mosquito® Crystal, mosquito® LCP and an active humidity chamber: essential tools for successful membrane protein crystallization

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introduction

Membrane proteins are involved in a wide range of physiological functions and abnormalities in the structures of these proteins can lead to many known diseases such as heart disease, depression, cancer and many others. However growing crystals of membrane proteins suitable for X-ray diffraction is still a problem for crystallographers.

In recent years developments in automation, miniaturisation and integration have made significant contributions to the membrane protein crystallization process. Automation of protein crystallization screening trials requires accurate placement of nanoliter volumes of protein and screen drops, in addition to the reproducible and accurate dispensing of solutions of varying viscosities. This is particularly important for the set-up of the highly viscous lipid mesophases in the Lipidic Cubic Phase (LCP) crystallization technique for membrane protein crystallization trials.

The use of liquid handling robots such as TTP Labtech’s mosquito® Crystal and mosquito® LCP has increased throughput and repeatability allowing for many more conditions to be screened. Although mosquito LCP can rapidly set up a crystallization screen, the use of TTP Labtech’s active humidity chamber ensures there is minimal evaporation of the LCP drops which ultimately increases reproducibility.

G-protein coupled receptors (GPCRs) represent one of the most important classes of protein due to their critical role in cell signalling in response to hormones and neurotransmitters. GPCRs represent 50-60% of the current drug targets and therefore play a crucial role in neurotransmitters. GPCRs represent 60% of the current drug targets and therefore play a crucial role in neurophysiological functions and abnormalities in the structures of these proteins.

1. mosquito® liquid handlers

<table>
<thead>
<tr>
<th>specifications</th>
<th>mosquito® Crystal</th>
<th>mosquito® LCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>pipetting range</td>
<td>25 – 1,200 nL</td>
<td>25 – 1,200 nL</td>
</tr>
<tr>
<td>primary SBS plate format</td>
<td>48, 96, 384</td>
<td>48, 96, 384</td>
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<tr>
<td>dead volume</td>
<td>&lt; 0.3 µL</td>
<td>&lt; 0.3 µL</td>
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<tr>
<td>optional extras</td>
<td>active humidity chamber</td>
<td>active humidity chamber</td>
</tr>
<tr>
<td>applications</td>
<td>protein crystallography set-ups e.g. additive screening, microseeding, microbatch, birefringence</td>
<td>lipidic cubic phase (LCP) screening plus all the functionality of mosquito Crystal</td>
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</table>

2. active humidity chamber

Sample evaporation is an issue when dispensing very low volumes or volatile samples, causing inconsistent drop sizes, especially when environmental conditions and local humidity vary.

TTP Labtech’s active humidity chamber can be fitted to any mosquito instrument and allows users to accurately control the relative humidity (RH) of each experiment. Up to 90% humidity can be achieved in 3-5 minutes.

3. sensitivity of lipidic cubic phase to evaporation

Lipidic cubic phase (LCP) allows membrane proteins to retain their activity and structural integrity and facilitates crystal nucleation and growth. This polymeric lipid phase structure requires optimal movement of hydrophobic and water-soluble proteins. Variations of temperature and water content can change the phase properties of lipidic structures. Too much evaporation can alter the phase properties of a LCP protein as illustrated in Fig 3.

4. optimizing crystal formation of a GPCR protein

β2 AR is a GPCR predominantly localised in heart tissue. Drugs that inhibit β1 and β2 receptor signalling are used to modulate heart function and are known as beta-blockers.

The aim of the experiment was to observe the effect of using an active humidity chamber on β2 AR crystal formation.

Initial screening demonstrated that β2 AR had a higher propensity to crystallise in humid conditions compared to conditions of low humidity.

Reproducibility of this observation was measured using 96 repeats of the same condition. This condition was selected for its ability to crystallise the corresponding target.

A final volume of 200 nL containing 100 nL of protein and 100 nL of condition, was pipetted into a 96-well plate under high and low levels of humidity at room temperature. Using the active humidity chamber, humidity levels were in the range of 50 – 70% during set-up. As a control a plate was set up without the use of the chamber and under an ambient relative humidity of 22%. The trays were stored in an incubator at 22°C and visualised with a microscope after 72 hours.

5. higher levels of humidity reproducibly increased crystal yield

Reproducing the same conditions for crystallization of β2 AR protein under different humidity levels resulted in a 13% greater yield of crystals with increased humidity levels:

- Low humidity yielded 59% crystals across 96-well plate
- High humidity yielded 72% crystals across 96-well plate

conclusions

This poster demonstrated that maintaining higher levels of humidity during LCP crystallization set-up reduced drop evaporation. The combination of TTP Labtech’s mosquito LCP and its active humidity chamber rapidly reduced experimental inconsistencies whilst increasing β2 AR crystallization success rates in a 96-well plate.

Other benefits of TTP Labtech’s active humidity chamber include:

- up to 90% adjustable humidity within 3-5 mins
- rapid ramping to 90% humidity levels
- precisely controlled from within mosquito software
- allows per protocol control to suit multi-user lab

acknowledgments

We are grateful to Fabrice Gorrec and Tony Warne, MRC-LMB, Cambridge, UK for their kind assistance in the work presented in this poster.

Fig 1. mosquito LCP with active humidity chamber

Fig 2. Vapor formed from the active humidity chamber

Fig 3. Schematic of the equilibrium temperature – composition phase diagram for the monoolen (9:9 MAG) – water system in the vicinity of 20°C. (Caffrey, M et al, 2013 Phil. Trans. R. Soc. B. 368:20130621)

Fig 4. β2 AR protein crystals. (a) an example of hit crystals in a LCP drop (b) a dehydrated LCP drop resulting in a lack of crystal formation