ChipX : Microfluidic chips for the crystallization and the structural analysis of biomolecules

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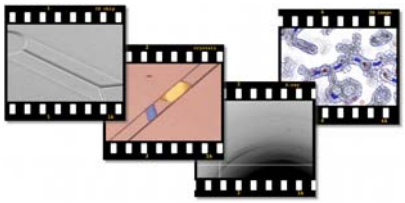
Project background

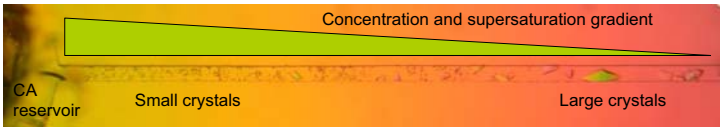
The ChipX project is focussed on the optimization of microfluidic tools for structural biology, structural genomics and drug design. It is a continuation of a pluridisciplinary research initiated in 2004 that associated a team of biochemists specialized in functional and structural studies of biomolecules, with two teams of physical-chemists experts in material science, microfabrication and microfluidics. The basic idea was to take advantage of the microfluidic technology to bypass the major difficulties encountered during biomolecule crystallization that is the most delicate and rate-limiting step of many structural biology projects. This collaborative work already led to a functional prototype that validates the concept of microfluidic chip based on counter-diffusion, a powerful crystallization method that is still seldom used [1].

ChipX concept: from the biomolecule to its 3D structure

In the present project we intend to implement our current chip concept in order to deliver a ready-to-use, user-friendly and cost-effective device designed for screening and optimizating crystallization conditions of biomolecules, as well as for monitoring crystal growth and performing X-ray diffraction analyses *in situ* in order to determine their 3D structure.



Optimization of on chip crystallization

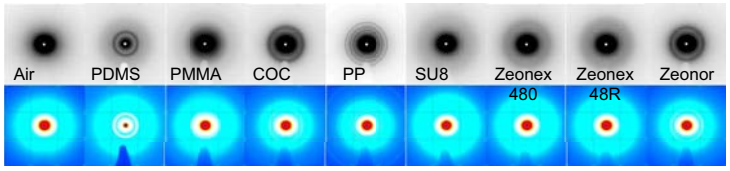


Chips were designed to implement the counter-diffusion method which is well known for its efficiency based on a self-optimizing process: the crystallizing agent (CA) diffuses through an elongated crystallization chamber (the microfluidic channel), creates a gradient of concentration and each assay samples a broad range of supersaturation states. The longer the channel, the broader the screening. This is why new chip generations include channels that are 3-4 longer than first prototypes (15 mm => 60 mm).

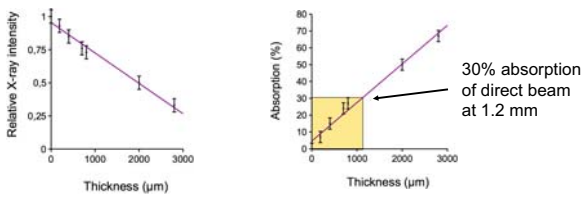
Optimization of on chip crystal analysis

The step following crystallization is crystal characterization by X-ray diffraction. Therefore, the chip material and its thickness must be compatible with this type of analyse both using synchrotron radiation and conventionnal laboratory X-ray sources. Extensive material tests (X-ray scattering and absorption) have been performed and PMMA and COC provide a good compromise (thickness/transparency *vs* chip rigidity) [2].

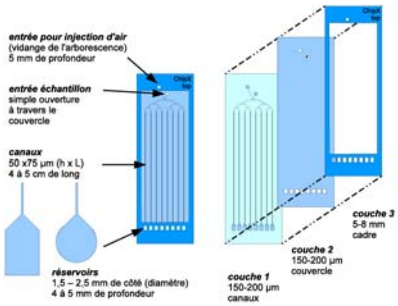
Material scattering background at λ=0.98 Å (FIP/BM30 beamline / ESRF)



X-ray absorption *vs* COC thickness at λ=1.54 Å (ARN – IBMC – lab source)



New ChipX design



ChipX-v0.3-5 – made at INL – hot embossing

2 or 3 COC layers:
- *Cover (2.7mm) + channels (2.7mm)*
- *Cover (2.7mm) + channels (0.7mm)*
- *Cover (0.7mm)+ channels (0.7mm) + Frame (2mm)*

Long channels for better screening:
5 cm x 75 µm x 75 µm
Reduced dead volumes
Integrated set of guide marks

ChipX-v1.0 – made at FEMTO – injection moulding

3 PMMA / PP layers :
cover (150-250 µm) + channel (400 µm) + frame (2-3 mm)
Long channels for better screening:
5 cm x 50 µm x 50 µm
Reduced dead volumes
Disconnected chambers
Integrated set of guide marks

Compatible with standard robotics
SBS format :
up to 4 chips on a SBS holder
To be used with pipetting stations
To be used for automated X-ray synchrotron analysis

ChipX deliverables

✓ Selection of appropriate materials	OK
✓ Optimized chip design	OK
✓ Fabrication by hot embossing	OK
✓ Fabrication by injection moulding	OK
✓ Large scale production	in progress
✓ Fonctional test on biological samples	in progress
✓ Negotiation with potential industrial partners	in progress

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* FR 06/06583 (July 19, 2006).

[2] K. Dhouib, C. Khan Malek, W. Pfleging, B. Gauthier-Manuel, R. Duffait, G. Thuillier, R. Ferrigno, L. Jacquamet, J. Ohana, J.L. Ferrer, A. Théobald-Dietrich, R. Giegé, B. Lorber & C. Sauter. Microfluidic chips for the crystallization of biomacromolecules by counter-diffusion and on-chip crystal X-ray analysis. Lab-on-a-Chip (2009), 9: 1412 - 1421.