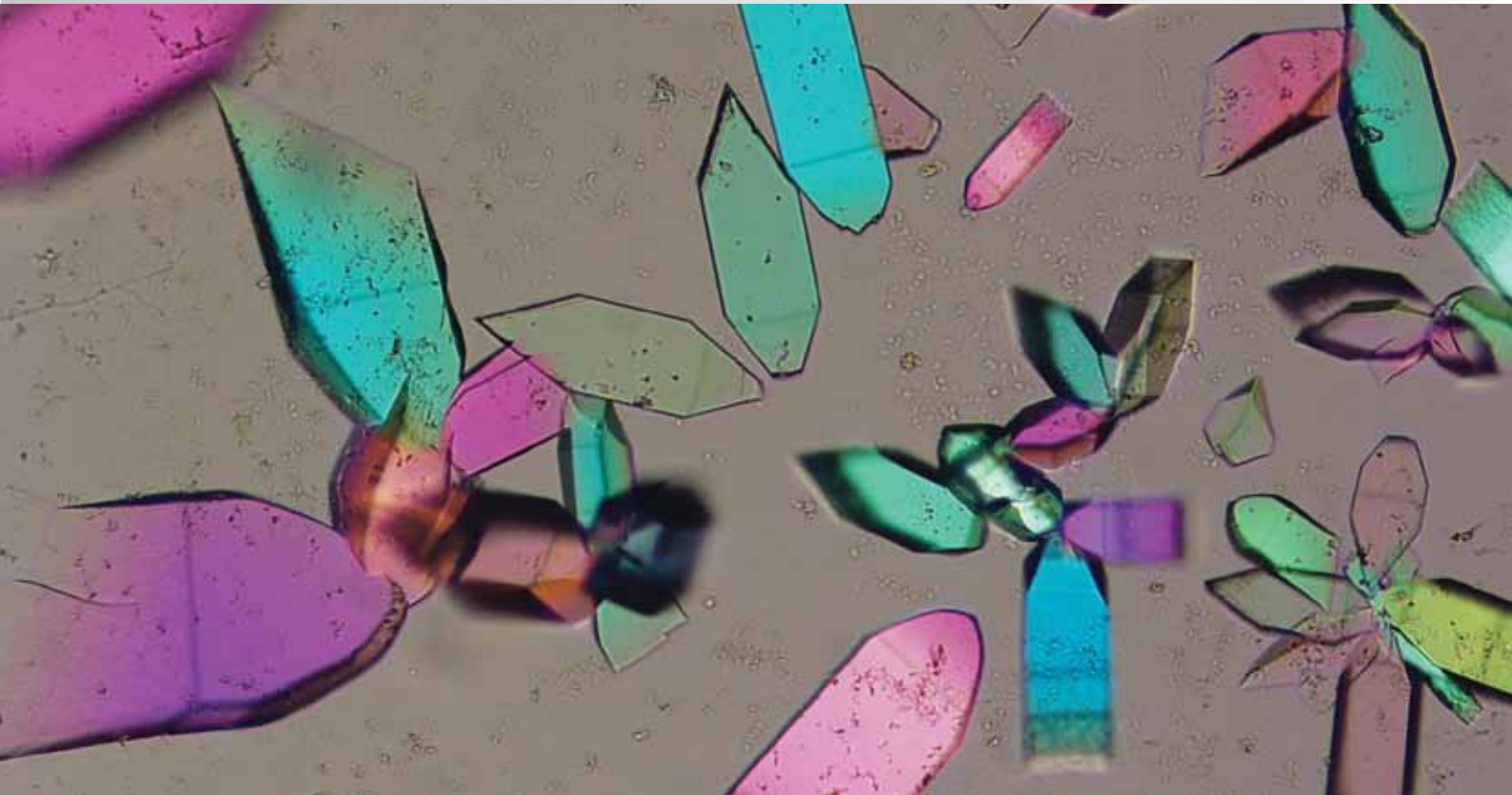


labcrystal

news from the world of protein crystallography



 **ttp labtech**

natural innovators

mosquito[®] Crystal: flexibility for protein crystallography, optimisation and scale-up

perfect drop, every time

TTP Labtech's mosquito[®] Crystal is the protein crystallographer's favourite instrument. Protein crystallisation screening has never been so fast, cost-effective and simple.

define the future of your protein research with mosquito

Using nanolitre volumes of valuable protein sample results in cost savings and allows more extensive screening.

- Zero cross-contamination from disposable pipettes
- Rapid set-up in less than 2 minutes per plate
- Save precious protein sample with low volume handling from 25 nL to 1.2 µL
- Easy set-up: ideal for first time users and multi-user environments

no need for down time between any experiment type

TTP Labtech's mosquito Crystal gives you the freedom to automate vapour diffusion protein crystallisation techniques such as hanging or sitting drop, seeding, microbatch or additive screening plate preparation without changing instrument settings.

accuracy and repeatability

Drops are placed centrally in the sub-wells of sitting drop plates every time, making protein crystals easier to identify by facilitating automated analysis. TTP Labtech's mosquito Crystal also allows you to create several multi-component drops per well, even in 96-well hanging drop set-ups, so you can assess different protein concentrations at the same time.

precise nanolitre handling even at high viscosity

mosquito's unique nanolitre liquid handling technology can perform multiple aspirations before a single dispense, which is essential for automating low volume additive screening and microseeding. You can even dispense a combination of solutions simultaneously – with additional mixing if required – not only ensuring perfect drop formation but also allowing volumes down to 10 nL to be handled for optimal protein crystallisation.

increase the volume with mosquito HV

In addition, TTP Labtech have recently launched mosquito HV to enable accurate dispensing of larger volumes required for effective scale-up and those studies requiring volumes between 500 nL - 5 µL. mosquito HV is as easy to program and run as a standard mosquito enabling fast and accurate automated set-up for crystallisation scale-up or seeding trials requiring larger volumes.

“The reproducibility and accuracy of mosquito Crystal during primary screen set-up and optimisation studies were crucial to obtaining well-diffracting crystals from a limited amount of sample in a time-efficient way.”

Ilka Muller, BioFocus. Read their story on page 11.

“Excellent liquid handling for setting up sitting drop crystallography experiments. It was fast! We set up over 4000 experiments with 8 proteins in only 6 hours. I had 27 ‘hits’ for crystallography conditions out of 480 mother liquor conditions.”

James Caldwell, San Diego State University, US

“We obtained crystals for a hard to get membrane protein literally within days after we had the mosquito in our lab.”

Dr Chris Ulens, Katholieke Universiteit Leuven, Belgium

Book a demo at sales@ttplabtech.com



Follow our mosquito Crystal group on LinkedIn for the most recent crystallography news!



Specifications	mosquito [®] Crystal
Dispense range:	25 nL – 1200 nL
Plate/ deck capacity:	2 or 5
Experimental set-up type:	hanging drop, sitting drop, microbatch, bicelle, additive screening and microseeding
Plate set-up time:	<2 mins
Dead volume:	<0.3 µL
Min accessible volume:	10 nL
Dimensions (w x d x h):	390 x 470 x 690 mm (15.5 x 18.5 x 27")
Weight:	27 kg (59 lbs)
Services:	110/220 V single phase 50/60 Hz
Noise:	64 dBA peak noise during operation
Optional extras:	humidity chamber

www.ttplabtech.com

mosquito® LCP: automate your membrane protein screening, optimisation and scale-up

Membrane protein crystallisation screening with TTP Labtech's mosquito® LCP overcomes the common problems encountered with highly viscous lipids. mosquito LCP allows you to fully automate LCP set-ups accurately and repeatably.

precise, high speed dispensing

A mosquito pipetting head (as used in mosquito Crystal) and an automated LCP syringe dispenser are combined in one compact instrument capable of highly accurate, precise and rapid plate set-up.

zero contamination

TTP Labtech's mosquito LCP employs our proven positive displacement mosquito tips for screen additions guaranteeing zero cross-contamination and highly accurate pipetting across a vast viscosity range.

unrivalled reproducibility down to 25 nL

mosquito LCP allows you to reliably dispense LCP, protein and screen solutions in volumes as low as 25 nL.

highly accurate drop positioning

automated calibration of syringe and accurate pipette positioning ensures precise drop-on-drop placement for easy automated imaging.

“ mosquito LCP is faster, more accurate, robust and user friendly. The positive displacement tips help to accurately manipulate liquids with the large range of viscosities ”

Dr Vadim Cherezov, The Scripps Research Institute, La Jolla, USA. Read his story in labcrystal on page 6-7!

full mosquito Crystal functionality

mosquito LCP includes all the functionality of mosquito Crystal for your automated vapour diffusion set-ups, allowing you to set up both LCP and traditional protein crystallisation experiments in any commercially available plate with no configuration changes or additional software/hardware.

additional components to enhance your screening

TTP Labtech also offers additional components to enhance your membrane protein crystallisation screening, such as an optional humidity chamber and an automated mixer for preparing LCP.

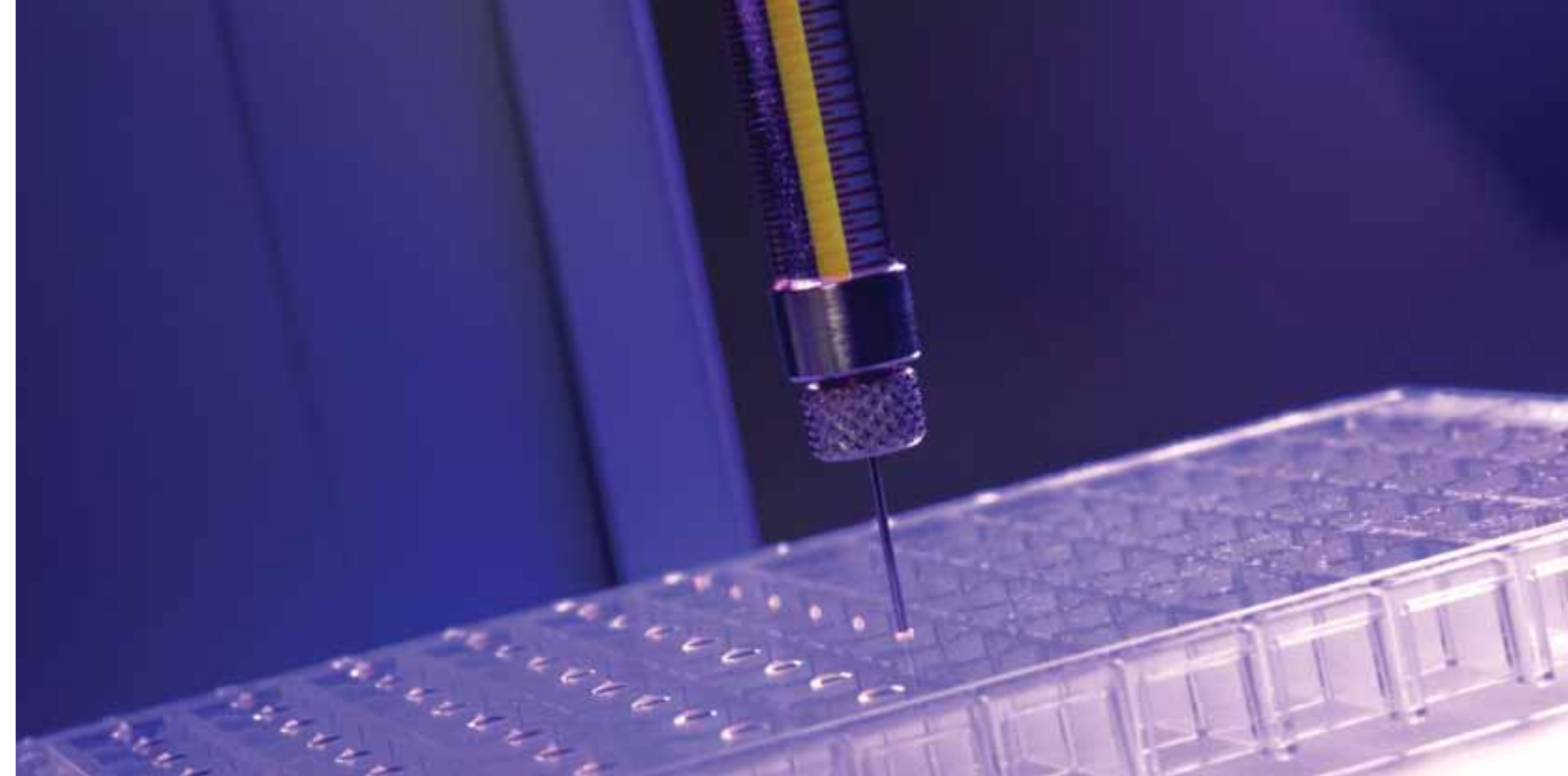
“ mosquito LCP boasts a small footprint. Tip location and reproducible and accurate tip return is of great benefit for the precise positioning of drops. An extremely user friendly instrument used for both ‘in meso’ and ‘in surfo’ methods of protein crystallography! ”

Professor Martin Caffrey, Trinity College, Dublin, Ireland



consumables

Check out our consumable list of pipettes, strips, plates and plate seals at: www.ttplabtech.com/consumables



iQ plate for sitting drop set-ups

To facilitate the sitting drop technique, TTP Labtech has developed a triple drop 96-well plate. The iQ plate has been created specifically for high throughput, low cost, low volume sitting drop protein crystallisation set-ups and is ideal for microseeding protocols. The plate has a reservoir range of 30–80 µL and, crucially, offers three identical sitting drop locations to facilitate high-density combinatorial experiments. The plate has been constructed from optically clear, low birefringence plastic suitable for UV imaging and has large area flat bottom wells for optimal drop formation and crystal viewing. With SBS standard dimensions, it is designed to fit all common holders including the mosquito plate deck, which makes it ideal for automated crystal screening.

Specifications	mosquito® LCP
Dispense range:	25 nL – 1200 nL
Plate/ deck capacity:	2 or 4
Experimental set-up type:	LCP, hanging drop, sitting drop, microbatch, bicelle, additive screening and microseeding
Plate set-up time:	<2 mins, LCP setup <5 mins
Dead volume:	<0.3 µL
Min accessible volume:	10 nL
Dimensions (w x d x h):	430 x 590 x 690 mm (17 x 23 x 27")
Weight:	34 kg (75 lbs)
Services:	110/220 V single phase 50/60 Hz
Noise:	64 dBA peak noise during operation
Optional extras:	humidity chamber, LCP mixer

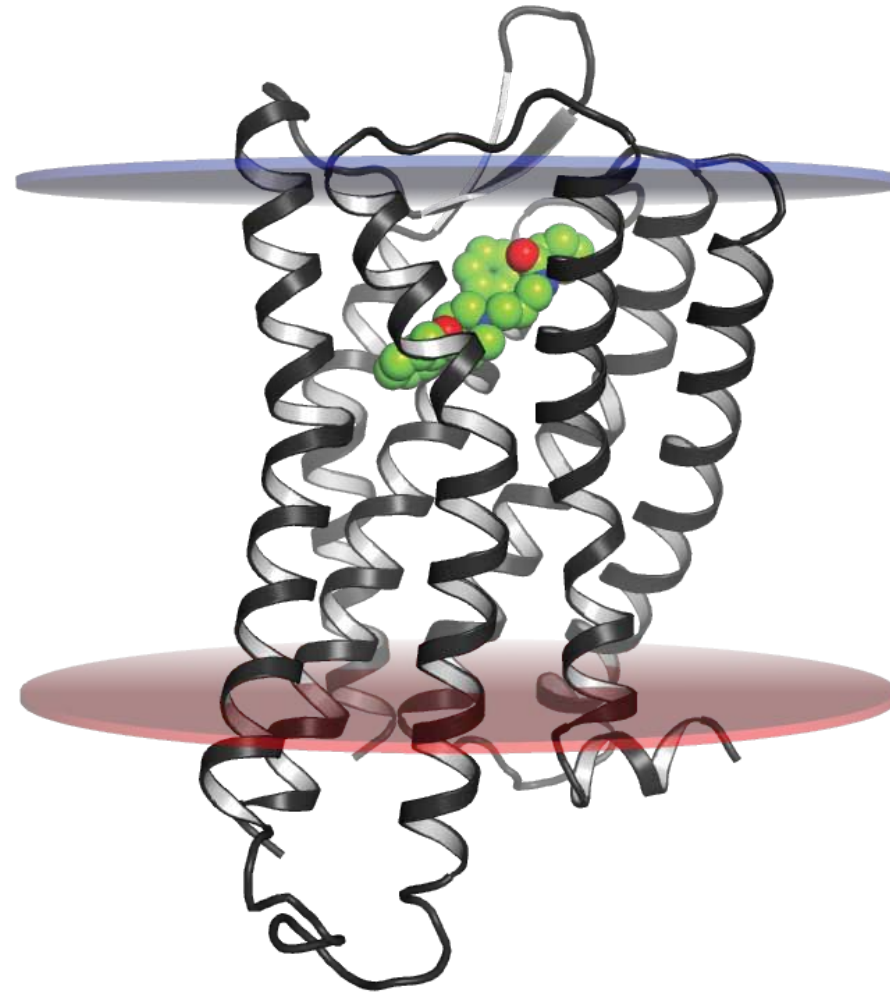
mosquito® LCP forms part of Scripps' LCP Toolchest

TTP Labtech's mosquito® LCP has recently been included into a research project referred to as the LCP Toolchest (1) which evaluates LCP instrumentation and methodologies for the crystallisation, stabilisation of membrane proteins and for structural studies thereof.

This project has been developed and hosted by Dr Vadim Cherezov at The Scripps Research Institute, USA, as a result of a project funded by the National Institute of Health Common Fund's Joint Center for Innovative Membrane Protein Technologies (JICMPT; <http://jicmpt.scripps.edu>). Its goal is to evaluate and disseminate information about crystallographic technologies to the broader scientific community, providing feedback to instrumentation companies in order to help them improve their products for the benefit of the membrane protein structural biology community.

Vadim Cherezov has been established in the field of membrane biophysics and protein crystallography for over 15 years; since his PhD from Moscow Institute of Physics and Technology in 1997 on characterising interactions between membranes and postdoctoral research in Martin Caffrey's group, Ohio State University. Starting as a research associate and staff scientist at The Scripps Research Institute in 2006, he quickly progressed to establishing his own group as an Assistant Professor in 2009. His group focuses on the structure and function of membrane proteins implicated in human health, with a strong interest in proteins in a lipid environment. Vadim has become an expert in the field of "in meso" or LCP protein crystallography.

A number of the research projects undertaken by this group have employed an LCP crystallisation robot. In early studies, Vadim had used a custom built LCP crystallisation robot which was designed by the Caffrey group at the Ohio State University (OSU) (2). However since acquiring a mosquito LCP in November 2011, he has found that it is faster, more accurate, robust and user friendly.



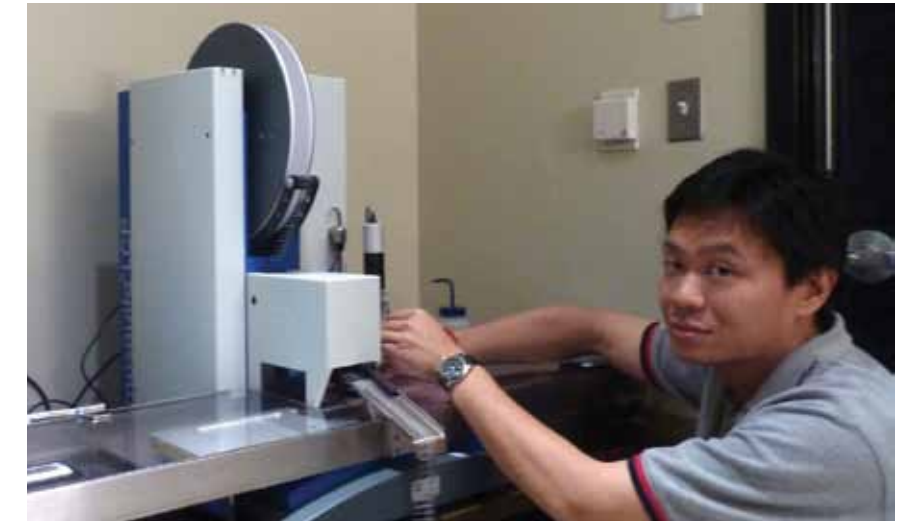
Structure of the human nociceptin/orphanin FQ opioid G protein-coupled receptor in complex with a mimetic peptide³

“ TTP Labtech's mosquito LCP provides us with an extremely reliable and accurate solution for setting up high-throughput crystallisation trials, and is virtually maintenance free. ”

Whilst comparing these robots, Vadim highlights that one of the advantages of mosquito LCP over other similar instruments is the use of disposable positive displacement tips for dispensing precipitant solutions. He highlights that positive displacement helps to accurately manipulate liquids with the large range of viscosities used as precipitant in crystallisation trials with disposable tips guaranteeing zero cross-contamination between wells.

Vadim's group focuses mainly on LCP crystallisation techniques in a microbatch mode. When setting up a microbatch LCP experiment, Vadim advises the establishment of a controlled humidity level, (preferably at or above 85 % RH).

Since having the mosquito LCP in his laboratory, Vadim's group has successfully determined the structure of a nociceptin/orphanin FQ receptor complex (3) in collaboration with the GPCR Network Center (<http://gpcr.scripps.edu>) and revealed β -domain structures of Escherichia coli O157:H7 intimin and Yersinia pseudotuberculosis invasin (4) in collaboration with Susan Buchanan from NIDDK – also a mosquito LCP user. mosquito LCP is also being used in collaboration with other labs at The Scripps Institute and within the GPCR Network Center to crystallise a number of membrane proteins. Its ease of use and robust nature, with no need to recalibrate between experiments or liquid classes, makes mosquito LCP ideal for specialist labs such as Vadim's, first time users, and multi-user environments alike.



Staff scientist Wei Lui, from the Vadim Cherezov laboratory, using mosquito LCP

What is your lab's story? Contribute to our next labcrystal and get a free spool of tips.

Send your brief abstract to labcrystal@ttplabtech.com

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from protein purification to crystallisation trial in one day

Professor Savvas Savvides is head of the Unit for Structural Biology at Ghent University, where his research group works towards the elucidation of structure-function relationships of soluble and membrane-associated proteins (and complexes thereof) of biomedical and biotechnological importance. Often working with prohibitively low amounts of recombinant proteins and protein complexes, mosquito[®] Crystal has enabled broad and effective screening to be performed.

Prof Savvides established the Unit for Structural Biology at Ghent University seven years ago, after moving from the USA where he spent 12 years at respected research centres such as Cornell University, Oregon State University, and Washington University School of Medicine studying structural biology. His track record reflects a commitment to high-impact interdisciplinary research in structural biology in the context of well-networked external collaborations with academic and industrial R&D partners.

His current research group focuses on the elucidation of structure-function relationships of key protein and protein-protein complexes within two main research domains: cytokines and receptors in the human haematopoietic and immune system, and bacterial secretion and molecular transport machineries in pathogenic bacteria. Consistently publishing his work in high impact journals, such as: *Nature Structural and Molecular Biology*, *PNAS*, *Blood*, *Molecular Cell*, and *EMBO Journal*, he is a recognised authority in the study of structure-function relationships of proteins of biomedical and biotechnological significance.

Following a very favourable evaluation of a demonstration instrument, and based on positive feedback by colleagues worldwide who are already proud users of mosquito Crystal, Savvas acquired his mosquito Crystal in May 2011. In the ensuing few months his group experienced a “dramatic change of experimental mentality regarding protein crystallisation”. Scientists in his group are now able to rapidly screen minute amounts of purified protein samples that often lie in the sub-milligram range. With mosquito’s fast set-up and ease of use, they are able to transfer recombinant proteins and complexes immediately post-purification into crystallisation trials using 50 nL or 100 nL droplets in a single day (often within the hour).

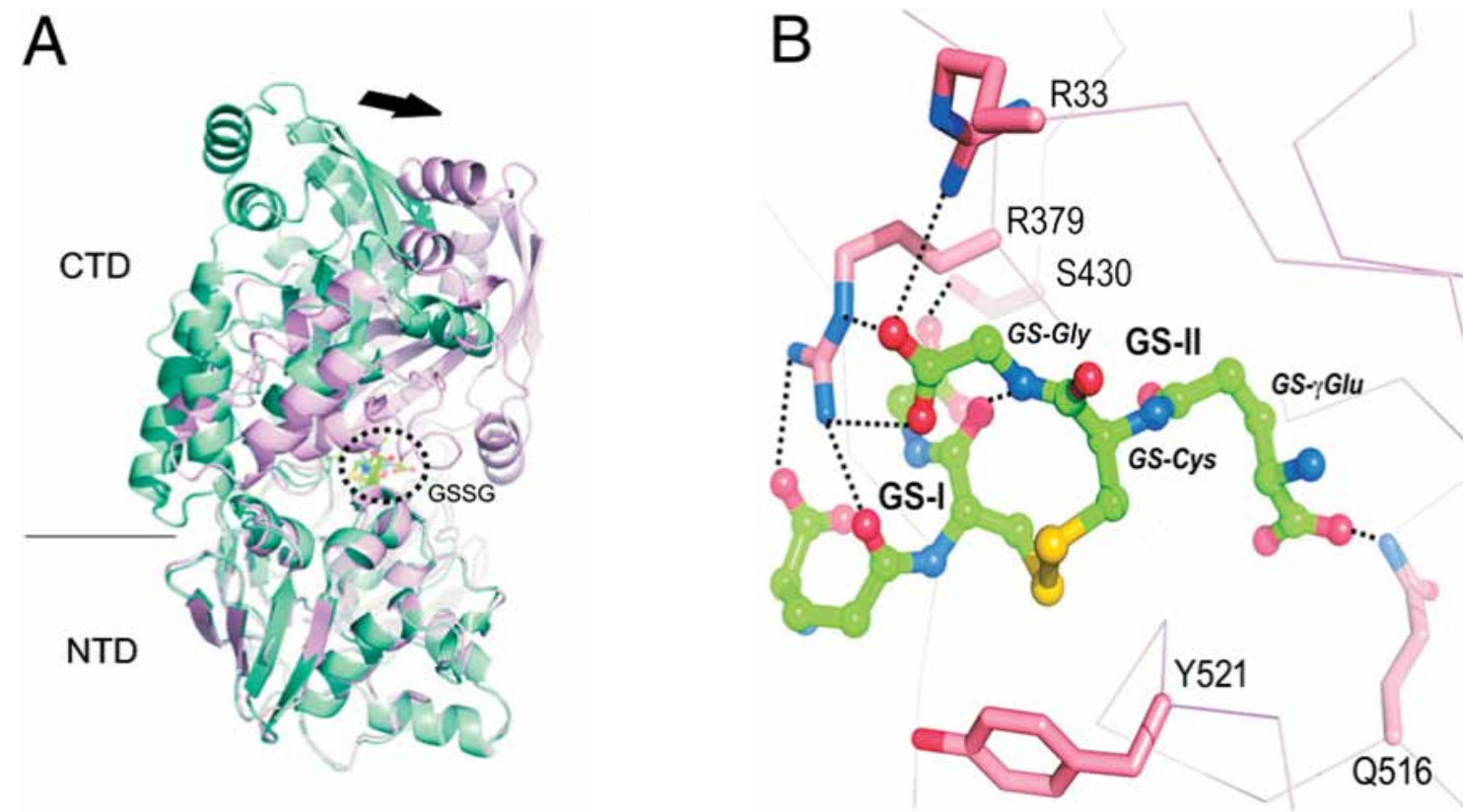
Before mosquito Crystal, Savvas highlighted that “Prohibitively low amounts (around or less than 0.5 mg of total protein) of recombinant proteins and protein complexes imposed tremendous constraints in the ability to screen the crystallisation space efficiently and broadly. In addition, it was often not possible to perform the optimisation of crystallisation leads using the same batch of recombinant protein that was used for the initial screen. mosquito Crystal’s nanolitre pipetting capabilities has allowed us to maximise screening conditions in addition to saving precious protein samples for optimisation studies using the same batch of recombinant protein that was used for the initial screen.”

Savvas’ research group houses 10 post graduate students and post-doctoral scientists, who combine structural information obtained via hybrid methods in structural biology (X-ray crystallography, Small-angle X-ray Scattering (SAXS) and Electron Microscopy (EM), with biochemical, biophysical, and cell biological studies). He has found mosquito Crystal to be “ideal for a multi-user environment, being extremely robust, with very few things that can go wrong and with no possibility for cross-contamination”. He states that is “ideal for medium-sized labs that do not have the means to run a crystallisation facility with the help of a dedicated lab technician”.

The ability of mosquito Crystal to help address complex biological questions in structural biology quickly and efficiently has been highlighted in his recent work, screening and optimising crystallisation conditions to elucidate the structure of a bacterial periplasmic binding protein HbpA that binds the redox tripeptide glutathione. This work was recently published in the leading journal *Proceedings of the National Academy of Science* (1).

More recently, the Savvides Lab was able to obtain diffraction-quality crystals of the viral decoy receptor BARF1 from the Epstein-Barr virus in complex with its target human cytokine. The work has led to unravelling this receptor structure, providing a possible mechanistic basis for immune system subversion by BARF1. These results will be appearing shortly in *Nature Structure and Molecular Biology* (2). mosquito Crystal proved to be a defining experimental platform in this endeavour allowing close to 2,000 crystallisation trials using a limited amount of sample. Notably, the crystals that ultimately led to this important crystal structure were obtained directly from these initial crystallisation screens. Savvas highlighted that the speed, less than 2 mins per plate, and reproducibility in pipetting 50 nL droplets without sample cross-contamination offered a clear experimental advantage.

“TTP Labtech’s mosquito[®] Crystal proved to be a tremendously efficient tool in setting up close to 2000 crystallisation trials using a limited amount of sample. The speed, less than 2 minutes per plate and reproducibility in pipetting 50 nL droplets, without sample cross-contamination offered a clear experimental advantage.”



(A) Ribbon diagram showing an overlay of unliganded Dpp (PDB entry 1DPE) with GbpA from *H. parasuis*. The structures were superposed with respect to their N-terminal domains (B) Binding of GSSG to the GbpA interdomain interface. The two glutathione legs are labeled as GS-I and GS-II. For clarity some interactions have been omitted. The figure was created with PyMOL (The PyMOL Molecular Graphics System, Schrodinger, LLC).

The Unit for Structural Biology at Ghent University houses state-of-the-art facilities to carry out front line research of molecular structural biology, leading to the elucidation of the structure-function landscape of a protein starting with the gene. “The recent addition of mosquito Crystal to the group’s instrument portfolio has proved to be a tremendously efficient tool in circumventing acute experimental bottlenecks, and its introduction into our experimental arsenal has radically improved our experimental efficiency.”



Find mosquito application notes at www.ttplabtech.com/products/mosquito/bibliography.html

In summary: the mosquito Crystal system provides a compact and very versatile crystallisation platform in a multi-user environment without the need to appoint a dedicated technician to supervise and/or execute crystallisation trials. In particular, the ability to go from protein purification to extensive crystallisation trials on the same day (often within 1-2 hours post-purification) using 50 or 100 nL crystallisation droplets offers a clear experimental advantage.

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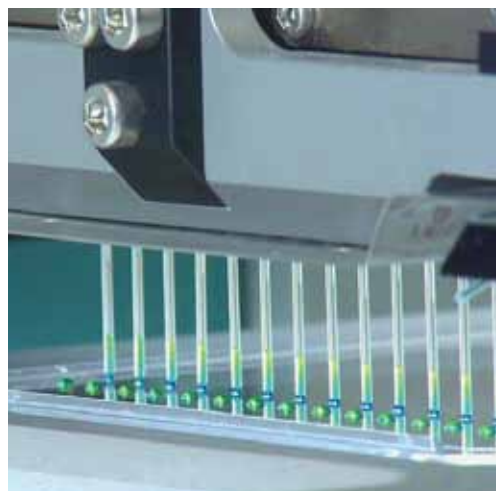
optimising crystal formation using microseeding

The process of obtaining structural information by protein X-ray crystallography plays a major role in drug design and discovery. However, the process of obtaining crystals suitable for data collection can represent a bottleneck in structure determination with lengthy, large scale trials required to determine optimal conditions for crystal formation.

The process of crystal formation can be divided into three discreet stages:

- initial screening to produce hits;
- optimisation to produce larger stable crystals; and finally
- scale-up for the production of larger crystals for data collection.

mosquito[®] Crystal and mosquito[®] LCP can be successfully employed throughout all of these stages to speed up the process of crystal screen set-up conditions, optimisation and final screen conditions. Once a set of screen conditions have been identified, it is essential to determine the optimal conditions for protein crystallisation, ensuring crystal stability and/or conformation to enhance conditions for ongoing crystal growth following successful nucleation.



mosquito can add extremely low volumes of seed stock (10 nL) together with screen solution.

Seeding or microseeding is a well established method used to improve the quality and reproducibility of crystals. Seeding takes advantage of the fact that crystal formation is a two step process involving nuclei formation and crystal growth. The initial step, nuclei formation, is more likely to occur if the protein solution is highly supersaturated. In contrast, crystal growth is maintained in the metastable zone. Seeding methods separate the two events of nucleation and crystal growth. In microseeding this separation is accomplished by transferring a seed, a submicroscopic crystal, from one condition where the level of supersaturation is high, to a similar condition at a lower level of supersaturation. In order to have lower levels of supersaturation either the protein or the precipitant concentration is lowered in a crystallisation set-up.

The determination of optimal conditions for successful crystallisation during the seeding process can be extremely time-consuming. Setting up plates with varying salt, buffer and precipitant ratios and concentrations for the crystal optimisation process can be tedious, repetitive and complicated. mosquito Crystal or mosquito LCP can help this process significantly, as both instruments are capable of automating hanging drop, sitting drop and microbatch set-ups without requiring instrument set-up changes. Using mosquito, it is easy to add extremely low volumes of seed stock together with screen solution to the protein drop to enable rapid elucidation of ideal scale-up conditions. mosquito's ability to multi-aspirate not only provides "in tip" mixing but also gives additional benefits by reducing the minimum dispense volume from 25 nL to 10 nL. Another key factor is the use of disposable positive displacement tips which will never clog when handling the seed stock 'paste'.

Example of microseeding a hanging drop screen set-up

The mosquito deck is loaded with an inverted hanging drop plate seal, a 96-well plate of screen buffers, and reservoirs of protein samples and seed stock.

- **Step 1.** mosquito aspirates protein from the protein reservoir and multi-dispenses 100 nL drops in columns across the plate seal
- **Step 2.** Next, mosquito is used to aspirate 10 nL of seed stock from the reservoir and then moves to the last column of the screen plate and multi-aspirates 90 nL of screen solution
- **Step 3.** This combined drop is dispensed accurately on top of the first column of protein drops on the plate seal
- **Step 4.** Steps 2 and 3 are repeated to create a mirror image of the screen plate on the plate seal.
- **Step 5.** Finally, the plate seal is inverted over the screen plate using a simple alignment jig so that each droplet of protein, seed stock and screen hangs over the corresponding screen well.

The entire procedure takes under 2.5 minutes per 96-well plate and eliminates both protein and seed stock wastage.

“TTP Labtech's mosquito is working well, we have set up a bit over 500 plates since it was installed, and we have had a lot of success with seeding.”

Alastair McEwen, PhD, Structural Biology and Genomics Technology Platform, Integrated Structural Biology, IGBMC

BioFocus shed light on Huntington's disease using mosquito[®] Crystal

The Structural Biology group at BioFocus have been using mosquito[®] Crystal since 2008. BioFocus is a contract research organisation, based on the Chesterford Research Park, near Cambridge, UK, which specialises in lead optimisation or fragment based discovery programs to deliver pre-clinical drug candidates for neurodegenerative and inflammatory diseases.

The Structural Biology Services group, headed by Dr Marike Lamers, has expertise in high throughput gene expression, automated protein purification, crystallisation and X-ray crystallography. The group consists of seven scientists with three X-ray crystallographers who routinely use mosquito Crystal in their screening trials.

As one of the early users of mosquito Crystal, Marike was involved in its development, and was impressed by its simplicity and the ability to achieve reliable and accurate dispensing of low volumes of protein for crystal screening set-up. She highlighted that one of the main advantages of this robot was its ability to accurately and consistently set 100 to 200 nL drops, thereby enabling small amounts of protein to be used – which was impossible using manual methods.

Recent work in collaboration with the CHDI Foundation, Inc. (Los Angeles, USA), on the crystallisation of apo-caspase-6 resulted in a publication in the Journal of Molecular Biology (1). The CHDI Foundation, a biomedical research foundation devoted to discovering Huntington's disease-modifying therapies, collaborated with BioFocus to crystallise apo-caspase-6 in a state amenable to drug design. Caspase-6 has been implicated in neuronal survival and apoptosis, is also linked to the generation of fragments that show neuronal toxicity (2). This paper describing the successful crystallisation of an active form of apo-capsase-6 and caspase-6 in complex with an irreversible covalent inhibitor Z-VAD-FMK used



Dr Marike Lamers, Head of The Structural Biology Services group at BioFocus



Caspase 6 (Additive H7)

mosquito Crystal for both screening trials and optimisation. It has also recently been summarised in a TTP Labtech application note entitled "Crystal structure determination of a key processing enzyme in Huntington's disease (HD)"

Marike's group find that the hanging drop technique is most efficient method for crystal screen set-up, providing more hits when compared to the sitting drop or microbatch methods. mosquito[®] is more accurate in setting up the drops compared to manual methods and increases the reproducibility in obtaining crystals.



Caspase-6 FMK (Wizard E62)

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“The reproducibility and accuracy of TTP Labtech's mosquito Crystal during primary screen set-up and optimisation studies is crucial in obtaining well-diffracting crystals from a limited amount of sample in a time-efficient way.”

coming soon: woodpecker[®]

Simple, fast and accurate screen optimisation specifically designed for today's crystallographer by TTP Labtech, the maker of mosquito[®]. TTP Labtech's woodpecker[®] gives the freedom to use any liquid type without the need for washing or liquid specification.

simple

- easy set-up and operation, just like TTP Labtech's mosquito
- minimal training needed, new users can be up and running in less than 10 minutes

fast

- less than 5 minutes to dispense a four ingredient gradient plate
- no need for liquid classification

accurate

- better than 5% CV at 1 μ L
- zero cross-contamination
- positive displacement, non-contact dispensing
- accurate dispensing of all types of liquids regardless of viscosity

clean

- non-contact disposable pipettes
- no washing cycles
- no chance of blocking or clogging

Specifications	woodpecker
Volume range:	0.5 μ L - 100 μ L
Dispense resolution:	200 nL
No. of dispense heads:	5 or 10
Plate format:	24, 48, 96
Dead volume:	< 0.5 mL
Dimensions (w x d x h):	600 mm x 555 mm x 630 mm 24" x 22" x 25"

Released 2012. Front cover illustration courtesy of Dr Alexey Rak, Sanofi R&D

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