

<b>ESRF</b>	Experiment title: Kinetic crystallography of respiratory oxidases	<b>Experiment number:</b> LS-784
<b>Beamline:</b> ID14/EH3	<b>Date of experiment:</b> from: 17 Dec.1997 to: 20 Dec. 1997	<b>Date of report:</b> 2/24/98
<b>Shifts:</b> 9 shifts	<b>Local contact(s):</b> Soichi Wakatsuki	<b>Received at ESRF:</b>

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**Report:** Due to the high profile and intense demand for the Cytochrome c reductase structure, we were unable to perform the kinetic crystallography work which was initially planned during this time. However, we believe the completion of cytochrome c reductase structure was a reasonable use of this beam time as this is one of the protein we intend to perform kinetic crystallography on. We hope to undertake the **the Kinetic crystallography of respiratory oxidases** under the next allocated time.

Cytochrome c reductase (also known as Complex III or *bc*<sub>1</sub> complex) is the middle segment of the mitochondrial respiratory chain which is crucial for aerobic metabolism. Defects in the respiratory chain frequently lead to several mitochondrial myopathies including many neuromuscular disorders such as Parkinson's disease. Bovine heart mitochondrial *bc*<sub>1</sub> complex, a rather large oligomeric enzyme, is composed of 11 polypeptide subunits having a total combined weight of 240 kDa. The primary sequences of all eleven subunits are known for the bovine heart system. Three of these subunits contain redox prosthetic groups, specifically, cytochrome *b*, cytochrome *c*<sub>1</sub>, and the Rieske iron-sulfur (2Fe-2S) cluster. Cytochrome c reductase is an integral membrane protein complex and is currently the largest membrane protein complex to be crystallized. So far, the high resolution structure of the Rieske iron-sulfur protein (Iwata et al., 1996, Structure 4,567 - 579) and preliminary structure of the complex (Xia, et al., 1997 Science 277,60-66) have been published. However, the structure of the whole the complex still remains to be completed.

We are currently working on two crystal forms of the *bc*<sub>1</sub> complex. One is the hexagonal bipyramid (P6522, a=b=212 Å c=342 Å) and the other is the hexagonal rod (P65, a=b=130 Å, c=720 Å). Our strategy is to obtain a MIR phase set from the bipyramid crystals and then extend the phase using multicrystal averaging and, finally, refine the structure using the rod form. In this experiments LS784, we have collected native and derivative data sets from the bipyramid form using the newly installed MarCCD (See the experiment report LS782). We were able to obtain a good native set which was isomorphic with the derivative sets collected at ID2 in September 1997. Using the data from both experiments, we calculated a MIR phase set up to 4.0 Å which has been extended to 3.0 Å by multicrystal averaging (Fig. 1.). We are currently working on an atomic model for the *bc*<sub>1</sub> complex in the both crystal forms.

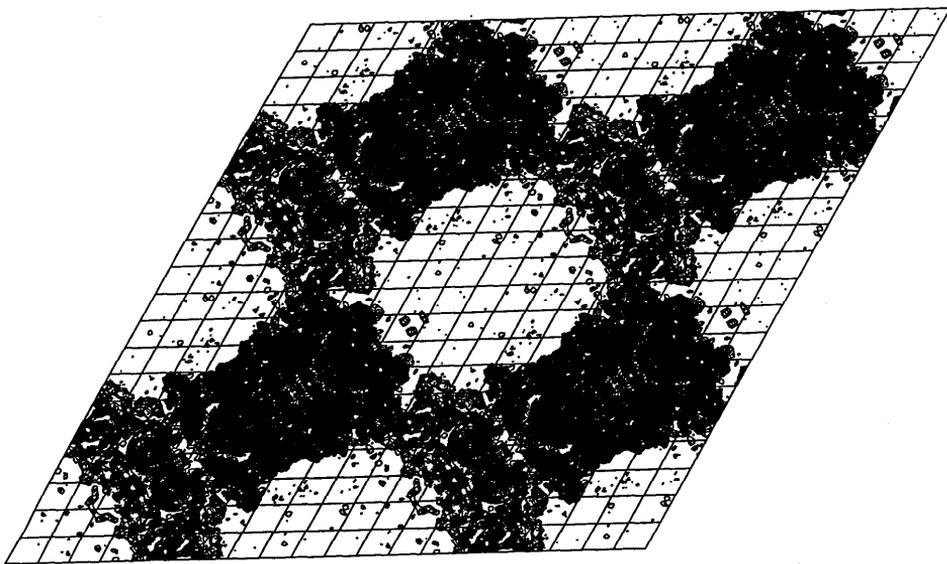


Fig. 1. MIR map of the cytochrome *bc*<sub>1</sub> complex at 4.0 Å resolution (bipyramid form). The figure is a projection map from the *c*-axis of the crystal. The length of the edges of the figure is 424 Å.