Infrastructure for Cryomicroscopy and Electron Diffraction Core Facility

CryoEM (cryo-electron microscopy) is a Nobel Prize-winning technique that revolutionised structural biology, accelerating progress in medicine, pharmacy, biology and biochemistry. This versatile method involves vitrification of samples in their native state by plunge freezing followed by imaging with a high-end transmission electron microscope (TEM) under cryogenic conditions. Since the "resolution revolution" (~2013), major breakthroughs in hardware and software such as more sensitive cameras and sophisticated data processing algorithms have transformed EM images into high-resolution molecular structures. Modern CryoEM microscopes equipped with the newest energy filters can yield atomic-level structures even close to 1 Å resolution (Nakane et al., 2020), often published in top journals such as Nature or Science.

In 2019 the University of Warsaw purchased and installed one of the first fully dedicated Cryo-TEM instruments in the country: 200kV Glacios (Thermo Fisher Scientific), located at CeNT UW in the **Cryomicroscopy and Electron Diffraction Core Facility**. The instrument is equipped with an autoloader holding up to 12 grids, a CCD Ceta16M camera, a direct electron detector camera Falcon3EC and a phase plate for contrast improvement. Such configuration allows **single particle analysis (SPA)** and **cryo-tomography (CryoET)**. SPA serves as a dominant tool for determining 3D atomic structures of biomolecules based on images containing projections of hundreds of thousands of randomly oriented individual particles vitrified on TEM grids. These projections are computationally classified and averaged and optimized classes are used to create a 3D structural model (Glacios enables resolution ~3 Å). CryoET provides 3D visualisations of biomolecules *in situ*, in a broader context such as an organelle or even a single cell. Here, a vitrified sample trimmed down to a thin lamella is tilted across a range of angles (e.g. –60° to +60°) to record individual projections needed to reconstruct the 3D model.

Besides these two main CryoEM methods there is another mode based on diffraction, currently unavailable in the CeNT microscope: **microcrystal electron diffraction** (**MicroED**) (Nannenga and Gonen, 2019). MicroED is a cutting-edge, rapidly developing variant of three-dimensional electron diffraction (3D ED) methods (Gemmi et al., 2019; Jones et al., 2018; Gruene et al., 2018), optimal for the CeNT's microscope. During a MicroED experiment, small crystals (≤1 µm) are embedded on EM grids. Once well-diffracting crystals are detected, MicroED datasets are collected by exposure of the sample to an electron beam in the diffraction mode during continuous rotation of the stage. MicroED data are recorded on a fast camera as a movie with each frame containing a diffraction pattern representing a fragment of the reciprocal space. MicroED is analogous to the standard single-crystal X-ray diffraction and the collected data can be directly processed by the existing X-ray crystallography software.

3D ED is ideally suited to study tiny crystals or their fragments since **electrons interact with matter ~10^4 times stronger than X-rays.** This allows to study crystal structures and properties of nano-sized crystals at atomic resolutions. These include materials for which it is difficult (or impossible) to obtain crystals of a size suitable for X-ray analysis even at synchrotrons, or which can only be investigated with X-ray free-electron lasers (XFELs). In addition, small crystals are less affected by defects and, therefore, may yield superior data quality. Moreover, 3D ED allows for easier identification of light atoms, such as lithium and hydrogen(!) when compared to X-ray diffraction, providing an alternative to neutron diffraction experiments. 3D ED is much more accessible in terms of crystal size and facility access. **Having in-house access to 3D ED is like having at hand a synchrotron station, an XFEL and a neutron diffractometer simultaneously**.

3D ED method is a powerful tool for structural identifications of a wide variety of crystal samples: soluble and membrane proteins (often still in lipidic mesophases), peptides (including those involved in neurodegenerative diseases), crystals of small organic and inorganic

molecules, pharmaceuticals, natural products, pigments, metal-organic frameworks, zeolites, semi-conductors and minerals (often still embedded in a solid matrix). The amount of material needed to prepare grids is extremely small, much less than a milligram, enabling even forensic studies. **Currently, 3D ED is one of the most promising techniques for analysis of nanomaterials and development of nanotechnologies.** The technique has already made important contributions to drug discovery by determining structures of protein-drug complexes and supra-resolution of small molecules and natural products, often directly from powders, or mixtures, therefore bypassing the costly and time-consuming crystallization experiments.

Access to the MicroED technology on the Ochota Campus opens up wide new opportunities for all research groups from different departments and institutes of our University. Possible applications include drug discovery and development, together with identification of natural products, protein structure determination or drug screening, studies of new minerals and micro- and nanocrystals produced by living organisms. MicroED may become a routine analytical tool for practicing organic chemistry, allowing for rapid, on-the-fly, structural determination from powders and seemingly amorphous materials. It will speed up development of important new organic and inorganic materials such as catalysts, drug candidates or liquid crystals. Exciting frontier research within the reach of the Ochota Campus research groups includes establishing the best models of electron scattering to obtain more accurate and precise structures from 3D ED, development of methods accounting for dynamic effects in MicroED, collecting subatomic resolution data to observe minute details of the electrostatic potential, including chemical bonds, electron pairs or determination of atomic charge states. Having access to MicroED technology will allow UW research groups to become the worldwide leaders, and the very first groups in Poland developing and using this technique.

As the CryoEM infrastructure is usually in very high demand, we foresee that similarly to those abroad it will work 24h/7days per week. Thus, it produces a very large amount of data: a single CryoEM run can record thousands of images and generate between 1 to 10 Terabytes (TB) of raw data. Next, the raw data needs to pass multiple processing steps to get 3D images of the studied biomolecules. The processing is highly intensive computationally and requires GPU processors (used for acceleration by CryoEM software, such as RELION or CryoSPARC). Additionally, in order to minimize the processing time, multiple GPU servers need to be used in a parallel way. Also, the storage needed for the CryoEM infrastructure should have PetaBytes (PB) of capacity to preserve the original data, which present a golden opportunity for big data analytics and novel modelling technologies. The proposed project will solve the above problems by providing the following **upgrade package** to modify the existing Glacios microscope: (a) a MicroED module: replacement of the Ceta16M camera with a dedicated Ceta-D camera for diffraction and a MicroED package field retrofit, (b) building a dedicated CryoEM data storage and using with GPU cluster-based data processing capabilities at the Biological and Chemical Research University Centre at the Ochota Campus, (c) purchasing individual workstations for processing data collected during SPA, CryoET, and MicroED experiments – one Windows-based for on-the-fly processing of data while being acquired, and 3 Unix machines for the Core Facility users. The upgrade will also include the necessary auxiliary equipment for CryoEM sample preparation: (d) GraFix (gradient fixation) station for purifying and stabilising macromolecular complexes for SPA, (e) a glow discharge instrument for preparing TEM grids prior to plunge freezing, (f) Vitrobot – an automated station for plunge freezing, (g) larger dewars for storing frozen grids together with a CryoEM starter kit with dedicated pucks, (h) smaller dewars for handling samples during plunge freezing, autogrid preparation and transfer to other labs, (i) 180L pressurised dewar for automatic LN2 refill of the Glacios, (j) consumables for Vitrobot, TEM grids and clipping accessories for preparing autogrids, (k) a cryo tool dryer for drying the CryoEM accessories.