dragonfly® crystal
application note

Fast and straightforward macromolecular crystal optimisation with dragonfly® crystal

introduction

X-ray crystallography is the most successful method employed to gain an understanding of macromolecule structures at the atomic level. Obtaining the required crystals from a purified sample of a protein, nucleic acid or any macromolecular complex poses significant challenges since tedious experimental set-ups with poor success rates are involved. First, an initial crystallisation screen is carried out on the purified sample with a broad range of commercially available conditions. Then, in-house custom optimisation screens are prepared in order to reproduce and optimise any initial leads. With optimised crystals, it is later possible to acquire quality X-ray diffraction data and hence solve high resolution structures.

Optimisation screens are usually prepared manually on traditional liquid handlers (i.e. a moving head with eight tips where pipetting is controlled with a system liquid). The associated protocols are time consuming (10–20 minutes per screen). In addition, typical stock solutions contain reagents with low surface tension and/or high viscosity such as the high molecular weight polyethyleneglycols (PEGs). This often results in pipetting inaccuracy and cross-contamination.

This study shows the optimisation of crystals from three different protein samples at the Laboratory of Molecular Biology (LMB, Cambridge, UK) with a novel automated liquid handling technology developed by SPT Labtech Ltd. (Melbourn, UK) called dragonfly® crystal (Fig 1). This novel instrument operates with positive displacement pipette tips and non-contact dispensing. Disposable tips ensure zero cross-contamination with CVs below 5% at 1 µL dispense for all liquid types including glycerol (Fig 2). dragonfly crystal enables screens to be prepared quickly and easily in any SBS plate format. A 96-condition screen can be prepared in 3–6 minutes (depending on the number and final volume of conditions prepared).

Fig 1. dragonfly® crystal. The robot has a small footprint and is maintenance-free. To make a screen, the stock solutions are poured into the troughs located on the main stage (up to 10 stock solutions). Syringes are attached to the head of the robot. Once the plate is in place, the formulation protocol is selected before launching the automated making of the screen into the wells of the plate.

materials and methods

Lysosyme, Concanavalin A and Catalase protein samples were prepared and initially crystallised as described elsewhere [1].

The initial condition selected for each sample was optimised by tuning up and down the concentrations of the precipitant (PEG) and the additive (e.g. propanediol) following the "4-corner solutions" protocol. This protocol produces concentration gradients for two reagents across a crystallisation plate, where each well of the plate receives appropriate volumes from four stock solutions individually [2]. 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).

key benefits

- **prepare a 96-condition screen in 3-6 minutes**
- **accurately handle reagents with low surface tension and/ or high viscosity**
- **up to 10 independent heads for simultaneous, non-contact dispensing**

The resulting conditions were homogenised with a microplate orbital shaker (750 rpm, 1 min.) and the crystallisation drops were set up using a mosquito® nanolitre dispensing technology (100 nL protein + 100 nL condition) [3]. Drops were observed daily and photographs of those containing crystals were taken after three weeks.

> "The effectiveness of dragonfly crystal for preparing crystallisation conditions into 96-well plates has been demonstrated, with efficient optimisation screens prepared three times faster than classic liquid handling technology."

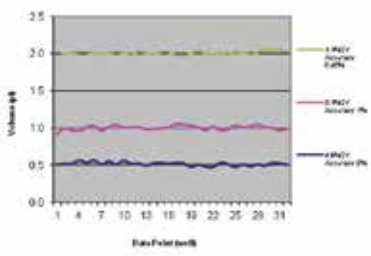

Fig 2. Graph showing CVs with 100% glycerol (measured by absorbance with Tartrazine)

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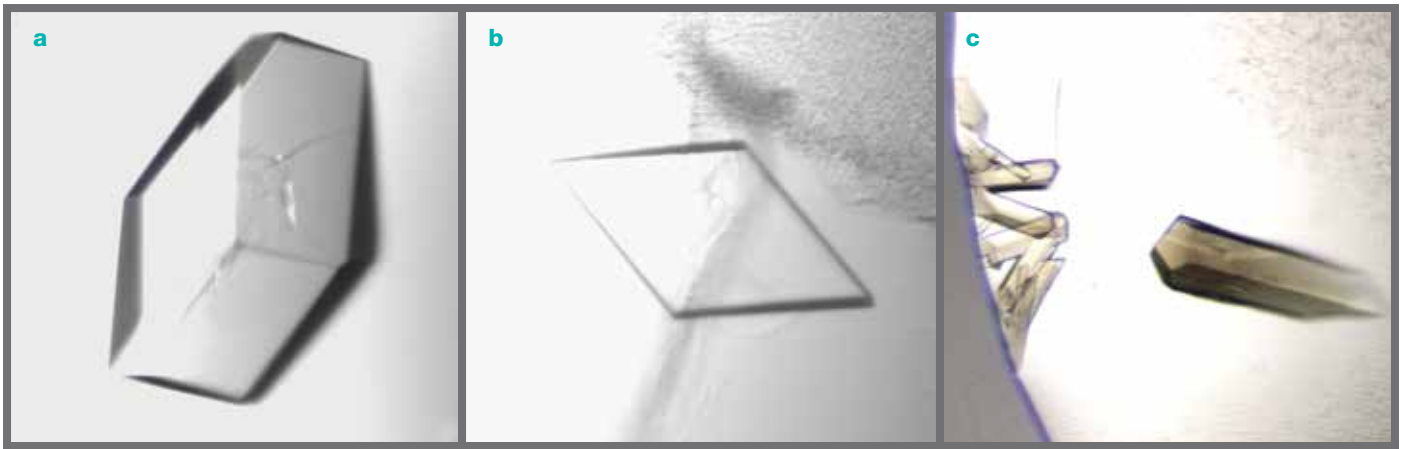

Fig 3. Light micrographs showing crystals of (A) Lysosyme, (B) Concanavalin A and (C) Catalase. Magnifications differ and crystal sizes vary between 150 and 400 µm.

results

Each optimisation screen was prepared in 4.5 minutes. Following seven days incubation after setting up the drops, between 3–15 crystallisation hits were observed across each plate. Fig 3 shows a selection of crystallisation hits with: A. Lysosyme crystal (18.2% w/v PEG 20000, 0.007 M sodium potassium tartrate, 0.15 M sodium citrate pH 6.0), B. Concanavalin A crystal (11.6% w/v PEG 20000, 10% v/v PEG 300, 0.15 M CAPSO pH 9.0), C. Catalase crystal (30% v/v PEG 550MME, 4.5% v/v propanediol, 0.15 M CAPSO pH 9.5).

Crystal nucleation and growth were investigated by optimising the concentrations of the reagents and the pH. As a result, large amounts of single and large crystals were produced. This is ideal for performing structure determination and ligand-binding studies which typically involve severe losses in crystals (e.g. during cryoprotection and soaking).

conclusion

The effectiveness of dragonfly for preparing crystallisation conditions into 96-well plates has been demonstrated, with efficient optimisation screens prepared three times faster than classic liquid handling technology.

dragonfly crystal's positive displacement, non-contact capabilities ensure accurate dispensing across a wide range of viscosities without the risk of cross-contamination due to the use of disposable tips. With straightforward set-up and operation, minimal training is required and hence a multitude of users will be able to operate independently. As an indicator of success, dragonfly crystal is now routinely employed for crystallisation screening at the LMB (see example Fig 4).

Fig 4. Crystals of a DNA-clamp target obtained with an optimisation screen prepared on the dragonfly crystal (with the permission of Rafael Fernandez Leiro and Meindert Lamers, details to be published elsewhere).

references

[1] Gorrec et al. (2011) "Pi sampling: a methodical and flexible approach to initial macromolecular crystallisation screening" Acta Crystallogr D Biol Crystallogr. 67, 463–470.

[2] Stock et al. (2005) "Robotic nanolitre protein crystallisation at the MRC Laboratory of Molecular Biology" Prog Biophys Mol Biol. 88, 311-27.

[3] Jenkins and Cook (2005) "mosquito®: an accurate nanoliter dispensing technology" J Lab Autom. 9, 257-261.

author

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