ESRF	**Experiment title:** Bovine mitochondrial F_1-ATPase	**Experiment number:** **LS 304**
Beamline: **BL4**	**Date of experiment:** from: 21 Feb. '96 to: 23 Feb. '96	**Date of report:** **26.8.96**
Shifts: **6**	**Local contact(s):** Ed Mitchell	*Received at ESRF:* *(1 L sly 13s6*

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Report:

Two datasets were collected, each from a single crystal that had been frozen in liquid propane which in turn was frozen in liquid nitrogen.

The first dataset was from crystals grown in the presence of ADP rather than the ATP analogue AMPPNP. This dataset was collected to 2.85 Å resolution. Integration of the images was not straightforward due to the presence of strong diffuse scattering, particularly beyond 3.2Å. This made determination of the true mosaic spread of the crystal particularly difficult. Some minor modifications to the integration algorithms in MOSFLM were required to optimise processing. More significantly, a three parameter model was introduced in the scaling program SCALA by P. R. Evans in an attempt to model, and eliminate, the effects of the diffuse scattering on the integrated intensities. The most obvious effect of the diffuse scatter was a very large, resolution dependent, partial bias, which could be as high as 30% in the absence of any correction. When the diffuse scattering correction was applied, the partial bias was reduced to 2% (7% in the highest resolution bin). The final dataset had an Rmerge of **6.3%,** and was 93.3% complete to 2.85Å resolution with a mean multiplicity of 2.6.

An initial difference electron density map based on the published model (which has AMPPNP bound to four subunits) showed strong negative peaks at the positions of they phosphates of the phasing model, confirming that this crystal form does indeed have ADP bound at the five nucleotide binding sites. In global terms, there have been small changes in the relative orientations of the subunits, and in particular the $\alpha 3\beta 3$ assembly appears to have rotated by about seven degrees relative to the γ subunit. In addition, there have been modest (˜1.5Å) movements of two arginine sidechains co-ordinating the nucleotide phosphate groups. The current model has a conventional R factor of 24% (Rfree 28%) and the mechanistic implications of the observed confirmational changes are currently being evaluated.

The second dataset was collected from a crystal soaked in ADP and A1F4, which is known to be a potent inhibitor of F1-ATPase and is believed to mimic the pentacovalent intermediate in the reaction. Processing the data posed the same problems as encountered with the ADP crystal, but of even greater magnitude because of the higher resolution and the consequent increase in the relative magnitude of the diffuse scattering. Nevertheless, using the diffuse scatter correction in SCALA, the final dataset gave an Rmerge of 6.0% to 2.65Å resolution, with a completeness of 94.8% and mean multiplicity of 3.0.

Unfortunately there was no indication in the resulting electron density map for bound A1F4. However this has provided a significant increase in resolution (from 2.85 to 2.65Å) over the published structure, and this will therefore form the reference structure to which other complexes (including the ADP form) can be compared. The refinement of this form is almost complete, with a current R-factor of 24% and Rfree of 29%.