high-throughput, low-volume soaking of protein crystals in the rapid screening of fragment libraries

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introduction

Growing protein-ligand complex crystals can be challenging, especially in cases where the affinity is poor and the solubility of the ligand in the crystallisation condition is low. Various methodologies are often trialled before obtaining a diffraction-quality protein-ligand crystal.

Co-crystallisation is a common method for producing protein-ligand complex structures. It is especially useful when drug-like compounds trigger conformational changes in proteins. This can result in variations in the growing conditions or crystal forms, and may necessitate wider screening strategies for co-crystallisation in general.

Alternatively, soaking protein crystals with ligands is the fastest route to produce high-throughput structures, as long as the starting crystal form is easy to grow reproducibly, able to accommodate the desired ligand and, is robust to physical and chemical changes.

This poster will describe a low-volume, high-throughput soaking method developed by Dr. David Hargreaves (AstraZeneca, UK) for screening a fragment-based lead generation (FBLG) library. TTP Labtech's mosquito® crystal enabled fast and accurate miniaturisation of the crystallisation set-up.

the protein crystallographer's favourite liquid handler

mosquito crystal is the protein crystallographer's favourite liquid handler. Each of TTP Labtech's disposable micropipette tips has its own individual piston – not an air gap or system liquid – offering true positive-displacement pipetting with no risk of clogging, corrosion or cross-contamination. It brings together speed, accuracy and high-precision to pipette nanolitre volumes.

- rapid and reliable automated plate set-up for all standard crystallisation techniques
- unrivalled reproducibility down to 25 nL
- cost saving uses smaller volumes of valuable protein and enables a larger number of screening conditions to be studied
- user- friendly no physical changes to complete different experiments
- flexible multiple aspiration before a single dispense
- unrivalled drop precision perfectly positioned drops

Fig 1. mosquito crystal (8- or 16-channel)

screening fragment-based lead generation (FBLG) libraries

Fragment-based drug discovery (FBDD) has been developed as an alternative strategy to high-throughput compound screening. It enables discovery of small, less complex compound fragments which can be linked or expanded to design potential drugs.

Dr. Hargreaves' automated screening method uses mosquito crystal to enable small volumes of fragment libraries (10-25 nL) to be prepared systematically.

method: low-volume, high-throughput soaking of 3-phosphogylcerate dehydrogenase (PHGDH) crystals

mosquito crystal was used to make up 96 soaking solutions in 1 µL volumes. These solutions contained fragment library cocktails of; high concentrations, high-affinities (low concentrations), and concentration gradients of compounds.

Having already grown PHGDH crystals in a subwell of a standard crystallisation plate, the soaking solutions were prepared and transferred quickly and automatically. This reduced potential errors and improved reproducibility. The process took place within the subwells of a MRC 96-well 2-drop crystallisation plate (Fig 2) using mosquito crystal to reproducibly pipette the low volumes at each stage of the process.

In brief, 30 nL of different compounds dissolved in DMSO (100 mM) was dispensed into well 3 followed by the addition of 500 nL of solution from well 1.

200 nL of the resultant solution in well 3 (approximately 6 mM) was then transferred to well 2 which already contained the crystals in approximately 200 nL of mother liquor. This operation resulted in 400 nL of compound solution containing PHGDH crystals at approximately 3 mM. The wells were then resealed using adhesive tape prior to incubation overnight.

Data were collected at Diamond i04 in a single 8 hour shift.

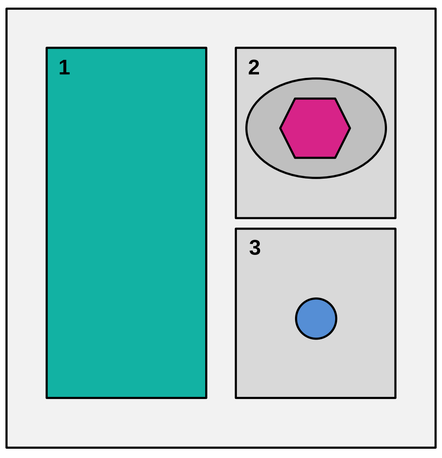


Fig 2. A schematic of a standard MRC 96-well 2-drop crystallisation plate illustrating the set-up for crystal soaking (1 = mother liquor solution, 2 = crystal to be soaked, 3 = concentrated compound).

results

32 fragment structures that bound to PHGDH were delivered to the project team followed up with 96 analogues selected from the compound collection (27 of which produced bound structures).

Interestingly, biophysical characterisation of the initial hits showed successfully bound fragments had Kds in the range of 400-2500µM (ITC) and 270-6000µM (NMR).

The mosquito crystal was invaluable to pipette the small volumes required for this method. It enabled accurate placing of drops and produced reproducible crystals.

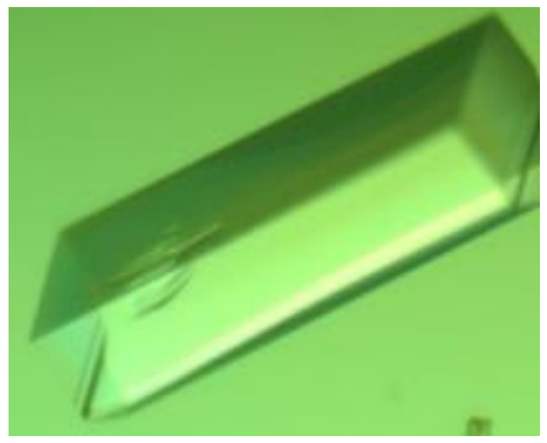


Fig 3. PHGDH crystal that has been soaked with 3 mM of compound in a well of a standard MRC 96-well 2-drop crystallisation plate.

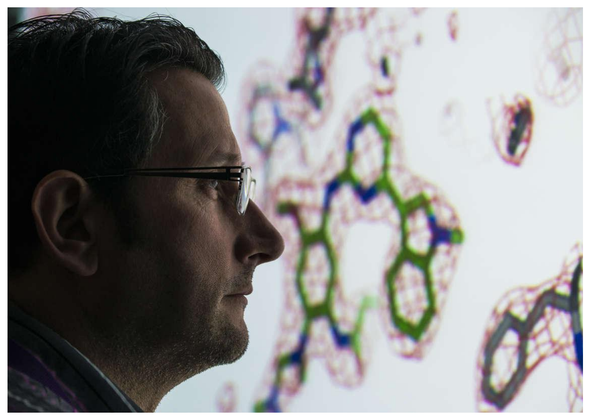
discussion: protecting crystals using 'drop first'

An obvious risk when transferring solutions into wells that already contain crystals is that of crystal damage.

Using the mosquito crystal unique 'drop first' setting and adjusting the subwell height ensured the drops were placed onto the existing drops rather than touching off in the plate as normal. In drops containing lots of small crystals it was not possible to see any specific mechanical damage caused by adding the soak solution.

With fragment libraries of compounds becoming commercially available in 96-well format plates, using the mosquito crystal to prepare and dispense soaking solutions lowers the risk of human error during dispensing.

The success of this soaking method relied on the accuracy and reproducibility of mosquito crystal's low volume pipetting which is in the range of 25 nL to 1,200 nL.



Dr. David Hargreaves, Associate Principal Crystallography Scientist at AstraZeneca, UK

conclusions

This poster demonstrates how the soaking method of crystallisation can be applied in high-throughput using very low volumes of protein solution

This was made possible using TTP Labtech's mosquito crystal liquid handler which pipettes in the range of 25 nL to 1,200 nL

This new method is:

- fast
- reproducible
- reliable
- cost-effective
- easy to perform

Making it a highly attractive initial screen for all structure-based compound screening.

"In our lab, the mosquito crystal is invaluable for crystallography, but is also used outside crystallography where small volume liquid handling is required."

Dr. David Hargreaves, AstraZeneca, UK

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