Acta Cryst. (2002). A**58** (Supplement), C301

STRUCTURE AND FUNCTION ANALYSIS OF A FAMILY OF CYSTEINE-RICH HYPOTHETICAL PROTEINS FROM THE HUMAN PATHOGEN HELICOBACTER PYLORI

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The genome sequence of the human pathogen Helicobacter pylori contains a family of cysteine-rich hypothetical proteins that so far lack a functional and three-dimensional description. Four members of this family, designated Helicobacter cysteine-rich proteins (Hcp), have been expressed recombinatly in *E. coli*, refolded and purified. It was shown that the proteins HcpA, -B, -C and -D are capable of binding and hydrolysing β -lactam antibiotics. The substrate profiles characterize these proteins as penicillinases rather than cephalosporinases. The structural analogy between β -lactams and D-Ala,D-Ala peptides suggests an implication of Hcp proteins in cell-wall biosyntesis. HcpB and -C were crystallized and the structures were refined at 1.95 Å and 2.50 Å resolution, respectively. The structures reveal modular architectures of α/ α-repeats that are distantly related to the tetratricopeptide-repeats. The HcpB structure also shows a bound ligand that was copurified with the protein. The electron density suggests that this ligand might be a N-acetyl-muramic acid (NAM), a ubiquitous compound of the bacterial cell-wall, that was validated by mass-spectrometry.

Keywords: CYSTEINE-RICH, β -LACTAMASE, HYPOTHETICAL PROTEIN

Acta Cryst. (2002). A**58** (Supplement), C301

AUTOMATION OF MACROMOLECULAR DATA COLLECTION - INTEGRATION OF DATA COLLECTION AND DATA PROCESSING

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The data collection programs at the SRS Daresbury (PXGEN++) and the ESRF Grenoble (ProDC) have been close coupled to Mosflm via TCP/IP sockets through an expert system and a linking server program to provide a high level of automation. A characterize crystal button on the data collection GUI initiates the automatic determination of appropriate data collection parameters for single crystals of previously unknown space group. The characterize crystal command collects two images from a crystal (at 0° and 90° in phi), and then sends an instruction to the Mosflm server which is forwarded to Mosflm to conduct the characterization. Mosflm proceeds to (i) autoindex each image individually and also both together, (ii) estimate the effective mosaicity, (iii) integrate the first image to determine the effective resolution limit, and (iv) calculate a suitable data collection strategy to give maximum completeness for both unique and anomalous data. An expert system developed jointly at SRS and ESRF then makes further decisions regarding data collection based on these results. The Mosflm server has been developed to provide an extendable interface to a next-generation GUI, and also gives simple access to the program for other interfaces. Improvements to Mosflm itself allow the appropriate sequence of operations to be carried out in a flexible and robust manner.

Keywords: AUTOMATED DATA COLLECTION DATA PROCESSING

Acta Cryst. (2002). A**58** (Supplement), C301

NEW HYDRA-PLUS-ONE SYSTEM FOR SPEEDY

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Robbins Scientific in collaboration with the TB Structural Genomics Consortium Crystallization Facility has developed a new, automated, high-throughput dispenser for the setup of protein crystallization trials. The New Hydra-Plus-One System is composed of a Hydra-PP System, equipped with a motorized XYZ-platform that holds two standard SBS footprint plates, 96 non-disposable precision glass syringes and a single channel microsolenoid dispenser. The single-channel dispenser, which is based on non-contact liquid-handling technology, transfers between 100nl to 50ul of protein solution with a dispense accuracy of greater than 90% and a dispense-precision variation of less than 7% into microplates. The protein samples are aspirated from a 0.5ml micro tube and dispensed at a speed of 60 seconds per 96-wells. Up to 300ul of premixed crystallization cocktails can be aspirated with the 96 syringe assembly from a bioblock and simultaneously dispensed into the reservoir and into the droplet wells at a dispensing speed of 60 seconds per 96 wells. Washing the tips is a separate process that can be carried out once plates are sealed, between changing proteins and screen blocks. The Hydra-Plus-One Systems modular design allows full integration with lab automation components available from different vendors. Embedded in automated process, the throughput of the system can reach as many as 50 96-well crystallization plates per hour. The Hydra-Plus-One System combines high presicion, reliability and speed in an ideally suited cost effective high-throughput protein crystallization system.
LLNL is operated by UC for US DOE under contract W-7405-ENG-48. Work was funded by NIH-P50-GM62410 center grant.

Keywords: CRYSTALLIZATION ROBOT HIGH THROUGHPUT

Acta Cryst. (2002). A**58** (Supplement), C301

A NEURAL NET APPROACH TO THE AUTOMATED ANALYSIS OF CRYSTALLIZATION TRIALS

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An important tool for the Structural Genomics initiative will be a means to automatically detect crystals in crystallization experiments, as well as classifying other non-crystallizing trials. Such information will not only relieve the many man hours of tedious visual inspection but may also provide directions to proceed in subsequent crystallization experiments and a metric for the design of better crystallization screens. A computer program, Crystallization Experiment Evaluation Program (CEEP), has been developed which carries out this procedure classifying crystallization experiments into 5 broad categories (invalid experiment, clear drop, homogenous precipitant, inhomogeneous precipitant, crystal hit). The program consists of two main parts, a preprocessor for determining the drop boundary and extracting features from within the drop and a classification engine in the form of a self-organizing Kohonen neural net. The algorithm has currently been tested on over 500,000 images derived from crystallization experiments performed with the entire proteome of Thermotoga maritima. Crystallization experiments were robotically generated in a submicrolitre, sitting drop vapour diffusion, 96 well format with 3 images taken over a period of 28 days for each individual experiment. The results and problems of the analysis are presented using various classification vectors as well as with the incorporation of time series information.

Keywords: STRUCTURAL GENOMICS, CRYSTALLIZATION, IMAGE ANALYSIS