

## MS03 Crystallization and biophysical characterization

MS3-03

Serial crystallography: a game-changer in crystallization and crystal handling

K. Rollet<sup>1</sup>, R. De Wijn<sup>1</sup>, P. Pachi<sup>1</sup>, L. Coudray<sup>1</sup>, P. Bénas<sup>1</sup>, C. Sauter<sup>1</sup>

<sup>1</sup>ARN - IBMC - CNRS - Unistra - Strasbourg (France)

### Abstract

Over the past decade, the advent of X-ray free electron lasers delivering ultra intense X-ray beams has revolutionized biocrystallography. With a brilliance a billion times higher than at synchrotrons, the XFEL beam destroys the sample just after the emission of its diffraction signal in a process called “diffraction before destruction”. While this firepower allows the characterization of smaller crystals than ever (micro or even nanocrystals), the sample needs to be refreshed after each shot and the collection of a full dataset requires series of thousands of crystals. Also, crystal cryocooling is no longer necessary and this type of analysis is mostly performed at room temperature. In these near-to-physiological conditions and thanks to the temporal resolution of XFEL pulses (<100 fs), the dynamics of biological systems (conformational changes, catalytic events) can be probed in crystallo. Similar protocols have been implemented at synchrotron facilities and are widely accessible. To take advantage of these new approaches, crystal growers need to adapt current protocols mainly devoted to the production of large single crystals, to the preparation of showers of microcrystals with homogeneous size and diffraction quality. Based on crystal growth principles and examples of alternative crystallization approaches including advanced crystallization control or microfluidics technologies [1,2,3], we will describe new routes of sample preparation and crystal delivery for serial crystallography.

### References

- [1] de Wijn, Rollet et al. Monitoring the production of high diffraction-quality crystals of two enzymes in real time using in situ dynamic light scattering. *Crystals* (2020), 10: 65-77.
- [2] de Wijn et al. A simple and versatile microfluidic device for efficient biomacromolecule crystallization and structural analysis by serial crystallography. *IUCrJ* (2019), 6: 454–464.
- [3] de Wijn, Rollet et al. Crystallization and structure determination of an enzyme:substrate complex by serial crystallography in a versatile microfluidic chip. *Journal of Visualized Experiments* (2021), 169: e61972.