TTP LABTECH

mosquito® LCP: Making membrane protein crystallization accessible to the research scientist

mosquito® LCP offers high throughput, high accuracy and unrivaled reproducibility to an otherwise challenging manual process. Its inherent flexibility, which allows it to perform the other vapor-diffusion methods for crystallography, provides the scientist with a wide range of options to suit any specific project. Its precision, reliability and ease of use offer accessibility of this novel membrane-protein crystallization technique to a wider community.

Integral membrane proteins such as G protein–coupled receptors (GPCRs) and ion channels represent key molecules involved in cell signaling, cell homeostasis, and human disease; one-third of all approved drugs target such proteins. The need to obtain high-resolution structural information to complement molecular and cellular data on membrane proteins is therefore of great importance to the scientific community and the drug discovery industry.

Membrane proteins are known to be difficult to purify and crystallize because of their instability and propensity to precipitate outside their native bilayer environment. As such, traditional methods of crystallography using aqueous solutions can prove unsuitable for their reconstitution. In 1996 an *in meso* lipidic cubic phase (LCP) technique was developed to crystallize bacteriorhodopsin[1] and has since revolutionized the process of crystallizing membrane proteins. This method provides a framework for the nucleation and growth of membrane proteins, thereby facilitating crystallization.

LCP crystallizations are carried out as a two-step process. First, the protein is mixed with the lipid, and a lipidic cubic phase forms spontaneously, producing a jelly-like, highly viscous material. To enhance crystallization, additives and precipitants are added, and depending on the conditions, crystals typically appear within days or weeks.

Crystallization trials usually involve a large number of conditions being tested with the sample. Efficient crystallization trials not only need to minimize sample volumes to ensure maximum utilization of valuable proteins and costly reagents, but also need to be automated in order to reduce laborious manual labor and speed up the plate-preparation process. Automation reduces manual pipetting errors and ensures sample uniformity.

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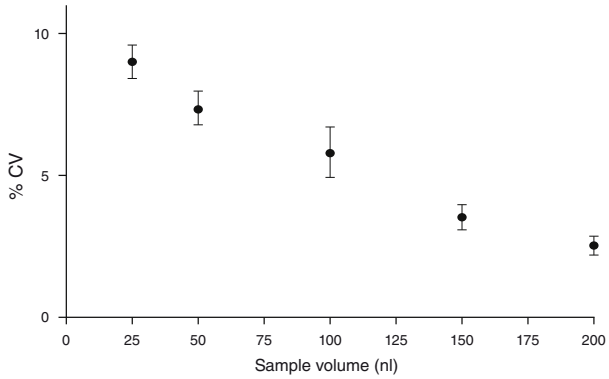

Figure 1 | The variation of dispensing accuracy from the mosquito LCP syringe is <10%. Accuracy increases to 6% at 100 µl, a volume commonly used in membrane-protein crystallography.

There are a number of technical issues associated with the LCP method that pose challenges to the automation of this process. The main problem, however, is the difficulty of accurately handling small volumes of the extremely viscous cubic phase material, which until recently has restricted successful use of this technique to experts.

Lipid/protein phase mixing and handling

The problem of mixing proteins and lipids was solved by Martin Caffrey, now of Trinity College, Dublin, who devised a method to couple two syringes, one containing lipid and a second containing protein, linked by a volume connector. Repeated mixing of the protein and lipid back and forth between the syringes results in a lipidic cubic mesophase being formed[2].

The mosquito LCP pipetting instrument was designed to automate nanoliter dispensing of LCP material and screening buffers. It was developed as a result of a collaborative program between TTP LabTech, Gebhard Schertler and Pat Edwards.

With an optional LCP mixer, the mosquito LCP offers a fully automated solution to the *in meso* (LCP) technique, thereby significantly

APPLICATION NOTES

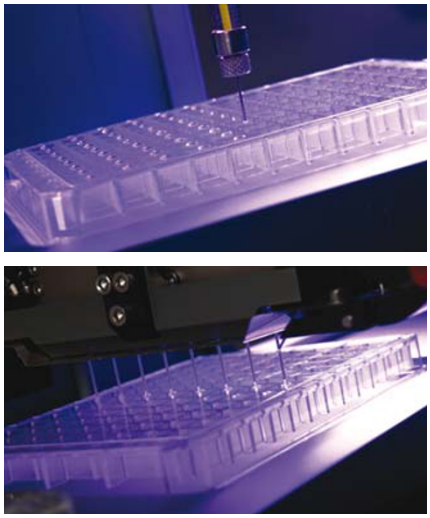

Figure 2 | Precise and repeatable positioning of drops in each well using TTP LabTech's mosquito® LCP. (**a**) The positive displacement syringe pump aliquots discrete 50 nl LCP drops to within 5-µm accuracy into 96-well plates. (**b**) Nanoliter volumes of precipitant aliquots are rapidly dispensed in column format directly onto the LCP drops using mosquito disposable tips, thereby reducing exposure of the LCP drop to air and environmental effects.

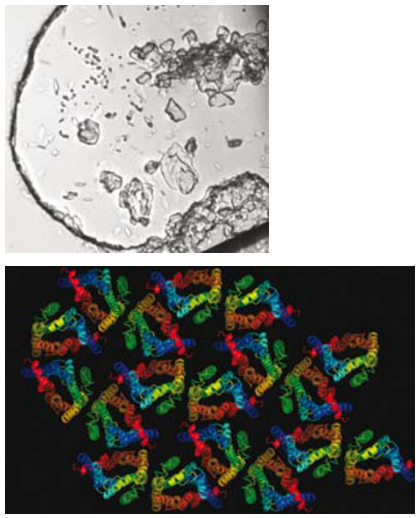

Figure 3 | Crystallization of β-adrenergic receptor crystals using TTP LabTech's mosquito® LCP. (**a**) β-adrenergic receptor crystals in a LCP setup imaged in transmission with 280-nm-wavelength light in an LCP sandwich plate. (**b**) Layer of β-adrenergic receptor molecules in an LCP crystal imaged roughly normal to the membrane plane. (Photo courtesy of T. Warne, P. Edwards and G. Schertler.)

increasing the speed of setting up screening plates. The ability to accurately dispense nanoliter volumes of LCP solution, at significantly lower volumes than with the manual method, saves valuable protein and allows a larger number of screening conditions to be evaluated.

LCP dispensing accuracy and repeatability

The credibility of the mosquito LCP is based on the ability of its syringe dispensing arm and mosquito pipette tips to accurately dispense LCP drops and screening solutions, ensuring consistent drop volumes and placement, hence eliminating manual error.

The mosquito LCP incorporates the features and functionality of the well-established mosquito® Crystal by combining a mosquito pipetting head with a micro-syringe dispenser that can accurately dispense 25–200 nl of LCP material onto glass slides or commercially available plates. mosquito LCP is compatible with all SBS-format plates, including a newly designed LCP plate developed by the Schertler group and SWISSCI that allows efficient imaging of crystals.

Analysis of the mosquito LCP dispensing volume demonstrates high accuracy, giving <10% variation for volumes down to 25 nl. **Figure 1** shows that the percent coefficient of variation (CV) of drop volume is <10% for all volumes tested, predictably decreasing gradually as the volume increases.

Precise and repeatable positioning of the drops in each well, within 5 µm, ensures accurate buffer placement on top of the LCP drop and efficient imaging of membrane-protein crystals (**Fig. 2**). Once a column of LCP drops has been dispensed (**Fig. 2a**), precipitant is rapidly placed directly onto these drops, reducing their exposure time to air and environmental effects to <2 s (**Fig. 2b**).

Precipitation and crystallization

The true benefits of an accurate automated pipetting system become apparent during the screening phase of optimization of the crystallization process. During this stage, numerous additives such as detergents, organics, salts and commercially available precipitants are studied to determine the optimal conditions for crystal formation.

The combination of the mosquito Crystal, mosquito LCP arm and pipetting head provides full automation of the dispensing of LCP drops, additives and precipitants to 96-well-plate setups in <5 min, and the unique positive-displacement tips permit multiple aspirations and precipitant additions. The disposable tips guarantee zero cross contamination, eliminating the need for time-consuming tip washing.

The ability to automate this process not only saves effort and time but also ensures reproducibility of both liquid volumes delivered and drop placement, which is necessary for the efficient crystallization of the membrane proteins. **Figure 3** shows the recent successful crystallization of β-adrenergic receptor crystals using TTP LabTech's mosquito LCP, permitting high-resolution structure determination of this membrane protein.

As with all protein crystallography, the effect of the environment (temperature and humidity) on the crystallization process can be crucial. This is true particularly for highly sensitive membrane proteins. The compactness and robustness of TTP LabTech's mosquito LCP instrument not only ensure that it will occupy minimal bench space but also allow the scientist to transport it to environmentally controlled rooms for the successful crystallization of membrane proteins.

1. Landau, E.M. & Rosenbusch, J.P. Lipidic cubic phases: a novel concept for the crystallization of membrane proteins. *Proc. Natl. Acad. Sci. USA* **93**, 14532–14535 (1996).
2. Cheng, A., Hummel, B., Qiu, H. & Caffrey, M. A simple mechanical mixer for small viscous lipid-containing samples. *Chem. Phys. Lipids* **95**, 11–21 (1998).