**MS63.28.5**
*Acta Cryst*. (2005). A**61**, C83
**Ribitol and Xylitol: Explaining the Differences in Physical Chemical Properties**
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The diastereomeric pentoses xylitol [1] and ribitol [2] show some remarkable differences in their physical properties in the solid state. Though xylitol has the lowest melting point (T = 93° C) it has a higher density (ρ = 1.540 g/cm³) than ribitol (T = 102° C, ρ = 1.488 g/cm³).

Based on accurate X-ray diffraction data we have performed experimental electron density studies and rigid-body TLS analyses, in order to analyse the interplay between entropy and enthalpy contributions to the free energy, and thereby explain the observed differences in physical properties.

Topological analyses of the electron densities show that the chemical bonds of the two pentoses are identical. Though the compounds have different hydrogen bond patterns, they most likely have very similar crystal packing energy. A result in accordance with calorimetric measurements and interaction energies derived from periodic DFT calculations.

Assuming that the translational and librational molecular normal modes are harmonic and uncoupled from the motion of neighbouring molecules we find the difference in vibrational entropy in the solid state to be 6 J $mol^{-1}$ $K^{-1}$, a result that accounts quantitatively for the difference in melting point.

[1] Kim H. S., Jeffrey G. A., *Acta Cryst*., 1969, B**25**, 2607-2613. [2] Kim H.S., Jeffrey G. A., Rosensten R. D., *Acta Cryst*., 1969, B**25**, 2223-2230.
**Keywords: charge density, chemical physical properties, rigid-body analysis**

## MS64 DIFFICULT PHASING AND DIFFICULT STRUCTURES IN STRUCTURAL BIOLOGY

***Chairpersons:*** Bi-Cheng Wang, Alexandre Urzhumtsev

**MS64.28.1**
*Acta Cryst*. (2005). A**61**, C83
**How to deal with Pathological Crystals of Macromolecules**
Alexander Wlodawer[a], Istvan Botos[a], Nicole LaRonde-LeBlanc[a], Zbigniew Dauter[ab], [a]*Macromolecular Crystallography Laboratory, National Cancer Institute, Frederick, MD, USA.* [b]*Biosciences Division, Argonne National Laboratory, Argonne, IL, USA*. E-mail: wlodawer@ncifcrf.gov

Although macromolecular crystallography is rapidly becoming largely routine due to advances in the methods of data collection, structure solution and refinement, difficult cases are still common. We have recently completed a number of structure determinations that utilized less than perfect crystals and these cases exemplify various difficulties faced by protein crystallographers. The structure of the proteolytic domain of *Archaeoglobus fulgidus* Lon was solved with crystals that contained superimposed orthorhombic and monoclinic lattices in a seemingly single crystal. Another, hexagonal crystal form exhibited unusually large degree of non-isomorphism that was not apparent in the analysis of the unit cell parameters. Crystals of the *A. fulgidus* Rio1 kinase exhibited both pseudosymmetry and twinning that masked the problems during analysis of intensity distribution. We will discuss the ways of identifying the observed phenomena and the approaches to solving and refining macromolecular stuctures if only less than perfect crystals are available.
**Keywords: pseudosymmetry, twinning, non-isomorphism**

**MS64.28.2**
*Acta Cryst*. (2005). A**61**, C83
**Phasing at Resolution higher than the Experimental one**
Rocco Caliandro, Benedetta Carrozzini, Giovanni L. Cascarano, Liberato De Caro, Carmelo Giacovazzo, Dritan Siliqi, *IC-CNR, Bari, Italy.* E-mail: rocco.caliandro@ic.cnr.it

We have developed a novel procedure which, combined with classical electron density modification (EDM) techniques, is able to:
a) extrapolate moduli and phases of non-measured reflections with resolution lower or higher than the experimental one;
b) actively use such moduli and phases in typical situations met in macromolecular crystallography:
1) *ab initio* phasing: data resolution ($RES_{obs}$) in the interval 1.5-1.0Å, an approximated electron density available (e.g., after the application of *EDM* procedures) with mean phase error ($MPE_{obs}$) in the range (25°, 60°);
2) *SAD-MAD*, *SIR-MIR*, *SIRAS-MIRAS* phases: $RES_{obs}$ in the interval 2.8-1.5Å, an approximated electron density available with $MPE_{obs}$ in the range (40°, 65°);
3) *ab initio* phasing, $RES_{obs}$ in the interval 1.5-1.0Å, no phase information available.

Our results [1,2] indicate that in case 3 extrapolation can make difference between success and failure. In cases 1 and 2 the extrapolation procedure is able to reduce the mean phase error of the measured reflections, provides sensible estimates (in modulus and phase) for additional reflections behind and beyond $RES_{obs}$, and increases the interpretability of the final electron density map.

[1] Caliandro R., Carrozzini B., Cascarano G.L., De Caro L., Giacovazzo C., *Acta Cryst.*, 2005, D, *in press*. [2] Caliandro R., Carrozzini B., Cascarano G.L., De Caro L., Giacovazzo C., *Acta Cryst.*, 2005, D, *submitted*.
**Keywords: macromolecular crystallography, extrapolated reflections, resolution**

**MS64.28.3**
*Acta Cryst*. (2005). A**61**, C83
**A Challenging 90 Residue Problem for X-ray Crystallography: the $^{2}$F1-$^{3}$F1 Module Pair of Human Fibronectin**
Enrique Rudiño-Piñera[a], Jennifer R. Potts[a], Raimond B. G. Ravelli[b], George Sheldrick[c], Elspeth F. Garman[a], [a]*Department of Biochemistry, University of Oxford, U.K.*. [b]*EMBL Grenoble Outstation, France*. [c] *Structural Chemistry Department, University of Göttingen, Germany.* E-mail: elspeth@biop.ox.ac.uk

Human fibronectin (Fn) is a large multidomain protein found in the extracellular matrix and plasma. It is involved in many cellular processes. The ability to bind Fn is a characteristic that has been demonstrated for a number of pathogens. Although the structures of two F1 module pairs have been determined by NMR, no X-ray structures have been reported so far.

Fibronectin crystals of the $^{2}$F1-$^{3}$F1 module pair diffracting to 1.7 Å were obtained but they exhibited symptoms of possible twinning (1). Initially, we attempted to solve the structure by MR using different ensembles of the NMR models, but after many different strategies failed, we moved on to MIR methods. A number of different derivative datasets were collected, and all showed partially occupied sites but did not give interpretable maps. In-house data were collected for the sulphur SAD method, but this also failed. RIP was tried next, and although a large signal from the breakage of the 4 disulphide bonds was obtained, the maps were again uninterpretable.

We then collected highly redundant S-SAD data to a highest resolution of 2.15 Å. A sulphur signal was measured, but yet again the maps were uninterpretable. Eventually, the structure was solved when the phase information from the S-SAD and RIP data were combined.

Data were collected at the ESRF, beamlines ID 14-4 and BM 14, and at the SRS, station 9.6.

[1] Rudiño-Piñera E., Schwarz-Linek U., Potts J. R., Garman E. F., *Acta Cryst.*, 2004, D**60**, 1341-1345.
**Keywords: fibronectin, RIP, sulphur SAD**

**MS64.28.4**
*Acta Cryst*. (2005). A**61**, C83-C84
**NCS and Normal Modes Ensembles Solve Difficult MR Problem**
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We describe a strategy that has been used to push further the molecular replacement limits by taking advantage of the

oligomerisation state of a given molecule both in solution and in the crystal.

This strategy combines the information coming out from experimental data such as the existence of a non crystallographic symmetry in the crystal, reinforced by electronic microscopy data of the oligomeric structure in solution.

Computational methods are then used to extract the orientations and relative positions of each molecule in the oligomer in order to build an oligomeric model to search for a molecular replacement solution.

The closest monomer model in the PDB has 26% identity and, although accurate enough to detect the crystal structure, more contrasted results are obtained by using normal mode analysis to generate a series of models, including NMR-like ensembles.

**Keywords: molecular replacement, normal modes, non-crystallographic symmetry**

**MS64.28.5**

*Acta Cryst*. (2005). A**61**, C84

**Disorder and Twinning: New Equations and Applications to the Structure Determination of Proteins from the Carboxysome Shell**

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Although crystal structure determination for proteins is sometimes routine, this is often not the case. Structure determination may be complicated by a number of factors, including certain kinds of crystal growth disorders, of which merohedral twinning is one particularly interesting type. Twinning can prevent successful structure determination if it goes undetected, but it is often treatable if it is correctly identified. The effects of twinning are sometimes masked by other phenomena, such as pseudo-centering, scattering anisotropy, and non-crystallographic symmetry. Recently developed intensity statistics equations for dealing with these complications will be discussed, along with the emerging structure of proteins from the carboxysome shell, determined from crystals suffering from merohedral twinning. The carboxysome is a polyhedral protein shell, which resembles a viral capsid and is found in many bacteria. Bacteria employ the carboxysome shell as a primitive organelle by enclosing RuBisCO and other enzymes in its interior, in order to carry out cellular $CO_2$ fixation in a sequestered environment. This work is supported by the NIH and the BER Office of the DOE.

**Keywords: disorder,twinning, structure determination**

## MS65 PROTEIN INTERACTIONS WITH OTHER BIOLOGICAL MACROMOLECULES

***Chairpersons:*** Zihe Rao, Brian W. Matthews

**MS65.28.1**

*Acta Cryst*. (2005). A**61**, C84

**Protein-protein Complexes in Cell Adhesion**

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Recent structural results will be presented for the extracellular region of receptor protein tyrosine phosphatase μ (RPTPμ). RPTPμ mediates stable, homophilic cell adhesion. Strategies to produce well-ordered crystals of the full, multidomain, extracellular region will be described and the resulting insights into homophilic adhesion discussed.

**Keywords: glycoprotein, receptor, protein-protein complex**

**MS65.28.2**

*Acta Cryst*. (2005). A**61**, C84

**Activating the Molecule of Mass Destruction**

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Proteasomes are 700 kDa complexes of 28 protein subunits that are assembled in a barrel-like architecture. They are abundant in the cytosol and nucleus of eukaryotic cells, where they degrade numerous protein substrates in order to perform housekeeping and regulatory functions. The proteolytic active sites are sequestered inside the hollow proteasome structure, thereby protecting inappropriate substrates from degradation. Proteasomes are activated in vivo by activators that bind to the end rings of alpha subunits. The best known of these is the 19S activator, which mediates degradation of polyubiquitylated substrates. Two other activators, 11S and Blm10/PA200, stimulate hydrolysis of small peptide substrates. Structural and biochemical data will be presented on the interaction of an 11S activator with proteasome, and the mechanism of opening the entrance port into the proteasome interior will be discussed. Preliminary studies on the Blm10 activator will also be presented.

**Keywords: proteins structure, proteasome, macromolecular structures**

**MS65.28.3**

*Acta Cryst*. (2005). A**61**, C84

**Structure and Mechanism of RecBCD**

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Double strand breaks in bacterial cells can result from a variety of things including collapsed replication forks or other DNA damage. One mechanism for repair of breaks involves the multifunctional enzyme complex, RecBCD. RecBCD comprises two distinct DNA helicase subunits, a number of differentially regulated nuclease activities, and the ability to recognise a recombinational hotspot called Chi. In order to understand more about the molecular basis of these activities we have determined the crystal structure of RecBCD complexed with DNA. The structure reveals the basis for the two different helicase activities and explains the regulation of nuclease digestion. The structure also suggests how the enzyme might be able to scan DNA for Chi sequences as the DNA passes through the protein complex.

**Keywords: helicase, recombination, structure**

**MS65.28.4**

*Acta Cryst*. (2005). A**61**, C84

**Molecular Basis for the Allosteric Inhibition of JNKs by the Peptide Fragment from the Scaffolding Protein JIP1**

Yong-Seok Heo, S.-K. Kim, C. I. Seo, Y. K. Kim, B.-J. Sung, H. S. Lee, J. I. Lee, S.-Y. Park, J. H. Kim, K. Y. Hwang, Y.-L. Hyun, Y. H. Jeon, S. Ro, Y. Kwon, J. M. Cho, T. G. Lee, *Department of Drug Discovery, CrystalGenomics,Inc. Seoul, Korea.* E-mail: ysheo@crystalgenomics.com

The c-jun N-terminal kinase (JNK) signaling pathway is regulated by JNK-interacting protein-1 (JIP1), which is a scaffolding protein assembling the components of the JNK cascade. Overexpression of JIP1 deactivates the JNK pathway selectively by cytoplasmic retention of JNK and thereby inhibits gene expression mediated by JNK, which occurs in the nucleus. Here, we report the crystal structures of human JNK1, 2, and 3 complexed with pepJIP1, the peptide fragment of JIP1, revealing its selectivity for JNKs over other MAPKs and the allosteric inhibition mechanism. The specific hydrogen bonds between JNKs and pepJIP1 can provide the selective regulation. Binding of the peptide also induces a hinge motion between the N- and C-terminal domains of JNKs and distorts the ATP-binding cleft, reducing the affinity of the kinase for ATP. Considering the value of JNKs as therapeutic targets for several diseases, the information from these structures can contribute to the optimization of JNK inhibitors of high affinity and specificity, which can be derived from pepJIP1. In addition, we also determined the ternary complex structures of pepJIP1-bound JNK1, 2, and 3 complexed with SP600125, an ATP-competitive inhibitor of JNKs, providing the basis for the JNK specificity of the compound.

**Keywords: JNK, scaffolding protein, JIP1**