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Services, Rules, Policies & Rates at the Cryo-Electron Microscopy Facility at National Institute of Chemistry (NIC), Ljubljana, Slovenia

The instruments in the Cryo-EM Facility represent a substantial investment and resource for NIC research community as well as other co-investors and potential other internal and external users, academic or industrial. To ensure that these resources are available at the optimum performance level and ensure minimum of downtime, the Internal Cryo-Electron Microscopy Advisory Committee (CEMAC) has established the following services, rules and policies. Please note that the services, policies, rules and rates will be reviewed periodically by the committee and are subject to change.

Internal Cryo-Electron Microscopy Advisory Committee (CEMAC):

- Assoc. Prof. Dr. Marjetka Podobnik, Department of Molecular Biology and Nanobiotechnology (D11), NIC
- Prof. Dr. Roman Jerala, Department of Synthetic Biology and Immunology (D12), NIC
- Assoc. Prof. Dr. Miha Pavšič, FKKT, UL

Core staff (facility manager): Dr. Matic Kisovec, Department of Molecular Biology and Nanobiotechnology (D11), NIC

Cryo-Electron Microscopy Facility at the NIC is separated into two parts:

- **Cryo-EM Sample Preparation Facility** located at the Department of Molecular Biology and Nanobiotechnology (D11), first floor of the main building, room S-150
- **Glacios Imaging Facility** located in the basement of the Pregl Research Center, Room PRC -1.17.2, which is also part of D11. The technical room for the microscope is in PRC -1.18.

  The Cryo-EM Sample Preparation Facility is a Bio-Safety Level 1 (BSL-1) facility, while Glacios Imaging Facility is not under the BSL-1 regime. External BSL-1 samples are brought to the Cryo-EM Sample Preparation Facility in a tight enclosure suitable for BSL-1. Work in BSL-2 and higher is currently not possible.

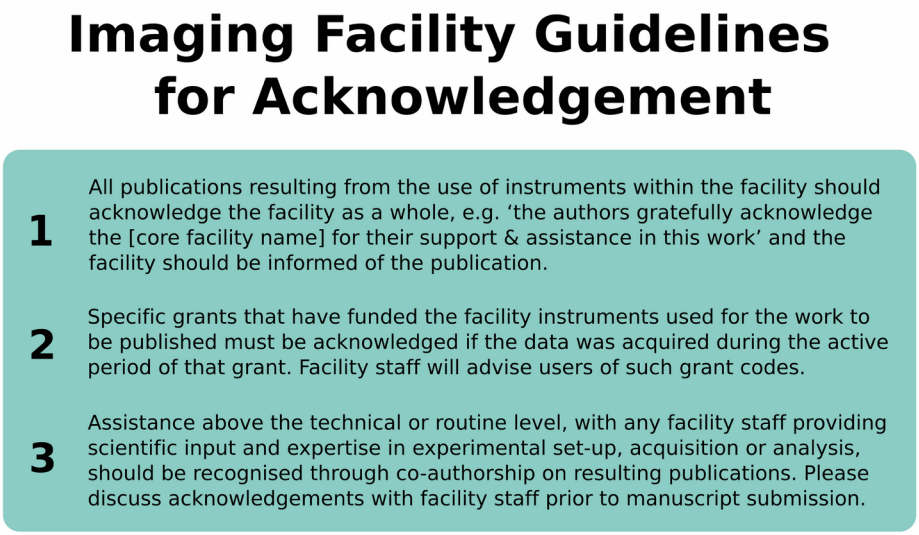
  The info about the BSL level of samples and the chemical nature and potential health risks of samples has to be provided to the facility staff (see the example of the Sample Declaration Form at the end of this document, empty template is provided on the Cryo-EM website - www.ki.si/en/glacios).

SERVICES OFFERED

Cryo-EM Facility:

- The Glacios Imaging Facility houses Glacios by Thermo Scientific (produced and installed in 2019), Falcon 3EC detector, Ceta-D camera (Micro-ED), Volta phase plate, Vitrobot MkIV+ cryo plunger, and all accessories needed to perform sample preparation for cryo-EM.
- All users have to be first trained to independence and proficiency in microscope operation and sample preparation. The ultimate goal of user training is to create independent users who require minimal assistance from the facility staff. These trained users can only be from the member institutions of the cryo-EM consortium (KI, FKKT, FFA, NIB).
- For external users, the data can be collected only by the experienced and trained users. All samples, internal or external, have to comply with the rules described here.
- The Cryo-EM Screening Facility can provide cryo-EM data processing and reconstruction services upon request and mutual agreement (see below).
- The use of the facility and contributions of the staff must be acknowledged: e.g. *Some of this work was performed at the National Institute of Chemistry Cryo-EM Facility, supported by Slovenian Research and Innovation Agency Infrastructure Programme IO-0003.*

- Please follow the guidelines below. Source: https://www.rms.org.uk/network-collaborate/bioimaginguk-network/imaging-facility-publication-guidelines.html



COLLABORATIONS

- Cryo-EM facility at NIC is also interested in direct collaborations with labs implementing cryo-EM experiments in their research.
- A thorough discussion of the theme and scope of the project will need to be addressed in detail with the Cryo-EM facility before agreement to the collaboration.
- Upon successful agreement, the participating staff members and their significant intellectual contribution to the project should be honoured and considered for co-authorship on all resulting publications.

TRAINING POLICIES

Hands on training is provided at our Facility, and the training is absolutely critical to learning the operation of the microscope. Cryo-EM facility staff will provide training on all of the equipment as needed.

Cryo-EM Sample Preparation and Glacios Imaging training:

1. Training is usually one-on-one and consists of at least four, 4-hour sessions.

a) During the 1st session, the core staff will walk you through the instrument's standard operating procedures, rules and (radiation) regulations and go over Vitrobot usage, glow-discharge usage, liquid nitrogen and liquid ethane handling and loading cryo samples into the nanocab.

b) During the 2nd session, the core staff will walk you through Glacios usage, low dose setup, alignments, image collection and Falcon III usage, and troubleshooting.

c) During the 3rd and 4th sessions, you are allowed to practice under the supervision/guidance of the staff. If additional training is required for a particular case, the user shall consult the staff and coordinate with their calendar.

2. The ultimate goal of any user is to be an **independent user** of the **Cryo-EM Facility.**

Definition of Independent User:

We define an independent user as one educated and capable to operate Glacios microscope in the Cryo-EM Facility during normal operational hours, as well as weekends, holidays and overnight without the supervision of staff. To be independent, individual users must demonstrate proficiency in:

1. Vitrobot usage and sample preparation,
2. loading and handling of cassettes and nanocabs,
3. microscope alignments, low dose imaging and data collection,
4. ending the data-collection session and
5. basic troubleshooting (and being able to assess what does/does not constitute “basic troubleshooting” vs problems that require immediate notification of the staff).

Users should be able to perform all necessary steps safely and effectively. Anything out of the ordinary should be communicated to Cryo-EM facility staff.

Independent users will be asked to sign a form that they are familiar with the facility’s ' Incident Response Plan' and ' Instructions for safe use of the cryo-electron transmission electron microscope'.

USAGE AND BILLING

All new users are requested to consult the Cryo-EM Facility web page (www.ki.si/en/glacios) for basic information and then contact the Cryo-EM facility (cryoem[at]ki.si) to get additional information and start the discussion. We kindly ask you to attach the Sample Declaration Form with at least the basic information with your first message. The Form is available on the same website.

Cryo-EM staff will schedule a time for a detailed discussion about the project feasibility, pricing, and goals.

When and how the services will be billed should be agreed upon before the start of the experiments.

CRYO-EM FACILITY

- All users must sign-up for instruments in the Cryo-EM Facility on the Cryo-EM facility internal reservation system (available to independent users). You can reserve up to 14 days in advance. You can modify or cancel your reservation up to 24-hours prior to your reserved time slot. Users will be charged for the entire reserved slot, even if the user finishes early unless a replacement user for this slot is found. If your sample is not viable and the session needs to be cancelled on the same day in the Cryo-EM Facility, please contact Cryo-EM staff as soon as possible. Failure to notify a Cryo-EM Facility staff in the first hours of the session will result in full financial responsibility for the session.
- The basic information on the nature of the sample and its quality has to be provided to the facility staff beforehand. The information provided should include: size, biological source, bio-safety info and instructions how to handle the sample and discard the sample when finished, sample purity (for example SDS-PAGE gels, size-exclusion chromatograms, DLS or similar) and buffer contents (salts, detergents, antifreeze agents, organic solvents, metals etc.)
- The Cryo-EM Facility staff will coordinate the training sessions with new users.
- The minimum length for a cryo-EM sample screening session on the Glacios microscope is 4 hours in the Cryo-EM Facility. 4-hour sessions can be reserved in the Cryo-EM Facility internal reservation system in the following time slots: 8 am - 12 pm and 12 pm - 4 pm. Additionally, an 8-hour screening session on Glacios is allowed (8 am - 4 pm). Sessions can be extended provided proper session extension is reflected in the reservation system. **Failure to obey these rules will result in a revocation of privileges to the Cryo-EM Facility.** Therefore, please sign up accordingly.
- Overnight and 24-hour data collection sessions are possible and have to be confirmed by the Cryo-EM Facility staff in advance.
- All users must clean up their work areas, return all used items to their proper storage location and leave all rooms, microscopes and accessory instruments in the Cryo-EM Facility in a condition such that they are ready for the next user by the end of their session.

DATA STORAGE AND ACCESS

- All the collected data will be available for transfer to external HDD or preferably downloaded by the user to a remote server. In case of download, users will get the necessary information from the Cryo-EM Facility staff.
- Data will be stored on the Glacios offload server for at least a month (or longer upon request). **After this period data may be deleted without any notice.** User is responsible for making personal copies and backups.

TREATMENT OF INSTRUMENTS/TOOLS/WORK SPACE

- The Cryo-EM Facility provides shared instruments and tools to the research community. The users and the quality of their research rely heavily on them and their pristine condition. Therefore, users must treat the cryo-EM equipment with care and cannot remove instruments/tools/chemicals from the facility at any time. If any of the tools are missing or damaged, it is the user's responsibility to report this to the facility manager as soon as possible at the beginning or during the user session.
- All users must follow the Cryo-EM Facility workflow and rules as instructed during training.
- All users must clean up their work areas after they have finished their session, return all used items to their proper storage location and leave all rooms, microscope and accessory instruments in the Cryo-EM Facility in a condition such that they are ready for the next user.
- Due to limited space, users should not store their samples, solutions, or other materials or equipment that were brought by them (grids, tweezers, etc.) in the Cryo-EM Facility unless authorized by the Cryo-EM staff or in the "grid motel" LN2 dewar for short- or mid-term grid storage.
- **VERY IMPORTANT:** If users experience any problems while operating the instruments (hardware issues or software/computer errors), the user should not attempt to resolve the problem on their own (unless specifically instructed by the facility manager). On the microscope: stop operations, close the column valves and contact the Cryo-EM facility manager immediately (24-hour contact is posted next to the microscope PC and in the Cryo-EM Sample Preparation Facility). Attempting to correct a malfunction and inadvertently damaging the instruments can void our service agreement with ThermoScientific (and the service company). If an error causes termination of the session the user will not be billed for the remainder of the booked time.

CURRENT RATES AT CRYO-EM FACILITY AT THE NIC

(overhead and VAT not included)

- **Vitrobot (cryo plunger) + glow discharge:**
  Internal independent + Instruct-ERIC consortium independent partners: **13 €/day**
  Academic Independent Use (other external): **19 €/h**
  Corporate Independent Use (External): **26 €/day**

- **Glacios microscope:**
  Internal independent + Instruct-ERIC consortium independent partners: **25.5 €/h**
  Academic Independent Use (other external): **88 €/h**

- **Training on Vitrobot, Glacios microscope at the Cryo-EM Facility:**
  Internal + Instruct-ERIC consortium partners: **25.5 €/h**
  Academic Independent Use (other external): **25.5 €/h**
  Corporate Use: **88 €/h**

- **Consumables**
  Cryo-EM grids: **8 – 35 € per piece** depending on the type of the grid
  C-clip ring: **9 € per piece**
  C-clip: **2 € per piece**

- **Operator time, data processing**
  Academic use - Operator support during freezing or data collection: **44 €/h**
  Corporate use - Freezing or data collection by Cryo-EM Facility staff: **100 €/h**

- **Equipment usage for data processing**
  Academic Independent Use: **1 € /h**
  Corporate Independent Use (External): **2 €/h**
  Academic use – Data processing by Cryo-EM Facility Staff: **38 €/h**
  Corporate use – Data processing by Cryo-EM Facility Staff: **76 €/h**

Prices are subject to change.
Instruct-ERIC consortium partners prices are based on yearly ARIS contribution to Instruct.SI.

CONTACT

Please contact us on: cryoem[at]ki.si

Table 1: Example of Sample Declaration Form. Excel spreadsheet file available at https://www.ki.si/en/glacios.

Cryo-EM Facility at the National Institute of Chemistry		
SAMPLE DECLARATION FORM		All applicable fields should be filled out. Return the form to cryoem[at]ki.si or to the person that sent it your way. This is also the contact address if there are any questions.
Date	**12. mar. 4567**	
Name		I, the user, declare that the information in this form is correct, to the best of my knowledge. I will take all steps to ensure safe and proper transport and storage of material when using the equipment in the NIC Cryo-EM Facility. I also acknowledge that I could be held liable for any false, incorrect, and misleading information made on this form. The contributions of the Facility and/or of the personnel will be properly acknowledged (Guidelines from the RMS).
Email address		
Affiliation/ Institution		
Supervisor/PI		
Number of samples		
SAMPLE 1		
	name/label	
	molecular weight/size	
	origin (species)	
	link to information, if available in any of the public databases/resources (PDB, UNIPROT, …)	
	bio-safety level (BSL)	
	natural or recombinant or synthetic	
	production organism	
	buffer composition	
	special safety requirements or sample requirements	
	additional notes	
SAMPLE 2 (copy and renumber if there are more than 2 samples)		
	name/label	
	molecular weight	
	origin (species)	
	link to information, if available in any of the public databases/resources (PDB, UNIPROT, …)	
	bio-safety level (BSL)	
	natural or recombinant or synthetic	
	production organism	
	buffer composition	
	special safety requirements or sample requirements	
	additional notes	