P.04.01.31
Acta Cryst. (2005). A**61**, C178
Molecular Mechanism of Ubiquitin Recognition by GGA3 GAT Domain
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GGA (Golgi-localizing, γ-adaptin ear domain homology, ARF-binding) proteins, which constitute a family of clathrin coat adaptor proteins, have recently been shown to be involved in the ubiquitin-dependent sorting of receptors, through the interaction between the C-terminal three-helix-bundle of the GAT (GGA and Tom1) domain (C-GAT) and ubiquitin. We report the crystal structure of human GGA3 C-GAT in complex with ubiquitin. At the center of the interface, three pockets on the hydrophobic Ile44 surface of ubiquitin accommodate three hydrophobic residues from helices α1 and α2 of C-GAT. Two distinct orientations of ubiquitin Arg42 determine the shape and the charge distribution of the third pocket of the ubiquitin Ile44 surface, leading to tight and loose binding modes of C-GAT. The flexibility of the third pocket explains why ubiquitin Ile44 surface can interact with structurally divergent ubiquitin binding modules. In addition, biochemical and NMR data suggest another hydrophobic binding site on C-GAT helices α2 and α3, opposite to the first binding site, also binds ubiquitin although weakly. The double-sided ubiquitin binding provides the GAT domain with higher efficiency in recognizing ubiquitinated receptors for lysosomal receptor degradation.
Keywords: ubiquitin system, membrane trafficking, complex structure

P.04.01.32
Acta Cryst. (2005). A**61**, C178
Expression and crystallization of Toxoflavin lyase(TflA) & TRP
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TRP (transthyretin-related protein) from soil bacterium *Bacillus subtilis* was suggested to be involved in the ureide pathway. In order to investigate its enzymatic role in the pathway, we have carried out structural study of TRP protein (121 amino acid). TRP gene from *Bacillus subtilis* was subcloned into the expression vector, pET15b and expressed as the His-tagged protein, followed by purification using immobilized metal affinity chromatography (IMAC). Crystals were formed within 6 days at concentration of 12 mg/ml with hanging drop.

TflA (222 amino acid) is an enzyme degrading a phytotoxin, toxoflavin. This toxin which is produced from *Burkholderia glumae* causes rice grain rot in rice nursery boxes. TflA gene subcloned into pET14b vector was overexpressed and purified by using IMAC and ion-exchange chromatography. Crystals of TflA were observed in 2M ammonium sulfate, 0.1M MES, pH7.2. Preliminary crystallographic data will be presented.
Keywords: TflA, TRP, enzyme mechanism

P.04.01.33
Acta Cryst. (2005). A**61**, C178
Protein Crystal Quality Studies using high Resolution X-ray Diffraction
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During the last years a number of papers were reporting about a positive effect of magnetic fields on the quality of protein crystals. All this findings are based on a limited number of experimental data.

To systematically investigate this proposed effect of homogeneous magnetic fields, crystals of 4 different proteins were grown in magnetic fields of up to 16 Tesla. Another application of magnetic fields in protein crystal growth is the crystallization of proteins in inhomogeneous magnetic fields. The effect of inhomogeneous magnetic fields on the crystal quality was studied on 3 model proteins.

The quality of the crystals grown with and without homogenous and inhomogeneous magnetic field was subsequently determined by means of high resolution rocking curve measurements. Comprehensive intensity data sets were taken as well. A detailed analysis of the results will be presented, based on measurements of more than 200 crystals.

The mosaicity measurements were carried out at the Petra-1 undulator beam line at DESY in Hamburg. The 6-circle diffractometer installed at the beamline allows the determination of the orientation matrix and the measurement of reflection profiles at arcsec resolution of 4 reflections each at three azimuthal angles in less than 20 minutes. It is therefore very well suited for comparative crystal quality studies.
Keywords: protein crystallization, high resolution X-ray diffraction, magnetic field

P.04.01.34
Acta Cryst. (2005). A**61**, C178
High-throughput Protein Crystallization at the Center for Eukaryotic Structural Genomics
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The Center for Eukaryotic Strucural Genomics (CESG) solves structures of selected, unique eukaryotic proteins. Here we report the status and performance of our integrated robotic WHITE ICE system (Wisconsin HI-Throughput Extensible and Integrated Crystallization Environment) consisting of a Tecan Genesis™ crystallization platform, CrystalScore™ and CrystalFarm™ imaging systems, and Sesame, our laboratory information management system. We also present a preliminary analysis of the Fluidigm Topaz™ microfluidic chip-based crystallization and imaging platform, and evaluate its performance relative to microliter-scale crystallization experiments. The relative performance of protein samples prepared by micro- and large-scale protein production pipeline methods is also evaluated. The screening success rate for CESG fold-space targets is over 30%, and ~80% for test targets. We report analysis of our initial screening strategy and results from a salvage pathway encompassing alternative screens, perturbation screening, reductive methylation, and mutagenesis. Supported by Protein Structure Initative NIH grant P50 GM64598.
Keywords: crystallization, structural genomics, protein crystallography

P.04.01.35
Acta Cryst. (2005). A**61**, C178-C179
The *Xtaldb* System for Project salvaging in high-throughput Crystallization
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Over the past 15 years, advances in protein crystallography have continuously redefined the concept of "low-hanging fruit". Salvaging proteins that do not crystallize in high-throughput environments has become increasingly important for structural coverage of major protein families with sufficient granularity to allow 3-D homology modeling. To address this problem, we have created *Xtaldb*, a scalable, cross-platform, networked expert system for tracking and statistical analysis of crystallization experiments. *Xtaldb* records detailed information about each crystallization, including chemical and biochemical data about reagents and macromolecules, crystal images, annotated observations, and diffraction data. These data are gathered with a minimum of researcher input with the aid of

touchscreens, barcode scanners, digital cameras, and other forms of automation.

Using these data, the *Xtaldb* system organizes experiments and overall target status information into projects and provides tools for data mining and statistical analysis of the crystallization data both on the project and database-wide levels. To test these tools, we used the system in the salvage of a group of ten targets that previously failed to produce a structure in the MCSG pipeline. To date, two structures have been solved and deposited in the PDB, and three others diffract natively: two to 2.7Å, and one to 3.6Å.

Keywords: crystallization of proteins, bioinformatics, structural genomics

P.04.01.36

Acta Cryst. (2005). A**61**, C179

GIXD Investigation of GlnB of *H. seropedicae* Adsorbed on Silicon

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Protein adsorption on solid surfaces has a wide range of applications[1]. The use of Grazing Incidence X-Ray techniques to investigate protein structure adsorbed on interfaces is a promising tool that may lead to the understanding of its function. In diazotroph microorganisms, GlnB of *H. seropedicae* signalizes levels of nitrogen for a series of proteins involved in the regulation of expression and activity of nitrogenase complex. The GlnB-HS structure was already determined by x-ray diffraction revealing a trimmer of (36kDa)[2].

The subject of this investigation is to understand the interaction of protein GlnB-Hs, a globular protein, on Si (111) and Si(100) surfaces under different conditions of deposition. The spin coating technique[3] was used to obtain a uniform thin film. This experiment was conducted on a Huber six-circles diffractometer, at XRD2 beamline(LNLS- Brazil), with energy tuned to approximately 7Kev. The results were used to obtain information on protein layer assembly. The initial scattering profiles of standard θ-2θ obtained in grazing incidence geometries showed signal of protein layers ordering corresponding to a d-spacing of 30Å in Si(111) and 40Å in Si(100) out of plane direction compatible with crystallographic data.

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Keywords: protein assembly, adsorption, grazing incidence diffraction

P.04.01.37

Acta Cryst. (2005). A**61**, C179

Using Multilayer Soft Lithography Formulator Chips to map Precipitations Diagrams of Proteins

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In the post human genome era, the focus has shifted from sequencing genomes to investigate the proteins that are encoded by the genomes. The structural genomics programs have different missions but they all share the fact, that they have put together a high throughput pipeline that make it cheaper, easier and faster to get from gene to the three dimensional structure of the encoded protein. In this pipeline there are several bottlenecks, but it is agreed in general that the most significant bottleneck is to get from the protein solution to protein crystals of diffraction quality.

We have implemented the method of Multilayer Soft Lithography to produce Formulator chips [1] to address the problem of protein crystallization. Using the Formulator chip, the solubility behaviour of a protein can be experimental characterised using minute volumes of sample. The protein is screened against 3000 chemical conditions using less than 10 μL of purified protein sample. Subsequently 30 to 50 chemical conditions from this sparse screen are selected for detailed mapping of the precipitation diagram, in which the concentrations of protein against precipitant are varied. Using the experimental precipitation diagrams tailor made crystallisations experiments are designed, maximizing the probability of producing crystals of the protein.

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Keywords: microfluidics , crystallization, phase diagrams

P.04.01.38

Acta Cryst. (2005). A**61**, C179

Metal Cations Effect on Membrane Crystallized Lysozyme

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Microporous hydrophobic membranes are innovative tools in protein crystallization [1]. In this work, effects of $CoCl_2$ and $CuCl_2$, used as precipitant agents, on membrane-crystallization of hen egg white lysozyme (HEWL), are described. The HEWL*Co^{2+} complex gave rise to a new $P2_12_12_1$ orthorhombic form (a= 36.81 Å, b= 77.56 Å, c= 80.38 Å) beside the ordinary tetragonal one. Literature reports only another similar case: a $P2_12_12_1$ orthorhombic form of an HEWL*Ni^{2+} compound, grown under strong magnetic field [2]. Membrane crystallization of HEWL with $CuCl_2$ allowed to observe new coordination positions of Cu^{2+} to lysozyme (Asp18, Asp87) respect to ones already described (Asp52, Leu129, Arg14) [3]. DSC tests showed cobalt coordination increases lysozyme stability, while copper binding by oxygen atoms is unfavourable and decreases crystals melting point. HEWL specific activity increases after crystallization owing to its further purification and seems to be more affected by copper coordination.

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Keywords: crystal growth apparatus design, bioinorganic chemistry, protein crystallization

P.04.01.39

Acta Cryst. (2005). A**61**, C179-C180

Mexicain, from the Crystal to the Structure: A Sixty Years Journey

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Mexicain is a cysteine protease from the tropical plant *Pileus Mexicanus* (now, also called *Jacaratia mexicana*) first described by Castañeda and co-workers in 1942 [1]. Previous crystallization trials of mexicain were reported but unfortunately the quality of the crystals was not good enough for accurate X-ray analysis [2, 3].

In this work we present the strategy to find crystallization conditions that produce crystals of the complex protein-inhibitor that are suitable for x-ray diffraction studies. We will present purification protocols and biochemical characterization of mexicain as well as the crystallization of mexicain bound to the inhibitor by vapor diffusion and counter-diffusion techniques. Crystals were cryo-protected with glycerol to a final concentration of 20%. Frozen crystals were analyzed using an X-ray rotating anode source and they diffracted to a resolution of 1.94 Å. The crystal belongs to the monoclinic space group $P2_1$ with unit cell parameters a = 57.36 Å; b = 90.45 Å; c = 80.39 Å; and β = 92.64°. The asymmetric unit contains four molecules of mexicain with a corresponding crystal volume per protein mass (*Vm*) of 2.24 and a solvent content of 45% by volume.