BAG MX-484 Beam time report.

Beam time March 6th ID14:4

Sample abbreviation	No of crystals tested	No of Datasets collected Native	Derivative	Phases from this exp.	Phases from MR	Typical resolution limit
SORT	40		3	No	No	3.2 Å
MALA	7		5	No	Yes	3.1 Å
MOR	8					4-7 Å
dCTPD	6					
MAN26A	3		1	No	Yes	2.5 Å
EJC	15	1				2.3 Å

Beam time May 19th 2006 ID23:1

Sample abbreviation	No of crystals tested	No of Datasets collected Native	Derivative	Phases from this exp.	Phases from MR	Typical resolution limit
RasGAPSH3	15		2	yes		1.8 Å
aFGF	6	3		no	yes	1.6 Å
12	2	2		no	no	3.5 Å
23	1	1		no	no	2.0 Å
34	4	4		no	no	3.3 Å
F12	2	2		no	no	2.8 Å
E1(Ca2) AMPPcP	3	1		no	yes	2.9 Å
4A3	2	1			yes	3.0 Å
EJC-AlF	4	1			yes	2.8 Å
Cub	3					7 Å
C3b	3					10 Å
hC5	6					9 Å

Beam time July 5th 2006 ID14:1

Sample abbreviation	No of crystals tested	No of Datasets collected Native	Derivative	Phases from this exp.	Phases from MR	Typical resolution limit
GluR5-ago1	12	2		no	yes	2.2 Å
GluR5-ant	11	1		no	yes	2.9 Å
Ig2	5	1		no	yes	1.0 Å
Np-23	9	1		no	yes	2.0 Å
MalA	4		1	no	yes	4.2 Å
EJC-AlF	5	1			yes	3.0 Å
RACK1	15			no	no	4.5 Å
SORT	5	1	1	no	no	3.5 Å
proSORT	10	1			no	4.5 Å

Results:

SeMet RasGAP SH3 domain.

Two useful SAD data sets were collected from two crystal forms of selenomethionine substituted protein. The crystals diffracted to 1.5 and 1.8 Å, respectively (PDB entries: 2j05, R-work/R-free: 17.7/19.9; 2j06, R-work/R-free: 20.8/24.0). A paper describing the structural results is under review in *FEBS Letters*. Structure solution by MR was attempted prior to this experiment and proved to be unexpectedly difficult. Only a single reasonable solution out of 1200 calculations emerged from a computer intensive screening using the program Phaser.

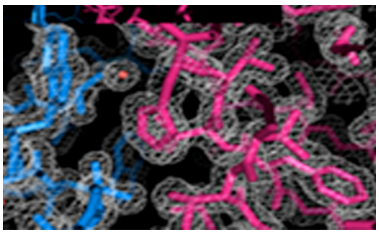

Figure: RasGAP SH3 domain. The electron density map produced from our experiment at ID23-1 was of high quality.

aFGF.

A native dataset was collected on rat acidic fibroblast growth factor to a resolution of 1.4 Å. The structure was solved by molecular replacement. Coordinates of the refined structure (R-work/R-free: 21.5/23.1) have been deposited (PDB entry 2j3p and a manuscript will soon be submitted.

GluR5 and nCAMs

Work is still in progress with data collected from the ligand-binding core of GluR5 in complex with one agonist and one antagonist, respectively, as well as with extracellular modules of neural cell adhesion molecules.

MalA

The structure of the *Sulfolobus solfataricus* αalpha-glucosidase MalA was determined recently [1]. Various MalA crystals soaked with the product glucose or the αalpha-glucosidase inhibitors deoxynojirimycin (DNM) and acarbose were tested. The datasets collected are generally affected by severe radiation damage. Statistics for the best datasets for

DNM and acarbose complexes are summarized in Table 1. The structures were solved by molecular replacement, and the refinements are still in progress.

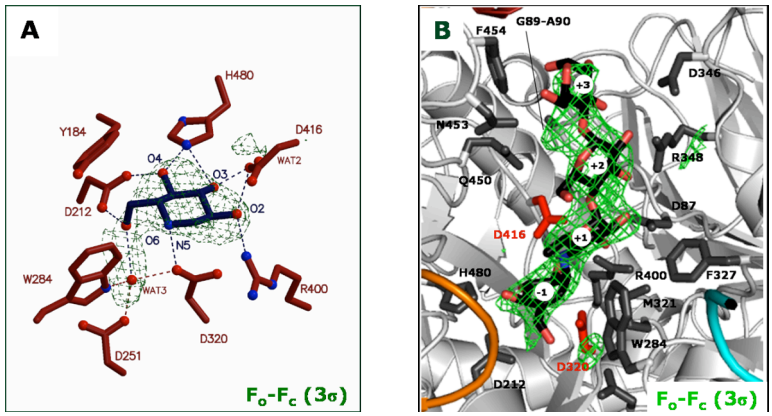

Figure 1 MalA complexes with the α-glucosidase inhibitors (A) DNM and (B) acarbose.

	MalA:DNM	**MalA:Acarbose**
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit cell (Å)	97.1, 192.5, 280.7	97.7, 191.5, 283.7
Resolution (Å)	35-3.3 (3.40-3.30)	35-2.8 (2.96-2.80)
R_{merge}	10.7 (31.0)	12.6 (42.6)
Completeness (%)	97.3 (95.1)	78.7 (78.6)
<I/σ>	11.0 (3.9)	6.8 (3.1)
R/R_{free}	21.6/23.8	21.5/23.5

Table 1 Data collection statistics for the MalA complexes

[1] Ernst, HA, Lo Leggio. L., Willemoës, M., Leonard, G., Blum, P. & Larsen, S. (2006). *J. Mol. Biol*., **358**, 1106-1124.

The Exon Junction Complex.

In higher eukaryotes a multiprotein exon junction complex is deposited on spliced mRNAs. The complex is organized around a stable core serving as a binding platform for numerous factors that influence mRNA function. We have determined the crystal structure of a tetrameric exon junction core complex containing the DEAD-box ATPase eIF4AIII bound to an ATP analogue, MAGOH, Y14, a fragment of MLN51, and a polyuracil mRNA mimic at 2.3 Å resolution based on data collected at the SLS synchrotron (Andersen *et al.*). A completely equivalent data set was later collected at ESRF id 14-3. eIF4AIII interacts with the phosphate-ribose backbone of six consecutive nucleotides and prevents part of the bound RNA from being double stranded. The MAGOH and Y14 subunits lock eIF4AIII in a prehydrolysis state, and activation of the ATPase probably requires only modest conformational changes in eIF4AIII motif I. The crystal structure of the free eIF4AIII was

determined at 3.3 Å resolution from data collected at ESRF id23-1. These data were collected from needle shaped crystal with a cross section of 20 μm. This structure demonstrates that a large conformational change involving a 160º rotation of a domain takes place upon incorporation of eIF4AIII into the EJC.

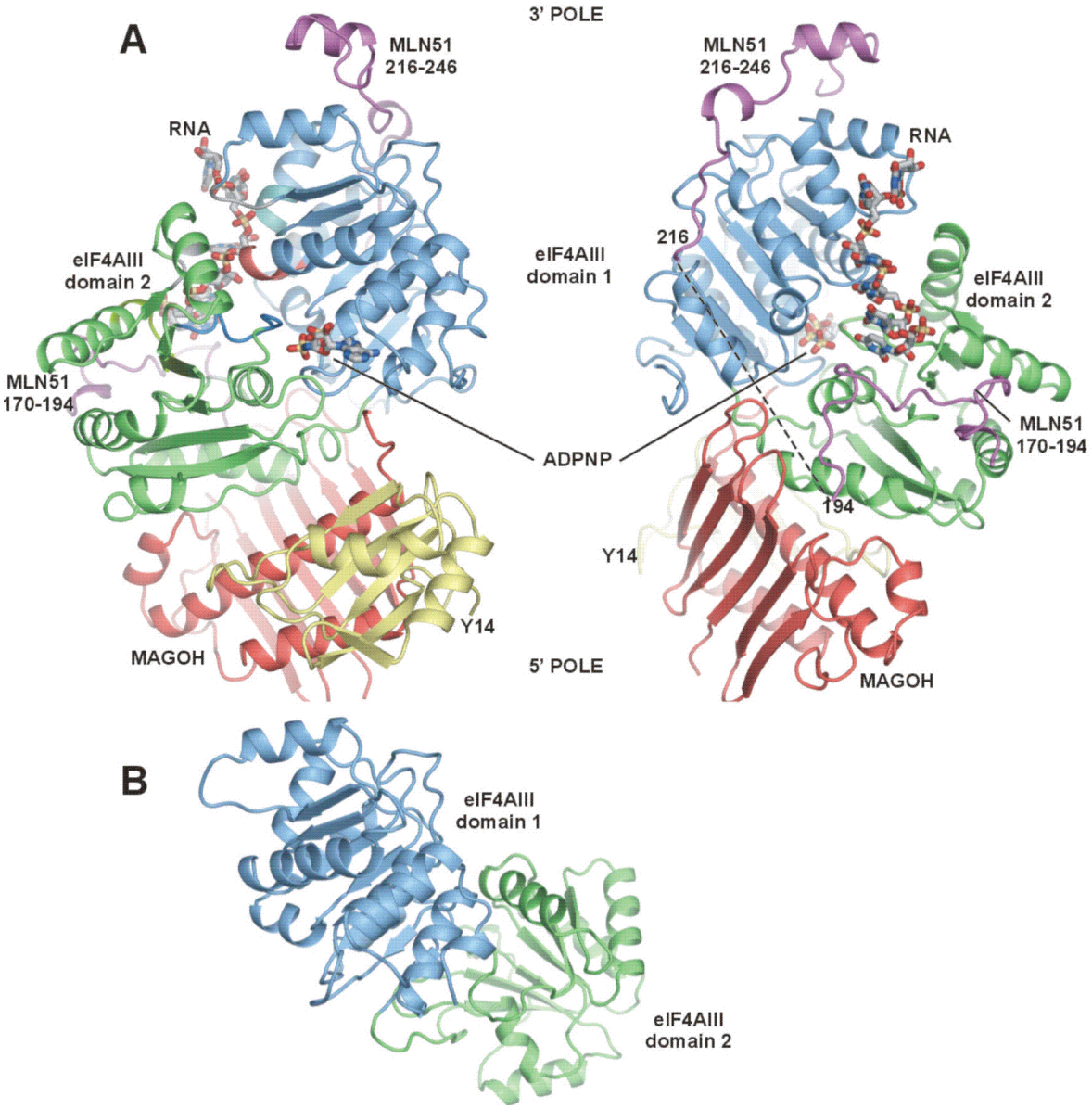

Figure 1. Structures of the Exon Junction Complex and free eIF4AIII. **A,** The EJC viewed from the ATP side (left) and the RNA side (right) with domains 1 and 2 of eIF4AIII coloured blue and green, respectively. MLN51 is shown in purple, Y14 in yellow, and MAGOH in red. The dotted line connects the two ordered fragments of MLN51. **B**, The open conformation of eIF4AIII with domain 1 in the same orientation as in the left panel of **A**.

Andersen, C. B. F., Ballut, L., Johansen, J. S., Chamieh, H., Nielsen, K. H., Oliveira, C. L. P., Pedersen, J. S., Seraphin, B., Le Hir, H., and **Andersen, G. R**. (2006) *Science, published online August 24*. Structure of the exon junction core complex with a trapped DEAD-box ATPase bound to RNA.

Sortilin

Sortilin is one of five members of the Vps10p_D receptor family. This family of type-1 receptors are mainly expressed in the central nervous system. It has been found that in the presence of proNGF, sortilin forms a complex with the neurotrophic receptor $P75^{NTR}$. This complex formation signals apoptosis to the cell.

Crystals of the extracellular part of sortilin has been obtained both in the apo form, in complex with neurotenisn and a neurotensin analogue NT69L. Also crystals of a mutant form possessing the propeptide has been obtained. A large number of heavy atom soaked crystals has been tested and some datasets have been collected.

Crystals of sortilin either in the apo form or in complex with neurotensin is found in two different C2 spacegroups. Within each of these spacegroups we find variations in cell dimensions of up to 6%. Even though data are collected at 100 K radiation damage is observed which makes sad phasing from a single derivative difficult. Recently crystals of the mutant has been obtained and was found to be of spacegroup R3.