ESRF	**Experiment title:** Cambridge MRC Block Allocation Group Yeast ATP synthase	**Experiment number:** LS 1669
Beamline: ID 14-2	**Date of experiment:** from: 5/3/00 to: 8/3/00	**Date of report:** 27/8/00
Shifts: 2	**Local contact(s):** Dr Julien Lescar	*Received at ESRF:*

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Report:

The experiment was mainly aimed at improving the resolution of the data obtained in February 1999 on ID20B from yeast mitochondrial ATP synthase crystals. The low resolution structure resulting from these previous data has meanwhile been published in: D. Stock, A.G.W. Leslie, J.E. Walker (1999) "Molecular Architecture of the Rotary Motor in ATP Synthase", *Science* **286**, 1700-1705.

Abstract:

ATP synthase contains a rotary motor involved in biological energy conversion. Its membrane embedded F_o sector has a rotation generator fueled by the proton motive force, which provides the energy required for the synthesis of ATP by the F_1 domain. An electron density map obtained from crystals of a subcomplex of yeast mitochondrial ATP synthase shows a ring of ten c-subunits. Each c-subunit forms an α-helical hairpin. The inter-helical loops of six to seven of the c-subunits are in close contact with the γ- and δ-subunits of the central stalk. The extensive contact between the c-ring and the stalk suggests that they may rotate as an ensemble during catalysis.

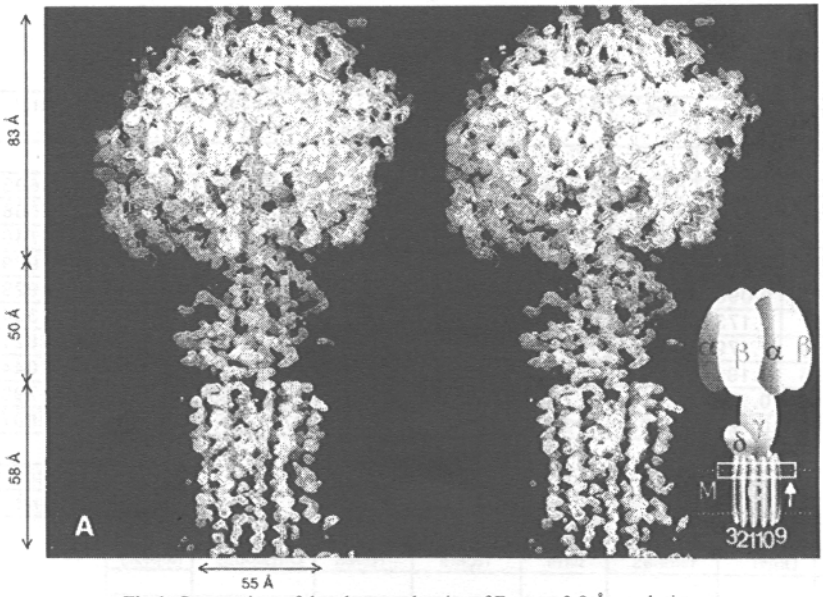

Fig.1: Stereo view of the electron density of F_1c_{10} at 3.9 Å resolution.

We now seek to understand the proton pathway through the transmembrane c ring and the function of the motor. The limited resolution (3.9 Å) of the published data did not allow allocation of side chains or the refinement of the model against the data to a reasonable crystallographic R-factor.

During this experiment we obtained a dataset to about 3 Å resolution. The crystals were cocrystallised with the F_1 specific inhibitor AlF_x, incubated with the F_o specific inhibitor dicyclohexylcarbodiimide (DCCD) and frozen in liquid nitrogen. The crystals are monoclinic ($P2_1$) with unit cell dimensions a = 135.9 Å, b = 174.1 Å, c = 139.2 Å, β = 91.5°. The dataset was collected in two 40° wedges with 0.3° Phi increment and 30 sec exposure time per image. During the second wedge the crystal suffered from radiation damage which reduced quality and completeness of the data considerably.

Data statistics:

N	Dmin (A)	Rfac	Rfull	Av_I	SIGMA	I/ sigma	sd	Mn(I)/sd	Nmeas	Nref
1	9.49	0.080	0.080	230089	32890.5	7.0	21804	25.1	11239	3603
2	6.71	0.081	0.080	128914	23114.7	5.6	11121	23.9	13922	5568
3	5.48	0.069	0.079	40156	4620.0	8.7	3672	14.1	14788	6746
4	4.74	0.079	0.079	39919	5256.0	7.6	4697	11.1	17450	7929
5	4.24	0.090	0.080	42128	6397.8	6.6	5930	9.4	19699	8929
6	3.87	0.173	0.086	26048	10266.3	2.5	5828	6.1	15799	7354
7	3.59	0.179	0.090	17719	5128.9	3.5	5710	4.5	18782	8821
8	3.35	0.196	0.092	10784	3635.0	3.0	4841	2.8	14499	7044
9	3.16	0.185	0.093	8772	2334.1	3.8	3695	2.4	9644	4799
10	3.00	0.184	0.094	6622	1701.8	3.9	2872	1.9	5214	2607

Overall:	0.094	0.094	53326	12941	4.1	6899	8.5	141036	63400
	Rfac	Rcum	Av_I	SIGMA	I/sigma	sd	Mn(I)/sd	Nmeas	Nref

N	Dmin	Nmeas	Nref	Ncent	%poss	Cm%poss	Mlplcty
1	9.49	11585	3949	240	95.6	95.6	2.9
2	6.71	15488	7134	274	96.0	95.9	2.2
3	5.48	17280	9238	260	96.4	96.1	1.9
4	4.74	20486	10965	269	96.6	96.3	1.9
5	4.24	23129	12359	282	96.5	96.4	1.9
6	3.87	19561	11116	218	78.2	92.0	1.8
7	3.59	23794	13833	276	89.8	91.6	1.7
8	3.35	20593	13138	237	79.5	89.4	1.6
9	3.16	15176	10331	71	59.1	84.5	1.5
10	3.00	10277	7670	14	41.5	78.2	1.3

Overall:	177369	99733	2141	78.2	78.2	1.8
	Nmeas	Nref	Ncent	%poss	Cm%poss	Mlplcty

The dataset allowed assignment of most side chains. The refinement of the 4000 residue model is still in progress.

During the second shift we obtained a data set of a F_1c_{10} crystal soaked in $HgCl_2$. The R_{merge} of this data set to 5 Å resolution is 7.1%. The aim was to label the accessible cysteines in the structure in order to improve phase information from molecular replacement. No heavy atom peaks could be located in the difference fourier map (R_{iso}: 14.7%).

Furthermore, bovine F_1 ATPase crystals soaked in the inhibitor DCCD were tested for diffraction to better than 2.5 Å, which could not be achieved.