Towards more dose efficient cryogenic electron microscopy of biological samples

Impact

As of March 2023, the number of cryo-EM labs worldwide has grown exponentially to more than one thousand (1056). The majority of these labs possess state-of-the-art equipment, such as the Titan Krios (355), JEOLs (42), Arctica (94), Glacios (208), Tundra (13), and Talos F200C (39) [322]. As a result, the cryo-EM market has become one of the fastest-growing markets in the scientific industry. A recent report projects that the entire cryo-EM market will drive growth of over 1 billion USD by 2027 [323]. Investing in cryo-EM technology provides companies with the opportunity to develop new products and stay ahead of the competition, leading to increased competitiveness in various industries. Cryo-EM technology has revolutionised the field of structural biology. In particular, cryo-EM SPA has allowed researchers to reconstruct 3D structures of macromolecules at atomic resolution [1,2].

However, it is worth noting that a top-end Titan Krios (> 5 M€) may not always be necessary for SPA, especially when imaging samples with a thickness less than 100 nanometres. In fact, lower-voltage cryo-EMs can produce higher-contrast images due to their more favourable elastic/inelastic cross-section ratio, as mentioned in **chapter 7.** One major advantage of lower-voltage microscopes is their relative affordability, making them more accessible to researchers and labs. The trend on the cryo-EM worldwide map already shows that more labs are obtaining 100 keV Tundra microscopes. The main constraint for obtaining high-resolution structures using a 100 keV electron microscope is the lack of an optimal detector. In **chapter 5**, the integration of a Timepix HPD detector, which works well at all energies, in imaging as well as diffraction mode, has shown promising results. Low-energy microscopes equipped with an HPD offer better affordability, making cryo-EM technology more accessible to researchers and labs in the fields of life and material sciences. This democratisation of the access to cryo-EM technology would allow more researchers to use cryo-EM to study various biological systems, potentially leading to new discoveries and advances in our understanding of complex biological processes.

As it is said by Werner Heisenberg "We have to remember that what we observe is not nature in itself, but nature exposed to our method of questioning." When imaging samples with electrons in the EM, there are multiple processes happening, such as radiation damage, hydrogen gas generation, beam-induced motions, and charging. Charging is an important and fundamental issue, as biological specimens are typically insulators. Charging will affect our observations and the interpretation of the results (**chapter 4**). While charging may not seem to have any significant social or economic impact, studying it is nonetheless crucial. For instance, during the space race between the US and the Soviet Union, the US government invested billions of dollars in its space program between 1957 to 1969, with a significant portion of the funds going towards fundamental research. This research had a substantial impact on many areas of science and technology, foreseen and unforeseen, such as GPS technology, miniaturised electronics, and environmental monitoring. Although the charging issue may not be as significant as exploring

space, it is a fundamental problem when imaging specimens using electrons. It is essential to remember that what we are observing is not the sample in itself, but rather the sample as affected by our method of questioning.

Currently, SPA cryo-EM employs a basic technique that involves irradiating the sample with a parallel flood beam to obtain its projections. This approach, however, comes with several limitations such as low SNR, charging, and radiation damage (although this is inevitable for EM). Quantum mechanics dictates that if we can obtain the wave function of an exit electron, we can potentially retrieve all the information about the sample. Unfortunately, the wave function collapses by the time the electron reaches the detector, limiting the amount of information we can obtain. Nevertheless, recent advances in measurement techniques, as discussed in **chapter 6**, have enabled more dose-efficient methods of data collection, e.g. by measuring all the characteristics of its wave. While some techniques, such as MPTEM and quantum sorters, may not be immediately applicable, others such as laser phase plates, holography, and ptychography hold evident promises for the near future. These methods could allow for more information to be extracted during the limited lifetime of a biomolecule within the electron beam, thus pushing the limits of resolution and enabling the study of the building blocks of life in unprecedented detail. This could lead to a better understanding of biological processes and potentially facilitate the development of new therapeutics to fight disease.