strategies for high-throughput ligand screening - automated co-crystallisation and soaking

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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 automated low-volume, high-throughput techniques for both types of crystallisation methods. These methods were developed by Dr. David Hargreaves (AstraZeneca, UK) and Dr. Jörg Benz, F.Hoffman-La Roche AG, Switzerland.

TTP Labtech's mosquito® crystal enabled fast and accurate miniaturisation of the crystallisation set-up for both techniques.

the protein crystallographer's favourite liquid handler

mosquito crystal is the protein crystallographer's favourite liquid handler (Fig 1). 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)

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

1. automated co-crystallisation

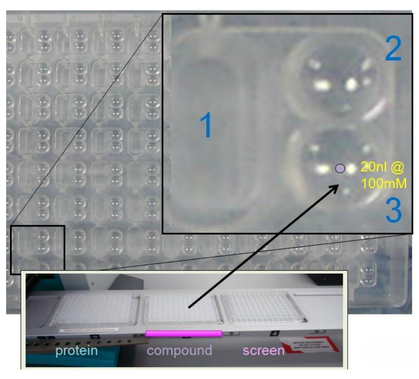
Automated co-crystallisation allows rapid and repeatable set up of protein ligand crystallisation experiments directly from compound stock plates.

method: optimised gradients for ligand/additive screening

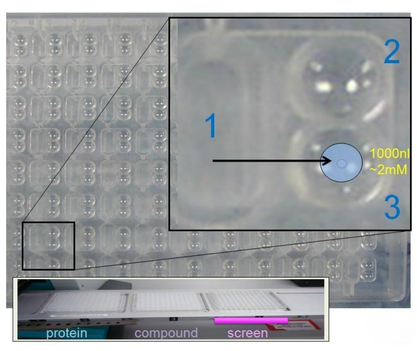
100 mM stock compounds in DMSO are diluted to an optimal 1mM in a low-volume, high-throughput assay using mosquito crystal.

The deck of mosquito crystal is then set up as follows left to right:

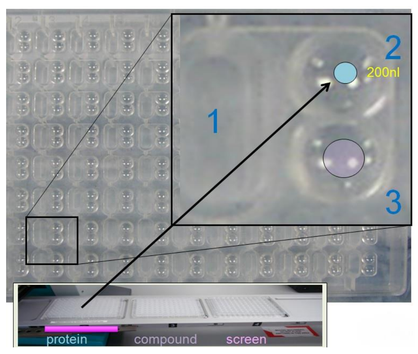
Plate 1	Protein source plate
Plate 2	Compound stock plate (100mM)
Plate 3	MRC crystallisation plate containing crystal screen



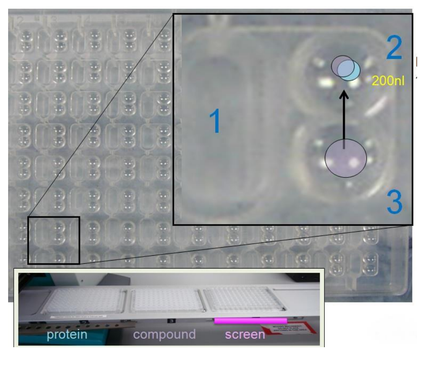
Step 1: Transfer 20 nL of a 100mM stock of ligand to subwell 3 of the crystallisation plate.



Step 2: Take 2000 nL of the optimised gradient (Fig 2) and dispense it on top of the ligand in subwell 3 – run a few mix cycles in the tip to ensure even dilution of ligand stock.



Step 3: Transfer 200 nL of the protein to subwell 2 of the crystallisation plate.



Step 4: Take 200 nL of the diluted ligand stock from subwell 3 and add it to the protein in subwell 2 to complete the crystallization drop.

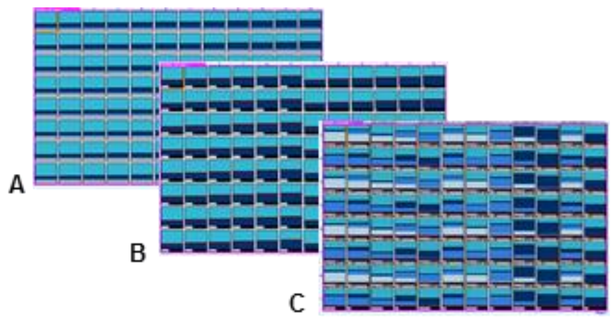


Fig 2. Options for optimised gradient screens across (a) 12 wells or (b) 2 x 6 wells for 8 conditions, and (c) 4 x 24 wells for 4 different compounds.

2. automated crystal soaking

A similar dilution method can be applied for automated crystal soaking using the mosquito crystal to enable small volumes of fragment libraries (10-25 nL) to be prepared systematically. This Fragment-based drug discovery (FBDD) approach has been developed as an alternative strategy to high-throughput compound screening.

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.

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. The process took place within the subwells of an MRC crystallisation plate using mosquito crystal to reproducibly pipette the low volumes at each stage of the process.

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 containing crystals (Fig 3). The wells are then resealed using adhesive tape prior to incubation.

Fig 3. An example of a crystal that has been soaked with 3 mM of compound in a well of a standard crystallisation plate.

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 400-2500 µM (ITC) and 270-6000 µM (NMR).

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

conclusions

- this poster demonstrates how the methods of co-crystallisation and soaking can both be automated for high-throughput screening using very low volumes of both protein and ligand solution
- these methods were made possible using TTP Labtech's mosquito crystal liquid handler which pipettes in the range of 25 nL to 1,200 nL
- these new methods are both:
  - fast
  - reproducible
  - reliable
  - cost-effective
  - easy to perform
- making either of these methods a highly attractive initial screen for all structure-based compound screening.

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