labCrystal

news from the world of protein crystallography

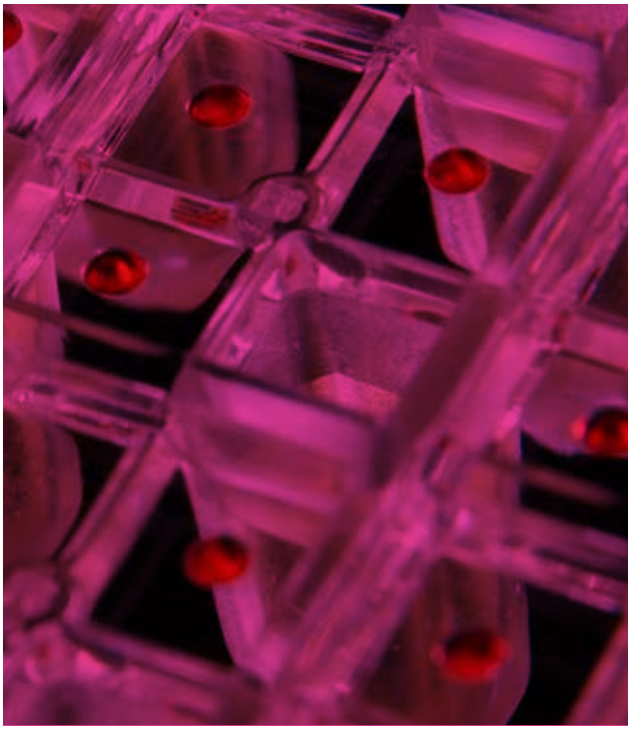
SPRING 2014

Working at the limits of crystallography

Optimal conditions for optimal crystals!

Application of the mosquito® liquid handler for crystallisation under oil

dragonfly®: automated optimisation of crystals



ttplabtech

natural innovators

welcome

After 100 years of development, X-ray crystallography has become the leading technique for studying the atomic structure and related properties of materials. There are still many challenges associated with obtaining the perfect crystal but new technologies have made the process more efficient. TTP Labtech provides crystallographers with the means to improve throughput and accuracy in setting up crystallisation experiments by automated, precise pipetting of low volumes. This edition of **labCrystal** includes the use of TTP Labtech's new dragonfly® by Fabrice Gorrec at the MRC LMB and innovative use of the established mosquito® Crystal by Michael Collazo at UCLA. It also highlights our support service and resources. We hope you enjoy reading **labCrystal** and have a great year of crystallography!

Dr Soheila Vaezeslami
field applications scientist

Dr Sarah Burl
scientific communications officer

contents

New dragonfly screen optimiser

dragonfly® – mosquito's ideal partner

dragonfly accurately aspirates and dispenses **any volume** (0.5 µL to 4 mL) of **any liquid** (low and high viscosity solutions). At the core of dragonfly are the syringes that can aspirate and dispense samples. Each dragonfly syringe is a positive displacement pipette that uses its own HDPE plunger inside the polypropylene body, as opposed to an air gap or system liquid. This ensures guaranteed zero cross-contamination of samples and removes the need for time-consuming and expensive solvent wash steps or liquid classification.

automated optimisation of crystals

dragonfly is the ideal system to complement TTP Labtech's mosquito range in the protein crystallisation workflow. Once the initial crystal 'hits' are identified, dragonfly optimises the set of conditions to grow better diffracting crystals. Screen optimisation is used in some form or another by all protein crystallography labs.

specifications	dragonfly®
Dispense range	0.5 µL – 4 mL (0.5 – 6 µL in a single shot)
Reservoir capacity	10 mL
Dispense resolution	100 nL
Plate set-up time	< 4-8 mins for 4-ingredient 96-well plate
Plate format	96, 48, 24,15 wells
Dead volume	< 0.5 mL
Dimensions (w x d x h)	575 x 600 x 645 mm (23" x 24" x 25")
Net weight	5 head: 45 kg (99 lb), 10 head: 55 kg (121 lb)

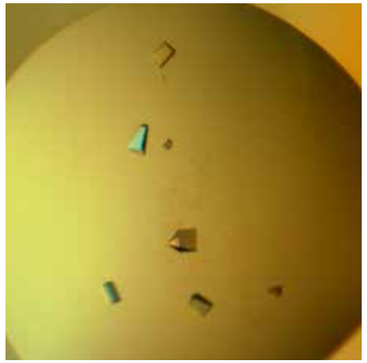
mosquito's **active humidity chamber**

This mosquito accessory reduces experimental inconsistencies caused by variation in the humidity in the environment, by allowing users to control the relative humidity (RH) of each experiment. Available for all new and existing mosquito instruments.

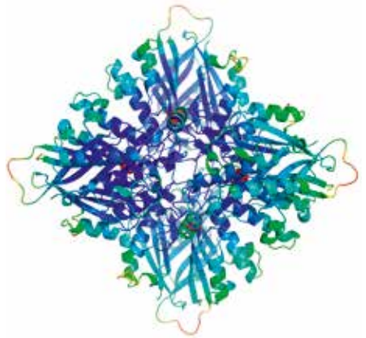
mosquito's active humidity chamber

Working at the limits of **crystallography**

Figure 1: AtzD protein



(A) Crystals of the AtzD protein set up at C3



(B) Ribbon representation of a tetramer of AtzD

mosquito® LCP overcomes the common problems encountered with accurately dispensing highly viscous materials used in membrane protein crystallisation. Australia's Collaborative Crystallisation Centre (C3) describes the use of mosquito LCP in determining the structure of cyanuric acid hydrolase (AtzD) and identifies the active substrate binding site of this enzyme.

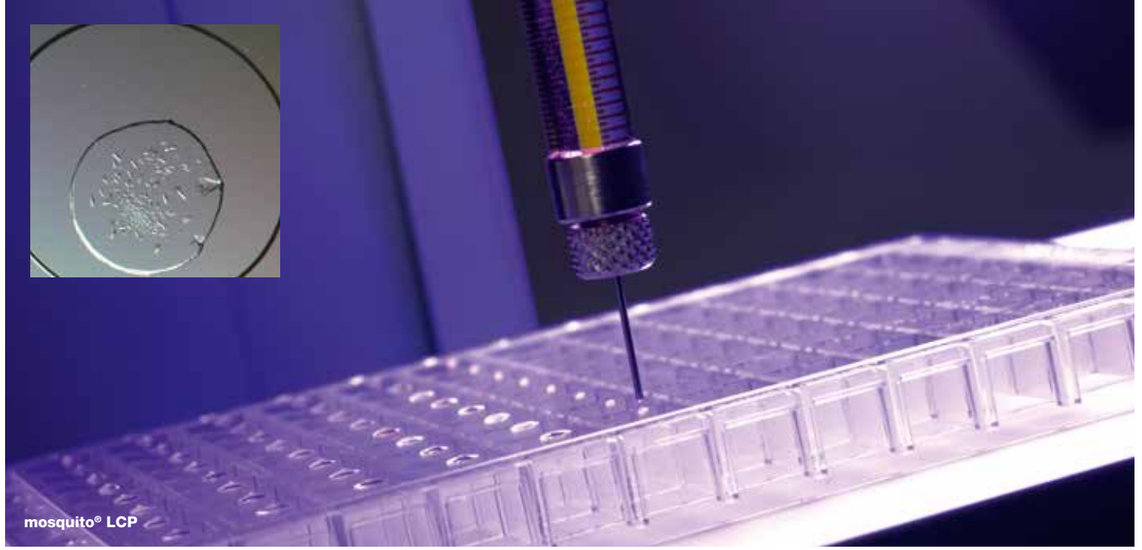
In a recent article published by the CSIRO structural biology group, mosquito LCP was employed to set up seeded crystallisation experiments of AtzD, an enzyme involved in the detoxification of the pesticide atrazine [1] (Figure 1A). The results demonstrated the first X-ray structure for this class of enzyme. This enzyme performed an interesting ring opening chemistry and displayed a fold that had not previously been observed in a protein structure (termed the 'Toblerone fold'). The high-resolution structure allowed the identification of the binding pocket residues that are involved in substrate specificity (Figure 1B).

In addition to the LCP experiments, C3 uses mosquito LCP for screening (including microseeding, soaking and additive screening), optimisation and also the scaling-up of experiments. A second mosquito LCP located in the cold room is used for bicelle crystallisation, which requires low-temperature dispensing. This second instrument is also used for setting up light and/or temperature-sensitive samples.

"mosquito: lovely software, really tidy footprint"

Dr Janet Newman

Dr Janet Newman, C3's facility manager, has found TTP Labtech's mosquito LCP to be a fundamental addition to the automated instruments used in C3. "The low-volume, positive displacement technology of mosquito ensures reproducible and rapid dispensing, accurate drop placement and zero cross-contamination. The disposable tips eliminate the need for time-consuming wash stages between the set-up of individual screening plates, which significantly speeds up the process of setting up crystallisation experiments and allows us to set up difficult samples without fear of them clogging the machine".



Highlighting the benefits of the multi-aspirate function of mosquito, Dr Newman adds that the ability to rapidly take up small volumes of multiple solutions significantly enhances throughput. For example, a small volume of a seeding solution (as low as 10 nL) can be aspirated followed by the crystallisation reagent, and then dispensed together directly into a sitting or hanging protein drop, with mixing if required.

As well as proving indispensable for the set-up of vapour diffusion, LCP and bicelle screening studies, mosquito LCP's novel dispensing and intuitive software has enabled the C3 group to set up experiments which fall outside the normal mandate of a crystallisation laboratory, for example, setting up lipid/solution mixtures for high-throughput lipid phase analysis [2].

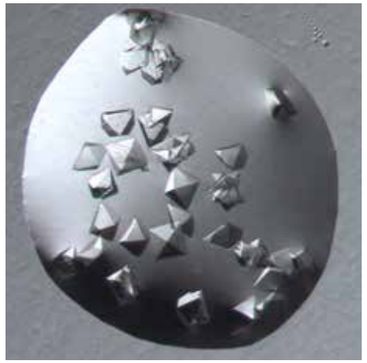
Dr Janet Newman, facility manager, C3

The Collaborative Crystallisation Centre (C3) was established in 2006, in partnership with CSIRO, Australia's Commonwealth Scientific and Industrial Research Organisation. It is Australia's only full fee-for-service crystallisation facility focused on high-throughput screening. In addition, academic and commercial collaborations allow external research groups to take advantage of C3's scientific experience in protein crystallisation and structure determination. As part of this service, C3 provides users with state-of-the-art technologies and instruments for protein crystallisation as well as enabling the development of novel technologies.

[1] Peat, T S *et al* (2013) Mol. Microbiol. 88(6): 1149-63
[2] Darmanin, C *et al* (2012) ACS Comb. Sci. 14(4): 247-52

Optimal conditions for **optimal crystals!**

Figure 1: Proteinase K crystals



(A) Original hit (200-250 µm)

(B) Optimisation condition (400 µm)

In a crystallisation experiment, determining the optimal crystallisation condition that will produce the best diffracting crystals relies on multiple experiments varying the conditions of each reagent. This gradient of components can be set up using a dedicated robot such as TTP Labtech's dragonfly®. However, following a method developed by Mike Collazo at UCLA, mosquito® Crystal itself is also capable of setting up optimisation experiments.This is faster, more accurate and saves on reagents compared to a manual set-up.

After an original crystallisation hit is obtained, the condition is often optimised further by setting up a new plate varying the concentrations of the components along the x- and y-axis in the reservoir. In one example, proteinase K crystals were optimised in a hanging drop format. A gradient of reagents was set up over a reservoir of the original condition using the multi-aspirate function of the mosquito Crystal. This multi-aspirate function allows several components to be aspirated into the same pipette tip and then be dispensed together. The y-axis can be varied by loading eight different solutions into a single column and concentrations of different components or pH can be varied in the x-axis. In this example, 1 µL drops (1:1 of protein to condition) were set up with the protein concentration altered in the y-axis (50 mg/mL to 30 mg/mL in 0.1 M Tris HCl buffer, pH 7.5 in 8 rows of a 96-well plate), and the concentration of ammonium sulphate (condition) in each drop varied from 0.3 M to 0.8 M across 12 columns in the x-axis. The drops were left to equilibrate over a reservoir solution containing the same components as the original hit (0.1 M Tris pH 7.5, 1.2 M ammonium sulphate). The plates were then imaged for crystal formation and the conditions that produced the best crystals were determined (Figure 1).

"The versatility of mosquito Crystal has enabled us to deviate from convention in our crystallisation set-ups"

Michael Collazo

Unlike traditional manual optimisation or optimisation with a dedicated robot (e.g. dragonfly) where the components are mixed in the source plate reservoir, the drops are equilibrating against an 'alternative' reservoir which is the original hit condition. The reagents are 'multi-aspirated' from a source plate and mixed directly with the protein (Figure 2).

In an additional experiment, crystals of a novel proteobacteria protein were optimised using this method. The protein was cloned and purified in collaboration with Nicole Wheatley of UCLA. The original crystals were obtained in a hanging drop format, in a 1:1 mixture of 25 mg/mL protein and a condition containing 4 M ammonium sulphate and 1 M Bis-Tris pH 5.5 (Figure 3A). In this optimisation the concentration of the protein was kept constant, while the pH and the concentration of the ammonium sulphate were increased in the y- and x-axes respectively, using the mosquito Crystal. Previously, both manual and optimisation under oil were set up, but only poor crystals were obtained where the needles were too thin to harvest.

Diffraction data was collected on some of the best single and sharp-edged optimised crystals at the UCLA X-ray crystallography core facility at a wavelength of 1.54 Å. The optimised crystals resulted in improving the resolution successfully from 2.5 to 2.1 Å. While the refinement resulted in larger 625 µm crystals compared to 200 µm in original hit the best diffracting crystals (Figure 3B) were not necessarily the largest ones. The optimised condition consisted of 0.7 M ammonium sulphate and 1.0 M Bis-Tris pH 5.7.

From left to right: Michael Collazo (crystallisation facility manager), Nicole Wheatley (graduate researcher), Michael Sawaya (crystallographer at Eisenberg lab), Duilio Cascio (crystallographer and X-ray facility manager)

Figure 2: Schematic of a sitting drop set-up

(A) The traditional method of crystallisation where different ratios of components are placed in each reservoir well and pipetted onto the sitting protein drop (pink)

(B) In this study each well contains the same components from the original hit condition (termed the 'alternative' reservoir) with varying components pipetted onto the sitting drop from a source plate

Figure 3: Crystals of a novel proteobacteria protein

(A) Original hit resulting in 2.5 Å data

(B) Optimised hit resulting in 2.1 Å data

The UCLA Macromolecular Crystallization Facility of the UCLA-DOE Institute of Genomics and Proteomics provides state-of-the-art, high-throughput, crystallisation services to all institutions. The full-service core offers access to sophisticated equipment and technologies, and technical assistance in sample preparation. The facility will provide screen preparation, plate set-up, automated image acquisition and analysis, and optimisation design. Our high-throughput set-up can produce up to 18,000 experiments in a single day. Our researchers have been very successful and have deposited 75 structures in the Protein Data Bank in less than 2 years. http://www.doe-mbi.ucla.edu/facilities/crystallization

Application of the mosquito® liquid handler for **crystallisation under oil**

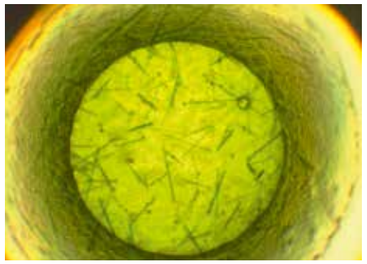
The ‘under oil’ (microbatch) method of protein crystallisation consists of a drop of protein sample combined with the crystallisation reagent (screen) of choice pipetted under a layer of oil. Advances in automation have facilitated the widespread adoption of high-throughput microbatch methods that include vapour diffusion and convection-free crystallisation. Consequently, rapid initial screening of several conditions using as little as 25 nL of protein can be achieved.

The original microbatch set-up, introduced by Chayen *et al* [1] was performed using a thin layer of paraffin oil, which is relatively water-impermeable and therefore reduces the dehydration rate of the crystallisation drop. In this case there is almost no vapour diffusion, and the concentrations of the protein and crystallisation reagents do not change significantly throughout the course of the experiment. This is in contrast to the vapour diffusion method that is a dynamic process in which conditions are changing throughout the crystallisation process. The relative stability of the under-oil method is the simplest model of crystallisation and provides a reliable history of crystal growth; however, it is often thought that the gradual change of conditions that occurs with the diffusion methods may be vital to crystal formation [2].

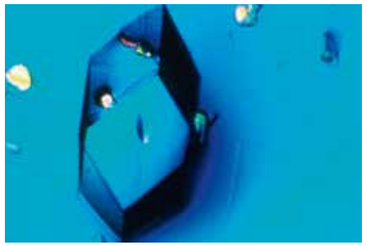
Alternatively, water-permeable oils or oil mixtures are sometimes used as a barrier between the reservoir and the crystallisation drop in a traditional hanging or sitting drop, called the ‘vapour diffusion rate controlled under oil’ method. In this method, drops equilibrate more slowly against the reservoir, resulting in slower nucleation and growth rates.

Following this development, Allan D’Arcy [2] modified the ‘microbatch under oil’ technique by using a 1:1 silicon oil and paraffin oil mixture (AI’s Oil). In this case the drops are not equilibrating against a reservoir and nucleation may happen at any time when the concentration of the protein falls into the nucleation phase of the process.

Figure 1: Microbatch under oil



(A) The first membrane protein crystallised using the microbatch technique



(B) Crystals of apocrusacyanin C1 from lobster carapace optimised using an oil barrier in hanging drop [5]

What oil to use? People have different choices for the oil. The mixture of oil that is used is dependent on a number of properties, e.g. protein drop behaviour, low X-ray background and cryo-protectant ability. Therefore the choice of oil is something that needs to be carefully considered and tested by trial and error for each protein.

One of the more interesting aspects of crystallisation under oil is its success with membrane proteins. Because these ‘under oil’ methods provide different kinetics of crystallisation, they have proven to be helpful in forming crystals that otherwise were impossible to obtain (e.g. chlorophyll binding protein 43 of the Photosystem II complex from spinach [3] (Figure 1A), ATPase F1c10 complex [4] and apocrusacyanin C1 from lobster carapace (Figure 1B: Naomi E. Chayen, Imperial College London, UK, personal communication).

There are several different ways that any microbatch experiment can be set up. Taking advantage of modern liquid handling robots it has been possible to automate the process. The drops can be set up at very low (nanolitre) volumes resulting in lower consumption of valuable protein. The mosquito liquid handler, which is based on true positive displacement and contact-based dispensing, is best suited for this purpose and is in widespread use (Figure 2). Here are just two common examples of how mosquito is used in research labs worldwide to set up the microbatch drops:

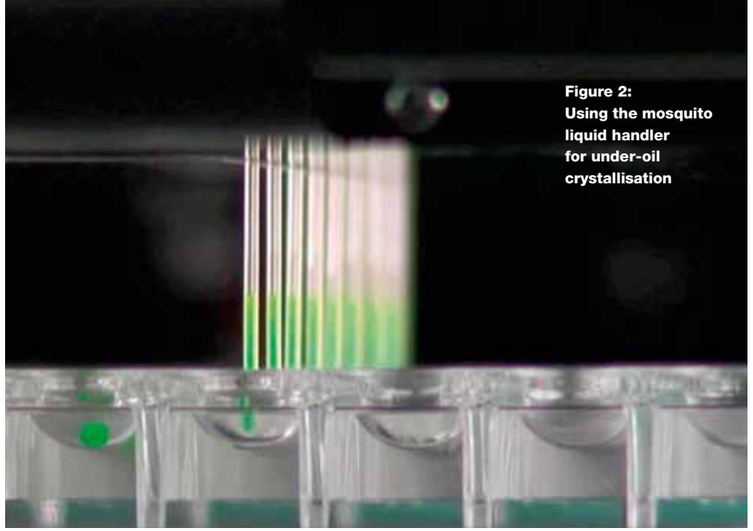


Figure 2: Using the mosquito liquid handler for under-oil crystallisation

Example 1: Using the multi-aspirate function of mosquito the protein and reservoir are aspirated in the same pipette tips and dispensed together underneath the oil.

Example 2: The protein is dispensed followed by reservoir solution on the plate and then the oil is dispensed on top. This can be done in either normal sitting drop or dedicated 96-well microbatch plates. Since mosquito is a contact-based dispenser and does not shoot the drops out of the pipette tips, it ensures that the drops are always placed on top of each other. The lack of mixing of the components during this method allows for less disruption of the equilibrium and often greatly improved crystal formation.

As discussed here, the mosquito liquid handler can be easily employed for automated microbatch screen set-up, ensuring greater throughput and accuracy.

Figure 1A and 1B images kindly supplied by Prof. Naomi E Chayen, Imperial College London, UK.

[1] Chayen, N E *et al* (1990) J. Appl. Cryst. 23(4): 297-302
[2] D’Arcy, A *et al* (1996) J. Cryst. Growth 168(1-4): 175-180
[3] Chayen, N E (2003) Methods and Results in Crystallization of Membrane Proteins. Iwata, S. Chapter 8 ed. (International University Line, USA): 131-139
[4] Stock, D. *et al* (1999) Science 286 (5445): 1700-1705
[5] Chayen, N E (1997) J. Appl. Cryst. 30:198-202

dragonfly®: optimisation optimised

Efficient optimisation screens can be prepared three times faster than classic liquid handling techniques using TTP Labtech's new dragonfly. The development of dragonfly is a result of close collaboration with the Medical Research Council's (MRC) Laboratory of Molecular Biology (LMB), Cambridge, UK which enabled TTP Labtech to gain an insight into its customers' needs.

The ability to crystallise proteins, nucleic acids or macromolecular complexes poses significant challenges to the protein crystallography community. Following the establishment of a successful primary screen, crystal optimisation is vital to ensure high quality X-ray diffraction data to determine high-resolution structures. This optimisation stage involves setting up a series of complex screen combinations in which the ratio of each component from the original screen is varied until the optimal conditions for crystal growth are found.

To facilitate this process, TTP Labtech has introduced the dragonfly screen optimiser to its successful liquid handling portfolio. dragonfly is a compact, low-cost addition to any crystallographer's bench, designed to ease the effort and tedium of the complicated plate set-ups required at the optimisation stage.

With its novel, non-contact dispensing technology, dragonfly ensures highly accurate, automated, positive displacement dispensing from disposable syringes directly into crystallisation plates. It provides rapid non-contact dispensing where each tip can dispense any volume (from 0.5 µL to 4 mL), of any liquid, into any well, with zero cross-contamination.

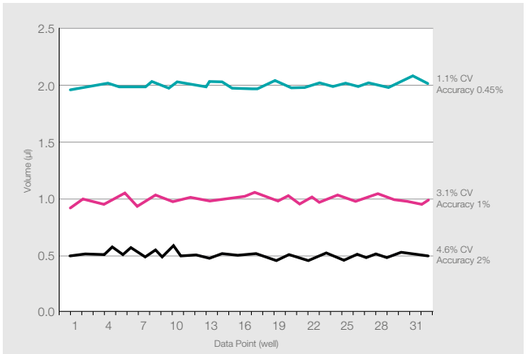


Figure 1: Graph represents % CVs of dispensing 50% glycerol using dragonfly (measured by absorbance with tartrazine)

For all crystallisation techniques optimisation screens can be accurately set up in any standardised microwell plate format. CVs below 5% have been demonstrated for 1 µL dispense of all liquid types, including glycerol (Figure 1). dragonfly also offers independent control of volumes in each pipette and simultaneous dispensing from up to 10 pipetting heads. This feature makes it a very fast dispenser allowing for a 96-well plate with four ingredients to be set up in less than 5 minutes.

dragonfly's easy-to-use software makes it possible to configure a required gradient profile and plate set-up for each screen solution, or to use a template screen design. Once screen reservoirs are filled, dragonfly automatically aspirates stock solutions and creates a 96-well screen within 3-6 minutes (depending on the number and final volume of conditions prepared).

Crystallisation automation scientist from the LMB, Fabrice Gorrec, was one of the first users of dragonfly. In a proof-of-concept study he optimised conditions for the formation of three well known proteins: lysosyme, concanavalin A and catalase, using the '4-corner solutions' protocol. Following selection of the primary conditions which resulted in crystal formation, Fabrice performed crystal optimisation by altering the concentrations of the precipitating agent (PEG) and the additive (propanediol). dragonfly was used to automatically produce optimisation screens modifying concentrations of the reagents and also the pH in nine different 96-well sitting drop plates (final volume in each well: 85 µL).

In this study, the resulting conditions were homogenised with a microplate orbital shaker and the crystallisation drops were set up using the mosquito Crystal (100 nL protein + 100 nL well solution). Drops were observed daily and photographs of those containing crystals were taken after 3 weeks (Figure 2A – C).

In this study, the effectiveness of dragonfly for preparing crystallisation conditions into 96-well plates was shown, with efficient optimisation screens being prepared three times faster than classic liquid handling technology. As an indicator of success, dragonfly is now routinely employed at the LMB for crystal screen optimisation.

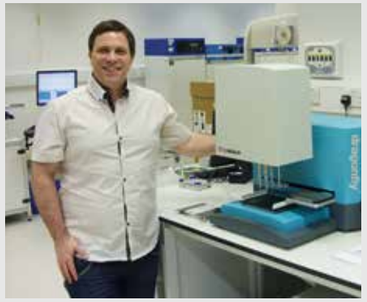
Figure 2: Light micrographs of crystal formations

(A) Lysozyme **(B) Concanavalin** **(C) Catalase**

Magnifications differ and crystal sizes vary between 150 and 400 µm.

"dragonfly is an exciting addition to TTP Labtech's successful liquid handling portfolio for crystallisation screening, allowing the rapid generation of crystals in the optimisation process."

Fabrice Gorrec



Fabrice Gorrec, crystallisation automation scientist with the dragonfly prototype at the MRC LMB

The Medical Research Council's (MRC) Laboratory of Molecular Biology (LMB), Cambridge, UK, is one of the world's leading research institutes. It is responsible for many pioneering techniques, such as methods for determining the three-dimensional structures of proteins and other macromolecules, the sequencing of DNA and the development of monoclonal antibodies providing the knowledge needed to solve key problems in human health.

The **mosquito** range for crystallography

TTP Labtech's mosquito liquid handling portfolio provides you with precise and repeatable nanolitre pipetting, every time, irrespective of liquid viscosity or environmental conditions. mosquito out-performs competing technologies due to its disposable tips and true positive displacement pipetting. This provides unrivalled accuracy and precision at low nanolitre volumes across a huge liquid viscosity and surface tension range, which is essential in protein crystallisation.

automating all your favourite crystallisation techniques

You can automate all the popular protein crystallisation screening techniques using both the mosquito Crystal and mosquito LCP without changing the set-up on the instruments:

- vapour diffusion screening set-ups: hanging drop, sitting drop, microbatch
- membrane protein screening set-ups: LCP (mosquito LCP only), bicelle
- advanced techniques: additive screening, seeding, microseeding
- optimisation
- scale-up

"90% of customers said throughput has increased since purchasing a mosquito"*

multiple aspirations, single dispensing

mosquito can perform multiple aspirations before a single dispense, which is essential for automating seeding and direct addition of additive screens to the drops. You can even dispense a combination of solutions simultaneously – with additional mixing if required – ensuring perfect drop formation for optimal protein crystallisation.

"100% of customers are either 'satisfied' or 'very satisfied' with the performance of their mosquito"*

reliable, robust and reduced running costs

TTP Labtech offers two versions of mosquito for protein crystallography market: mosquito Crystal and mosquito LCP.

Both mosquito versions offer the following key benefits for protein crystallography:

- accuracy and precision at nanolitre volumes
- flexibility to address a range of set-up techniques
- fast
- easy to use – perfect for multi-user labs
- optimised sample use
- unrivalled reputation for reliability

*Results from the TTP Labtech 2013 Customer Survey

mosquito Crystal – the market leader for crystallisation screening set-ups

With TTP Labtech's mosquito Crystal, you can use smaller volumes of precious protein sample with no risk of cross-contamination.

Precise drop placement ensures drops are positioned centrally in the sub-wells of sitting drop plates every time and facilitates automated plate imaging, thus making your protein crystals easier to identify. It also allows you to create several multi-component drops per well in 96-well hanging drop set-ups, enabling you to assess different protein concentrations, ligands or complexes at the same time.

These are just some of the reasons mosquito Crystal has become the protein crystallographer's favourite liquid handler.

mosquito LCP – the market leader for LCP crystallisation screening

The mosquito LCP combines the mosquito pipetting head (as used in mosquito Crystal) with an additional integrated lipidic cubic phase (LCP) syringe dispensing arm with automatic calibration.

It possesses all the functionality of the mosquito Crystal but also provides accurate and precise pipetting of highly viscous LCP mixtures used in membrane protein crystallisation.

mosquito® Crystal

mosquito® LCP

specifications	mosquito® Crystal	mosquito® LCP
Dispense range	25 nL – 1,200 nL	25 nL – 1,200 nL
Plate / deck capacity	2 or 5	2 or 4
Experimental set-up type	Hanging drop, sitting drop, microbatch, bicelle, additive screening and microseeding	LCP, hanging drop, sitting drop, microbatch, bicelle, additive screening and microseeding
Plate set-up time	< 2 mins	< 2 mins LCP set-up < 5 mins
Dead volume	< 0.3 µL	< 0.3 µL
Min. accessible volume	10 nL	10 nL
Dimensions (w x d x h)	390 x 470 x 690 mm (16" x 19" x 27")	430 x 590 x 690 mm (17" x 23" x 27")
Net weight	Approx. 27 kg (59 lb)	Approx. 34 kg (75 lb)
Services	110/220 V single phase 50/60 Hz	110/220 V single phase 50/60 Hz
Noise	64 dBA peak noise during operation	64 dBA peak noise during operation
Optional extras	Humidity chamber	Humidity chamber, LCP mixer

service and support

TTP Labtech's quality engineering is backed by high-quality service, support and advice to ensure that customers receive fast, reliable and effective solutions.

Support services

provided by TTP Labtech include:

- technical engineering (in-house and field-based support)
- application (application and assay enquiries)
- relocation (includes inspection, decommissioning and transportation requirements)
- training (basic and advanced operator training)

Service agreements

TTP Labtech offer customers the very best in instrument support services with various technical support options available to suit most budgets and requirements.

The optimal Full Service contract provides complete peace of mind by maintaining the same high level of all-inclusive cover that applies during the instrument's warranty period.

- 4-hour priority response to email/call
- telephone support
- unlimited remote application and technical support including secure dial-up access to instrumentation
- 48-hour on-site response
- annual preventative maintenance visit
- unlimited site visits
- parts, labour and expenses included
- unlimited software updates
- discounts are applicable to multiple instruments and contracts spanning multiple years

TTP Labtech also have technical assistance agreements in place with partner companies in the US, Japan, China, Russia, Korea and Australasia to provide a complete product support service for customers working outside our normal European office hours.

"100% of customers were either 'satisfied' or 'very satisfied' with the level of support received since installation of mosquito"*

*Results from the TTP Labtech 2013 Customer Survey

Want to know how we can help you improve your crystallography experiments? Want to see how our instruments work? For many valuable resources for crystallographers, check out our website **http://ttplabtech.com/resources**

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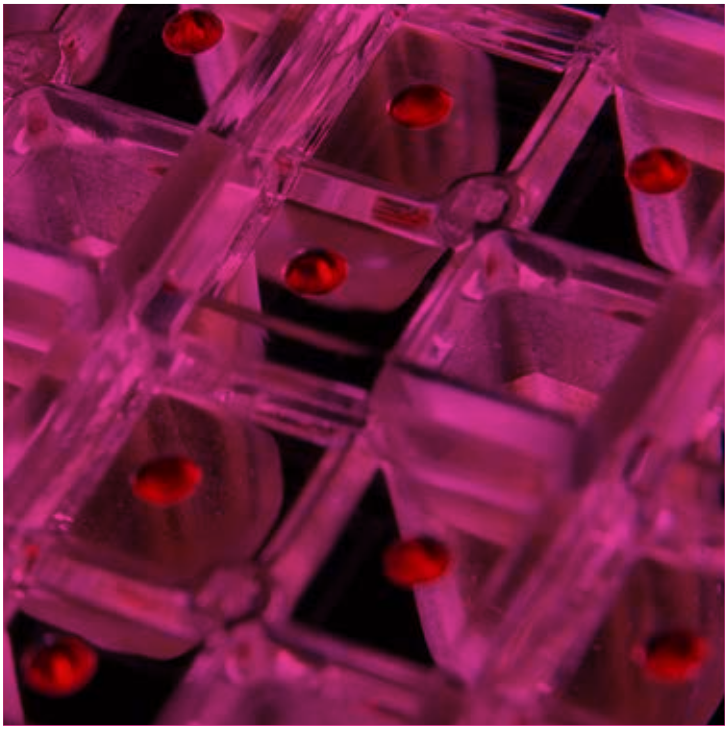

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