

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 P3₁21 with cell dimensions a = 97.02Å and c = 127.59Å. A quick soak of a crystal in a K₂PtCl₄ 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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[1] Iyer L.M., Aravind L., *BMC Genomics* 2002, 3, 33-33. [2] Liu, et al., *Acta Cryst. Section D*, 2005, in press.

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 archaea 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, glyconeogenesis and glycogen metabolism, while having a positive regulatory effect on glycolysis[3].

[1] Rodriguez-Sainz M.C., Hernandez-Chico C., Moreno F., *Mol. Microbiol.*, 1990, 4, 1921. [2] Murayama N., Shimizu H., Takiguchi S., Baba Y., Amino H., Horiuchi T., Sekimizu K., Miki T., *J. Mol. Biol.*, 1996, 256, 483. [3] Romeo T., *Mol. Microbiol.*, 1998, 29, 1321.

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 acid. 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