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