	Experiment title: BAG-LEBS-2003-2	Experiment number: MX-204
Beamline: ID14-1	**Date of experiment**: from: 21/11/2003 at 8h00 to: 22/11/2003 at 8h00	**Date of report**: 17/02/2003
Shifts: 3	**Local contact(s)**: Dr. E. Micossi	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

***Guillaume Hible~ (Ph.D. student), *Louis Renault (research assistant, CR1)~, Valérie Biou~, *Corine Mas#, *Renaud Dumas# ~LEBS, 1, Av. de la Terrasse, 91190 Gif-Sur-Yvette, France, # laboratoire de physiologie cellulaire végétale DRDC/CEA-Grenoble, France**

Béatrice GOLINELLI* and Stéphane MOUILLERON*, LEBS, CNRS, Gif sur Yvette

Report:
G. Hible, L. Renault : Structural studies of bacterial Guanylate Mono-Phosphate Kinase (GMPK) and of regulatory GTP-binding proteins (1.25 shift):

We used 1.25 shift beam time on ID14-1 to collect monochromatic data sets and study diffraction of new small crystals or of poorly diffracting crystals on three projects.

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 enzyme 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 already collected at ESRF two different conformational states of a bacterian guanylate kinase bound to guanine nucleotides. On ID14-1 we collected one data set to 2.7 Å of the unliganded form of this guanylate kinase (Rsym=34% for the resolution shell from 2.8 to 2.7 Å). The molecular replacement has been completed.
Before the end of refinement of the structure, optimized crystals allowed the collect of a complete dataset by Andrew Thompson and Eric Girard on ID29 (15/12/2003) at 2.1Å resolution, which exhibit the same space group and unit cell parameters. Actually the refinement of the 2.1Å structure (Rfree=28%) and structures comparison are in process.

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 Arf1and 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 ID14-1 one data set to 1.5 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.

Structural study of the GTPase mechanism of the large GTP-binding protein GBP1:

GBP1 is a 67kDa multi-domain GTP-binding protein which has an antiviral activity poorly understood and hydrolyzes GTP into GDP and GMP. Our aim is to characterize its unique GTPase properties of the protein (Prakash B. et al. (2000), Nature 403, 567-71) and its regulation mechanism by solving the different intermediate states of the GTP and GDP hydrolysis reaction for which crystals have been obtained.

We have tested on ID14-1 new small GBP1 crystals obtained with a non-hydrolyzable GDP analogue. No data set were collected since all crystals showed diffraction patterns heavily splitted.

Corine Mas, Renaud Dumas, Valerie Biou: complexes of threonine synthase (TS) (1 shift)

Threonine synthase is an allosteric enzyme in plants. It is activated by Sadenosyl methionine (SAM) and uses a pyridoxal phosphate (PLP) as cofactor. We have solved the structure of the apo-enzyme and are now seeking to characterise the allosteric movement. During this experiment, we have tested 15 crystals and measured data on 2 of them. Below is the scaling statistics for the best data set.

N	1/resol^2	Dmin	Nmeas	Nref	%poss	Mlplcty	Rmeas	(Rsym)	PCV	PCV0
1	0.013	8.85	3894	989	99.8	3.9	0.052	0.044	0.068	0.068
2	0.026	6.26	7652	1668	100.0	4.6	0.051	0.045	0.060	0.060
3	0.038	5.11	10018	2114	100.0	4.7	0.057	0.050	0.068	0.068
4	0.051	4.43	12020	2493	100.0	4.8	0.051	0.045	0.061	0.061
5	0.064	3.96	13557	2782	100.0	4.9	0.060	0.053	0.072	0.072
6	0.077	3.61	15089	3075	100.0	4.9	0.083	0.074	0.102	0.102
7	0.089	3.35	16459	3338	100.0	4.9	0.128	0.114	0.160	0.160
8	0.102	3.13	17638	3568	100.0	4.9	0.204	0.182	0.260	0.260
9	0.115	2.95	18642	3769	100.0	4.9	0.346	0.308	0.450	0.450
10	0.128	2.80	19830	3998	100.1	5.0	0.496	0.443	0.652	0.652
	Overall		134799	27794	100.0	4.8	0.085	0.075	0.103	0.103

	Nmeas	Nref	%poss	Mlplcty	Rmeas	(Rsym)	PCV	PCV0

Even though the structure could be solved using molecular replaceement from the apo enzyme, the refinement does not proceed well. We are now crystallising selenated protein to try and obtain independent phases.

Béatrice GOLINELLI* and Stéphane MOUILLERON* (0.75 shift)

A few crystals of glucosamine 6 P synthase (GlmS) have been tested but a lot were twinned. However, we succeeded to record a 3.1 A data set of GlmS in complex with 6-diazo-5-oxo-norleucine (DON) and 6-deoxy-amino-mannitol (Space group P1, a =83,45 b=87,36 c=184,28 α=90,44 β=89,90 γ=89,61). The completion is 84,3 % (80,3 % in the last shell). The structue is being solved by molecular replacement and contains the ligands.