Soutenance

THESE

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Auditorium ESRF

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Time-resolved monochromatic synchrotron crystallography of a plant photoreceptor domain

Thèse de Doctorat de l'Université de Grenoble

Time-resolved crystallography (TRX) enables the identification at a near atomic detail of the progressive structural changes of a protein fulfilling its function, thus leading to the establishment of genuine molecular movies. Laue (polychromatic) diffraction at 3rd-generation synchrotrons first managed to obtain such movies at a 100 ps time scale, and more recently, the 4th generation X-ray sources XFELs (X-ray Free Electron Lasers) achieved a 100 fs time scale using a monochromatic beam. The goal of this thesis was to investigate the possibility of performing TRX on a macromolecular monochromatic crystallography beamline, by taking advantage of the latest technological developments. We envisaged three different photosensitive proteins, for which I first developed various crystallogenesis strategies in order to control the size and shape of the crystals. I also considered various ways of presenting the crystals of the beam at room temperature, either a single crystal mounted in a loop and kept in a wet air stream, or microcrystals moving in a grease jet passing through the beam. We then investigated how specific radiation damage could affect the structures of intermediate states at room temperature, given how sensitive they can be at cryogenic temperature. We concluded that it constitutes a much lesser concern at room than at cryogenic temperature. After initial characterization of the various proteins and sample environments, we focused on a time-resolved crystallography approach on crystals of the plant photoreceptor domain *At*Phot2LOV2 (the LOV2 domain of the blue-light photoreceptor photropin-2 from *Arabidopsis thaliana*). The dark state *At*Phot2LOV2 converts in microseconds into the signalling state, or light state, which relaxes in hundreds of seconds. Using a fast pixel X-ray detector, we characterized the structural decay of the light state, and showed that it proceeds via a space group conversion over a 20 min time course. We then slowed down the rate of the light state in crystals by limiting the quantity of photons, and we were able to monitor the process of light state population build-up with a 63 ms time resolution using an approach recording full data sets on less than 100 crystals, which we called TR-SOX for time-resolved serial oscillation crystallography, which takes advantage of merging partial data sets. Overall, our work paves the way for time-resolved crystallography on MX monochromatic synchrotron beamlines on regular-sized crystals of proteins that undergo structural rearrangements on the millisecond time scale.