

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application**:

<http://193.49.43.2:8080/smis/servlet/UserUtils?start>

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.



	Experiment title: Structural characterization of the cytoplasmic dynein heavy chain from human and <i>Dictyostelium discoideum</i>	Experiment number: MX 1096
Beamline: ID23-2	Date of experiment: from: May 4 th 17:00 to: May 5 th 8:00	Date of report: June 1 st 2010
Shifts: 1	Local contact(s): Mrs. Elspeth Gordon	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): Dr. Martin Kollmar and Christian Eckert MPI for Biophysical Chemistry Am Fassberg 11 37077 Göttingen, Germany		

Report:

Our goal is to solve the structure of various constructs of the 550 kDa cytoplasmic dynein-heavy-chain from human and the cellular slime mold *Dictyostelium discoideum*.

Two DHC-constructs formed crystals. One consists of the linker- and the AAA1-domain (referred to as construct 1), both of which are essential for dynein motor activity. The other one represents the part from the stalk, which includes the microtubule-binding domain, to the C-terminal part of the DHC (referred to as construct 2). Both constructs were expressed as myosin-fusion-proteins, so that the MWs are 180 kDa and 250 kDa, respectively.

First diffraction measurements of the crystals were performed at DESY in Hamburg (Max-Planck beamline), because the crystals were too small for in-house measurements.

Unfortunately, we had to find out in Nov. 2009 that the crystals were even too small for DESY and did not show any diffraction.

For this reason we applied for beamtime at the ESRF in Grenoble, where we were awarded 1 shift at the microfocus beamline ID 23-2 for scattering experiments.

Results:

In fact, the crystals of both constructs diffracted at ID 23-2. Crystals of construct 1 have dimensions of 5x10x10 μm , and one of them showed reflections to a resolution of around 3 Å. However, although we tried crystal annealing, the crystal showed high mosaicity. Because of radiation damage, we were not able to collect a complete, high-resolution data set. Also, because we could not test these crystals at DESY, we could not test cryoconditions in

advance of the ESRF experiments. The obtained crystal parameters are: P222, 118 x 134 x 140 Å, consistent with one molecule per asymmetric unit.

Crystals of construct 2 (about 10x10x100 µm) diffracted at best to about 10 Å. However, during our measurements we noticed that the cryoconditions were everything but optimal, and have to be improved. Nevertheless, most of the tested crystals diffracted. The obtained crystal parameters are: R32, 288 x 288 x 147 Å, consistent with one molecule per asymmetric unit.

In addition, we tested a few crystals of a subcomplex of dynactin, the activator of cytoplasmic dynein, that we obtained just 10 days before beamtime. Luckily, these small needles (10x10x300 µm) diffracted to about 2 Å, and we were able to collect two complete datasets using the helical data collection method at ID23-2. The parameters for the best dataset are: P4, 124 x 124 x 77 Å, 100% completeness, $I/\sigma I = 2.05$, resolution: 1.99 Å.

In conclusion, the beamtime at ID23-2 was a major breakthrough in dynein research, showing for the first time diffracting crystals of the catalytic domain and the major part of the motor domain. The diffraction quality of one construct would be good enough for a high-resolution structure, although the crystals and the cryoconditions have to be improved. The crystals of construct 2 are not diffracting good enough yet, and also need improvement. In addition, we got very good datasets for a subcomplex of dynactin.

