

working at the limits of crystallography

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).

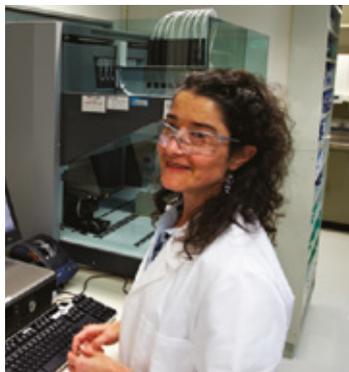
In addition to the LCP experiments, C3 uses mosquito LCP for screening (including microseeding, soaking and additive screening), optimisation and also the scaling-up of experiments. A second mosquito LCP located in the cold room is used for bicelle crystallisation, which requires low-temperature dispensing.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

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".

Dr Janet Newman, facility manager, C3



mosquito: lovely software,
really tidy footprint

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.

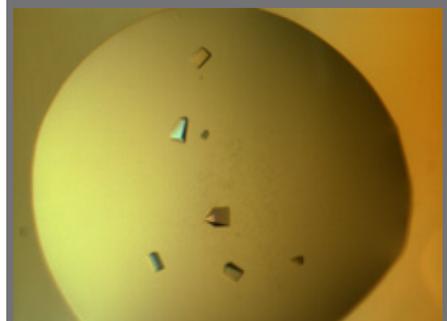
key benefits

The low-volume, positive displacement technology of mosquito ensures:

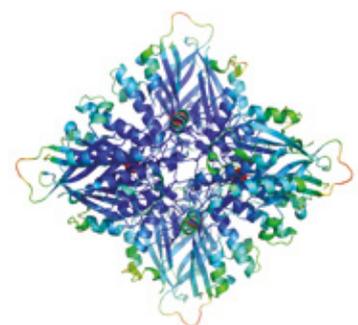
- reproducible and rapid dispensing
- accurate drop placement
- zero cross-contamination.

The disposable tips eliminate the need for time-consuming wash stages between the set-up of individual screening plates.

Fig 1. AtzD protein



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



(B) Ribbon representation of a tetramer



mosquito® LCP

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].

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 highthroughput 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.

References

- [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



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