The CRYSTALHARP™ -

an advanced high throughput capillary plate for protein crystallization

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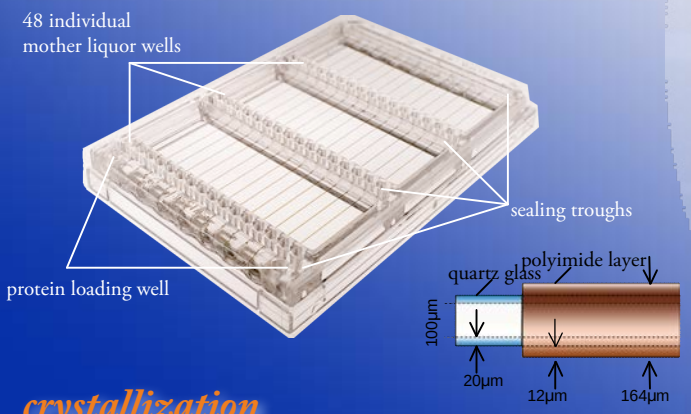
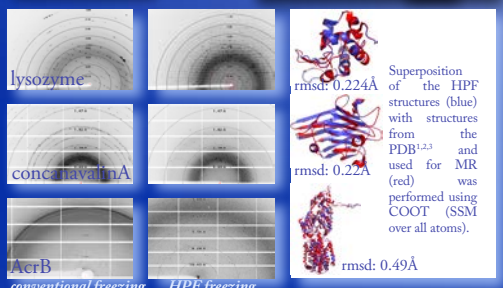


The CrystalHarp™ is a capillary crystallization plate based on the counter-diffusion method. It is designed for 48 high throughput-screening experiments, which require only 20 ul of protein. Furthermore, the CrystalHarp™ can be used to simultaneously screen for optimal crystal growth conditions, for the incorporation of anomalous scattering atoms, and for the addition of cryo protectant in a single capillary tube. Due to it's SBS format the plate is compatible with any incubation and imaging systems and allows *in situ* X-ray diffraction measurements. Alternatively, an individual capillary can be removed from the CrystalHarp™ and the crystal can be analysed 360° *in situ*.

crystallization

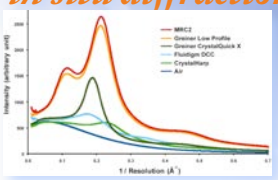
More than 10 soluble and 3 membrane proteins were successfully crystallized based on known vapour-diffusion conditions. In one case the CrystalHarp™ was also successfully used for screening new conditions, showing that the plate is applicable for screening and focussing of crystallization conditions. Additionally, cryo protetcant solutions were added to the precipitation wells simply pipetting through the oil layer. *In situ* diffraction analysis of the CrystalHarp™ shows very low background scattering rendering the plate applicable for plate screening (e.g. synchrotron/PX scanner). Alternatively, a single capillary can be mounted to a magnetic base hold in place with a metal tube and a drop of water.

high pressure freezing

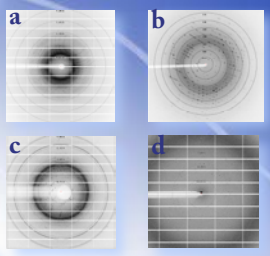


conventional freezing, no cryo protectant — *HPF freezing, no cryo protectant*

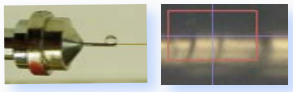
in situ diffraction



Background scattering of various crystallization plates. Figure taken from Bingel-Erlenmeyer et al., CG&D, 2010



Diffraction pattern of a Thaumatin (a), Lysozyme (b), ConcanavalinA (c) and AcrB (d) crystals grown in the CrystalHarp with cryo protectant.



Crystals in capillary of 8 different proteins were high pressure frozen (>200 MPa, Leica EM HPM100) without the addition of cryo protectant and the diffraction compared to conventionally snap frozen capillary grown crystals. After HPF, the capillaries were mounted at -160°C using a microtome (Leica Ultracut EM UC6/FC6). In all cases the abolishment of ice rings and often an improvement in resolution and mosaicity was observed.

Counter diffusion crystallization in capillary is practical and cost-effective method to grow crystals suitable for *in-situ* X-ray analysis. Counter diffusion has been used to simultaneously screen for optimal crystal growth conditions and mix in cryogenic solutions in a single capillary tube. The CrystalHarp™ offers the advantage of *in-situ* diffraction screening and hence reduces problems harvesting crystals and difficulties in transportation to a bare minimum. We show, that crystals grown in capillary diffract to at least the same resolution as the ones grown by vapour diffusion. Studies on HPF protein crystals without addition of cryo protectant result in diffraction pattern devoid of ice rings. These results are congruent with the finding of Kim *et al.*[4]. The development of this plate and its applications constitutes a major step forward towards implementing automated *in-situ* high throughput crystal diffraction screening into a synchrotron beam line environment.

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References
[1] Carpentier *et al.*, 2010, Structure 18(11)
[2] Ahmed *et al.*, 2007, Acta Cryst D 63
[3] Drew *et al.*, 2008, Mol. Membr. Biol. 25
[4] Kim *et al.*, 2005, Acta Cryst D 61