

Miniaturization of Protein Crystallization

Honeybee Technical Note 101

Overview

The study of protein structure and function has been greatly accelerated by the development of X-ray crystallography of protein crystals and co-crystallization of proteins and inhibitors. Preparation of protein crystals is notoriously difficult forcing scientists to test relatively large numbers of potential crystallization conditions prior to production of crystals suitable for X-ray analysis. In many cases the protein of interest is not abundantly available so these preliminary tests must be performed on a small scale or the number of conditions screened is restricted. The ability to reduce the volume of protein used in each experiment allows the number of conditions that can be screened to be maximized ensuring a greater chance of crystal growth.

With manual pipetting and indeed many automated liquid handlers the reduction of volume is limited to 1 μ L. Digilab dispensing technology from Digilab enables miniaturization of protein crystallization experiments to nanolitre volumes.

Determining optimal growth conditions

Experiments to determine optimal growth conditions involve combining the proteins in solution together with small molecular weight reagents (precipitants) also in solution. The concentration of protein and precipitant are slowly increased to the point at which protein crystals begin to grow. Increasing the concentration involves loss of water by vapor diffusion or transfer of precipitant from a higher concentration by liquid-liquid diffusion or free interface diffusion. The vapor diffusion experiments are further described by the physical arrangement of the protein-precipitant combination: sitting drop, hanging drop and microbatch (diffusion of water through an oil).



Challenges Facing Miniaturization

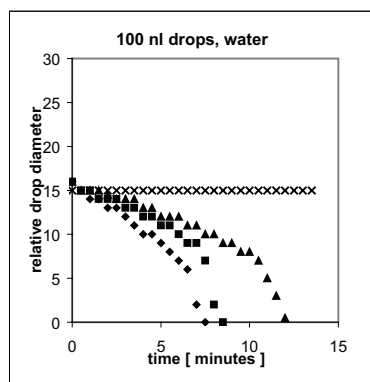
Viscosity

The major challenges to preparing protein crystallization experiments at low volume are related to the viscosities of the precipitant solutions involved. As the drop size decreases below 1 μ L the surface area to volume ratio increases and the effects of surface-active agents such as PEGs become dominant. Maintaining accuracy and precision becomes a challenge.

Evaporation

When dealing with sub-microlitre drop sizes, evaporation of the precipitant and protein droplet becomes a major concern (figure 1).

Figure 1: Effect of environmental conditions on rate of evaporation



- From a flat surface (◆)
- Greiner crystallization plates without reservoir (■)
- Greiner crystallization plates with reservoir (▲)
- Greiner crystallization plates with reservoir and humidity cover (×).

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Positioning Precision

A major challenge to dispensing nanolitre volumes for protein crystallization is the precision of drop positioning. It is important that the protein drop is positioned directly on top of the precipitant to achieve optimum conditions for crystallization.

Miniaturization Solutions

The Digilab dispensers use proprietary technology to effectively address the issues arising from miniaturization of protein crystallization.

Viscosity Control

The Digilab dispensers are capable of accurate and precise dispensing of small drops in the 20 nL to 5 µL range. These dispensers use patented technology which controls dispensing parameters to maintain high accuracy and precision at sub-microlitre volumes with viscous liquids such as PEG. Their ability to dispense highly viscous solutions at low volume makes Digilab dispensers ideal for miniaturization of crystallization experiments (figure 2).

Sources of control over liquid handling parameters include:

- Aperture of the dispensing tip
- Speed of dispense
- Valve opening time
- High precision syringe pumps

*Patented synQUAD technology
in the Honeybee 81*



SOLUTION	% PEG (MW)	N	% CV
Crystal Screen 41	20 (4K)	96	5.48
Crystal Screen 31	30 (4K)	96	5.49
Natrix 48	30 (4K)	96	6.00
Wizard I 31	20 (8K)	96	7.06
Wizard I 17	30 (8K)	96	7.62

Nominal 100 nL of each solution containing 25 µM Na-Fluorescein dispensed into 2 rows of 1536 plates

Figure 2: Digilab Dispensing of PEG-Containing Crystallization Screening Solutions

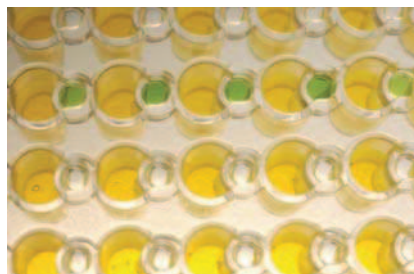
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Evaporation control

Altering the environmental conditions can control the rate of evaporation. In order to reduce the risk of evaporation to a minimum either the plate should be prepared in less than 2 minutes or humidity control should be used. The range of Digilab dispensers provides solutions to this problem with all instruments featuring either rapid plate preparation or humidity control.

Control of Positioning Precision

High resolution x-y-z motors within the Digilab dispensers ensure accurate positioning of the protein drop on top of the precipitant.

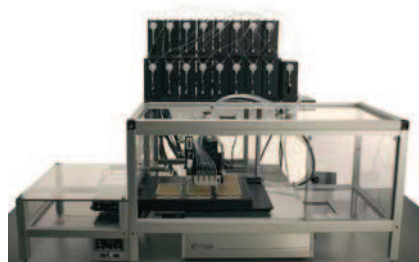


Honeybee Systems For Protein Crystallization

All of the Digilab dispensers can be used for protein crystallization but the Honeybee Systems have been developed specifically for this application. The requirements of every protein crystallization project can be met using an instrument from the Honeybee range.



Honeybee 961



Honeybee 161

WHICH HONEYBEE SYSTEM TO USE?

Model	No of Crystal Plates	Speed	Precipitant			Protein			Humidity Control	Technique		
			Dispense Channels	Source		Dispense Channels	Source			Sitting Drop	Hanging Drop	Micro Batch
				Deep Well Block	Pre-filled Reservoir		Well	Vial				
961	2	+++	96	✓	✓	1	✓	✓	-	✓	-	-
81	4	+	8	-	✓	1	✓	✓	Yes	✓	✓*	✓
161	4	++	16	-	✓	1	✓	✓	Yes	✓	✓*	✓
Automated	30	++	16	-	✓	1	✓	✓	Yes	✓	✓	✓

*requires Neuroprobe™ plate

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SynQUAD Technology for Protein Dispensing

Using Digilab SynQUAD technology for protein crystallization has many benefits. Non-contact dispensing and efficient tip cleaning ensure elimination of carry-over during crystallization experiments. In addition to reducing the amount of protein required per experiment, the minimum aspiration volume achieved using SynQUAD technology (1 μ L) means efficient use can be made of even the smallest protein sample. The fluidic inertia necessary for non-contact dispensing of the protein ensures efficient mixing of the protein and the precipitant even at nanolitre volumes.

Co-crystallization Studies

Traditional protein crystallization experiments use only one protein solution and a single precipitant. The ability to determine structure from co-crystallized proteins, co-factors and inhibitors is of great advantage to the drug discovery process. Many of these inhibitors have low solubility, a potential problem when dispensing sub-microliter volumes. The liquid handling flexibility of the SynQUAD technology in the Honeybee systems provides the researcher with the capability to run co-crystallization experiments. The SynQUAD channels are tolerant of DMSO, in which many inhibitors are stored due to their low solubility..

Summary

The SynQUAD range of low volume Digilab liquid handling dispensers overcomes the obstacles to miniaturization of protein crystallization. These dispensers enable Digilab to offer a complete solution for low volume protein crystallization.

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The logo for Digilab, featuring the word "DIGILAB" in a bold, blue, sans-serif font. The letter "I" is stylized with a grey triangle pointing upwards, and a registered trademark symbol (®) is located to the right of the "B".