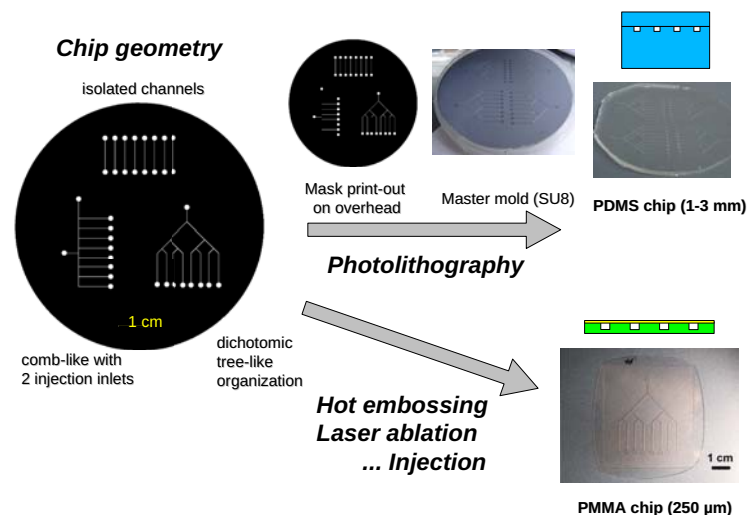


Project background

The ChipX project deals with the optimization of microfluidic tools for structural biology, structural genomics and drug design. It is the continuation of a pluridisciplinary research initiated in 2004 and associated a team of biochemists specialized in functional and structural studies of biomolecules, with two teams of physical-chemists experts in material science, microfabrication and microfluidics. The idea is to take advantage of the microfluidic technology to facilitate biomolecule crystal production that is a rate-limiting step in structural biology projects. This collaborative work has already led to a functional prototype that validates the concept of a microfluidic chip based on counter-diffusion, a powerful crystallization method that is still seldom used [1,2]. In the present project we intend to implement the current chip concept in order to deliver a ready-to-use, user-friendly and cost-effective device designed for screening and optimization of crystallization conditions, as well as for monitoring crystal growth and performing X-ray diffraction analyses *in situ*.

Chip design & fabrication

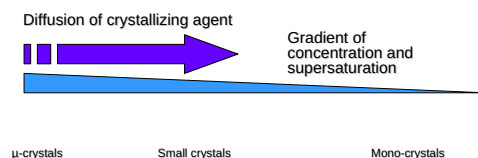
Several channel geometries were tested and chips were manufactured using different materials. Polydimethyl-siloxane (PDMS) was employed first to enable fast prototyping by photolithography. Other polymers were used, e.g. polymethyl-metacrylate (PMMA) or cyclo-olefin copolymer (COC), to produce thinner devices by either laser ablation or hot-embossing. The next step will involve injection molding.



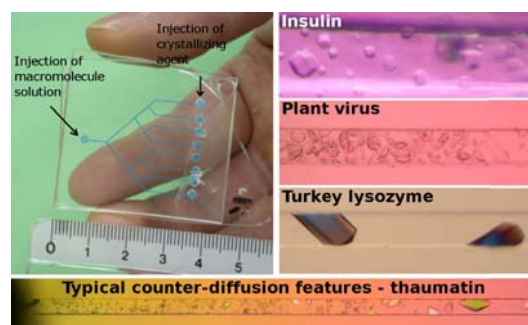
Growing crystals by counter-diffusion

Chips were designed to implement the counter-diffusion method which is well known for its efficiency based on a self-optimizing process [5]. The protein is filled in an elongated crystallization chamber (capillary or channel). By diffusing a crystallizing agent through it, one creates a gradient of concentration and a broad range of supersaturation states.

Principle



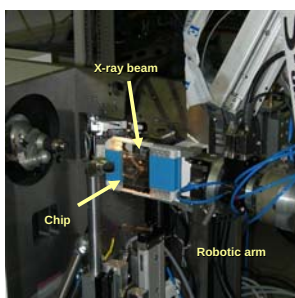
Examples



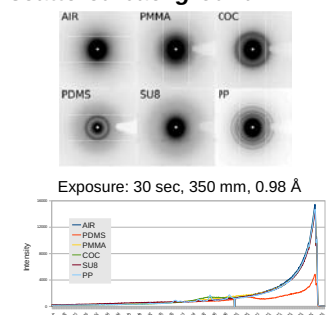
In situ crystallographic analysis

X-ray analyses are performed on beamline FIP-BM30A at ESRF, Grenoble. Samples (materials or chips) are held in the beam by the arm of a robot (Stäubli, France) dedicated to crystal analysis in microplates. Material composition and thickness determine the characteristics of the chip such as rigidity, X-ray absorption and diffusion (see scattered background).

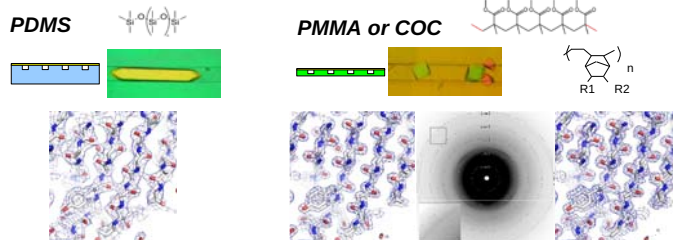
Beamline setup



Chip materials: comparison of scattered background



Effect of chip material on thaumatin crystal analysis



Data collection (~25°C)

Chip	PDMS	PMMA	COC
Nb of images	20	20 + 20	100
Distance	300 mm	300 mm	250 mm
Oscillation	2°, 180 sec	1°, 30 sec	0.5°, 20 sec
Resolution range	2.8 – 20 Å	1.85 – 20 Å	1.65 – 20 Å
Mosaicity	0.09°	0.07°	0.07°
Completeness	84.2 % (87.3 %)	95.9% (86.5%)	91.4% (88.5 %)
Rsym	12.9 % (22.6 %)	5.7% (15.1%)	10.2% (47.8 %)
I/σ	9.0 (5.3)	13.4 (4.9)	8.9 (2.2)

References

[1] C. Sauter, B. Lorber, A. Théobald-Dietrich, R. Giegé, C. Khan-Malek, B. Gauthier-Manuel, G. Thuillier, R. Ferrigno. Dispositif microfluidique pour la cristallisation et l'analyse cristallographique de molécules. *French patent application* FR 06/06583 (July 19, 2006).

[2] C. Sauter, K. Dhoubi and B. Lorber (2007). From macrofluidics to microfluidics for the crystallization of biological macromolecules. *Crystal Growth Des.* 7, 2247-2250.

Perspectives...

- ✓ Optimization of chip design and production => cheap and user-friendly device
- ✓ Automation of chip setup => standardization for high throughput screening
- ✓ Automation of *on chip* crystal analysis => "FEDEX" crystallography