



Structrual Biology Cryo-EM Platform

University of Cologne



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vesion: 2024

User Agreement

Mission and objectives

The main tasks of the **STRUBiTEM** (StrukturBiologie cryo- TransmissionsElektronenMikroskopie) platform are to provide the infrastructure and expertise to help scientists analyse their biological macromolecules using cryo-electron microscopy (cryo EM). Cryo EM has the potential to solve structures of proteins smaller than 200 kDa with a resolution of approximately 3 Å. A particular focus of the **STRUBiTEM** platform is on analysing the protein dynamics caused by conformational changes.

The aim of these user rules is to create a framework for all users of this platform to enable fair, open and transparent collaboration. These user rules cover many aspects of cooperation between users and the platform, but there are also points that are not covered in detail. There will always be a certain amount of leeway in order to do justice to scientific work. Anything that is not set out in writing here will be regulated via personal communication.

Structure and organization

The **STRUBiTEM** platform is part of the Institute of Biochemistry/Department of Chemistry at the University of Cologne. The platform was established in 2017 through two major DFG grants (German Research Foundation).

The **STRUBiTEM** platform is operated by specialised staff. The core tasks of the platform are sample preparation for electron microscopy (EM) (both negative contrasting and vitrification), the acquisition of "single particle" data sets (room temperature and cryo conditions) as well as the pre-processing and quality control of the acquired images. In addition, the staff provides expertise in protein purification for cryo EM, helps to develop strategies for data analysis and assists in the interpretation of results. Training is available for users to learn how to prepare samples and operate the microscopes independently. Complete projects can also be taken on as part of scientific co-operations. The scientists of the **STRUBiTEM** platform also pursue their own scientific projects and are active in teaching at the Institute of Biochemistry.

Personnel

For a list of personnel see appendix Personnel

The **STRUBiTEM** platform has a head who is responsible for all matters relating to the platform. The head is supported by a scientific director who advises on scientific issues. A half-time technical assistant position is available to assist with sample preparation and microscopy. Invoicing and invoice processing is carried out by secretarial staff.

User Meeting

A user meeting is held annually at the Institute of Biochemistry, to which all users are invited. The purpose of the user meeting is to promote dialogue between users and their continuing education.

Equipment

See appendix Equipment

Operational Concept

Access rules

For all applications, a project description must be submitted to **STRUBiTEM** (to strubitem-contact@uni-koeln.de). Based on this project proposal, the project will be evaluated in terms of its scientific relevance, expected results and current progress to determine at which level the project is and how much measurement time will be allocated at Krios. Possible access levels:

Level 1 projects

Applicants are guided through the experimental design and supported in sample preparation and data acquisition. The first phase serves to inform the applicants about the requirements and to characterise the sample by means of negative contrasting. If successful, the second phase aims to establish plunge freezing conditions and obtain preliminary cryo-EM data - in particular 2D classes and an initial 3D model.

Level 2 projects (application for beam time)

On request, applicants can be guided through the experimental design and supported in sample preparation. Applicants are supported in data acquisition. The aim is to collect sufficient data for a high-resolution 3D reconstruction. For this purpose, one or more screening sessions are required to determine optimal samples. The data will be made available within the RRZK infrastructure. Support for 3D reconstruction is available on request.

Level 3 projects (application for beam time)

Applicants are supported in the collection of data. The aim is to collect sufficient data for a high-resolution 3D reconstruction. The data will be made available within the RRZK infrastructure.

As the measurement time at Krios and the capacity of the platform employees are a limiting factor, the projects are assessed every three months by a Project Reading Committee (PRC). The PRC's rating and the capacity of the facility are used to determine the reading time for each project for the next 3 months.

Proposals are kept strictly confidential and are read only by the PRC and the facility director.

Proposals must be submitted for each 3-month measurement period. For continuing projects, resubmission of the previous proposal is possible, but in this case the last section "continuing projects" must be completed. Priority in applying for measurement time will be given to projects with solid preliminary data, especially preliminary negative contrast data, or, if only negative contrast is applied for, with solid data on protein purification and activity. Projects will also be weighted according to the applicant's project progress to date. [STRUBiTEM](#) reserves the right to de-prioritise projects from groups/PIs that do not comply with the rules of the institution or, in repeated cases, not to accept applications.

Users will be informed of the PRC's decisions by the head of the [STRUBiTEM](#) platform.

Before submitting project proposals, projects can also be discussed informally with the head of the facility.

As long as the centre is not fully occupied (waiting time for a measurement period of less than 12 weeks), all projects will be carried out.

Training

Sample preparation

[STRUBiTEM](#) offers training courses for negative contrasting and vitrification of samples. Groups of up to two people can be trained together. After successful training, the relevant equipment can be booked and used independently by the users.

Microscopy

Krios training consists of two units. Users are only instructed individually. After the training and if the platform management deems the skills to be sufficient, users are allowed to screen samples, set up data recordings and carry out basic alignments on the microscope. This permission is only valid during office hours from Mon-Fri 9am-4pm. If users have shown that they can work independently, they may also use the Krios microscope outside office hours. The platform management grants permission for the respective activities. Damage to platform property caused by exceeding the authorised activities may result in the user being excluded from the platform.

Talos training consists of two sessions with a maximum of two people. After the training, users are authorised to use Talos independently during office hours from Mon-Fri 9am-4pm. After the users have shown that they can work responsibly and independently, they may also use the Talos outside office hours.

The platform management grants permission for the respective activities. Damage to platform property caused by exceeding the authorised activities may result in the user being excluded from the platform.

Booking

Equipment bookings can be made via the platform's online booking tool. Bookings can only be made by users with the relevant training courses. Invoices are issued on the basis of the booked usage times. If platform staff are required, this must be agreed in person with the platform management.

Krios/Talos

Microscope times, especially for data acquisition, are a limiting factor. A certain contingent of measurement times is reserved for the users who are named in the DFG funding for the establishment of the platform.

Due to service times, the platform management can block the microscopes at any time.

Cancelling policies

If a booked measurement time cannot be used, the platform management must be informed immediately. Cancellation can be made free of charge up to 48 hours before the start of use, otherwise the costs will be charged if no other user can be found who would like to take over the time.

User Fees

User fees are charged for the measurements on the microscopes, which are calculated from the running costs of the platform. The user fees are recalculated each year on the basis of the previous year's expenditure.

See separate document User fees

Data Management

The platform has the right to use the measured data for quality control and to generate metadata (CTF, drift). Project-relevant data is temporarily stored within the [STRUBiTEM](#) platform. The data can be forwarded to the data centre of the University of Cologne for further processing and processing on data centre resources. Data will not be shared with third parties without the request of the project leader.

The rules of Good Scientific Practice (GWP) require research data to be archived for at least 10 years. In the context of the [STRUBiTEM](#) platform for molecular cryo-EM, these are in particular the recorded movie frames.

The data produced in the [STRUBiTEM](#) facility can be stored in accordance with the GWP in coordination with the Cologne Competence Centre for Research Data Management C3RDM. Data will only be uploaded on request if appropriate documentation of the data is available for the [STRUBiTEM](#) platform. Users are responsible for their own data.

Security

Electron microscopes are potentially dangerous. They are operated with high voltage and electrons are emitted. Potentially dangerous X-rays are emitted. It is therefore forbidden to modify the hardware of the microscopes, especially the enclosures that shield the X-rays and the components that are under high voltage. Liquid nitrogen is used for sample preparation and for operating the microscopes under cryogenic conditions. According to German law, it is mandatory to receive annual safety training from the safety officer for handling liquid nitrogen and for working with X-rays.

Authorship and publications

Acknowledgements

The DFG evaluates the influence and status of a platform based on how often it is named or cited in scientific publications and how often it is involved as a co-author in a publication. To ensure that the [STRUBiTEM](#) platform can continue to provide the best service, train its staff and provide new equipment and software, it is important that all users adhere to the following recommendation:

Once data have been recorded or processed using the [STRUBiTEM](#) platform, the role of the platform or platform personnel should be mentioned in the acknowledgements of the publication. This applies to publications,

presentations, bachelor's and master's theses as well as doctoral theses. Please let us know if you mention the platform in an acknowledgement.

The STRUBiTEM platform can be acknowledged as follows:

We gratefully acknowledge access to the infrastructure of STRUBiTEM, the platform for molecular cryo-EM of the Institute of Biochemistry at the University of Cologne (funded by DFG grant INST 216/949-1 FUGG)

Coauthorship

Central platforms must charge for their services in order to maintain equipment in good working order, retain staff, purchase software updates and provide consumables for users. Irrespective of these fees, the usual rules for co-authorships also apply to STRUBiTEM platform staff if they make a significant intellectual or experimental contribution to a project. Individual solutions for co-authorships should be discussed in person.

Note on GWP

To ensure the integrity of the scientific data generated, we follow the rules of good scientific practice to the best of our knowledge and belief and expect the same from all users.

Appendix

Personnell

Head of STRUBITEM:
Dr. Monika Gunkel

Scientific director:
Prof. Dr. Elmar Behrmann

Technical assistant:
Jennifer Lange

Secretary:
Melanie Schmitz

Equipment

Electron Microscopes:
Titan Krios G3i (Thermo Fisher)
Talos L120C (Thermo Fisher)

Plunge Freezer:
Vitrobot MarkIV (Thermo Fisher)
CP3 (Gatan)

Nutzerordnung für Projekt ID:

Projektverantwortliche/-r

Die/der Projektverantwortliche bestätigt, dass sie/er die Nutzerordnung gelesen und verstanden hat und den darin festgehaltenen Regeln folgen wird. Darüber hinaus, stellt sie/er sicher, dass Nutzer/-innen unter ihrer/seiner Verantwortung diesen Regeln folgen werden.

Unterschrift Projektverantwortliche/-r: _____

Nutzerliste für dieses Projekt:

Mit ihrer/seiner Unterschrift bestätigt der/die Nutzer/-in die Nutzerordnung gelesen und verstanden zu haben und den darin festgehaltenen Regeln Folge zu leisten.

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Unterschrift Plattformleiterin STRUBiTEM:

Dr. Monika Gunkel: _____