visualize ambiguous bonding electrons between aplyronine A and actin.

Keywords: aplyronine A, antitumor effect, maximum entropy method

P.04.19.6

Acta Cryst. (2005). A**61**, C254

Crystal Structure of SSL11, a Superantigen-related Toxin from *Staphylococcus Aureus*

Heather M. Baker[a], Matthew Chung[b], John D. Fraser[b], Edward N. Baker[a], [a]*School of Biological Sciences, University of Auckland, New Zealand.* [b]*Department of Molecular Medicine, University of Auckland, New Zealand.* E-mail: h.baker@auckland.ac.nz

The pathogenic bacterium *Staphylococcus aureus* expresses a large number of protein toxins, which contribute to serious human disease. Analysis of the s. aureus genome identified a cluster of genes, encoding what are now called "staphylococcal superantigen-like" (SSL) proteins [1]. We report structural and functional studies on one of these proteins, SSL11.

SSL11 was expressed in *E. coli*, purified and crystallized from 0.2M NaH_2PO_4, 20% PEG3350. The crystals are monoclinic, space group $P2_1$, with a=52.2, b=100.9, c=79.6 Å, β=91.2°, with 4 molecules in the asymmetric unit. The crystal structure was solved by molecular replacement, using the structure of the related SET3 (SSL5) [2] as search model, and is being refined at 2.2 Å resolution (R=0.308, R_{free}=0.337). The fold of SSL11 very closely resembles that of SSL5 and shows that it belongs to the wider superantigen family.

Functional studies show that SSL11 does not have superantigen activity, but instead binds with high affinity to the human IgA receptor. This suggests a role in human disease. Intriguingly, SSL11 forms a dimer in the crystal that closely resembles that formed by SSL5 (but not by superantigens), suggesting a functional importance.

[1] Lina G., Bohach G.A., Nair S.P., Hiramatsu K., Jouvin-Marche E., Mariuzza R., *J. Infect. Dis*., 2004, **189**, 2334. [2] Arcus V.L., Langley R., Proft T., Fraser J.D., Baker E.N., *J. Biol. Chem*., 2002, **27**, 32274.

Keywords: bacterial toxin, crystal structure, dimerisation

P.04.19.7

Acta Cryst. (2005). A**61**, C254

Crystal Structure of 5-Aminolevulinate Synthase of *Rhodobacter capsulatus*

Jörg O. Schulze[a], Isa Astner[a], Dieter Jahn[b], Wolf-Dieter Schubert[a], Dirk W. Heinz[a], [a]*Division of Structural Biology, German Research Centre for Biotechnology (GBF).* [b]*Institute of Microbiology, Technical University Braunschweig, Germany.* E-mail: jos@gbf.de

5-Aminolevulinate synthase (ALAS) is the first and rate-limiting enzyme of heme biosynthesis in humans, animals, other non-plant eukaryotes and α-proteobacteria. It catalyzes the synthesis of 5-aminolevulinic acid, the first common precursor of all tetrapyrroles, from glycine and succinyl-coenzyme A.

eALAS (e for erythroid) is one of two isoforms of ALAS expressed in mammals and is responsible for approximately 90 % of body heme production. Naturally occurring mutations in human eALAS directly cause a hereditary disease known as X-linked sideroblastic anemia (XLSA). These disorders are characterized by inadequate formation of heme and accumulation of iron in erythroblast mitochondria.

We solved the crystal structure of ALAS of *Rhodobacter capsulatus*, 50 % identical by sequence to its human counterpart, at a resolution of 2.1 Å. Additional structures with each of the substrates glycine and succinyl-CoA reveal the active site organization and provide new insight into the enzyme mechanism.

We can now locate most naturally occurring XLSA mutations with high precision and interpret the clinical XLSA-cases in terms of the three-dimensional structure of the enzyme involved. Thus new impetus is given to finding ways of treating XLSA.

In addition the structure of ALAS completes the structural analysis of enzymes in heme biosynthesis.

Keywords: heme biosynthesis, pyridoxal 5'-phosphate, anemia

P.04.19.8

Acta Cryst. (2005). A**61**, C254

Structures of *B. burgdorferi* OspB Alone, and in Complex with a Bactericidal Fab

Michael Becker[1], Jonas Bunikis[2], Barbara D. Lade[1], John J. Dunn[1], Alan G. Barbour, Catherine L. Lawson[3], [1]*Biology Department, Brookhaven National Laboratory, Upton, NY.* [2]*Department of Microbiology & Molecular Genetics and Department of Medicine, University of California at Irvine, College of Medicine, Irvine, CA.* [3]*Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ, USA.* E-mail: mbecker@bnl.gov

Certain antibody Fab fragments directed against the C-terminus of outer surface protein B (OspB), a major lipoprotein of the Lyme disease spirochete, *Borrelia burgdorferi*, have the unusual property of being bactericidal even in the absence of complement. We report here X-ray crystal structures of a C-terminal fragment of *B. burgdorferi* outer surface protein B (OspB) both alone and in a complex with the bactericidal Fab H6831. The H6831 epitope is topologically analogous to the LA-2 epitope of OspA and is centered around OspB Lys 253, a residue essential for H6831 recognition. A 8-sheet present in the free OspB fragment is either disordered or removed by proteolysis in the H6831-bound complex. In both crystal structures, OspB C-terminal fragments form artificial dimers connected by *inter*molecular 8-sheets. OspB structure, stability, and possible mechanisms of killing by H6831 and other bactericidal Fabs are discussed in light of the structural results.

Keywords: Lyme disease, antibody antigen interactions, cell surfaces

P.04.19.9

Acta Cryst. (2005). A**61**, C254

Crystal Structure of Botulinum Neurotoxin Type G Light Chain

Joseph W. Arndt, Wayne Yu, Faye Bi, Raymond C. Stevens, *Department of Molecular Biology, The Scripps Research Institute, La Jolla, USA.* E-mail: arndt@scripps.edu

The seven serotypes (A-G) of botulinum neurotoxins (BoNTs) block neurotransmitter release through their specific proteolysis of one of the three proteins of the soluble N-ethylmaleimide-sensitive-factor attachment protein receptor (SNARE) complex, which is essential for membrane vesicle fusion. BoNTs have stringent substrate specificities that are unique for metalloprotease in that they require exceptionally long substrates. In order to understand the molecular reasons for the unique specificities of the BoNTs, it is essential to expose the molecular differences in their structures that give rise to their unique characteristic. Therefore, structures of all serotypes are required, and toward achieving this goal here is reported the crystal structure of the catalytic light chain of *Clostridium botulinum* neurotoxin type G (BoNT/G-LC) that has been determined to 2.35 Å resolution. The structure of BoNT/G-LC reveals a C-terminal β-sheet, which is critical for LC oligomerization, is unlike that seen in the other LC structures. Serotype structural differences observed in the pool of LC structures reveal residues in BoNT/G-LC that are likely to be involved in substrate recognition of the P1' residue and a second remote exosite for recognition of a SNARE motif.

Keywords: botulinum neurotoxin, light chain, substrate recognition

P.04.20.1

Acta Cryst. (2005). A**61**, C254-C255

Integration of XAS and NMR Techniques for the Structure Determination of Metallo-proteins. Examples from the Study of Copper Transport Proteins

Stefano Mangani[1,2], Lucia Banci[1], Ivano Bertini[1], [1]*Magnetic Resonance Center CERM and Department of Chemistry, University of Florence, Via Luigi Sacconi 6, 50019, Sesto Fiorentino, Florence, Italy.* [2]*Department of Chemistry, University of Siena, Via Aldo Moro – 53100 Siena – Italy.* E-mail: Mangani@unisi.it

NMR is a powerful technique for protein structure determination in solution. However, when dealing with metallo-proteins, NMR

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.

[1] Banci L., Bertini I., del Conte R., Mangani S., Meyer-Klaucke W., *Biochemistry* 2003, **42**, 2467. [2] Arnesano F., Banci L., Bertini I., Mangani S., Thompsett A.R., *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 3814.
Keywords: NMR, XAS, structural genomics

P.04.20.2
Acta Cryst. (2005). A**61**, C255
Unraveling the Structures of Antizyme and its Complexes
Marvin Hackert, Donald Carroll, Maria Martinez, David Hoffman, *Dept of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX, USA*. E-mail: m.hackert@mail.utexas.edu

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.

[1] Kern A.D., Oliveira M.A., Coffino P., Hackert M.L., *Structure*, 1999, **7**, 567. [2] Almrud J.J., Oliveira M.A., Kern A.D., Grishin N.V., Phillip, M.A., Hackert M.L. *J. Molec. Biol.*, 2000, **295**, 7.
Keywords: antizyme, enzyme inhibition, polycations

P.04.21.1
Acta Cryst. (2005). A**61**, C255
Structural Studies of a Novel Phosphotriesterase Capable of Degrading Soman
Sonia Larsen, Kiira Ratia, Bernard D. Santarsiero, Andrew D. Mesecar, *Center for pharmaceutical Biotechnology, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, IL 60607, USA*. E-mail: slotti1@uic.edu

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

P.04.21.2
Acta Cryst. (2005). A**61**, C255
Studies of Heme Proteins by Time-resolved Crystallography: Allosteric Action and Structural Relaxation
Vukica Šrajer[a], Reinhard Pahl[a], James Knapp[b], William Royer[b], Marius Schmidt[c], [a]*The University of Chicago, Chicago (IL), USA.* [b]*University of Massachusetts Medical School, Worchester (MA), USA.* [c]*Technische Universität München, Germany.* E-mail: v-srajer@uchicago.edu

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).

[1] Šrajer V., Ren Z., Teng T.-Y., Schmidt M., Ursby T., Bourgeois D., Pradervand C., Schildkamp W., Wulff M., Moffat K., *Biochemistry*, 2001, **40**, 13802. [2] Schotte F., Lim M., Jackson T. A., Smirnov A. V., Soman J., Olson, J. S., Phillips G. N. J., Wulff M., Anfinrud P., *Science*, 2003, **300**, 1944. [3] Anderson S., Šrajer V., Pahl R., Rajagopal S., Schotte F., Anfinrud P., Wulff M., Moffat K., *Structure*, 2004, **12**, 1039. [5] Schmidt M., Pahl R., Šrajer V., Anderson S., Ren Z., Ihee H., Moffat K., *Proc. Natl. Acad. Sci.*, 2004, **101**, 4799. [6] Rajagopal S., Anderson S., Šrajer V., Schmidt M., Pahl R., Moffat K., *Structure*, 2005, **13**, 55.
Keywords: time-resolved Laue diffraction, hemoglobin allostery, protein motions

P.04.22.1
Acta Cryst. (2005). A**61**, C255-C256
Crystal Structure of Conserved Hypothetical Protein YBEY from *Escherichia Coli*
Alexander Fedorov, Elena Fedorov, Wuxian Shi, Udupi Ramagopal, Steve Almo, *Department of Biochemistry, Albert Einstein College of Medicine, New York, USA*. E-mail: fedorov@aecom.yu.edu

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