Final Report of MX332 at ID29

We performed two MAD experiments on Se-Met derivative of the cytochrome c insertion gene H product (CcmH): one at 2.5 Å resolution and one at 2.3Å resolution. Both experiments were successful and the crystals survived for the three complete data collections. We analysed the data with the program SOLVE obtaining a FOM of ~ 0.60 and a FOM after RESOLVE of ~ 0.8 for both data sets. These statistic allowed us to obtain a clear and connected experimental electron density map (Fig. 1). Building of the model is in progress.

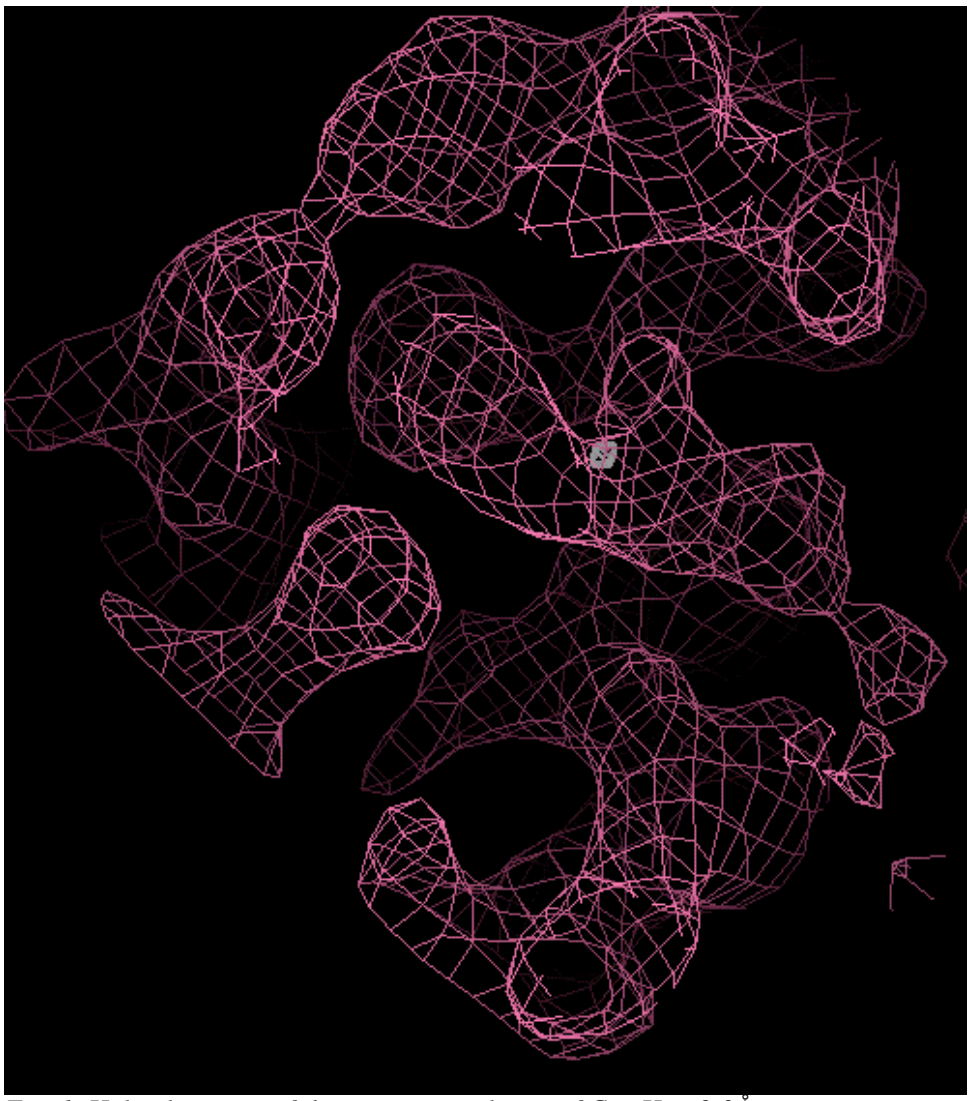

Fig. 1. Helical portion of the experimental map of CcmH at 2.3Å.

After the two MAD experiments we moved the wavelength to 1.7Å to reach the edge of the Manganese ion, to perform a SAD experiment. The protein under study is an endoribonuclease from *X. laevis* (XendoU), which recognises and cuts a stretch of poly Uridines. XEndoU crystals can grow in three different space groups, with intrinsic

different diffraction limits and high internal anisotropy. Its structure has been solved with a combination of MIR, MIRAS and MR. A clear density in the putative active site with a coordination for a cation was visible in data collected on crystals without the substrate. Therefore, in order to prove the nature of this density as the cofactor Mn^{2+}, a SAD experiment on the Mn edge was attempted.
The protein has been co-crystallised with Mn^{2+} and UMP (part of its substrate). Unfortunately most of the crystals dramatically decayed before a decent multiplicity could be reached. The only one who survived long enough is the one whose data are summarised in the Table below.

	Overall	Outer Shell
Low resolution limit	68.52	2.42
High resolution limit	2.30	2.30
Rmerge	0.137	0.536
Rmeas (within I+/I-)	0.161	0.632
Rmeas (all I+ & I-)	0.161	0.632
Fractional partial bias	-0.095	-0.296
Total no. of observations	149919	20582
Total number unique	42400	6022
Mean(I)/sd(I)	8.6	2.4
Completeness	98.1	95.9
Multiplicity	3.5	3.4

The data are actively being worked with, at present.

After having brought back the wavelength to 1.0052Å, and having experienced a few drawbacks, among others the fall of the beamstop (kept in position just with plasticine!), we collected one data set of the cytp450 EryK in complex with the inhibitor clotrimazole. These co-crystals, although grown in the same condition, were not isomorphic to the native ones (collected at ID14-3 the day before).
A data set spanning 200° was collected up to 1.9Å resolution. The data failed to be indexed with the same orthorhombic cell of the un-complexed protein. In particular the long axis appears to be shorter by almost 1/3. Data are being processed at the moment and will be dealt with, once a reliable model of the protein alone, coming from the data collection of ID14-3, will be refined and validated.

The first tiny crystals of the aromatase/cyclase WhiE from *S. coelicolor* were tested and one data set collected at a maximum resolution of 2.6Å and scaled to an orthorhombic space group. The data are under active processing.

Towards the end of the shift, we tested a few other crystals of the bacterial GST soaked with the antibiotics ryfamicin and tetracycline, which were not all tested at ID14-3. Unfortunately none of them diffracted reasonably over 4Å resolution, therefore no data were collected.