**New avenues on target characterization and nanotools design from advancements in**

**cryo-electron microscopy**

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Single particle cryo-electron microscopy (SP Cryo-EM) has recently experienced a “Resolution Revolution” and it has emerged as one of the leading techniques for high-resolution structural determination of biomacromolecules, representing a breakthrough in structural biology and finding relevant applications in nanotechnology. In SP Cryo-EM, few microliters of homogeneous solution containing the biological system of interest are sufficient to reconstruct its structure up to the atomic resolution and in only few weeks of work.

This has opened the possibility to address complexes that it was not possible to investigate with crystallography or NMR, since by SP Cryo-EM very large and flexible assemblies can be analyzed. The resulting three-dimensional structures can account for dynamic rearrangements and allow to determine multiple structures mapping the evolution of catalysis and molecular recognition.

All the technical advances achieved in Cryo-EM, spanning from the sophisticated methodologies used for specimen preparation, the increased performance of the modern microscopes, the recent introduction of direct electron detectors, the availability of automated data collection systems and the improvement of computational tools for single-particle image processing will be herein discussed.