The development of acoustic levitation for time resolved protein crystallography experiments at XFELS

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ABSTRACT

This body of work is building on the systems described in a recent publication by Tsujino and Tomizaki [1]. They describe the application of acoustic levitation to support protein crystals for X-ray diffraction experiments carried out at Synchrotron light sources. For clarity, a typical acoustic levitation system generates intense acoustic waves which are reflected back from a mirroring surface set at a distance matched to the frequency of sound being used. In air the reflected acoustic waves generate locations of minimum pressure or 'nodes' which act as wells for micron sized particles in the solution such as crystals. Multiple nodes can be generated and the nodes can also be made to process. This system can localise crystals periodically in air and therefore have enormous potential as a crystal sample delivery system. The system described in this work is a varient from this , described by Marzo et al [2]and uses multiple transducers and there is no requirement for a mrror surface as the sound from multiple tranducers generates the accoustic trap.

These acoustic traps also have the significant benefit of eliminating potential beam attenuation due to support structures or microfluidic devices. There is an additional need to eliminate sample environments when similar experiments are carried out using an X-ray Free Electron Lasers (XFEL) such as the LCLS at Stanford California as any sample environment would not survive the exposure to the X Ray beam. At an XFEL the Xrays are a billion times brighter than synchrotron radiation sources.

The application for this system will be to exmine turn over in Beta lactomase proteins. The system will allow for diffraction data to be collected before and after turnover. For clarity this protein is responsible for bacteria becoming antibiotic resistant and there for of significant importance to future world health

Keywords: X FEL, Sample delivery, Time resolved, Protein crystallography, Accoustic levitation

1 INTRODUCTION

We are using this technology to develop a sample delivery mechanism for conducting X-ray crystallograpic experiments at primarily X-FEL light sources.The devices are capable of levitating a single drop with the ability to rotate it to facilitate multi axis diffraction paterns to be achieved from the crystals suspended within it. In essence we have developed an accoustic goneometer capable of multi axis data collection from a single crystal.

The system operates at room temperature in a helium back filled environment to reduce beam attenuation so it automatically lends itself to being further developed for time resolved studies. With the inclusion of Induction Based micro Fluidics (IBF) reagents and substrates can be launched into the already suspended crystal sample to initiate the reaction.

2 THE BIOLOGY

A significant barrier in conducting these experiments currently is co-ordinating the presentation of the crystal, the mixing of the substrate with the crystals and then illuminating the crystal with X-rays. In our system we propose to restrain the crystals with an acoustic trap and 'fire' a drop of substrate solution into the crystal prior to X-ray radiation using IBF. We are currently using this system to explore the mechanism of the beta-lactamases; several families of proteins which catalyse the hydrolysis of beta-lactam antibiotics reducing the effectiveness of antibiotic treatment.

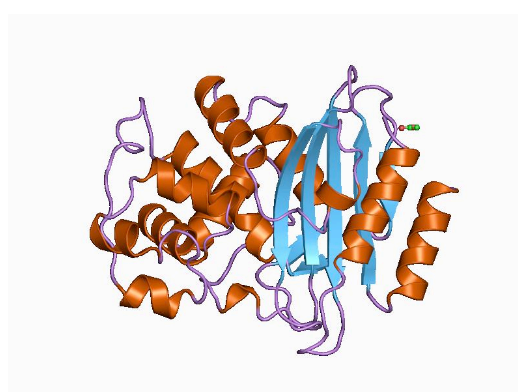

Fig 1 protein structure of 'Streptomyces albus beta-lactamase'

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These proteins are ideal for this task, not only because the biological question is exceptionally relevant in a post-antibiotic world, but also several substrates undergo a colorimetric change during catalysis so the X-ray diffraction data can be obtained whilst monitoring the activity of the enzyme.

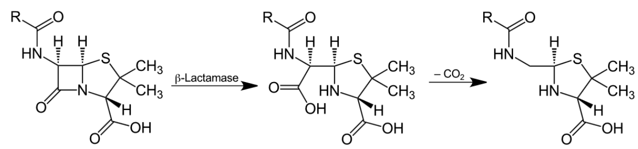


Figure 2 Action of β-lactamase and decarboxylation of the intermediate

The same colour change can also be used to study the efficiency of the mixing taking place.

The technical challenge this work is aiming to overcome is the reduction in crystal size the system can support as smaller protein crystals allow for quicker substrate diffusion. The size of the trapping potential has a direct relationship to the frequency of the acoustics and moving to higher frequencies is not trivial.

The development of a system will also see us develop induction based microfluidics (IBF) to introduce additional reagents or substrate to the suspended protein crystals already suspended.

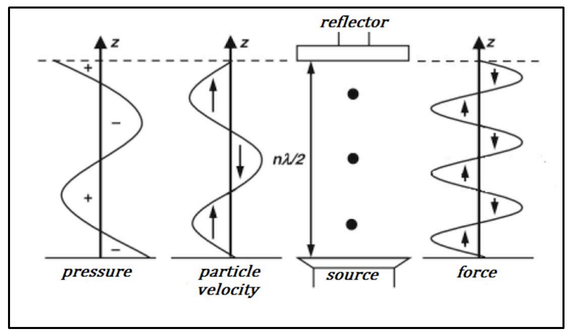
3 ACCOUSTIC LEVITATION

The concept was first conceived by Kundt leading to the development of the Kundt's tube in 1866, initially to look at the speed of sound in gases. The tube was a transparent horizontal pipe which contained a small amount of a fine powder. At one end of the tube was a source of sound at a single frequency (a pure tone). The other end of the tube was blocked by a movable piston which was used to adjust the length of the tube. A sound generator was turned on and the piston adjusted until the sound from the tube suddenly increased in intensity indicating that the tube was at resonance. This meant the length of the round-trip path of the sound waves, from one end of the tube to the other and back again, was a multiple of the wavelength λ of the sound waves. Therefore the length of the tube was a multiple of half a wavelength. At this point the sound waves in the tube were in the form of standing waves, and the amplitude of vibrations of air are zero at equally spaced intervals along the tube, called the nodes. The powder was caught up in the moving air and settled in little piles or lines at these nodes, because the air was still and quiet there. The distance between the piles was one half wavelength λ/2 of the sound. By measuring the distance between the piles, the wavelength λ of the sound in air can be found. If the frequency f of the sound is known, multiplying it by the wavelength gives the speed of sound c in air:

$c = \lambda f$ {\displaystyle c=\lambda f\,}

The detailed motion of the powder was in fact actually due to an effect called acoustic streaming caused by the interaction of the sound wave with the boundary layer of air at the surface of the tube

A bi-product product of such a system however, was the fact that you can manipulate and place particles within the tube. The interest in this technology for this application focuses on using the acoustic energy to do exactly this, thus positioning samples without introducing any material into the beamline. Depending on the frequency there can be a single node or a multiple nodes where there is zero pressure and particles/droplets can be suspended within these zones.



Figuure 3 Acoustic levitation principals

In recent years, other workers have explored using acoustics in this way **[3]**including multiple transducers and mirrors so that the location and motion of particles can be more specifically manipulated in more than 1 dimension by controlling the relative phase of the acoustic signals. A slight variant to this technology has been reported in Jan 2015 by Andrade **[4]** which allows for the distance between the reflector and the transducer to be varied on the fly into non-resonant conditions offering additional flexibility.

The varient that is the focus of this work however is to build on the work of Marzo et al **[2]** where by building a system with multiple tranducers focused to a single spot they can develop an acoustic trap without the need for an acoustic mirror as the configuration uses attractive forces also. With careful control of the multiple tranducers the particle suspended can be moved around, rotated and the whole system can be inverted.

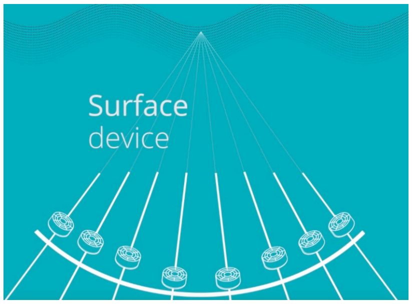


Fig 4 tractor sound beams suspending a particle **[2]**

IBF technology can kinetically project drops to targets of all types, and facilitate dynamically directing the liquids in flight to targets (a required, trait for small volumes of liquids). It works by biasing the droplet to be launced and steering it using an electric field to its target, in this case the levitating droplet containing the crystals. This is the workers technique for instigating time resolved reactions. In the following figures a suspended drop can be seen before and after a launched sample has been launched to meet the suspended drop. For reference the launched sample is 400nL.

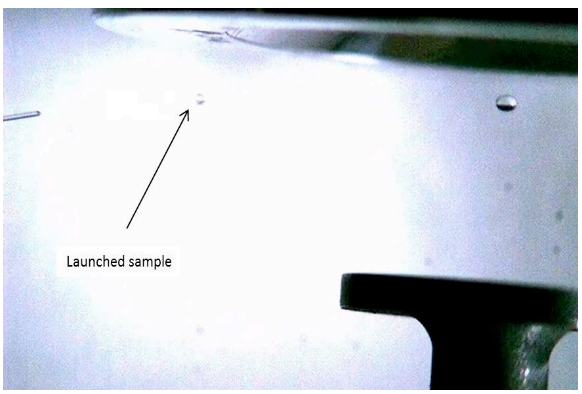


Figure 5 IBF launching of an additional reagent **[5]**

` Figure6 At the point of mixing

4 CURRENT SYSTEMS ~ LIQUID JETS

The majority of the XFEL data collection involves liquid jets as a delivery system. By creating a high pressure sheath around a central channel liquid jets of droplets containing crystals can be ejected and presented to the X-ray Beam.

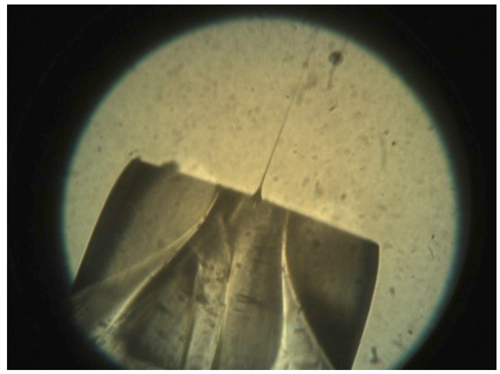


Figure 7: In vacuum jetting of a capillary with asymmetric bore inside a square ID glass tube. Since the cone tip of the liquid capillary is centered inside the gas aperture, the jet emerges straight. The liquid cone attached to the glass cone tip is visible. Square glass tube OD: 0.6mm (wall to wall). Gas exit aperture diameter ~100 micron**. [6]**

Typical flow rates of sample anywhere between 10 – 20 microliter/minute and the jet diameter typically about 6 micrometer. When the liquid jet is running, the vacuum in the experimental chamber is < 3x10-5Torr. However although the tip that develops the liquid jet is small the infrastructure to support the jet can is significant (see figure 8)

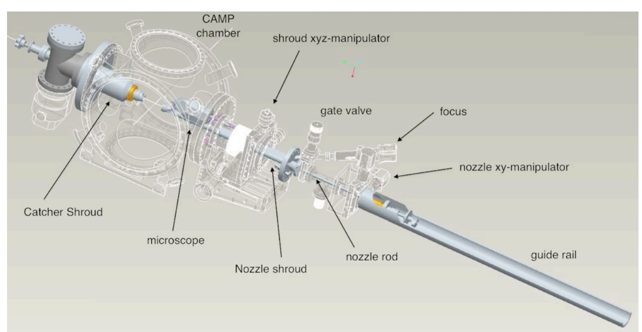


Figure 8: Differential pumping system for the liquid jet injector. It consists of two parts, the catcher shroud and the nozzle shroud, which can be decoupled and retracted via a bayonet coupling. This allows the use of fixed samples without breaking the vacuum

5 CHALLENGES AND ADVANTAGES

One of the biggest challenges to the development of the system is droplet size reduction. Although the size of the droplet supported can be reduced as frequency is increased a droplet is still required to be of a nominal size. The phenomenon is described by its Bond number. This is the ratio between the liquids surface tension, density and its own size. If this number is too low the drop will burst. Sound intensity is also another variable to be explored as it can distort the geometry of the droplet being suspended.

In addition from these fundemenrtal overruling principles the system will need to become compatible with Xray facilities such as the LCLS and Diamond's Beamlines. These limitations include space and the requirement to operate in a helium environment.

6 INITIAL SETUP

Our initial set up moves away from traditional accoustic levitation and uses sound to levitate the sample using descrete tractor sound beams. Sample may be added to the device using IBF techniques. for time resolved studies. The device uses the same configuration described by Marzo et all. **[2]** to enable both suspension and manipultation of the droplet.

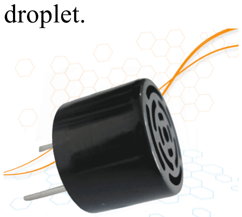


Figure 9 Multicomp MCUST16P40B12RO tranducer (2cm diameter)

With careful control droplets can be suspended rotated and moved in space. By mounting the transducers in a 3D printed dished structure the system can suspend , rotate and grip the suspended drop even when inverted. A microcontroller is used to generate different phases of the ultrasound frequency which provide the input signal to motor driver chips that in turn drive the ultrasound transducers. Marzo et al demonstrated that a simple two channel arrangement was adequate to allow the trapped particles to be moved upwards in increments of 500µm. The low power also enures the bond number for the droplet size is also maintained to a sustainable level. An image of the device can be seen in Figure 10. The transducers used in this system are Multicomp MCUST16P40B12RO (see figure 9) and are only a few bucks to buy. The mounting plate is readily printed using most standard 3D printers.

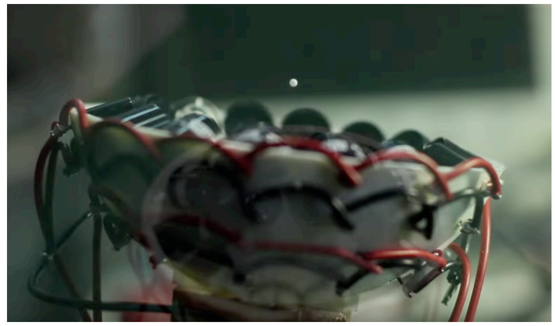


Figure 10 tractor sound beam system levitating polystyrene bead

7 CONCLUSION

This methodology for handling microcrystals in mother liquor for presentation to Xray beams at both synchrotron and XFEL light sources is showing great promise. In its simplest format IT offers an airborne goniometer with unlimited angular access for obtaining multiple angular rotations and their diffraction patterns. With the inclusion of the ability to unite multiple droplets and therefore instigating room temperature mixing we have an excellent sample environment for time resolved experiments. As the reaction takes place in space there is no contamination between samples which in turn could be dosed with greater and greater amounts of reagents. The other big win for the system is the sample is stationary unlike with liquid jets allowing for reduction in waste and multiple angles and potentially a structure being solved from a single crystal. This system particularly lends itself toward the study of Beta lactamases as it can suspend the protein crystals, add appropriate substrate initiate mixing and then rotate to collect data all at room temperature.

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