ESRF	**Experiment title:** Functional microtubule motor proteins: crystallographic study of ***Drosophila*** ncd and kinesin proteins	**Experiment number:** IS-908
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Report:

Kinesin is a microtubule associated motor protein that was first isolated in 1985. Genetic and immunological methods have since led to the discovery of many similar proteins, collectively known as the kinesin-like family. These motors are believed to play many essential roles within eucaryote cells. In particular, important aspects of modern cell biology concern the role of these motors in organising the distribution of organelles within the cytoplasm, in intracellular traffic, and in mitosis and myosis. These proteins have therefore attracted considerable interest as model systems for understanding how molecular motors work. Native kinesin is a 380 kDa heterotetramer with two heavy and two light chains. The heavy chains have a globular motor domain -340 amino acids long, a helical region that forms a coiled-coil with the second heavy chain, and a globular distal region that interacts with the light chain.

The aim of the present project is to understand how these motors move along microtubules in an ATP dependent way. We are obtaining medium resolution 3D maps of motor-microtubule complexes using electron cryomicroscopy in view of defining, at medium resolution, the conformations and interactions with microtubules of motors during ATP hydrolysis. The molecular envelopes obtained in this way are being combined with atomic resolution structures of the motors and of tubulin obtained by X-Ray or electron crystallography.

We aim to compare two motors, kinesin and ncd, that move in opposite directions along the polar microtubule pathway and have started crystallisation studies of dimers of these two proteins (from ***Drosophila,*** overexpressed in *E.Coli*)
We have recently obtained small crystals of an ncd dimer from ***Drosophila*** in presence of ATP, ATP-bromide, ATP-γ-S and of the selenomethionyl protein (13 Se sites per monomer). The space group parameters are:
C2221 with a= 110, b= 146, c= 260 Å with two dimers per asymmetric unit.
Data have been previously collected on native protein crystals (ATP-bromide, ATP native and selenomethionyl protein on BM02 and IDl4-EH3 (see previous report).

- Search for heavy atom derivatives:
First trials by soaking crystals in solutions containing heavy atoms derivatives were unsuccessful. We have undertaken cocrystallization in solutions containing heavy atom derivatives. Crystals of the selenomethionyl protein were obtained in solutions containing 0.1 mM mercury acetate and data collection was carried out on ID14-EH3 on two crystals in the following conditions:
Detector-crystal distance=209 mm, λ=0.9475 Å, step per frame=0.5 °, exposure time 40 s
crystal 1: resolution up to 4 A, high mosaicity, only 11446 usable reflexions (overlaps), Rsym = 7.8%.
crystal 2: resolution up to 3.5 A, 138030 measured reflexions, 29135 unique reflexions, Rsym = 6.6%.
Data were also collected in the same conditions on a native crystal from the selenomethionyl protein:
resolution up to 3.7 A, 79193 measured reflexions, 23282 unique reflexions, Rsym = 8.8%.
Difference Patterson maps did not reveal any site for the heavy atom.
We believe that the difficulties to find heavy atom derivatives arise from the crystallization conditions in presence of 2M NaCl. We are trying now to find soaking conditions where the crystals stay stable in a lower concentration of NaCl and the binding of the heavy atoms should be easier.

-The structure of the ncd monomer has been previously solved (Fletterick and coll.)(I222, a=127 Å, b=122 Å, c=68Å), one molecule per asymmetric unit) but the coordinates are not published. We have also collected data on a monomer ncd crystal in the following conditions:
distance detector-crystal 118 mm, increment per frame 0.5°, exposure time 20 s, range 50°, resolution up to 2.7 Å, 56784 measured reflexions, 13595 unique reflexions, Rsym = 3.2 %. This experiment should allow us to have the structure and use the ncd model of the monomer for molecular replacement.