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Tackling Large Macromolecular Complexes: The Interplay of Electron Microscopy and Protein Crystallography

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Abstract

Large macromolecular assemblies which play a fundamental role in many cellular processes are often too large, flexible and dishomogeneous to be amenable to X-ray crystallography and NMR. Due to the recent technical and computational improvements, single particle (cryo)electron microscopy (EM) has become a powerful complementary method to determine the structure of large complexes at sub-nanometer resolution.

The combination of EM reconstruction with X-ray crystallography enables structural biologists to gain a much more comprehensive understanding of the target molecule than could possibly be achieved with any single technique. EM reconstructions can be interpreted in greater precision by fitting an X-ray model into an EM density map. On the other hand, an EM reconstruction at low resolution can be used as a phasing model in solving crystallographic structures of viruses or macromolecules, as well as provide the architectural framework to understand the role of a subunit within a larger assembly.

The talk will present an overview of the methods and a critical analysis of the relative advantages and disadvantages of both techniques.

As a case study we will analyse how the interplay of the two techniques has provided information to unravel the architecture and function of the MCM replicative helicase. Before cell division, the DNA needs to be faithfully and completely replicated. MCM proteins have a key role in eukaryotic cells, acting as a molecular motors to unwind the DNA double helix before replication. MCM proteins are present only in proliferating cells and are highly expressed in malignant human cancers cells and pre-cancerous cells and are therefore ideal diagnostic biomarkers and possible targets for drug development. A detailed knowledge of their structure and function is a pre-requisite for the full exploitation of their potential in cancer diagnostic and therapy.