Unità Infrastrutturale CRYO-EM

A PROPOSAL PRESENTED BY A STUDY GROUP FROM THE STRUCTURAL BIOLOGY NATIONAL COMMUNITY

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Foreword

Following the results of a two-stage national consultation over the past two years and the approved development schemes (Convention, article 3.4), the national structural biology community, represented by forty leading scientists in the field, discussed the needs and ideas that could lead to the establishment of a national cryo-EM facility (Cryo-Electron Microscopy Infrastructure Unit at the Human Technopole).

This document presents the outcomes of these discussions, highlighting the main objectives and operations of the anticipated Cryo-EM facility.

During the brainstorming exercise (five distinct meetings, held between December 2022 and May 2023), the availability of a cryo-EM facility capable of facilitating substantial screening throughput for grid preparation and preliminary analyses on medium-level cryo-electron microscopes was identified as a high national priority. Considering the pressing need to enhance scientific culture and capabilities, particularly in the field of single-particle cryo-EM analysis, the document also emphasizes the requirements for training programs, support laboratories, personnel, and user management.

The following pages outline the conclusions reached by seven working groups that focused on the following specific areas:

1. Cryo-EM hardware
2. Computing and storage resources, software
3. Cryo-EM grid preparation laboratory setup
4. Rooms and laboratory space
5. On-site technical support
6. Site access applications
7. Training

Please note that, although an estimate at current costs was considered, this document does not provide any assessment of the costs associated with individual initiatives, as such evaluations would be either disproportionate or premature at this time.

Martino Bolognesi
Emeritus Professor of Biochemistry
University of Milano
24 May 2023

WORK GROUP ANALYSES

WG1: main cryo-EM hardware installation

WG1 investigated possible technical solutions for the selection of suitable transmission electron microscopes operating at cryogenic temperatures (cryo-TEMs) hosting automated sample loading.

Description

Microscope hardware

We recommend that the cryo-TEM(s) to be purchased should:

- be fitted with a field-emission gun (FEG) to generate an appropriate operational electron beam with high brightness, high coherence, and high stability for high-resolution imaging. The intensity of the beam should be stable for at least two days;
- be equipped with a "constant power" objective lens that minimizes thermal drift and hysteresis for optimal resolution and contrast in single particle analysis, tomography, and electron diffraction applied to the study of biological samples;
- be capable of modifying the acceleration voltage to any value between 80 and 200 kV in minutes;
- be equipped with an oil-free vacuum system.

Sample loading system

- be equipped with an automated contamination-free loading system for cryo-grids. It must load up to 12 autogrids per cassette in a single vacuum-interruption action;
- utilize a system for ice-free exchange of grids that is highly reliable and fast (order of a few minutes);
- utilize sample holders that must be compatible with those of other microscopes available at HT and at most international facilities (Krios operating at 300 kV);
- utilize grids compatible with cryo-DualBeam systems dedicated to the preparation of thin, electron-transparent lamellas for high-resolution cryo-electron tomography or microED of micro-crystals.

Performance under cryo-conditions

- during its use under cryo-conditions loss of electron transmission owing to ice formation on the sample should be below 5% over 24 hours (approx 0.2 nm/hour)
- the sample stage should be extremely stable under cryo-conditions as limited drift improves imaging. Drift should be less than 0.25 nm/s 30 mins after grid exchange and less than 0.05 nm/s 60 mins after grid exchange
- ice-quality of frozen hydrated samples should not decay for at least 24h in the microscope.

Detector

- the cryo-TEM must be equipped with a post-column electron detection system at high DQE. Ideally, it should be a 4k×4k pixel direct electron detector (DED).
- the camera should allow for lossless data compression with electron-event representation (EER).
- the detector should be equipped with its own storage unit of at least 60 Tb connected to the camera via fiber optics.

- pixel size should be at least 14 x 14 μm^2.
- internal and external frame rate of the detector should not be less than 320 fps.
- DQE at 200kV should not be less than 0.91 (0) an 0.62 (half Nq)
- we also suggest the installation of a post-column energy filter for improved SNR. The availability of a such electron optical system makes a Volta phase plate largely redundant.

Conclusions

We suggest the purchase of **two 200 kV Glacios 2 autoloading cryo-TEMs** equipped with **Falcon 4i direct electron detector** and **Selectris X energy filter.** These instruments represent an excellent technical solution fulfilling all the required criteria and ensure maximum compatibility with equipment already available at HT and at the international cryo-EM facilities.

Roberto STEINER (U. Padova), Silvia ONESTI (ELETTRA, Trieste), Beatrice VALLONE (U. Sapienza, Roma)

WG2: computing/storage resources and software provision

WG2 will explores the hardware developments required for the establishment of two computing access points that will allow grid preliminary analyses, but also on site structure determination, if needed. The computing resources are expected to be complemented by adequate storage capacity and by provision of state-of-the-art software packages. The computing branch of the facility in principle could be made available (*via* remote access) to the national community before completing the whole facility.

Aims
Each Cryo-EM research project requires intense data analysis and storage. Therefore, it is estimated that the UI platform should hold at least 5 PB for data analysis and storage to guarantee access to the national research groups. It is our firm opinion that, thanks to end-user training and automated data collection workflows (SerialEM, EPU, etc.), cryo-EM will be accessed by an increasing number of research groups, even from research fields different form the most traditional ones.

Description
This work package comprises short- and long-term goals.

1) Short-term goals:
   - one full-time staff figure to manage the CryoEM computing facility (for day-to-day maintenance, installation of new software, data analysis support, consultation with users and training);
   - cryo-TEM stations with installed EPU and SerialEM;
   - provision for on-site and remote data collection;
   - short-term data storage (2 months from the day of collection; 300 TB, fast connection for data transfer, e.g. 10-GbE);
   - a backup unit for the short-term storage (for raw movies and motion-corrected micrographs);
   - long term data storage (12 months from the day of collection, then to be deleted; at least 5 PB, fast connection for data transfer, e.g. 10-GbE). Free storage is limited to 100 TB per research group. In the future, it is wished that the UI platform will allow heavy users to purchase additional storage on site that will be administered and networked by HT;
   - data movement service (e.g. Globus data transfer service that includes end-to-end encryption, or Aspera);
   - network upgrades to ensure fast data transfer (10-GbE connection);
   - 2 processing workstations equipped for data processing, including:
     - 4 x GPU
     - 2 x 28-core CPU (or more)
     -10-GbE connection to data storage server
     -software packages such as RELION and cryoSPARC, and integrated with machine learning software for particle picking, such as crYOLO, TOPAZ (already integrated in cryoSPARC).

2) Long-term goals:

- creation of a web interface that illustrates the steps to take along the pipeline of cryoEM acquisition, processing (this is useful to assist new users of the facility and in general to guide users new to CryoEM) and transfer;
- creation of a web-based access to consultation with the IT manager (and possibly a similar helpdesk for other staff members of the facility can be created);
- considering the rapid evolution of software for cryoEM, SPA and cryoET, we propose to create a platform to run cryoEM jobs. The aim is to eliminate the burden of software installation and updating for individual research groups. Two existing platforms, one public and one commercial, appear to be suitable templates for the UI platform:

  - COSMIC2 (http://cosmic-cryoem.org), a public web-based platform of open-source software for cryoEM. Regarding the HT-platform, allocation of cloud computing resources and time can be internal to a supercluster at HT or external (such as CINECA). Such a web platform could run similarly to the PXSOFT package installed at ESRF and dedicated to X-ray crystallography (https://www.esrf.fr/UsersAndScience/Experiments/MX/Software/PXSOFT).

  -Another interesting service, albeit commercial, is provided by SBGrid (https://sbgrid.org), a consortium that provides users with a suite of structural biology software preconfigured to run. SBGrid *per se* cannot be implemented in the UI platform because it targets individual research groups, and it charges a large annual fee. However, we list it here because it offers a different solution for the CryoEM community.

Conclusions

This work-package outlines the hardware developments necessary to maximise the support to training and access to the CryoEM national platform by interested scientists focusing on diverse biological questions. The computing resources are expected to be complemented by adequate storage capacity, cutting-edge software packages and experienced personnel.

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WG3. Cryo-EM grid preparation lab

WG3 explores all the small equipment, materials, and reagents needed for the proper operation of the facility at the experimental and training levels. To support the grid preparation lab described below, we recommend the availability of a sample optimization and characterization lab to further assess sample quality before vitrification. However, this extension is not considered in this section.

Aims
The following list was designed to meet the requirements of a cryo-EM sample preparation laboratory conducting two sample preparation experiments simultaneously for single-particle cryo-EM. In the near future, it will also cater to cryo-ET.

Description
The materials are schematically grouped in sections and summed-up as follows:

- Vitrification:
  One high-throughput vitrification system: Chameleon - SPT Labtech, or VitroJet – CryoSol; two state-of-the-art automatic plunge freezers: Mark IV Vitrobot – TFS, and/or Automatic Plunge Freezer EM GP2 – Leica.
- Small equipment:
  One glow discharge apparatus with the possibility to use air/solvent vapor, as EMS Globe Plus glow discharge (Quorum), one refrigerated centrifuge, one Nanodrop, one vortex, one low-temperature oven, one air dryer, one heating plate, one aspiration hood for ethane operation, two magnifying glass light lamps and one 4ºC fridge with -20ºC freezer.
- Ethane:
  Two bottles of REG HBS.V 200-10-3,5 NF E, each containing 5L of ETHANE N45 Cy-S reg 5/99- Valve AFNOR E, to have the capacity to prepare liquid ethane in two experiments independently.
- LN2 dewars:
  A complete system of grid storage with capacity for 1200 grid-boxes as Cryo-EM HC35 Complete Grid Storage and Shipping Puck System – Mitegen. 4x 1 L Enamelled steel dewars, 4x 4 L Dewars, 2x Foam Dewars, one 25 L Dewar with wheels, as Cryolab 25 – Agar scientific and two funnel.
- Autogrid preparation tools:
  2x AutoGrid Assembly Workstations, 2x Loading Stations, 4x Capsules, 4x Cassettes, 24x C-Clip Insertion Tools, a stock of 4’000 AutoGrid Rings and C-Clips, 6x Grid Container Tools, a stock of at least 120x grid-boxes for storage and 12x hybrid grid boxes for routine vitrification.
- Grids:
  A grid stock of 2’000 grids composed of: 50% Quantifoil grids (1.2/1.3, 0.6/1, 2/1, ultraAu 1.271.3, UltraAu 0.6/1), 20% C-Flat, 10% Continuous C, 10% Lacey carbon, 10% GO 1.2/1.3 Quantifoil grids.
- Tweezers:
  30x Soft grip tweezers 115mm extra fine tips for grid and small things handling, 4x Large Blunt Tweezers, 10x thermally isolated Tweezers as Dumont Ergonomic ESD Tweezers Style 5 Anti-Magnetic , 12x Cryo Tweezers for the vitrobot, 12x Autogrid Tweezers, 2x Cassette Tweezers.

- Safety:
  4x Waterproof Cryogenic Protective Gloves, Cryo safety kit, 10x 3M™ Visitor Specs, Cryogenic Protective Face Shield.
- Various:
  25ml of Amylaminefor evaporation during glow discharging, nanobeads fiducials for cryo-ET, one detergent screen, amphipols and VitroEase™ Buffer Screening Kit for sample optimization during vitrification,Microscope Slides (100x stock), Scalpels (20x), Parafilm, filter paper, Standard Vitrobot Filter Paper, General Purpose Scissors (2x), Tape, Glass bottles, eppendorf tubes, 15ml and 50ml falcon tubes and gloves (s, m, l).

Conclusions

The above list presents the materials needed to conduct two independent vitrification experiments simultaneously. A wide range of materials has been considered to provide the highest flexibility to the users. The lab was designed to store vitrified grids. This list includes an estimate of the stock needs for proper operation, taking into account potential delays that may occur in grid purchase. A partial stock has been considered to replace damaged materials used, especially during training sessions.

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WG4: lab(s) layout and supporting rooms

WG4 provides a detailed description of the expected layout of the lab(s) and supporting rooms required to accommodate the instrumentation and operators (including external users and facility members) of the future CryoEM UI facility.

Objective

The aim of WG4 is to emphasize the key features of the lab(s) layout and supporting rooms, including space and equipment, needed to effectively house the requested instrumentation and ensure optimal working conditions.

Description

• Microscope rooms:

- Two separate rooms to house the microscopes requested by WG1. These rooms should be electrically and mechanically isolated using the same technology employed in the microscope rooms at HT, such as a Faraday cage embedded in the walls and a concrete platform to support the microscopes (as necessary).
- Two rooms to accommodate the workstations responsible for controlling the microscopes. Each workstation room should have communication capabilities with the respective microscope room. For safety reasons related to the LN2 cryo-cooling system used in the microscopes, the workstation rooms should be equipped for visual inspection of the microscopes.

• Grid preparation lab:

- One dedicated room for sample preparation. This room should have the capacity to accommodate the four vitrification systems requested by WG3, along with the necessary supporting equipment. It is highly recommended to install two laminar flow hoods for the two Vitrobots (as mentioned in WG3).
- One room designated for Dewar storage. Placing this room near one of the sample preparation rooms is recommended. Additionally, visual inspection of the room is desirable for safety purposes. Note: All the rooms described above require systems and equipment to control temperature, humidity, and oxygen sensors.

• Computing/storage rooms:

- One room specifically allocated for the data storage system (as mentioned in WG2).
- One room to house two workstations dedicated to on-site data analysis (as mentioned in WG2). It is important to consider support for two operators per workstation: one external user and one facility member, when necessary.
- One room designated for training, as outlined in WG7. Therefore, the installation of necessary equipment for both theoretical and practical training is requested (e.g., projector and associated workstation, large screen, and approximately twenty workstations to accommodate the estimated number of on-site users).

Note: The rooms described above also require systems and equipment to control temperature and humidity.

Conclusions

After carefully considering the requests put forth by WG1, WG2, WG3, WG5, and WG7, the present WG has compiled a list of the necessary lab(s) layout and supporting rooms to fulfill these requests in terms of space and equipment.

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WG5: **Technical support sought on site**

The Cryo-EM UI facility will operate under the technical assistance of onsite staff that will supervise the microscopes, the grid plunging and preparation lab, data collections, and the computing equipment, including software maintenance. WG5 identifies the professional profiles, the number of personnel units and the technical support required.

Aim

The primary objective of the Italian cryo-EM research community is to contribute to the establishment of the UI facility for cryo-EM grid preparation and screening, and support for complete data collections. Given the considerable amount of time, commitment, and expertise required by the service provided, we envisage the recruitment of a dedicated Senior Scientist assisted by a team of Junior Scientist/Senior Technicians.

Description

The **Senior Scientist** (with > 5 years of research experience after a PhD) should be a researcher with proven experience and know-how in cryo-EM techniques, namely single particle reconstruction and tomography. By **Junior Scientists/Senior Technicians** in what follows we mean researchers with proven experience in Cryo-EM sample preparation (i.e., grid preparation and vitrification) and data processing and analysis.

We outline the staff needed during the cryo-EM facility development process:

1. **Current setup/short time plans of Phase-1:**
   During facility implementation **(Phase-1, ~year 1)**, **1 Senior Scientist** and **1 Junior Scientist/Senior technician** will be required. Taking advantage of the existing resources at HT, the staff will set up the biolab, instrumentation, and equipment. At the same time, the staff will support users in sample preparation, data collection, analysis and processing, according to guidelines drafted by HT in consultation with the Italian Structural Biology Community.

   During Phase-1, the Senior and the Junior scientists will have access to the currently available instrumentation at HT, which we envisage as: (a) a *Krios* 300 kV cryo-TEM; (b) a *Glacios* 200 kV cryo-TEM; (c) a *Talos* 120 kV TEM; (d) an *Aquilos* 2 for lamellae sample preparation; (e) and to all the ancillary instrumentation for sample vitrification.

   The Senior Scientist will be responsible for assisting the users and managing their projects through all stages of the experiment (including overall design, grid preparation and vitrification, grid screening and optimization, preliminary data analysis, data storage). The same Senior Scientist will also provide training for the junior scientist/technician. With this setup, the Facility should be operational and able to offer monthly one slot/shift for *Glacios* measurements and another one for vitrification and screening to each of the dozen Italian groups with an active project in its early stages.

2. **Mid-term expansion Phase-2 (years 2-3):** once the planned instrument acquisitions take place and the facility has two additional, operative, intermediate-level cryo-TEMs, we anticipate the need of at least **2 additional Junior Scientists/Senior Technicians**.

   By the time the facility will be fully open to users, the **Senior Scientist** recruited in Phase-1 will serve as the head of the facility and represent the primary point of contact for the operation and maintenance of all the equipment (laboratory, microscopes, computing equipment and setup, and data storage), as well as for the training and management of other staff members. They will also be the main point of contact for external users. Their expertise will be essential to all users during the screening sessions, which are the most time-consuming and challenging stages in the cryo-EM structure determination process. They will evaluate the quality of samples and grids and will be crucial for guiding the users to the next steps of the experiment (i.e., data collection and/or sample/vitrification optimization).

   The **Junior scientists/Senior technicians**, once trained, will support the Senior Scientist in all operations and work closely with the users during sample vitrification. They will manage the laboratory, keeping track of all the materials that need to be purchased and/or prepared for each experiment.

3. **Fully operating facility Phase-3 and long-term plans (years 3-4):** After the start-up phase, we expect the Italian Community of Structural Biologists will need a new 300kV high-end cryo-TEM, and thus another **additional Junior Scientist/Senior Technician**, according to user requirements. The access to this microscope will be allowed, after a strict selection, to users who are able to provide sound preliminary data and grids already vitrified and screened. Therefore, measurements and maintenance at this microscope could be reasonably carried out by such a high level technician.

Conclusions

At full capacity we envisage the employment of at least one **Senior and four Junior Scientists/Senior Technicians**. We cannot exclude that when the Italian Cryo-EM user community grows, additional units of personnel may be needed. Other considerations:

- **None of these positions ought to be short term contracts**. Career support should be provided, following principles such as the ones expounded for example in the UK Concordat to Support the Career Development of Researchers: https://researcherdevelopmentconcordat.ac.uk/. Individually tailored career development plans should be put in place, spanning the range of careers from Academic to Technical Support.
- **The personnel must be routinely involved in the scientific projects** - with co-authorship granted to the facility personnel on any peer-reviewed publications directly stemming from or describing work done at the facility.
- **Training of users** to be carried out by all the above personnel, through user shadowing / residency stages that can extend up to days or weeks.

We acknowledge advice from: Matteo Ardini (Univaq, L'Aquila), Antonio Chaves Sanjuan (Unimi, Milano), Giuseppe Cannone (MRC-LMB, Cambridge, UK), TJ Ragan and Joanna Fox

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Linda Celeste MONTEMIGLIO (CNR-IBPM, Roma); Gianpiero GARAU (IIT-NEST, Pisa); Pietro ROVERSI (CNR-IBBA, Milano); Gabriele GIACHIN (U. Padova)

WG6: Application modalities, reporting, user service, hosting

WG6 describes the essential requirements for an efficient management of machine-time applications, from the submission/evaluation of project proposals to the reporting steps, including users support, hosting and safety rules to enter the CryoEM@HT facility. The coordination of the incoming users and of machine-time allocation must rely on a permanent User Office (USO) that will also provide accommodation options and transport information. The proven experience developed at synchrotron sites facilities in Europe represents a leading reference point.

Aims

WG6 aims to build the prerequisites for a widely accessible and well-organized facility for the Scientific community. Major goals include: *i.* Establishing transparent procedures for users' access, based on clear application rules, well-defined access priorities, evaluation steps, reporting protocols; *ii.* Setting the basic requirements for a well-organized user support with defined access rules, safety protocols and hosting services.

Description

Application forms and reporting

Users will apply for access to the CryoEM UI facility using a CryoEM adapted version of the **SMIS platform**, which is well known to the Italian scientific community regularly accessing the European Synchrotron Facilities. SMIS is the Portal where users can apply for beam time, complete online safety training, and provide personal information for travel, accommodation, and refund programs (https://smis.esrf.fr/).

Access to the facility will be granted in steps based on the project quality and advancement status. Projects can concern all areas of medicine, biology, chemistry, biotechnology, green science, environmental and food science. Submitted projects can refer to basic or applied research and must be sound for scientific and technological impact. In the first phase, the PI must guarantee the production of the samples necessary to access **levels I, II or III,** as defined below. Sufficient proof of the sample's quality can be included with the proposal at this stage, comprising previous results obtained at other facilities.

Approved projects will benefit from waiving of the **Experimental Costs** (bench and instrument fees) independently to the levels of access.

The **Safety of the samples** must be evaluated by the HT Safety officer. The safety level is the standard one foreseen for the HT. Access to the laboratory by users should follow the current procedures existing for HT personnel, through adequate training. User training for laboratory safety should be established according to HT polices, mainly in the form of safety training courses regularly performed by online platforms (see WP7).

Project application for access to level I (see below) is annual, with reporting of results of previous applications. Upon first time application per project, no reporting of results of previous applications is needed. The review process is handled by an **International Scientific Committee** nominated by the Director General.

The International Scientific Committee should be established on purpose, to guarantee regular evaluation sessions. The committee should be ideally composed by a facility member, at least one scientist from the CryoEM community active at Human Technopole, and two external experts in Structural Techniques. The Committee should be periodically renewed to ensure adequate rotation.
Access requests to Levels II and III, are subject to: 1) project application approval (Level I); 2) presentation of evidence to fulfil the requirements for the level of interest. Request for access to Levels II and III is open all year.
Reporting will be contextual of the yearly project application. The report will provide a list of the shift used, results obtained, and foreseen outcome. The facility will provide a user feed-back form on the quality of the services provided.

- **Levels of access**

We envisage three levels of access:
Level I: Once positive assessment has been granted on project quality, the user will be given access to Sample Characterization Facility (SCF). SCF is the laboratory where it is possible to characterize the sample. The aim of the quality control is to confirm the suitability of the sample for CryoEM.
Level II: Access to the Sample preparation facility where the sample will be tested through negative staining at the given microscope and/or vitrified. Negative staining can be avoided when sufficient justification is provided and test CryoEM images can be collected at the given microscope.
Level III: Given positive validation of the sample at level II, access to a high-end microscope for data collection(s) is granted.

Management support (users service & hosting)
We foresee that the CryoEM UI facility will be visited by a maximum of 15 scientists per week, with a stay of 3 days in average per week (2 visits per day of 2 people (max)/shift). To enable and coordinate the User Service we foresee the establishment of a **User Office (USO)** model like the one already in place at European Synchrotron Facilities such as ESRF, Grenoble (FR) and EMBL, Hamburg (DE), with two administrative staff members (2 FTE). The USO will coordinate the beamtime application process and the application review system and support the HT scientists in allocating the experiment shift. The USO will communicate dates to the users, coordinate the logistic for sample delivery and user travel, including booking of the guest house and provide the framework for travel expenses reimbursement. **Travel and Accommodation Costs** are paid by the facility, following the European Synchrotron Facilities model. To facilitate users hosting, we foreseen the establishment of a **Local Guesthouse** with 20-30 beds (also for guests of training events).

Conclusions and costs evaluation
The organization and regulation of users access to the CryoEM UI facility is fundamental to pursue the objectives of the present initiative. It implies allocation of resources to maintain infrastructures and dedicated personnel, to support:
User office (USO) costs (2 FTE positions and equipped office);

HT Guesthouse (20 rooms) and Travel and Accommodation;
International Scientific Committee costs;
Safety Committee, Safety training and protocols (refer to HT safety officers and HT organization for further evaluation).

Coordinator: Michele CIANCI (U. Politecnica Marche), with Laura CENDRON (U. Padova), Paola STORICI (ELETTRA, Trieste), Luca MAZZEI (UNIBO).

WG7: ***Training Scheme***

Following the 3rd meeting of the National Community on the Cryo-EM Facility @HT, the Working Group 7 gathered and formulated a proposal of the training activities that Human Technopole should offer to the Structural Biology community in the context of the National Platforms.

Aims

The principal goal of the training scheme here proposed is to foster scientific culture regarding cryo-EM methodologies, and specifically disseminate information and protocols regarding the infrastructures and the scientific activities ongoing at the CryoEM UI.

Description

The training scheme envisioned by the WG7 combines online and onsite activities, as outlined below.

Online: The increasing importance of CryoEM methodologies in integrative structural biology approaches and the fast development of the associated technologies call for the development of appropriate tools to make the theoretical knowledge in the field and the best-practice protocols available to the widest scientific community, with no delays.

- To this aim, the CryoEM UI will develop, maintain and update an *online training platform* with a collection of virtual courses and tutorials on topics including EM theoretical bases, sample preparation, data collection, and processing. The courses will be open to the entire scientific community, free at any time, organized in sessions of about 20 minutes each, which can be followed individually. They will be integrated with self-assessment modules, and accessible with a free registration.
- The educational plan will be complemented with the establishment of a *digital forum* where scientists will be able to interact with each other and with HT technologists, and a *repository* where technical reports, experimental protocols, etc. linked to experiments carried out at the CryoEM UI facility can be published in a free format, on a voluntary basis, without peer review, possibly assigned with a DOI (and always open to comments/ review) in open access.

Most importantly, these online dissemination plans will mainly focus on instrumentation and practices at the CryoEM UI. In this respect, they will be unique for the international community and will be instrumental to pave the way for users' access to the Structural Biology platforms.

Onsite: Similar emphasis will be given to onsite activities intended to form scientists and technologists in all aspects of the EM pipelines through dedicated in person courses and workshops. Specifically, onsite activities will be organized as described below.

- *Workshops for PhD students and Post-doctoral fellows*: they will consist of one-week courses once or twice a year for three years, with hands-on practical sessions in which students will learn fundamentals of sample preparations, data collections and processing in a propaedeutic manner. Students will enroll ideally at the first year and follow the sequential courses throughout the PhD/Postdoctoral period. After each course, selected students with interesting samples will be invited to prolong their staying at the facility, to proceed with the analysis pipeline.
- *Workshops for EM facility managers and technologists*: these one-week courses will be tailored for personnel working in EM facilities present or establishing in the incoming years. As such, beside the basics of EM, they will cover aspects of working and maintenance of instrumentation, as well as users' support.

- *Workshops of EM data processing*: these courses will illustrate the theoretical basis and practical usage of software used for image processing and model building, with focus on newly developed algorithms. They will be one-week long, with practical sessions.
- Training is also envisioned as *users' support* during the users' visits at the CryoEM UI facilities in the areas of EM sample preparations, grid screenings and data collections,
- S*abbatical training*: once a year, a scientists or EM facility manager will be allowed to flank an HT facility manager for 2-3 weeks to understand how real-life challenging projects are tackled.

Considering the ongoing activities in the Italian Structural Biology community, the training scheme will focus initially on Single-Particle-Analysis pipelines, and later be completed with analogous activities for tomography (ET).

Conclusions

The training scheme proposed by WG7 aims at disseminating knowledge on CryoEM approaches, with focus on experimental activities ongoing at HT/UI that will become accessible to Italian Scientists. It envisions a combination of ***online and onsite activities***, the latter of which will be mainly delivered through one-week courses for students, scientists and technologists operating in the CryoEM field.

To run the proposed activities, ***two dedicated Tutors*** will be required. The first, with a strong theoretical background, will oversee the online platform and will manage the dissemination of the PN-related training activities ongoing at HT. He/She will also assist in the organization of the onsite workshops. The second Tutor will have a more technical profile and will participate in the practical training sessions.

The training activities outline above will require at least one ad-hoc ***training lab*** equipped with work-stations.

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