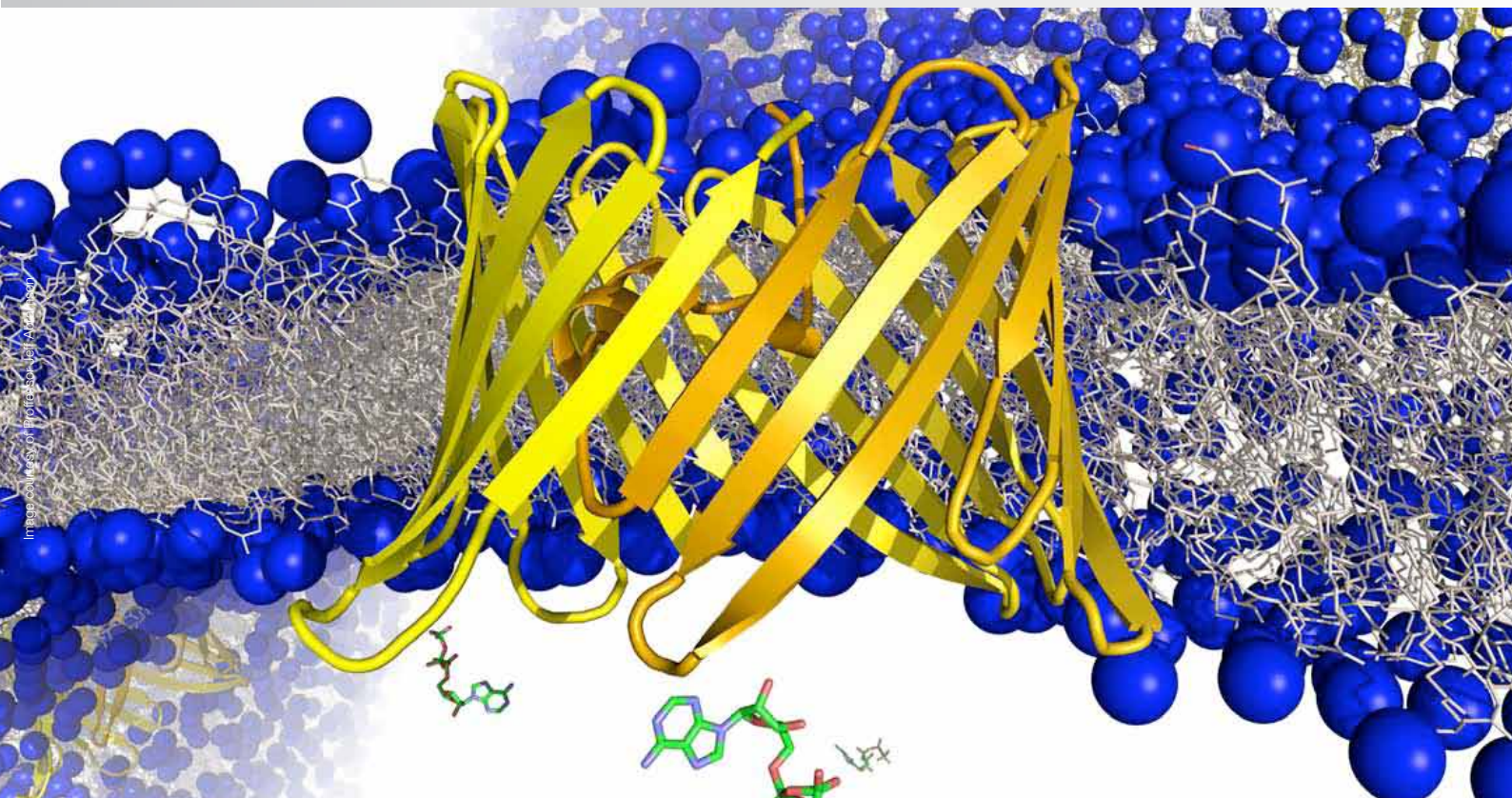


labcrystal

news from the world of protein crystallography



 **ttplabtech**

natural innovators

mosquito[®] Crystal and mosquito[®] LCP: for your protein crystallisation needs

TTP Labtech's mosquito[®] Crystal is the protein crystallographer's favourite instrument. It makes protein crystallisation screening faster, more cost-effective and quite simply easier than ever before.

With the addition of mosquito[®] LCP to its portfolio, TTP Labtech offers a fully automated solution to lipidic cubic phase (LCP) screening, making membrane protein crystallisation available to every research scientist in this field. Used by a high number of eminent protein crystallographers worldwide, mosquito Crystal and mosquito LCP's unique features include:

low volume liquid handling

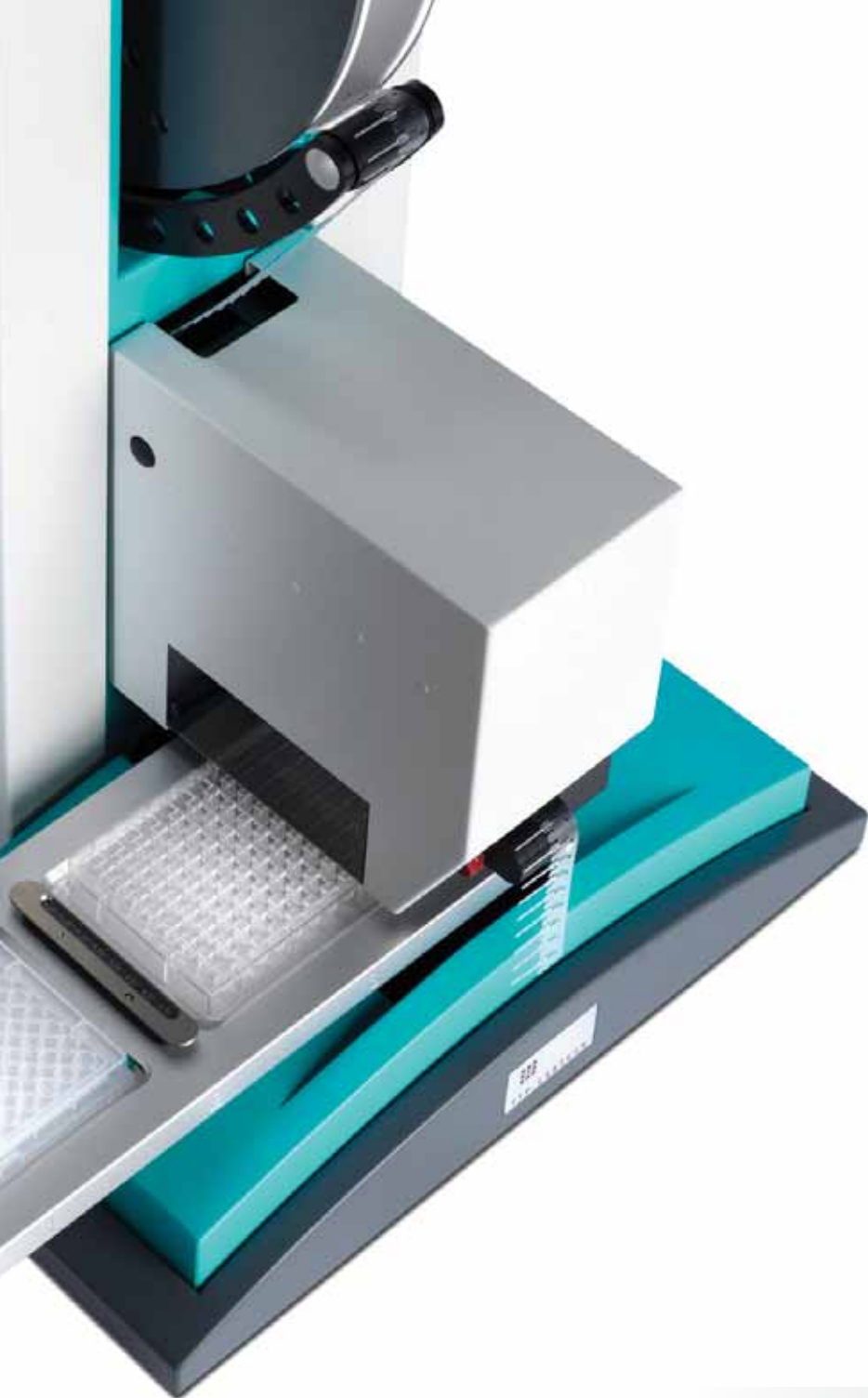
- Accurate and reproducible pipetting throughout the 25 nL to 1.2 μ L range
- Precise low volume sample handling across a vast viscosity range
- Disposable low volume tips, guaranteeing zero cross-contamination and eliminating the need for tip washing.

flexibility and precision

mosquito is a flexible accurate nanoliter pipettor capable of fast automated set ups for a range of crystallisation techniques.

- Rapid set up times of under 2 minutes/plate
- Precise, low volume pipetting from positive displacement tips
- Highly accurate drop positioning.





robust and reliable

mosquito can reliably dispense all liquid types with unrivalled accuracy and repeatability

- Ideal for first time users and multi-user environments due to it's ease of set up and use
- No need to recalibrate between experiments or different liquid classes
- Unrivalled reliability
- Perfect drop dispensing every time.

With over 10,000 users to date, mosquito is the instrument of choice for protein crystallisation.

Contact us to arrange a demo at sales@ttplabtech.com

automation speeds up crystallisation screening

What advantages do TTP Labtech's mosquito® Crystal and mosquito® LCP give you?

The automation of protein crystallisation screening has contributed significantly to the rapid progress of crystallography-based structural biology. mosquito® Crystal and LCP are able to perform accurate, repetitive dispensing of highly viscous solutions used in protein crystallography. These instruments offer time savings, consistency between replicates and reproducibility between experiments which were previously unachievable using manual screening methods.

By overcoming the challenges of both the accurate pipetting of solutions of varying viscosities and precise drop positioning, mosquito Crystal enables the automation of a range of vapour phase diffusion methods (sitting and hanging drop) and microbatch set up without instrument configuration change.

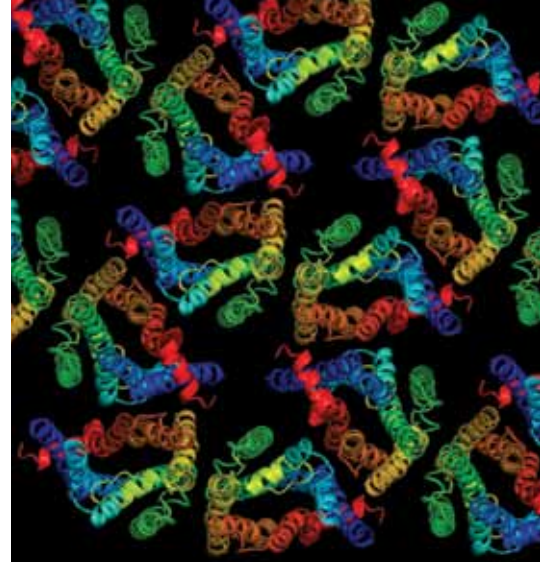
In addition, mosquito's accuracy of drop placement allows users to set up multiple drops in a well, maximising the number of screening conditions per plate. This allows different constructs, volume ratios or protein concentrations to be assessed simultaneously and can yield up to 288 conditions in a single sitting or hanging drop plate in under four minutes. With reliable and robust hardware, together with simple user-friendly software, mosquito Crystal offers flexibility for the crystallographer to perform screening, optimisation (including microseeding) and scale up effectively.

The rate and ease at which crystallisation of proteins have been achieved using mosquito Crystal has aided significantly in the study and

elucidation of structure activity relationships (SARs) for a number of proteins. Two recent papers published in P.N.A.S. and the Journal of Molecular Biology have described the successful crystallisation of proteins using this robot.^{1,2} In the first study a periplasmic heme-binding protein HbpA was successfully crystallised from *Haemophilus influenzae* Rd enabling its role to be re-defined as a glutathione import protein. The latter study highlights mosquito's ability to perform rapid screening and optimisation of the crystallisation of a novel enzyme, apo-caspase-6, a key component of Alzheimer's disease.

The addition of mosquito LCP and an optional LCP mixer to the product range, for the automated crystallisation of lipophilic membrane proteins, has proved extremely successful in a number of laboratories. By incorporating a microsyringe dispenser that can accurately dispense low volumes of the highly viscous cubic phase used in the 'in meso' or LCP technique, this is the ultimate instrument for any protein crystallography laboratory. mosquito LCP is not only capable of carrying out the 'in meso' and increasingly popular bicelle methods of membrane crystallography but also of all the 'in surfo' techniques described for mosquito crystal.

Both mosquito Crystal and mosquito LCP are ideal for multi-user environments due to their robust nature and ease of use, with no need to recalibrate or wash between experiments.



β -adrenergic receptor molecules in an LCP crystal image roughly normal to the membrane plane.

conclusion

In conjunction with biological studies, the knowledge of the crystallographic structure of a protein can provide important information about protein conformation and its active site. The successful automation of crystallisation using robots such as mosquito Crystal and LCP has significantly improved the process of protein crystallisation. By maximising the number of screening conditions per protein sample, optimising crystallisation conditions and enabling faster scale up, the structural biologist can address complex biological questions quickly and efficiently. These robots enable crystallisation techniques to be made available not just to a select group of specialist scientists but to be used by multi-disciplinary teams working in the field of structural and molecular biology.

References

1. Vergauwen, B., Elegheert, J., Dansercoer, A., Devreese, B. and Savvides, S.N. (2010) Glutathione import in *Haemophilus influenzae* Rd is primed by the periplasmic heme-binding protein HbpA. P.N.A.S. 107: pp13270-5.
2. Muller, I., Lamers, M.B., Ritchie, A.J., Park, H., Dominguez, C., Munoz-Sanjuan, I., Maillard, M. and Kiselyov, A. (2011) A new apo-caspase-6 crystal form reveals the active conformation of the Apoenzyme. J. Molecular Biol. 410: pp307-15.

mosquito[®] Crystal for crystallographic studies of RNA protein complexes

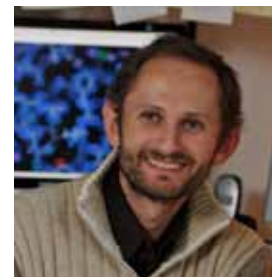
RNA plays a key role in a variety of cellular activities and in many cases its biological function is conferred by its three-dimensional structure.

The understanding of both RNA activities and the proteins and factors involved in RNA processing mechanisms requires the knowledge of the structure and conformational dynamics of these molecules. X-ray crystallography is a method of choice for determining high resolution structures of these molecules.

Dr Sébastien Fribourg's structural biology group at the European Institute of Chemistry and Biology and INSERM Unit 869, in Pessac, France focus on the structural study of protein complexes involved in RNA processing mechanisms (Pol III transcription initiation, mRNA and rRNA maturation), with an aim to obtain insights into the basic mechanisms underlying RNA processes and their relationship with associated human diseases.

As a biochemist with a strong background in structural biology, Dr Fribourg employs a range of methods such as Small angle X-ray scattering (SAXS), nuclear magnetic resonance (NMR) and electron microscopy (EM) with a focus on X-ray crystallography, leading to numerous publications in journals such as RNA, Structure, Nat. Struct. Mol. Biol. and Nucleic Acids Research.

Despite having a mosquito Crystal for only 10 months he has successfully obtained crystals from five protein samples using the hanging drop technique. Previous crystallisation studies using manual methods posed many difficulties in reproducing the exact conditions during screening. In particular inaccuracies in manual pipetting made it impossible to maintain consistent volumes, especially when using nanolitre quantities.



“ Any worry about cross contamination is a zero problem, we can study point mutants within the same plate without fear of contamination. ”

DR S. FRIBOURG
EUROPEAN INST. CHEM AND BIOL.

Dr Fribourg invested in this instrument after hearing about its robustness, ease of use and fast set up time from Professor Elena Conti's lab when she was working at EMBL. The use of mosquito Crystal ensured accurate, repeatable nanolitre pipetting, maximising the use of valuable protein sample and reducing the use of expensive screening solutions.

Fribourg's group have found that mosquito Crystal is a versatile instrument, which is robust, easy to use and to calibrate if needed. In addition it benefits from no cross-contamination allowing fast and reproducible screening.

membrane protein crystallisation the American way!

Professor Jeff Abramson and his group in the Physiology Department of the David Geffen School of Medicine, University of California, Los Angeles (UCLA) were one of the first research groups to employ TTP Labtech's mosquito® Crystal in 2005.

Prof. Abramson's scientific career has been devoted to determining the structure of integral membrane proteins in order to establish a molecular basis for their function. His group specialise in the development of innovative tools and approaches for determining the 3D structure of membrane proteins¹.

In 2005, in collaboration with Joby Jenkins, the mosquito product manager, and Dr Janet Newman (now at the Bio Collaborative Crystallisation Centre, Melbourne, Australia) they successfully popularised the automation of additive screening using mosquito crystal.

This automated crystal optimisation technique is now routinely used to determine protein crystal structures in crystallography groups worldwide.

With a prolific publication history in high impact journals such as Nature, Science and P.N.A.S., Prof. Abramson's research group employs mosquito Crystal with a range of biochemical and biophysical tools to determine the biological basis of membrane transport^{2, 3, 4}. He recognises that membrane protein crystallography can be a daunting task, stating that "It is absolutely essential to understand the fundamental mechanisms behind human physiology and disease. With membrane proteins currently being

the target of over 50% of all marketed drugs, a detailed understanding of their structure and function is vital."

Prof. Abramson's group have been successful in the determination of a number of membrane protein structures, being the first to reveal the structure of a member of the Sodium Solute Symporter family (the sodium/galactose symporter, vSGLT, from *Vibrio parahaemolyticus*)^{5, 6} as well as a murine Voltage Dependent Anion Channel, a eukaryotic β -barrel protein from the outer mitochondrial membrane^{3, 7}.

It is widely known that the crystallisation of membrane proteins can often present practical challenges. Residing in a phospholipid bilayer and being hydrophobic in nature make membrane proteins difficult to express, purify and crystallise.

Detergent-based crystallisation techniques are most commonly used for membrane protein crystallography, however due to the instability of membrane proteins in this environment the use of 'in meso' lipidic cubic phase (LCP) is becoming a popular alternative and has been successfully automated using the mosquito LCP.



“ mosquito® Crystal has been recognised for its robustness and ease of use for a while now, with very few things that can go wrong and with no possibility for cross-contamination.”

JEFF ABRAMSON, UCLA

Prof. Abramson, however, is using his mosquito crystal to automate the bicelle method of membrane protein crystallisation (see description on next page). By maintaining a cold environment and pre-chilling the sample holder block it is possible to use mosquito Crystal to accurately and easily pipette the bicelle solution, thereby facilitating the automation of screening for this lipidic based technique of membrane protein crystallisation.



In a recent JoVE video (entitled “High-throughput crystallization of membrane proteins using the lipidic bicelle method”), Prof. Abramson details how to easily generate and automate bicelle crystallisation using mosquito Crystal, demonstrating that this technique is easily adoptable for all membrane protein crystallisation projects.

References

- ¹ Hsieh, J.M., Besserer, G.M., Madej, M.G., Bui, H.Q., Kwon, S., and Abramson, J. (2010) Bridging the gap: a GFP-based strategy for overexpression and purification of membrane proteins with intra and extracellular C-termini. *Protein Sci.* 19: pp868-80.
- ² Besserer, G.M., Ottolia, M., Nicoll, D.A., Chaptal, V., Cascio, D., Philipson, K.D., and Abramson, J. (2007) The second Ca^{2+} -binding domain of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger is essential for regulation: crystal structures and mutational analysis. *Proc. Natl. Acad. Sci. U S A.* 104: pp18467-72.
- ³ Ujwal, R., Cascio, D., Colletier, J.P., Faham, S., Zhang, J., Toro, L., Ping, P., and Abramson, J. (2008) The crystal structure of mouse VDAC1 at 2.3 Å resolution reveals mechanistic insights into metabolite gating. *Proc. Natl. Acad. Sci. U S A.* 105: pp17742-7.
- ⁴ Chaptal, V., Ottolia, M., Mercado-Besserer, G., Nicoll, D.A., Philipson, K.D., and Abramson, J. (2009) Structure and functional analysis of a Ca^{2+} sensor mutant of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. *J Biol Chem.* 284: pp14688-92.
- ⁵ Faham, S., Watanabe, A., Besserer, G.M., Cascio, D., Specht, A., Hirayama, B.A., Wright, E.M., and Abramson, J. (2008) The crystal structure of a sodium galactose transporter reveals mechanistic insights into Na^+ /sugar symport. *Science.* 321: pp810-4.
- ⁶ Watanabe, A., Choe, S., Chaptal, V., Rosenberg, J.M., Wright, E.M., Grabe, M., Abramson, J. (2010) The mechanism of sodium and substrate release from the binding pocket of vSGLT. *Nature.* 468: pp988-91.
- ⁷ Choudhary, O.P., Ujwal, R., Kowallis, W., Coalson, R., Abramson, J., and Grabe, M. (2010). The electrostatics of VDAC: implications for selectivity and gating. *J. Mol. Biol.* 396: pp580-92.

the bicelle method for membrane protein crystallisation

Although the LCP method of membrane protein crystallisation is a highly successful and well established technique, in the absence of a specialised robot such as mosquito[®] LCP, the technical drawbacks of this ‘in meso’ method often limit the number of trials that can be performed in a day.

Such drawbacks not only include the handling of the highly viscous lipidic cubic phase but the lengthy incubation and mixing times required to combine the protein/lipid components. As a result, membrane protein crystallisation in lipid-based bicelles and nanodiscs is currently seeing an increase in popularity.

Popularised for crystallisation by Faham and Bowie¹, bicelles are small bilayer discs that form lipid/amphiphilic structures at low temperatures (4°C) and a perforated lamellar phase at room temperature and above. On mixing the membrane protein solution with these bicelles at low temperatures, the protein enters the discs which act as a pre-existing “scaffold” for crystal formation. At room temperature and above, the mixture forms a viscous gel conducive for membrane protein crystallisation. At lower temperatures (4°C), the bicelle mix remains in a less viscous, liquid form. Consequently, protein in bicelles can be handled easily at low temperatures in the same manner as proteins in detergent. This enables crystallisation trials to be performed using mosquito Crystal.

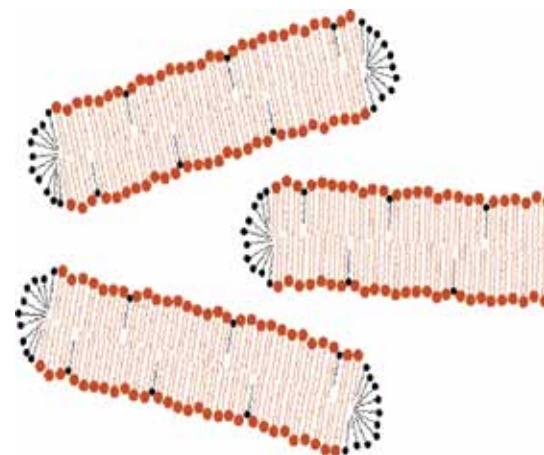
A typical bicelle mixture is formed by mixing the lipid dimyristoyl phosphatidylserine (DMPC) and the detergent, 3-(cholamidopropyl)

dimethylammonio-2-hydroxy-1-propanesulfonate (CHAPSO). In a standard crystallisation trial, a concentrated membrane protein solution (10 mg/mL) is mixed with the bicelles (a 1:4 ratio of bicelle:protein mix is recommended to start with) by gently pipetting the contents up and down at 4°C or on ice until the mix appears homogeneous.

Following mixing and 30 minute incubation at this temperature, this bicelle/protein mix can be easily dispensed in sitting or hanging drop format using mosquito’s positive displacement pipettes for screening and/or optimisation studies.

The bicelle/protein mix remains in a less viscous, liquid form at temperatures between 0 and 22°C so it is essential that the sample is contained in a pre-chilled holder block on the deck of mosquito. Bicelle crystal formation occurs above 22°C where the solution changes from its more liquid like to highly viscous form providing a strong pre-existing scaffold for crystal formation.

Similarly to the “in meso” method, the bicelle technique has been found to form type I crystals and has been successfully used to crystallise several membrane proteins^{1,2,3} offering considerable versatility in combination with practical ease of use with mosquito.



References

1. Faham, S., and Bowie, J.U. (2002) Bicelle crystallization: a new method for crystallizing membrane proteins yields a monomeric bacteriorhodopsin structure. *J. Mol. Biol.* 316: pp1-6.
2. Rasmussen, S.G., Choi, H.J., Rosenbaum, D.M., Kobilka, T.S., Thian, F.S., Edwards, P.C., Burghammer, M., Ratnala, V.R., Sanishvili, R., Fischetti, R.F., Schertler, G.F., Weis, W.I., and Kobilka, B.K. (2007) Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. *Nature.* 450: pp383-7.
3. Vinothkumar, K.R. (2011) Structure of rhomboid protease in a lipid environment. *J. Mol. Biol.* 407: pp232-47.

optimising crystal formation

Successful protein crystallisation is a difficult and labour intensive task, involving extensive numbers of screening conditions and variations thereof. One of the many challenges facing the protein crystallographer is growing crystals of sufficient size and quality to successfully determine the protein's structure (typically requiring crystals of around 100–300 μm).

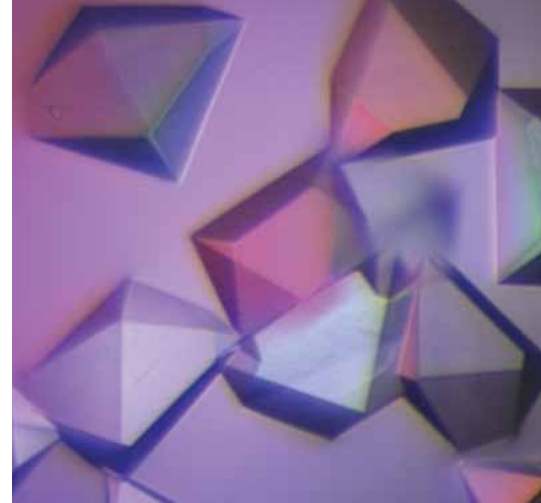
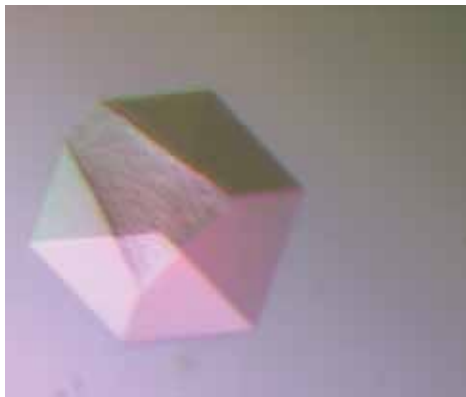
The process of protein crystallisation can be divided into three discrete stages: initial screening to produce hits; optimisation to produce larger stable crystals and define conditions for reproducibility of the crystal; and, finally, scale up to produce larger crystals for X-ray data collection.

Following the initial screening phase to establish one or more conditions for successful crystal growth, optimisation may include the incorporation of additives¹ to enhance crystal stability and/or conformation and seeding or microseeding² to enhance conditions for ongoing crystal growth following successful nucleation.

The time-consuming nature of additive screening and microseeding is easily overcome with mosquito Crystal and LCP using either hanging drop, sitting drop and microbatch set-ups. The ability to multi-aspirate not only provides 'in tip' mixing, but also gives additional benefits by reducing the minimal dispense volume. In addition, it has been found that by keeping the sample holder block chilled, sample evaporation can be reduced during longer, more complex, protocols.

Following the successful optimisation of protein crystal conditions, these can be rapidly transferred to scale up to produce larger more defined structured crystals for X-ray data collection.

The scale up process can also be successfully automated using TTP Labtech's range of mosquito robots including the mosquito HV (launched in February 2012) for highly accurate microlitre pipetting (from 0.5 to 5.0 μL). Successful scale up from sitting drop hits has been routinely achieved using larger volumes in the 48 well MRC Maxi plate using mosquito HV. Furthermore, simply using mosquito Crystal or LCP at the upper end of their volume range effectively allows 1 + 1 μL drops to be placed using a 96 well format for hanging drop hits rather than moving to 48 or 24 well plates.



“ We obtained crystals for a hard to get membrane protein literally within days of installing a mosquito[®] Crystal in our lab.”

DR C. ULENS, KU LUEVEN

- ¹. Additives can stabilise or engender conformity by specific interactions within the protein molecule. Additives include a range of small molecules such as glycerol or divalent cations such as magnesium. Detergents and screening with a range of concentrations of such molecules can be expensive and labour and reagent intensive. The ability to automate additive screening using low nanolitre volumes has enormous benefits reducing the use of costly reagents and precious protein sample.
- ². During the process of crystal formation, often optimal conditions for nucleation of a crystal may not be the same as that required for its subsequent growth. Nucleation occurs when the solution is supersaturated, whereas ordered crystal growth is optimal in a state of lower supersaturation, "a metastable solution". As a result, in order to optimise the quality and reproducibility of crystals it is often beneficial to seed crushed early phase crystal samples from one set of conditions into a second set of conditions. Seeding or micro-seeding provides a general method of decoupling crystal nucleation from crystal growth and is easily achieved and extremely effective using mosquito Crystal or LCP.

the study of GPCRs at Heptares Therapeutics

Heptares Therapeutics specialises in the study of G-protein coupled receptors (GPCRs) and employs its proprietary StaR[®] technology to purify these membrane proteins in stable conformations for SAR analysis.

This technique has enabled unique structure based drug discovery approaches to design GPCR-targeted medicines against previously undruggable targets in disease areas such as Parkinson's disease, sleep disorders, Schizophrenia and Alzheimer's disease.

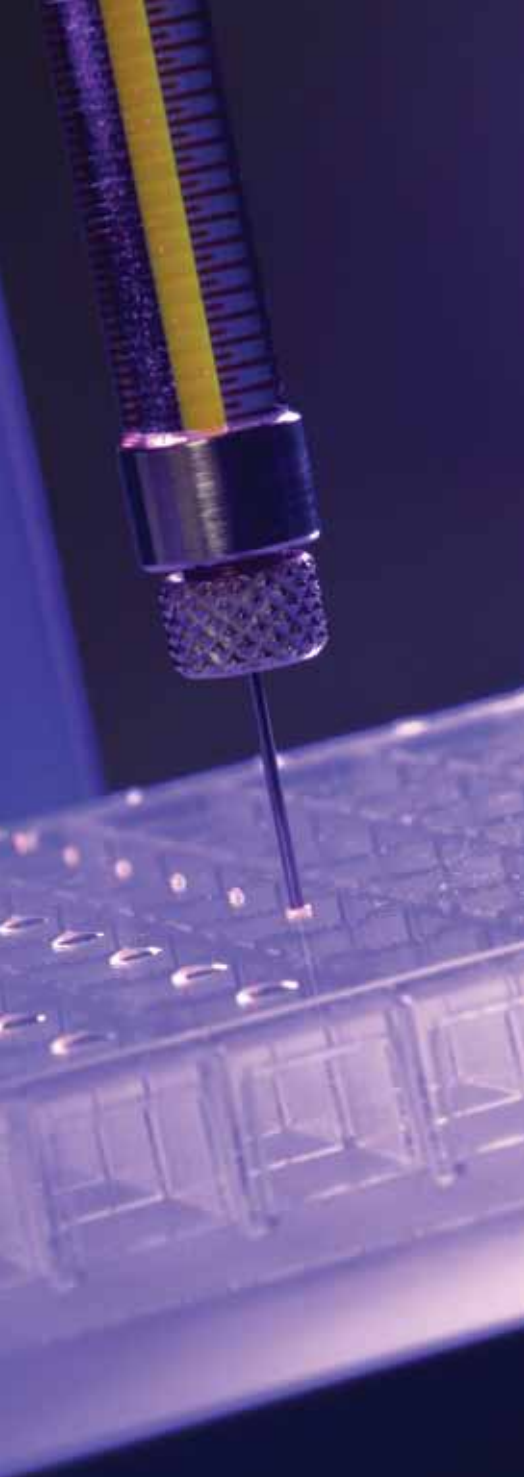
Dr Andrew S. Doré, a senior crystallographer from Heptares Therapeutics Ltd. who completed his DPhil in Sir Tom Blundell's laboratory, has been following the design and manufacture of the mosquito[®] LCP since its development in collaboration with Professor Gebhard Schertler (now at the Paul Scherrer Institute in Switzerland), and Dr Pat Edwards of the Structural Studies group at the MRC's Laboratory for Molecular Biology (LMB), Cambridge in early 2010.

Heptares was one of the first companies to invest in a mosquito LCP in August 2010 and has already successfully crystallised a number of new GPCR targets (see crystal picture on opposite page). They selected a number of new GPCR targets with the instrument for its accuracy of deployment in addition to its dual use capacity for vapour diffusion and "in meso" (or LCP) experiments. Prior to using the mosquito LCP, Heptares was using a manual ratchet dispenser set up for LCP crystallisation screening assays.

“Following the introduction of mosquito[®] LCP into our laboratory, we have found this robot to require minimal maintenance producing reliable and reproducible results.”

(DR ANDY DORÉ, HEPTARES THERAPEUTICS)





“mosquito[®] LCP is one of, if not the, fastest for crystallisation trial set up and deployment in both vapour diffusion and LCP. I would not entertain any other instrument.”

DR ANDY DORÉ, HEPTARES THERAPEUTICS

This manual method was not only time consuming but posed numerous difficulties in the accuracy of drop dispensing and placement. Dr Doré highlighted that “the mosquito LCP has significantly increased throughput, and the ability of the mosquito LCP to deliver reproducible LCP boli at 50 nL volumes in less than 3 minutes per 96 well plate is great”. Its disposable tips guarantee zero cross-contamination between samples and positive displacement technology enables mosquito LCP to cope with a range of viscosities and surface tensions of solutions used in crystallisation.

Heptares is also currently employing the increasingly popular bicelle method of membrane protein crystallography using the mosquito LCP. Dr Doré has recently successfully defined conditions for the crystallisation of a number of proteins using this method. The bicelle technique has been found to be favourable for type I crystal formation as with the Lipidic Cubic Phase technique, but initial hits can be far easier to identify. The use of mosquito LCP has enabled faster throughput, maximising the number of screening conditions per sample. In addition, it has helped with optimising crystallisation conditions and enabling faster scale up so that the structural biologist can address complex biological questions quickly and efficiently.

mosquito[®] Crystal and mosquito[®] LCP

Specifications	mosquito [®] Crystal	mosquito [®] LCP
Dispense range:	25 nL – 1200 nL	25 nL – 1200 nL
Plate/ deck capacity:	2 or 5	2 or 4
Experimental setup type:	hanging drop, sitting drop, microbatch, bicelle	LCP, hanging drop, sitting drop, microbatch, bicelle
Plate set up time:	<2 mins	<2 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 (15.5 x 18.5 x 27")	430 x 590 x 690 mm (17 x 23 x 27")
Weight:	27 kg (59 lbs)	34 kg (75 lbs)
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

Front cover illustration courtesy of Professor Jeff Abramson, UCLA. Crystal Images courtesy of T. Warne, P. Edwards and G. Schertler LMB, University of Cambridge, UK, the Paul Scherrer Institute, Switzerland and the York Structural Biology Laboratory, University of York, UK.

TTP Labtech Ltd

Melbourn Science Park
Melbourn
Hertfordshire SG8 6EE
United Kingdom

tel: +44 1763 262626

fax: +44 1763 261964

sales@ttplabtech.com

TTP Labtech Inc

One Kendall Square
Suite B2303
Cambridge MA 02139
United States

tel: +1(617) 494 9794

fax: +1(617) 494 9795