** 1100-1103.
4. Donella-Deana A., Cesaro L., Sarno S., Brunati A., Ruzzene M. and Pinna L.A., *A*utocatalytic tyrosine-phosphorylation of protein kinase CK2 alpha and alpha' subunits: implication of Tyr182, *Biochem. J.* 2001, **357** 563-567.
5. Battistutta R., Structural bases of protein kinase CK2 inhibition, *Cell. Mol. Life Sci.* 2009, **66** 1868 – 1889.

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BIOCHEMICAL AND STRUCTURAL CHARACTERIZATION OF AN IMMUNE MODULATORY PROTEIN COMPLEX (UL141-TR2) PARTICIPATING IN TNF-R SIGNALING PATHWAYS

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Recently, a number of proteins belonging to the tumor necrosis factor (TNF) and TNF receptor (TNF-R) families were cloned and characterized in our laboratory. Protein UL141, encoded by human cytomegalovirus (Human CMV), belongs to the tumor necrosis factor family UL14. It is implicated in viral immunoevasion and downregulates cell surface expression of PVR (CD155), which is a ligand for NK cell-activating receptors. UL141 binds human PVR in the endoplasmatic reticulum and prevents its maturation and transport to the cell surface [1, 4]. The members of the TNF-R family are characterized by cysteine rich domains (CRD) containing three disulfide bridges with a cysteine knot topology [2, 3]. The number of CRD ranges from four to six in TNF-R1 and TNF-R2 to two or even three in TRAIL receptor 2 (TR2 or DR5). Those CRD form probably the most important ligand-binding region in the complex of UL141-TR2.

TRAIL-R2/DR5 is a death receptor of the TNFR superfamily that promotes apoptosis of both transformed and viral-infected cells. We have shown UL141 can directly bind to TRAIL-R2 and inhibit its cell surface expression in human CMV infected cells, desensitizing them to TRAIL-mediated killing. Elucidating the structure of this non-canonical interaction between UL141 and a human death receptor should shed significant light upon the mechanism of action employed by HCMV to target this signaling pathway.

Both proteins, UL141 and TR2, were expressed in insect cells using the baculovirus expression system. His-tagged UL141 and TR2-Fc fusion protein were purified from the culture supernatant by affinity chromatography using Ni^{2+}-NTA and Protein A columns, respectively. As part of studies to determine the structure of UL141, the complex formation, crystallization and preliminary X-ray diffraction analysis of the UL141-TR2 complex are described. It was found that the UL141-TR2 complex comprises one UL141 dimer and one TR2 monomer. This was confirmed and purified by size-exclusion chromatography and followed by SDS-PAGE analysis. So far, crystals of UL141 protein were obtained in presence of dioxane using calcium acetate and polyethylene glycol as a precipitant. Well-shaped tetragonal bipyraminds grew within several days at 22°C. The co-crystallization of UL141 together with TR2 is currently in progress.

1. Dolan, A. et al.: *J. Gen. Virol.,* 85 (2004) 1301-1312.
2. Banner, D. W. et al.: *Cell*, 73 (1993) 431-445.
3. Naismith, J. H. and Sprang, S. R.: *Structure,* 4 (1996) 1251-1262.
4. Ma, Y. P. et al.: *Arch. Virol.,* 151 (2006) 827-835.