

The three-dimensional structure of biomacromolecules by X-ray crystallography

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X-ray crystallography has provided a unique contribution to the development of the structural biology and of the structure-function paradigm in biosystems. Starting from the pioneering experiments of Astbury,¹ this area has been disseminated by a countless number of fundamental studies performed by exploiting the interaction of the X-ray with samples of biomacromolecules either in fibrous or crystalline states. In this scenario, it is not surprising that as many as 17 Nobel prizes in chemistry or medicine have been awarded to protein crystallographers.

The overall aim of the present lecture is to provide a global view of the structure determination process by X-ray crystallography². In particular, the main steps of the macromolecular crystallography pipeline (sample preparation, crystallization, generation of the X-ray radiation, data collection and processing, structure refinement and validation) will be described. The specificities of the fiber and single crystal approaches will be highlighted. Moreover, strengths and weaknesses of the methodology will be illustrated in the framework of current structural biology challenges.

A particular emphasis will be given to the problems related to the refinement of biomacromolecular structures and of the indicators commonly used to assess the success of the refinement protocol. The impact of the cross-validation in improving the accuracy of structures determined by X-ray crystallography will be also described. The need for the setup of effective validation protocols will be stressed through the illustration of major debacles of protein crystallography. A chronological account of the actions that have been undertaken over the last three decades to solve this problem will be presented. A survey of the different parameters that are commonly monitored in protein structure validation protocols will be also presented. A detailed description of the wwPDB validation protocol (<https://validate.rcsb.wwpdb.org/>)³ will be given. Finally, I will also illustrate structure quality assessment approaches based on the evaluation of protein fine structure (QuiProQua - <http://study.ibb.cnr.it/quiproqua/>)⁴.

¹Astbury, W.T.; Street, A; *Trans. R. Soc. Lond.* **1931**, A230, 75-101.

²Wlodawer, A.; Minor, W.; Dauter, Z.; Jaskolski, M.; *Febs Journal* **2008**, 275, 1-21.

³Gore, S. et al.; *Structure.* **2017**, 25, 1916-1927.

⁴Balasco, N.; Esposito, L.; Vitagliano, L.; *Acta Cryst.* **2017**, D73, 618–625..