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]

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Keywords: RNA-binding proteins, structure, protein crystallography

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Crystal Structure of PilF from *Pseudomonas aeruginosa*

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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

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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

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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

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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.

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Keywords: SERCA1a, phospholamban, cocrystallisation

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Structural Studies on Collagen binding Integrin αI Domains

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Integrins are a large family of cell adhesion receptors that mediate

cell-cell or cell-extracellular matrix interactions by bidirectional signaling. They are involved in a number of physiological processes such as platelet aggregation, inflammation, tumor metastasis and other diseases. The αβ heterodimeric glycoproteins are composed from 19 different α subunits and 8 different β subunits [1]. The collagen binding integrin family consists of four collagen receptors that have a common β1 subunit non-covalently bound either to α1, α2, α10 or α11 subunit. They all have a 200 amino acid inserted domain (I-domain) in the N-terminal region of the αsubunit, which is responsible for recognition of the ligand [2]. The αI domain folds into a "Rossman fold", which forms a metal ion-dependent adhesion site, referred to as MIDAS [3].

The crystal structure of α1I and α2I, and also the complex structure of α2I bound to a collagen-like peptide, have been solved [4,5,6]. Comparison of the α2I in complex with a collagen-like peptide (open conformation) and α2I without ligand (closed conformation) showed that conformational changes occur, when the ligand is bound [6].

We have shown that two peptides, CTRKKHDC and CARKKHDC, bind to α1I and competitively inhibit collagen binding. We have modeled the open conformation of α1I in complex with a collagen-like peptide and characterized the binding of the ligand and the structural changes that are caused [7]. Our aims are to further study the binding of collagen-like peptides to α1I and to characterize the conformational changes that might occur.

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Keywords: integrin, I-domain, collagen-binding

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The Structure of the ParC Subunit of Topoisomerase IV from *Streptococcus pneumoniae*

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Topoisomerases relieve torsional stress in DNA within cells by breaking one or both strands of DNA, then either winding or unwinding the DNA helix, followed by strand closure.

TopoIV (whose subunits are encoded by *ParC* and *ParE*) is a decatenating enzyme that resolves interlinked daughter chromosomes following DNA replication. TopoIV uses a double-strand passage mode as does Gyrase (which controls DNA supercoiling by relieving topological stress arising from the translocation of transcription and replication complexes bound to DNA) by a different mechanism: Gyrase wraps DNA around itself, while TopoIV does not. The difference in DNA-wrapping between Gyrase and Topo IV contributes to their different functional roles within cells.

The ParC subunit of TopoIV has been crystallised in tetragonal and hexagonal forms. Analysis of the tetragonal crystals (diffracted significantly better than hexagonal ones) showed that they were internally twinned. Present in the crystal are two *I222* crystal lattices aligned in opposite directions and forming pseudo 4-fold symmetry.

The structure of ParC subunit was solved by Molecular Replacement using a model build on the basis of the homologous GyrA subunit from *E.coli*[1] (1AB4 deposited in the PDB) as a model. Analysis of packing of the protein molecules in the unit cell shows screw axes || to ***c***, going in opposite directions.

[1] Cabral J.H.M., Jackson A.P., Smith C.V., Shikotra N., Maxwell A., Liddington R.C., *Nature*, 1997, **388**, 903.

Keywords: topoisomerase IV, ParC, twinning

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A Proposal to Engineer Protein Crystallization through Metal Ions

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A topic of current interest is engineering surface mutations in order to improve the success rate of protein crystallization. This report explores the possibility of using metal-ion-mediated crystal-packing interactions to facilitate rational design. *Escherichia coli* apo acyl carrier protein was chosen as a test case because of its high content of negatively charged carboxylates suitable for metal binding with moderate affinity. The protein was successfully crystallized in the presence of zinc ions. The crystal structure was determined to 1.1 Å resolution with MAD phasing using anomalous signals from the co-crystallized Zn(II) ions. The case study suggested an integrated strategy for crystallization and structure solution of proteins via engineering surface Asp and Glu mutants, crystallizing them in the presence of metal ions such as Zn(II) and solving the structures using anomalous signals [1].

[1] Qiu X., Janson, C.A., *Acta Crystallogr D*, 2004, **60**, 1545.

Keywords: crystallization, crystal packing, anomalous signals

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The Crystal Structure of Human CDK7 and Its Protein Recognition Properties

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CDK7, a member of the cyclin-dependent protein kinase family, regulates the activities of other CDKs through phosphorylation on their activation segment and hence contributes to control of the eukaryotic cell cycle. CDK7 also assists in the regulation of transcription as part of the transcription factor TFIIH complex. For maximum activity and stability, CDK7 requires phosphorylation, association with cyclin H, and association with a third protein, MAT1.

We have determined the crystal structure of human CDK7 in complex with ATP at 3 Å resolution. The kinase is in the inactive conformation, similar to that observed for inactive CDK2. The activation segment is phosphorylated at Thr170 and is in a defined conformation that differs from that in phospho-CDK2 and phospho-CDK2/cyclin A. The functional properties of the enzyme against CDK2 and CTD as substrates are characterized through kinase assays. Experiments confirm that CDK7 is not a substrate for kinase-associated phosphatase.

Keywords: cyclin-dependent kinase, cell cycle, structure

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Novel Vapour Diffusion Method

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We present a new method for the crystallization of biological macromolecules, combining advantages of the vapour diffusion method with advantages of the micro-batch method.

The classic vapour-diffusion, either in its hanging-drop or sitting-drop configuration is still the preferred method by many crystallographers. This is mainly due to its inherent dynamics and the final endpoint of the diffusion. The new method uses a combination of two oils. As in the micro batch method, the total system is protected from evaporation by paraffin oil (first phase). However, the protein/precipitant mixture (fourth phase) is able to equilibrate with a reservoir solution (third phase) via a second layer of oil (second phase), present under the upper layer of paraffin oil. The second oil is not miscible either with the paraffin oil, nor does it interact and influence the properties of both phases three and four. Procedures for