

introduction

Protein crystal optimization is vital to ensure high quality X-ray diffraction data for the solving of high resolution structures. This process involves the set-up of a series of complex screening combinations where the ratios of the individual components identified from primary crystallization studies are varied.

In order to reduce the effort and tedium of this process, TTP Labtech have designed **dragonfly®** for crystallization screening as an addition to their successful mosquito® liquid handling portfolio.

Enzymes that are essential for the growth of ***Mycobacterium tuberculosis*** (*Mtb*, the bacterium that causes tuberculosis) are valuable drug targets. During the hit validation step of drug discovery, there is a requirement for structure determination of the target. However crystallization of the hit protein continues to remain the bottle neck of this process sometimes taking many months to achieve a suitable crystal.

The use of reliable, low volume automated systems such as TTP Labtech's mosquito® Crystal has improved the time and accuracy of primary screening.

This poster will describe how Dr. Michal Blaszczyk at Cambridge University, UK has used dragonfly to optimize the conditions for the crystallization of target enzymes involved in the growth of *Mtb*.

1. dragonfly

TTP Labtech's dragonfly is a liquid handler for simple, fast and accurate crystal screen optimization.

dragonfly's positive displacement technology ensures highly accurate dispensing, from 0.5 µL up to 4 mL, across a wide range of viscosities. Its rapid plate preparation is uniquely combined with non-contact dispensing to ensure zero cross-contamination. Dispense resolution is 0.1 µL, allowing very fine gradients to be created.

benefits

- easy to use software
- rapid plate preparation (96 wells in < 5 mins)
- accurate dispensing of all types of liquids regardless of viscosity
- dispense any volume from 0.5 µL upwards, into any well, from any syringe
- less than 5% CVs at 1 µL
- zero cross-contamination
- no blocking or clogging
- low dead volume aspiration
- choice of 5 or 10 independent dispensing heads
- compatible with 15-, 24-, 48-, 96- and 384-well SBS plate formats
- small bench top footprint
- easy creation of complex optimization experiments

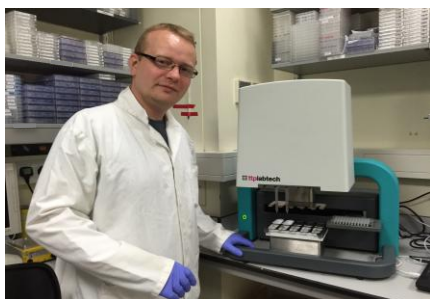


Fig 1. Dr. Michal Blaszczyk at work with his dragonfly

2. crystallization of target protein involved in growth of *Mycobacterium tuberculosis*

Tuberculosis (TB) remains a global healthcare problem with a nearly 2 million deaths a year. In many countries the BCG vaccine is not effective allowing latent infection of *Mycobacterium tuberculosis* (*Mtb*) to remain. Reducing the growth of *Mtb* would be a significant step in reducing the world's burden of tuberculosis. Enzymes responsible for the growth of *Mtb* are valuable drug discovery targets.

Initial screening of possible drug targets creates a large number of hits that then require validation. Information on these hits is provided by crystallization of the target to characterize its ligand interactions.



Fig 2. A micrograph of *Mtb* (image obtained from Centre for Disease Control (CDC), USA)

3. from crystal hits to workable crystal system

Dr Michal Blaszczyk is a part of Prof. Tom Blundell's group that uses Fragment Based Drug Discovery (FBDD) to develop novel drugs against TB. The pipeline of FBDD consists of three main steps:

1. small molecular weight fragment library screen
2. validation of binding by NMR and X-ray crystallography
3. characterization of binding by isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR)

Good quality crystals are required for this process to be successful. Obtaining such crystals can be very challenging and time consuming and is considered as the major bottleneck for all FBDD projects.

initial screening

The vapor diffusion sitting drop technique has become increasingly popular for initial screening. This is mainly due to increasing numbers of commercially available crystallization screens and advances in automated technology.

Extensive screening using TTP Labtech's mosquito® Crystal increases the throughput and accuracy and therefore provides rapid validation of targets. These hits will then go on to be optimized.

optimization

The hit conditions are optimized for pH and concentrations of precipitant, salt, additive, DMSO and protein.

Automation of this step with TTP Labtech's dragonfly® is very important as it allows for systematic check of multidimensional chemical space e.g. a single 96-well plate can contain 4 sets of optimization conditions to form a 3D map of optimal components.

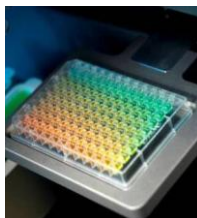


Fig 3. 96-well MRC plate gradient set up with dragonfly

4. efficient crystal optimization

Extensive screening of enzymes involved in the growth of *Mtb* produced six initial hits that went on to be optimized by Dr. Blaszczyk. Twenty different optimizations were required to obtain nicely diffracting crystals (Fig 4).

high throughput

Using the dragonfly four optimizations per 96-well plate (4 x 24 optimization) to form a 3D matrix of conditions.

reduced volumes of reagents and sample

This optimization method saved on amount of sample utilised but also reduced the capacity needed on the crystal imager. When the process was coupled with mosquito Crystal approximately 20 µL of protein sample was used per 96-well plate.

In addition, the volume of condition media was reduced by nearly two thirds, mainly due to the ability to perform the screen in 96- rather than 24-well plates.

faster and more accurate pipetting

Optimization of six conditions using the dragonfly took one week rather than the more usual time of 1-2 months. These conditions consistently produced crystals demonstrating the accuracy of dragonfly and mosquito's pipetting.

simplicity

Optimization protocols can be saved for future use making it easy to set up several optimizations at one time and ensuring consistency between experiments.

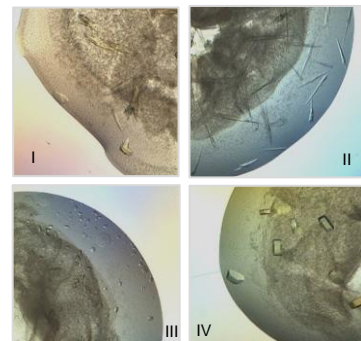


Fig 4. Stages of optimization for a target protein involved in growth of *Mtb*. Stages I - IV took 1 week using dragonfly screen optimizer as apposed to 1- 2 months if done manually

conclusions

This poster demonstrates how Dr. Blaszczyk increased the efficiency of his optimization process and obtained better diffracting crystals with the use of dragonfly screen optimizer. Efficiencies were gained in time, money and sample volumes used. Improvements in accuracy and precision were also gained from using TTP Labtech's automated liquid handlers.

dragonfly is a valuable, compact, low cost addition to the crystallographer's bench.

dragonfly is an essential tool for optimal crystal optimization that eliminates lengthy and complicated plate set-up.

dragonfly enables more optimization conditions to be tested with minimal amounts of sample thereby increasing the chance of obtaining viable drug targets.

"dragonfly is very flexible and easy to adapt to specific user's needs"
Dr. Michal Blaszczyk