HUMAN TECHNOPOLE

NATIONAL FACILITY FOR STRUCTURAL BIOLOGY

CALL FOR ACCESS

25-SB-ROUND1

1. INTRODUCTION

The Access of Researchers affiliated with Universities, *Istituti di Ricovero e Cura a Carattere Scientifico* (IRCCS), and Public Research Entities to Fondazione Human Technopole (HT) National Facilities (NFs) is regulated by the NF Access rules available on the NFs dedicated webpage (link).

Services offered by NFs are available through regular open calls for Access that are published yearly on the HT website (link) and are free of charge for the project (or aspects of the project) approved for Access.

The open call for Access is aimed at supporting Access to the technologies offered by the NFs and it is not meant to provide direct funding to the Applicant. The costs for the activities to be performed at the NFs will be fully covered, including shipment of relevant material from and to the Applicant's laboratory as well as travel and accommodation for the Applicant and/ or Applicant's team member(s) (User) while accessing the NF. Project-related costs (personnel, consumables, and other costs) at the Applicant's laboratory are not funded.

The User Access workflow comprises different steps, spanning from the initial submission of the application to evaluation and Access approval, Access to the performance of the service(s) and Access conclusion. A detailed description of the workflow is available on the NFs dedicated webpage (link).

1.1 Access modalities

Three different Access modalities can be requested. Their availability will vary, based on the service specifics of each NF:

- **"Simple" Access to NF or individual instruments thereof**: This modality is intended for Users involved in projects requiring technologies that are available at the NF for **direct Access by User**. This Access modality requires prior expertise with the technology of interest. After an initial introductory training aimed at defining the level of expertise of the User, **the use of the instrument with limited supervision by NF staff is authorised**. For defined NFs/ instruments/ services this Access modality may be restricted or not available.
- **Access to NF services**: This procedure entails the provision of **services performed by NF staff on behalf of the User**. NF services may include both standard services as well as, when foreseen by the technology development specifics of each NF, bespoke services conceived and discussed with the User. To allow the NF staff to best align the experimental activity to the research objective, the User may be invited, if needed, to assist the NF staff while performing the project or aspects of it.
- **Access to NF services including training**: This procedure **entails training by NF staff** to provide Users, in addition to or alternatively to the services described in the previous modality, with training courses and/or programs, aimed at transferring the expertise necessary for the independent use of the specific technology. In this case, technical and/or experimental activities are conducted with the active participation of the User. Training can be provided by NF staff while performing the service(s) or in a dedicated session. This type of Access is also aimed at researchers who want to acquire expertise for subsequent independent use of a specific technology in other laboratories.

2. TERMS AND DEFINITIONS

2.1 Access

"Access" refers to the authorised use of the NF and of the services offered. Such Access can be granted for sample preparation, set-up, execution and dismantling of experiments, education and training, expert support and analytical services, among others. Access to the NFs includes all infrastructural, logistical, technical and scientific support (including training) that is necessary to perform the aspects of the project approved for Access.

2.2 Researcher

"Researcher" is a professional engaged in the conception or creation of scientific knowledge. They conduct research and improve or develop concepts, theories, models, techniques, instrumentation, software or operational methods.

2.3 Principal Investigator

"Principal Investigator" (PI) is the Researcher affiliated with an eligible Institution with the role of independent Group Leader, who is responsible for coordinating the research activities conducted within the framework of the submitted project.

The PI shall hold a primary appointment as Group Leader at an eligible Institution, with the following requisites:

- Coordinate an independent research team.
- Have a supervisory role towards junior and/ or senior Researchers.
- Their Group has an autonomous budget sufficient to cover their current research expenses.
- Be the recipient of independent research funding as PI or co-PI.

Junior PI: Up to 6 years from their first appointment in an independent Group Leader position.

The period specified above may be extended beyond 6 years in the event of adequately documented career breaks, occurring before the submission of the application and resulting from:

i. Maternity leave: The time limit is increased by 18 months for each child born after their first appointment in an independent group leader position; if the Applicant is able to document a longer total maternity leave, the period of eligibility will be extended by a period equal to the documented leave, taken before the submission of the application. Maternity status must be documented by submitting the birth certificate of the child or children.

ii. Paternity leave: The time limit is increased by the actual amount of paternity leave taken before the application submission deadline for each child born after their first appointment in an independent group leader position. Paternity status must be documented by submitting the birth certificate of the child or children.

iii. Long-term illness of more than 90 days, or national service: The time limit is increased, for each eligible event occurring after their first appointment in an independent group leader position, by the actual amount of leave from which the Applicant has benefited prior to the application submission deadline.

Established PI: More than 6 years from their first appointment in an independent group leader position.

2.4 Applicant

"Applicant" is the Principal Investigator who applies to a NF open call for Access and who is responsible for the submitted project. They can be of any nationality and must be affiliated with an eligible Italian Institution, as detailed in section 4.

2.5 User

A "User" is intended as a Researcher affiliated with an eligible Institution who accesses, physically or remotely, the NFs to perform the approved activities or to support the National Facility staff while performing the approved service.

If requested by the Applicant, the User of the NF can also be a separate member of their research team.

3. APPLICATION TYPE

Applicants shall select the type of application they want to submit, choosing between two options:

a. **Standard** application for projects that are technically mature.

b. **Proof-of-concept** application for:

i. Projects with high scientific potential but with insufficient technical maturity or preliminary data.

ii. Projects aimed at setting up the experimental conditions required for a standard project, including methods or technology development projects.

iii. Time-limited Access projects (e.g., to acquire data to complete a manuscript, or preliminary data needed for a grant application, or single microscopy session).

4. ELIGIBILITY AND ADMISSIBILITY

PIs, as defined in section 2.3 of this call, affiliated with an eligible Institution are eligible to apply. The Applicant's role as a PI shall be confirmed by their Institution in a mandatory letter of Institutional endorsement (Template available in Annex I).

Applications from Researchers who are not independent should be submitted by their Group Leader. Applicants are strongly encouraged to support NF Access by young Researchers (R1 and R2 profiles of the European Framework for Research Careers, link) who are part of their group. In this case, the Applicant shall indicate in the application form that the NF User is a member of their group, specifying User's career stage.

Below are the links to the relevant lists of eligible Institutions:

Universities: This category includes Institutions recognized by the Ministry of University and Research (link). In detail:

i. State funded public universities, listed under the following link.

ii. Specialized superior graduate schools or Institutions, listed under the following link.

iii. Legally recognized non-public universities, listed under the following link.

iv. On-line universities, listed under the following link.

Istituti di Ricerca e Cura a Carattere Scientifico (IRCCS): this category includes Institutions recognized by the Ministry of Health and listed at the following link.

Public research entities: this category includes:

a) Institutions recognized by the Ministry of University and Research and listed at the following link.
b) Area di Ricerca Scientifica e Tecnologica di Trieste - Area Science Park;
c) Agenzia Spaziale Italiana - ASI;
d) Consiglio Nazionale delle Ricerche - CNR;
e) Istituto Italiano di Studi Germanici;
f) Istituto Nazionale di Astrofisica - INAF;
g) Istituto Nazionale di Alta Matematica "Francesco Severi" - INDAM;
h) Istituto Nazionale di Fisica Nucleare - INFN;
i) Istituto Nazionale di Geofisica e Vulcanologia - INGV;
j) Istituto Nazionale di Oceanografia e di Geofisica Sperimentale - OGS;
k) Istituto Nazionale di Ricerca Metrologica - INRIM;
l) Museo Storico della Fisica e Centro Studi e Ricerche "Enrico Fermi";
m) Stazione Zoologica "Anton Dohrn";
n) Istituto Nazionale per la Valutazione del Sistema Educativo di Istruzione e di
o) Formazione - INVALSI;
p) Istituto Nazionale di Documentazione, Innovazione e Ricerca Educativa - INDIRE;
q) Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - CREA;
r) Agenzia Nazionale per le Nuove Tecnologie, l'energia e lo Sviluppo Sostenibile - ENEA;
s) Istituto per lo Sviluppo della Formazione Professionale dei Lavoratori - ISFOL (a decorrere dal 1° dicembre 2016 denominato Istituto nazionale per l'analisi delle politiche pubbliche - INAPP);
t) Istituto Nazionale di Statistica - ISTAT;
u) Istituto Superiore di Sanità - ISS;
v) Istituto Superiore per la Protezione e la Ricerca Ambientale - ISPRA, ferme restando le disposizioni di cui alla legge 28 giugno 2016 n. 132;
w) Istituto nazionale per l'assicurazione contro gli infortuni sul lavoro – INAIL.

Eligible Institutions/ Institutes are strongly encouraged to limit the number of applications submitted to this call for Access to the very best two, with at least 50% coming from Junior PIs.

Such indication does not represent an eligibility criterion but rather a guideline aimed at ensuring the widest distribution of Access among Institutions in the Country.

Applicants shall declare that they have not received funding to perform the submitted project (limited to the aspects included for Access to the NF) in their own laboratory, home Institution or elsewhere. Applicants shall confirm the economic and scientific feasibility for the aspects of the project to be performed outside the NFs.

Applicants cannot request Access for the same service if an approved Access is ongoing. Before submitting a new application for the same service, Applicant shall consult with the NF staff and confirm that the ongoing Access will be completed before the end of the next evaluation round. A clear motivation for the request must be provided.

A PI submitting an application to this call for Access cannot request access to other NFs (i.e., cannot participate to other 2025 - ROUND 1 calls for Access). If more than one application is submitted, **ALL will be rejected** during administrative review. Applicants who have an application under evaluation are not allowed to submit another application before receiving notification of the results.

Applications must be written in English and must be complete (i.e., consist of all the requested elements and information) and respect all administrative and technical requirements (e.g., proposal or CV format, mandatory declarations, technical requirements of the services, sample availability, sample requirements, including number of samples to be analysed). Incomplete applications or applications that do not meet the requirements will be considered not admissible and will be rejected at the administrative review stage.

5. APPLICATION CONTENT AND FORMAT

The application, to be submitted through the online portal PICA (link) consists of six components:

1. **Applicant's general information**.
2. **Justification for requesting Access to the NF**.
3. **Abstract** to be inserted in the dedicated section on the application portal (Max 1500 characters including spaces).
4. **Project proposal**, to be uploaded in PDF format in the dedicated section on the application portal, shall include the following sections:
    a. *Title*
    b. *Significance.*
    c. *Innovation.*
    d. *Approach, including aims, preliminary data in support of the proposed experiments, experimental design and anticipated results.*
    e. *Environment, including facilities and resources available to support the aspects of the project to be performed elsewhere (i.e., outside the NF).*

    Below, the mandatory format for the proposal:

    Standard application: Max 3 pages (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom) figures included, references excluded. Accepted file formats: PDF. Max size: 30MB - Name the file as APPLICATION ID_PROPOSAL_Surname

    Proof-of-Concept application: Max 2 pages (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom) figures included, references excluded. Accepted file formats: PDF. Max size: 30MB - Name the file as APPLICATION ID_PROPOSAL_Surname

Proposal template is available in Annex II of this call.

Applications that do not meet the format requirements will be considered not admissible and will be rejected at the initial administrative review stage.

5. **Applicant's CV in NIH biosketch format**. The CV, to be uploaded in PDF, shall be drafted in English, using the template available at this link and following the mandatory format: max 4 pages, page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom. For support in drafting the CV, please refer to NIH website: Create Biosketches | NIAID: National Institute of Allergy and Infectious Diseases (nih.gov).

   Applications that do not meet the format requirements will be considered not admissible and will be rejected at the administrative review stage.

6. **Letter of Institutional Endorsement**, addressing the following points:

   a. *Confirmation of the Applicant's role at their Institution, and their eligibility under the category of PI (see section 2.3).*

   b. *Confirmation that relevant authorisations, declarations and accreditation from the competent authority(ies) have been obtained or will be obtained no later than two (2) months after Access approval, in order to process samples and data through the NFs.*

   c. *Justification of the request for Access – including a statement on why the project cannot be performed at the Applicant's Institution.*

   d. *Confirmation that the Applicant has not received funding for performing the submitted project, for the aspects to be performed at the NFs, in their own laboratory, home Institution, or elsewhere.*

   e. *Confirmation of the project's economic and scientific feasibility for the aspects to be performed at the host Institution.*

   f. *Acceptance of NF Access Rules.*

   The Letter of Institutional Endorsement, to be uploaded in PDF or p7m in the dedicated section on the application portal, shall be drafted using the facsimile available as Annex I of this call.

7. **Technical information**, to be filled in in the dedicated section(s) of the application portal, indicatively including:

   a. *Requested service(s), as described in Annex III of this call.*

   b. *Sample technical information.*

   c. *Requested preliminary data for technical feasibility analysis (if applicable).*

   d. *Whether the entire sample set is already available (otherwise indicate the date of availability of the entire sample set).* ***It is mandatory that samples and relevant authorisations are available at the moment of application or no later than two (2) months from receiving Access approval****.*

   e. *Resources and expertise to receive and process the products – data (e.g. Cryo-EM micrographs) or reagents (e.g. human iPSCs) – generated by the NF.*

f. *Research data management plan and bioinformatics support for data analysis, specifying (mandatory when the project output includes research data - e.g., genomics or proteomics data, bioimages from microscopy services, among other):*

   i. *How the bioinformatics analysis of the data generated by the NF will be performed (if such analysis is not provided by the NF for Data Handling and Analysis).*

   ii. *How the data generated by the NF will be handled during and after the end of the project.*

   iii. *Whether and how the data will be shared/ made Open Access.*

   iv. *How data will be curated and preserved, including after the end of the project.*

Details and format of the technical information to be provided are available in the dedicated section of the application portal.

Information provided in sections 1 and 6 are used for the eligibility and admissibility check.

Information provided in section 7 is used for assessing the technical feasibility of the aspects of the project to be performed at the NF.

The entire application is evaluated by the Standing Independent Evaluation Committee (SIEC) to assess its scientific merit.

6. APPLICATION SUBMISSION METHODS, CALL DEADLINE AND EVALUATION PERIODS

Applications shall be submitted exclusively through the application portal PICA managed by CINECA and accessible at this link, according to the indicated terms and methods.

This call for Access (Call ID: 25-SB-ROUND 1) will open on the 15th of February 2025 (13:00 CET) and will close on the 31st of May 2025 (13:00 CET).

A comprehensive list of services, available equipment and the technical requirements for Access as well as terms and conditions are available on the dedicated NFs webpage (link).

The complete list of offered services and technical requirements are available in the Annex III of this call.

Samples as well as relevant authorisation for their use, shall be available at the moment of submitting the application or not later than two (2) months after Access approval. When the project foresees the analysis of more than one batch of samples, the first batch shall be available when the application is submitted or not later than two (2) months after Access approval.

7. EVALUATION OF APPLICATION

The evaluation procedure is conducted by the SIEC that is supported by a Panel of independent external Reviewers (Review Panel) selected by the SIEC on the basis of their scientific expertise.

Each Review Panel is composed of 2 SIEC members, who will act as Chairs, plus 10 appointed external Reviewers, with the relevant expertise.

Below is a scheme describing the evaluation steps and timeline.

There are four application categories that are evaluated and ranked separately:

- Junior PI – Standard application
- Established PI – Standard application
- Junior PI – Proof of Concept application
- Established PI – Proof of Concept application

The NF User Access Office first performs an administrative review of the application to ensure that all the requested components have been provided, and that all eligibility criteria have been met. Incomplete applications or applications that do not meet all the requirements will be considered not admissible and will be rejected at the administrative review stage.

The application is then sent to the Review Panel for assessing scientific merit and technical feasibility.

If the number of applications exceeds by a factor of 4 the estimated capacity of the NF, a triage will be applied within each application category by the relevant Review Panel.

Triage criteria will include:

a. Justification for requesting Access to the NF.

b. Field-Weighted Citation Impact (FWCI).

c. Track record in securing research funding.

The application will remain confidential throughout the entire evaluation process. Reviewers will be asked to declare that they do not have any conflict of interest, and they will be bound by a Confidentiality Agreement.

The application will be individually evaluated by three Reviewers who are part of the relevant Review Panel.

Proposals will be evaluated and ranked based on their average score, within each category.

An on-line meeting of the Review Panel may be requested by the Chairs if deemed necessary (for example to discuss proposals with highly discrepant scores).

At least 50% of the available Access will be allocated to applications from the two Junior PI categories.

7.1 Evaluation criteria

The scientific merit of the project is assessed based on the following criteria:

- **Significance**: Overall scientific merit of the proposed research. If all the experiments proposed are successful, how will the resulting knowledge advance the field?
- **Innovation**: Degree of innovation (conceptual and/ or technological), and ambition of the proposed study compared to the state-of-the-art in the relevant field.
- **Approach**: Appropriateness of proposed methodology, preliminary data in support of proposed experiments, and project feasibility.
- **Environment**: Facilities and resources available to support the aspects of the project to be performed elsewhere (i.e., outside the NF).
- **Justification for requesting Access to the NF**: Explanation on why the service cannot be performed at the host Institution, at a cost which is deemed affordable for the applicant.
- **Applicant**: PI's scientific background and expertise.

7.2 Scoring system

A numeric score between 1 (exceptional) and 9 (poor) is provided for each of the six evaluation criteria. Moreover, an overall project score including a short descriptive comment is provided as feedback to the Applicant.

- **HIGH**:
  - **Score 1 (Outstanding)** – The proposal successfully addresses all relevant aspects of the criterion. There are no weaknesses.
  - **Score 2-3 (Excellent - Very Good)** – The proposal addresses the criterion exceptionally well, aside from a small number of minor weaknesses.
- **MEDIUM**:
  - **Score 4-6 (Very good - Good)** – The proposal addresses the criterion well, but a number of weaknesses are present.
- **LOW**:
  - **Score 7-8 (Fair - Poor)** – The proposal broadly addresses the criterion, but there are significant weaknesses.
  - **Score 9 (Poor)** – The criterion is inadequately addressed, or there are serious inherent weaknesses.

7.3 Technical feasibility analysis

During the evaluation, the relevant experts from SIEC will receive a report from NF staff who will perform a comprehensive analysis of the proposed project's technical feasibility. Technical

feasibility also includes an evaluation of the fulfilment of the technical requirements in terms of capacity to receive and process the research data generated by the NF, as described in the research data management plan. This latter evaluation is performed in consultation with the NF for Data Handling and Analysis.

Based on the technical maturity of the project, the application can be assessed as Feasible/ Not Feasible/ Proof-of-Concept study required.

7.4 Evaluation results and Access approval

NF staff provides the SIEC with information on the resources needed (cost and time) to perform the highest ranked projects. Applications with the highest scientific score that fulfil all technical requirements are approved for Access by the SIEC, based on the capacity of the NF. NF staff schedules Access. A selected number of applications may be placed on a waiting list (in case of cancellations).

Evaluation results – Access granted, Access conditionally granted, Access waitlisted, Access not granted – are communicated to the Applicant through the Access portal.

Applicants whose applications are placed on the waiting list will receive additional information advising whether the project can be Access approved or should be resubmitted within the subsequent application window.

8. AFTER ACCESS HAS BEEN APPROVED

After Access approval, a kick-off meeting is organised and the Applicant is invited to meet NF staff to discuss the experimental design of the project and to finalize the project plan.

Once the project plan has been agreed and the relevant ethical and legal authorisation(s) for the use of the samples has(have) been provided, the NF User Access Office coordinates the signature of the required formal Agreements (e.g., Access Agreement, Collaboration Agreement, other) and the project can commence.

9. AFTER ACCESS HAS BEEN COMPLETED

At the end of the activities carried out at the NF, and not later than 3 months thereafter, if not differently agreed with the NF User Access Office, the Applicant must submit a short report on the results obtained and the impact of the service on their research. Moreover, a final report to be published on the NFs website and describing the impact of the Access to the NF on the research project for which the service has been requested, shall be provided upon publication of the relevant results. Applicants who will not be able to demonstrate the consistency and relevance of the activities carried out at the NF with the research project for which Access was requested will be considered not eligible to participate in the subsequent calls for Access.

Moreover, the Applicant will be asked to fill in a brief, mandatory survey regarding their experience, providing feedback and suggestions for further service improvement.

The Applicant must communicate to the NF User Access Office (via email to national.facilities@fht.org) any publication acknowledging the NF.

Research data obtained during Access shall be made available to the scientific community following the FAIR principles. Applicant must inform the NF User Access Office (via email to national.facilities@fht.org) when and how the data are made public.

10. CONTACTS

Requests for information and/or clarifications concerning the application procedure may be sent to the dedicated e-mail address national.facilities@fht.org, indicating the call ID in the subject line.

11. REFERENCES

NF Access Workflow_Convenzione (link)

NF Access Rules_Convenzione (link)

NF Access Agreement_Convenzione (link)

12. CHANGES TO THE CALL

Any changes or additions to this notice will be communicated through publication on the NFs website (link).

ANNEX I: LETTER OF INSTITUTIONAL ENDORSEMENT TEMPLATE

(Print on paper bearing the official letterhead of the host Institution)

Endorsement letter of the host Institution

To whom it may concern:

I, the undersigned, …….. (*name of legal representative or special attorney*), born in ……. (*city*) on ……..(*date*), as legal representative *(or special attorney, by means of special power of attorney identified by ……..*) and on behalf of ……(*name of the host Institution*), legal residence in *(referred to the host Institution*) ……(*city*), address …….., regarding the project ID (*refer to the ID allocated to the application on the PICA portal*)…….., presented by ……(*Applicants's first name and surname*), as Principal Investigator on the call for Access to Human Technopole National Facilities…..(ID *of the call*),

Declare

- That the host Institution is among those eligible to participate in the call for Access as it belongs to the following eligible category: (select among University, IRCSS, Public Research Entities);
- That the Applicant, Dr ……. (*Applicant's first name and surname*) is an independent group leader (Principal Investigator) affiliated with a primary appointment at the host Institution and that they meet the eligibility criteria as indicated in the call;
- That the Applicant has not received funding for performing elsewhere, the aspects of the project for which they are seeking here support from or Access to Human Technopole National Facilities;
- That the services requested here cannot be performed by the Applicant at the host Institution, at a cost which is deemed affordable for them;
- That relevant authorisations, declarations and accreditation from the competent authority(ies) have been obtained or will be obtained within two (2) months after the approval of the Access in order to process samples and data through Human Technopole;
- That, if applicable, biological specimens have been obtained with the corresponding approval of the Bioethics Committee and appropriately signed 'informed consent', both for their collection and their use, including conservation, manipulation, derivation and processing to be carried out by Human Technopole National Facilities;
- That, if samples were obtained from subjects who signed an 'informed consent', said informed consent allows that sequencing data and results are included in secure controlled Access databases and accessed/ used by authorised third parties;

and is committed

- To accept the terms and conditions to Access Human Technopole National Facilities as described in the National Facilities Access rules (link);
- To sign the Access Agreement should the project be approved (link)

For the host Institution (Applicant legal entity/beneficiary):

Date ……..

Name and Title ……

Email and Signature of legal representative or delegated person …….

ANNEX II: PROJECT PROPOSAL TEMPLATE

Mandatory proposal format

Standard application*: Max 3 pages (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom) figures included, references excluded. Accepted file formats: PDF. Max size: 30MB - Name the file as APPLICATION ID_PROPOSAL_Surname*

Proof-of-Concept application*: Max 2 pages (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom) figures included, references excluded. Accepted file formats: PDF. Max size: 30MB - Name the file as APPLICATION ID_PROPOSAL_Surname*

PLEASE REMOVE THE INFORMATION ABOVE BEFORE SUBMITTING

Proposal content:

1. TITLE

2. SIGNIFICANCE

3. INNOVATION

4. APPROACH

5. ENVIRONMENT

6. REFERENCES (Optional)

ANNEX III: SERVICE LIST

HUMAN TECHNOPOLE

NATIONAL FACILITY FOR STRUCTURAL BIOLOGY

CALL FOR ACCESS

25-SB-ROUND1

SERVICE LIST

Table of contents

1. INTRODUCTION

The NF for Structural Biology stands as a one-of-a-kind scientific and technological hub for integrative structural biology. NF for Structural Biology consists of six Infrastructural Units (IU) working together to (i) provide and support conventional practices, and (ii) promote and establish innovative workflows in the field of integrative structural biology. The Cryo-Electron Microscopy Unit (IU1) aims at identifying, visualizing, and characterizing biological players of interest, both isolated and within their cellular compartments. The Biomass Production Unit (IU2) provides Access to different cell lines for protein expression and performs scale-up of bioprocesses for large-scale productions. The Biophysics Unit (IU3) is a technological platform for biophysical characterization of macromolecules and their interactions. The Structural Proteomics Unit (IU4) relies on crosslinking mass spectrometry (XL-MS) to provide topological and structural restraints on protein-protein interactions in samples ranging from purified protein complexes to cellular fractions. The Dynamic Single-molecule Unit (IU5) provides tool to visualise biological processes in real-time with single-molecule sensitivity thanks to cutting-edge instruments that combine optical tweezers with fluorescence and label-free detection modules. Finally, the Technology Development Unit (IU6), which is expected to become operational in 2026-2028, will serve the purpose of fostering technological innovation in the field of integrative structural biology.

The strategic mission of the NF for Structural Biology is to expand and share its current expertise and technology with the entire Italian research community. The great majority of the instrumentation present in the NF is not available elsewhere in Italy, making it an attractive venue for all domestic researchers, from commercial and academic entities alike. In particular, the Cryo-EM Unit (IU1) will grant Access to high-end microscopes capable of determining structures of macromolecules to high resolution and providing detailed information on their cellular context. Notably, the Cryo-EM Unit is, as of now, the best if not the only laboratory in the country equipped to perform cryo-CLEM and cryo-ET as part of the workflow. The resources offered by the Biomass Production Unit (IU2), such as bioreactors and fermenters, are complemented with expertise in scaling up bioprocesses for large-scale protein production across several cell types, ranging from bacteria to mammalian cells. The Italian research community will also benefit from Access to a library of different cell lines and the necessary knowhow for their maintenance in culture. Furthermore, within the Biophysics Unit (IU3), investigators will in turn be able to combine different techniques to thoroughly and efficiently characterize their sample from a biophysical and biochemical perspective, resulting in an optimized if not ideal specimen for subsequent structural biology experiments. Finally, the Structural Proteomics Unit (IU4) will provide topological information at the single amino acid level, so as to be able to identify and characterize proximal and direct interactors *in vitro* and *in situ*. To the best of our knowledge, IU4 is unique in Europe offering XL-MS technology as a service. Similarly, the instrumentation within the Dynamic Single Molecule Unit (IU5) is, within the Italian landscape, the only one which will be Accessible to external Users.

2. SERVICE LIST

(IU1) Cryo-Electron Microscopy Unit

SID: SB-IU1-A – Negative Stain EM Screening

Services description: We offer sample preparation by negative stain EM followed by TEM imaging at 120 kV. A maximum of 4 service units (i.e. 4 days of screening and sample preparation on 1 specimen or 4 different ones) can be requested. For each session a maximum of 8 grids can be prepared and processed. Imaging will be provided for a maximum of 8 continuous hours per unit of service. An optional "polishing" size-exclusion chromatography (SEC) step can be performed on thawed material by the Biophysics Unit, as and if considered necessary by NF Staff. 400 mesh copper grids with amorphous carbon film layer will be used as support. Glow discharging will be performed by a Pelco EasyGlow device. Staining will be performed with a 2% (w/v) uranyl acetate aqueous solution. Imaging in TEM mode at 120 kV will be performed on a Thermo Scientific Talos L120C equipped with CETA 16M camera. Imaging conditions requested by the User, if provided, must be compatible with NF practices. This service will only be performed by NF staff.

Technical requirements: Biological specimens must have a total molecular weight greater than 150 kDa; glycerol, sucrose, phosphate (i.e., PBS) or detergents must not be present in the sample; concentration of purified biological sample must be higher than 0.01 mg/ ml and the sample volume must be greater than 50 µl; the User should provide 10 ml of final sample buffer to allow preparation of dilutions; the sample is required to have demonstrated purity (i.e., SDS-PAGE) and homogeneity (e.g., elution profile from SEC).

Information to be provided in the technical requirements table for each sample:

- Sample name
- Uniprot ID(s)
- MW (kDa), total in case of complex
- Buffer Composition
- Sample Concentration (mg/ml)
- Final Volume of purified sample (µl)
- If a representative SDS PAGE is included in application
- If a representative SEC chromatogram is included in application
- Additional info

Access modality available: Access to NF services.

SID: SB-IU1-B – Cryo-EM Screening

Services description: This service provides sample preparation by plunge-freezing, grid clipping for autoloader system and cryo-TEM imaging at 200 kV. User can either provide the purified sample in solution, the sample already vitrified on EM grid, or the vitrified sample on an already clipped (i.e. Thermo Scientific autoloader compatible) EM grid. User might request cryo-EM screening on a maximum of 4 different samples per application. For each sample, a maximum of 3 Cryo-EM Screening sessions can be allocated. For each Cryo-EM screening session a maximum of 8 grids can be prepared and processed. An optional "polishing" SEC

step can be performed on thawed material by the Biophysics Unit, as and if considered necessary by NF Staff. Glow discharging will be performed by either a Pelco EasyGlow, a Quorum GloQube, or Solarus II plasma cleaner device. Plunge-freezing will be performed on a Thermo Scientific Vitrobot Mk IV or a Leica EM GP2. Applicants may provide TEM grid supports of preference, otherwise NF staff will use holey TEM grid supports available. Imaging will be provided for a maximum of 8 continuous hours per unit of service. In case of grids showing optimal particle distribution and ice quality, this service can be extended for overnight data collection, if compatible with NF staff working hours. Cryo-TEM imaging will be performed only in EF-TEM mode at 200 kV on a Thermo Scientific Glacios equipped with a Falcon 4i direct electron detector and Selectris X energy filter. Imaging conditions requested by the User, if provided, must be compatible with NF practices. In case of User-provided already-clipped grids, these will be inspected by NF staff prior to acceptance. This service will only be performed by NF staff. This service does not cover for buffer screening (i.e. detergents, pH, salt, additives screening or else) or any other grid modifications (i.e. graphene oxide, pegylation, affinity grids or else).

Technical requirements: Biological specimens must have a total molecular weight greater than 150 kDa; glycerol, sucrose, or detergents must not be present in the sample; concentration of purified biological sample must be equal or higher than 0.35 mg/ml and the sample volume must be greater than 50 µl; the User should provide 10 ml of final sample buffer to allow preparation of dilutions; the sample is required to have demonstrated purity (i.e., SDS-PAGE) and homogeneity (e.g., elution profile from SEC). For a specimen to be imaged by cryo-ET, the area of interest must have a maximum thickness of 200 nm; Grids will be inspected by NF staff prior to acceptance: damaged or bent grids will not be loaded on the microscope.

Information to be provided in the technical requirements table for each sample:

- Sample name
- Sample provided as [Purified Sample \ Vitrified Grid \ Vitrified and Clipped Grid]
- Sample type [Protein/ Cell/ Other]
- Uniprot ID(s)
- MW (kDa), total in case of complex
- Buffer Composition
- Concentration (mg/ml)
- Volume of purified sample (µl)
- Representative SDS PAGE included in application
- Representative SEC Chromatogram included in application
- Additional Info

Access modality available: Access to NF services.

SID: SB-IU1-C – High-resolution Cryo-TEM Imaging

Services description: This service provides high-resolution cryo-TEM data collection of vitrified specimens. To ensure efficient usage of high-end microscope time this service is exclusively dedicated to EM grid ready for data collection at the time of application (i.e. user cannot request this service together with service SB-IU1-B – Cryo-EM Screening). A maximum of 2 samples for data collection can be requested per application. For each sample a maximum of 48hr of microscope time can be requested per application. According to instrument availability and experimental needs, data collection will be carried out either on 200kV or 300kV microscope systems. The User can provide cryo-TEM grids either unmounted or mounted on a Thermo Scientific cartridge. In case of an unmounted grid, clipping of the specimen in Thermo Scientific cartridges will be performed by NF staff. In case of User-provided already-clipped grids, these will be inspected by NF staff prior to acceptance. Imaging at 200 kV will be performed on a Thermo Scientific Glacios while imaging at 300 kV will be performed on a Thermo Scientific Titan Krios G4, both equipped with a Falcon 4i direct electron detector and a Selectris X energy filter. Imaging conditions (i.e., dose, pixel size, magnification, etc.), if requested by the User, must be compatible with the NF best practices. Microscope time includes all steps from clipping to loading and TEM alignments and according to NF staff availability. For single-particle acquisition, User might opt for beam-image shift assisted data collection (~ 450 – 600 movies/hour) or stage movement for each hole (~ 100 – 250 movies/hour). This service will only be performed by NF staff.

Technical requirements: Only for grids ready for data collection at time of application, cannot be requested together with Cryo-EM Screening service; Preliminary data must be provided, including representative micrographs and, if available, any 2D and 3D class averages or reconstructions, respectively; Not for screening purposes; Only for biological specimens with a total molecular weight greater than 150 kDa; glycerol, sucrose or detergents must not be included in the sample buffer unless specifically required for vitrification and/ or particle distribution purposes; For specimens to be imaged by cryo-ET, the area of interest must have a maximum thickness of 200 nm; Grids will be inspected by NF staff prior to acceptance: damaged or bent grids will not be loaded on the microscope.

Information to be provided in the technical requirements table for each sample:

- Experiment Type [Single-Particle Analysis/ Cryo-Electron Tomography]
- Sample name
- Sample provided as [Vitrified Grid \ Vitrified and Clipped Grid]
- Sample type [Protein/ Cell/ Other]
- Uniprot ID(s)
- MW (kDa), total in case of a complex
- Buffer Composition
- Concentration (mg/ml)
- Representative micrographs included in application
- and 3D preliminary data, if available, included in application
- Days of data collection requested (max 2)
- Additional Info

Access modality available: Access to NF services.

Analysis of generated data (i.e. model building) can be provided as a combined service by the National Facility for Data Handling and Analysis. Please select SID: NF61.02.01 - Cryo-electron microscopy analysis. For more details please refer to Appendix 1.

SID: SB-IU1-D – Volume Electron Microscopy

Services description: This service is designed to cover imaging on large volumes and includes sample preparation by either high-pressure freezing or chemical fixation, freeze substitution, resin embedding, section preparation by ultramicrotomy and S/TEM imaging at 300kV. The User can provide specimen at any stage, so being already fixed, stained, embedded as well as already sectioned and made ready for EM acquisition. A maximum of 2 specimens can be processed per unit of service, and 4 samples maximum (i.e., replicates) can be prepared per specimen. High-pressure freezing will be performed on a Leica EM ICE. Freeze substitution will be performed on a Leica AFS2, and a User might specify a substitution protocol of preference, which will be utilized if compatible with NF practices. Similarly, for chemical fixation, the User is allowed to specify their protocol of preference, which will be adopted if compatible with NF practices. For resin-embedding, the User could provide a resin of preference, or alternatively, the currently available resin be used. Sections of resin-embedded sample will be prepared on Leica UC7. A maximum of 1 resin-embedded sample can be processed for unit of service, to be sectioned in ribbons, and to be applied to a maximum of 4 TEM support grids. User might specify thickness of final sections, and these recommendations will be followed if compatible with the NF practices. User might provide TEM grid support of preference, otherwise the currently available grid support will be used. Imaging will be performed an a Thermo Scientific Spectra 300 kV either in TEM, equipped with CETA 2 camera, or STEM mode in BF/DF/HAADF. Imaging conditions, if requested by the User, must be compatible with NF practices. Microscope Access is granted for a maximum of 5 days per unit of service, including all steps from sample loading to alignments and collection, and according to NF staff availability. All steps of this service will only be performed by NF staff. To maximise efficient use and Access to high-end TEM instruments, priority will be given to applicants specifically requesting parts of this service (e.g. only sectioning, or only S/TEM imaging of already prepared sections).

Technical requirements: Only for biological specimen with total thickness smaller than 150 µm; Only BSL-1 or BSL-2; User must provide 50 ml of final sample buffer to allow preparation of dilutions and for sample handling; for EM imaging, final sections must have maximum thickness of 250 nm for TEM and 500 nm for STEM.

Information to be provided in the technical requirements table for each sample:

- Sample(s) Description
- Sample provided as [Unfixed \ Chemically Fixed \ Fixed and Sectioned]
- Sample preparation details (if already fixed, embedded, sectioned)
- Further information useful for analysing the technical feasibility of the submitted request

Access modality available: Access to NF services.

Analysis of generated data (i.e. image segmentation, denoising) can be provided as a combined service by the National Facility for Data Handling and Analysis. Please select SID: NF61.03.01 - Volume electron microscopy analysis. For more details please refer to Appendix 1.

SID: SB-IU1-E – Cryo-FM Imaging

Services description: This service provides cryo-Fluorescence Microscopy imaging including sample preparation by plunge-freezing and grids clipping. A maximum of 2 specimens can be processed per unit of service, maximum 4 grids (i.e. replicates) can be prepared per specimen. Glow discharging will be performed by either a Pelco EasyGlow, a Quorum GloQube, or Solarus II plasma cleaner device. Plunge-freezing will be performed on a Thermo Scientific Vitrobot Mk IV or a Leica EM GP2. Applicants may provide TEM grid supports of preference, otherwise the NF staff will use the available TEM grid supports. Widefield imaging will be performed on a Leica Thunder cryo-CLEM system. Confocal imaging will be performed on a Leica Stellaris 5 cryo-CLEM system equipped with white light laser. Both microscopes are equipped with a 50x / 0.9 NA lens. This service is provided for a maximum of 8 continuous hours per unit of service, including plunging, clipping, and imaging. In case of User-provided already-clipped grids (in Thermo Scientific cartridges), these will be inspected by NF staff prior to acceptance. This service will only be performed by NF staff.

Technical requirements: Only for biological specimens with thickness smaller than 15 µm; Only for cells in suspension, and in case of adherent cells, User must provide already plunge-frozen grids; Only BSL-1 or BSL-2; User must provide 50 ml of final sample buffer to allow preparation of dilutions and for sample handling.

Information to be provided in the technical requirements page of the application:

- Sample Description
- Sample provided as [To be vitrified \ Already Vitrified]
- Sample preparation details
- Further information useful for analysing the technical feasibility of the submitted request

Access modality available: Access to NF services.

(IU2) Biomass Production Unit

SID: SB-IU2-A – Protein Expression In Insect Cells

Services description: The service aims to cover the entire process of Baculovirus protein expression, starting from a Bacmid. Initially, commercial Sf9 cells are transfected with the Bacmid, supplied by the applicant, which contains the gene of interest and a fluorescence marker. The initial stage results in a low-titre Baculovirus suspension (P1 generation), followed by a second amplification (P2 generation) to produce a high-titre suspension. The P2 Baculovirus suspension could be directly used for protein expression or another round of Baculovirus amplification is performed before infecting commercial High Five insect cells (at 0.5x10^6 cells/ml). The virus amount used depends on the construct and might need

optimization in initial small-scale infections. Protein expression is monitored indirectly by checking for the fluorescence marker (carried by the Bacmid) and cell viability. Finally, cells are harvested via centrifugation, and the pellet or supernatant is made available.

Technical requirements: 1. Sterile, endotoxin-free Bacmid, free from phenol and sodium chloride containing the gene/s of interest. Please note that 20 µl of purified bacmid per P1 generation requested (indicative DNA concentration of 5 µg/µl) is typically sufficient for 5 to 8 litres of protein expression; 2. Bacmid should have a fluorescence reporter gene (e.g. GFP, YFP) to monitor its expression; 3. Preliminary test or feasibility information supporting the production of recombinant Baculovirus and protein expression of interest.

Information to be provided in the technical requirements page of the application:

- Required volume of the desired protein expression (consider that commercial High Five insect cells are infected at 0.5x10^6 cells/ml)
- Preliminary tests or feasibility information from literature supporting the Baculovirus production of the protein of interest

Access modality available: Access to NF services, Access with Training.

SID: SB-IU2-B – Protein Expression In Mammalian Cells In Suspension

Services description: The service offers dedicated pipelines for small- and large-scale cell cultivation and protein expressions:

- Small scale: utilizing commercial Expi293F cells, transient transfection is conducted with a plasmid containing the gene/s of interest (potentially with a fluorescent marker), provided by the applicant. Cell cultivation and transfection take place in Erlenmeyer flasks within dedicated shaker incubators; typically, 500 ml of cell culture per 2-liter flask is utilized up to a total cultivation volume of approximatively 10 litres. Transfection of an Expi293F cell culture is performed at approximately 2 to 3 x10^6 cells/ml, following a modified protocol with polyethylenimine (PEI). While no expression test will be performed, the efficiency of transfection can be readily assessed if the plasmid includes a fluorescent marker. Transfected cells are subsequently harvested by centrifugation, and either the frozen pellet or the refrigerated supernatant will be provided.
- Large scale: bioreactor cultivation and protein expression are performed either with commercial cell lines (e.g., Expi293F cells) transiently transfected with applicant-provided plasmids, or with any mammalian cell line adapted to suspension cultivation supplied by the applicant. Preliminary tests are normally performed using a 0.5 litres scale bioreactor to define and optimize bioreactor cultivation conditions. Subsequently, a 10 litres scale bioreactor enables large-scale cell cultivation and protein expression, employing both transient transfection protocols and stable cell line expressions. Moreover, the implementation of perfusion, utilizing an acoustic retention device, may be explored to increase the biomass production and, consequently, protein expression. Similar to small-scale operations, no expression test will be performed. Instead, monitoring of transfection efficiency for transient expression can be undertaken if the plasmid carries a fluorescent marker or fluorescently tagged protein of interest.

Technical requirements:

For transient protein expression:

1. Sterile, endotoxin-free plasmid DNA, free from phenol and sodium chloride containing the gene/s of interest.
2. Plasmid DNA should have the following specifications: Amount of 1.1 mg for each litre of cell expression culture (consider an approx. cell concentration of 2 to 3 10^6 cells/ml at the transfection); The DNA concentration should be greater than 1 µg/µl with a ratio Abs260 nm/Abs280 nm between 1.7 and 2.1.
3. The plasmid should contain a fluorescence reporter gene (e.g. GFP, YFP) if monitoring of transfection efficiency is required.
4. Preliminary test or feasibility information supporting the successful transfection using a mammalian cell line (including adherent cells) and proof of actual protein expression should be provided.

For stable cell line expression:

1. Cell line (BSL-1 or BSL-2), mycoplasma free, adapted for suspension cultures
2. Culture conditions should be provided.
3. Successful trials of suspension cultures conducted in flasks with volumes exceeding 100 mL, validated by confirmed protein expression.

Information to be provided in the technical requirements page of the application:

- Required volume of the desired protein expression experiment
- Preliminary tests or feasibility information from literature supporting the expression in mammalian cells of the protein of interest (for both transient and stable cell line expression)
- For request involving bioreactors, any pre-existing protocol that should be followed
- Localization of protein of interest (intracellular, membrane-embedded, or extracellular)

Access modality available: Access to NF services, Access with Training.

SID: SB-IU2-C – Protein Expression In Yeast and Bacteria Cells

Services description: The service offers dedicated pipelines for small- and large-scale cell cultures and expressions:

- Small scale: 15 litres fermenter useful for initial trial test before moving to larger scale fermenter or small expression using both bacteria and yeast cells. Typical culturing strategies are available such as batch and fed batch (up to 2 feeds). Max working volume of 10 litres.
- Large scale: 150 litres fermenter dedicated to large cultivation and protein production. Typical cultivation strategies are available, including batch and fed batch (up to 2 feeds). Max working volume of 100 litres.

In both cases, cells will be collected and separated from the supernatant using dedicated superspeed centrifuges or a High-Speed Tubular Centrifuge. Upon request, the cells can be lysed using a continuous flow cell disruptor or by a high-capacity cryogenic grinder.

Technical requirements: 1. Bacterial or yeast strains (BSL-1 or BSL-2); 2. growth/ cultivation conditions available; 3. expression protocols available.

Information to be provided in the technical requirements page of the application:

- Required volume of the production
- Growth conditions
- Preliminary tests or feasibility information from literature supporting the production requested
- Any pre-existing protocol that should be followed
- Localization of the product of interest (intracellular or extracellular)

Access modality available: Access to NF services, Access with Training.

(IU3) Biophysics Unit

SID: SB-IU3-A – Characterisation of Macromolecular Samples

Services description: Structural biology and biochemical assays often require sample optimization to achieve stability and homogeneity, while understanding the oligomerisation state and precise composition of macromolecular complexes is crucial for unravelling their architecture. To address these needs, the Biophysics Unit offers a service leveraging various instruments to determine the molecular weights of species in solution and their thermal stability parameters. Techniques utilized include mass photometry, SEC-MALS, dynamic light scattering, and nanoDSF.

Technical requirements: Technical requirements vary based on the number of techniques employed, ranging from as little as 20 µl to several hundred µl of sample, at roughly 10-50 µM concentration. More concentrated and abundant samples facilitate comprehensive characterisation through repeated experiments, typically necessary for in depth analysis. Initially, SEC elution profiles of the sample and the corresponding SDS-PAGE gel must be provided.

Information to be provided in the technical requirements page of the application:

- Uniprot ID(s)
- MW (kDa) total in case of complex
- Ligands
- Buffer Composition
- Concentration (µM)
- SDS PAGE
- SEC chromatogram
- Volume of purified sample (µL)

Access modality available: Simple Access, Access to NF services, Access with Training.

SID: SB-IU3-B – Measurement of Affinity Constants

Services description: The Biophysics Unit is equipped with instruments designed to determine the strength and biophysical properties of macromolecular or protein-ligand interactions, including association and dissociation constants, as well as stoichiometry of binding. Available techniques include isothermal titration calorimetry, microscale thermophoresis, and bio-layer interferometry (which can also be utilized for quantifying known components in complex mixtures).

Technical requirements: Technical requirements will depend on the chosen technique, but to ensure complete binding saturation in the assay at least one of the two components should be at a concentration approximately 10-20 times greater than the dissociation constant (Kd). Selection of the technique, and consequently sample requirements, will be influenced by factors such as strength of association, availability of the sample, its homogeneity, and overall biophysical properties of the system under investigation.

Information to be provided in the application:

- Uniprot ID(s)
- MW (kDa) of interacting species
- Rough estimate of Kd
- Buffer Composition
- Available concentrations of samples (µM)
- SDS PAGE (if available)
- SEC chromatogram
- Volume of purified sample (µL)

Access modality available: Simple Access, Access to NF services, Access with Training.

SID: SB-IU3-C – Sample Check/Optimisation for Structural Biology Workflows

Services description: Many workflows available in other Infrastructural Units of the National Facility for Structural Biology may require a rapid sample quality control verification (if not performed by the users) or a step of sample optimisation, for instance via size-exclusion chromatography just prior to experiments. These services, which differ from the full characterisation of the macromolecular sample because of their ancillary role with respect to other IU workflows, may only be requested in combination with such workflows.

Technical requirements

Information to be provided in the technical requirements page of the application The minimal requirements and information to be provided for these services will be those dictated by the other IU workflows they are intended to support.

Access modality available: Access to NF services, Access with Training.

SID: SB-IU3-D – Protein Purification

Services description: We offer access to expert support in isolating and purifying proteins from a variety of biological sources. We offer customisable workflows, including cell lysis via sonication or homogenization, depending on any given sample's specific requirements. The service can accommodate both purification from standard small and medium-volume lysates, or isolation from large-volume sample preparations, ensuring flexibility for different experimental needs. Our purification techniques include affinity, ion exchange, and size-exclusion chromatography, delivering (when possible) high-quality proteins suitable for downstream applications such as structural analysis or functional assays.

Technical requirements: The minimal requirement for the purification of proteins is the biological source from which the proteins will be purified (which could be generated by the IU2 - Biomass Production Unit). It is also essential to provide the complete polypeptide sequence of the target protein. Additionally, any further details provided regarding purification strategies, existing protocols, protein stability, buffer preferences, or functional information would be greatly beneficial to optimise the purification process.

Information to be provided in the technical requirements page of the application:

- Uniprot ID(s)
- Exact sequence of the target protein construct, comprising tags
- Information on purification protocols, if available
- SDS PAGE of previous purification attempts (if available)

Access modality available: Access to NF services, Access with Training.

(IU4) Structural Proteomics Unit

SID: SB-IU4-A – Crosslinking MS Acquisition of Purified Protein Complex without Crosslinking Reaction Optimisation

Services description: The Structural Proteomics unit will perform crosslinking MS on a purified protein complex to identify protein-protein interactions and map residue distances. This service does not include preliminary crosslinking reaction optimisation, which should be performed by the User at their own institution (in consultation with NF staff). This service will only be performed by NF staff.

Technical requirements: The User must provide at least 80 µg of a purified protein complex (recombinant or and/ or isolated to high purity from endogenous sources), with amounts estimated by Bradford, BCA or by absorbance if the A260/A280 ratio is not suggestive of nucleic acid contamination. The sample must be in a buffer free of primary amines (e.g., Tris should be avoided). For complexes of less than 4 proteins and a mass of under 200 kDa, 40 µg are sufficient. Ideally, the sample should be crosslinked in the user's home laboratory and prepared upon consultation with our NF and shipped as a protein precipitate, gel bands or frozen, as indicated by NF staff on a per project basis.

Information to be provided in the application:

- Uniprot ID(s)

- Buffer composition
- Amount available for single experiment (in µg)
- Can the experiment be repeated, if needed?
- SDS PAGE (if available)

Access modality available: Access to NF services, Access with Training.

SID: SB-IU4-B – Crosslinking MS Acquisition of Purified Protein Complex with Crosslinking Reaction Optimisation

Services description: The Structural Proteomics unit will perform crosslinking MS on a purified protein complex to identify protein-protein interactions and residue distances. This service includes preliminary crosslinking reaction optimisation and proteomics acquisitions. These can be performed by our NF staff provided the protein sample, or by a visiting User with our assistance.

Technical requirements: The User must be able to produce at least 2 preps of 80 µg of a purified protein complex (recombinant or and/ or reconstituted from endogenous sources), with amounts estimated by Bradford, BCA or by absorbance if A260/A280 ratio are suggestive of no nucleic acid contamination. The sample must be in a buffer free of primary amines (e.g., Tris should be avoided). For complexes of less than 4 proteins and a mass of under 200 kDa, 2 preps of 40 µg are sufficient. Ideally, if stability is not a concern, the sample should be prepared fresh and shipped on ice, or on dry ice if the sample can withstand freeze/ thawing. In case of freezing, please notify us of the glycerol (or other cryoprotectant) concentration (if present) and provide us with buffer to exchange this out.

Information to be provided in the technical requirements page of the application:

- Uniprot ID(s)
- Buffer Composition
- Amount available for experiment (in µg)
- Can the experiment be repeated, if needed?
- SDS PAGE (if available)

Access modalities available: Access to NF services, Access with Training.

SID: SB-IU4-C – Crosslinking MS Acquisition of Immuno-precipitate From Tagged Overexpressed Bait

Services description: The Structural Proteomics unit will perform crosslinking MS to characterize the interactome of a particular target protein *in situ*. Crosslinking reactions can be performed on cells, in lysate or after competitive or otherwise non-denaturing (native) elution. As this experiment requires a careful and at times tricky design, preliminary results will be shared with the User and guidance provided to set or optimize the experimental conditions. This service will be performed by NF staff if the crosslinking reaction occurs at the home institution with guidance from us, or by a visiting User under our direct supervision if the entire workflow is requested.

Technical requirements: The User must have a working immunoprecipitation of their target protein with a visible band on a western blot and a known yield in the eluate (excluding antibody amount). Overexpressed protein with affinity enrichment followed by elution by tag cleavage or competition is recommended. For example, a protein overexpressed with FLAG tag, eluted with FLAG peptide. The peptide is then removed by dialysis and protein concentration is estimated by BCA or Bradford. The goal is to have a setup that can achieve over 100 µg of the eluted protein excluding the antibody or the competition peptide.

Information to be provided in the technical requirements page of the application:

- Cell type utilized and species
- Uniprot ID of bait
- Yield of IP and number of cells/amount of biomass utilized for the experiment
- Western blot (if available)
- SDS PAGE stained with Coomassie (if available)

Access modality available: Access to NF services, Access with Training.

SID: SB-IU4-D – Crosslinking MS Acquisition of Immuno-precipitate From Endogenous Material Or of Cellular Fraction

Services description: The Structural Proteomics unit will perform crosslinking MS to characterize the topology of the protein-protein interactome of a particular target protein or enriched cellular fraction *in situ*. Examples include: interactome of pulldowns with an endogenously tagged protein of interest; interactome of vesicles/ cellular compartments; interactome of bacterial cells or virus/ host interactions after pulldown of specific virulence factors. Crosslinking reactions can be performed on cells, on lysates or after competitive or otherwise non-denaturing (native) elution. As these experiments require careful design, the User will be granted Access to preliminary acquisitions to characterise the sample and set the correct reaction conditions, or guidance to perform the optimization at their home institution. This service will be performed by NF staff in collaboration with a visiting User.

Technical requirements: The User must have a working immunoprecipitation or enrichment of their target protein with a visible band on a western blot and on an SDS-page Coomassie-stained gel, or a biochemical or other type of enrichment of a cellular fraction of interest. The User must also perform their experiments in a system that can be scaled up in terms of biomass: as an example, hundreds of millions of cells are commonly needed to enrich for the protein and peptides of interest when these experiments are performed in HEK293T cells. The amount of material required will depend on the natural abundance of the protein or fraction of interest. The Biomass unit may be used to produce the required amounts. The goal is to have a setup that can yield over 150 µg of the fraction of interest.

Information to be provided in the technical requirements page of the application:

- Cell used and species
- Uniprot ID of bait if IP was performed
- Yield of IP in µg and number of cells/amount of biomass used to obtain it. Alternatively, typical amount of cellular fraction material available in micrograms of protein.
- Western blot (if available)

- SDS PAGE stained with Coomassie (if available)
- Availability of the User to visit the NF in person

Access modality available: Access to NF services, Access with Training.

SID: SB-IU4-E – Integrative Modelling with Crosslinking MS and Cryo-EM Data

Services description: The Structural Proteomics unit will perform integrative structural modelling combining medium/ low resolution (from 7 to 30 Å) cryo-EM densities and crosslinking MS data acquired at our NF. In case of very large systems, negative stain data may also be used. This does not refer to model building in cryo-EM densities, but to calculations of localization of subunits or of areas not observed in EM maps. This task can be performed on data from services SB-IU4-A or SB-IU4-B only. Example software: DisVis, integrative modelling platform (IMP), AlphaLink/ AlphaLink2. This service will be performed by NF staff, but training will be available if requested, provided basic bioinformatics skills of the User (bash terminal, python).

Technical requirements: The User must already have the density map and have or be planning to obtain a crosslinking MS dataset for their protein complex in this evaluation round. The data must be on a recombinant or purified system. For data acquired at our NF, only services SB-IU4-A or SB-IU4-B can be integrated with this service.

Information to be provided in the technical requirements page of the application:

- Uniprot ID(s)
- Resolution of available EM data
- Type of available EM data (single particle cryo, cryo-ET, negative stain)
- List any other EM data in the literature
- List any crosslinking MS data if available or in literature, if possible specifying number of self and heteromeric crosslinks

Access modality available: Access to NF service, Access with Training.

SID: SB-IU4-F – Proteomics acquisition on Orbitrap Astral – high load

Services description: This service comprises 8 hours of equipment time on the Orbitrap Astral LC-MS. The configuration of the system is: Vanquish NEO HPLC in trap and elute configuration. Mobile phase A is 0.1% formic acid in water, mobile phase B is 80% acetonitrile in water with 0.1% formic acid. The MS is set up with an EasySpray source. The MS is operated for high- load, using EasySpray pepMap NEO columns (50cm, 75µm diameter, 10nm pore size). It is possible to use the FAIMS filter to improve sensitivity or fractionate samples online. Overhead on measurements due to sample loading and column washing is about 10 minutes per injection. Typical gradient length 30 minutes (no gains are typically observed beyond this gradient length due to the speed of the Astral analyser). Both DIA and DDA proteomics workflows are available. Each proposal can request up to 9 sessions for a total of 3 days of MS time. The service does not comprise sample preparation from proteins to peptides.

Technical requirements: Dried peptide samples purified from in solution or in gel digestion via C18 desalting and quantified. Minimum 50ng of material per injection. Absence of PEG or other contaminants (e.g., lipids, surfactants, dyes). Presence of large amounts (>5e8 in TIC intensity) of contaminants will cause the measurement session to be terminated early. This setup is not suitable for low load or single cell applications. The configuration of the instrument cannot be modified.

Information to be provided in the technical requirements page of the application:

- Species of origin
- Sample preparation protocol including protease used and any cleanup/fractionation step
- Number of total injections
- ng of peptides per sample
- Are preliminary MS data available? If yes, include in proposal text.
- Is there already a suggested gradient/method?

Access modality available: Access to NF services.

SID: SB-IU4-G – Proteomics acquisition on Orbitrap Astral – high throughput

Services description: This service comprises 8 hours of equipment time on the Orbitrap Astral LC-MS. The configuration of the system is: Vanquish NEO HPLC in trap and elute configuration. Mobile phase A is 0.1% formic acid in water, mobile phase B is 80% acetonitrile in water with 0.1% formic acid. The MS is set up with an EasySpray source. The MS is operated for high- throughput, with EasySpray pepMap columns (15cm, 150µm diameter, 10nm pore size). It is possible to use the FAIMS filter to improve sensitivity or fractionate samples online. Overhead on measurements due to sample loading and column washing is about 2 minutes per injection. Typical gradient length 15 minutes (no gains are typically observed beyond this gradient length due to the speed of the Astral analyser and the operation in high flow). This configuration allows for up to 100 samples per day. Both DIA and DDA proteomics workflows are available. The service does not comprise sample preparation from proteins to peptides.

Technical requirements: dried peptide samples purified from in solution or in gel digestion via C18 desalting and quantified. Minimum 100ng of material per injection. Absence of PEG or other contaminants (e.g., lipids, surfactants, dyes). Presence of large amounts (>5e8 in TIC intensity) of contaminants will cause the measurement session to be terminated early. This setup is not suitable for low load or single cell applications. The configuration of the instrument cannot be modified. Each proposal can request up to 6 sessions for a total of 3 days of MS time.

Information to be provided in the technical requirements page of the application:

- Species of origin
- Sample preparation protocol including protease used and any cleanup/fractionation step
- Number of total injections
- ng of peptides per sample
- Are preliminary MS data available? If yes, include in proposal text.

Access modality available: Access to NF services.

(IU5) Dynamic Single-Molecule Unit

SID: SB-IU5-A – Assay Development

Services description:

This service enables researchers to explore the potential of single-molecule techniques and develop new protocols and workflows. Prior experience with dynamic single-molecule (DSM) approaches is not required.

The unit is equipped with two state-of-the-art instruments:

1. C-Trap 'Dymo' utilizes confocal scanning and is primarily used for imaging in solution.

2. C-Trap 'Edge' offers the flexibility to switch between wide-field fluorescence and TIRF imaging. Additionally, it allows label-free imaging via IRM, making it ideal for studies close to the surface.

The unit provides support for a wide range of experimental approaches.

Examples of experiments performed in solution:

1. KYMOGRAPHS:
    - Characterization of protein binding to DNA (or possibly RNA)
    - Observation of protein diffusion or directed motion along DNA
    - Analysis of protein-protein interactions upon DNA binding
    - Identification of optimal DNA substrates for protein binding (e.g., ssDNA vs. dsDNA, specific sequences, or structural motifs)
2. FORCE SPECTROSCOPY:
    - Detecting structural changes in DNA upon protein binding (e.g., melting, looping, compaction)
    - Unfolding experiments for RNA hairpins or other secondary structures
    - Monitoring protein enzymatic activity (e.g., transcription)

Examples of surface assays:

- Motility assays involving motor proteins
- Mechanical characterization and manipulation of cells, larger sub-cellular structures, or assemblies (e.g., microtubules)

This service will only be performed by NF staff, ideally accompanied by the User. As part of this service, the NF staff will check sample quality, troubleshoot, and define optimal conditions for data acquisition. If possible, an automation protocol for the experiment can be developed to streamline future data collection. The User can spend up to 10 days in the NF, testing various samples. The work can be split into two visits. The outcome of this service is an established protocol that can be used for 'data acquisition' service.

Technical requirements:

The User should have a clear understanding of the methodology and provide the anticipated assay design in the proposal. The User should specify in the application which probes are already available. The unit provides standard coated polystyrene beads and biotinylated DNA that can be used for the experiments. Typically, about 500 µl of 10 nM fluorescently labelled protein is required to perform an experiment. Ideally, small aliquots (5-10 µl) of concentrated protein should be provided. Dyes excited with 488 nm ("blue"), 561 nm ("green"), 638 nm ("red") can be used. All other assay-specific components must be provided by the User.

Information to be provided in the technical requirements page of the application:

- Information expected from the single molecule data in the project and expected added value offered by the single molecule approach compared to conventional methods
- Description of previous experience with single molecule techniques. (e.g. no previous experience / experiments performed at home build instrument in my lab / experiments performed at home build instrument in collaboration / experiments performed at commercial instrument in collaboration / other.
- Description of the type of single-molecule experiment you intend to perform, providing as many details as possible (e.g. protein binding to DNA (specific or nonspecific), transcription (assisting or opposing force), motility assay (type of filaments, surface- or bead-attached), or unfolding experiment)
- Reference or protocol of assay development
- Details about existing sample: protein name(s), MW, concentration, binding characteristics (binding partners, Kd if known) etc.
- Protein labelling (type of fluorophore, labeling strategy, and degree of labeling, if known)
- Project step of most significant troubleshooting (e.g. protein labelling, preparing appropriate DNA template, optimising binding / protein activity conditions etc)

Access modality available: Access to NF services, Access with Training.

SID: SB-IU5-B – Data Acquisition

Services description: This service is dedicated to projects where specific single molecule assays have been already established or, alternatively, should be combined with 'assay development' service as a simple 'package'. Examples of experiments currently performed routinely by the unit: (i) detecting changes to DNA structure upon protein binding e.g., melting, looping, compaction; (ii) characterizing protein behaviour upon binding to DNA (diffusion, direct motion, interactions with other proteins). This service can be performed by the NF staff or by a trained User. The training can be provided by the unit as part of this service. Full training typically requires 3-5 days. The User can spend up to 10 days at the NF. The work can be split into two visits. Original data will be handed to the User as .h5 files. The unit will assist the User in Accessing the files using Pylake. In case of kymographs, NF staff can provide a short training on existing tools such as Lakeview.

Technical requirements: The User should have a clear understanding of the methodology and provide a detailed description of the assay. In the application, the User should specify the

level of experience and provide preliminary data that will be reproduced during the project. Only standard reagents can be provided.

Information to be provided in the technical requirements page of the application:

- Summary of the existing data?
- Established protocol or publication with assay details
- Changes that may be made to the assay that has been developed or published
- Outcome to be extracted from the data; measurable parameter, details on the number of repeats, different conditions, statistical methods.

Access modality available: Simple Access, Access to NF services, Access with Training.

APPENDIX 1: Description of the Data analysis services available in combination with NF for Structural Biology

Table 1: *Overview of the NF for Structural Biology services which can be combined with the data analysis services provided by the NF for Data Handling and Analysis*

Category	Service code	Service name
Cyro-Electron Microscopy	NF61.02.01	Cryo-electron microscopy analysis
Volume-Electron Microscopy	NF61.03.01	Volume electron microscopy analysis

A detailed description of each IU1 service is found in the remainder of this section.

NF61.02.01 Cryo-Electron Microscopy Analysis

Service description

Cryo-Electron microscopy analysis encompasses the analysis of cryo-electron microscopy data, both single particle and tomographic reconstruction. This service includes, but is not necessarily limited to, the following use-cases:

- **Single-particle analysis (SPA)**: Development of image processing pipelines for the reconstruction of single particle 3D density maps, starting from cryoEM raw datasets or pre-processed micrographs/particles. Map validation.
- **Atomic Model Building**: De novo model building from reconstructed 3D density maps, fitting of existing atomic structures and refining of atomic models. Model validation.
- **Analysis of Flexibility and Heterogeneity**: Development of image processing pipelines for local reconstruction and refinement of flexible regions and evaluation of the conformational heterogeneity landscape of the macromolecules.
- **Tomography reconstruction**: Development of image processing pipelines for the reconstruction and analysis of tomograms, starting from tilt-series containing fiducial markers or fiducial less. Segmentation of the tomograms and sub-tomogram averaging (STA).
- **Custom pipeline development:** Construction of a computational pipeline combining two or more individual steps.

While these are examples of the services we can provide, we anticipate that most projects will require some combination of tools and services and so we will work with successful Applicants to craft pipelines that fulfil their analysis needs, as well as provide training and support in their future use. Our ethos is to work openly and transparently with our Users in the spirit of scientific collaboration. During the application phase, it will only be necessary to describe the analysis

goals; the precise details of the analysis will be discussed with the users upon selection of the project.

Access modality available

- Access to facility service
- Access to facility service including training

Requested inputs from users

For this service, we require a detailed project description outlining the analysis goals, and the expected data to be analyzed. A full analysis plan will be developed in collaboration with the successful applicants and the National Facility for Structural Biology as the project proceeds. The data will be transferred directly from the National Facility for Structural Biology to the National Facility for Data Handling and Analysis upon successful completion of the data acquisition phase of the project.

Technical requirements

Applicants must ensure that the samples meet the quality standards of the National Facility for Structural Biology. The National Facility for Data Handling and Analysis will coordinate with the National Facility for Structural Biology to ensure that the data acquired is of sufficient quality and quantity to ensure that the desired reconstruction can be achieved.

Results

Upon successful completion of the selected project, results will be delivered in a format of the Users' choosing and depending on the project needs (typically .mrc or .pdb, but other formats or intermediate files may be delivered depending on User preferences). In addition, we will provide whatever software, code, and support is required for the User to reproduce the analysis at their home institute.

The facility will also assist the user in submitting raw data to public repositories, as stipulated in the *National Facilities Access Rules*.

Combined services

This service can be combined with the following services offered by the National Facility for Structural Biology:

SB-IU1-C – High-resolution Cryo-TEM Imaging

To access the combined services, please submit an application to the National Facility for Structural Biology requesting data analysis.

NF61.03.01 Volumetric EM analysis

Service description

The Volumetric EM service provides segmentation and analysis of structures in serial EM section data. This service is restricted to segmentation and analysis of a finite number of structures per image (ie, mitochondria, organelles, vesicles, other similar objects of study). The proposal must include specific examples of the structure(s) of interest in order to judge feasibility. This service includes, but is not necessarily limited to, the following use-cases:

- **Data pre-processing:** de-streaking, alignment, contrast adjustment.
- **Data annotation:** generating dense labels covering structures of interest for the purpose of training AI algorithms.
- **Model Development**: Training and deployment of AI segmentation algorithms specific to the research question.
- **Downstream Analysis**: Analysis of segmented structures, morphology, number, distribution.

While these are examples of the services we can provide, we anticipate that most projects will require some combination of tools and services and so we will work with successful Applicants to craft pipelines that fulfil their analysis needs, as well as provide training and support in their future use. Our ethos is to work openly and transparently with our Users in the spirit of scientific collaboration. During the application phase, it will only be necessary to describe the data and the desired form of the analysis result; the precise details of the analysis will be discussed with the Applicants upon selection of the project.

Access modality available

- Access to facility service
- Access to facility service including training

Requested inputs from users

For this service, we require a detailed project description outlining the analysis goals, and the data to be analyzed. A full analysis plan will be developed in collaboration with the successful applicants and the National Facility for Structural Biology as the project proceeds. The data will be transferred directly from the National Facility for Structural Biology to the National Facility for Data Handling and Analysis upon successful completion of the data acquisition phase of the project.

Technical requirements

Applicants must ensure that the samples meet the quality standards of the National Facility for Structural Biology. The National Facility for Data Handling and Analysis will coordinate with the National Facility for Structural Biology to ensure that the data acquired is of sufficient quality and quantity to ensure that the desired reconstruction can be achieved.

Results

Upon successful completion of the selected project, results will be delivered in a format of the Users' choosing and depending on the project needs. In addition, we will provide whatever software, code, models, and support is required for the User to reproduce the analysis at their home institute. The form will depend on the specifics of the project and the needs of the Users.

To reduce the burden of access for our Users, we will use open-source software tools during the NF projects.

The facility will also assist the user in submitting raw data to public repositories, as stipulated in the *National Facilities Access Rules*.

Combined services

This service can be combined with the following services offered by the National Facility for Structural Biology:

SB-IU1-D – Volume Electron Microscopy

To access the combined services, please submit an application to the National Facility for Structural Biology requesting data analysis.