	Experiment title: **BAG - CNRS gif sur Yvette** b-glucosyl transferase, catalytic antibodies...	**Experiment number**: LS 1928
Beamline: **ID14-H1**	**Date of experiment**: from: 20/06 to: 22/06	**Date of report**: 31/08/2001
Shifts: 6	**Local contact(s)**: Hassan Belrhali	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Solange Morera* CNRS Laurent Larivière* DEA
B. Golinelli*, CNRS
L. Renault*, CNRS S. Pasqualato* Ph. D. Student
Stéphane Duquerroy*, Felix A. Rey, Berend Jan Bosch*

Report:

S. Morera

β-Glucosyltransferase (BGT) is a DNA-modifying enzyme encoded by bacteriophage T4 which catalyses the transfer of glucose from uridine to 5-hydroxymethylcytosine in double-stranded DNA. We have shown that BGT cleaves the glucose of UDPG. To identify the glucose position, inhibitors of BGT mimicking UDPG were diffused in native BGT crystals. We collected 4 data sets, three with BGT + inhibitor and one with BGT + UDPG. All the structures are under refinement process.

B. Golinelli

A crystal of an antibody possessing peroxidase activity in the presence of a porphyrine cofactor was soaked in the presence of the cofactor. The crystals belong to space groupe C2 with a= 56.1 Å, b=62.9 Å, c=113.6 Å, β=91.8 and a mosaicity of 0.8 that necessitated a $\Delta\varphi$=0.5. Whereas the crystals diffracted to only 3.1 Å resolution at LURE, a full data set at 2.1 Å resolution was recorded on ID14-1. The structure has been solved by molecular replacement and has been refined to Rfree=29.9 %, R= 29.6 %.
Data from another crystal of a different porphyrin antibody possessing peroxidase activity have also been collected but are not yet fully processed. These belong to space group C2 with a=263.8 Å, b=55.4Å, c=147.1Å, β=122.4.

Antibody 4B2 catalyses an allylic rearrangement. Its structure in the presence of its hapten (PNAS, 2000, 97, 9892) has shown that a hapten containing a positively charged amidiniuml function is able to induce a carboxylate in the active site of an antibody. The structure at high resolution of the antibody in the presence of a substrate analogue is needed to confirm that the carboxylate acts as a general base in catalysis and validate this new strategy. New crystals of this antibody have been tested in different cryoconditions but they did not diffract beyond 4 Å.

L. Renault, S. Pasqualato

We have used 1 shift of beam time on beam line ID14-EH1 on 21/6/2001 to collect a data set from a crystal of an apoptotic fragment from proEMAP2 (Endothelial Monocyte-Activating Polypeptide 2). ProEMAP2 is associated with several aminoacyl-tRNA synthetases and becomes an independent domain with inflammatory

cytokine activity upon apoptotic cleavage from the p43 component of the multisynthetase complex. We collected a complete data set from a twinned crystal that diffracted to 2.2 Å.

Reflections could be reduced and scaled with DENZO and Scalepack from 24 to 2.5 Å with the following statistics: Rsym = 7.9 % (23 % from 2.59 to 2.5 Å), average I/_ = 11, redundancy = 6, mosaicity = 0.34°, B(Wilson)= 58 $Å^2$. Crystals belong to space group I222 or $I2_12_12_1$ with a=43.95, b=94.78 and c=95.90 with one molecule in the asymetric unit.

The structure was solved by molecular replacement in space group I222 from the crystal structure of a shorter EMAP2 fragment that we solved this year [Renault L. et al., 2001, EMBO J. 20, 1-9]. Refinement of the structure is in progress with CNS (current R-cryst and R-free, 28% and 34%). Although the quality of the data is sufficient to model small conformational changes in the EMAP2 portion, the additional domain is not visible in the electron density and refinement is blocked, which we attribute to the twinning problem of the data.

Stéphane Duquerroy, Felix A. Rey, Berend Jan Bosch

Herpesviruses possess a lipid envelope in which are anchored eleven different glycoproteins. Among them, glycoprotein gD is responsible for receptor recognition and binding, an important step which precedes fusion of the viral membrane with that of the target cell, leading to infection. gD is therefore indispensable to the viral cycle and indeed, some monoclonal antibodies to gD are known to neutralise HSV1 in cell culture. gD is a typical type I membrane protein of about 400 amino-acids with a large N-terminal ectodomain and a small C-terminal endodomain. Amino-acids 1-317 of gD from Herpes Simplex Virus 1 (HSV1) (containing two putative N-linked glycosylation sites) was cloned and expressed in CHO cells as a fusion protein with an Fc fragment from an antibody. In collaboration with E. A. Stura, we have obtained crystals of this fusion protein (gD-Fc). The crystals also contain an Fab produced from the cleavage of a rheumatoid factor (RF), that binds to the Fc part of the construct. Those crystals will therefore yield not only the structure of gD, but also that of this RF. This too will be of interest, as rheumatoid arthritis is an important human disease.

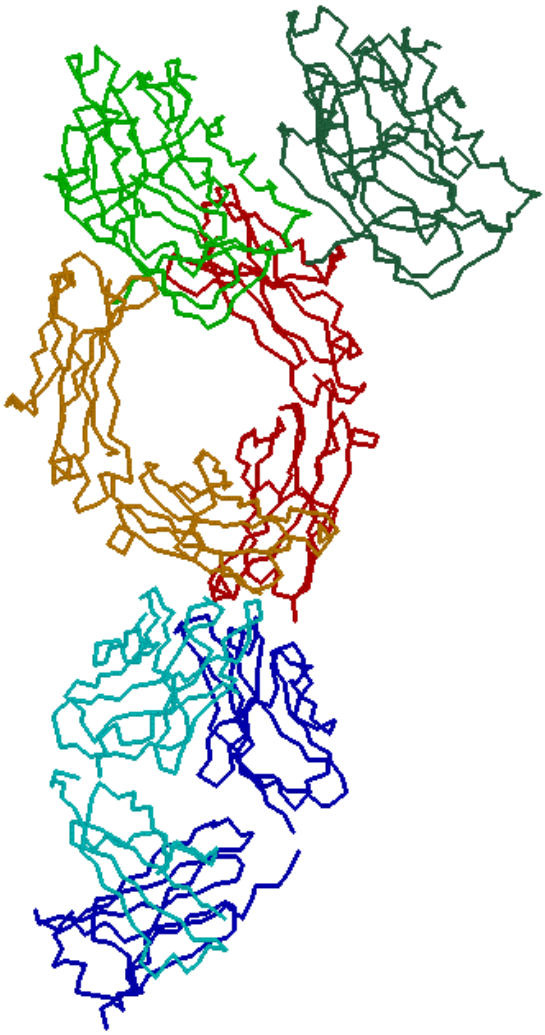

***Figure** : Initial model of the gD-Fc fusion protein in complex with a Fab fragment.*
The Fab molecule (bottom of the figure) produced from the cleavage of a rheumatoid factor recognizes the base of the Fc molecule (middle of the figure). At the top of the figure, two gD molecules.

A first X-ray diffraction experiment provides a complete dataset extending to 3.1 Å resolution (space group C2, a=243.6 Å b=76.4 Å, c=102.8 Å and β=91.5°). Molecular Replacement solutions for Fab Fc and gD models produced the first electron density maps. R factor is 42 % after rigid body refinement of this solution. In order to reduce model bias and to improve this initial maps we also collected two prospective heavy atom derivatives. The two crystals belong to the same space group C2 (a=256.2 Å, b=75.9 Å, c=103.0 Å and β =101.4°, 3 Å resolution and a=225.4 Å, b=75.3 Å, c =102.0 Å and β=92.0, 3.5 Å resolution respectively) but were not isomorphous to the first one.

MR solutions for these two other crystal forms have been found for the Fab-Fc complex but not for gD. Initial R factors are 42%, 45% and 48% respectively for the three crystal forms after rigid body refinement of the MR solution. Crystal averaging provided enhanced electron density maps. They clearly show Fab and Fc parts of the model but electron density is more disordered for gD molecules.

Refinement is underway starting with the Fc and Fab and will be be ended by gD molecules reconstruction.