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Shifts: 3 shifts	**Local contact(s):** Andrew THOMPSON	*Received at ESRF :* 0 1 MAR. 1999

Names and affiliations of applicants (*indicates experimentalists):

BAG main proposer: Otto DIDEBERG
Eva PEBAY-PEYROULA, IBS*
Sylvestre GRIZOT, IBS *
Claudine COHEN-ADDAD, IBS*
Frank KOZIELSKI, IBS*
Richard WADE, IBS*
Damien FLEURY, IBS*
Salvatore DE BONIS, IBS
IBS: Institut de Biologie Structurale, 41 av. des Martyrs, 38027 Grenoble Cedex 1

Report:

Two projects were investigated during the 3 shifts.

A- NADPH oxydase complex

This complex is present in neutrophile cells, interacts with the membrane and plays a key role in the defense system of vertebrates. It is composed of two one membrane protein component and and several cytosolic proteins, necessary for the activation of the whole complex.

We want to understand the protein-protein associations within this macromolecular complex and how the soluble proteins interact with the membrane part to induce the production of superoxyde ions, responsible for the destruction of bacteria.

A complex of two of the cytosolic proteins, a small G-protein, rac1, associated to a rhoGDI protein has been crystallized for the first time.

Several crystals were tested (data collected at 100K). The best crystal diffracted up to 2.7 Å. The results are summarized below.

Crystal-detector distance=250 mm, λ=0.9465 Å, step per frame 1 °, exposure time=2.5 s, resolution 2.7 Å, orthorhombic space group $P2_12_12$, a=155.6 b=89.9 c=62.9 Å, Rsym=5%, completness=85%.

B- Microtubule motor proteins, tubuline and associated proteins

Kinesin is a microtubule associated motor protein and play many essential roles within eucaryote cells (distribution of organelles within the cytoplasm, intracellular traffic, mitosis and myosis). The aim of the project is to understand how these motors move along microtubules in an ATP dependent way by combining the atomic structures obtained by X-ray diffraction with the medium resolution 3D maps of motor-microtubule complexes using electron cryomicroscopy. We want to obtain the crystal structure of the motor proteins in the different nucleotidic states.
We have previously solve the structure of ncd dimer (see report on experiment LS1062). We have now obtained crystals of different kinesines from *Drosophila* (ADP state). and these crystals were tested during the present experiment.
The results are summarized below.

- ncd dimer with a long coil-coil region, crystals difficult to be frozen, weak diffraction observed up to 8 Å at room temperature, very high mosaicity, cell dimensions ~ 90, 200, 290 Å.

- Kinesin from *Drosophila,* monomeric, ADP state, tiny crystals (40x40x300 microns) hexagonal space group a=134.8 c=85.7 Å, uncomplete data collected up to 3.5 Å resolution (detector-crystal distance=250 mm, λ=0.9465, step per frame=0.5°).

- Kinesin from *Drosophila,* short dimeric, ADP state, tiny crystal (30x30x200 microns), resolution limit 3.6 Å, cell parameters ~420x420x100 Å.

- Different tests on the previous ncd dimer (data previously collected up to 2.9Å); no improvement of the resolution could be obtained.
This project is a long-term project. In the next future, we will work to the improvement of the size and the quality of the crystals and will also start crystallization work with a strictly non-hydrolysable analogue of ATP.

- Other tests were also performed on crystals of tubuline complexed with SCG10, a neurone-specific protein that plays a role in the assembly-desassembly of microtubules. Very tiny crystal (10x10x100 microns) diffracted at 7 Å (room temperature)