

Cryoprotection without cryoprotectant

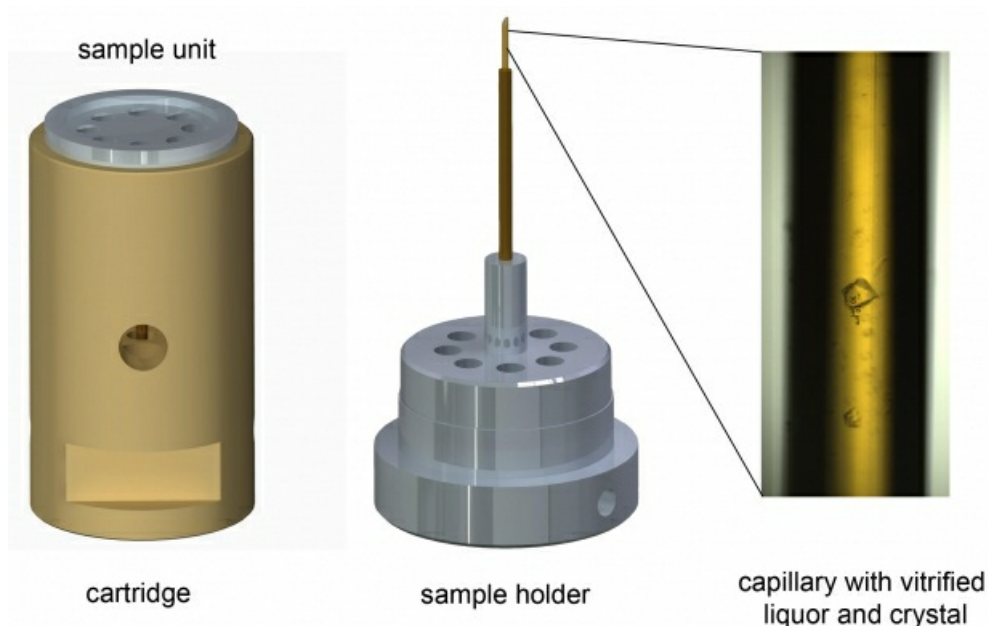
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Once a protein has been crystallized successfully the crystal needs to be cryopreserved for data collection. This task can be very time consuming and may be another obstacle in the already very complex crystallization process, especially for membrane proteins. Alternatively high pressure cooling (HPC) could be used to simplify the workflow with one general concept.

In cooperation with Leica Microsystems Vienna a setup was created for reliable and very fast high pressure cooling of protein crystals in capillaries. The setup consists of a cylindrical cartridge as support that configures the nitrogen flow during vitrification. And a sample unit with a copper pin that holds the crystal in a polyimide capillary. The assembly of cartridge and sample unit with capillary is cooled and dropped into a dewar with liquid nitrogen (on the left in the Figure below). After cooling the sample unit is elongated and separated from the cartridge, simultaneously. The complete sample holder (middle position below) with crystal can now be stored as usual within a cryovial. The vitrified mother liquor and crystal appear completely clear (right position below). The samples can be automatically mounted at the synchrotron as they fit the SPINE standard. Crystals of hen egg white lysozyme and concanavalin A were successfully frozen at 200 MPa and yielded data sets to a resolution of 1.45 Å and 1.35 Å with mosaicities of 0.165 ° and 0.173 °, respectively.

Extensive analysis was done to describe the useful working range of HPC in capillaries with 250 µm inner diameter. Three different 96 well crystallization screens that are most frequently used in our ESFRI-Instruct Core Centre were analyzed for the formation of amorphous ice in the freezing setup described above. More than 85 % of the screening conditions were directly suited for HPC. This means a drastic improvement for most of all crystals that suffered from improper freezing before.

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