HUMAN TECHNOPOLE

NATIONAL FACILITY FOR STRCUTURAL BIOLOGY

CALL FOR ACCESS

24-SB-PILOT

Amendment II

The present amendment is aimed at *i.* revising the eligibility and admissibility process described in paragraph 4 and the evaluation of application process described in paragraph 6 of the Call for Access and *ii.* updating the service description and technical requirements for selected services.

1. The sentence *"Applications must be written in English and must be complete (i.e., consist of all the requested elements and information). Incomplete applications will be considered not eligible and will be rejected at the administrative review stage"* is revised as follows:

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, declarations, technical requirements of the services, sample requirements). Incomplete applications or applications that do not meet the requirements will be considered not admissible and will be rejected at the administrative review stage.

The sentence *"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" is revised as follows:*

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 the requirements will be considered not admissible and will be rejected at the administrative review stage.

2. Update service description and technical requirements for selected services.

(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.

Services available in combination with the NF for Data Handling and Analysis: SID: NF60.002 - CryoEM Analysis.

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.

Services available in combination with the NF for Data Handling and Analysis: SID: NF60.002 - CryoEM Analysis.

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.

(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 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.