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Improving and automating preparation steps of protein crystals for X-ray diffraction

Thèse de Doctorat de l'Université Joseph Fourier

Crystallography is from far the most contributing technique for the structure analysis of macromolecules at atomic resolution. In this thesis, instrumentation developments to improve and accelerate experimental procedures for X-ray diffraction experiments are tackled. Indeed the preparation steps of protein crystals for X-ray diffraction data collection are the main causes of forming a bottleneck towards automated pipelines from protein crystallization to structure resolution.

Firstly, an emerging method in today macromolecular crystallography is the room temperature in situ X-ray diffraction of protein crystal samples in their crystallization drops, with proven benefits in crystal screening and also structure resolution. However, it requires a great number of crystals to be centered and diffracted in a row. Thus a fully automated system answering to this requirement is presented and assessed in this manuscript as one of the results of these PhD studies. Secondly, in this manuscript studies and developments on automating harvesting, cryo-protecting and flash-cooling steps of protein crystals preparation are reported, as well as assessment experiments and results. With new robotic approach, crystals are manipulated with a micro-gripper on a 6-axis robotic arm to prepare and to analyze them with 360° rotation possibility for cryo-temperature single wavelength X-ray diffraction. Lysozyme and Nika Fe-EDTA protein crystals has been prepared and diffracted with this new method. Structural comparisons show no differences between the new methodology and the manual one, while robustness, repeatability and experimental time are significantly improved. At last, different integration scenarios of the presented methodologies, highlights their interest in fully automated macromolecular crystallography pipelines.