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.
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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