methods are unable to directly determine the structure of the metal site and its coordination geometry. The capability of X-ray absorption spectroscopy (XAS) to provide the structure of a metal ion bound to a protein is then perfectly suited to complement the process of structure determination. This aspect is particularly relevant in structural genomic projects where high throughput of structural results is the main goal.
We have recently exploited the synergism of the two techniques in the structure determination of bacterial copper transport proteins [1,2]. The synergism extends, in favourable cases, to the detection of metal-mediated protein-protein interactions leading to the formation of functional protein complexes. Examples will be provided about proteins involved in the assembly of the Cu_A and Cu_B sites of cytochrome *c* oxydase.

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Keywords: NMR, XAS, structural genomics

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Unraveling the Structures of Antizyme and its Complexes
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Polyamine levels are regulated in multiple ways, including the role of a novel protein inactivator, antizyme (AZ), that targets ornithine decarboxylase (ODC) for degradation by the 26S proteasome. We have previously reported the X-ray structures of mouse [1] and human [2] ODCs. An extension of these studies deals with understanding the novel method of regulating ODC activity through the action of antizyme (AZ), in concert with another protein, antizyme inhibitor (AZI). Full length AZ-1 from rat has resisted crystallization, thus we have been working with several modified forms of the protein. An AZ-1 fragment encompassing amino acid residues 87-227 has been prepared in a highly soluble, stable form that is amenable to structural analysis by multi-dimensional NMR methods. This fragment retains its ODC binding activity. Many elements of the AZ secondary structure have been identified. Current efforts are focused on the determination of the tertiary structure of this AZ fragment and the characterization of its complexes with ODC and AZI using a variety of biophysical techniques. The status of these projects will be reported.

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Keywords: antizyme, enzyme inhibition, polycations

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Structural Studies of a Novel Phosphotriesterase Capable of Degrading Soman
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Phosphotriesterase (OPH) is an enzyme that is capable of hydrolyzing organophosphorus neurotoxins such as those commonly found in a variety of insecticides and chemical warfare agents. This enzyme is naturally found in a variety of organisms including bacteria, squid, insects and humans. We have cloned, expressed, purified and determined the x-ray structure of an OPH enzyme isolated from an extremophile that has increase thermostability and solubility compared to the most commonly studied enzyme from *Pseudomonas diminuta*. More over, our enzyme has increase activity toward soman gas. Unfortunately, none of the enzymes studied to date have activity toward the most lethal and abundant chemical warfare agent on earth, Russian VX-gas. For this reason, we are looking to re-engineer our enzyme to broaden its substrate specificity range by means of site-directed and saturation mutagenesis, as well as other directed evolution approaches.

In order to be successful, we are using x-ray crystallography to map the reaction coordinate of the enzyme and to identify residues that play important roles in catalysis. We have determined the high resolution structure of OPH in complex with an intermediate analog by using monochromatic x-rays. We have also used polychromatic x-ray methods to determine the structures of 3 separate time points (T = 0, 30, 60 minutes) on a single crystal that was subjected to a slow-reacting substrate in a flow cell. All data sets were taken at BioCARS at the Advanced Photon Source (Argonne National Laboratory). The final structures and progress in analysis of the data will be presented.
This research is supported by Office of Naval Research award N000140210956.
Keywords: time-resolved, organophosphorus hydrolase, directed-evolution

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Studies of Heme Proteins by Time-resolved Crystallography: Allosteric Action and Structural Relaxation
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Time-resolved macromolecular crystallography has reached a mature phase with demonstrated ability to detect small structural changes on ns and sub-ns time scale [1-5] and with important advances in the analysis of time-resolved crystallographic data, such as the use of Singular Value Decomposition method to determine the structures of intermediates and elucidate the reaction mechanism [5-6]. We present results of ns time-resolved crystallographic studies of heme proteins: allosteric action in real time in cooperative dimeric hemoglobin and structural relaxation processes in myoglobin. Studies were carried out at the BioCARS beamline 14-ID at the Advanced Photon Source (USA).

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Keywords: time-resolved Laue diffraction, hemoglobin allostery, protein motions

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Crystal Structure of Conserved Hypothetical Protein YBEY from *Escherichia Coli*
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The crystal structure of protein YBEY of unknown function from Escherichia Coli was determined by X-ray crystallography methods. The structure was solved by SeMet MAD method and refined to Rcryst=0.234, Rfree=0.273 at 2.7A resolution. Diffraction data sets were collected at NSLS beam lines X29A and X9A.

The protein YBEY is a member of uncharacterized protein family UPF0054 consisting of 70 similar sequences. The fold of the protein consists of one central helix surrounded by a four-stranded sheet and four other helices. The structure revealed fold similarity to matrix mettalloproteinases. They share a conserved zink-binding motif, which represents the active site of metalloproteinases. The Zn position is occupied by Ni in YBEY structure. Details of the structure and

presumptions about possible function of protein YBEY will be presented.

Keywords: structural genomics, NYSGRC, metalloproteinase

P.04.22.2

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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

P.04.22.6

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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