SWISSMX CM Supports are designed for micro-crystal sample mounting at synchrotrons and XFELs. The MXCM chip offers low background and a simple loading procedure for both small-scale vapour diffusion or large-scale batch grown crystals. For serial crystallography applications, the ideal sample is a clean (precipitate-free) dispersion of homogeneous 10-20 µm crystals (longest dimension) at a concentration of $1x10^6$ crystals/mL. However, a wide range of samples can be loaded for other applications with a lower size limit of approximately 2 µm. The chip format is primarily designed for cryogenic use at synchrotrons or XFELs, however, room-temperature is possible under a humidity stream, or by the addition of an enclosure.

Contents of this pack:

- 10 SWISSMX CM Support assemblies. Includes the SWISSMX CM Support attached to a base and pin.
- 10 cryo-vials.
- Blotting paper strips (Whatman Chromatography paper 1CHR).

Other materials necessary:

- Micropipettes and tips, typically P10 or P2.
- Sample: Protein crystals in solution .
- Any buffers or cryoprotectants as necessary for a given sample.
- Liquid Nitrogen and Cryoware for freezing samples.
- A pin wand
- A light microscope

The supports have two distinct sides [Figure 1(a)]: well and flat. These side are created by the manufacturing process. These sides can be distinguished by reflecting light from the chip surface. On the well side only the film window will reflect light, whereas the on the flat side, the entire chip surface is reflective [Figure 1 (b and c)]. The importance of the sides is that, as a by-product of the way that the chips are manufactured, the well side of the chip is highly hydroscopic i.e. it naturally draws all available solution from the surrounding region to it. Therefore, it is easier to load crystals on the flat-side and blot away excess solution from the well-side. Although, crystals can be successfully loaded onto both sides of the support.

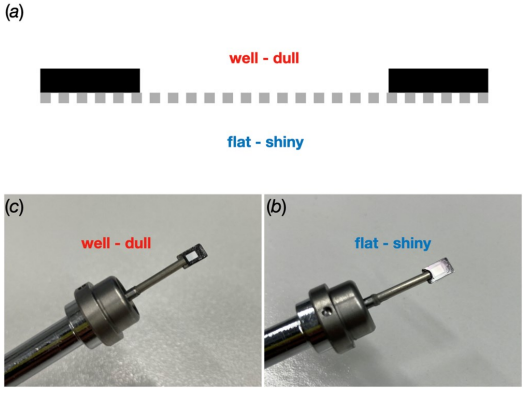

Figure 1: Overview of SWISSMX CM support. (*a*) Schematic of support with side labelled. (*b*) and (*c*) Images showing the reflective properties of the dull and shiny chip sides, respectively.

Loading—from Batch

- Place 1 µL of the crystallisation buffer, i.e. the combination of protein and precipitant solution that the crystals grew in plus cryo-protectant if required, onto the well-side of the chip [Figure 3(b)].
- Place 1 µL of resuspended crystal slurry with cryo protectant, if needed [Figure 3(c)]. Use the pipette tip to ensure that the entire chip window is covered in solution taking care not to touch the film surface. An ideal crystal concentration will depend on the crystal size, for best results $1x10^6$ crystals/mL for 10-20 µm crystals (longest dimension).
- Depending on the buffer components, the solution on the well-side will begin to draw the excess solution from the flat-side [Figure 3(d) and (e)]. Aid this transfer by gentle removing solution from the well-side with blotting paper until no more solution remains on either surface.
- Solution typically collects in the corners of the well on the well-side [Figure 1(e)].
- Ensure that all this solution has been blotted away [Figure 3(f)]. Once this has been achieved, plunge cryo-cool in liquid nitrogen.
- The whole loading procedure for a single chip should take 80-100 seconds depending on the components in the crystallisation solution.

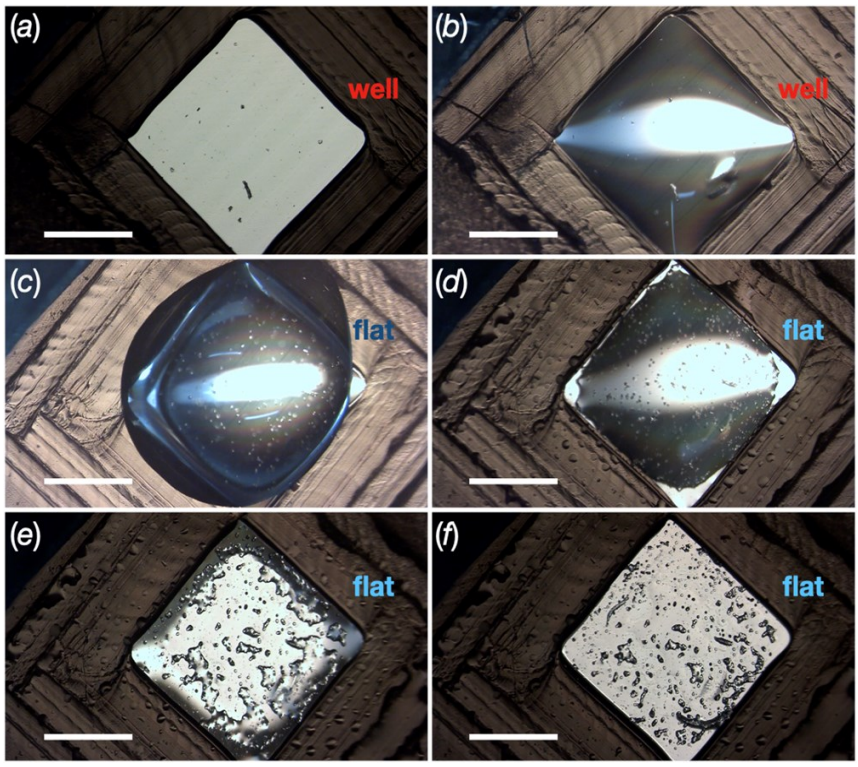

Figure 3. Blotting HEWL micro-crystals grown in batch onto the flat-side. An empty chip (*a*), crystallisation buffer loaded to the well-side (*b*), micro-crystal slurry loaded to flat-side (*c*), blot and hydroscopic force removes most of buffer [(*d*) and (*e*)], blotted chip ready for cryo-cooling (*f*). The scale bar is 1 mm.

Loading—from Vapour Diffusion

- Re-suspend crystals grown in a vapour diffusion crystallisation plate with 1 µL of crystallisation buffer, i.e. the combination of protein and precipitant solution that the crystals grew in, plus cryo-protectant if required, [Figure 4(b)].
- Remove 1 µL of crystal slurry.
- Place 1 µL of the same buffer used in Step 1 onto the well-side of the chip [Figure 4(c)].
- Place the 1 µL of crystal slurry from Step 2 onto the flat-side of the chip [Figure 1(c)]. Use the pipette tip to ensure the entire chip window is covered in solution taking care not to touch the film surface.
- Depending on the buffer components, the solution on the well-side will begin to draw the excess solution from the flat-side [Figure 4(d)]. Aid this transfer by gentle removing solution from the well-side with blotting paper [Figure 4(e)] until no more solution remains on either surface.
- Solution typically collects in the corners of the well on the well-side. Ensure that all this solution has been blotted away [Figure 1(f)]. Once this has been achieved, plunge cryo-cool in liquid nitrogen.

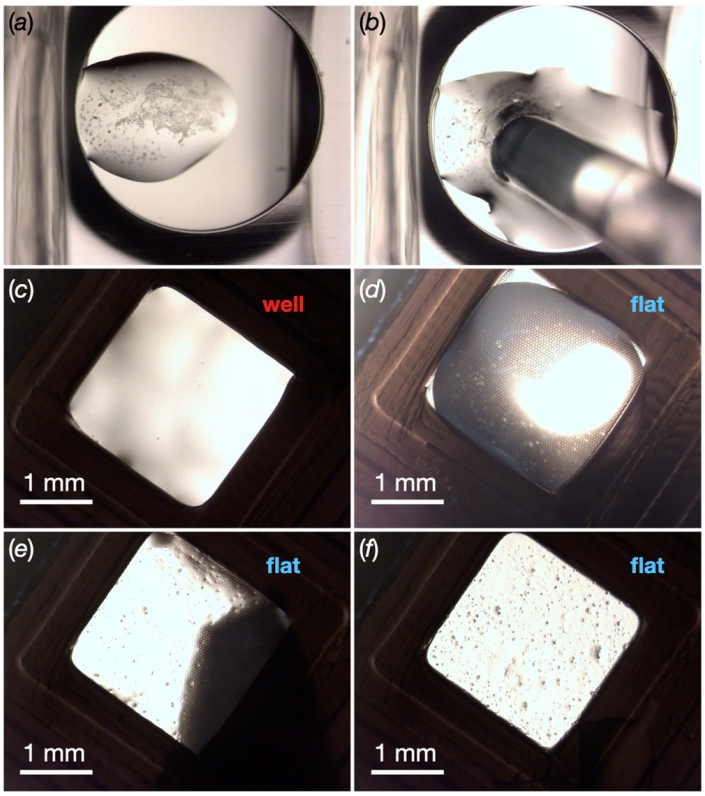

Figure 4. Blotting 3CL micro-crystals grown in 96-well, sitting-drop, vapour diffusion plates onto the flat-side. Crystals in the well in crystallisation plate (*a*), add crystallisation buffer to re-suspend the crystals (*b*), crystallisation buffer loaded into the well-side (*c*), micro-crystals from the plate loaded onto the flat side (*d*), blotting away solution from the well-side (*e*), blotted chip ready for cryo-cooling (*f*).

Practical Tips and tricks:

- Removing all solution from the edges of the chip is essential to prevent the chip cracking upon cryo-cooling.
- A comfortable range of deposition volume on a chip is between 0.5 and 2 µL. The crystal density can be increased by multiple depositions if required.
- A positive displacement pipette can facilitate deposition.
- The blotting time can depend on the viscosity of the solution. Visual observation, using a light microscope, of the surface of the drop as it recedes through the membrane during the blotting is key.
- 0.1 % DDM to the buffer components can aid blotting.
- Preparation time estimate: by plunge freezing, a Unipuck can be filled in about 10-15 minutes.

Trouble shooting quick reference

Issue	**Causes and Solutions**
Ice rings, streaks, brownish areas on the chips	Cryoprotection is insufficient, possibly due to: A thick liquid layer left on the chip caused by insufficient blotting Too low concentration of cryoprotectant Precipitate retaining too much liquid, increasing cryoprotectant amount may help
Protein crystals are dried out	Blotting too long Waiting too long before cryo-cooling
Poor diffraction	Cryoprotection may be insufficient The blotting may be inadequate Crystals may have dried out Crystals may need a thicker liquid layer with more cryoprotection
Background is high, diffuse rings	A precipitate may retain liquid on the chip Blotting may be insufficient or did not happen at all

Reference:
Agnieszka Karpik, Isabelle Martiel, Per Magnus Kristiansen, Celestino Padeste, Fabrication of ultrathin suspended polymer membranes as supports for serial protein crystallography, Micro and Nano Engineering, Volume 7 (2020),100053, ISSN 2590-0072.