



Institute of Biochemistry
Structural Biology Transmission
Electron Microscopy

University of Cologne



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User Agreement

Preamble

The aim of this user agreement is to create a framework for a fair, open and transparent treatment of all users of the facility. This agreement covers a lot of points concerning the collaboration between the users and the facility but it is far from exhaustive. There will always be a certain range of flexibility to meet the needs of scientific work. Everything that is not nailed down in this agreement is communicated personally.

An overview of available equipment can be found on the STRUBiTEM webpage: strubitem.uni-koeln.de/infrastructure

General Description

The imaging facility STRUBiTEM (structural biology cryo-transmission electron microscopy) is part of the Biochemistry institute at the University of Cologne. The facility was founded 2017 with the help of two major DFG (German Research Foundation) grants.

The main objective of STRUBiTEM is to provide both, the infrastructure as well as expert knowledge to enable users to analyze biological macromolecules by cryo-electron microscopy (cryo-EM). Depending on the samples, cryo-EM has the potential to solve the molecular structures of proteins smaller than 200kDa to resolutions better than 3 Å. A specialty of STRUBiTEM is the analysis of dynamic proteins assuming multiple conformations.

The hardware of STRUBiTEM is maintained by dedicated staff. Our core business is the preparation of electron microscopy (EM) samples (including both negative staining as well as vitrification), the acquisition of single particle data sets (both at room temperature and under cryo conditions), and pre-processing as well as quality assessment of micrographs. Beyond these, we also provide expert knowledge on protein purification for cryo-EM applications, data analysis strategies and data interpretation. Moreover, we train selected users for independent sample preparation and microscope usage, either supervised or independent. As part of a scientific cooperation, we can also provide complete workflows for specific targets. The staff of STRUBiTEM also pursues independent scientific projects, and participates in the teaching of the institute of Biochemistry.

Facility Access Rules

For all applications a project proposal has to be submitted to STRUBiTEM. This project proposal will be used to evaluate the project according to its scientific relevance, expected output and current progress in order to determine the level and how much beam time at the Krios is going to be allocated. Possible entrance levels:

Level 1 Projects

Applicants will be guided in experimental design, and assisted in sample preparation and data collection. The first aims are to inform applicants about requirements and to characterize the sample by negative stain microscopy. If successful, the second phase aims at establishing freezing conditions and obtaining preliminary cryo-EM data – especially 2D classes and an initial 3D model.

Level 2 Projects (application for beam time)

On request, applicants will be guided in experimental design and assisted in sample preparation. Applicants will be assisted in data collection. The aim is to collect enough data for a high resolution 3D reconstruction. For this, one or more screening sessions will be required to identify optimal sample grids. Data will be made available inside the RRZK infrastructure. Assistance in 3D reconstruction is possible on request.

Level 3 Projects (application for beam time)

Applicants will be assisted in data collection. The aim is to collect enough data for a high resolution 3D reconstruction. Data will be made available inside the RRZK infrastructure.

As beam time at the Krios and the capacity of the facility is a limiting factor the projects will be ranked by a Project Reading Committee (PRC) every 3 month. The ranking of the PRC and the capacity of the facility will be used to determine the beam time for every project for the next 3 month

the template for the project proposal can be found on STRUBiTEM webpage: strubitem.uni-koeln.de/access

please send project proposals as pdf to strubitem-contact@uni-koeln.de.

period. **Proposals will be kept strictly confidential and only be read by the PRC and the head of the facility.**

Proposals have to be submitted for every 3 month measurement period. For continued projects, a resubmission of the previous proposal is possible, however in this case the last section 'continued projects' has to be filled out. Priority for beam time will be given to projects with solid preliminary data, especially preliminary negative-stain data, or if only negative-stain EM is requested solid data on protein purification and activity. Projects will also be weighted based on the prior project history of the applicant/applicant's PI. STRUBiTEM reserves the right to down-weight the priority of projects from groups/PI not complying with the facility rules, or ultimately not accept applications in repeated cases. Users will be informed about the decisions of the PRC by the head of the facility.

Current members of the Proposal Reading Committee evaluating the projects are listed on the STRUBiTEM webpage:
strubitem.uni-koeln.de/access

Before submitting project proposals, projects can also be discussed beforehand with the head of the facility on an informal basis.

Biological and chemical hazard statement:

If necessary, a biological and chemical hazard statement has to be handed in to estimate the security level of the samples (S1). If the project has to be considered S1, a copy of the formsheet Z has to be provided to the facility as part of the project proposal.

Training

Sample Preparation

STRUBiTEM offers training sessions for plunge freezing and negative staining. Groups up to 4 people can be trained together. Sample preparation machines can be booked and used independently after instruction.

Microscopy

Krios training consists of at least 2 sessions. Only 1 user is trained in a session. After the training and prove of their abilities, users get the permission to screen samples, to setup data acquisition, to do basic alignments on the microscope within office hours (Mo-Fr. 9-16h). After proving their ability to work independently, users can also work outside office hours. The permission is given by the head of the facility. Crashing things because users did not stick to their permissions, might lead to exclusion from the facility.

Talos training consist of at least 2 sessions with a maximum of 2 persons. After instruction people are allowed to operate the microscope within the office hours (Mo – Fr 9–16 h) independently. After proof of their abilities, users can also work outside office hours. The permission is given by the head of the facility. Crashing things because users did not stick to their permissions, might lead to exclusion from the facility.

Booking

Booking of Equipment is possible via the online booking tool of the facility. Equipment can only be booked by users who had the relevant training. The booked time slots are used for calculating fees. If staff support is required, availability has to be confirmed with the head of the facility personally.

Access to the booking tool "booked" is given by the head of the facility
Strubitem-contact@uni-koeln.de

Krios

Data acquisition slots are a limiting factor. A dedicated amount of acquisition slots is reserved for parties that are named in the DFG grant that founded the facility. Basing on this reserved times and the ranking of the PRC, the head of the facility will distribute the available microscope time between the different projects. Users will be informed about the measuring time dedicated for them.

The head of the facility can always block the microscopes if service is required.

Cancellation Policies

If booked microscope slots cannot be used you should inform the platform staff immediately. You have to cancel your slot at least 48h before usage, otherwise you can be charged with the costs if the microscope time cannot be used otherwise.

Documentation

Usage of all instruments, plunge freezers and microscopes, has to be documented in the logbooks specified by the head of the facility. If documentation is lacking, the head of the facility can block further usage of the instruments.

User Fees

See separate document.

Data Policies

The facility has the right to monitor data for quality controls and to generate meta-data (CTF, drift). Project relevant data will be temporarily stored within the EM facility. Data can be handed over to the University IT-Department for further data handling and processing on IT-resources. Data are not shared with third parties, only on request.

Long term storage of data

According to the rules of good scientific practice (GSP) scientific data have to be archived at least 10 years. In connection to the STRUBiTEM facility for cryo-EM these are mainly the acquired movie-frames.

If not explicitly negotiated by the user, scientific data that are produced in the StruBiTEM facility, will be stored in coordination with the Cologne Competence Center for Research Data Management (C³RDM), according to the rules of GSP. The costs for the long term storage will be invoiced to the user by the STRUBiTEM facility. The actual costs will be published in the actual price list.

At the moment the infrastructure for the long term storage is still under construction and will be most probably available 1st quarter of 2022. Until then, the users are responsible for a GSP conform storage of their data.

DFG guideline for good scientific practice
<https://wissenschaftliche-integritaet.de/en/>

Cologne Competence Center for Research Data Management
<https://fdm.uni-koeln.de/home>

Safety Policies

Electron microscopes are potentially dangerous. They operate at very high voltages and are electron emitters. They might emit harmful x-rays. It is forbidden to manipulate the hardware of the microscopes, especially the housings that shields the x-rays and the high voltage parts.

The cryo-EMs are operated under liquid nitrogen conditions and sample preparation for cryo-EM requires liquid nitrogen handling.

Adhering to German legislation it is mandatory to have a safety introduction by the responsible safety officers for x-ray and liquid nitrogen at least once a year.

Publication Policies

Acknowledgments

The DFG evaluates the impact and importance of facilities based on their number of acknowledgments, citations and co-authorships. To ensure that the cryo-EM facility can continue to provide the best service possible, maintain staff and acquire new equipment and software, it is important that all users adhere to the following guidelines.

As soon as data were acquired or analyzed within the cryo-EM facility or by facility staff, their role has to be cited within the acknowledgments. This includes publications, talks, as well as Bachelor, Master and PhD theses. Also, please let us know whenever you acknowledge the cryo-EM platform!

Co-Authorships

Facilities have to bill for their services to keep the equipment in excellent condition, maintain staff, pay for software updates and consumables to obtain an optimal working environment for their users. Despite these fees, common rules for co-authorship also apply to cryo-EM facility staff members (substantial intellectual or experimental contributions). Individual solutions for authorships should be discussed.

Short description of the complete procedure

- For every project a project proposal with all relevant information is required
- EM equipment can be booked via online booking tool
- Access to the equipment is given only after instruction by the facility staff
- Usage of EM equipment has to be documented in the specified logbooks
- Booked equipment, measuring time or staff support can be cancelled 48h before usage. Otherwise you can be charged for the costs
- Users agree to treat the equipment properly and careful. They have to follow the instructions of the facility staff, otherwise they can be excluded from the EM labs. Users agree to leave equipment properly and avoid biological and chemical contaminations. If there are problems or defects you have to inform the facility immediately
- Data have to be stored in the indicated project directories. Data have to be named clearly with project ID, responsible person, running number. Data that are not clearly named will be deleted. Data are temporarily stored for 1 week on facility storage afterwards they will be deleted. Data can be further processed on University IT server.
- Project supervisors request access for users and therefore are responsible for the proper usage of the EM infrastructure by the supervised person. To minimize the personal risk we recommend a third party liability insurance.
- We presume that users follow good scientific practice.

User Agreement for Project ID

by the general supervision of

The supervisor confirms that he/she has read and understood the user agreement and agrees to follow all rules and regulations within. Moreover, he/she will ensure that each user from his/her group will follow all the rules within.

Signature Supervisor: _____

Users listed for the project:

With their signature, each user confirms that he/she has read and understood the user agreement and agrees to follow all rules and regulations within.

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Confirmed by the head of the STRUBiTEM facility:

Dr. Monika Gunkel: _____