binding protein (SBP) component of the high-affinity manganese ABC-type transport system from the cyanobacterium *Synechocytis* sp.PCC 6803 (*Syn*) to 2.9Å by combined MAD/molecular replacement. The metal ion binding site containing Mn^{2+} has a distorted tetrahedral geometry, with Glu220 and Asp295 situated closer to the ion than His89 and His154. This geometry may be due to a disulfide bond between Cys219 and Cys268.

Sequence homology comparisons show that only putative cyanobacterial manganese SBPs contain these conserved cysteines, suggesting the MntC has a special role in manganese mobilization into the photosynthetic apparatus. We show that reduction of the disulfide bond *in vitro* releases bound manganese. We propose that *in vivo* reduction of the disulfide bond by a redox active protein, alters the position of Glu220 thereby modifying the affinity towards the bound metal. We have identified a homologous gene from the thermophilic cyanobacterium *T. vulcanu*s. The final full length clone (GenBank accession code AAV65297) was sequenced and found to be 54% homologous with the *Syn mntC* and it contains the conserved cysteins. The gene was cloned into an expression vector and the expressed protein has been purified and crystallized. Preliminary ICP-MS measurements show that this protein binds Mn^{2+}, and we thus propose that this gene encodes for the MntC homolog in these species. We are now in the process of fine tuning the crystallizing conditions of this protein in order to determine its crystal structure.

Keywords: ABC transporter system, photosynthesis, redox

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3D Structure Determination of the Cpn60-2 Protein from *Mycobacterium tuberculosis*

Anat Shahar[1], E. Dobrovetsky[1], M. Melamed-Frank[1], Y. Kashi[2], N. Adir[1], [1]*Department of Chemistry and Institute of Catalysis Science and Technology, Technion, ISRAEL.* [2]*Department of Biotechnology and Food Engineering, Technion – ISRAEL.* E-mail: manat@tx.technion.ac.il

Heat shock proteins (HSP) are a large super-family of proteins which are highly conserved throughout evolution and are necessary for the correct folding of proteins inside the cell. Cpn60-2 from *Mycobacterium tuberculosis (Mt)* belongs to the HSP60 family which is also called Chaperonins. These proteins are involved in folding of a large number of proteins in an ATP dependent manner. In addition, Cpn60-2 is one of the most immunogenic of all *Mt* proteins, eliciting a significant immune response when whole cells are used in vaccination. Due to its high immunogenity, Cpn60-2 has a medical importance.

We have isolated Cpn60-2 by over expression of the cloned gene encoding for Cpn60-2 into pQE60 vector to enable metal chelate affinity purification. The recombinant protein was shown to protect *E. coli* cells from heat shock stress. Crystals of His-Cpn60-2 grow in 2-14 days and were improved by different methodologies. The crystallization conditions are 10% 2-propanol, 20% PEG 4K, 0.1M Hepes pH 7.5. Crystallographic analysis shows the crystals to be monoclinic ($P2_1$) with unit cell parameters of a=58.460Å, b=112.209Å, c=77.5Å, β=95.482° and containing a dimer in the asymmetric unit. We have collected a complete 2.75Å data on ESRF beamline ID14-1. The structure has been solved by the molecular replacement method using a lower resolution model recently published. At present, the structure of the Cpn60-2 has been refined to R/R_{free} factors of 23.69/31.79%.

Keywords: protein crystallography chaperones, stress, bacteria

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Crystal Structure of OXA-24, a Novel Class D β-lactamase with Carbapemenase Activity

Elena Santillana[a], Germán Bou[b], Antonio Romero[a], [a]*Centro de Investigaciones Biológicas, CSIC, Ramiro de Maeztu, 9, 28040-Madrid (Spain).* [b]*Complejo hospitalario universitario Juan Canalejo; A Coruña (Spain).* E-mail: esh@cib.csic.es

One of the main concerns in Medicine is the presence of microorganisms causing infections which harbour antibiotic resistance mechanisms. Among the different mechanisms associated with antibiotic resistance, much attention is currently being focused on the presence of β-lactamases. Oxacillinases are Ambler class D β-lactamases that possess active site serine groups like class A and class C β-lactamases. These enzymes are characterized by their hydrolytic activity for isoxazolyl, penicillins, methicillin and aztreonan significantly, sparing most extended-spectrum cephalosporins.

Six oxacillinases with carbapenem-hydrolyzing activity have been sequenced from *Acinetobacter baumannii*. OXA-24 shares 40% identity with a group of oxacillinases consisting of OXA-5, -7, -10 and -11. Despite these similarities, some interesting and differing features exist between previous oxacillinases and OXA-24. Thus, OXA-24 lacks hydrolytic activity against oxacillin, cloxacillin, and methicillin but displays a moderate level of resistance to carbapenemes. Crystals of OXA-24 from *A. baumannii* were grown using the vapour diffusion technique. They belong to space group $P4_12_12$, with cell dimensions a=b=102.2 Å, c=86.1 Å and one molecule in the asymmetric unit, which diffracted beyond 2.5 Å. It was possible to locate the position of the enzyme in the unit cell using molecular replacement with the coordinates of OXA-10 as a search model. The three dimensional structure of OXA-24 could establish the molecular basis to explain the relevance of the substitutions in its hydrolytic activity. The structure is currently undergoing refinement.

Keywords: β-lactamases, antibiotic resistance, protein crystallography

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Crystallographic Studies of Human Methionine Adenosyltransferase (MAT)

Christina L. Rush, M. Kotb, S. White, *Molecular Sciences, University of Tennessee, Memphis, TN 38163 and Department of Structural Biology, St. Jude Children's Research Hospital, Memphis, TN 38105.* E-mail: christina.rush@stjude.org

Methionine adenosyltransferase (MAT) catalyzes the formation of the key enzymatic cofactor, S-adenosylmethionine (AdoMet), from ATP and methionine. AdoMet is important because of it's involvement in various biochemical pathways including polyamine synthesis as well as the methylation of nucleic acids and lipids. MAT activity, in mammals, is regulated by a β subunit which lowers the Km of MAT for L-methionine and renders the enzyme more susceptible to feedback inhibition by AdoMet. This regulatory subunit has been modeled and is currently in crystallization trials. Furthermore, to better understand the role of the β-regulatory subunit, the three dimensional structure of the human MAT complexed to its β subunit will be determined through crystallographic studies.

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Optimization of Crystallization of the Flavoprotein WrbA by using Additives

Julie Wolfova[a], Jannette Carey[b], Ivana Kuta Smatanova[a,c], [a]*Institute of Physical Biology, University of South Bohemia Ceske Budejovice, Zamek 136, 373 33 Nove Hrady, Czech Republic.* [b]*Chemistry Department, Princeton University, Washington Rd and William St, Princeton, NJ 08544-1009, USA.* [c] *Institute of Landscape Ecology, Academy of Science of the Czech Republic, Zamek 136, 373 33 Nove Hrady, Czech Republic.* E-mail: julinka.w@tiscali.cz

Tryptophan (W)-repressor binding protein A, WrbA, is an *Escherichia coli* stationary-phase protein. Its predicted influence on the binding interaction between DNA and the tryptophan repressor (TrpR) wasn't proved [1] and thus its physiological function remains unclear. According to sequence analysis and homology modelling, WrbA was identified as the founding member of a new protein family, sharing the open, twisted α/β fold typical for flavodoxins [2]. The biochemical and biophysical studies of purified WrbA apoprotein [1]

revealed some unique properties of the WrbA family: lower affinity for its cofactor - the flavin mononucleotide (FMN) - and the multimeric character of protein in solution. WrbA protein is apparently the first characterized case in which multimerization is associated directly with the flavodoxin-like domain itself. In all other multimeric flavodoxins the flavodoxin-like domain is fused to a multimerization domain [3]. WrbA protein and its homologs thus present a unique family among the typical flavodoxin-like proteins. Structural analysis may aid in understanding these unique properties and may reveal the physiological role of WrbA in the living organisms. This was a motivation for searching of diffraction-quality crystals.

WrbA apoprotein crystals grown by standard and advanced crystallization techniques consisted of twinned plates. The quality of crystals was successfully improved by using additives and gelling protein solution for crystallization. Crystals suitable for X-ray diffraction measurement were measured at synchrotron DESY, beamline X13 (Hamburg), at cryotemperature. Crystals diffracted to 2.2 Å. Solving of protein structure is in progress.

Limited proteolysis [4] of WrbA apoprotein led to preliminary identification of folded substructures and flexible parts of protein structure.

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Keywords: flavoproteins, macromolecular crystallization, optimization

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Improving the Growth of Biomacromolecular Crystals for Neutrons and X-rays

Monika Spano-Budayova, Francois Dauvergne, *EMBL/ILL Grenoble, 6, rue Jules Horowitz, BP 181, 38042 Grenoble Cedex 9, France*. E-mail: spano@embl-grenoble.fr

The bottleneck in biomacromolecular crystallography still remains the growth of single crystals with good crystal quality and size. Whereas development of third generation synchrotron sources has allowed X-ray protein structures to be solved from crystals of a few 10^{-4} mm^3, a major hurdle to neutron protein crystallography is that unusually large crystals (~1mm^3) are required to compensate for the weak flux of available neutron beams [1].

We have invented a novel method for the crystallization of proteins allowing alteration and optimisation of the conditions in order to get crystals that are appropriate for X-ray and neutron diffraction analysis. We propose a rational physico-chemical approach of crystallization based on knowledge of the phase-diagram [2]. We have constructed a device, which enables the phase diagram to be investigated, the nucleation and crystal growth of biological macromolecules to be controlled, and the solubility of seeded H/D-labelled biological macromolecule crystals to be manipulated. This semi-automated crystallization tool is also intended for *in situ* observation by optical microscopy and allows sequential image acquisition, processing and storage. We report here our first experimental results obtained with "real" protein systems.

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Keywords: phase-diagram, crystal growth, solubility

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Crystallization of the TOM Complex from *Neurospora crassa* together with Monoclonal Antibodies

Patrick Schreiner[a], Simone Schmitt[a], Michael Groll[a], [a]*Adolf-Butenandt-Institut of Physiological Chemistry, University of Munich, Germany*. E-mail: patschrein@gmx.de

The majority of mitochondrial proteins is nuclear-encoded and synthesized in the cytosol. Transport of these proteins into mitochondria is mediated by translocation machineries located in the outer and inner membrane. The multisubunit TOM complex (Translocase of the Outer membrane of Mitochondria) is responsible for protein sorting and translocation of proteins across the outer mitochondrial membrane. Our aim is crystallization and structure determination of the TOM complex in order to elucidate its architecture, functional mechanism and regulation. The low number of existing crystal structures of membrane proteins reflects the difficulties in obtaining good quality crystals of this class of proteins. The TOM complex can only be isolated in its native state from the outer membranes of mitochondria, which makes its purification a difficult and challenging task.

The filamentous fungi *Neurospora crassa* turned out to be an excellent model organism for studying the TOM complex due to its fast growth rate and simple manipulation procedures. We have been able to purify and crystallize the TOM complex from *Neurospora*, comprising Tom40, the major pore forming protein, Tom22, Tom5, Tom6 and Tom7. The obtained crystals already diffract to a resolution limit of 6 Å. Currently, we have been working on improvement of the reflection qualities of the TOM complex crystals. Crystallisation in complex with antibody fragments has been reported to facilitate the crystallization of membrane proteins and to improve the diffraction quality of such crystals. The binding of Fv or Fab antibody fragments to the epitops on the protein surface enlarges the hydrophilic part of integral membrane proteins, thereby providing additional surface for crystal contacts. We are working on the production of murine monoclonal antibodies against the TOM complex to improve the resolution of the TOM crystals.

Keywords: crystallization, membrane proteins, antibodies

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Crystallisation and Functional Analysis of Procarytic and Eukaryotic Rhomboid Proteases and Hsp70 Chaperones

Christian Körner, Martin Sichting, Michael Groll, *Institute of physiological cemistry, University of Munich. Butenandtstr. 5, 81377 Munich, Germany*. E-mail: christian.koerner@bio.med.uni-muenchen.de

Protein degradation and processing is an essential cellular process, which is performed by various intracellular proteases. Most proteases are localized in the cytosol. However, there exist also few examples of membrane proteases, which differ in their architecture, mechanism, regulation and function. Recently a new family of transmembrane proteases termed rhomboid proteases was discovered. They cleave their substrates within their transmembrane domains. Members of this family belong to the class of serine proteases. They are distributed among all three kingdoms of life and are located to the inner membrane of bacteria, to the membrane of the Golgi apparatus and to the inner membrane of mitochondria. All rhomboid proteases possess multiple transmembrane domains. We try to purify and crystallise the rhomboid protease from various bacteria and archaea to reveal its proteolytic mechanism. Additionally we are developing an in vitro assay to identify possible substrates of rhomboid proteases and look for knock out phenotypes.

The second main subject of our work is revealing the three dimensional structure and possible mechanism of prokaryotic and eukaryotic members of Hsp70 chaperones. Although the structure of different domains of Hsp70 proteins have been already published, no crystal structure of the whole protein exists so far. The orientation and spacial arrangement of the N-terminal nucleotide binding domain and the C-terminal substrate binding domain in the structure of the whole