P.04.01.31
Acta Cryst. (2005). A**61**, C178
Molecular Mechanism of Ubiquitin Recognition by GGA3 GAT Domain
Masato Kawasaki[a], Tomoo Shiba[a], Yoko Shiba[b], Yoshiki Yamaguchi[c], Naohiro Matsugaki[a], Noriyuki Igarashi[a], Mamoru Suzuki[a], Ryuichi Kato[a], Koichi Kato[c], Kazuhisa Nakayama[b], Soichi Wakatsuki[a], [a]*Struct. Biol. Res. Cen., Photon Factory, Inst. Mater. Struct. Sci., KEK, Japan.* [b]*Grad. Sch. Pharm. Sci., Kyoto Univ., Japan.* [c]*Dept. Struct. Biol. Biomol. Engin., Grad. Sch. Pharm. Sci., Nagoya City Univ., Japan.* E-mail: masato.kawasaki@kek.jp

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
Sangkee Rhee, Woo-Suk Jung, Du-kyo Jung, *Department of agricultural biotechnology, Seoul National University. Seoul, Korea.* E-mail: srheesnu@snu.ac.kr

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
Alke Meents[a,c], Bernd Reime[a], Heiko Schulz-Ritter[a], Dieter Elefant[c], Rainer Harf[a], and Edgar Weckert[a], [a]*HASYLAB@DESY, Hamburg, Germany.* [b]*IFW, Dresden, Germany.* [c]*present address: Paul Scherrer Institut, Swiss Light Source, Villigen, Switzerland.* E-mail: alke.meents@psi.ch

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
George N. Phillips Jr., Craig A. Bingman, Simon T.M. Allard, Eduard Bitto, Byung Woo Han, Jason G. McCoy, Janelle T. Warrick, Gary E. Wesenberg, *University of Wisconsin, Madison, USA*. E-mail: phillips@biochem.wisc.edu

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
Matthew Zimmerman[a], Maksymilian Chruszcz[b], Katarzyna Koclega[a], Zbyszek Otwinowski[b], Wladek Minor[a], [a]*Mol. Physio. & Biol. Physics, University of Virginia, Charlottesville,VA.* [b]*U.T. Southwestern Medical Center at Dallas,TX.* E-mail: mzimmerman@virginia.edu.

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