ESRF	**Experiment title:** BAG-LEBS-2003-2	**Experiment number**: 30-01-627
Beamline: BM-30 A	**Date of experiment**: from: 15/11/2003 at 8h00 to: 16/11/2003 at 8h00	**Date of report**: 11/12/2003
Shifts: 6	**Local contact(s)**: Dr. Sonia Fieulaine / Philippe Carpentier	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

During our beam time it was not possible to do MAD experiments on BM30 with automatic wavelelength tuning and optimisation due to problems on the beam line. Furthermore computer network and computing on the beam line were very slow during our experiment.

G. Hible, L. Renault : Structural studies of bacterial Guanylate Mono-Phosphate Kinase (GMPK) and of the Arf regulatory GTP-binding protein activation (3 shifts):

We used 3 shift beam time on BM30A to collect monochromatic data sets and study diffraction of new small crystals.

Structural study of a bacterial Guanylate Kinase:

Guanylate Kinase (GMPK) is a nucleoside monophosphate kinase that is essential for the biosynthesis of GTP and dGTP by catalyzing the reversible phosphoryl transfer from ATP to (d)GMP to yeld ADP and (d)GDP. In addition, antiviral prodrugs like Aciclovir, and anticancer prodrugs, are dependent of this enzymze for their activation. Our aim is to characterize the catalytic intermediates and use the structures as models for the activation of antiviral prodrugs.
We have investigated different nucleotides in complex with a bacterial GMPK (23,5kDa).

We tested several crystals of two complexes of GMPK bound to nucleotide.

- One complex gives rod-shape crystals which exhibit anisotropic diffraction, with a resolution limit of 3,4Å. Furthermore the crystals were generally twinned.
- The other complex gives crystals that diffract to 3,5 to 4Å, depending on the nature of the cryoprotectant, with big unit cell dimension. So the overlap between the spots is very important even with small oscillations (0,5°). As the estimation of the Matthews parameters give 6 to 8 molecules per asymmetric unit, the resolution of the structure by molecular replacement will be very difficult

So we are thinking of producing heavy-atom derivatives in order to solve the structures by MAD or MIRAS.

Structural study of the GEF-catalysed activation of Arf small GTP-binding protein:

Arf G proteins functions as binary switches in regulating transport vesicle budding in endocytosis and exocytosis and phospholipase D activation by cycling between inactive cytosolic GDP-bound and active membrane-anchored GTP-bound states. Like many other regulatory G proteins, the conversion of Arf-GDP to Arf-GTP is intrinsically very slow and is catalyzed by a guanine nucleotide exchange factor (GEF) along a complex multi-step reaction which is poorly understood at the molecular level. This reaction involves transient binary and ternary complexes between G protein, guanine nucleotide, and GEF. We have crystallized and solved recently two different transitory complexes between Arf1 and the Sec7 domain of ARNO GEF trapped by a mutation in the GEF active site or by the use of the inhibitor Brefeldin A (Renault, et al. (2004) Nature 426, 525-530) and try now to complete the view of additional steps of the reaction.

We collected on BM30A one data set to 1.8 Å of a complex between Arf1 and mutated ARNO-Sec7 for which we try to access to a step of the reaction by crystal back–soaking. Molecular replacement is in process to see if the ligand has been dissociated.

Paola Llinas (Ph. D. student, CEA-Saclay), Marie-Helene Ledu (Research Assistant, CEA-Saclay) : Structural studies of human receptor involved in metastasis proliferation (3 shifts):

The next 3 shifts beam time have been lent to the group of Marie-Helene Ledu from the CEA-Saclay for SAD experiments at the Os LIII absorption edge or the W LIII absorption edge. Her experimental report is written below:

Malgré le problème informatique nécessitant des changements de longueur d'onde manuels, la session s'est bien déroulée.
Nous avons collecté un jeu de données SAD au pic de l'osmium, mais les intensités sont très faibles (problème de taille du cristal).

Nous avons collecté trois jeux de données SAD incomplets au pic du tungstene, dont un est inexploitable. Les deux autres ont été rassemblés à un jeu de données collecté préalablement, menant à une complétude de 60%. Le tungstène semble absent.

Nous avons collecté un jeu de données MIR avec un dérivé uranium, et le traitement est toujours en cours.

Pour ce projet, il est toujours nécessaire d'effectuer de nombreux tests avant de trouver un cristal qui diffracte, et les temps d'exposition se situent autours de trois minutes / image.