

Automated protein crystal optimisation with TTP Labtech's dragonfly™



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introduction

The ability to crystallise proteins, nucleic acids or macromolecular complexes pose significant challenges to the protein crystallography community, from large scale screening assays for the determination of initial crystallization conditions, screen optimisation and final screen set-up.

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

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

This poster demonstrates that "dragonfly" is a valuable, compact, low cost addition to the crystallographer's bench. It eliminates lengthy and complicated plate set-up at the optimisation stage of crystallisation.

1. dragonfly: optimisation optimised



TTP Labtech's dragonfly screen optimizer

dragonfly by TTP Labtech is a low cost, bench top, automated gradient maker, enabling the rapid generation of screen well plates for protein crystal optimisation.

Novel dispensing technology enables highly accurate, automated, non-contact, positive displacement dispensing from disposable syringes directly into crystallography plates.

dragonfly is capable of dispensing a wide range of fluid viscosities, ethanol to 100% glycerol; with a minimum dispense volume of 1 µL from a 10 mL reservoir with zero cross contamination. Independent volume control and simultaneous dispensing from up to 10 pipetting heads allows the creation of optimisation screens directly into crystallisation plates in less than 5 minutes.

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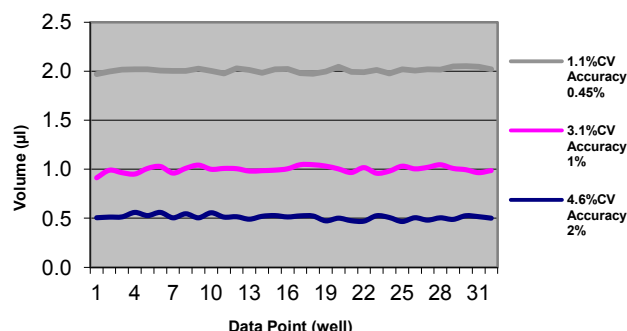
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2. accuracy and performance

dragonfly has an impressive dispensing performance. It offers CVs of < 5% for all liquid types down to 2 µL without any liquid classification being necessary.



100% Glycerol (measured by absorbance of Tartrazine)

3. proof of concept study

Conditions for the optimisation of the crystal formation of three well known proteins, Catalase, Concanavalin A and Lysosyme were explored using dragonfly for screen set-up.

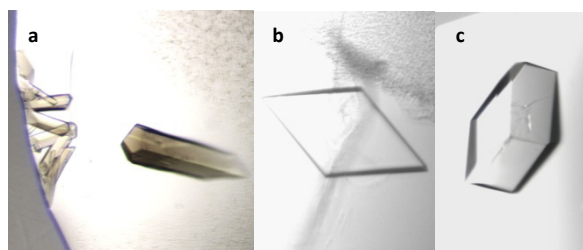
Following selection of the primary conditions which resulted in crystal formation, a 4-corner solutions protocol was employed in a 96-well plate, where concentrations of the precipitant (PEG) and the additive (propanediol) were varied based around the initial screen conditions. This protocol produced concentration gradients across a crystallisation plate, where each well of the plate receives appropriate volumes from four stock solutions individually.



A 4 corner gradient plate for protein crystal optimisation created using TTP Labtech's dragonfly.

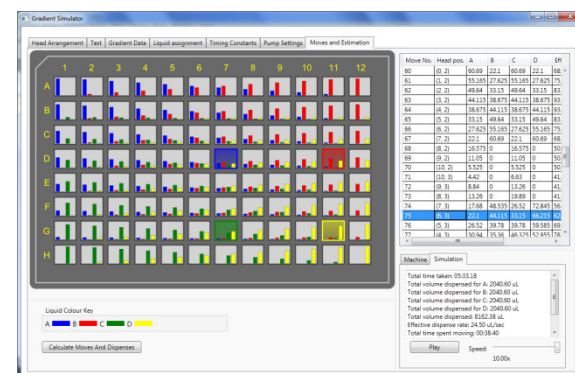
Overall 9 optimisation screens were automatically produced in 96-well sitting drop plates (final volume in each well: 85 µL) using dragonfly since three similar screens at different pHs were made for each sample (0.5 unit above and below the pH of the initial condition).

In this study, the resulting conditions were homogenised with a microplate orbital shaker (750 rpm, 1 minute) and the crystallisation drops were set up using a mosquito Crystal (100 nL protein + 100 nL condition). Drops were observed daily and photographs of those containing crystals were taken after three weeks.



Light micrographs showing crystals of a, Catalase, b, Concanavalin A and c, Lysosyme. Magnifications differ and crystal sizes vary between 150 and 400 µm. Images kindly provided by Dr F Gorrec, MRC LMB, Cambridge, UK.

4. set up and running



Setting up dragonfly to run an optimisation screen directly into a 96-well crystallography plate couldn't be easier:

set up...

Select plate type, enter the individual screen solutions and assign them to the dispense heads –using an intuitive graphical menu. Fill the screen reservoirs direct from standard storage tubes and dragonfly will aspirate directly when required – no need to load bottles or fill syringes.

design...

Using a graphical display, click and drag to design the required gradient profile for each screen solution or you can simply select a pre-defined screen design, such as the '4-corner method'.

run...

Just press go and watch the live feedback of the plate make-up as it happens (see above screenshot).

With its easy to use, intuitive interface, dragonfly's software enables the crystallographer to rapidly and easily set up and determine their own conditions for efficient optimisation studies.

dragonfly is a fast, simple and accurate solution to screen optimisation for today's protein crystallographer.

conclusion

TTP Labtech's dragonfly screen optimiser offers:

- automated, non-contact dispensing
- auto-aspiration from easy to fill reservoirs
- positive-displacement syringes provide reliable and accurate dispensing
- disposable syringes: - zero cross-contamination
- no slow wash cycles
- high speed; fills a 96-well plate < 5 minutes
- minimum dispense volume <= 0.5 µL
- dispensing resolution: 200 nL
- dispenses an extensive viscosity range from alcohols through to glycerol with incredible precision (< 5%CV)
- no requirement for liquid classification
- up to 10 independent and modular liquid channels
- removable reservoirs for simple loading
- less than 0.5 mL dead volume
- compact, small bench-top footprint
- low cost

any volume, any liquid, any well