SMP: Protein

Project: Structure Determination of Human Proteins

Udo Heinemann - Max Delbrück Center for Molecular Medicine Berlin-Buch (MDC) – heinemann@mdc-berlin.de

Introduction

In the context of the Systematic Methodological Platform (SMP) "Protein" the Protein Structure Factory (PSF) aims at systematically investigating the tree-dimentional structures of medically relevant human proteins as well protein-protein, protein-DNA or protein-ligand complexes *via* X-ray analysis. Crystal structures of proteins are a key to understanding the molecular mechanisms of disease and may serve as starting points for rational drug design. In the course of this work, the molecular function of these proteins will be illuminated by linking the structural data with the proteomics data from SMP "Protein" partner laboratories. We are primarily interested in elucidating the mode of protein-protein interactions involved in disease-related cellular signalling.

The task includes high-throughput protein crystallization in 96-well format, experimental diffraction data collection at the third-generation synchrotron BESSY and generation/refinement of atomic protein models based on the collected data.

Screen development, storage, visualization and crystal annotation is accomplished to a large extent with the help of robots, which allow an extensive throughput. All experiments are precisely documented in a database. Fixed Standard Operating Procedures (SOP) are used to perform manual experiments.

Results/Project Status

Protein target lists were assembled and proteins or expression clones already available at the Protein Structure Factory were identified. Protein targets suggested by SMP "Protein" partner laboratories were annotated and categorized according to criteria that predict the success of crystal structure analysis. Thus, the absence of predicted transmembrane regions, extended coiled-coiled regions or unfolded segments from all suggested target proteins was verified by standard bioinformatics tools. Proteins of major interest containing these elements may be trimmed down to their ordered parts in order to permit structure analysis. The results of the bioinformatic analysis are included in the PSF database and accessible by all SMP "Protein" partner laboratories.

Some immediately available target proteins were expressed from prouction clones available at the PSF and purified for crystallographic studies, whereas most of the potential target proteins have to be cloned in suitable expression vectors by the SMP "Protein" partner Konrad Büssow.

The purified proteins were pre-characterized by dynamic light scattering and tested for optimal protein concentration by a self-developed precipitation test suitable for crystallization setups. Protein-protein interactions were verified by a native polyacrylamide gel electrophoresis and quantitated by mass spectrometry by the SMP "Protein" partner Johan Gobom.

Subsequently, the proteins undergo a primary crystallization screen with ~ 400 different buffer/precipitant conditions based on the commercially available screens from Hampton Research (USA). Once cryrstalline structures are found (needles, microcrystals, etc.) additional fine screens are designed and executed to improve the crystal quality and size for X-ray diffraction experiments using the PSF beamlines at the third-generation synchrotron ring BESSY, Berlin-Adlershof.

For protein co-crystallization, the same basic methodology is used, but the protein-protein or protein-ligand complexes are set up at the appropriate stoichiometry.

Crystallization

Three human proteins were crystallized, and their 3D structures were determined by X-ray diffraction methods. The first protein TPC6 (Figure 1) is a subunit of the transport protein particle (TRAPP) complex, a tethering complex playing an important role for the attachment of ER-derived transport vesicles at the Golgi membrane (1).

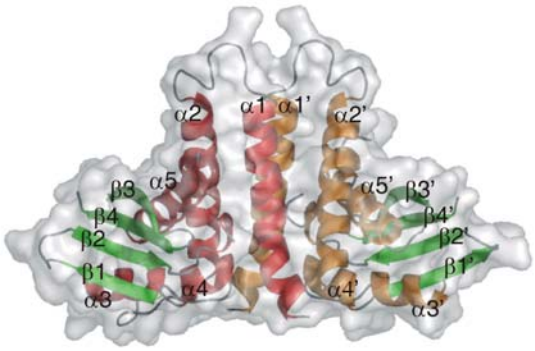

Fig 1: *Overall structure of TPC6. Schematic representation of labeled helices (red and orange) and strands (green) of the TPC6 dimer with a semitransparent molecular surface.*

The crystal structure of TPC6 allowed the computer modeling of a binary protein complex formed by TPC6 and the homologous TRAPP subunit BET3 (Figure 2). This TPC6/BET3 heterodimer is regarded the first functional subcomplex of TRAPP and of any tethering complex described so far.

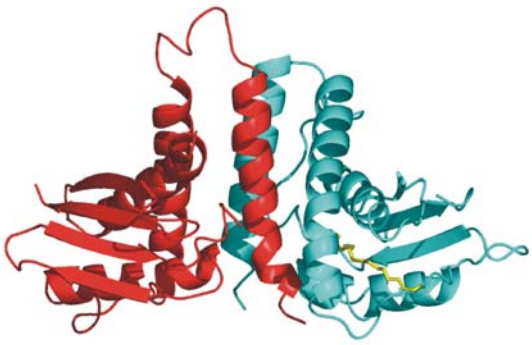

Fig 2: *Computer model of a TRAPP sub-complex formed by TPC6 (red) and palmitoylated BET3 (blue).*

The second protein, the N-terminally truncated product of the aortic preferentially expressed gene-1 (APEG-1) is a novel specific differentiation marker of arterial smooth muscle cells that possibly plays a role in regulating growth and differentiation of these cells (Figure 3) (2).

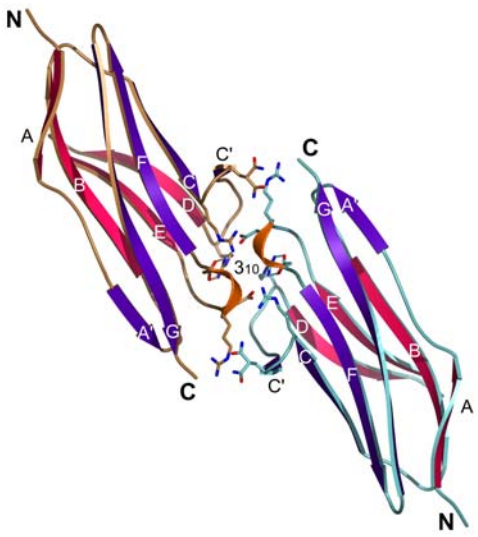

Fig 3: *Ribbon diagram of the ΔAPEG-1 homodimer. Bi-dentate salt bridges are represented by broken lines. Residues lined up at the binding interface are depicted.*

PTD012 is a third protein whose structure could be determined recently (Figure 4). PTD012 adopts a zinc-containing novel fold and displays ester hydrolase activity on the synthetic test substrate *p*-nitrophenyl acetate (3).

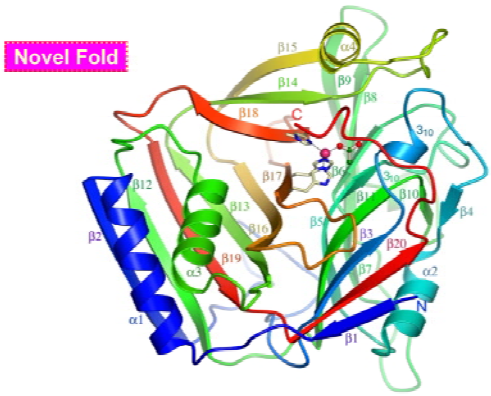

Fig 4: *Ribbon diagram of PTD012 colored by sequence position (progression through the visible spectrum from blue to red starting at the N-terminus). A bound acetate buffer molecule is depicted as a ball-and-stick model. A Zn^{2+} ion bound at a site predicted to support the enzymatic activity of PTD012 is shown as pink sphere.*

A further human protein, GADD45γ, forms nice crystals which were suitable for X-ray data collection (Figure 5). GADD45γ is a member of a family of genes that are induced by DNA damage and function in negative regulation of cell growth. This activity of GADD45γ is mediated by interactions with cellular proteins such as the proliferating-cell nuclear antigen (PCNA) and protein kinases. The structure determination is in progress.

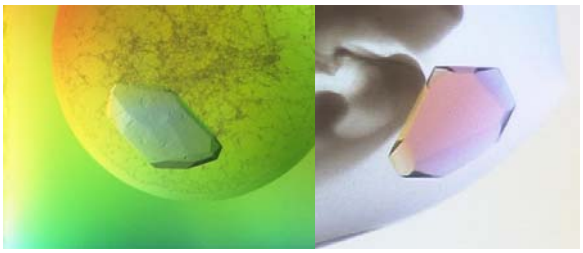

Fig 5: *Crystals of GADD45γ used for data collection.*

Additional five human proteins show crystals which have to be improved for data collection and structure analysis. First protein-protein co-crystallization experiments were started and are under progress.

Bioinformatics

At the website of the Protein Structure Factory, a web browser based demand was installed, which affords a rapid and confidential data exchange with SMP-Protein partner sites. All current target lists are available there for all project partners. For representation of all protein-protein interactions and the associated LIMS-based product monitoring, a new version of an advanced barcode tracker was developed. Partner molecules such as ligands or other proteins can be included in this barcode system. Thus, using this barcode system, any experiment for any target is trackable at any time.

Technical infrastructure

The high-throughput storage with a *nl*-droplet dispensing system is routinely in use. The existing camera system (necessary for annotation of crystallization experiments) could be improved and, together with a newly developed software, a local crystal observation is possible.

Outlook

In the next future, the crystallization of two further protein-protein complexes will be initiated. The expression and purification of additional 10 human proteins for the co-crystallization of five protein-protein complexes in the next round is planned and in preparation. Five single human proteins showed highly soluble small-scale expression and the clones are available for large-scale expression, purification and crystallization.

Furthermore, many more clones with soluble protein expression are expected from the SMP "Protein" partner Konrad Büssow for additional cocrystallization experiments.

Lit.: ***1.*** *Kümmel D, Müller JJ, Roske Y, Misselwitz R, Büssow K, Heinemann U (2005) Structure of the TRAPP subunit TPC6 suggests a model for a TRAPP subcomplex. EMBO Rep. 6: 787-793* ***2.*** *Manjasetty BA, Scheich C, Niesen FH, Roske Y, Götz F, Behlke J, Sievert V, Büssow K, Heinemann U (2005) X-ray structure and dimerisation of engineered human aortic preferentially expressed protein-1 (APEG-1). Submitted.* ***3.*** *Manjasetty BA, Büssow K, Fieber-Erdmann M, Roske Y, Gobom J, Scheich C, Götz F, Niesen FH, Heinemann U (2005) Crystal structure of Homo sapiens PTD012 reveals a zinc-containing novel fold. Submitted.*