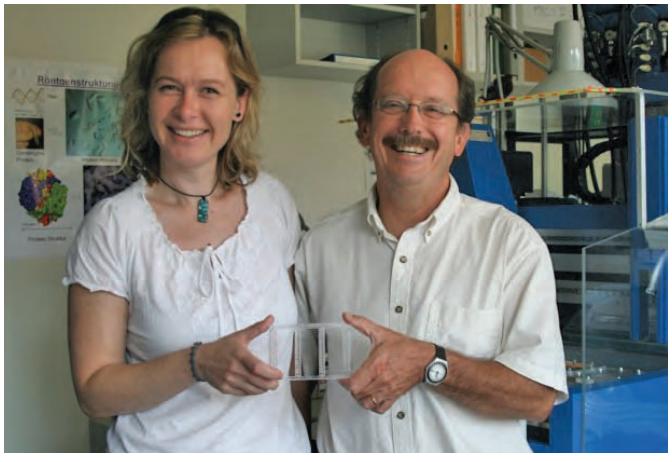
COUNTER DIFFUSION

HIGH QUALITY DIFFRACTION DATA IS A PREREQUISITE TO DETERMINE PHASES OF SUFFICIENT QUALITY AND TO OBTAIN A STRUCTURE FROM A CRYSTALLIZED MACROMOLECULE

Dr Mareike Kurz

The most common method to obtain diffraction data is to take crystals grown by vapour diffusion and manually manipulate them during the steps of cryo protection, plunge cooling and mounting for data collection [1-4]. The manual manipulation steps can be inappropriate for fragile and difficult to handle crystals and might even prevent the acquisition of useable diffraction data.

An alternative is offered by the method of counter diffusion, in which the crystallization is performed in long capillaries, using highly concentrated precipitating solutions to provoke successive nucleation events approaching equilibrium [5-7]. The method of counter diffusion, especially in restricted geometry, allows protein crystallization under nonconvective conditions and therefore mimics microgravity experiments. During the equilibration of the protein and precipitating solution a supersaturated spatiotemporal gradient along the capillary is formed. This creates a continuous nucleation front, which travels along the protein solution and allows crystallization at a favourable concentration at any position in the capillary. This unique nature of the counter diffusion method has the advantage of a much broader screening of variables in one single experiment **(Figure 1)**.



Dr Mareike Kurz and Dr Beat Blattmann, University of Zurich, Switzerland.

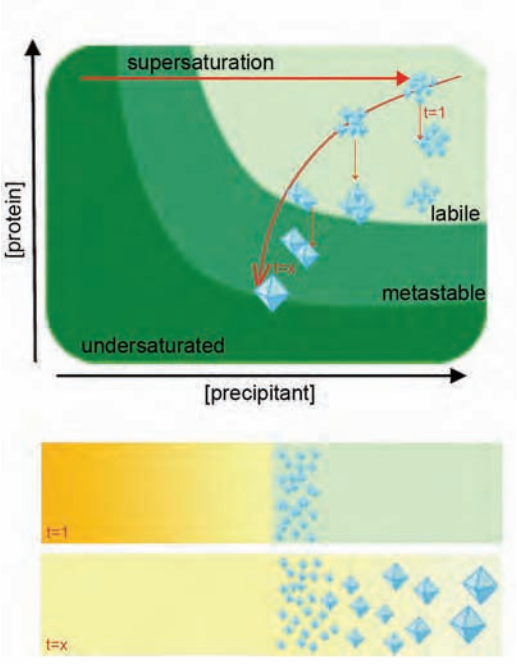


Figure 1. shows the solubility diagram for counter diffusion crystallization. During crystallization, the protein solution is driven to the supersaturated region by reducing the solubility of the macromolecule from the solvent (top). The nucleation moves into the labile region, which reduces the solubility of the protein solution until the metastable region is reached. In a counter diffusion experiment (bottom), the protein and precipitant solutions are placed in front of each other inside a capillary. The two solutions diffuse against each other, forming a spatial–temporal gradient of supersaturation and a continuous nucleation front that travels along the capillary. Figure adapted from (7).

Additionally, the counter diffusion process can be used to screen for optimal conditions for protein crystal growth, to incorporate strong anomalous scattering atoms as well as to add cryogenic solutions in a single capillary tube. Moreover, problems related to harvesting crystals and difficulties in transportation are eliminated.

Over the last decades several tools and devices combining counter diffusion crystallization in capillaries with in-situ diffraction analysis and post-crystallization treatments have been developed and implemented in structural genomic pipelines. Counter diffusion based crystallization tools include microofluidic devices [8-13], microcapillary-based microbatch plastic tubings [14, 15] as well as glass capillaries [5-7, 16, 17]. Furthermore, counter diffusion based crystallization devices that couple visualization and automation for in-situ X-ray diffraction analyses to address high throughput screening have been developed. Worth mentioning are the COC based microchannel crystallization plates, which can be assembled to fit in SBS-format frames [18].

However, most methods and tools available to date (for a review see [19]) are not practical, need large amounts of protein and are far from being useful for today's state-of-the-art nanolitre scale high throughput crystallization facilities. The newest capillary counter diffusion based crystallization device commercially available is the CrystalHarp™ **(Figure 2)**. In comparison to all other devices, the CrystalHarp™ (i) allows counter diffusion crystallization in 48 individual experiments (ii) consumes only 500 nl protein solution for one individual capillary experiment, (iii) can easily be loaded with protein solution and offers simultaneously screening for new and optimal growth conditions, incorporation of anomalous scatterers and addition of cryogenic solutions in a single capillary, (iv) allows addition of precipitating buffer using standard dispensing robots and (v) due to its SBS format can be imaged with standard imaging systems used for sitting drop vapor diffusion experiments. The CrystalHarp™ allows in-situ X-ray diffraction analysis of a crystal by any conventional X-ray source. Otherwise, an individual capillary can be removed from the crystallization plate and the crystal can be analyzed 360° in situ by X-ray diffraction.

An additional advantage of capillary counter diffusion crystallization is the possibility to perform high pressure freezing, hence omitting the tedious procedure to search for a cryo protectant to avoid hexagonal ice formation. Rapid freezing of aqueous specimens at a pressure of 210 MPa (high pressure freezing) reduces or prevents the formation of cubic or hexagonal ice phases and thus avoids ice rings in X-ray diffraction experiments [20-23].

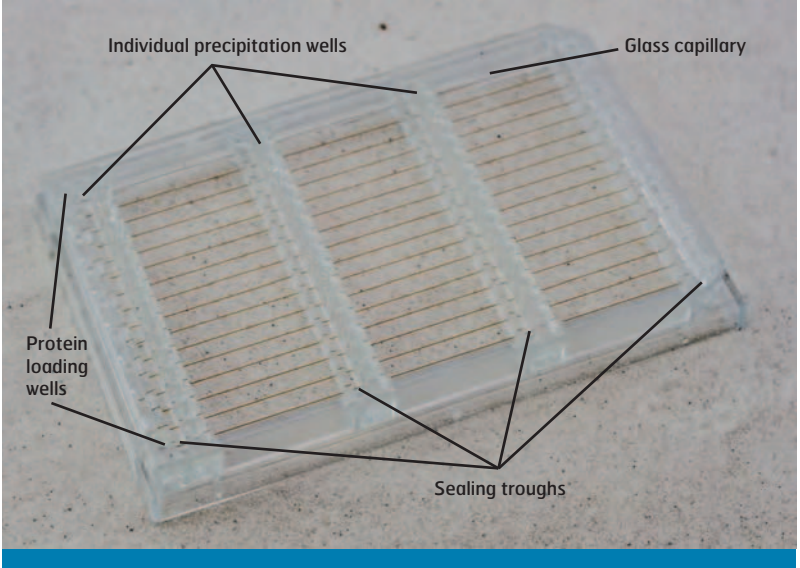
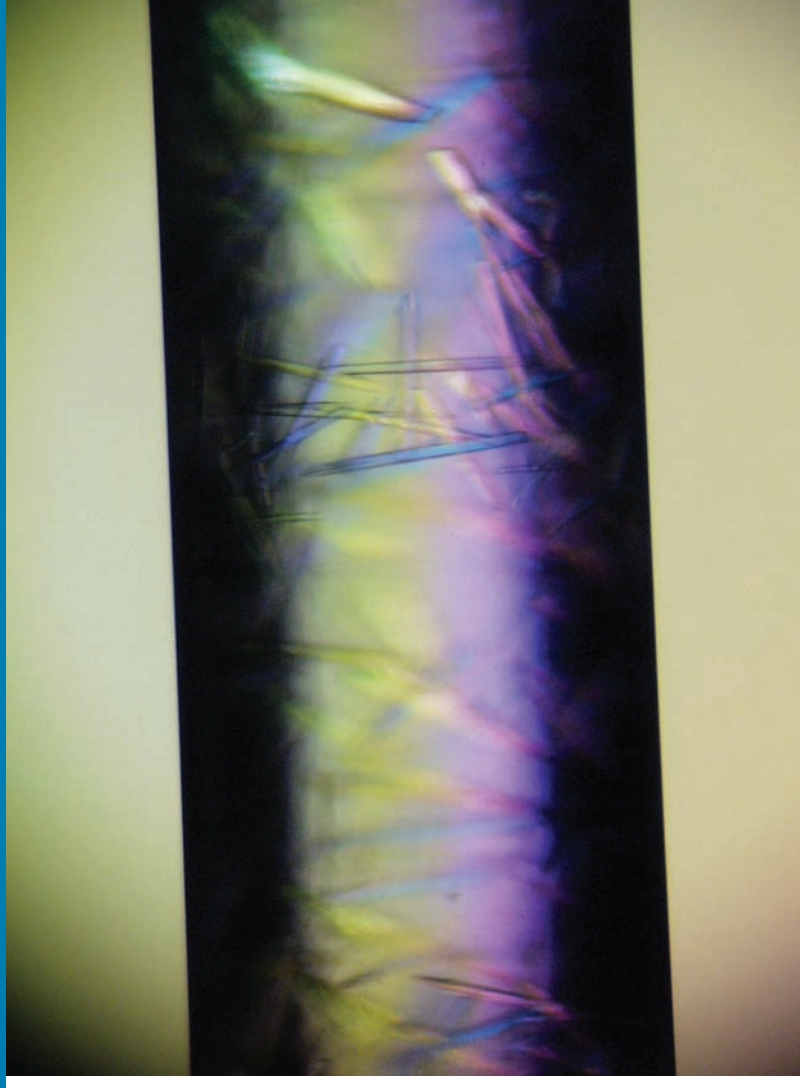


Figure 2. shows the CrystalHarp™ plate, designed for 48 counter diffusion crystallization experiments. The indicated protein loading wells, the 48 individual precipitation wells and the four cutting and sealing troughs to create the single experiments are indicated. Picture taken from (23).



References

1. Berry, I. M., Dym, O., Esnouf, R. M., Harlos, K., Meged, R., Perrakis, A., Sussman, J. L., Walter, T. S., Wilson, J., and Messerschmidt, A. (2006) SPINE high-throughput crystallization, crystal imaging and recognition techniques: current state, performance analysis, new technologies and future aspects, Acta Crystallogr D Biol Crystallogr 62, 1137-1149.
2. Weber, P. C. (1997) Overview of protein crystallization methods, Macromolecular Crystallography, Pt A 276, 13-22.
3. Pflugrath, J. W. (2004) Macromolecular cryocrystallography--methods for cooling and mounting protein crystals at cryogenic temperatures, Methods 34, 415-423.
4. Garman, E. F., and Owen, R. L. (2006) Cryocooling and radiation damage in macromolecular crystallography, Acta Crystallogr D Biol Crystallogr 62, 32-47.
5. Garcia-Ruiz, J. M., and Ng, J. D. (2007) Counter-Diffusion Capillary Crystallization for High-Throughput Applications, In Protein Crystallization Strategies for Structural Genomics (Chayen, N. E., Ed.), pp 111–126, La Jolla: International University Line.
6. Gavira, J. A., Toh, D., Lopez-Jaramillo, J., Garcia-Ruiz, J. M., and Ng, J. D. (2002) Ab initio crystallographic structure determination of insulin from protein to electron density without crystal handling, Acta Crystallogr D Biol Crystallogr 58, 1147-1154.
7. Ng, J. D., Gavira, J. A., and Garcia-Ruiz, J. M. (2003) Protein crystallization by capillary counterdiffusion for applied crystallographic structure determination, J Struct Biol 142, 218-231.
8. Hansen, C. L., Skordalakes, E., Berger, J. M., and Quake, S. R. (2002) A robust and scalable microfluidic metering method that allows protein crystal growth by free interface diffusion, Proc Natl Acad Sci U S A 99, 16531-16536.
9. Zheng, B., Roach, L. S., and Ismagilov, R. F. (2003) Screening of protein crystallization conditions on a microfluidic chip using nanoliter-size droplets, J Am Chem Soc 125, 11170-11171.
10. Zheng, B., Tice, J. D., and Ismagilov, R. F. (2004) Formation of Arrayed Droplets by Soft Lithography and Two-Phase Fluid Flow, and Application in Protein Crystallization, Adv Mater 16, 1365-1368.
11. Zhou, X., Lau, L., Lam, W. W., Au, S. W., and Zheng, B. (2007) Nanoliter dispensing method by degassed poly(dimethylsiloxane) microchannels and its application in protein crystallization, Anal Chem 79, 4924-4930.
12. Gerdts, C. J., Tereshko, V., Yadav, M. K., Dementieva, I., Collart, F., Joachimiak, A., Stevens, R. C., Kuhn, P., Kossiakoff, A., and Ismagilov, R. F. (2006) Time-controlled microfluidic seeding in nL-volume droplets to separate nucleation and growth stages of protein crystallization, Angew Chem Int Ed Engl 45, 8156-8160.
13. Du, W., Li, L., Nichols, K. P., and Ismagilov, R. F. (2009) SlipChip, Lab Chip 9, 2286-2292.
14. Kalinin, Y., and Thorne, R. (2005) Crystal growth in X-ray-transparent plastic tubing: an alternative for high-throughput applications, Acta Crystallogr D Biol Crystallogr 61, 1528-1532.
15. Yadav, M. K., Gerdts, C. J., Sanishvili, R., Smith, W. W., Roach, L. S., Ismagilov, R. F., Kuhn, P., and Stevens, R. C. (2005) In situ data collection and structure refinement from microcapillary protein crystallization, J Appl Crystallogr 38, 900-905.
16. Garcia-Ruiz, J. M. (2003) Counterdiffusion methods for macromolecular crystallization, Methods Enzymol 368, 130-154.
17. Ng, J. D., Stevens, R. C., and Kuhn, P. (2008) Protein crystallization in restricted geometry: advancing old ideas for modern times in structural proteomics, Methods Mol Biol 426, 363-376.
18. Ng, J. D., Clark, P. J., Stevens, R. C., and Kuhn, P. (2008) In situ X-ray analysis of protein crystals in low-birefringent and X-ray transmissive plastic microchannels, Acta Crystallogr D Biol Crystallogr 64, 189-197.
19. Otalora, F., Gavira, J. A., Ng, J. D., and Garcia-Ruiz, J. M. (2009) Counterdiffusion methods applied to protein crystallization, Prog Biophys Mol Biol 101, 26-37.
20. Fourme, R., Girard, E., Kahn, R., Dhaussy, A. C., and Ascone, I. (2009) Advances in high-pressure biophysics: status and prospects of macromolecular crystallography, Annu Rev Biophys 38, 153-171.
21. Kim, C. U., Chen, Y.-F., Tate, M. W., and Gruner, S. M. (2008) Pressure-induced high-density amorphous ice in protein crystals, J Appl Crystallogr 41, 1-7.
22. Mishima, O., Calvert, L. D., and Whalley, E. (1984) 'Melting ice' I at 77 K and 10 kbar: a new method of making amorphous solids, Nature 310, 393 -395.
23. Kurz, M., Blattmann, B., Kaech, A., Briand, C., Reardon, P., Ziegler, U., and Gruetter, M. G. (2012) High-throughput counter-diffusion capillary crystallization and in situ diffraction using high-pressure freezing in protein crystallography, J Appl Crystallogr 45, 999-1008.