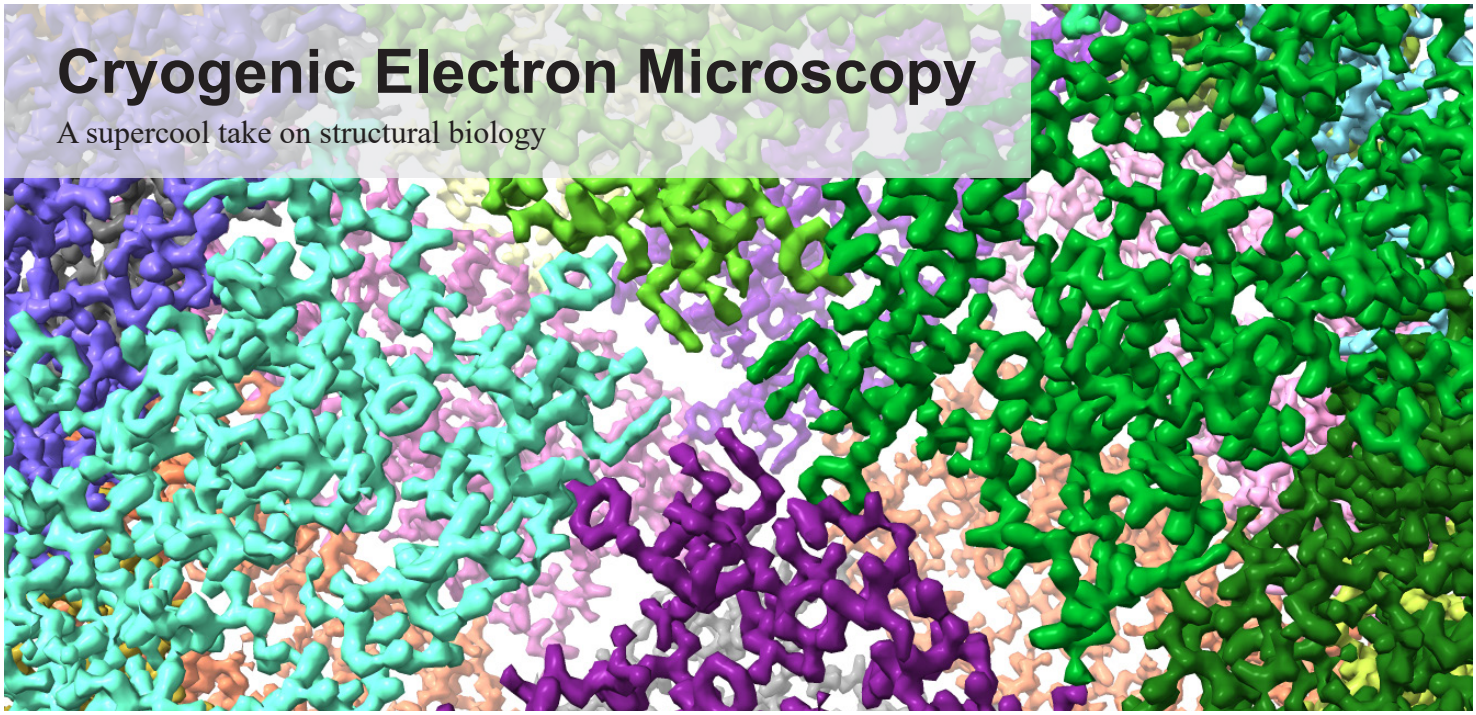
# LIFE SCIENCE

NEWSLETTER OF THE UNIVERSITY RESEARCH FACILITY IN LIFE SCIENCES, THE HONG KONG POLYTECHNIC UNIVERSITY / IS10 / FALL 2023



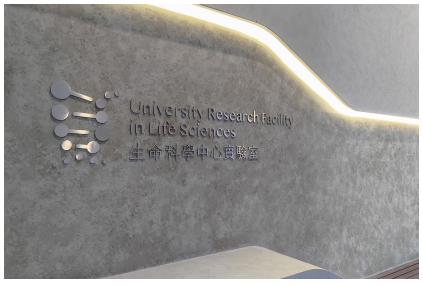
## Cryogenic Electron Microscopy

A supercool take on structural biology

## GRAND OPENING OF THE ANIMAL IMAGING CENTRE

The Animal Imaging Centre (AIC) of the ULS was officially open to users in mid-2023. Being the first dedicated animal imaging core facility in Hong Kong, the AIC aspires to support preclinical research studies at PolyU, and to advance cutting-edge, multidisciplinary and collaborative research in bioimaging and neuroscience. The establishment of the AIC was generously supported by the University Grants Committee (site preparation), Research Grants Council (CRF equipment funding) and the University (internal equipment funding).

Two major categories of ULS equipment are available at the AIC, namely Preclinical Animal Research and Fluorescence Microscopy. Animal holding rooms are also available for temporary holding during the course of a longitudinal study.




The Animal Imaging Centre of the ULS on Y Podium.

### Preclinical Animal Research

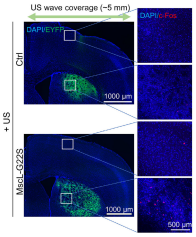
One major focus of the AIC is to support longitudinal and multimodal imaging of small rodents, including mice and rats. The **Bruker BioSpec 70/20 USR MRI System** supports noninvasive and high-resolution imaging of soft tissues in live rodents, while the **Fujifilm Ultrasound/Photoacoustic Imaging System** allows users to examine various physiological parameters, as well as perform tissue and molecular imaging *in vivo*. To obtain high-resolution volumetric images of mainly hard tissues, users may access our **Bruker SkyScan 1276 Micro-CT Scanner**. Our **PerkinElmer IVIS Lumina III *In Vivo* Imaging System**, on the other hand, is a popular piece of equipment for cancer biology studies, where researchers may trace metastatic cancer cells using fluorescence or bioluminescence.

### Fluorescence Microscopy

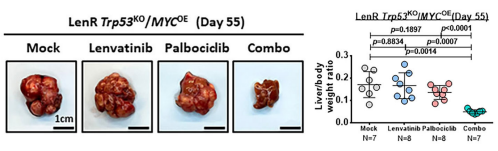
The newly installed **Nikon AX R MP Microscope** supports high-speed, intravital multiphoton imaging, as well as optogenetic photostimulation. Our **Logos X-CLARITY Tissue Clearing System** allows users to perform automated tissue clearing of such samples as mouse organs or even embryos for subsequent volumetric fluorescence imaging using our **Zeiss Lightsheet 7 Microscope**, or other confocal or multiphoton microscopes.

## POLYU RESEARCH

**1.** Prof. Sun Lei's group (BME) demonstrated a novel sonogenetic stimulation approach to control behaviour in freely moving mice with high circuit specificity and subsecond temporal resolution. Ultrasound was shown to stimulate neurons in dorsal striatum in MscL-G22S mutants to activate a defined neural pathway. The Leica SP8 and Nikon T*i*2-E Microscopes were used in this study.




*Above:* MscL-sonogenetic stimulation led to neuronal activation in dorsal striatum, as shown by the c-Fos marker. *PNAS* **120(22)**, e2220575120 (2023).
*Below*: CDK6 inhibition by palbociclib sensitises HCC cells to lenvatinib treatment in a lenvatinib-resistant HCC mouse model. *Nat. Comm.* **14**, 6699 (2023).




**2.** Using the lysine-targeted sulfonyl fluoride chemical probe XO44, Prof. Terence Lee's group (ABCT) observed ERK/YAP1 pathway-mediated CDK6 upregulation in hepatocellular carcinoma (HCC) cells. Suppression of CDK6, on the other hand, showed synergistic effects with lenvatinib treatment *in vitro*. This suggests CDK6 is a promising target for treating lenvatinib-resistant HCC. The BD Aria III Cell Sorter, C6 Flow Cytometer, IVIS *In Vivo* Imaging System and Leica SPE Microscope were used in this study.

## CRYOGENIC ELECTRON MICROSCOPY

*A supercool take on structural biology*

Cryogenic electron microscopy (cryo-EM) has brought about the so-called resolution revolution in the recent decade. The technique has gained much popularity amongst biologists after Jacques Dubochet, Joachim Frank and Richard Henderson were awarded the Nobel Prize in Chemistry "for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution" in 2017. To allow PolyU researchers to have access to such technology, the ULS began planning for the establishment of our Cryo-EM Centre in 2018. The Centre was officially open in 2023, and currently houses 4 pieces of major equipment, and a collection of sample preparation equipment to support the cryo-EM and cryo-electron tomography (cryo-ET) workflows. In this article, a brief overview of the technique and our Cryo-EM Centre will be offerred. A more detailed introduction to the single-particle analysis (SPA) and cryo-ET techniques will be available in subsequent newsletter issues.

The major advantages of cryo-EM are that only a small amount of biological sample is required, and that crystals are not a prerequisite. Cryo-EM has enabled researchers to obtain near-atomic resolution structures of proteins that could not previously be solved by X-ray crystallography. In a typical single-particle cryo-EM experiment, a purified protein sample is first checked for its purity and homogeneity by negative staining and subsequent transmission electron microscopy (TEM) imaging at room temperature **(Fig. 1a)**. An ideal sample shall be homogenous and free of aggregates. The sample would then be subjected to further screening and data collection at cryo-temperature.

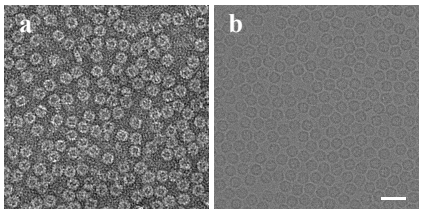



**Figure 1.** TEM images of **(a)** negatively stained apoferritin and **(b)** vitrified apoferritin sample captured using the ThermoFisher Talos L120C 120 kV TEM and Glacios 200 kV Cryo-TEM, respectively. Scale bar is 25 nm.

The protein sample would at this point be vitrified (*i.e.*, frozen rapidly) using a special equipment called a Vitrobot in a sample preparation room with extremely low relative humidity (typically below 20%) to avoid ice contamination. The vitrification process traps individual protein molecules in an exceedingly thin layer of amorphous ice in a sample-grid for subsequent screening and imaging.

A ThermoFisher Glacios 200 kV Cryo-TEM and a Krios G4 300 kV Cryo-TEM are available at the ULS Cryo-EM Centre for screening and high-resolution data collection after vitrifying the sample. Sample screening at cryo-temperature is essential for a successful cryo-EM workflow, as the sample would be checked for the quality of the biochemistry as well as vitrification **(Fig. 1b)**. The sample could then be transferred directly to the Krios for imaging. The Krios is equipped with the latest-generation Falcon 4i direct electron detector and the Selectrix energy filter, which greatly enhance the data acquisition speed and signal-to-noise ratio, respectively. Both the Glacios and Krios Cryo-TEMs are equipped with an autoloader, enabling automated data collection over an extended period of time.

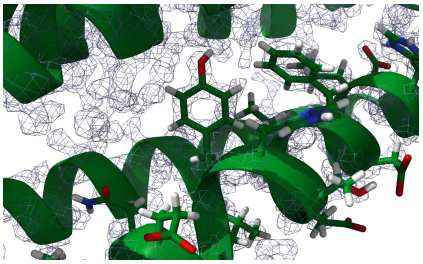


**Figure 2.** Reconstructed 3D model of apoferritin at 1.5 Å resolution using the ThermoFisher Krios G4 300 kV Cryo-TEM System at the ULS Cryo-EM Centre.

Individual protein molecules are expected to have different orientations as appeared on each of the hundreds or thousands of micrographs obtained in a dataset with a cryo-TEM system. Specialised software would be used for data pre-processing, alignment, particle picking and classification, and finally 3D reconstruction **(Fig. 2)**. The ULS is in the process of acquiring an NVIDIA A800 GPU workstation to support cryo-EM and cryo-ET data analysis workflows. The workstation will also support on-the-fly data processing, enabling researchers to further improve the efficiency of their experiments.

## COMING SOON

The Nikon AX R MP Upright Multiphoton Microscope will be upgraded with the new NSPARC detector in Q1 2024, enabling researchers to perform intravital or deep-tissue super-resolved multiphoton imaging.

## GET IN TOUCH

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## ULS EQUIPMENT AT A GLANCE

**Mass Spectrometry**
- Bruker AmaZon Speed Ion Trap-ETD MS
- Bruker UltrafleXtreme MALDI-TOF/TOF MS
- Agilent 6460 Triple Quadrupole LC/MS
- Agilent 6540 Quadrupole-TOF LC/MS
- SCIEX 6500⁺ QTrap LC/MS
- ThermoFisher Orbitrap IQ-X LC/MS
- Waters UPLC with QDa Mass Detector

**Cryo-Electron Microscopy**
- ThermoFisher Krios G4 300 kV Cryo-TEM System
- ThermoFisher Glacios 200 kV Cryo-TEM System
- ThermoFisher Talos L120C 120 kV TEM System
- ThermoFisher Aquilos 2 Cryo-FIB System
- ThermoFisher Vitrobot Mark IV

**Fluorescence Microscopy**
- Abberior STED Super-resolution Microscope
- Leica SPE Confocal Microscope
- Leica SP8 Multiphoton/Confocal Microscope
- Nikon T*i*2-E Live-cell Imaging System
- Nikon SIM/STORM/A1 SR/Confocal Microscope
- Nikon SMZ1270i Fluorescence Stereomicroscope
- Nikon AX R MP Upright Multiphoton Microscope
- Zeiss Lightsheet 7 Microscope
- Zeiss Lattice Lightsheet 7 Microscope

**Preclinical Animal Research**
- Bruker BioSpec 70/20 USR MRI System
- Bruker LF90II Body Composition Analyser
- Bruker SkyScan 1276 *in vivo* Micro-CT Scanner
- PerkinElmer IVIS *in vivo* Imaging Systems
- Fujifilm Vevo LARZ Ultrasound/Photoacoustic Imaging System
- Promethion Metabolic Cage System

**Cell and Molecular Biology**
- BD FACSAria III Cell Sorter
- BD FACSymphony A3 Cell Analyser
- BD Accuri C6 Cell Analyser
- BD FACSVia Cell Analyser
- Roche LightCycler II qPCR System
- Applied Biosystems QS 5/7 Flex qPCR Systems
- Seahorse XF$^{e}$24 Extracellular Flux Analyser
- Logos X-CLARITY Tissue Clearing System
- Invitrogen Countess II FL Auto Cell Counter

**Biochemical Analysis**
- Bio-Rad Bio-Plex 200 Suspension Array System
- Bruker Sierra SPR-32 Pro Analyser
- Jasco J-1500 Circular Dichroism Spectrometer
- Jasco CPL-300 CPL Spectrometer
- Malvern MicroCal Automatic ITC System
- Tecan Automatic Liquid Handling System

**Genomic Science**
- Agena MassARRAY Analyser 4 System
- Agilent 2100 Bioanalyser System
- Covaris ME220 Focused-ultrasonicator
- 10× Chromium iX Single Cell Analysis System
- Illumina MiSeq NGS System
- Illumina NextSeq 2000 NGS System
- Nanopore GridION Mk1 Sequencing System

• New in 2023 • Upgraded in 2023