MS2-P4 Ultrathin membrane chips as X-ray transparent supports for serial crystallography

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Free electron lasers (FELs) allow collection of crystal diffraction data in serial femtosecond crystallography experiments (SFX), which are promising for studies of dynamic phenomena occurring in the femto- to nanosecond range. As the very intense radiation interacting with the sample leads to its destruction data need to be collected in the so-called "diffract-before-destroy" regime [1,2].

Protein crystals usually contain high amounts of water. Loss of the solvent leads to the deformation of the well-ordered structure of the crystal and reduced quality of the diffraction patterns. Therefore, protection from dehydration during measurements is essential. This can be achieved *via* flash cooling, keeping the sample in humidified atmosphere or by enclosure in watertight packaging.

In situ crystallization on microfabricated silicon chips with ultrathin silicon nitride membranes was used to produce protein crystals placed at defined position in required yields. The nanoliter volume cavities in the silicon chip can be filled with crystallization solution in manual or automatic manner (subnanoliter precision dispensing unit). Using vapor diffusion-based crystallization we obtained required size of crystalline sample for time-resolved tests at an FEL, which also allowed screening experiments of different crystal habits on one single chip. Assembly of two chips sealed with double-sided adhesive to form an array of sample chambers proved to be sufficient to preserve high quality of the grown crystals for the FEL experiments [Fig. 1.]. *In situ* crystallization is a promising alternative to the deposition of the crystal suspension on the fixed target support [3] or liquid-jet/injector based methods.

References:

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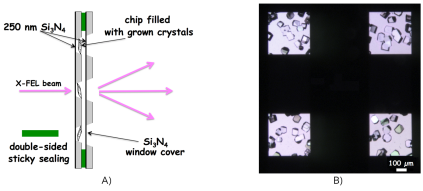

Figure 1. A) Solid support asymmetric sandwich for FEL data collection, B) *In situ* grown lysozyme crystals on nanomembrane chip. Scale bar: 100 µm. The density of the crystals has been optimized for a time-resolved measurement on defined size sample at room temperature.

Keywords: serial femtosecond crystallography, microfabricated silicon chips, in situ protein crystallization