ESRF BLOCK ALLOCATION GROUP PROGRESS REPORT

BAG RESPONSIBLE: **So Iwata**
EXPERIMENT NO: **MX-491**
LAST REVIEW DATE: **Sep.2005**

Shift usage since last Biennial Review:

Allocated	27	Used	21	Cancelled by Users	0	Cancelled by ESRF	0
Total Number of Visits		6	Total Number of Visitors	10			

BAG Principle Investigators (indicate by # those left since last review, * those new since last review.)

Principal Investigator	Institute
So Iwata	Imperial College London
Bernadette Byrne	Imperial College London
Xiaodong D. Zhang	Imperial College London
Momi Iwata	Imperial College London

Total Number of PDB submissions from data from ESRF beam lines since last report	11
Total Number of Publications resulting from data from ESRF beam lines since last report	7

List below the <u>five</u> most important publications directly resulting from data recorded either wholly or partially on ESRF beamlines (you must indicate [1] ESRF data only; [2] data from more than one source):

1. H.Makyio *et. al*., (2005) Structure of a central stalk subunit F of prokaryotic V-type ATPase/synthase from Thermus thermophilus. *The EMBO Journal* **24**, 3974-3983[2].
2. R. Horsefield *et al*., (2006) Structural and computational analysis of the quinone-binding site of complex II (succinate-ubiquinone oxidoreductase): a mechanism of electron transfer and proton conduction during ubiquinone reduction. *J. Biol. Chem*., **281**, 7309-7316[1].
3. O. Mirza *et al*., (2006) Structural evidence for induced fit and a mechanism for sugar/H+ symport in LacY. *EMBO J*., **25**, 1177-1183[2].
4. Suno R *et. al*., (2006) Structure of the whole cytosolic region of ATP-dependent protease FtsH. Mol Cell., **22**, 575-85[2].
5. Rappas *et.al.,* (2006) Structural basis of the nucleotide driven conformational changes in the AAA+ domain of transcription activator PspF. *J Mol Biol*., **357**, 481-92[2].

Summary (<u>250 words maximum</u>) of the results obtained since last biennial review:

We have obtained the following results: (1) The structures of two components of the V0V1-ATPase have been determined: (A) the subunit F structure revealed two conformations and allowed it to be modelled into the EM maps of the entire V0V1-ATPase complex (published) and (B) the A3B3 subcomplex (3.0 Å; refinement in progress). (2) We have reported the structure of the Complex II (SQR) from E. coli co-crystallized with a specific inhibitor. The inhibitor-bound structure allowed a mechanism for quinone reduction to be proposed. (3) Two novel ligand-free structures of the lactose permease (LacY) from E. coli, determined at acidic and neutral pH have been reported. The structures allowed a model for the mechanism of coupling between lactose and proton translocation to be proposed. (4) The structure of the polysulfide reductase (Psr) from T. thermophilus has been solved. The structures of the native enzyme and a quinone-inhibitor complex are currently being refined. (5) The structure of an ATP-dependent protease, FtsH, which digests misassembled membrane proteins and short-lived soluble proteins in order to control their cellular regulation has been reported. Two structures were reported: the protease domain and the entire cytosolic region containing both AAA+ ATPase and protease domains. (6) We determined the AAA+ domain structures with and without various substrates of the phage shock protein F (PspF), a member of bacterial transcription activator proteins. These structures permit a description of the atomic details underpinning the origins of the conformational changes during ATP hydrolysis.
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Beamtime Request Justification: No more than half an A4 page. If the number of shifts requested in your current application is significantly higher or lower than that requested in the previous year, please comment here on the reasons for this.

The proposal requests beamtime for projects, which fall into two main categories: membrane proteins (including respiratory complexes and membrane transporters) and macromolecular assemblies. Crystals of membrane proteins are commonly characterised by high solvent contents and are therefore weakly diffracting. In addition, they often have high levels of mosaicity, are small in size and suffer rapidly from radiation damage. Consequently, in the majority of cases, the diffraction properties of crystals of membrane proteins cannot be screened using 'in-house' sources. Access to beamlines at ESRF equipped with robotic sample changers is essential, allowing the diffraction properties of large numbers of crystals to be screened rapidly. In addition, the measured diffraction from membrane protein crystals can vary greatly depending on the properties of the X-ray beam used to collect the data. For example, the diffraction properties of crystals of one of the targets of the current proposal, the human Cl-/HCO3- exchanger Band 3 protein, depend on the size of the X-ray beam used to collect the diffraction data. In this case, the high mosaicity of the crystals mean that a microfocus beam is required. Rapid radiation damage requires that the crystal must be translated in the beam during data collection; a microfocus beam means that this can be achieved with smaller crystals. Many of the targets of the current proposal are respiratory complexes, which contain intrinsic metal ions, making them amenable to phasing by MAD or SAD methods. In fact, many crystals of membrane proteins are too fragile for soaking in heavy atom solutions, so MAD or SAD phasing using either the intrinsic metals or Se-Met derivatives are the most applicable phasing methods. Regular access to tunable beamlines at ESRF is therefore essential.

Beam line performance: No more than half an A4 page. Please comment on the beam line performance during your visits, together with any constructive suggestions about possible enhancements to the facilities.

ID14/EH4 : Good beamline, but slightly disadvantageous for us because of the limitaion of the available wavelengths as we work on a number of metalloproteins containing Fe.
ID23-1and ID29 : Most suitable beamlines for our group, the beam is reasonably small and strong enough to collect data.
ID23-2: An excellent microfocus beam, it is very useful for some of our crystals. It is difficult to collect a good data set because of severe radiation damage, we need to collect a number of dataset to find an optimum condition for each type of crystals. Fixed wavelenght is disadvantageous. Although we would like to have a constant access to this beamline, a consequtive 6 shifts at this beamline (ex. 17-19 June, 2006) was not really useful, as not all crystals are suitable to this beamline.

Introduction of the automatic sample changers to the all beamlines has hugely improved the efficiency of our experiments .