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

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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

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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

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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