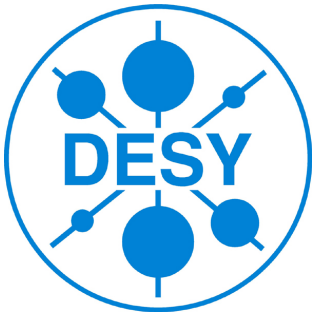

Developing a Tape-Based Delivery System for Serial Synchrotron Crystallography

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Serial protein crystallography is a method for understanding the structure of protein crystals. Performing this high resolution imaging requires a source of radiation in the X-ray regime and a technique which is being explored is serial synchrotron crystallography in which single diffraction patterns are collected and merged into a three dimensional reflection intensity set. The delivery system which enables the protein crystal flow across the beam path can significantly affect the quality of the synchrotron data. A tape-based mechanism has successfully been developed to efficiently deliver protein crystal samples to the synchrotron beamline at PETRA III (P11), and its performance in delivering lysozyme has been characterized.

Contents

1 Introduction

1.1 Serial Protein Crystallography at Synchrotron Beamlines and The Conveyor System

Obtaining structural information about proteins is the focus of structural biology. Using synchrotron radiation rather than ultra fast, femtosecond ranged free electron laser pulses has many advantages for crystals below the size of 10μm for which the synchrotron beam focus size is well matched to the sample. The quality of the diffraction data is good and produces clean patterns and a high number of partial data sets can help solve for the structure of the crystal. In serial crystallography thousands of microcrystals are exposed to X-ray beams and only the single diffraction patterns are retained for eventual indexing and reconstruction into a detailed three-dimensional structural model.

A conveyor type system is being developed at DESY for these protein crystallography experiments. The achievable transport speed matches the expected exposure time. Protein crystal is dropped onto a variable speed conveyor of polyimide (Kapton) tape with holes of 3-5μm in width. The microcrystals are lodged into the holes (Figure 1) which allow for a reduced background from the Kapton in the collected patterns. Kapton is used due to its X-ray transparency and that its intensity peaks do not coincide with the protein samples that are being tested. The the resolution range of the Bragg peaks of Kapton is 17.5$\mathring{A}$ for an exposure time of 60s while with similar conditions lysozyme is 5.5$\mathring{A}$. In this study, lysozyme is the main sample used for testing because of its well known diffraction pattern. Lysozyme provides a good benchmark to use in the study

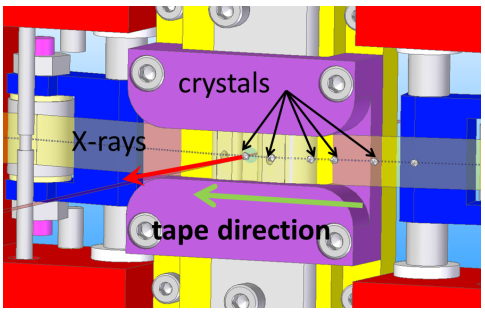


Figure 1: The crystals will be lodged in the holes on the tape and then exposed to a 3μm X-ray.

of synchrotron serial protein crystallography. A simple conveyor belt system is devised which runs a Kapton polyimide tape on it for lysozyme delivery. The tape drive is an efficient method because it can achieve a wide range of repetition rates.

The tape-drive system is projected to be used at the PETRA III Beamline P11 which operates between 5.5 and 30 keV. The focus size of the beam is in the range of 3X4μm which match the holes produced by the laser on the tape.

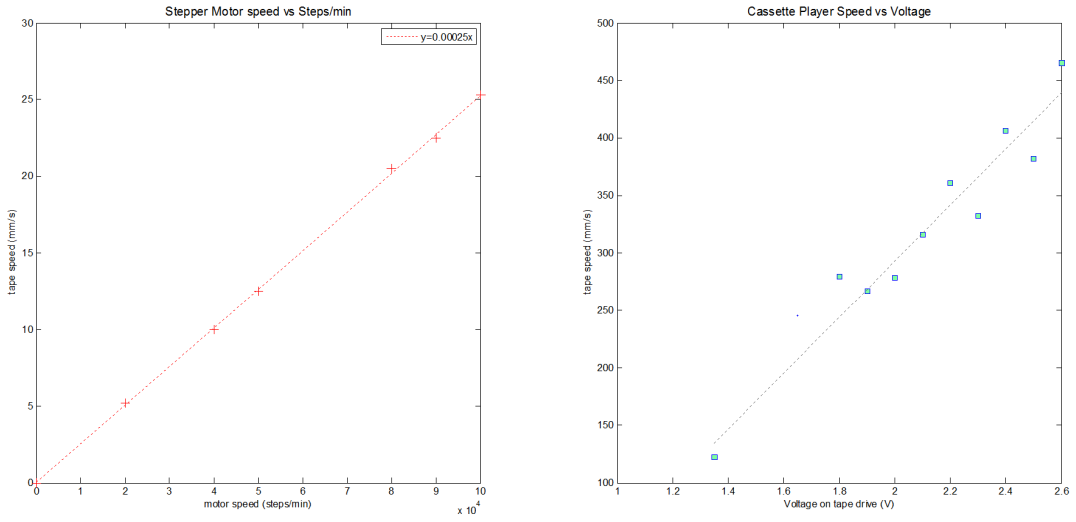
2 Methods and Experimental Setup

2.1 Prototype Tape-Drive

Two types of conveyor system tape-drive prototypes were setup for the studies made in this experiment. The first was devised from a cassette player using three power supplies for the motor to operate. For our purposes, only two were used; the fast forward and reverse function and one for activation and deactivation of the solenoid which connects the motor to the tape. To reverse the direction of the tape the polarity of the supply input on the play function was switched. Lysozyme at the synchrotron beam requires a transit time of about 3ms which would require the tape to move at 10mm/s. The fast forward and reverse function of the cassette player was tested by varying the voltage input. It was found that this prototype runs about 10X too fast at its minimum speed for an accurate simulation of the delivery system, and that the voltage to tape speed relation is not linear and not easily repeatable (Figure 2b).

The second prototype involved using a rotational stepper motor (Lexium SD3) which divides each rotation in an equal number of steps combined with the mount component of the cassette player in order to preserve the fast forward and rewinding control. A software was designed with Python to control the steps per minute of the motor. At 40,000 steps/min this motor runs at a tape speed of 10$\pm$2mm/s which is accurate for the purposes of this experiment (Figure 2a). A regular 90minute audio cassette was modified and its magnetic tape was replaced with polyimide tape that is 6.5mm in width.

Two tape types are tested; regular polyester plastic film with magnetic coating and Kapton polyimide tape.



(a) Stepper Motor (b) Cassette Player

Figure 2: Relationship between speed controls and actual cassette speed for both prototype systems. The stepper motor has a y=0.00025x fit for its steps/min to actual speed in mm/s relation.

2.2 Goniometer and Nozzle Mobility

For control of sample delivery onto the tape, a glass capillary is mounted into a goniometer. The goniometer is from Kleindeik Nanotechnik (MM3A-EM micromanipulator) which provides flexible and precise movement with three degrees of freedom on the glass capillary and movement.

This is important because different pressure and distances between nozzle and tape affect the delivery of the sample. There is integrated fine and coarse displacement in one drive and the goniometer can operate at a high velocity. Its holding force is of up to 1N with ranges down to nanometer precision for all three movements. The glass capillary is mounted onto the goniometer via a custom printed 3D piece which was designed with SolidEdge Software and printed with FormLabs SLA Printer shown in Figure 4. The inner diameter of the section where the nozzle is inserted into in the printed extension is 1.6mm due to the resolution and precision of the printer and to give rotational mobility to the capillary tip. A photograph of the setup is shown in Figure 3 and Figure 4.

2.3 Nozzles and Capillaries

A wide range of nozzles and capillaries were tested for sample delivery. All capillaries used are made of glass and have a polyimide coating with an outer diameter of 360μm and inner diameters ranging from 30-200μm. Anything larger than 200μm becomes too fragile to use. The capillaries are ground into nozzle shape by use of a fiber polishing device (UltraTec Polisher) which uses sanding paper with a rotating capillary insert at a

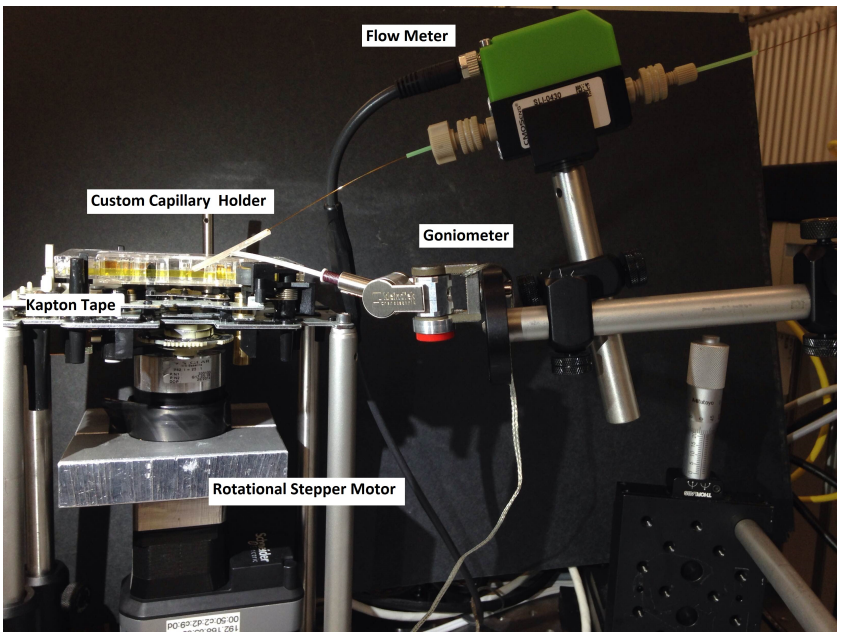


Figure 3: The 3D printed extension allows for control of the capillary with the goniometer. A 200μm inner diameter capillary connects the flowmeter to the syringe pump (not shown) which is 10cm above the plane of the flowmeter.

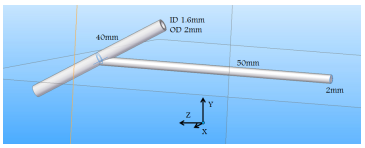


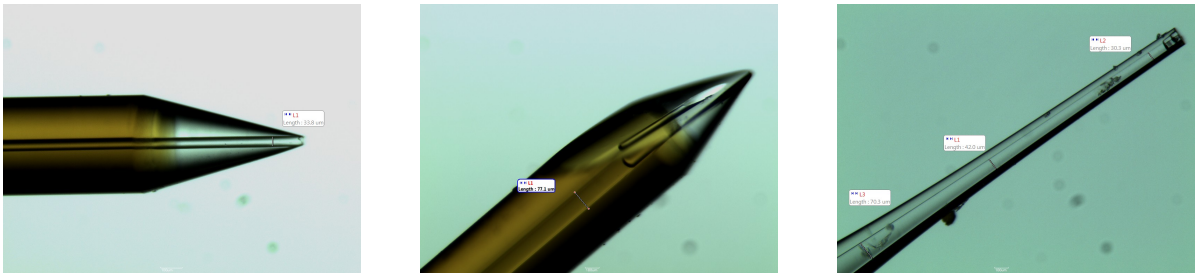
Figure 4: Custom capillary holder printed in white resin has an outer diameter of 2mm and an hollow inner diameter of 1.6mm. The goniometer extension is at 30°with the capillary holder and is solid with an outer diameter of 2mm.

specified and variable angle to create tips. The tip is ground symmetrically at an angle of 70° (Figure 5a). One capillary that was additionally ground asymmetrically (on one side only) to expose an oval opening of the inner capillary at 75°was also tested (Figure 5b).

Lastly, a tip pulling method was used (Figure 5c). With a Sutter Instument Co. Model P-2000 Pipette Puller, the glass capillaries are pulled apart by puller bars and a class 1 CO_2 laser focused at its center. The result is generally a capillary which has tip opening to be much smaller than the inner diameter of the original capillary. This allows for a very precise dosage of samples and for usage of capillaries with large inner diameters. There are five variable parameters on the capillary puller on scales of 0 to 900: heat specification of the output power of the laser (energy supplied to glass), filament scanning pattern, velocity at which the puller bar must be moving before the final pull is executed, the delay which controls the timing of the final pull and the final pull force. The ideal settings for the glass capillaries used is to set filament and pull to

zero, heat settings to 220-500 range, velocity of 30 and a delay of 128. It is also possible to run cycles as to heat and pull single capillaries more than once.

The puller produced tips which have an inner diameter of down to 5μm at the tip. The slanted tip (Figure 5b) is sensitive to its positioning angle; on the setup a rotation control piece was connected to the end part of the capillary for a portion of the experiments.



(a) Evenly ground capillary. ID=30μm.

(b) Ground at 75°. ID=50μm.

(c) Pulled capillary. ID=75μm.

Figure 5: Three nozzle types used for testing sample delivery.

2.4 Pumping Methods and Flow Rates of Sample Delivery

To ensure a constant flow of the sample onto the tape, two pumping systems were tested and chosen based on the amount of pressure needed for sample flow and the prevention of clogging due to the microcrystalline composure of lysozyme. The first was a set of piezo micropumps (Bartels Mikrotechnik). These have a piezo ceramic mounted on a brass membrane that is compressed when a voltage is applied, resulting in a downstroke of the liquid passed through the pump. Because these are extremely low pressure pumps (they support a maximum of 250mbar at 100Hz with water), two piezo pumps were first set up in series so that enough pressure could be achieved. For the piezo pumps, amplitude, wave type and frequency could be controlled. For lysozyme a resonance frequency of about 160Hz produces the maximum output for a given amplitude. Amplitudes of 40-250 volts from peak-to-peak were tested. A SLI-Sensirion FlowMeter that has sensibility $<$ 0.01μL/min was installed between the delivery point and the piezo pumps to monitor the flow rate of the sample. Flow rates lower than 1μL/min are ideal for sample preservation and to achieve minimized stream sizes onto the tape. The piezo pump system proved to show little reproducibility with respect to flowrate, and high frequencies tend to wear out the control box.

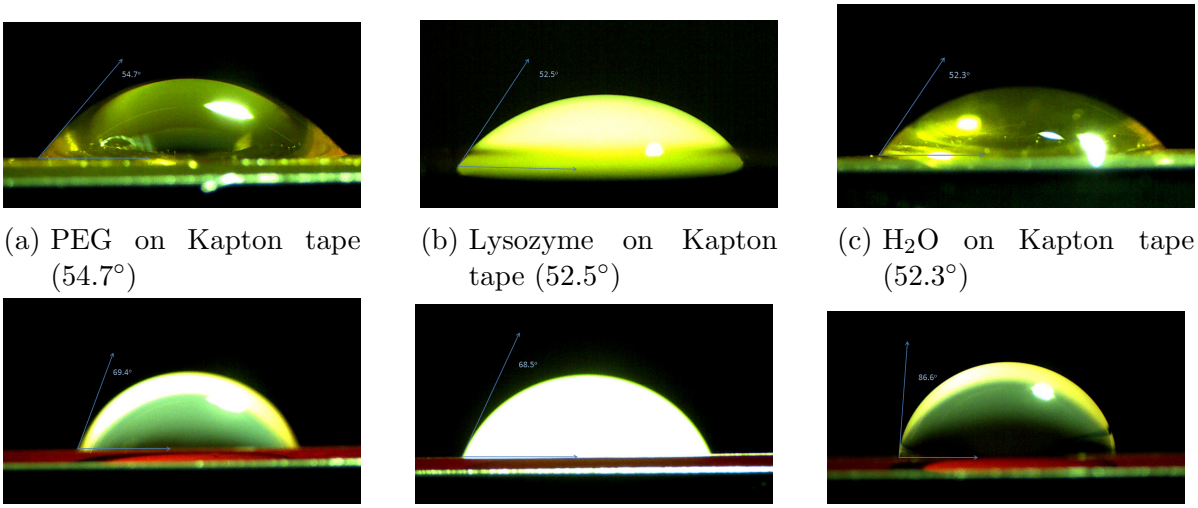
The pumping system which proved most reliable and to show strong correlations with stream width of sample onto the tape was the low pressure Cetoni GmbH neMESYS Syringe Pump. The syringe has a 100μŁ volume capacity. A small volume over a long length is ideal for precise control of the pump. This system makes use of variable sized syringes and a software that allows stable speed and flow rate control. A switch alternates between pumping for sample delivery and refilling the syringe with sample from a reservoir. This syringe pump can achieve flow rates as low as 0.01μL/min which

means that very little sample is consumed during deliveries and that the size of the stream on the tape is minimized.

A second type of syringe pump was tested to avoid settling of the lysozyme micro-crystals: the magnetic stir pump which proved to be difficult to use. The configuration of the syringe includes an opening at the position of the stir magnetic which allows for air to enter when refilling the syringe, subsequently resulting in a foam as the magnet stirs the lysozyme.

2.5 Sample Types

The main sample analyzed is lysozyme, however this was compared to deionoized H_2O and 10% concentrated PEG 4000 (polyethylene glycol). The hydrophobicity affects the width of the stream formed onto the tape; both the sample type and the tape type are variable parameters.



(a) PEG on Kapton tape (54.7°)

(b) Lysozyme on Kapton tape (52.5°)

(c) H_2O on Kapton tape (52.3°)

(d) PEG on magnetic tape (69.4°)

(e) Lysozyme on magnetic tape (68.5°)

(f) H_2O on magnetic tape (86.6°)

Figure 6: Contact angle of regular magnetic coated tape and polyimide tape for three sample types.

The lysozyme was prepared with a precipitant of 4M sodium bromide and a 50mM acetate buffer. The lysozyme to precipitant ratio is 1:3 and is crystallized at 4°C. This yielded crystals of about 4-5μm (Figure 7). Subsequently the lysozyme is vortexed and filtered with a 10μm filter to ensure no crystals aggregate and create clogs in the tubing and nozzle. The contact angle of the three samples with regular magnetic cassette tape and polyimide tape were measured and the magnetic tape shows higher hydrophobicity therefore the polyimide has higher wettability as shown in Figure 6.

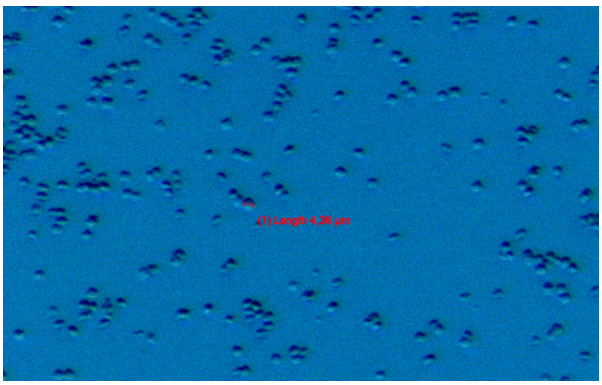


Figure 7: Microscope image of lysozyme crystals used for testing sample delivery. Crystals are 4-5μm in size.

3 Results

3.1 Flow Theory of the Deposited Stream

Several relations between the varied parameters in this experiment were found. The main focus is to analyze the width of the stream produced on the tape, which is given by the meniscus formed at the contact point between the nozzle and the delivery tape. A simple model was calculated to describe this relation between the fluid flow and the stream width. Assuming the stream to be a rectangular parallelepiped where x is the width and y the thickness, the flow (f) at a speed (v) in one dimension moving through its cross-section (A) is given by

$$f = Av \tag{1}$$

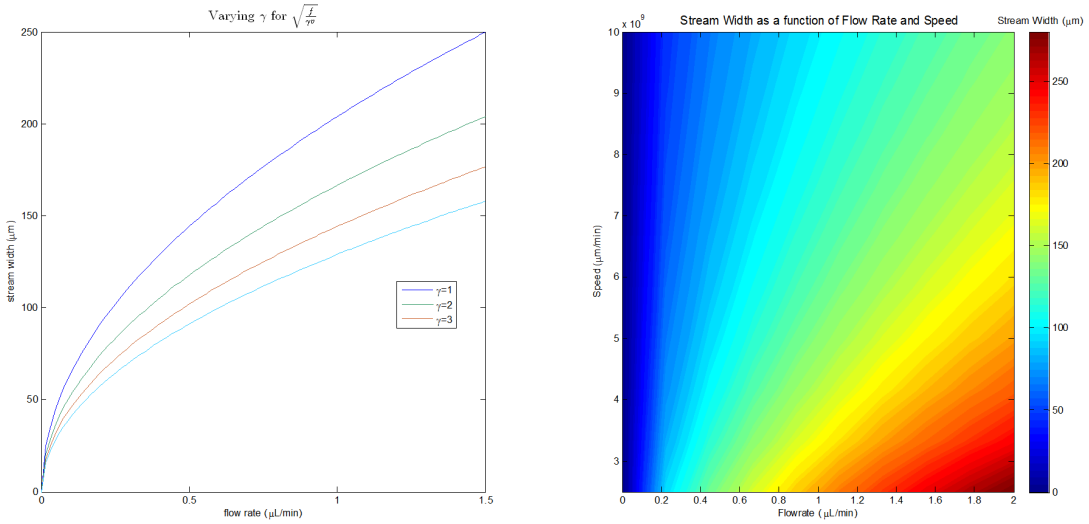
and by keeping the relationship between y and x is fixed, let $\gamma = \frac{y}{x}$. Then

$$x = \sqrt{\frac{f}{\gamma v}}. \tag{2}$$

The parameter γ and the stream width have an inverse square root proportionality. In the following experiments γ is characterized by viscosity, surface tension and contact angle. By varying the gamma factor one can examine how the stream width changes (Figure 8).

3.2 Experimental Results

The experiments of depositing lysozyme sample onto the Kapton tape with the prototype tape drive were carried out at a contact angle of 45°between tape and nozzle due to the PETRA III beamline structure. The relation between flowrate and stream width is first analyzed. This stream must be larger than the crystals and the laser drilled tape holes. To measure the stream width produced by the sample on the tape, a microscope and camera setup (Moticam 3.0MP) was used and positioned face-on with respect to the



(a) Gamma was varied and plotted according to Eq (2) with respect to flow rate and stream width.

(b) Keeping gamma fixed, a plot is made by varying speed and flow rate. Units on the order of experimental quantities are shown.

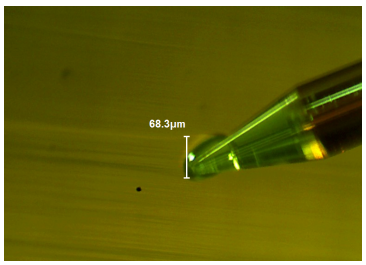
Figure 8: Varying parameters γ and f from Eq (2).

tape and images were later calibrated and measured using ImageJ Software as shown in Figure 9. For flow rate control, the syringe pump is initially set at about 1.5μL/min and then lowered to 0.05μL/min. The response read by the flow meter near the tip , and therefore the actual flowrate onto the tape is delayed by about 15 minutes. In this time images are captured for analysis and when the flowrate reaches a minimum it is stabilized for a considerable duration of the tape. Experimental runs without clogging have a duration of 15-40 minutes depending on the amount of available tape. The data retrieved have been plotted and fit functions were calculated based on the relation derived in Eq (2) and Figure (11).

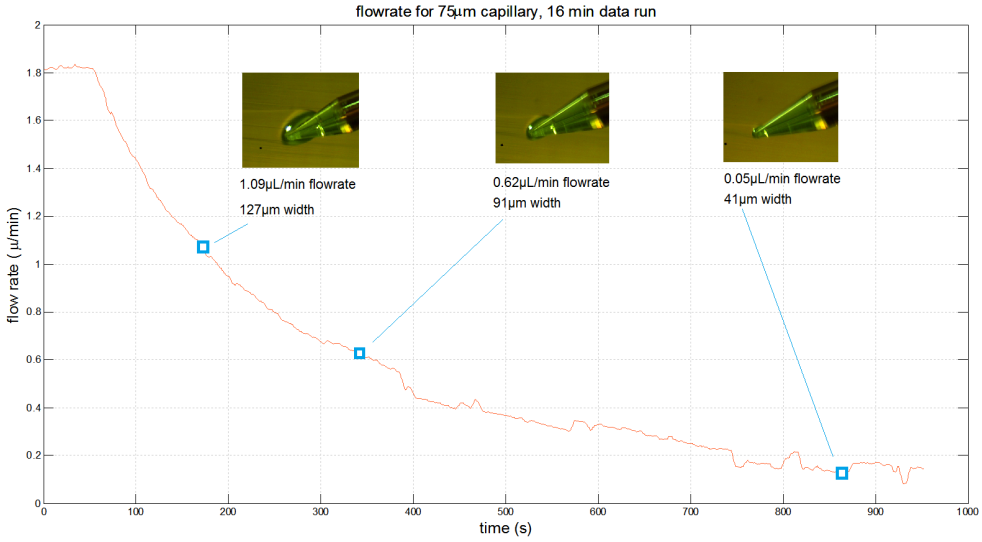
It is shown that for lower flowrates the stream width decreases. The selected nozzles for experimentation with lysozyme are a 30, 50 and 75μm inner diameter capillary tips that were ground evenly and the 30μm inner diameter pulled capillary. The tip ground at an angle was not used due to the excessively large meniscus formation on the tape. First the experiment with lysozyme is shown; flowrates start at about 1μmL/min and lowered to 0.01μmL/min (Figure 10).

This experiment is repeated for 30μm pulled and regularly ground tip for H_2O and PEG 10%, 4K (Figure 11).

It is also found that the stream width is related to the speed at which the tape is running. Five speeds were chosen, which correspond to 25mm/s, 20mm/s, 15 mm/s, 10 mm/s and 5mm/s. A fixed flowrate of 0.18$\pm$0.02μL/min is maintained (Figure 12). The variation of speeds experiment was carried out for possible future applications of the tape drive to beamlines with higher repetition rates.



(a) The stream was measured close at the meniscus. For velocity data measurements were taken at a fixed flow rate and speed and then averaged.



(b) Flowrate vs time for lysozyme.

Figure 9: Measurement example is shown as well as a time sequence of the flow rate over time and selected corresponding images of the capillary on the Kapton tape.

The data of the experiments and the theoretical model follow similar trends; an inverse square root relation between stream width and velocity and a square root relation between stream width and flowrate of sample. It is important to note that the square root function fits on the data have a offset; for zero flowrate a nonzero stream width is shown. This is due to systematic error in the experimentation. When the syringe pump is set to 0μL/min flowrate, there is residual pressure in the system and the sample continues to flow for some time.

A final analysis was made on the relation between γ and the dynamic viscosity (ν) and contact angle (θ) of the samples.

Sample	ν (P)	θ on polyimide
Lysozyme	21.43	52.5°
dH_2O	6.09	52.3°
PEG 10% 4K	29.43	54.7°

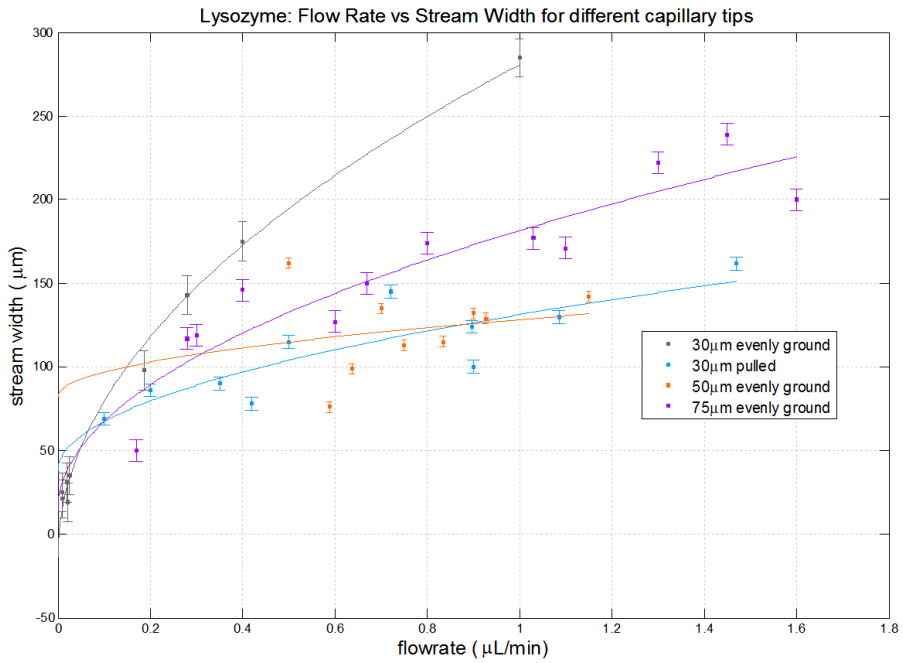
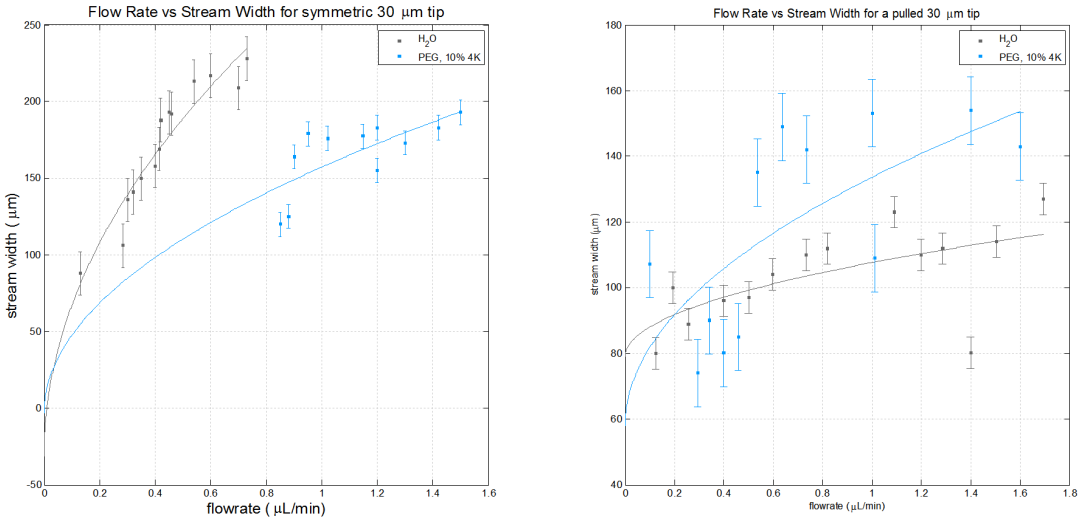


Figure 10: Meniscus width and flow rate decrease for all nozzle types; the pulled capillary achieves lowest stream widths.

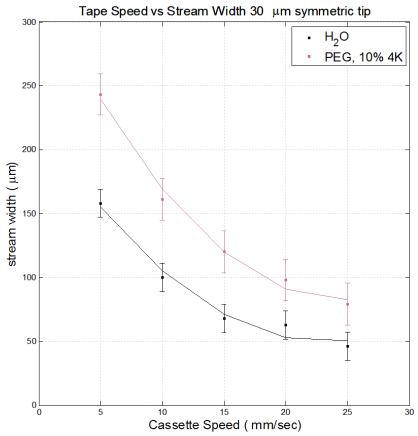
The viscosities of the samples were measured with a viscometer (Fs Technologie Flucon) and were averaged over 100 readings. The units are in poises (P). By using Eq (2), γ values are found at 0.1μL/min flowrate and a tape speed of 10mm/s. These are plotted against viscosity and contact angle to reveal possible relations between these quantities and define γ=f(ν, θ) (Figure 13).



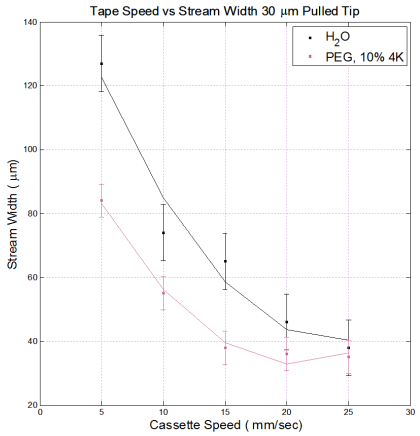
(a) Flowrate vs stream width for symmetric 30μm tip.

(b) Flowrate vs stream width for pulled 30μm tip.

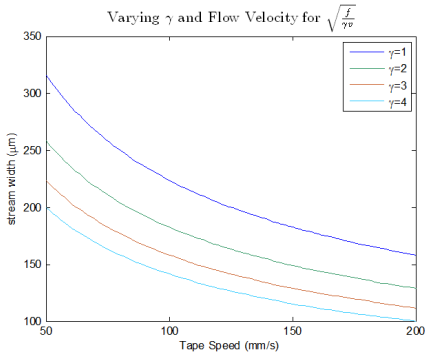
Figure 11: Flow rate vs stream for different samples.



(a) Stream width vs tape speed for H_2O.



(b) Stream width vs tape speed for PEG.



(c) Theoretical calculation of flow speed vs stream width for varying γ factors.

Figure 12: Correlation between tape speed and stream width for H_2O and PEG with a 30μm inner diameter pulled and evenly ground capillary tip. Figure (c) shows the theoretical plot for velocity for trend comparison.

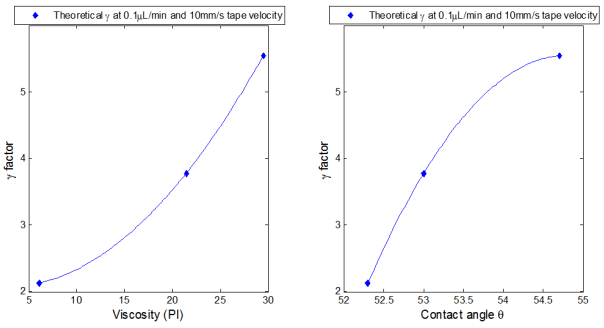


Figure 13: Calculated γ plotted against viscosity and contact angle for three samples at a flowrate of 0.1μL/min.

4 Conclusions and Further Work

The results from the experiments designed for the PETRA III beamline P11 synchrotron show that a minimized stream width can be achieved by variation of flow rate and tape speed. The most reliable tips for lysozyme are evenly ground nozzles because they are not sensitive to rotational position, allowing for the removal of an extra component and complication in the setup, and the short tip presents least clogging of the microcrystals. In the case of PEG and H_2O all tips are easy to use. From theoretical calculations, the γ factor can be further studied and related to the contact angle, which tells us the surface tension properties of the samples and the viscosities. Higher viscosity presents lower stream widths and for future applications the viscosity of the lysozyme or other protein crystal samples can be controlled and optimized in their buffer concentrations of PEG. Flowrates of 0.1μL/min are optimal for small streams and can be achieved by starting from a higher flowrate on the syringe pump after a period of 10-15 minutes.

An improvement in the system could include a drop on demand mechanism, in which the capillary tip is replaces with a vibrating piezo nozzle that has controlled frequency to eject droplets onto the Kapton tape. This would minimize the rate of sample consumption, although the synchronization of the beam pulse to the droplet ejection and laser holes on the tape can be challenging.

A further improvement is to change the contact angle between the tape and capillary tip; this would minimize the adhesive forces between the sample and the tape and produce smaller menisci, however due to the limited space and setup of the beamline at PETRA III, these experiments were carried out at 45°. Another possibility for future improvement is to coat the capillary tips with a hydrophobic coating.

It was observed that the protein crystal size is important for stability in the system; the crystals must be larger than the size of the holes on the tape, however crystals larger than 5μms tend to clog the capillaries. The sample must be prefiltered before being introduced into the reservoir (in this case, the low-pressure syringe pump) with a 5 or 10μm filter to avoid clumps in the crystals. This also resolves settling issues; smaller crystals settle over longer periods of time which is ideal for long running experiments.

The theoretical model is very approximate in this analysis; a circular section for the cross section of the stream can be used rather than a rectangle, which results in an arcos relation between stream width and γ factor and this could be explored further to make theoretical fits closer to the experimental data.

Lastly, it was observed that running the tape in the opposite direction of the tip inclination also produced a reliable stream. This could benefit the efficiency of experimentation at beamlines because it eliminates the need to rewind the tape, however the meniscus cannot be observed due to the fact that it is beneath the tip and if a camera was setup perpendicularly to the tape rather than face on, the measurements would be distorted and difficult to interpret.

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