ESRF	**Experiment title:** Crystal Structure of the Semliki Forest Virus glycoprotein spike.	**Experiment number:** LS958
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Shifts; *9*	**Local contact(s):** Julien Lescar *	*Received at ESRF :* 01 SEP. 1998

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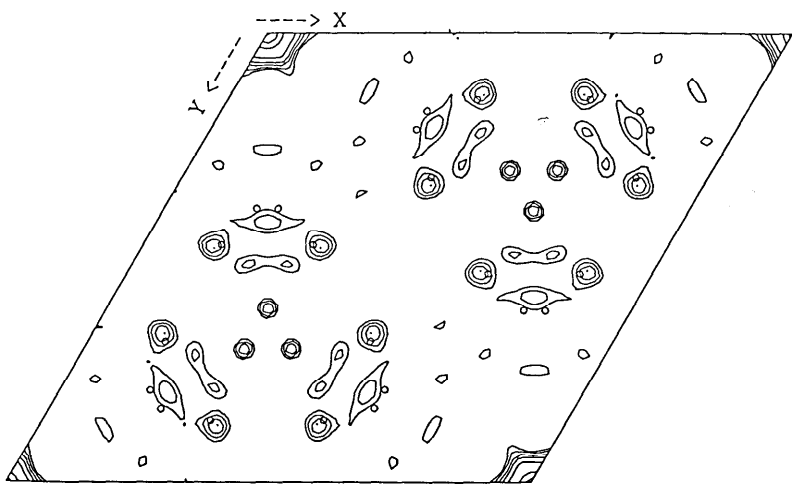
Report:

The aim of this project is to determine the crystal structure of the glycoprotein spike from Semliki Forest Virus (SFV). The SFV crystals belong to the hexagonal space group P6222 or its enantiomorph P6422, with unit cell dimensions a=80.18 Å c=335.09 Å, and diffract X-rays rather weakly. On the ID2 beamline, using a collimated beam of 0. lx 0.1 mm2, we were able to collect diffraction data to about 2.8 A along the c* axis and to only about 3.4 Å along directions perpendicular to it, for the largest crystals (about 0.1x0.1x0.3 mm^3). This typically required exposure times of 30s with an oscillation range of 0.5°. In addition, rapid degradation of the diffraction resolution (specially in the plane perpendicular to c*) required the use of several crystals for collecting the higher resolution data sets.

Since the presence of a whole El-E2 heterodimer within the asymmetric unit would have implied a solvent fraction of about 35%, N-terminal microsequencing of irradiated crystals has been performed. This showed the presence of only the El glycoprotein (Mw of 45 kDa). This result which gives a solvent content of 62%, is consistent with the long time needed for initial crystal growth (about 6 weeks) and the formation of a precipitate within the crystallizing medium, presumably formed by the E2 protein. Subsequently, we have modified our purification protocol and crystallized only the El glycoprotein. Three related crystal forms have been obtained: using the El glycosylated fraction (C form), after treatment with glycosidases (A form) or the original mixture (B form).

On ID2, we have collected complete data sets for native crystals and also for several putative derivatives at medium (3.5-4Å) or low (5-6Å) resolution for the B and C form crystals which happen to diffract best. Two heavy atom compounds (K_2PtCl_4) and ($K_3UO_2F_5$), proved to be the most promising. The Pt derivative allowed us to define the outline of the molecule, though this derivative alone was not sufficient for high resolution phasing. Interpretation of the Uranyl isomorphous difference Patterson maps at 4.5 A resolution revealed one major site in general position and a cluster of sites near a special position of the crystal. Later on, we have collected this same derivative in Hamburg, at the wiggler beam-line BW7A, at the Uranyl LIII absorption edge. Anomalous Patterson maps calculated at 4.5 Å resolution confirmed the interpretation of the Uranyl heavy-atom structure (fig 1).

Thus, our current efforts aim at producing a 4.5 Å resolution map by combining phase information from both the Pt and U derivatives. These efforts are made difficult by the significant lack of isomorphism we observe between different data sets, which can be illustrated by unit cell dimension changes of about 2% in the dimension of the a axis (a=80.18 A for the native C form, and a=78.60 for the Uranyl derivative). Thus, our goal is now to determine the structure by the MAD method using crystals derivatized with this Uranyl compound.



Scale = 1.0000 mm/A Section 60
20-5 A c9 anomalous excluding FC9<3sig and DANO > 270