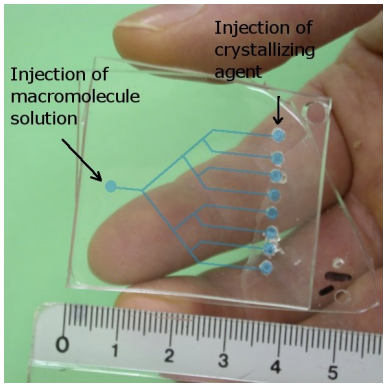
Experimental report:

Data collection with crystals inside a microfluidic chip

Claude Sauter & Bernard Lorber
ARN – UPR 9002 du CNRS
Institut de Biologie Moléculaire et Cellulaire
15 rue René Descartes – 67084 Strasbourg

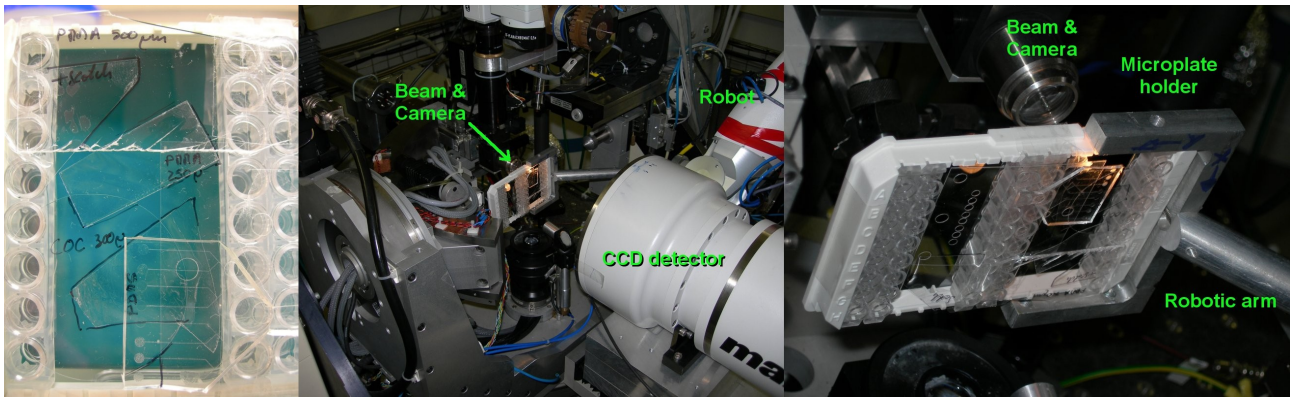


Here we report the results of experiments conducted in April 2006 in the frame of the development of microfluidic chip prototypes (see figure) for biomolecule crystallization. The first goal of the analysis was to compare the background scattering generated by different polymers. The second was to collect diffraction data of crystals grown inside such a microfluidic chip using the Stäubli robot on the CRG FIP-BM30A beamline.

Evaluation of chip materials

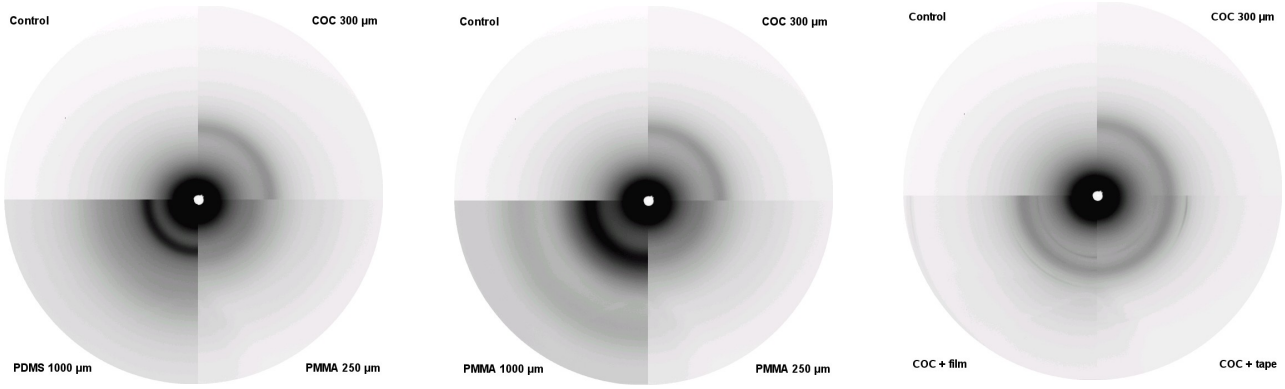
The microfluidic device is made of a polymer layer that contains the channels. These channels are closed by another material that is either a film (ViewSeal film - Greiner BioOne) or a sealing tape (CrystalClear - Hampton Research). X-ray scattering of these materials was analysed. Therefore, sheets of polymers were placed in the x-ray beam either alone or together with a film or a tape. Frames were collected using identical conditions (exposure of 30 sec, distance of 200 mm, λ of 0.800 Å) and compared to the diffuse background recorded in the absence of any material. Following samples with minimal thickness currently used for chip manufacturing were tested:

PolyMethyl MetAcrylate (PMMA) of 1 mm and 250 µm
Cyclo Olefin Copolymer (COC) of 300 µm
Poly DiMethyl Siloxane (PDMS) of 1 mm



The samples (on the left hand side) were taped on a standard microplate (NUNC plate with 96 removable wells) held by the FIP robotic arm (on the right hand side) and placed in the beam at a distance of 200 mm from a MAR CCD detector (center).

Results of the analysis are summarized in the three collages of images below. In the two first views each quarter corresponds either to another material or another material thickness. The third one compares the effect of film and tape on the scattering of COC.



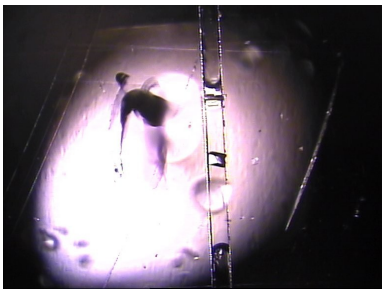
As expected, the thicker the material the stronger the x-ray scattering. 200-300 µm thick COC and PMMA give a comparable but weak background with respect to the control. A priori both are compatible with an x-ray analysis of protein crystals. The presence of a additional layer such as a sealing tape or film does not significantly increase the signal of the material.

Protein crystal analysis

Crystals of hen egg white lysozyme were prepared at IBMC (Strasbourg) using a fast batch protocol. Chips were made of PDMS and PMMA. The microfluidic channels were sealed by CrystalSeal tape. The crystallization conditions designed to produce crystals within 10-20 minutes were the following:

lysozyme (40 mg/ml) in 100 mM Na-acetate-Na pH 4.6, 0.3 M NaCl, 10 % PEG 3350, 0.3 % octyl-glucoside (m/v)

Crystals grown in 2-3 mm thick PDMS devices (see picture) were first analyzed but they didn't yield any diffraction signal. The cause for the loss of diffraction properties was likely crystal dehydration because of the high porosity of PDMS (as suggested by the reduction of the volume of mother liquor and the presence of air in the microfluidic channel). This highlights another disadvantage of PDMS, in addition to its flexibility and its strong interaction with x-rays.



Crystals were also prepared in PMMA chips of the same geometry but with a 10 fold thinner polymer (i.e. 250 µm). After three days, 3 large crystals (filling almost the 100 µm large channels) did not show any sign of dehydration. Two of them were analyzed and diffracted x-rays to ~2 Å resolution.

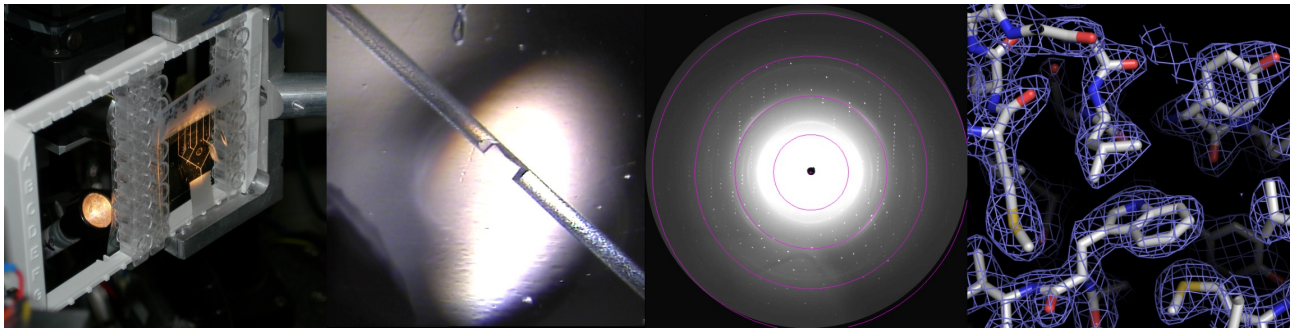
A series of 30 images was collected at room temperature corresponding to a total angle of 30°, the largest rotation achievable with the available experimental setup. This was

sufficient to solve the structure by molecular replacement with a 70% complete dataset. We look forward to using the new FIP/BM30A setup to collect data over a greater angle (120-150°) and to achieve a higher completeness.

X-ray data summary

Wavelength	0.799 Å
Distance	200 mm
Temperature	~25°
Exposure	15 sec
Oscillation	1°
Nb images	30
Unit cell	a = 79.1 Å. c = 38.8 Å
Space group	P43212
Resolution	2.15 – 20 Å
Nb reflections (uniques)	12115 (5040)
Completeness	72.4 % (72.7 %)*
Rsym	7.4 % (20.1 %)*

*High resolution shell: 2.15-2.21 Å



Example of "on chip" crystal analysis. From the left to the right: PMMA prototype taped to a microplate held in the beam by the robotic arm; lysozyme crystal in a microfluidic channel seen by the BM30A alignment camera; diffraction image (oscillation of 30 sec, and 1°, distance of 200 mm, room temperature) with diffraction shells indicated by pink circles at 2.1 Å, 2.8 Å, 4.3 Å and 8.5 Å, respectively; electron density map at 2.15 Å resolution with a partially refined model.

Conclusion

This series of experiments enabled us to identify two materials that are more compatible with the x-ray analysis than PDMS originally chosen for easy and rapid prototyping. It also demonstrated the feasibility of "on chip" data collection. This aspect needs now to be generalized to a greater number of biomolecules. This will to find a way to cryo-cool the crystals. Finally, our results open the possibility to design new prototypes f automated crystal analysis on a synchrotron beamline using a handling robot. These points are included in our proposal submitted in December 2006.