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.

[1] Ahuatzi D., Herrero P., de la Cera T., Moreno F., *J. Biol. Chem.*, 2004, **279**, 14440. [2] Kuser P.R., Krauchenco S., Auntenes O.A.C., Polikarpov I., *J. Biol. Chem.*, 2000, **275**, 20814.

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+}.

[1] Olesen C, Sørensen, et al., *Science*, 2004, **306**, 2251. [2] Toyoshima C., et al., *Nature*, 2000, **405**, 647.

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

three specific uridine bases in *Escherichia coli* ribosomal 23S RNA to pseudouridine: bases 955, 2504, and 2580 in the case of RluC and 1911, 1915, and 1917 in the case of RluD. Both have an N-terminal S4 RNA binding domain. While the loss of RluC has little phenotypic effect, loss of RluD results in a much reduced growth rate. We have determined the crystal structures of the catalytic domain of RluC, and full-length RluD. The S4 domain of RluD appears to be highly flexible or unfolded and is completely invisible in the electron density map. Despite the conserved topology shared by the two proteins, the surface shape and charge distribution are very different. The models suggest significant differences in substrate binding by different pseudouridine synthases. [1]

[1] Mizutani K., Machida Y., Unzai S., Park S.-Y., Tame J.R.H., *Biochemistry*, 2004, **43**, 4454-4463.

Keywords: RNA-binding proteins, structure, protein crystallography

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Crystal Structure of PilF from *Pseudomonas aeruginosa*

Kyunggon Kim, Jongkil Oh, Youngsoo Kim, *Division of Genomic Medicine, College of Medicine, Seoul National University. 28 Yongon-Dong, Seoul, 110-799 South Korea.* E-mail: biolab@snu.ac.kr

The tetratrico peptide repeat (TPR) is a structural motif present in a wide range of proteins. It mediates protein-protein interactions and the assembly of multiprotein complexes. TPR motifs have been identified in various different organisms, ranging from bacteria to humans. Proteins containing TPRs are involved in a variety of biological processes, such as cell cycle regulation, transcriptional control, mitochondrial and peroxisomal protein transport, neurogenesis and protein folding. Type IV pilus biogenesis protein, PilF of *Pseudomonas aeruginosa* consists of 253 amino acids and makes up 3 tandem TPR motifs. It is known to require for correct fimbrial biogenesis. We could express the PilF of *Pseudomonas aeruginosa* in an *E.coli* expression system and produced selenomethionie-substituted crystal, which diffract to 2.5 Å. It belongs to P222 space group and unit cell is a=68.4 Å, b=70.0 Å, c=138.1 Å. This structure of the full sized TPR protein will lead to the first step in study of TPR interaction.

[1] Stover C.K., Pham X.Q., *Nature*, 2000, **406**, 31. [2] Watson A.A., Alm R.A., *Gene*, 1996, **180**, 49.

Keywords: PilF, TPR domain, crystal structure

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Crystallization and Data Collection of *Xanthomonas citri* Maltose-Binding Protein

Andrea Balan[a], Cristiane S. Souza[a], Luís Carlos S. Ferreira[a], Beatriz G. Guimaraes[b], Javier F. Medrano[b], João A. Barbosa[b], [a]*Department of Microbiology, University of São Paulo.* [b]*National Laboratory of Synchrotron Light, Campinas, Brazil.* E-mail: abalan@usp.br

In this work we report the crystallization and analysis of prelliminary data of the periplasmic maltose-binding protein (MBP) of the plant pathogen *Xanthomonas citri*, responsible for the canker disease affecting citrus plants all over the world. The 50,1 kDa protein has been overproduced in *Escherichia coli*, purified, and crystallized in complex with its substrate maltose. The crystallization of MBP using the sitting-drop vapour-diffusion method with PEG 20000 as precipitant is described. Crystals belong to the orthorhombic space group P2(1)2(1)2(1), with unit-cell parameters a = 105,83, b = 105,21, c = 262,32 Å. X-ray diffraction data were collected to a maximum resolution of 3.2 Å using a synchrotron-radiation source. Structure refinement is in progress.

Structural analysis, in combination with ongoing biochemical characterization, will assist the elucidation of the structure-activity relationship in regulating the uptake of maltose in this bacteria.

Keywords: MBP, *Xanthomonas citri*, crystallization

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Crystal Structure of Ubiquitin-like Domain of Murine Parkin

Koji Tomoo[a], Yasuhiro Mukai[a], Seiji Okubo[b], Heisaburo Shindo[b], Toshimasa Ishida[a], [a]*Department of Physical Chemistry, Osaka University of Pharmaceutical Sciences.* [b]*School of Pharmacy and School of Life Science, Tokyo University of Pharmacy & Life Science. Japan.* E-mail: tomoo@gly.oups.ac.jp

Parkin, which has been identified ubiquitin ligase, is the gene product of autosomal recessive juvenile parkinsonism (AR-JP). Parkin which consists of 464 amino acid residues has three domains; an N-terminal ubiquitin-like domain(ULD) and two RING finger-like domains. Parkin has important role in recognition of the target proteins and addition of the ubiquitin in proteasome system. In order to elucidate the fully function of Parkin, we have started the structure analysis of Uld of murine Parkin.

The recombinant murine Uld was expressed as inclusion body from *E.coli.* system. After refolding and purification, we crystallized Uld by hanging-drop vapor diffusion method. Under the condition of 0.1M acetate buffer(pH4.5) and 3M NaCl as a precipitant. The crystal belong to the hexagonal system, with unit cell dimensions of a=b=45.57 Å, c=64.75 Å, γ=120°. Diffraction data were collected up to 1.8 Å resolution at beam line BL24XU of SPring-8. The initial structure was determined by molecular replacement by using the solution structure of Uld as start model. Refinement of structure is currently in progress.

Keywords: ubiquitin system, crystallization, structure analysis

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SERCA1a and Phospholamban Cocrystallisation

Laura Fonso[a], Elisabetta Dalla Libera[a], Ernesto Damiani[c], Roberto Battistutta[a,b], Giuseppe Zanotti[a,b], Oriano Marin[a], Ernesto Carafoli[a], [a]*VIMM, Padua.* [b]*Department of Chemistry, University of Padua.* [c]*Department of Experimental Biomedical Sciences, University of Padua.* E-mail: laura.fonso@unipd.it

The Sarco(Endo)plasmic Reticulum Ca^{2+}-ATPase (SERCA) is a membrane Ca^{2+}-pump with a crucial role in the relaxation/contraction mechanism of the muscular cells.

SERCA1a has been purified from Sarcoplasmic Reticulum vesicles, isolated from rabbit fast twitch muscles. Ca^{2+}-ATPase concentration was increased within SR vesicles using different techniques: high ionic strength was employed to eliminate myosin and many membrane proteins and vesicles were treated with EDTA with the same purpose. Furthermore SR membranes have been purified by an extraction with low concentration of deoxycholate. Purified membranes were solubilised using a non-ionic detergent, $C_{12}E_8$, at 1.8 mg/ml final concentration. The supernatant was directly used for crystallization. Crystals of E1 SERCA1a grew in few days at 19°C with the hanging drop technique, using a precipitant solution containing: 15% (w/v) PEG 6000, 4% (v/v) tert-butanol, 15% (v/v) glycerol, 5 mM β-mercaptoethanol, 200 mM sodium acetate [1].

Synthesized PLB was solubilised in a solution containing chloroform/methanol with a ratio of 1/2 to a 31 mg/ml final concentration. SERCA1a and PLB were mixed to a 1:5 final molar ratio. Cocrystals grew in approximately a week, using the same precipitant utilized in SERCA1a crystallization.

[1] Sorensen T.L., Moller J.V., Nissen P., *Science*, 2004, **304**, 1672.

Keywords: SERCA1a, phospholamban, cocrystallisation

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Structural Studies on Collagen binding Integrin αI Domains

Anna-Maria Brandt, J. Santeri Puranen, Yvonne Nymalm, Tiina Salminen, Mark S. Johnson, *Department of Biochemistry and Pharmacy, Åbo Akademi University, Turku, Finland.* E-mail: abrandt@abo.fi

Integrins are a large family of cell adhesion receptors that mediate