Advanced high throughput capillary plate for protein crystallization

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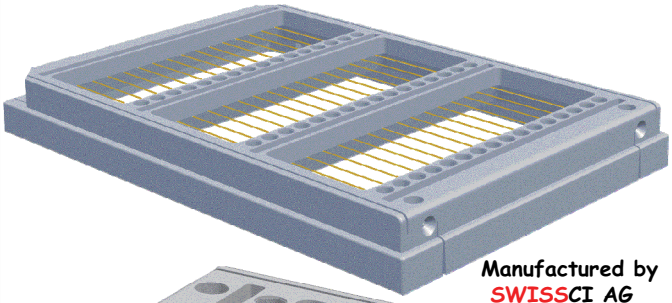
Aims:

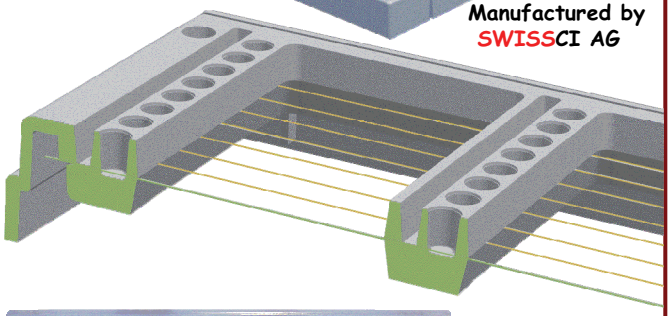
- capillary crystallization plate based on the counter-diffusion method[1]
- applicable for high throughput screening X-tal experiments
- as well as for individually designed X-tal experiments
- compatible with any incubation and imaging system
- easy to work with (loading protein/ solutions, mounting of X-tals).

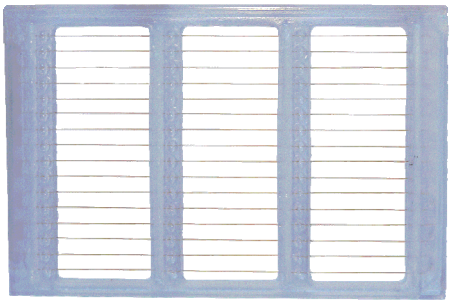
Solution: **Crystalharp**

- SBS formatted capillary plate designed for 48 counter diffusion X-tal experiments
- Manufactured and sold by SWISSCI AG



Manufactured by SWISSCI AG

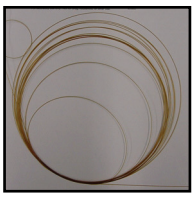




A detailed description on plate handling and plate loading can be found on the leaflet (see below).

Polyimide coated quartz capillary

Capillary internal diameter: 0.1 mm
Capillary length on a plate: 200 cm
Capillary length / experiment: 30 mm
V= 240 nl / experiment
Total of <16 µl of protein needed

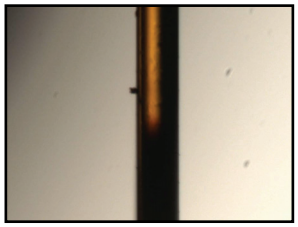


Crystallization using Crystalharp
Plates tested on different proteins
Crystals were taken to the SLS for diffraction analysis

		Diffraction
Membrane Protein (AcrB)		3.7Å
Membrane Protein (X)		10Å
Lysozyme		1.4Å

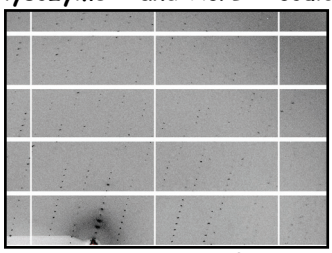
Capillaries used in a cryo stream - no ice formation

Capillaries are sealed and mounted in a glass sleeve on a standard magnetic CrystalCap.

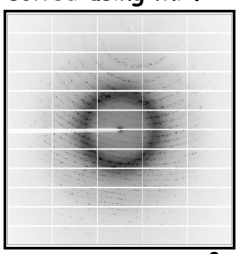


Cryo solution diffused partly through the capillary, cryo-protected part stays clear, non cryo-protected part turns intransparent on freezing.

Data collection was performed under cryocooling in a N_2-gas stream at the PX(X06SA) beamline at the SLS (Villigen, CH). Using the data collected from crystals grown in capillary the structures of lysozyme[2] and AcrB[3] could be solved using MR.



AcrB 3.7Å



lysozyme 1.4Å

Summary and conclusion

Counter diffusion crystallization of macromolecules in capillary is an easy, cost-effective, and practical procedure for obtaining protein crystals suitable for in situ X-ray data analysis. The counter diffusion process has been used to simultaneously screen for optimal conditions for protein crystal growth, and mix in cryogenic solutions in a single capillary tube. Problems harvesting crystals and difficulties in transportation are reduced to a bare minimum. We can show, that crystals grown in capillary diffract to at least the same resolution as the ones grown by vapour diffusion. A 1.4Å dataset for lysozyme and a 3.7Å dataset for AcrB were collected and the structures solved by molecular replacement. Additionally, we observed that capillary grown crystals can be flash-frozen without the need of a cryo protectant. The observation of ice rings was reduced to a bare minimum.

(1) Garcia-Ruiz JM, Methods in Enzymology, 1997, Vol. 368
(2) Cianci M, Helliwell JR, Suzuki A., Acta Crystallogr D, 2008 Dec;64
(3) Pos KM, Schiefner A, Seeger MA, Diederichs K.,FEBS Lett. 2004 Apr 30;564(3)

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