FA1-MS04-P01

Structure determination of the B-repeat, a domain of the invasion protein InlB. Maria Ebbes and Hartmut Niemann *Department of chemistry, Bielefeld University, Germany.*
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Internalin B (InlB) is a surface protein of the pathogenic bacterium *Listeria monocytogenes*. This protein binds the extracellular domain of the human receptor tyrosine kinase c-Met to induce uptake of the bacteria by phagocytosis into non-phagocytic cells. InlB consists of different domains, only one of which is involved in the binding of c-Met. We are working on the B-repeat, a domain in the middle of InlB, to which no function is allotted at the moment. We obtained a native data set with a resolution of 1.3Å. No obvious homologs exist, so we had to solve the phase problem experimentally. To this end, we used SeMet-crystals. From these crystals we measured a four-wavelength MAD data set at DESY, X12. The structure was solved with ShelxD. The crystals contain four molecules per asymmetric with one internal and the N-terminal Se-Met per molecule. ShelxD located five out of these eight Se atoms. Solvent flattening in ShelxE including the native data produced an excellent experimental electron density. Here we present the structure of the InlB B-repeat and its initial analysis including fold comparison and the prediction of binding sites for potential interaction partners.

Keywords: MAD phasing, protein structure, bacterial pathogenesis

FA1-MS04-P02

Phasing glycosyltransferase PimB' – *P*1 symmetry and radiation damage. Klaus Fütterer, Sarah Batt, Gurdyal S. Besra. *School of Biosciences, University of Birmingham, UK.*
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Phosphatidylinositol mannosides (PIMs) are glycolipids in the cell wall of *Mycobacterium tuberculosis* (and related species) that modulate the host immune response and help establish a long-lasting latent infection, a hallmark of the pathophysiology of tuberculosis. Decoration of the inositol ring of phosphatidylinositol (PI) with α-D-mannose occurs through consecutive action of a series of related mannosyltransferases of the GT-B superfamily of glycosyltransferases. The enzyme PimB' catalyses transfer of α-D-mannose from GDP-mannose to the 6-hydroxyl of the inositol moiety. Using recombinant protein of *Corynebacterium glutamicum*, a non-pathogenic relative of *M. tuberculosis*, we obtained plate-like crystals of PimB' that were in space group *P*1, diffracting up to 2.2 Å resolution with two copies of PimB' in the asymmetric unit. Phasing by molecular replacement, using low-identity search models of related glycosyltransferases, failed to produce an interpretable map, while the 10 µm-thin crystals did not tolerate heavy metal soaking. Crystals of SeMet-derivatised PimB' rapidly suffered from radiation damage, even in a much attenuated beam, limiting data redundancy and anomalous dispersion, while data completeness was restricted by unusable reflection profiles in two 60º segments over a 360º degree sweep. We eventually succeeded in phasing this structure through combining 4 individual SeMet-SAD data sets with data of PimB' bound to brominated GDP-mannose in a phasing run with SHARP. Map interpretation rested on phase improvement by NCS-averaging and matching experimentally found Se-positions, using SHELXD, with the Met-residues in a homology model of the conserved, Rossmann fold-like C-terminal domain of PimB'. Iterative model building and refinement could then proceed in a straightforward fashion.

Keywords: *Mycobacterium tuberculosis*, GT-B glycosyltransferase, SAD phasing

FA1-MS04-P03

Protein tags as phasing tools? Christian Große, Georg Michael Sheldrick. *Dept. of Structural Chemistry, Georg-August-Universität Göttingen, Germany.*
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During the last decade, several affinity protein tags have been developed, which are included as an additive protein sequence. Their highly specific strong affinity affords an one-step purification with minimal effect on biological activity. On the other hand, protein tags are often flexible and disordered in the crystal structure. However, tags are often cleaved and can not be used for experimental Phasing of macromolecules by anomalous dispersion. Those kind of experiments requires well ordered atoms in the crystal lattice. A preorganized protein tag with metal chelating properties can bind anomalous scatterers in a determinate way. As a welcome side-effect such protein tags could adopt stable conformation in crystals and help the crystallization process. Here we report polypeptides synthesized by solid phase peptide synthesis which may prove useful for both metal affinity chromatography and macromolecular phasing as well. A distinct secondary structure in terms of β-hairpins could be confirmed by circular dichroism spectroscopy and NMR. As expected folding becomes stronger and the melting point increases due to additional metal ions. Their chelating properties could be proved by Ni, Zn, Co and Cu affinity columns and some candidates show as high chelating power as a classical His tag peptide. Fusion proteins made of Maltose-binding protein (MBP) fused with our polypeptides were used to check IMAC behavior under real conditions and to validate crystal growth promotion. We observe improved crystallization in the present of metal ions and solved first crystal structures from native protein.

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Keywords: His-tag, experimental phasing, β-hairpin

FA1-MS04-P04

Combined high-resolution X-ray and neutron analysis of porcine elastase. Takayoshi Kinoshita[a], Taro Tamada[b], Ryouta Kuroki[b], Kazuo Kurihara[b], Motoyasu Adachi[b], Takashi Ohhara[b], Toshiji Tada[a]. [a]*Graduate School of Science, Osaka Prefecture University,* [b]*Quantum Beam Science Directorate, Japan Atomic Energy Agency, Japan.*
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To help resolve long-standing questions regarding the catalytic activity of the serine proteases the structure of porcine pancreatic elastase has been analyzed by high-resolution neutron and X-ray crystallography. In order to mimic the tetrahedral transition intermediate a peptidic inhibitor was used. Neutron and X-ray diffraction data have, for the first time, visualized a SIHB formed between catalytic residues His57 and Asp102, as well as the oxyanion located at the oxy-anion hole, and has identified strong hydrogen bonds as contributing to inhibitor recognition in PPE. These are fundamentally important structural data for the catalytic reaction and molecular recognition of an enzyme.

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Keywords: Neutron analysis, X-ray analysis, elastase

FA1-MS04-P05

MR-SAD: Phasing employing the NMR structure without model bias. Andrea Thorn[a], Franz Kerek[b], Carlos Eduardo Lima da Cunha[a], Isabel Usón[c], George M. Sheldrick[a]. [a]*Institute of Structural Chemistry, Georg-August University Goettingen, Germany.* [b]*DoNatur GmbH, Martinsried, Germany.* [c]*ICREA at Instituto de Biología Molecular de Barcelona (IMBMB-CSIC), Barcelona Science Park, Spain.*
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Molecular replacement (MR) with an NMR model is an appealing method for macromolecular phasing. However, it inevitably introduces model bias, or even errors, into the X-ray structure. In addition, the correct MR solution may be poorly discriminated from false ones. [1]
Here, we apply MR-SAD [2] to Hellethionin D from *Helleborus purpurascens*.
Molecular replacement was conducted with the NMR structure [3] as search model in a multi-solution PHASER [5] approach. Only a small fraction of all residues could be found by MR since the average rmsd between the NMR structure and the final model was 1.9 Å^2, and there were seven protein copies in the asymmetric unit. The correct solution could be discriminated by automatic chain expansion in SHELXE [4], an approach similar to ARCIMBOLDO [6]. After expansion, 36 sulfur atom positions were determined and the rest of the structure was discarded: Only the sulfur positions were used for a new run of density modification and subsequent expansion in SHELXE. The final trace contained 311 of 322 residues, with no misplaced residues present.
Despite an anomalous signal unsuitable for initial SAD phasing and only 19% of all residues traceable by MR, the methods described here resulted in an almost complete, model-bias free trace of all seven protein chains.

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Keywords: phasing methods, NMR, NCS

FA1-MS04-P06

The CCP4 software suite - current status and future developments. Martyn Winn[a], Charles Ballard[b], Ronan Keegan[b], Georgios Pelios[b], Natalie Zhao[b], Eugene Krissinel[b]. [a]*STFC Daresbury Laboratory, Warrington, WA4 4AD, UK.* [b]*STFC Rutherford Appleton Laboratory, Didcot, OX11 0QX, UK.*
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CCP4 exists to produce and support a world-leading, integrated suite of programs that allows researchers to determine macromolecular structures by X-ray crystallography. CCP4 aims to develop and support the development of cutting edge approaches to experimental determination and analysis of protein structure, and integrate these approaches into the suite.
The current CCP4 software suite is on release series 6.1.x. A particular focus of these releases is the automation of significant parts of the structure solution process, including XIA2 for data processing, Crank for experimental phasing, MrBUMP and Balbes for Molecular Replacement, and Buccaneer for model building. There are also a number of new programs, including Pointless for Laue group and spacegroup determination, the new iMosflm interface, Parrot for density modification, and PISA for identification of protein-protein interfaces. We will give an overview of the additions to the CCP4 suite, as well as an update on established programs.
A major overhaul of the CCP4 suite is under development. A new graphical front-end will provide easier control of the suite, and considerable help with interpreting and evaluating the results. At the core, there will be in-built support for automation, making straightforward structures simple to solve, while continuing support for more challenging projects. Finally, usage of the suite will be underpinned by better data management, with support for database back-ends.
CCP4 also aims to enhance its functionality related to the maintenance and use of data on small molecules (ligands). Firstly, a considerably larger library of chemical compounds will be provided with the Suite. Extended search functions will be provided to allow for efficient retrieval of known compounds or their close analogs. Secondly, existing functions for generating restraint data for new ligands will be enhanced by the inclusion of relevant software, such as ProDRG, into the Suite, as well as by the development of new methods for structure reconstruction on the basis of partial similarity to structures in the library. Functionality will be available through a graphical front-end application, JLigand.

Keywords: protein crystallography, crystallographic software, automation