Tackling macromolecular recognition through single particle cryo-EM

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Electron microscopy (EM) is rooted on the dual charged-particle/wave nature of electrons (De Broglie equation) that allows exploring the organization of matter through an instrument that is conceptually very similar to an optical microscope. Based on this principle, EM has been developed for the past 90 years, since the pioneering work of E. Ruska and collaborators (ca. 1930). As compared to materials science, where EM reached atomic resolution decades ago, the application of EM to biological matter has seen a much slower development due to the inevitable radiation damage linked to exploration of the sample with accelerated electrons. The past decade has seen a dramatic improvement in the experimental conditions that allow studying 3D structures of protein, nucleic acids, and in general of macromolecular biological adducts, thanks to the synergistic action of three factors. On one hand, cryo-preservation techniques have been developed that allow mitigating radiation damage. On the other, the development of electron-counting-detectors, i.e. detectors that can indeed count each electron hitting their CMOS surface, allows using extremely low irradiation doses. Finally, the development of advanced software tools allows data reduction out of the collected micrographs with increased accuracy and within acceptable computing times. The theory and practice behind EM data collection and image reconstruction will be briefly discussed.

Based on the above considerations, cryo-EM has seen a true revolution over the past 4-5 years, particularly opening the way to the study of 3D structures of targets that 'traditionally' are poorly amenable to crystallographic or NMR approaches; among them membrane proteins, very large assemblies (e.g. the ribosome), and amyloid fibrils. Examples of such successful applications of cryo-EM have been flooding the literature and are witnessed by the exponential growth of the Electron Microscopy Data Bank (https://www.ebi.ac.uk/pdbe/emdb).

Cryo-EM studies from our lab will be presented, in the field of amyloid fibril core structure (from immunoglobulin light chains; in collaboration with G. Merlini et al., Pavia), a CRISPR-Cas9/Protein inhibitor complex (with C. Cambillau et al., Marseille), and the structure of 1.4 MDa complex of Glutamate synthase (with A. Vanoni et al., Milano).