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*

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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

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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

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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*

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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

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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