FA1-MS05-P01

High-end Solution for In-house Protein Crystallography. Marianna Biadene[a], Matt Benning[b], Vernon Smith[a]. [a]*Bruker AXS GmbH, Karlsruhe, Germany.* [b]*Bruker AXS Inc, Madison, WI, USA.*
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The ability to collect quality diffraction data in-house provides a an improvement in productivity and efficiency, reducing the reliance on synchrotron sources. High-end in-house solutions provide the maximum flexibility for the scientist. Due to advances in optics and the introduction of microfocus rotating anode generators, there has been a remarkable increase in the performance of home laboratory X-ray systems. When combined with an ultra sensitive CCD detector, these systems can produce data comparable to that collected at synchrotron beamlines. The overall performance of these solutions allows for a number of experiments which are hardly possible on many of the currently installed equipments:

- data sets suitable for in-house SAD phasing exploring the anomalous signal
- high quality, high-resolution data set with a resolution better than 1.2 Å
- complete data collection within less than a minute X-ray exposure time

We will present data on crystals of a number of number of proteins to demonstrate the exciting capabilities of a high-end systems such as the X8 PROTEUM (figure 1).

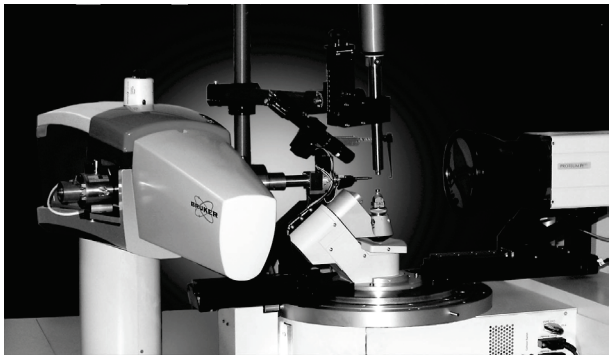

Figure 1: High-end In-house solution (X8 PROTEUM).

Keywords: SAD-phasing, high-resolution data, data collection time

FA1-MS05-P02

How do S-layers bind to bacterial cell walls. [a]A. Đordić[a], T. Pavkov[a], E. M. Egelseer[b], U. B. Sleytr[b], W. Keller[b]. [a]*Institute of Molecular Biosciences, K.F. University Graz, Austria.* [b]*Center for Nanobiotechnology, University of Natural Resources and Applied Life Sciences, Vienna, Austria.*
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Monomolecular paracrystalline surface layers (S-layers) are composed of a single (glyco)protein and are the most commonly observed cell surface structures of bacteria and archaea. Because of their diverse properties S-layers have various potential applications in nanobiotechnology [1]. However, detailed structural information on S-layer proteins is very scarce. In order to determine the structure-function relationship of SbsC, the S-layer protein from *Geobacillus stearothermophilus*, deletion mutants were produced. It was shown that the N-terminal part is responsible for binding to the secondary cell wall polymer (SCWP) and that the C-terminal part is essential for self-assembly [2]. Recently, the crystal structure of the C-terminally truncated form $rSbsC_{(31-443)}$ was solved to 2.4 Å [3].
To further characterize the interaction of SbsC with SCWP, binding studies with ITC and Thermofluor were performed. For some of the derivatives strong binding could be shown. To structurally describe the exact binding site(s), soaking and co-crystallization experiments of potential $rSbsC_{(31-443)}$-ligand complexes were set up. Crystals were obtained by co-crystallization with one of the SCWP derivatives. The complex crystallizes in the same space group as the native $rSbsC_{(31-443)}$ ($P2_1$) but with some changes in the unit cell parameters. The resolution could be improved to 1.8 Å and the structure was solved by molecular replacement. Comparing the two structures, with and without ligand, we observed a slight domain movement, which is necessary for the binding of the ligand to take place. The residues of $rSbsC_{(31-443)}$ involved in ligand binding are accommodated in domain I and positioned along the positively charged cleft. The crystal structure of the SbsC-ligand complex confirms the previous prediction of the SCWP binding site within the N-terminal domain of SbsC protein.

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Keywords: S-layer, co-crystallization, protein-ligand complex

FA1-MS05-P03

A Comparison of Models for Giant Haemoglobins Derived from Different Techniques. Helmut Durchschlag[a], Peter Zipper[b], Angelika Krebs[c]. [a]*Institute of Biophysics and Physical Biochemistry, University of Regensburg, Germany.* [b]*Physical Chemistry, Institute of Chemistry, University of Graz, Austria.* [c]*Bioinformatics and Structural Biology, Medical University of Graz, Austria.*
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Extracellular haemoglobins (Hb) found in many invertebrates are giant multisubunit molecules of hexagonal bilayer (HBL) appearance [1]. The most prominent example is *L.t.* Hb; it is a 3.6 MDa haemoprotein, a 180-mer consisting of 144 globin and 36 linker chains. Existence, nature and complexity of these large oxygen carriers have been enigmatic for nearly two centuries. The most intriguing questions addressed in the last years refer to the central cavity within the HBL structure, the precise composition of haeme-containing globins and haeme-free linkers, the exact 3D structure of the complex, its constituents and preferentially bound water molecules, and the biological significance of such huge assemblies.
The 2.6 Å resolution crystal structure of the dodecameric subunit of *L.t.* Hb and data for the 3.5 Å architecture of the complex became available recently [2, 3]. The combination of previous physicochemical data [electron microscopic (EM) reconstructions, SAXS, hydrodynamics] and currently available crystal data allows to scrutinize earlier established

anhydrous and hydrated models for the complex, the subunits, and the assembly from the constituents [4-8]. Moreover, the crystallographic availability of this MDa complex provides the opportunity to apply modern scattering and hydrodynamic modeling approaches to such huge entities, including assemblage of the complex from its constituents, and to check the extensive reduction steps to be adopted for modeling.
We tackled the following problems: (i) comparison of the SAXS- or EM-based conventional or *ab initio* models for the HBL complex with up-to-date crystallographic data, (ii) modelling the HBL complex from constituents, (iii) realistic assumptions or predictions regarding the contribution of hydration, (iv) search for any discrepancies between solution and crystal data. For modelling, primarily the programs DAMMIN, HYDRO, HYDCRYST and several modifications of the approaches were applied, in addition to usage of templates and superimpositions; results were checked by prediction of structural and hydrodynamic data. The most serious problems arose from amino acids missing in the crystallographic data base. We eventually managed to explain the observed discrepancies by the residues absent in the crystal structure of the linker chains; these residues are obviously located in the central core of the complex.

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Keywords: complex protein models, data reduction, hydration

FA1-MS05-P04

A Hybrid Pixel Detector in the Home Laboratory: Prospects for Better Data. Joseph D. Ferrara, Colin Acheson, Angela Criswell, Pierre Le Magueres, James W. Pflugrath, Katsunari Sasaki. *Rigaku Americas Corp., The Woodlands, TX, USA.*
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We have begun using a hybrid pixel detector (HPD), specifically the Dectris Pilatus 100K, in home lab single crystal X-ray diffraction experiments. In order to assess the utility of such a device for the home lab, we have studied the performance of this device for both small molecule and protein data collection experiments with copper radiation. We will present results comparing HPD data collection to conventional CCD data collection as well as results comparing conventional data collection to “shutterless” data collection in terms of data quality and increased throughput.

Keywords: area detector, hybrid pixel detector, data collection methods

FA1-MS05-P05

Bacterial conjugation in Gram-positive bacteria: Impact of Tra proteins from plasmid pIP501. N. Gössweiner-Mohr[a], E.-K. Çelik[a,b], M.Y. Abajy[a,b], K.Arends [b], E.Grohmann[b], W.Keller[a]. [a]*Institute for Molecular Biosciences, Karl-Franzens-University Graz, Humboldtstrasse 50/III, 8010 Graz, Austria.* [b]*Environmental Microbiology/Genetics, Technical University Berlin, Franklinstr. 29, 10587 Berlin, Germany.*
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Conjugative plasmid transfer is an important way for horizontal gene spread (e.g. antibiotic resistance genes) [1]. It can lead to the increase of bacteria with multiple antibiotic resistances. The plasmid conjugation process in Gram-negative bacteria has been studied in detail, whereas little information is available about the corresponding mechanisms in Gram-positive bacteria [2]. The transfer region of our Gram-positive multiple antibiotic resistance plasmid pIP501 is organized in an operon encoding fifteen putative transfer proteins. The transfer region of pIP501 encodes a putative simplified type IV secretion system (T4SS), as three pIP501-encoded Tra proteins show significant sequence similarity to the Agrobacterium tumefaciens T-DNA transfer system proteins. The homologues are an ATPase (ORF5 homologue of VirB4) [3], a coupling protein (ORF10 homologue of VirD4) and a lytic transglycosylase (ORF7 homologue of VirB1) [4].
One priority of the project is to determine the structure of ORF11 and ORF14, two members of the T4SS for whom neither homologues exist in the Agrobacterium tumefaciens T-DNA transfer system, nor detailed structure information is available. The focus on these exclusive members of the conjugation complex will further facilitate the understanding of the bacterial conjugation process in Gram-positive bacteria. 7xHis-fusion proteins of ORF11 and ORF14 have already been successfully expressed, purified and used in first crystallisation- and optimization screens. The purified proteins have also been examined for their secondary structure content, folding status and temperature stability by far UV circular dichroism.
As the aim of solving the structure of some individual key tra-region proteins gets closer, we will start to focus on the interaction of the core complex members and the detailed study of the complex structure as a whole.

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Keywords: crystallization, conjugation, pIP501