ESRF	**Experiment title:** Structure of the Macromolecular Complex between Nuclear Transport Factor 2 (NTF2) and Ran	**Experiment number:** **LS840**
Beamline: ID14-EH3	**Date of experiment:** from: 10-DEC-97 to: 12-DEC-97	**Date of report:** 21-AUG-98
Shifts: 5 shifts	**Local contact(s):** Dr Wim Burmeister	*Received at ESRF:* 28 AOUT 1998

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Report:

Note on beamtime allocation:

We were allocated 3 shifts on BM14 for project LS800 in the initial round of beamtime allocation, and were subsequently offered 5 shifts on IDl4-EH3 for project LS840, with only one week's notice, immediately prior to the time allocated for project LS800. However, we were not able to produce crystals of the macromolecular complex between Nuclear Transport Factor 2 (NTF2) and Ran at such a short notice. After consultation with Dr W. Burmeister, it was agreed that we should bring instead crystals of the dimerization domain of the *Dictyostelium* gelation factor (ABP120) and of the N-terminal domain of the Ras GTPase activating proteins and use these instead of the NTF2-Ran complex. Moreover, because we needed to collect a MAD data set for the ABP120 crystals, it was decided to use BM14 for these crystals and to instead collect the data for the motile major sperm protein (MSP) crystals on ID14-EH3.

Structure of helical subfilaments formed by the motile major sperm protein (MSP) of Ascaris

Ascaris MSP forms P212121 orthorhombic crystals constructed from helical arrays of MSP dimers that closely resemble the filaments formed to generate amoeboid cell motility in vivo. However, these crystals have a 465 Å c-axis and high mosaic spread, which has made data collection difficult. After inspecting the diffraction pattern from a number of flash-frozen crystals, a crystal was found that diffracted to 3.5 Å with low mosaicity, and indeed gave the best diffraction yet seen from these crystals. A full 90 degrees of data were collected with the large 465 Å cell edge completely resolved. However, about 45 degrees through the data collection, a fault with the goniometer control delayed data collection for two hours. Although the diffraction appeared, at the time, to be unaffected by this interruption, the data after this point were unable to be processed upon returning to Cambridge.

Structure of N-terminal domain of Ras GTPase Activating Protein Binding Protein, homologous to NTF2

Flash-frozen crystals of the N-terminal domain of Ras GTPase Activating Protein Binding Protein, with space group P6122 and unit cell dimensions a=91 A, c=194.5 Å diffracted to 2.4 Å, a substantial improvement on the 3 Å data collected on a rotating anode generator at the MRC. We collected a full native data set for these crystals together with two derivative data sets, one using uranium and the other mercury. The native data and the mercury derivative were complete to 2.4 A, while the uranium derivative diffracted further than the native, enabling a full data set to 2.2 Å to be collected. These data have enabled the structure of the Ran GTPase activating protein N-terminal domain to be solved by MIR and refined to a resolution of 2.4 A. The domain forms clear dimers and overall is structurally homologous to Nuclear Transport Factor 2 (NTF2), a soluble component of the nuclear protein import mechanism which interacts with the Ras-family GTPase, Ran.