

Diffraction-Capable Microfluidic Crystallization Chips for Screening and Structure Determination

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Abstract

We have produced a series of prototype Diffraction-Capable (DC) microfluidic crystallization chips for in situ x-ray diffraction data collection. In parallel, we have developed tools and hardware for rapid and simple collection of high-quality diffraction data from these devices.

Introduction

The identification of diffraction-quality crystals remains one of the main bottlenecks in crystal structure determination. Despite this, there have been few attempts to combine screening of crystallization reagents with collection of diffraction data. We are developing devices that will allow users to interrogate samples with X-rays without having to manipulate them manually. This will allow rapid access to diffraction data, whilst minimizing the amount of time required for harvesting and mounting of the crystals. Decisions on projects can then be made on the basis of diffraction quality rather than relying on arbitrary judgements of crystal quality by visual inspection.

Material Selection for Device Construction

Commercially available TOPAZ screening chips are made from PDMS, a silicone elastomer. This material absorbs and scatters x-rays strongly. Comparison of the scattering properties of PDMS with a number of carbon-based plastics led to the identification of alternative materials with superior scattering properties. For example, it can be seen from Figure 1 that the scattering profile of plastic 1 is very similar to that of paratone-N oil, a commonly used cryoprotectant. In addition, the scattering profiles of 25% glycerol and water suggested that chips should be designed to minimize the amount of background solvent present in the x-ray beam path.

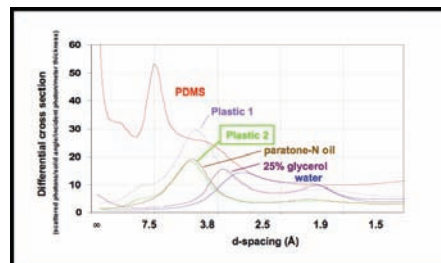


Figure 1. The x-ray scattering properties of candidate plastic materials for chip construction were compared with solutions commonly encountered during x-ray data collection.

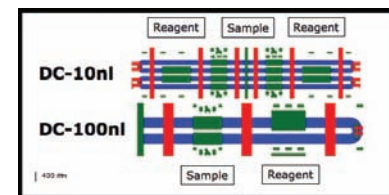
Prototype Chip Fabrication

Using designs based broadly on the commercially available Topaz crystallization chips, we have developed processes for building chips containing chambers of different dimensions.

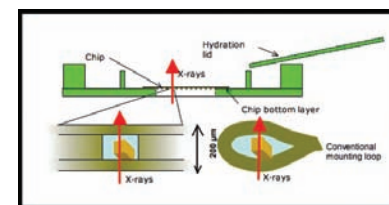
	Thickness	Chamber volume	Max. Crystal dimension in beam
DC-100nl	220 µm	100 nl	100 µm
DC-10nl	180 µm	10 nl	40 µm
X.96	5mm	0.75 nl	10 µm

Figure 2. Comparison of cross-sectional dimensions and sample volumes of Diffraction-Capable chips and Topaz x.96 chips

Diffraction-Capable Chips



Diffraction-capable chips have been designed and fabricated with protein chamber volumes ranging from 10nl-100nl. Crystallization takes place through free-interface diffusion and controlled dehydration

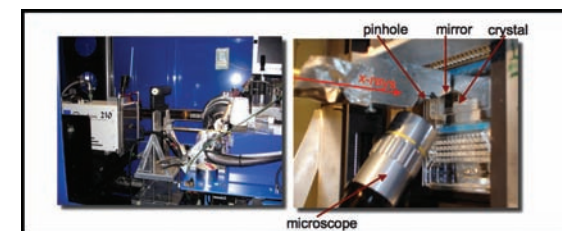


"Hands-free" Data Collection

The chips are mounted on modified TOPAZ carriers with a section removed. Hydration of the chip is maintained through the use of appropriately chosen hydration solutions loaded into a hydration chamber that surrounds the chip. The hydration lid is removed before diffraction scanning of the chip. For rapid diffraction-scanning of crystallization experiments, the chip and carrier are mounted in the plate goniometer.

Hardware for Diffraction Screening

The "plate goniometer" can be readily installed and removed without significant modification to the existing PX endstation at Beamline 8.3.1 at the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory (LBNL). The device occupies the space immediately "down beam" from the conventional crystal mount, so there is space for it in most PX beamlines. The motorized stage will accept SBS-compliant trays that can be turned on their side. A mirror mounted to the frame reflects the image of the crystal into the optical microscope. This arrangement is parallax-free and the crystal is viewed by the microscope from the "point of view" of the x-ray beam.



Example Diffraction Data

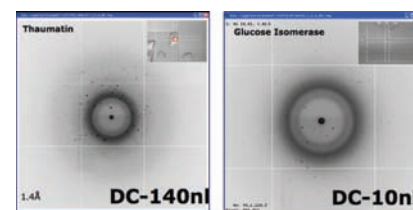


Figure 6. Example room temperature data collected using DC chips mounted on the plate-goniometer on BL 8.3.1. at the ALS. Still shots were taken using 20sec exposure times and a 50µm beam. The red box on the crystal images indicates the position of the beam. Minimum Bragg spacing at the edge of each image is indicated. Data were collected from 190 crystals from 7 chips in 4 hours.

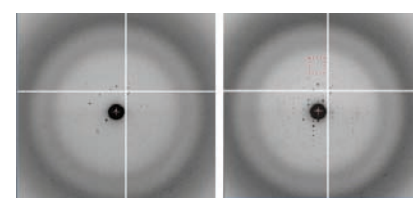


Figure 7. Diffraction data collected from crystals of a bacterial replication protein grown in the presence of A) GTPγS, B) GDP ALFx. Protein was prepared in three different nucleotide states and screened using DC-10nl chips. 170 crystals from over 60 different reagent conditions were screened for diffraction properties in ~3.5 hours, enabling the identification of a narrow region of reagent and ligand space in which diffraction-quality crystals were obtained. Optimization of these conditions is underway

Oscillation Data Collection from Cryo-Cooled Crystals

For collection of oscillation data, sections of chips can be removed for mounting in modified cryopins. Cryoprotectant can be added to the reagent chambers and allowed to diffuse into protein chamber before the chip and pin are cryo-cooled. Crystals can then be aligned in the x-ray beam and data collection takes place using the standard setup at the beamline.



Figure 8. A section of chip mounted in a modified cryopin. Each section of chip contains up to 4 chambers. Each chamber typically contains between 1 and 20 crystals, enabling the simultaneous mounting of ~4-80 crystals per pin.

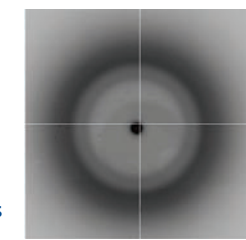


Figure 9. Diffraction image from 5-keto-4-deoxyuronate isomerase. Data, complete to 3.0Å, were collected in-chip at 100K and the structure was solved by molecular replacement

Structure determination from SeMet-derivatized protein



Crystal	Resolution (Å)	I/sigma	Rmerge (%)	Redundanc	% complete
1	30-2.3	15.2 (1.95)	12.0 (53.1)	4.6 (3.1)	87.3 (63.1)
2	30-2.5	16.1 (2.90)	8.2 (42.2)	4.7 (4.7)	90.3 (89.0)
1+2	30-2.5	19.7 (3.34)	11.5 (44.8)	8.3 (6.9)	97.2 (92.8)

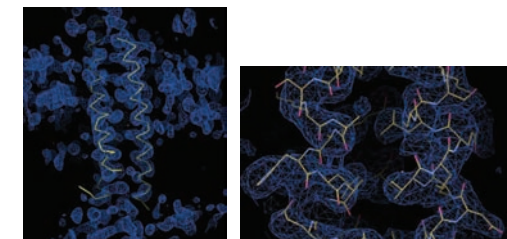


Figure 10. Anomalous diffraction data were collected at the NE-CAT microfocus beamline at the APS using a 20µm beam. Data were scaled and merged from 2 separate SeMet derivatised, crystals of GCN4-N16A located within the same chip section. Crystals were cryo-protected by diffusing glycerol into the sample chamber. Se sites and phases were calculated using SHELX as implemented by HKL2MAP. Figures show solvent-flattened (DM) experimentally phased electron density maps (30-2.5Å) with coordinates from PDB (1rb6) rigid-body refined into the map.

Summary

Prototype microfluidic devices for in-chip diffraction data collection have been developed. Chip materials and fabrication processes have been chosen to minimize background scatter.

Diffraction patterns from completely undisturbed crystals in-chip have been collected using a "tray goniometer" at ALS beamlines 8.3.1 and 12.3.1.

Diffraction scanning of crystals in-chip permits identification of conditions that produce best-diffracting crystals rather just the best-looking crystals

High resolution, complete data can be collected directly from cryocooled crystals located within sections of chip

SeMet anomalous data collected in-chip has been used to calculate high-quality experimental electron density maps