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

molecule will provide insights for the mechanism of action of this class of proteins.

Keywords: proteases, rhomboid proteases, Hsp70 chaperones

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Preliminar Diffraction Study of the Full-lengh Protein Hexokinase 2 of *Saccharomyces cerevisiae*

Laura Roces[a], Pilar Herrero[b], Fernando Moreno[b], Santiago García-Granda[a], [a]*Departmento de Química Física y Analítica.* [b]*Departamento de Bioquímica y Biología Molecular, Universidad de Oviedo. Spain.* E-mail: lrf@fq.uniovi.es

Hexokinase 2 (Hxk2) is, with the protein Mig1, the mayor mediator of glucose repression in *Saccharomyces cerevisiae*. It has been recently reported that both proteins interact to generate a repressor complex located in the nucleus of *S. cerevisiae* during growth in glucose medium [1]. The Lys6-Met15 decapeptide of Hxk2 was found to be necessary for interaction with the Mig1 protein.

The crystal structure of a fragment of Hxk2 containing residues 18-486 is deposited in the Protein Data Bank [2], though there is no structural information about the first 17 residues of the N terminus, where the Hxk2 decapeptide interacting with Mig1 protein is contained. Moreover, it is in this N terminus where the specific regulatory capacity of *S. cerevsiae* hexokinase 2 resides. The aim will be to define the three-dimension full-lengh protein Hxk2 fold, in order to get new hits and be able to explain the formation of the repression complex.

We report here the crystallization of the full-lengh protein Hxk2 using the microbath under oil method and the preliminar diffraction patterns obtained. The *S. cerevisiae* Hxk2 crystals have an hexagonal plate shape (different from the elongated bipyramidal shape reported for the Hxk2 fragment). The crystal dimensions are about 0.2 x 0.2 x 0.05 mm.

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Keywords: crystallization, protein folding, regulation

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A new Crystal Form of the SR Ca^{2+}-ATPase in the Ca_2E1 State

Anne-Marie Lund Jensen, Thomas Lykke-Møller Sørensen, Jesper Vuust Møller, Poul Nissen, *Dep. of Molecular Biology, University of Aarhus.* E-mail: amlj@bioxray.dk

The sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) is responsible for the re-uptake into the sarcoplasmic reticulum store of cytosolic Ca^{2+} released during muscle contraction. SERCA and the other cation pumps belong to the P-type ATPase family, whose functional cycle is fuelled by ATP hydrolysis via formation of a covalent aspartyl-phosphoanhydride intermediate. Several crystal structures representing different states of the functional cycle of the Ca^{2+}-ATPase have now been solved as recently updated [1]. The first structure to be solved was the Ca_2E1 state by Toyoshima et al. [2], which in comparison to later determined structures reveals an open arrangement of the cytoplasmic domains. We have obtained a new crystal form of the Ca_2E1 state in space group P1 with two molecules in the unit cell. Data were collected from a double crystal, allowing the processing and scaling of two independent datasets at 3.0 Å resolution, and phases from molecular replacement were refined by averaging. The structure appears to be almost identical to the original Ca_2E1 structure, indicating that the open domain arrangement is not the result of crystal packing effects. This provides further support to the use of this structure in describing the mechanism of activation upon binding of cytosolic Ca^{2+}.

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Keywords: Ca^{2+}-ATPase, crystallization macromolecular, reaction mechanisms

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Phase Behavior and Protein Interactions

Neer Asherie, *Department of Physics and Department of Biology, Yeshiva University Belfer Hall 1412 2495 Amsterdam Avenue, New York, NY 10033-3312, USA.* E-mail: asherie@yu.edu

Proteins in solution crystallize, form coexisting liquid phases, aggregate and gel. As a case study of protein phase behavior, I will present the gamma crystallins, a family of proteins from the mammalian lens. I will describe the phase behavior of several native and mutant gamma crystallins and talk about the connection between this behavior and human cataract.

The phase behavior provides information about the interactions between proteins. I will show that the general features of the phase diagram of globular proteins, such as metastable liquid-liquid coexistence, can be explained by modeling proteins as simple colloids, i.e. spherical particles with short-range, isotropic attraction. I will also discuss the aspects of the phase behavior which require more realistic models and explain how such models may be useful for protein crystallization.

Keywords: phase diagram, liquid-liquid phase separation, crystal solubility

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Crystallization Study of Photosynthetic Proteins from *Pisum sativum*

Ivana Kuta Smatanova[a,b], Jose A. Gavira[c], Pavlina Rezacova[d], Frantisek Vacha[a,e], Juan M. Garcia-Ruiz[c], [a]*Institute of Physical Biology, University of South Bohemia Ceske Budejovice, Nove Hrady, Czech Republic.* [b]*Institute of Landscape Ecology AS CR, Nove Hrady, Czech Republic.* [c]*Laboratorio de Estudios Cristalografico, Edificio BIC-Granada, Spain.* [d]*Institute of Molecular Genetics AS CR, Prague, Czech Republic.* [e]*Institute of Plant Molecular Biology AS CR, Ceske Budejovice, Czech Republic.* E-mail: ivanaks@seznam.cz

Crystallographic studies of photosystem II (PSII) proteins have given the first description of the structure of PSII, but these models are not absolutely complete as yet. The fact that membrane proteins are often unstable, highly temperature and light sensitive together with their complicated composition are responsible for difficult crystal growing and solving their structure.

Here we report a new approach for crystallization of monomeric photosystem II core complex using the counter-diffusion technique. The core complex of PSII was isolated from *Pisum sativum*, purified and prepared for crystallization trials. The protein crystallized in green needle-shaped crystal form from PEG4000 and MPD in MES pH 6.50 at 291-293K. Protein character of PSII crystals was confirmed by laser spectroscopy, and by X-ray diffraction measurement at the synchrotrons in Hamburg and Grenoble.

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Keywords: membrane proteins, photosystem II, macromolecular crystallization

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The Crystal Structures of the Pseudouridine Synthases RluC and RluD

Kenji Mizutani[a], Yoshitaka Machida[a], Kanako Sugiyama[a], Satoru Unzai[a], Sam-Yong Park[a], Jeremy R. H. Tame[a], [a]*Protein Design Laboratory, Yokohama City University, Suehiro 1-7-29, Tsurumi, Yokohama 230-0045, Japan.* E-mail: mizutani@tsurumi.yokohama-cu.ac.jp

The most frequent modification of RNA, the conversion of uridine bases to pseudouridines, is found in all living organisms and often in highly conserved locations in ribosomal and transfer RNA.

RluC and RluD are homologous enzymes which each convert