	Experiment title:	**Experiment number**:
	Stathmin – tubulin complex; Fusion domain of the spike protein of Mouse Hepatitis Virus	LS 1928
Beamline: ID14-EH4	**Date of experiment**: from: 01/05/01 to: 2/5/01	**Date of report**:
Shifts: 3	**Local contact(s)**: Raimond Ravelli	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Stéphane Bressanelli*, Laboratoire de Génétique des Virus, CNRS, Gif-sur-Yvette

Stéphane Duquerroy*, same affiliation A. Rey, same affiliation Berend Jan Bosch*

Benoit Gigant *, Marcel Knossow * LEBS CNRS Gif sur Yvette

Report:

Benoit Gigant, Marcel Knossow

Stathmin is a 17 kDa ubiquitous phosphoprotein that has been proposed to be a relay integrating diverse intracellular signalling pathways. It has recently been identified as a microtubule destabilising factor likely to be implicated in various microtubule dependant cellular functions in interphase or mitosis. Stathmin influences microtubules dynamics *in vivo* and *in vitro* either by preventing assembly or promoting disassembly of microtubules in a concentration-dependant manner. This effect is reduced upon stathmin phosphorylation by kinases.

Stathmin interacts with the αβ tubulin heterodimer to form a ternary complex, one stathmin for two tubulins. This complex has proven to be stable enough to be crystallised, whereas no good-diffracting crystal of tubulins has been reported up to now. The only near-atomic structural informations of tubulin come from electron microscopy of tubulin protofilaments. We have determined the 4 Å x-ray structure of a complex of GDP-tubulin with the stathmin-like fragment of RB3, a stathmin family protein (PDB ID code : 1FFX). This structure was solved by molecular replacement using the electron microscopy model and this work is now published (Cell, Vol. 102, 809–816, September 15, 2000).

While this structure is a milestone in the crystallography of tubulin, more precise data are required for a molecular description of the biological action of stathmin family molecules as well as of the interaction of small ligands of tubulin which interfere with microtubule dynamics. The last period was devoted to this aim. With bigger crystals than previously tested of tubulin in complex with a Se-met form of RB3, we were able to locate the four methionines of RB3. These data on

their own give a lot of informations on the RB3 part of the complex (orientation and a quite precise sequence assignment of the α-helix of RB3, localisation of its N-terminal part), which cannot be gained from the molecular replacement structure.

Felix Rey et al.

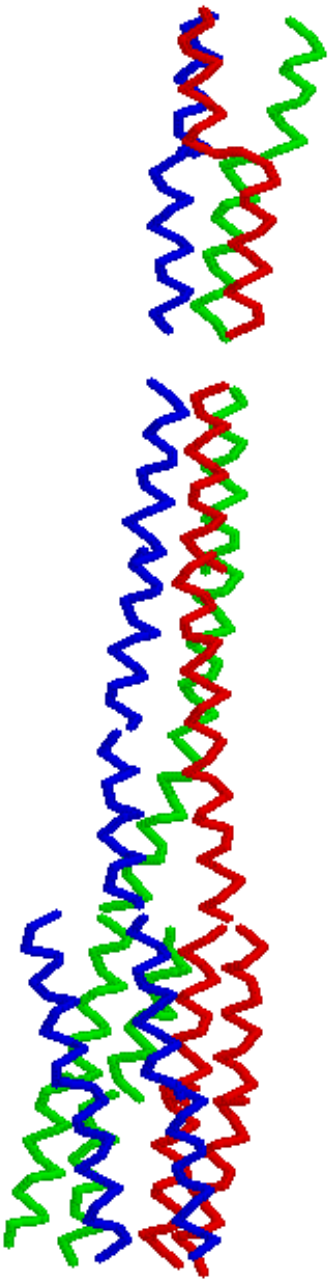

Figure 1 : Initial model of the membrane fusion domain of MHV. An extended three helix coiled-coil constitutes the core of the structure. It is surrounded by three smaller helices, which is the typical fold of type I fusion protein like influenza hemagglutinin.

Coronaviruses are large, enveloped, plus-strand RNA viruses. They cause highly prevalent diseases in humans and domestic animals and include human respiratory coronavirus (the "common cold" virus) and feline infectious peritonitis virus, among others. They cause systemic or localized infections, depending on whether or not they are restricted to a few cell types. Host specificity and membrane fusion are mediated by the spike glycoprotein, S. Mouse hepatitis virus (MHV) has been the object of intense study as a convenient model for coronaviruses. An accurate structure of the fusion domain of MHV–S could prove an important insight into the virus-cell interactions. On the practical side, it could lead to the design of antiviral drugs that interfere with the function of the spike protein. Indeed, it is known that virus infectivity can be neutralized by inhibition of membrane fusion (e.g., with monoclonal anti-S antibodies).

The complete S protein is a membrane protein of some 1600 residues. Of those, residues 953-1048 and 1216-1254 form the membrane fusion domain. This domain was reconstituted by producing the two polypeptides in *E. coli* and assembling them *in vitro*. They form a highly stable heterodimer that was crystallised both in the native form and as a selenomethionine derivative.

We collected a MAD dataset (three wavelengths) on the Se-Met derivative of a first crystal form. The crystals belong to spacegroup R32 with cell parameters a=b=55 Å, c=298 Å, alpha=beta=90°, gamma=120°. Diffraction was anisotropic, extending to 2.3 Å along c* and to about 3.5 Å along a*, b*. The resulting electron-density map allowed building of part of the model (Figure 1). It clearly shows typical fold of fusion type I molecules with a three stranded extended coiled-coil in the center of the trimer and a smaller flanking helix.

We also characterized a second crystal form (space group R3, a=b=62.6 Å and c=149.2 Å) Diffraction is still very anisotropic, extending to 3.0 Å among c* and only to 3.8 Å in other directions. Cross-crystal averaging failed to produce better electron density maps. Better phases might be obtained with the Se-Met derivative of this second crystal form, though. Model construction is underway but side chain building is still challenging due to the limited quality of electron density maps. This is probably the consequence of the strong anisotropy of all the data we recorded so far.