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Delivery methods for free electron lasers: Direct protein crystallization on solid supports economizes sample consumption in serial femtosecond crystallography

Nadia L Opara^{1,2}, Isabelle Martiel¹, Stefan A Arnold², Thomas Braun², Henning Stahlberg² and Celestino Padeste^{1,2}¹Paul Scherrer Institute, Switzerland²University of Basel, Switzerland

Classical crystallography methods based on synchrotrons usually require crystals of relatively large dimensions, i.e. above 5 micrometres. The recent availability of X-ray free electron laser sources providing femtosecond X-ray pulses of ultrahigh brightness facilitate the investigation of nanocrystals. However, in this case data collection must be performed in the mode of the serial crystallography in so-called diffraction-before-destruction regime because the probed area of the sample is destroyed after the interaction with ultra-intense radiation. As thousands of crystals must be provided sequentially to the XFEL beam, selection of an efficient sample delivery system is crucial to minimize protein consumption during data collection. Delivery methods applied so far include steady streaming liquid jets of the crystal suspension. The application of more viscous media like lipidic cubic phase, agarose or hyaluronic acid matrices has also been demonstrated. However, all these methods use significant amounts of the precious protein, which cannot be recovered even if not directly probed. Recent developments of drop of demand methods or fixed targets allow overcoming this problem. But still, handling of the fragile crystals should be gentle or at best avoided. Microfabricated silicon chips with ultrathin Si₃N₄ membranes provide the possibility to regularly position crystals on precisely defined spots by direct crystallization using classical vapor diffusion method. The sample consumption is minimal since crystal growth takes place in nanolitre volume cavities. No additional sample transfer is needed, because X-rays are probing the crystals at the spot where they grew on the X-ray-transparent ultrathin amorphous silicon nitride membranes. Assembly with a second chip to form a hermetically sealed sandwich protects specimens from dehydration and facilitates *in situ* diffraction data collection at room temperature, as demonstrated in a synchrotron experiment providing high-resolution patterns.

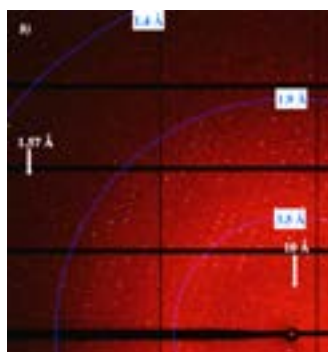


Figure1: a) Lysozyme crystals enclosed in-between silicon nitride membranes and mounted on a synchrotron beamline goniometer. b) High resolution diffraction pattern obtained by *in situ* exposure with synchrotron radiation from the crystal as indicated in (a).

Biography

Nadia L Opara joined the CINA group at the Biozentrum University of Basel and the LMN at PSI in Switzerland in 2014 in the frame of SNI PhD school program, to work on a project aiming at improving sample preparation methods for XFEL-based protein nano-crystallography. Beforehand she completed her Bachelor studies in chemistry and Master program in Molecular Biotechnology at the Lodz University of Technology in Poland.

nadia.opara@gmail.com

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