ESRF	**Experiment title:** Cambridge MRC Block Allocation Group **The structure of components of the clathrin-mediated endocytosis pathway**	**Experiment number:** **LS-1525**
Beamline: ID14-4	**Date of experiment:** from: 15 Sep 99 to: 18 Sep 99	**Date of report:** 25 Feb 00
Shifts: 3	**Local contact(s):** Sean McSweeney	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Phil Evans*

Marijn Ford*

MRC Laboratory of Molecular Biology
Hills Road
Cambridge CB22QH, UK

Report:

AP180 N-terminal domain

AP180 is a protein required for clathrin mediated endocytosis. Its exact function is not known, but it binds clathrin and stimulates assembly of clathrin cages, and is implicated in defining the cage size. Like many proteins involved in endocytosis, it consists of a number of domains, perhaps with separate functions. The N-terminal domain has been reported to bind phosphoinositol lipids, and thus may serve to anchor the protein on the membrane during the assembly of clathrin cages.

The N-terminal domain of about 30kD has been crystallised in a large cubic cell, spacegroup I23 or $I2_13$, with a=308 Å, containing up to about 16 molecules in the asymmetric unit. These crystals diffract weakly to about 3.5 Å resolution on beamline ID14-4, and on this

trip we collected three datasets, from two native crystals, and one crystal soaked the the mercury compound EMTS. The mercury soak caused a reduction in the symmetry of the crystals to orthorhombic, spacegroup I222 or $I2_12_12_1$, with now up to 48 molecules/ asymmetric unit. Attempts to solve the structure from these data has been shelved for the time being. Since this trip, a new orthorhombic crystal form with only about 8 molecules/ asymmetric unit has been found, and a close homologue has been crystallised in a more favourable form. In future, we hope to solve one or more of these crystal forms by molecular replacement.

β-adaptin atomic resolution

The C-terminal appendage domain of β-adaptin has been solved to 1.7 Å resolution using data from SRS Daresbury. During the model-building, an unidentified ligand was found in the putative binding site which serves as a partial mimic of the true protein ligand. On this trip we tried to collect atomic resolution (beyond 1.5 Å) in order to identify this ligand, but unfortuately the crystals had deteriorated slightly on storage, and the datasets collected were no better than the earlier 1.7 Å dataset. The ligand was subsequently realized to be oxidised DTT.