ESRF	**Experiment title: Herpesvirus Envelope Glycoprotein gD** (Data collected as part of the BAG CNRS -Gif sur Yvette coordinated by M. Knossow)	**Experiment number**: LS 1798
Beamline: ID14-EH1	**Date of experiment**: from: 08/02/01, 19.00 to: 09/02/01, 07.30	**Date of report**: 27/02/01
Shifts: **1**	**Local contact(s)**: Ed Mitchell	*Received at ESRF:*

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Report: 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. We are also working with an anti-gD antibody that neutralises HSV1 in cell culture. We have crystals of the Fab cleaved from this antibody.

The crystals of gD-Fc:RF diffracted to 5 Å on EH1 under cryoconditions. We are working on growing better crystals (figure 1).

We collected a 1.9 Å dataset on an Fab derived from a neutralizing anti-gD antibody. Spacegroup is $P2_1$ with cell parameters a=45.75, b=67.91, c=71.14, beta=106.4, completeness 98.7%, multiplicity 3.1, R_{sym} 4.4%. The structure is now being refined.

We collected a 2.5 Å dataset on a complex between an Fab and a mutant of protein L

from *Peptostreptococcus magnum*. Space group is $P2_12_12_1$ with cell parameters a=77.25 b=100.39 c=148.70, completeness 90.6%, multiplicity 2.2, R_{sym} 7.7%. The structure has been solved by molecular replacement and we can confirm that this mutant does indeed utilize a single site on the antibody whereas the wild type uses two binding sites to complex to the antibody light chain (Graille, Stura *et al.*, submitted). Complexation with protein L is a general method for antibody-antigen crystallization since protein L (and its single-binding-site mutant) bind outside the antigen combining site. This method is to be applied to the crystallization of the complex of gD with its neutralising antibody.

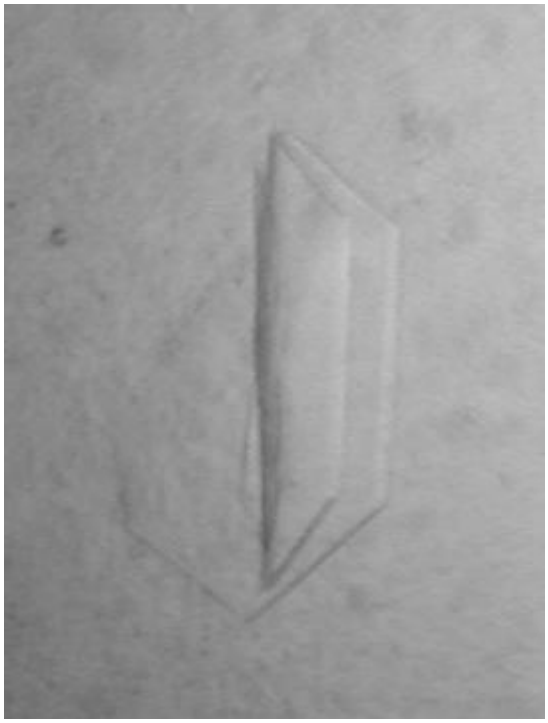

Figure 1: Crystals of RF:gD-Fc after deglycosylation with PNGase F