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ESRF

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.

ESRF	**Experiment title:** Structural characterization of the cytoplasmic dynein heavy chain from human and *Dictyostelium discoideum*	**Experiment number**: MX 1164
Beamline: ID14-4	**Date of experiment**: from: Sep 1th 17:00 to: Sep 2th 8:00	**Date of report**:
Shifts: 2	**Local contact(s)**: Mr. Edward Mitchell	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

This is one report for both experiments, MX 1165 (two shifts at ID 23-2) and MX 1164 (two shifts at ID 14-4), that were applied for and allocated at the same time.

Our goal is to solve the structure of various constructs of the 550 kDa cytoplasmic dynein-heavy-chain (DHC) from human and the cellular slime mold *Dictyostelium discoideum*. Very little is known about the high-resolution structure of the cytoplasmic DHC. So far only the small microtubule binding domain (about 20 kDa) and a short part of the stalk, which connects the microtubule domain to the motor domain, has structurally been determined. By characterizing the structure of the cytoplasmic DHCs from human and *Dictyostelium discoideum* using X-ray-crystallography, we want to contribute to the understanding how these large proteins are organized and function at atomic resolution.
Two of our DHC-constructs formed crystals. One consists of the linker- and the AAA1-domain (referred to as construct 1), which are essential for dynein motor activity. The other construct 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 fusion-proteins, so that the MWs are 180 kDa and 250 kDa, respectively.

First crystals of construct 1 had dimensions of 5x10x10 µm. Diffraction measurements of the crystals were performed at DESY in Hamburg (Max-Planck beamline), but the crystals were too small for DESY and did not show any diffraction. For this reason we applied for beamtime at ID23-2 in Grenoble. In May 2010 one of the crystals showed reflections to a resolution of around 3 Å. However, although we tried crystal annealing, the crystal showed high mosaicity and ice rings. 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. 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 had to be improved.

Therefore, we improved the crystals in size, we grew crystals in several slightly different conditions (different additives), and we froze crystals under different cryo-conditions. While blocks of 100 μm in size could be obtained for construct 1, the crystals of construct 2 could be improved so that they did not appear hollow or slightly cracked any more. During flash-freezing, both types of crystals seemed to be stable and happy.

We applied for beamtime at **ID 14-4** because we hoped that we could get better and complete data from the crystals of construct 1 that showed strong radiation damage at ID 23-3 in May 2010. We applied for beamtime at **ID 23-2** because the crystals of construct 2 were needles, that were (visibly) strongly improved compared to May 2010 but that were still very thin and might therefore show, if at all, strong anisotropic diffraction at ID 14-4. At ID 23-2, complete data from tiny needles could be obtained using the helical data collection method. In addition, ID 23-2 offers the possibility to use the device for controlled dehydration of protein crystals.

Results:

For construct 1 two different crystal forms were obtained. The crystals grew much larger than the original ones and had dimensions of about 30x60x60 μm. However, although different cryo-protectants were used, none of the optimized crystals diffracted to better than 8 Å at ID 23-2. Thus we could not reproduce the initial crystal that diffracted to about 3 Å in May 2010 at ID 23-2. The initial crystal was flash-frozen without any cryoprotectant because these initial crystals behaved unstable in the first cryo-conditions tried and we wanted to try something different. The “optimized” crystals seemed to be stable in the crystallization drops and under various cryo-conditions and thus we focused on screening crystals grown under varying conditions. Because the initial crystal conditions did not contain any cryoprotectants we thought we could not get wrong with finding cryo-conditions in which the crystals behave happily.
In addition we had the opportunity to make use of the dehumidifier at ID23-2 to test the effect of controlled dehydration on the crystals. But unfortunately crystal dehydration did not result in improved crystal diffraction. The dehumidifier might be suitable for another try as soon as we get reproducibly better diffraction.
In contrast to the crystals of construct 1 the diffraction quality of those from construct 2 could be improved from around 10 Å to 7 Å. Again different cryo-conditions were tested, but no further improvement in diffraction could be observed. The same holds true for crystal dehydration attempts.

Comparing ID 14-4 and ID 23-2, the crystals of both constructs diffracted reproducibly to “higher” resolution at ID 23-2.

Backup-project:
In addition, in May 2010 we tested a few crystals of a subcomplex of dynactin, the activator of cytoplasmic dynein. 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(1), 124 x 124 x 77 Å, 100% completeness, I/sigmaI = 2.05, resolution: 1.99 Å. Subsequently we were successful in co-crystallizing the protein with a ligand. The crystals diffracted to around 2.6 Å and we collected three complete datasets with the helical data collection method at ID23-2. The best dataset has the following parameters: P4(1), 124 x 124 x 77 Å, 100% completeness, I/sigmaI = 2.10, resolution: 2.58 Å. The structure of the apo-CapZ has been solved (publication in preparation). Unfortunately, the supposed to be CapZ-PIP2 co-crystals did not seem to contain PIP2.