Student abstracts

P1

EXPRESSION, PURIFICATION AND CRYSTALLIZATION OF EPIGENETIC READER OF THE NUCLEOSOME: THE PHD ZINC FINGER DOMAIN OF BAZ1A

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The plant homeodomain (PHD) fingers are small protein domains found in several nuclear factors that interact with chromatin. In particular they have been found to function as epigenetic readers of histone tails post-translational modifications (PTMs). Sequence analyses of the human genome have revealed a prevalence of human proteins containing PHD fingers adjacent to Bromodomains and other epigenetic domains to form tandem units.

The PHD zinc finger domain is constituted of about 60 residues and it is present in several eukaryotic proteins engaged in the control of gene transcription and chromatin dynamics. It is characterized by a Cys_4-$HisCys_3$ conserved zinc binding motif. It has been proven that this domain recognizes unmodified as well as post-translational modified residues on H3 tails and, in some cases, it has been found to interact with non-histone proteins. The PHD activity is the result of a combination of several different epigenetic interactions. Furthermore, it is noteworthy a linkage between the activity of some PHD zinc fingers and the onset of some human affections as cancer, immunodeficiency syndromes and neurological disorders.

We are interested to study the PHD zinc fingers domains of the human protein BAZ1A. This protein, belonging to BAZ family, contains other important domain as WAC, WAKZ motif and C-terminus-bromodomain important for assembling the chromatin remodelling complex of HuCHRAC. The biological and therapeutic importance of this protein is related to the fact that BAZ1A seems implicated in male fertility in accord with its high expression in male germ cells, indeed its deficiency causes male-specific sterility. BAZ1A may represent a novel target for male contraceptive development.

The purpose of the project is elucidating the structure-function relationship of PHD-histone interaction and, eventually, targeting this interaction using fragment-based approaches thereby paving the way for the design of small molecule chemical probes as novel modulators.

The PHD BAZ1A construct has been generated using the PCR-based cloning technology. For enhancing the solubility and stability during the expression and purification of the protein we have used a pCri11b vector, containing Sumo tag in addition to N-terminal 6-histidine tags. We have established and optimized a protocol for the expression and purification, comprising four-steps procedure including first Ni-affinity chromatography, followed by cleavage of the Sumo tag, a second Ni-affinity chromatography to remove the cleaved tag, an additional cation exchange chromatography and last polishing step performed by size exclusion chromatography.

Following the protocol described we are able to achieve high level of purity and we are currently exploring crystallization trials for PHD BAZ1A.

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P2

STRUCTURAL DETERMINATION OF 5' UTR RNA MOTIFS

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5' untranslated regions of mRNA contain cis-regulatory elements frequently forming secondary structures including IRESes, binding sites for RNA binding proteins, uAUGs and uORFs. These sequences play an important role in translation regulation by promoting or inhibiting translation initiation, affecting mRNA's stability and also acting as riboswitches. As impairment of this regulation machinery perturbs cellular metabolism, leading to various physiological abnormalities studying it at a structural level seems to be an important research subject.

The aim of my research is to determine the structure of 5' UTR RNA motifs using mainly X-ray crystallography method, in combination with low-resolution structural probing methods and theoretical structure prediction. Although X-ray crystallography method for structure determination is widely used for protein structure determination it can be also used for RNA. X-ray crystallography technique employs single crystal X-ray diffraction (SXRD) to unambiguously determine the three dimensional structure of large biological molecules at atomic resolution. The strength of this method lies in the high level of accuracy it provides and lack of the size limitation for the molecules that are analyzed. The steps involved in this technique consist of sample preparation, crystallization, X-ray diffraction and structure determination. But as the surface of RNA molecules is dominated by a poor differentiated regular array of negatively charged phosphates, the crystallization of RNAs remains a formidable experimental challenge which makes low-resolution structural probing methods like SHAPE, CD etc. and theoretical structure prediction also important.

Structural insight obtain using different structural characterization methods will help in understanding the different mechanisms of translation regulation, impact of 5' UTR structure on gene expression and linkage between mutations in UTR coding sequence and expression abnormlities.

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P3

BIOCHEMICAL STUDIES OF THE NOVEL DIMERIZATION DOMAIN WITHIN DROSOPHILA CTCF PROTEIN

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Drosophila CTCF is conserved genomic insulator protein able to support long-distance (at 5kb) genomic interactions. Obviously self-association activity of the protein is involved in this process. Analysis of recombinant deletion derivatives by chemical cross-linking revealed presence of dimerization domain in the N-terminal part of Drosophila CTCF. Limited proteolysis followed by MALDI-TOF MS analysis allowed us to determine precise position of dimerization domain. No homology with known protein domains was found. Despite the small coiled-coil region is predicted within the domain, itself it is not sufficient to support dimerization. N-terminal sequences which are not required for dimerization contributes to the overall stability of the recombinant protein. Large-scale bacterial expression and purification scheme was established. Analysis of the recombinant protein by both size-exclusion chromatography and DLS revealed apparent molecular weight of the protein corresponding to the tetramer rather than to the dimer discovered by cross-linking. These differences can be explained by the elongated shape of the molecule. A primary screening of crystallization conditions for recombinant CTCF N-terminal domain was performed.

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P4

X- RAY DIFFRACTION ANALYSIS OF THE VIRULENCE-RELATED PERIPLASMIC CHAPERONE, SURA, FROM *BURKHOLDERIA PSEUDOMALLEI*

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In Gram-negative bacteria the Outer Membrane (OM) delivers a physical protection to adverse compounds and is crucial for microbial pathogenicity. SurA is a peptidyl-prolyl isomerase chaperone that participates in the translocation pathway of outer membrane proteins (OMPs). Additionally, SurA is thought have a central role in the assembly of the majority of OMPs contributing for the folding and insertion of OMPs in the OM [1,2]. Furthermore, it has been reported that mutations on SurA gene have diminish the virulence of *Salmonella enterica*, *Shigella flexneri* and uropathogenic *E. coli*, suggesting SurA as a new suitable therapeutic target against several pathogens [3]. However, the only available molecular structures for this family of proteins come from *E. coli*. The *E. coli* structures offer only a partial view of the role of the SurA. Structure determination of SurA from additional clinically relevant species would have an important impact in understanding the biological function of this protein.

In E. coli, SurA was reported to be constituted by three separate domains (N/C, P1, and P2) [4], with the N/C-P1 domain displaying significant enzymatic activity [5]. In this study, the N/C-P1-domain of SurA from *Burkholderia thailandensis*, an avirulent relative of *Burkholderia pseudomallei* [6], was expressed, purified and screened for crystallisation.

The successful crystallisation and molecular structure determination of SurA N/C-P1-domain could have a critical impact in our understanding of the biology of this protein family. This may have important implications in the development of new microbiological therapeutic strategies in an era of increasing antibiotic resistance, with the urgent need for new therapeutic strategies.

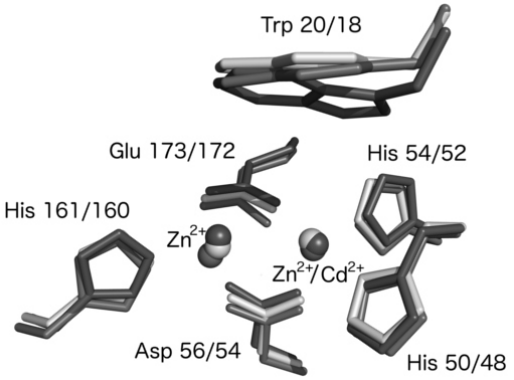

Figure 1. The active site of KynB is highly conserved. Superposition of *Ba*KynB (dark grey), *Bc*KynB (grey) and *Pa*KynB (light grey) active sites structures.

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P5

STRUCTURES OF BACTERIAL KYNURENINE FORMAMIDASE

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Kynurenine formamidase (KynB, EC 3.5.1.9) is the second enzyme involved in the aerobic degradation of L-tryptophan to anthranilate [1]. This pathway provides precursors for biosynthetic pathways such as anthranilate catabolism, 2-oxo-glutarate and quinolinate formation. Kynurenine formamidase catalyses the hydrolysis of *N*-formyl-L-kynurenine to L-kynurenine and formate [2]. This enzyme

is present in eukaryotes, which is called KNase, but is not related to bacterial KynB.

To assess the characterization of the bacterial KynB three different structures were determined; *Bacillus anthracis* KynB (*Ba*KynB, 4CO9, 4CZ1), *Burkholderia cenocepacia* KynB (*Bc*KynB, 4COG) and *Pseudomonas aeruginosa* KynB (*Pa*KynB, 4COB). The structures show a novel amidase fold and a crowded binuclear Zn^{2+} catalytic site that is a key feature for the reaction catalysis. The overall structure is highly conserved between the Gram-negative and Gram-positive bacteria as well as the active site pocket amino acids (Figure 1). KynB is a homodimer showing a -barrel-like domain in each subunit. The active site can only exist with the amino acid contribution of both subunits. The two Zn^{2+} are located in a rigid active site and present a coordination number of 5 or 6. The presence of these ions favours an acidic amidase reaction. The *Ba*KynB structure complexed with 2-aminoacetophenone (4CZ1) defines substrate recognition and suggests a reaction mechanism. In the case of the *Bc*KynB structure, one of the Zn^{2+} was replaced by a Cd^{2+} (Figure 1), which was present in the crystallization condition.

Additionally, kynurenine formamidase activity for *Ba*KynB, *Bc*KynB and *Pa*KynB was confirmed using a spectrophotometric assay [1]. K_m values were comparable to the *Bacillus cereus* KynB [3] and substrate inhibition was not observed.

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P6

STRUCTURAL CHARACTERIZATION OF INTERACTIONS BETWEEN ACCUMULATION ASSOCIATED PROTEIN (AAP) AND SMALL BASIC PROTEIN (SBP)-TWO ESSENTIAL PROTEINS GOVERNING *STAPHYLOCOCCUS EPIDERMIDIS* BIOFILM FORMATION

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A score of biotic and abiotic surfaces can be infected by a single or mixed microbial species by forming biofilms, a meshwork of unicellular organisms enclosed in an extracellular bacterial derived matrix composed of proteins, polysaccharides and nucleic acid [1,2]. *Staphylococcus epidermidis,* a coagulase negative opportunistic bacterium is a leading pathogen in nosocomial infections [3].The establishment of biofilm by *S. epidermidis* takes place by a two-step process, a primary attachment and an accumulation maturation phase [4].

The accumulation phase starts with cell to cell adhesion and two types of polymers: proteins and polysaccharides intercellular adhesins are involved in this process. The accumulation associated protein, the Aap, consists of two domains A and B [5]. Once, the domain A is proteolytically cleaved, the C- terminally located domain B becomes activated and takes part in cell to cell adhesion [6].Very recently it has been found that an 18kDa small basic protein consisting of 513bp, is involved in the interaction with Aap and contributes to biofilm formation (Decker *et al.,* submitted).

The Aap protein is suggested to play an important role in biofilm formation and Sbp has been found to be involved in interacting with Aap. However, the functional and structural characteristics of the interaction, which are not known to date, have to be investigated to understand the biofilm formation.We have established the protocols for the expression and purification of Sbp successfully. The purified protein is in soluble and monodispersed as demonstrated by Dynamic Light Scattering. In order to get an idea about the secondary structures of the protein, Circular dichroism spectroscopy was applied, showing that Sbp mainly contains ß-sheets along with some coiled structures and alpha helices. Importantly, DLS and analytical gel chromatography suggest that Sbp forms multimers when in solution. Small angle scattering analysis as well as Sbp resolution of crystal structure are now necessary to understand the biology of Sbp in great details. In addition, work will focus on the analysis of Sbp interactions with Aap Domain B. To this end, sub-domains of repetitive B domain have been cloned into E. coli expression vector and work is in progress to express and purify them successfully. These proteins will be used to characterize Sbp-Aap interactions by using in surface Plasmon resonance (SPR) and microscale thermophoresis (MST). Since an interacting structural characterization between the two proteins is required, co-crystallization techniques will be applied. The obtained structural data will help to understand the crucial function of Sbp in Aap dependent biofilm formation.

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P7

XYLOGLUCAN ENDOTRANSGLYCOSYLASE (XET) FROM GERMINATING NASTURTIUM SEEDS (*TROPAEOLUM MAJUS*)

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Xyloglucan endotransglycosylase (XET, EC 2.4.1.207) is plant cell wall-modifying enzyme acting on the cellulose-xyloglucan network. In recent years it has been found, that this enzyme is able to catalyze the fragments transfer between saccharides of different type, so called heterotransglycosylation [1], [2].

Therefore, the aim of my work was the detection of heterotransglycosylating activity of the crude protein extract from the germinating nasturtium seeds. For this purpose, we used fluorescently labelled oligosaccharides and different fluorimetric methods [3]. Moreover, the methods of molecular biology and cDNA sequencing were used for determination of primary structure of unspecific nasturtium XET in order to propose the tertiary structure of the protein by homology modeling.

Transglycosylating enzyme/enzymes able to catalyze the *in vitro* formation of covalent bonds between saccharides of different type were detected in the nasturtium seeds, by HPLC analyses and paper method. The enzyme action led to heterotransglycosylating reaction between xyloglucan and all tested fluorescently labelled oligosaccharides. Even after the change of donor substrates, the hybrid product formation catalyzed by nasturtium seed extract was observed. Interestingly, the fragments of hydroxyethyl cellulose were also incorporated to typical yeast saccharides, ß-(1,6)-D-glucooligosaccharides [4]. Although the microscopic analyses showed certain differences in the binding sites of fluorescently labelled oligosaccharides inside the nasturtium cells, the method largely confirmed the presence of unspecific transglycosylases.

We successfully gained the structural information about unspecific XET (pI 6,3) from the germinating nasturtium seeds. The complete cDNA is 1078 bp long without poly(A) tail and it contains the ORF coding for 280 amino acid residues (http://www.ebi.ac.uk/ena/data/view/HF968473). The 3D model of XET6.3 created on the basis of obtained primary structure and already described specific XET, has the typical structural features of the GH16 enzymes. The amino acids potentially responsible for so broad unspecificity of nasturtium XET6.3 were proposed.

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P8

INTERACTION OF THE NON-CODING RNA 7SK, A REGULATOR OF HUMAN TRANSCRIPTION ELONGATION, WITH ITS STABILIZING PARTNER LARP7

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The non-coding 7SK snRNA, an abundant RNA discovered in human nucleus, regulates transcription by RNA polymerase II [1]. It sequesters and inhibits the transcription elongation factor P-TEFb which, by phosphorylation of RNAPII and the pausing factors NELF and DSIF, switches transcription from initiation to processive elongation and relieves pauses of transcription [2]. This regulation process depends on the association between 7SK and protein HEXIM, neither isolated partner being able to inhibit P-TEFb alone. LaRP7, a protein with a La domain, has been shown to bind 7SK specifically, and ensure its stability [3].

In order to clarify how 7SK structural organization with its partners supports its functional relationship and assess how is triggered the regulation, we use a multiple approach, combining biochemical and structural methods. We showed previously by NMR mapping combined with mutagenesis and EMSA experiments that the HEXIM interaction relies upon a repeated GAUC motif [4]. Binding triggers an opening of the GAUC motif and stabilization of an internal loop.

We focus now on the interaction of LaRP7 with 7SK, in order to highlight which elements of 7SK are responsible for the specificity of the interaction. Footprinting assays show that on the whole, LaRP7 does not change dramatically the 7SK reactivity profile, but increases the sensitivity of the core region to V1 RNAse. Electrophoretic mobility shift assays performed with 7SK or its sub-domains show that the N-terminal region of LaRP7, comprising the homology domain with protein La binds the 3'-end of 7SK, a stretch of uridines. Specificity is provided by another domain, at the C-ter of the protein. Our recent results will be presented.

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P9

STRUCTURAL CHARACTERIZATION OF CATHEPSIN D PROTEASE FROM THE HARD TICK *IXODES RICINUS*

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The hard tick *Ixodes ricinus* is the major vector of Lyme disease and tick-borne encephalitis in Europe. Host blood proteins serve as the ultimate sources of nutrients for its growth, development and reproduction. IrCD1, a gut-associated cathepsin D-type protease from *I. ricinus*, is critically involved in blood protein digestion, namely it is responsible for the initial step of this process. IrCD1 represents a potential anti-tick vaccination target. Crystal structures of both inactive IrCD precursor (zymogen) and fully active mature IrCD were determined at 2.3 Å and 1.88 Å resolution, respectively, which allowed us to study the process of IrCD activation. Furthermore, two different mechanisms of inhibition of IrCD activity were investigated using crystal structures of complexes with an active-site peptidomimetic inhibitor (solved at 1.46 Å) and a propeptide-derived inhibitor binding to an exosite (solved at 1.81 Å).

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P10

STRUCTURAL STUDIES OF THE 14-3-3/PDC COMPLEX USING NMR AND SAXS

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Phosducin (Pdc), a highly conserved 30 kDa phosphoprotein, regulates visual signal transduction by interacting with the beta and gamma subunits of the retinal G-protein. Besides its well-established role in the regulation of the G-protein signaling, Pdc is also involved in the transcriptional control and the modulation of blood pressure. The function of Pdc is regulated through its phosphorylation and a binding to the regulatory 14-3-3 protein. The 14-3-3 proteins are 57 kDa (dimer) scaffolding molecules that regulate the function of other proteins through a number of different mechanisms. The exact role of the 14-3-3 protein in regulating of Pdc function is still unclear, but it is entirely possible that 14-3-3 either sterically occludes and/or affects the structure of Pdc. Both 14-3-3 binding motifs are located within the N-terminal domain of Pdc, which participates in the binding to the beta and gamma subunits of the retinal G-protein as well as contains the SUMOylation site Lys-33.

Our previous study revealed that phosphorylated Pdc and the 14-3-3 protein form a stable complex with 1:2 molar stoichiometry. Complex formation with 14-3-3 affects the structure and reduces the flexibility of both the N- and C-terminal domains of dpPdc, suggesting that dpPdc undergoes a conformational change when binding to 14-3-3. To further investigate this interaction and mainly the 14-3-3 protein-mediated conformational changes of Pdc, we performed structural studies using NMR, SAXS and tryptophan fluorescence. NMR studies confirmed the intrinsically disordered behavior of the N-terminal domain of Pdc and properly folded C-terminal domain. From SAXS we obtained low-resolution envelopes for both Pdc alone and the complex.

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P11

PHR1 AND PHR2 TRANSGLYCOSYLASES OF THE *CANDIDA ALBICANS* CELL WALL - THE BIOCHEMICAL CHARACTERIZATION AND SUBSTRATE SPECIFICITY

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The yeast cell wall is an essential component that defines cell shape, preserves the cell´s osmotic integrity, is involved in flocculation, sporulation and mediates cell-cell interactions. Moreover, in fungal pathogens the cell wall plays an essential role in the interaction with the host cells and in virulence. Inner layer of the yeast cell wall consists mainly of polysaccharide polymers. The external layer of the cell wall is formed by mannoproteins. The major structural component of the polysaccharide polymers is -(1,3)-glucan, next -(1,6)-glucan and minor but significant amount of chitin. These individual components are mutually linked by covalent bonds into large macromolecular complexes, which are based on -(1,3)-glucan backbone. Our attention is focused on transglycosylases, specifically to pH-regulated enzymes Phr1 and Phr2 (family GH72) from *Candida albicans*, which catalyze the linking of part of -(1,3)-glucan to another molecule of 1,3)-glucan.

The proteins Phr1 and Phr2 were heterologously expressed in *Pichia pastoris*. The transglycosylation activities of Phr1 and Phr2 were determined by a fluorescent *in vitro* assay and size-exclusion chromatography. Laminarin and laminarioligosacharides were used as the donors and the oligosaccharides labeled by sulforhodamine (SR) as the artificial acceptors. The wide spectrum of (SR)-oligosaccharides (OS-SR) was tested as acceptors: laminari-

OS-SR; N-acetyl-chito-OS-SR, cello-OS-SR and SR-oligosaccharides derived from xyloglucan, mixed-linkage -(1,3/1,4)-glucan; -(1,6)-glucan (pustulan), (1,4)-linked mannan and -(1,4)-glucan (starch). Results based on relative rates of transglycosylation and elution profile of the reaction mixtures measured by SEC have shown that -(1,3)-linked laminarioligosaccharides were only acceptors in reactions catalyzed by Phr1 and/or Phr2. The rate of transglycosylation mediated by Phr2 was higher than by Phr1. The pH optimum was 5,6 for Phr1 and 3 for Phr2. The optimum temperature was 30°C for both. The products of the transglycosylation reactions were identified by MALDI-TOF as the molecules composed of the acceptor and portions of the donor molecule attached to its non-reducing end.

P12

NUTRIENT UPTAKE BY UBIQUITOUS MARINE BACTERIA

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Oligotrophic oceanic gyres are the largest ecosystems on earth and profoundly affect global biogeochemical cycles. The microbial community of these ecosystems is dominated by the SAR11 group of -proteobacteria and *Prochlorococcus* cyanobacteria [1, 4]. Their success in these nutrient depleted habitats is based on a variety of high affinity substrate transporters. Even at nanomolar nutrient concentrations these transporters are capable of a rapid uptake [5, 9, 8] which gives a distinct advantage in the extreme competition for multiple nutrients in oligotrophic systems.

Generally, nutrients enter the periplasm via porines through the outer membrane. They are then transported into the cytoplasm by either low affinity ion driven transport systems or high affinity ABC-Transport systems which are induced at very low nutrient level [6, 2]. ABC transporter consist of a high-affinity substrate-binding protein (SBP), two hydrophobic transmembrane domains (TMD) and two nucleotide-binding domains (NBD) [3]. It has been shown that high-affinity SBPs dominate the metaproteome of SAR11 bacteria [7]. We will use protein crystallography to understand the underlying molecular mechanisms of primarily phosphate and iron uptake systems of SAR11 and *Prochlorococcus*. The microbial turnover rates of these nutrients will be measured by radioactively labelled precursors in the field. Until now, we have been able to purify the SBP for Iron (FutA) and Phosphate (PstS) of *Prochlorococcus* as well as solving the crystal structure of PstS. This model shows considerable structural consistency compared to other known PstS. To answer the central question why the nutrient uptake of these bacteria is so highly efficient we will have to regard each nutrient uptake system in its entirety, measure affinities and determine regulatory processes.

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P13

MANGANESE HOMEOSTASIS IN DEINOCOCCUS RADIODURANS: MEMBRANE PROTEINS, EXPRESSION, PURIFICATION AND CRYSTALLIZATION STRATEGIES

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Deinococcus radiodurans (*Dr*), known as the world's toughest bacterium, is extremely resistant to several oxidation agents such as ionizing radiation or hydrogen peroxide. This robustness is mainly due to efficient and precise DNA repair processes and a strong oxidative stress resistance mechanism that protects proteins from oxidative damage [1].

Recently, it was found that *Dr* is so susceptible to double strand DNA damage as all other species. However, the *Dr* Proteome revealed to be better protected to oxidation when compared with radiation sensitive species. The level of susceptibility to protein oxidation seems to be an indication of the bacterial survival rate [2].

The antioxidant defence in bacteria could be mediated by enzymes and/or non-enzymatic scavengers, as for instance divalent manganese complexes or carotenoides. Manganese complexes were identified as the most powerful reactive oxygen species scavenger in *Dr* [2, 3]. The *Dr* intracellular manganese concentration is around 20 times higher when compared for instance with *E. coli* [3, 4]. The understanding of the manganese homeostasis in this bacterium could give some clues about its high resistance to oxidation agents.

Two Mn-dependent transcriptional regulators (DR2539 and DR0865) were identified to regulate the Mn homeostasis, while, two ABC-type transporters (DR2523 and DR2238), a NRAMP family Mn transporter (DR1709) and an efflux protein (DR1236) are involved in Mn transport [1].

Dr2539 is a negative regulator of Mn transporters and positive regulator of Fe-dependent transporters while DR0865 is thought to regulate the Mn-ABC transporters [5]. DR1709 is up-regulated when the cells are exposed to radiation and the DR1236 gene deletion leads to cell susceptibility to high Mn concentration [1,6].

Nowadays, the heterologous expression, purification and crystallization of membrane proteins are one of the big challenges. In this work, we optimized a protocol to overexpress, solubilise and purify the efflux protein DR1236. The use of a new protocol in which the protein is cloned into a GFP-fusion vector revealed to be an asset in overexpression, solubilisation and purification steps [7].

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P14

THE COMPUTER MODELING OF PLANT LIPOXYHENASES-SUBSTRATES COMPLEX

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Lipoxygenases (LOX) are the first enzymes of lipoxygenase pathway in all living organisms, catalyzed the stereo- and regiospecific formation of unsaturated fatty acids hydroperoxides. There are a few theories of specificity product formations of lipoxygenases. Based on molecular dynamic and docking data we proposed a new model of plant lipoxygenases specificity. In this research we use high resolution X-ray structure of (13S)-specific soybean lipoxygenase-1 (GmLOX1) and homology modeling (9S)-specific maize lipoxygenase-3 (ZmLOX3). Our data suggest different shapes of lipoxygenase substrate-binding pockets. The Y-shape of ZmLOX3 active site is divided into three parts, flanked by the S272 (part I), W518 (part II) and G515 (part III) amino acids side chains. Similar to ZmLOX3 GmLOX1 active site is also divided into three parts flanked by homology amino acids (T257 for part I, W498 for part II and S567 for part III). Part III of GmLOX1 active site is turned to another side of protein.

The alignment of substrate on amino acids site chains is similar with some exceptions. For example, the alignments of native substrate (linoleic acid) in the active sites are similar in the 1,4-cis,cis-pentadiene systems orientations. The aliphatic chain of substrate is placed in the part II and III, its carboxyl chain is only in part III of the ZmLOX3 active

site. Its penetration is restricted by the W518 side chain. The aliphatic chain of substrates is placed in part I, II and III, its carboxyl chain only is in part III of the GmLOX1 active site. The GmLOX1 active site is wider and longer in comparison with ZmLOX3 active site. The shape of GmLOX1 active site allows to penetrate the arachidonic acid with two conjugated 1,4-cis,cis-pentadiene systems inside it, in contrast to ZmLOX3. Also we revealed two different ways of free fatty acids penetration into active sites. The entrances of active sites are in different parts of lipoxygenase. For the ZmLOX3 it is near the G515 and R727 amino acids. For GmLOX1 it is near the S567 and Q495 amino acids. The molecular dynamic simulations revealed the mobility of the amino acids side chains in those parts. The next stage of our investigation is to get the crystal structure of (9S)-specific lipoxygenase (ZmLOX3).

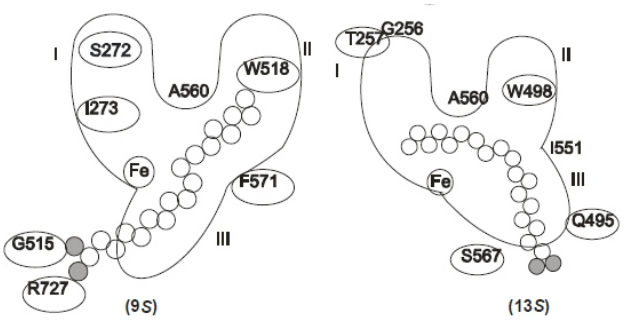

Figure 1. The schema of ZmLOX3 (9S) and GmLOX1 (13S) active sites . The substrate (linoleic asid) is present as white ball (carbon) and grey ball (oxygen); its hydrogens are not present. The amino acids side chains are present as ovals with its type and numbers. The iron is present as marked as Fe ball.

P15

ANALYSIS OF SUBSTRATE SPECIFICITY AND MECHANISM OF GLPG

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Membrane proteins of the rhomboid-family are evolutionarily highly conserved and include rhomboid intramembrane serine proteases and rhomboid-like proteins. The latter have lost their catalytic activity in evolution but retained the ability to bind transmembrane helices. Rhomboid-family proteins play important roles in intercellular signalling, membrane protein quality control and trafficking, mitochondrial dynamics, parasite invasion and wound healing. Their medical potential is steeply increasing, but in contrast to that, their mechanistic and structural understanding lags behind. Rhomboid protease GlpG from *E.coli* has become the model for the rhomboid family proteins and the main model intramembrane protease - it was the first one whose X-ray structure was solved. GlpG cleaves single-pass transmembrane proteins, but how substrates bind the enzyme and what determines substrate specificity is still unclear. Based on the structure of the empty enzyme and on biochemical analyses, substrate access into the active site was proposed to occur between transmembrane helix (TMH) 5 and TMH2, but molecular details have long remained obscure. We have recently succeeded in solving the crystal structures of rhomboid with a fragment of the substrate. They reveal the S1 to S4 subsites of the enzyme (which determine the kcat of the reaction), and elucidate some aspects of the mechanism. Furthermore, our biochemical analyses reveal the importance of the transmembrane helix of the substrate in its recognition by GlpG. We find that substrate's TMH contributes significantly to the binding affinity to the enzyme, hence to cleavage efficiency, but it also plays a role in cleavage site presentation to the active site of GlpG. Moreover, we identify four residues in transmembrane domains 2 and 5 of GlpG, whose mutations shift substrate specificity of GlpG, which means that they most likely interact with the topologically corresponding region of the substrate – its transmembrane helix. Taken together, our data support the model of the enzyme-substrate interaction where the initial contact between the two occurs at an intramembrane exosite of GlpG, which facilitates and is followed by the binding of the scissile-bond region of the substrate into rhomboid active site in a sequence-dependent manner. To obtain a full structural description of the intramebrane proteolytic reaction, our next goal is to solve the structure of GlpG in complex with a full transmembrane substrate.

P16

POTENTIAL ROLE OF MGM101 PROTEIN FROM *CANDIDA PARAPSILOSIS* IN MAINTENANCE OF MITOCHONDRIAL TELOMERES

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The mitochondrial DNA (mtDNA) molecules are organized into nucleoprotein complexes termed nucleoids. These structures play a crucial role in th mtDNA maintenance and segregation, but their organization and the role in the mtDNA replication and dynamics remain unknown. In this study we investigated the protein Mgm101 associated with the nucleoids in yeast mitochondria. In *Saccharomyces cerevisiae* Mgm101 protein is essential for the maintenance of the wild-type circular-mapping mtDNA and it is thought to be involved in the initiation of mtDNA replication and the repair of oxidatively damaged mtDNA molecules. To analyze role(s) of Mgm101 in yeast with a linear mitochondrial genome employing a distinct replication strategy, we investigated biochemical properties of Mgm101 protein in *Candida parapsilosis*. Here we demonstrate that although *CpMGM101* complements defects associated with *mgm101-1*^{ts} mutation in *S. cerevisiae* and is associated with mitochondrial nucleoids in *C. parapsilosis*, it exhibits several peculiar differences compared with its *S. cerevisiae* counterpart. Interaction of *Cp*Mgm101 with DNA is sequence non-specific and the protein is able to bind to single- and double-stranded DNA or DNA with blocked 3' end. *Cp*Mgm101 forms homooligomers with molecular weight of about 85-kDa corresponding to a trimer. Results from transmission electron microscopy of the complex of *Cp*Mgm101 with DNA demonstrated that *Cp*Mgm101 forms homogeneous trimeric ring-shaped structures at single-stranded ends of mitochondrial telomere-like sequence. In addition, we used small-angle X-ray scattering analysis for determination of the quaternary structure of *Cp*Mgm101 trimer and built an *ab initio* model of Mgm101 oligomer and its complex with DNA.

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P17

SPECIFIC RECRUITMENT AND ACTIVATION OF MYOSIN VI BY ITS CELLULAR PARTNERS

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Myosin VI is unique among all of the myosin superfamily members expressed in metazoans in that it traffics in the reverse direction on actin. Reverse directionality obviously creates the potential to serve unique roles in cells that cannot be accomplished by any other myosin [1]. Important studies have identified several partners for this myosin [2,3] and have highlighted its role in the maintenance of actin-rich ultra-structures of the cell and in different steps of membrane traffic necessary for cell division, cell migration and autophagy [4,5]. Given its directionality, it has been proposed that Myosin VI could facilitate endocytosis by serving a transport role of endosomes away from the plasma membrane [6]. For most of its suggested cellular functions, it may in fact function as a load-dependent anchor that can pull on structures and hold them in close opposition to actin filaments [7]. The unconventional myosin VI motor can exist as both a monomeric and dimeric form and some partners are thought to promote dimerization to activate the motor.

The main challenge of this project is to investigate how features of Myosin VI and how its binding partners can specify its function as either an anchor, a transporter or a membrane tensioning/shaping motor in diverse cellular processes. To describe the active form of Myosin VI and how different partners shape the motor upon its recruitment for specific actions depending on the cellular context, high-resolution structures of its C-terminal globular domain bound to different partners will be determined. The goal is thus to gain insights on the structural and mechanistic features that specify motor activity and on the precise

architecture of the partner/motor complexes to design *in vitro* and cell studies that will identify the specific roles the motors serve in participating in different cellular processes.

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P18

STRUCTURE AND MECHANISM OF BACTERIAL ABC-TYPE ECF TRANSPORTERS

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Energy coupling factor (ECF) transporters are the new class of ATP- binding cassette (ABC) transporters, mainly found in prokaryotes and also in Gram positive bacteria. The ECF transporters are responsible for vitamins and essential micronutrients uptake. Recently, two crystal structures of ECF transporters from *Lactobacillus brevis* have been reported [1] [2]. Both reported structures are substrate and nucleotide free and shows overall similar structure. The stoichiometry ratio for this complex is 1:1:1:1 for Ecf A, Ecf A', Ecf T and Ecf S . EcfA and Ecf A'are nucleotide binding subunit, EcfT is an energy coupling module and Ecf S is the substrate binding protein. The S-components can bind different substrates and were also crystallized with the respective substrates [3][4][5]. Very intriguingly, the position of S-component in the full complex is very unusual as it is nearly parallel to the membrane plane. But to analyze how relevant this position and to understand transport mechanism, more different states are essential, and this is our aim to get the high resolution crystal structures of ECF transporters in different states (substate bound-nucleotide bound, etc.) by X-ray crystallography. We will present preliminary results and our progress in this continuation.

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P19

STRUCTURAL INSIGHT INTO INTERACTION BETWEEN STEFIN B AND AMYLOID BETA PEPTIDE

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Stefin B is a cysteine protease inhibitor (cystatin). Its structures have already been determined [1-4]. It has been reported that it localizes to amyloid plaques of various origin [5]. We have studied the interaction between stefin B and amyloid beta peptide (Aß peptide). The inhibition of Aß peptide amyloid fibril formation (due to interaction between them) is stefin B oligomeric state dependent. Stefin B Y31 isoform, which is dimer only (domain-swapped dimer), inhibits Aß peptide amyloid fibril formation completely. We have also been able to define the stoichiometry of the complex by mass spectrometry. They interact in the ratio 1:1 (one stefin B dimer with one Aß peptide molecule). Stefin B E31 isoform (which is so called wild type protein) forms set of oligomers, from monomers, dimers and tetramers to even higher oligomeric species. Only tetramers (consist of two domain-swapped dimers) inhibit Aß peptide amyloid fibril formation, higher oligomers show partial inhibition, while monomers and dimers have almost no effect on the process [6]. The structure of domain-swapped dimer is not the one inhibiting the process, since we tried to see the same effect on Aß peptide amyloid fibril formation by the domain-swapped dimers of other cystatins [7].

We will try to determine the structure of the complex between stefin B Y31 isoform and Aß peptide to further clarify the interaction.

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P20

MINING THE ZINC PROTEOME – INTERPROTEIN ZINC BINDING SITES

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Although zinc is the most abundant transition metal in the cell, the information on zinc-metalloproteins is limited to DNA binding structures and a few enzymes, where Zn^{2+} ion plays either structural or catalytic role [1]. Mining the data from structural databases on interprotein metal binding sites, where Zn^{2+} is a mediator of ternary complex, is still a challenge, since many of such interactions could be unstable or transient, as most likely they play a regulatory role in living cells. Furthermore, a number of structures contains Zn^{2+} ion as an crystallization artifact, with no biological importance [2]. Searching sequence databases is also inefficient, since metal-binding residues are derived from two or more protein subunits. All things considered, the only way to study interprotein zinc-binding sites is to determine them experimentally, followed by verification of their biological significance.

Hence, we plan to identify and characterize metalloproteins capable of binding to immobilized Zn^{2+} ion with organic and peptide chelators on IMAC-like column, serving as molecular "baits" for proteins with surface zinc-binding site. All proteins selected on the column will be identified by MudPIT technology [3]. Their Zn^{2+}-binding thermodynamics and possible Zn^{2+}-induced structural changes will be investigated, as well as structure, stability, or biological function in case of previously unknown proteins. The most promising metalloproteins will be characterized by biocrystallography and/or solution NMR.

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