cooperation with the flavoprotein, NADH oxidase. Here we report the crystal structure of *A. xylanus* AhpC in its oxidized form. The enzyme forms a ring-like (α2)5-decamer, the structure of which is similar to those of the previously reported Prxs, and especially to that from *Salmonella typhimurium*. The dimer-dimer interface of the decamer exhibits moderate and conserved hydrophobic interactions, which have been proposed to dissociate at the physiological ionic strengths. In the crystal, electron densities of small molecules were observed between the decamers and were shown to play a unique role in the crystallization by bridging the decamers via the hydrogen bonds.

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Keywords: protein structures, antioxidants, oligomers

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Structures of CFTR NBD1 Suggest a Molecular Mechanism for Cystic Fibrosis

Hal Lewis[a], [a]*Structural GenomiX, San Diego, CA USA*. E-mail: hal_lewis@stromix.com

Cystic fibrosis (CF) is caused by genetic defects in the cystic fibrosis transmembrane conductance regulator protein (CFTR), most commonly through omission of residue Phe-508 (ΔF508) in the first nucleotide-binding domain (NBD1), resulting in misfolded, non-functional CFTR chloride channel. The structure of NBD1 was solved in order to better understand the effect of this deletion.

Initially, the structure of wild-type (WT) mouse NBD1 was determined [1]. This revealed a largely conventional NBD fold, compared with those of similar bacterial proteins, with the exception of additions in the N- and C-terminal regions of the protein with phosphorylatable and potentially regulatory function. Residue Phe-508 was seen to be surface exposed in a loop region and not of obvious importance to the NBD1 fold.

Recently, the structure of human NBD1 with ΔF508 present was determined [2], which revealed that the deletion did not alter or disrupt the fold of this domain. This observation was supported by thermodynamic measurements on WT and ΔF508 protein that showed the stability of NBD1 is unaffected as well. The effect of the deletion then appears to be the disruption of interactions of NBD1 with other domains of CFTR, most likely with the first membrane spanning domain (MSD1). This new understanding of the molecular mechanism of dysfunctional ΔF508 CFTR will lead to improved drug discovery efforts for CF.

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Keywords: disease-related structures, cystic fibrosis, nucleotide-binding domain

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Amyloidosis: Structure of a λ 6 Light Chain Antibody Fragment

Eduardo Horjales, Paula Gonzálezrubio-Garrido, Enrique Rudiño-Piñera, Baltazar Becerril, Luis Del Pozo, *Instituto de Biotecnología, UNAM, Mexico.* E-mail: horjales@ibt.unam.mx

It has been well evidenced the preferential association of the lambda VI antibody light chain subgroup with antibody light chain amyloidosis. We have generated an engineered light chain variable region domain (rVL6aJL2) whose 99 amino terminal residues are encoded by the germline VI gene segment 6a and the complementary segment of 12 residues encoded by the gene segment JL2. *In vitro* fibril formation assays demonstrated that the rVL6aJL2 is able to slowly aggregate itself as amyloid-like fibrils under physiological conditions. The recombinant protein expressed in *E.coli*, was purified and crystallised using 1.4 to 2.0 M sodium acetate, 100mM MES pH 6.5. Crystals diffracted up to 1.9 Å resolution. The crystals grew in three different space groups and all contained fibrilar structures assembled into the crystal. These structures have helicoidal shape with a 93 Å long pitch, and a section perpendicular to the axis, 45 Å wide, with a squared shape. The molecule that generates this structure through a 4_1 symmetry, is a dimer built as that formed by light and heavy chains in functional antibodies. These dimer-dimer contacts found, comprises a surface larger than 1600 Å^2 and the amino acids involved has been shown to participate in fibrillogenesis. As in other structures from fibrillogenic antibody light-chains, no significant conformational changes have been observed. The question we have so far is: have or not the helicoidal structures we found over 3 different space groups relation with the atomic structure of amyloidotic fibers?

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Keywords: amyloidosis, fibres, crystallographic structure

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Structural and Functional Study of the Bloom Syndrome Protein

Pascal Rigolet, Sophia Guo, Xu Guang Xi, *Laboratoire de Biotechnologies et Pharmacologie Génétique Appliquée CNRS UMR 8113, Ecole Normale Supérieure (ENS) Cachan, 61 avenue du Président Wilson, 94235 Cachan cedex, France.* E-mail: pascal.rigolet@lbpa.ens-cachan.fr

Bloom Syndrome (BS) is an autosomal recessive human disorder characterized by genomic instability and a predisposition to a wide variety of cancers. The gene mutated in BS encodes a three domains enzyme, the Bloom Syndrome Protein (BLM), which C-terminal extension can be divided in two subdomains: RecQ-Ct and HRDC.

We report herein that the RecQ-Ct domain, responsible for DNA unwinding, contains a zinc finger motif. In order to understand the role of this motif in BLM, we constructed a series of mutations altering its highly conserved residues. Experiments done with these mutants showed that they were severely impaired in DNA binding and for the subsequent ATPase and helicase activities, revealing the importance of the zinc finger motif for all the functions of the enzyme. We computed the three dimensional structure of the RecQ-Ct domain by homology modeling using the template structure of the RecQ helicase from *E. coli*. This model allowed us to study the consequences of mutations observed in the Bloom Syndrome Protein when associated to a cancer. The mutant enzymes have been expressed in *E. coli* and their activities have been compared to the wild type enzyme. In order to get new insight in the molecular basis of Bloom Syndrome disease, we underwent the crystallization of the RecQ-Ct and HRDC domains in presence of various DNA substrates.

Keywords: cancer, DNA-protein interactions, structure-function

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Molecular Basis for Antitumor Effect of Actin-aplyronine A Complex

Kunio Hirata[a], Shin Muraoka[a], Kiyotake Suenaga[b], Hiroshi Tanaka[c], Masaki Yamamoto[a,d], Masaki Takata[a,e], Hideo Kigoshi[b], [a]*JASRI/SPring-8.* [b]*Dept. of Chemistry of Tsukuba univ.* [c]*Dept. of materials science of Shimane Univ.* [d]*RIKEN/SPring-8.* [e]*CREST/JST.* E-mail: hirata@spring8.or.jp

Aplyronine A is a promising candidate of anticancer agent isolated from *Aplysia kurodai*. The complex structure of actin-aplyronine A is determined via a synchrotron X-ray analysis at 1.45Å resolution. Aplyronine A binds to actin in the similar manners with the trisoxazole toxins and gelsolin segment 1 around its aliphatic tail part which should play an important role for F-actin depolymerizing activity. In contrast, the structural aspects relating to peculiar interactions between aplyronine A and actin can be found around the macrolide ring part which should be a key to the potent antitumor effect of aplyronine A. Actin-aplyronine A complex structure should suggest that each complex comes to have ability to effect other biological reactions which are essential for deaths of cancer cells. The precise charge density map was also visualized with the maximum entropy method to construct the structure model of a disordered stereoisomer of a peculiar side-chain of aplyronine A as well as to

visualize ambiguous bonding electrons between aplyronine A and actin.

Keywords: aplyronine A, antitumor effect, maximum entropy method

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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.

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Keywords: bacterial toxin, crystal structure, dimerisation

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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

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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

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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

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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