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# A crystallization plate for controlling evaporation in hanging drops

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The modification and use of a crystallization plate for controlling the evaporation in hanging-drop trials is described. In this plate, known as the Nextal Crystallization Tool, each well is sealed by a screw cap that incorporates the cover-slip. Graduation marks were introduced on the outer circumference of the wells to enable accurate and reproducible adjustment of the tightness of the seal on the well. This design allows variable amounts of evaporation without exposing the drop. Crystallization experiments of $\alpha$-crustacyanin from lobster shell, for which controlled evaporation was applied to regulate the number and size of the crystals, are presented.

## 1. Introduction

Nucleation is the key to crystallization. It is often the case that either no nucleation occurs at all or that excess nucleation takes place in crystallization trials. It is therefore necessary to devise methods for inducing and controlling nucleation to obtain crystals of diffracting size and quality.

Several means (other than seeding) have been devised, mainly using the microbatch and vapour-diffusion methods, to control the amount of nucleation in crystallization trials (*e.g.* Saridakis *et al.*, 1994; Bray *et al.*, 1998; Chayen, 1997; Jovine, 2000; Saridakis & Chayen, 2003).

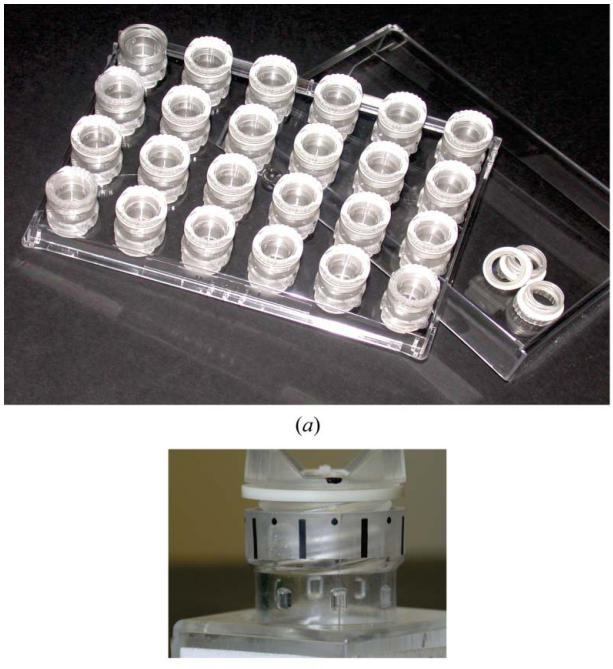

**Figure 1**
(*a*) Standard Nextal plate with screw cap. (*b*) The outer circumference of each well is marked with graduations so that the extent of the seal can be accurately set for each individual reservoir.

The Nextal Crystallization Tool (Fig. 1*a*) consists of wells that are sealed by a screw cap, which incorporates the cover-slip. This tool is particularly useful for setting up trials for decoupling the phases of nucleation and growth. This is achieved by transferring cover-slips holding hanging drops from reservoirs under conditions that normally give many small crystals to reservoirs at concentrations that normally yield clear drops. The screw-cap design affords easy transfer of drops from one reservoir to another. This is in contrast to Linbro plates where the reservoirs are generally sealed with grease that makes it difficult to remove the cover-slip after a period of time and to be certain of the effectiveness of the seal over the new reservoir.

The standard Nextal plate has now been modified to enable a further use that facilitates controlled evaporation.

Jovine (2000) reported experiments in vapour diffusion whereby trials were set up at lower precipitant concentrations than those required for crystallization. Nucleation was successfully induced by opening the cover-slip over each experiment for 2 min and was then arrested when the cover-slip was closed. However, crystallization trials that require longer exposure times could result in contamination of the drop when the cover-slip is open, and more importantly, the drops would dry out. If the cover-slips are positioned to cover just part of the well, there is no reliable measure of the extent to which the well has been opened, thus affecting reproducibility. The extent of opening and the length of exposure time are critical factors.

## 2. Design of the plate

Control of evaporation in hanging drops was performed efficiently and quantitatively using a modified Nextal Crystallization Tool, to which a new feature has been added as shown in Fig. 1(*b*). The outer circumference of each well has been marked with graduations so that the extent of the seal can be accurately set for each individual reservoir. The graduations on the well are used to adjust the tightness of the seal in order to allow variable amounts of evaporation without exposing the drop. The graduations also ensure reproducibility of the experiments.

**Table 1**
The effect of different evaporation times on the number and size of $\alpha$-crystacyanin crystals.

| Observation interval (days) | Length of evaporation time (h) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Sealed | 0.5 | 0.75 | 1.0 | 3.0 | 4.0 | 17 |
| 1 | Clear | Clear | Clear | Clear | Clear | Clear | Microcrystals and precipitate |
| 4 | Clear | Clear | Clear | Clear | One crystal >50 µm | ~22 crystals <50 µm | Hundreds of microcrystals |
| 5 | Clear | Clear | Clear | Clear | Six crystals | ~22 crystals | Hundreds of microcrystals |
| 7 | Clear | Clear | Clear | Clear | Six crystals | ~22 crystals | Hundreds of microcrystals |
| 8 | Clear | Clear | Clear | Clear | – | – | Hundreds of microcrystals |
| 13 | Clear | One crystal <10 µm | Two crystals | Three crystals | – | – | Hundreds of microcrystals |

## 3. Experimental

$\alpha$-Crystacyanin from lobster carapace was prepared at 10 mg ml$^{-1}$ in 0.1 *M* Tris HCl and 1 m*M* of EDTA at pH 6.1 (Chayen *et al.*, 2003). 2 µl drops were set up over 750 µl of reservoir solution by mixing of 1 µl of the protein solution plus 1 µl of well solution containing 6% PEG 550 MME, 20 m*M* zinc chloride and 0.1 *M* MES at pH 6.1. Trials were incubated at 283 K. Under these conditions the drops remain clear if the wells are sealed throughout the experiment. When higher concentrations of protein and/or precipitant were applied, numerous small crystals were obtained in 3–5 days.

The experiments involved the loosening of the screw caps above the wells at the time of setting up the trials and the subsequent sealing of the caps at specific time intervals. The tightness of the seal was set using the graduation marks on the circumference of the well, this being the most accurate way of setting equivalent extents of seal across the plate. The screw thread of the cap is one-quarter turn of the cap from the point at which the cap and the well engage to when the cap is sealed tightly. When the cap is just engaged with the top of the well, there is a gap of about 2 mm, which narrows down to a non-measurable amount when the cap is half sealed. All of the caps were set to the division that equated to the half-sealed position, so that there was no apparent gap. Control experiments run in parallel were sealed throughout. The drops were observed at regular intervals.

The extent to which the trial is allowed to evaporate is defined by the length of time that the reservoir was left unsealed (see above). Seal tightness is set such that vapour can escape from the trial without the trial being openly exposed to the atmosphere. The amount of dehydration from the drop and the reservoir has not been measured, since this is practically very complex; however, owing to the large difference in volume between the drop (2 µl) and the well (750 µl), it is likely that the loss of liquid takes place mainly from the drop. We believe that the evaporation rates in each trial are similar because the setup of the trials was identical. The composition and volumes of drop and reservoir, the temperature and the tightness of the seal were the same in each trial. The only variable was the length of the evaporation time.

## 4. Results

Table 1 demonstrates the results of a typical evaporation trial showing that crystal size and number are dependent on the length of time that the trials have been unsealed. Evaporation for 3 h gave the best results, yielding 1–8 single crystals with dimensions above 50 µm.

Evaporation times of less than 1 h result in crystals appearing only after 8 days, while times in excess of 3 h result in showers of microcrystals. Drops that were sealed throughout the experiment remained clear.

## 5. Conclusions

The modified plate saves material and the experimenter's time, since a variety of conditions can be explored in a single drop for both screening and optimization.

In the case of screening, the caps can be loosened if drops remain persistently clear. In the case of optimization, which involves setting up many experiments where a number of parameters need to be varied in turn, this evaporation method allows a range of parameters to be explored performing fewer trials.

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