presumptions about possible function of protein YBEY will be presented.

Keywords: structural genomics, NYSGRC, metalloproteinase

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Crystal Structure of *Pfu* 838710: the First Model of a Pfam CYTH Domain

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Pfu-838710 is a 21.4kDa protein from *Pyrococcus furiosus,* a hyperthermophile, belongs to a Pfam family [1] which includes the catalytic domains of CyaB-like adenylyl cyclase and thiamine triphosphatase (CYTH). The structure reported here represents the first structure for this Pfam.

Pfu-838710 crystallized in space group $P3_121$ with cell dimensions a = 97.02Å and c = 127.59Å. A quick soak of a crystal in a K_2PtCl_4 solution produced a platinum derivative as determined by Patterson analysis. The initial 2.6Å phases and electron density map were generated from single wavelength anomalous scattering data (λ= 1.5418) using the SCA2Structure pipeline [2]. The model was built using XFIT and refined against a 2.3Å resolution data set collected at SER-CAT (www.ser-cat.org), Sector 22 APS. The protein contains an 8-stranded anti-parallel β barrel that forms a closed tunnel. The structure has been refined to R = 22.3%, R-free = 25.8% (PDB ID 1XKC).

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Keywords: CYTH domain, *Pyrococcus furiosus*, Sca2Structure

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Structural Studies of Hyperthermophilic Enzymes from *Pyrococcus horikoshii*

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Pyrococcus horikoshii is hyperthermophilic archaeva that grow at temperatures between 88°C and 104°C with the optimal growth temperature of 98°C. The proteins synthesized by this organism have exceptional heat resistance properties and thus, may be used in different industries including pharmaceutical, food, chemical, paper and others. Structural genomics approach has been applied to determination of crystal structures of a number of these enzymes.

Gene fragments that encode target proteins have been amplified by PCR from cDNA of *P. horikoshii* OT3, complemented with N- or C-terminal His-tags and integrated into pET30a expression plasmid. The resulting constructs have been transformed into *E. coli* strain Rosetta-gami B (DE3) for protein production.

Four out of total nine enzymes have good expression levels. Purification protocols based on metal affinity and size exclusion chromatography have been developed. Typically 50 mg of pure protein suitable for crystallization can be produced from 2 liters of culture. Crystallization trials using nanotechnology robotics have produced encouraging results. Progress on the project will be reported.

Keywords: *P. horikoshii*, thermophilic enzymes, structural genomics

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Crystal Structures of pmbA and CsrA: Both Reveal New Folds

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The crystal structure of pmbA reveals a new fold. PmbA, which is encoded by the TM0727 gene of *Thermatoga maritima*, functions in the production of the antibiotic peptide microcin B17[1]. Additionally, pmbA is a putative modulator of DNA gyrase that may function with carbon storage regulator A (CsrA)[2]. The structure was determined using MAD phasing, and two monomers were refined to 1.95Å. The pmbA monomer is composed of two domains, with the N-terminal domain forming a long anti-parallel six-stranded β-sheet, and the C-terminal domain containing three anti-parallel β-sheets, five α-helices and regions of extended coil.

The crystal structure of the carbon storage regulator A (CsrA) gene of *Pseudomonas putida* also reveals a new fold. The structure of dimeric CsrA was determined with MAD phasing and refined to 2.05Å. Each monomer is composed of five consecutive anti-parallel β-strands and one α-helix, with the dimer formed by the intertwining of a pair of β-strands. *E. coli* CsrA is an RNA binding protein which, in conjunction with CsrB-RNA, negatively regulates glycogen biosynthesis, glyconeogenisis and glycogen metabolism, while having a positive regulatory effect on glycolysis[3].

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Keywords: structural genomics, new fold, MAD phasing

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Structure of the Bacterial YhcH Protein, a Putative Copper Aminosugar Epimerase

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Nine-carbon sugars sialic acids are located at the end of a glycan chain in vertebrate glycoconjugates and involved in molecular and cellular recognition. Bacteria can use sialic acid of the host cell as the sole carbon or nitrogen source and as a source of amino sugars for cell wall synthesis. In many pathogenic bacteria, proteins involved in sialic acid catabolism are encoded by the *nan* operon that includes a specific transporter, lyase, kinase, epimerase, and the *yhcH* gene of unknown function. The crystal structure determination of YhcH from *Haemophilus influenzae* was undertaken as part of a structural genomics effort in order to assist with the functional assignment of the protein. The structure was determined at 2.2 Å resolution by the MAD method. The protein fold is a variation of the double-stranded β-helix. Two antiparallel β-sheets form a funnel opened at one side, where a putative active site contains a copper ion coordinated to two histidines and an aspartic adic. Comparison to other proteins with a similar fold, and analysis of the genomic context suggest that YhcH may be a sugar isomerase involved in degradation of exogenous sialic acid.

Keywords: structural genomics, cupin fold, copper protein

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Crystallographic Studies of Several Essential Proteins concerning the Nucleotide Metabolism in *Bacillus subtilis*

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By using bioinformatics methods, 33 genes that related to *Bacillus subtilis* nucleotide metabolism were chosen in this study. By using *B. subtilis* genomic DNA, the genes were amplified by PCR and cloned with TOPO/GATEWAY systems. 22 proteins were expressed successfully and 16 soluble proteins were purified by Ni chelating and size-exclusion chromatography. So far, 8 diffractable crystals were

obtained and 6 structures were determined. Among them, Bs139 protein functions as phosphoribosylglycinamide formyltransferase (GART), an important enzyme in the de novo pathway of purine biosynthesis. Bs139 crystal diffracted to 2.5 Å resolution at home X-ray source and the structure was determined by molecular replacement (MR). Bs154 protein is a putative deoxyuridine 5'-triphosphate nucleotidehydrolase (dUTPase), which plays important role in DNA replication. Se-YosS crystal diffraction datasets were collected at Beijing Synchrotron Radiation Facility (BSRF) and the structure was determined by multi-wavelength anomalous diffraction (MAD) method.

Keywords: structural genomics, *Bacillus subtilis*, nucleotide metabolism

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ACTOR Gets an AGENT: Automation for Multiple Instruments

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Laboratory automation has improved the efficiency and capabilities of the modern crystallographer. To date, robotic methods are used for all steps of the crystallographic pipeline, from preparing solutions for crystal growth all the way to mounting crystals on goniometers and collecting diffraction data. Additionally, data processing has been automated to provide nearly immediate results to the scientist. With the need for crystal transport and data collection maturing, there has arisen the need to evaluate crystals and then choose to move collection worthy crystals to the most appropriate diffraction setup for full data set collection. ACTOR Gantry Enabling Numerous Targets, AGENT, has been added to the crystallographer's tool chest to fill this gap. AGENT allows ACTOR to mount crystals on multiple adjacent diffractometers. Crystals can then be evaluated, ranked, and, if they exceed a quality threshold, data can be collected. Based on the crystal diffraction properties, the best instrument and experimental parameters can be selected for data collection. Not limited to a single detector or generator, AGENT can outsource samples to multiple detectors on multiple generator platforms. Thus, AGENT provides the ultimate in high-throughput technology, while still retaining the scheduling flexibility required for optimal crystallographic data collection.

Keywords: automation, robots, structural genomics

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Crystal Structures of Sortase B from *Staphylococcus aureus* and *Bacillus anthracis* Reveal Catalytic Amino Acid Triad in the Active site

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Surface proteins of Gram-positive bacteria perform critical biological functions during the pathogenesis of human infections. These functions are only carried out when they are attached to the cell wall envelope. The anchoring process of the surface protein is accomplished by sortases via a transpeptidation reaction involving a C-terminal sorting signal containing a conserved five-amino acid motif. Sortase B recognizes NPQ**T**N in *S. aureus*, and NPKTG in *B. anthracis*, cleaves the polypeptide after the Thr residue and attaches the mature protein to the cell wall peptidoglycan. The catalytic mechanism for similar reaction has been proposed. Questions whether a thiol ion pair intermediate plays a key role in the sortase-catalyzed reaction and which residues constitute the active site remain unsolved.

In this paper, we report 1.6 and 2.0 Å resolution crystal structures of SrtB from *B. anthracis and S. aureus,* respectively, provide a first detailed view of the active site and enables the design of new experiments with a goal to target the protein for new class of drugs that would inhibit cell wall anchoring in gram-positive bacteria.

Keywords: sortase, *Bacillus anthracis*, structural genomics

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The Joint Center for Structural Genomics: A Multi-tiered Approach to Structural Genomics

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The JCSG (www.jcsg.org) has made substantial progress in the ultimate goal of HT structure determination by truly automated means. We have implemented a 3-tiered pipeline strategy where targets are characterized, categorized by behavior, and then processed in parallel by appropriate methods. Tier 1 is focused primarily on gathering experimental data on the proposed targets and is heavily reliant on complete automation and the processing of a large number of targets through initial crystallization trials. Targets are then either advanced towards structure determination by MAD or MR (Tier 2), or enter an appropriate salvage pathway (Tier 3). Salvage pathways have been developed which attempt to customize processing of smaller sunsets of targets through parallel processing methods. Such pathways include NMR and DXMS screening, mutagenesis, protein refolding, protein co-expression, baculovirus protein expression and orthologs. These strategies have been successfully applied to a prokaryote (*T. maritima*) and eukaryote (mouse) proteomes.

Collaborations with the scientific community are an important part of the development, production and dissemination aspects of the JCSG. These collaborations are created, supported, and dynamically managed to match the programmatic needs while maximizing the leverage of available resources. (NIGMS/PSI (P50-GM 62411).

Keywords: structural genomics, automation, high-throughput

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When Structures of Unknown Proteins are Determined, What is next?

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As part of Midwest Center for Structural Genomics (MCSG), our current aim is to solve high-resolution protein structures with less than 30% sequence identity to known structures. This approach unavoidably brings the realization that a large fraction of protein targets will be functionally uncharacterized. Three-dimensional structures of such proteins may furnish insight into their function. In the following case study we present recently determined x-ray crystallographic structures of proteins representing this category.

The RBSTP1166 protein from *Bacillus stearothermophilus* consists of 216 amino acids and related sequences appear to occur in a very small range of species. Preliminary structural comparisons suggest the protein may be a glycoside hydrolase.

YfiT, a hypothetical protein from *Bacillus subtilis* is found to have a divalent cation bound by three conserved histidines.The localization of the metal, its coordination geometry, the surrounding residues and the ligands involved suggest that YfiT might function as a peptidase or hydrolase.

An outer surface protein from *Bacillus cereus* has a two-domain