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A short story of the long road to cryo-EM in Portugal

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Proteins are fundamental to life, frequently assembling into complexes that play crucial roles in cellular functions. When proteins or their complexes exhibit dysfunction, they can contribute to the onset of various diseases, thereby making them viable targets for drug development. In this context, a comprehensive understanding of their 3D structures is crucial for the design of effective therapeutic agents.

Macromolecular crystallography has long been the principal method for determining 3D structures of protein. Even today it remains the predominant technique, contributing the highest number of protein structures to the Protein Data Bank (PDB; Berman *et al.*, 2000, 2003). However, many biologically relevant proteins and complexes are recalcitrant to crystallization. Advances in cryo-electron microscopy (cryo-EM) have revolutionized structural biology, enabling the structural elucidation of large macromolecular complexes at near-atomic resolution. Cryo-electron tomography has also allowed the visualization of cellular structures with unprecedented detail. Since 2011, the number of structures solved using electron microscopy has steadily increased. According to official statistics from the PDB, 10 086 structures determined using X-ray methods were released in 2025, compared with 7240 structures determined by electron microscopy. It is expected that in the near future the number of structures solved by electron microscopy will reach parity with those determined by X-ray methods. This increase is related to the resolution revolution era (Kühlbrandt, 2014), stemming from major technological advancements in cryo-EM, such as the development of digital direct electron detectors (see, for example, McMullan *et al.*, 2009; Milazzo *et al.*, 2005), improved computational methods with enhanced 2D classification algorithms (see, for example, Elad *et al.*, 2008; Scheres *et al.*, 2007) and advances in sample vitrification (see, for example, Dubochet *et al.*, 1981, 1988). This progress was recognized with the award of the 2017 Nobel Prize in Chemistry to Jacques Dubochet (University of Lausanne, Switzerland), Joachim Frank (Columbia University, New York, USA) and Richard Henderson (MRC Laboratory of Molecular Biology, Cambridge, UK). Their contributions, alongside those of numerous other researchers, have significantly advanced the visualization of macromolecules, making cryo-EM a powerful tool in structural biology.

Before these breakthroughs, electron microscopy was often referred to as 'blobology' due to its limited resolution. Nowadays, high-resolution 3D structures of proteins and their complexes can be obtained by single-particle analysis, even for proteins smaller than 100 kDa (Herzik *et al.*, 2019; Merk *et al.*, 2016). Additionally, collaboration between the electron microscopy and macromolecular crystallography communities has driven further automation and advances in the cryo-EM field.

Macromolecular crystallography is a key research area at ITQB NOVA and iBET (Instituto de Biologia Experimental and Tecnológica) in Oeiras, NOVA FCT (Faculdade de Ciências e Tecnologia) in Caparica and IBMC (now part of i3S–Instituto de Investigação e Inovação em Saúde) in Porto.

In 2017, we acknowledged the need to integrate cryo-EM to expand the portfolio of experimental methodologies applied in our structural studies. However, at the time no cryo-EM facility existed in Portugal and, although such facilities could be accessed abroad via collaborations or through INSTRUCT–ERIC, the latter required preliminary results that were impossible to obtain in Portugal.

To overcome this difficulty, we pursued a dual strategy, first by initiating discussions and negotiations to create a National Facility equipped with a cryo-electron microscope. ITQB NOVA played a crucial role in this, convening researchers from across the country and from diverse research fields in life sciences, ranging from structural to cell biology, to assess the national demand for cryo-EM. It soon became clear that Portugal could afford only one mid- to high-end microscope to serve the entire scientific community, including

both the structural and cell biology communities. Initially, we aimed to acquire a 300 kV cryo-transmission electron microscope (cryo-TEM), but due to its high purchase price and substantial annual maintenance costs, this option proved to be unfeasible. After extensive discussions, it was agreed to install the microscope at the International Iberian Nanotechnology Laboratory (INL) in Braga in northern Portugal, as it was the most suitable site for housing this equipment, due to an already existing infrastructure hosting several electron microscopes dedicated to materials science.

Secondly, we considered hiring an experienced cryo-EM researcher at ITQB NOVA to support internal knowledge transfer. However, we soon realized that this would be difficult to achieve, since it would likely be very challenging to attract an experienced cryo-EM specialist to Portugal at a time when no cryo-electron microscope was available in the country. As an alternative, we decided to move forward with an in-house training strategy in cryo-EM techniques, and to achieve this, we gained a Twinning project under the Horizon 2020 Research and Innovation programme (GA-857203), named IMpaCT – Imaging life from Molecules to Cells, aimed at building expertise in cryo-electron microscopy methodologies at ITQB NOVA. The mission was to improve our proficiency in cryo-EM for structural studies of proteins relevant to human health, including those involved in cancer, bacterial and viral infections, from individual molecules to large macromolecular complexes.

The project ran from 1 September 2019 to 28 February 2023 and was structured along three key pillars: data processing in collaboration with José Maria Carazo and Carlos Oscar Sorzano from the National Centre for Biotechnology of the Spanish National Research Council in Madrid, Spain, single-particle analysis in partnership with Sarah Butcher from the Institute of Biotechnology at the University of Helsinki in Finland, and cryo-electron tomography in collaboration with Michael Elbaum and Sharon Wolf from the Weizmann Institute of Sciences in Rehovot, Israel. Two pharmaceutical companies were also engaged: AstraZeneca (UK), which integrates cryo-EM into its daily pharmaceutical research, and Bluepharma (Portugal), which aims to incorporate cryo-EM methodologies into its workflow. Additionally, a Scientific Advisory Board (SAB) provided essential guidance throughout the project. The SAB members were Marta Carroni (SciLifeLab, Head of the Swedish Cryo-EM National Facility), Peter Peters (Maastricht University, a specialist in cryo-electron tomography and cryo-EM equipment development) and Werner Kühlbrandt (Max Planck Institute of Biophysics, Frankfurt, a renowned expert in cryo-EM, focusing on the structure and function of large membrane-protein complexes using single-particle analysis).

A solid work programme was designed around these three key pillars, and thus three hands-on workshops were organized: 'Data Processing in Cryo-EM applied to Macromolecular Complexes: from proteins to cells' (17–20 September 2019) organized by the CNB–CSIC at ITQB NOVA, 'Single Particle Analysis, hands on' (2–5 November 2020) organized by the University of Helsinki as mainly online due to the SARS-CoV2 pandemic and 'Cryo-Electron Tomography, hands-on', split into two sessions, the first being online (23–25 November 2020) and the second in person (21–24 March 2022), organized in Israel by the Weizmann Institute of Science. These workshops were enriched by complementary activities, including seminars led by renowned cryo-EM experts, cryo-EM biotech seminars and industry engagement through a mini-symposium and specialized seminars. The main goal of involving industry and organizing these activities was to promote knowledge transfer regarding the different applications using cryo-EM. Additionally, we supported various short-term exchange visits for PhD students and early-stage post-doctoral researchers to the partner laboratories, fostering hands-on training and project development. Expert visits from partner laboratories to ITQB NOVA were crucial in advancing our scientific projects and knowledge.

Our training activities were primarily aimed at PhD students and postdoctoral researchers at ITQB NOVA. However, we considered the establishment of a cryo-EM community in Portugal to be fundamental, therefore participation was extended to all researchers at the national level who were interested in these activities. For instance, researchers and students from iBET (a Portuguese private nonprofit institution with a broad research and development portfolio in biotech and pharma), IGC (Gulbenkian Institution of Sciences in Oeiras, now part of GIMM, the Gulbenkian Institute of Molecular Medicine), i3S and also INL participated in our training activities and seminars.

The IMpaCT project was instrumental in establishing cryo-EM expertise at ITQB NOVA, fostering knowledge exchange, strengthening our structural biology capabilities and enhancing Portugal's capabilities in cryo-EM. The project's activities also aimed to provide training to as many participants as possible so that they could then use this expertise in their research and at the very least create further interest for these methodologies at their home institutions.

In the meantime, in August 2019 the National Advanced Microscopy Network for Health and Life Sciences (CryoEM-PT) was established in Portugal. This network consists of a central node at the INL in Braga, and several regional nodes distributed throughout the country, including the University of Minho in Braga, i3S in Porto, the University of Coimbra, the University of Beira Interior in Covilhã, ITQB NOVA and IGC (now GIMM-Oeiras) in Oeiras, IST and IMM (now GIMM-Lisbon) at the University of Lisbon, UCIBIO at NOVA FCT in Caparica, MED at the University of Évora and CCMAR at the University of Algarve in Faro. In 2022, with the support of other network nodes, the INL secured a grant from the North Regional Development Commission (CCDR-N) that allowed the purchase of a 200 kV cryo-TEM. The purchase was the object of an international tender, and both ThermoFisher and Jeol applied. The selection commission awarded the contract to ThermoFisher due to its lower price.

It is worth noting that our training in cryo-EM methodologies through the IMpaCT project significantly contributed to establishing the CryoEM-PT network. Moreover, there were very fruitful discussions between the IMpaCT partners and

SAB with members of the CryoEM-PT scientific commission that also included visits to INL. As a symbolic corollary of IMpaCT, its final meeting took place on 1 February 2023 at the INL in Braga, followed by the kick-off meeting and official inauguration of the CryoEM-PT on the next day, 2 February 2023, also at the INL (Fig. 1).

The CryoEM-PT National Facility is equipped with a ThermoFisher Scientific Glacios cryo-TEM, which is crucial for sample screening and preliminary data collection before accessing higher-end facilities in Europe. This cryo-TEM is equipped with a high-brightness field electron gun (X-FEG), a 12-grid autoloader, a Falcon 4i direct electron detector and a CETA-D 16M CMOS camera for micro-electron diffraction. At the time of purchase, the Falcon 4i was arguably the best camera on the market for its intended applications, namely single-particle and tomography applications.

This microscope enables a variety of applications, including single-particle analysis (SPA) of macromolecules and their complexes, cryo-electron tomography of bacteria, thin cells and sections, and micro-electron diffraction (MicroED) from nanocrystals of chemical compounds and proteins. Since the inauguration of the Central Node at the INL, different Portuguese users have been accessing the microscope. A total of 60 users have collected data there, including researchers who had not previously used cryo-EM in their studies, from institutions such as the University of Minho, the University of Algarve and the University of Porto. In addition, iBET and Bluepharma also used the Glacios cryo-TEM in their R&D pipelines. The use of this instrument by the private sector was one of the main pillars supporting the investment by the CCDR-N.

Figure 1
Inauguration of the CryoEM-PT on 2 Februery 2023. From left to right: Célia Romão (ITQB NOVA), Sharon Wolf (Weizmann Institute of Sciences), Michael Elbaum (Weizmann Institute of Sciences), Pedro Matias (ITQB NOVA), Oliver Schraidt (INL), Enrique Carbó-Argibay (INL), Werner Kühlbrandt (Max Planck Institute of Biophysics), Peter Peters (Maastricht University), Sarah Butcher (Institute of Biotechnology, University of Helsinki), José Maria Carazo (National Centre for Biotechnology, Spanish National Research Council), Marta Carroni (SciLifeLab) and Ana Malheiro (INL).

The regional nodes play a key role in sample preparation and data processing, ensuring efficient use of the national facility. The ITQB NOVA node is dedicated to supporting researchers from different parts of the country. It is equipped for sample preparation and data processing, with plans for future upgrades to enhance its capabilities.

Throughout this process, INSTRUCT–ERIC and the ESRF have been instrumental in providing access to cryo-EM infrastructures via research proposals. INSTRUCT–ERIC grants access to INSTRUCT centres for preliminary data collection and data processing, which is often a prerequisite for the use of high-end microscopes. ESRF provides access through the 'Cryo-EM Solution-to-Structure Pipeline' and the Iberian Cryo-EM BAG (Block Allocation Group), further supporting the Portuguese cryo-EM community.

This brief overview outlines the development of cryo-EM in Portugal and our contributions to its advancement. Looking forward, the effective use of the CryoEM-PT National Facility requires the development of a robust cryo-EM research community. In the meantime, at the national level, other groups also started to develop their capabilities in cryo-EM, namely at i3S in Porto and NOVA FCT in Caparica. An important development in this context was the ERA Chair awarded to NOVA FCT in 2023, which allowed funds for the establishment of a cryo-EM laboratory at the NOVA FCT. In addition, ITQB NOVA will acquire a 120 kV electron microscope with cryo capabilities. This instrument represents a significant step towards the consolidation of a national cryo-EM community and will help to advance cryo-EM-based research projects. This will be the second electron microscope at the national level with cryo-capabilities for life sciences, and it will play a crucial role, especially in the Lisbon area, since it will allow grid screening, enabling a more rational and cost-effective use of the microscope time for data collection on 200 or 300 kV cryo-TEMs.

However, for the continued building of national capabilities in cryo-EM it is of the utmost importance that the Fundação para a Ciência e Tecnologia (FCT–IP, now AI^2), Portugal's main research funding agency, should implement a funding program dedicated to supporting the access of researchers to the CryoEM-PT National Facility, enabling them to address critical scientific questions, as is already the case for the supercomputing infrastructure. Sustained investment, collaboration efforts, knowledge exchange and infrastructure development will be essential to ensure a promising future for cryo-EM in Portugal. By fostering a vibrant cryo-EM research community, Portugal is now equipped to make significant contributions to cellular and structural biology, with implications for both fundamental research and applications in health and biotechnology.

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