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New Developments of the IMPAX Small-Volume Automated Crystallization System

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Abstract

Recent developments of the IMPAX system for automated crystallization are presented. A five-channel microtip has been introduced into the system thereby providing an extra degree of freedom for carrying out experiments. A new mouse-driven program for screening has been introduced, which creates a much wider scope for designing and executing screens covering new conditions of crystallization. The hardware has been adapted so that the system can also be used to set up vapour-diffusion trials. A simple design of a vapour-diffusion vessel, suitable for sitting drops of 2–15 μl, using smaller reservoir volumes (up to 100 μl), facilitates large-scale systematic trials.

Introduction

We have previously described a technique for conducting rapid crystallization surveys, which reduces labour and consumes very small quantities of materials (Chayen, Shaw Stewart, Maeder & Blow, 1990). A microdispenser comprising a bank of Hamilton syringes driven by stepper motors under computer control (IMPAX) is used to set up samples for crystallization. The system can be used to screen a wide range of arbitrarily chosen conditions and also to optimize the precise conditions for obtaining the best crystals.

Crystallization experiments are routinely set up as microbatch samples dispensed under oil where the components (macromolecule solution, precipitating agent, buffer and additives) are mixed in their final concentrations and dispensed through a multi-bore microtip into oil-filled wells of tissue-culture plates under the oil. The oil prevents evaporation and protects the samples (Chayen, Shaw Stewart & Blow, 1992). Protein crystals of diffraction size and quality have been grown in 1–2 μl drops (*e.g.* Wilson, Chayen, Hemmings, Drew & Pearl, 1991).

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This paper presents recent, novel developments in both hardware and software of this automated system.

Five-channel dispensing

To optimize conditions for crystallization the IMPAX system has to date been operated by mixing four solutions through a four-channel microtip. The channels typically contain the following reagents: channel 1: the macromolecule to be crystallized; channel 2: precipitating agent; channel 3: additive; and channel 4: buffer as a diluent to make up the volume of the trial.

In many instances, mixing four solutions does not provide enough degrees of freedom to carry out an experiment. For example, one may wish to vary the concentrations of the precipitating agent (channel 2) and the additive (channel 3) simultaneously, while keeping the protein concentration (channel 1) constant. If a buffer is to be included in channel 4 (which is used to make up the volume of the trial) its concentration may vary from well to well. In cases where a non-ionic precipitant such as PEG is used, this may lead to significant variations in the total ionic strengths.

This problem has now been solved by the addition of a fifth motorized syringe and valve assembly, used with a five-channel microtip. The fifth channel always contains water, and is used to make up the volume of the trial thereby allowing the concentrations of four materials to be independently controlled.

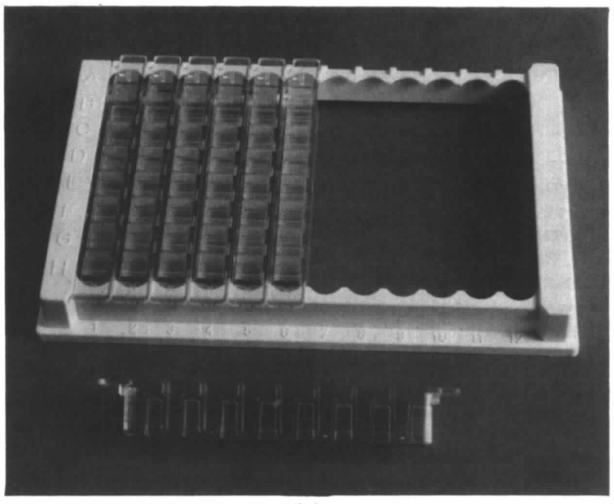
Adaptation to vapour diffusion

The uniqueness of the IMPAX crystallization system lies in its capacity for microbatch crystallization. However, in order to increase the flexibility of the system, the hardware can be adapted to perform vapour-diffusion trials. The set up of the hardware remains largely unchanged. The same Hamilton syringes (100 μl) which are used for dispensing

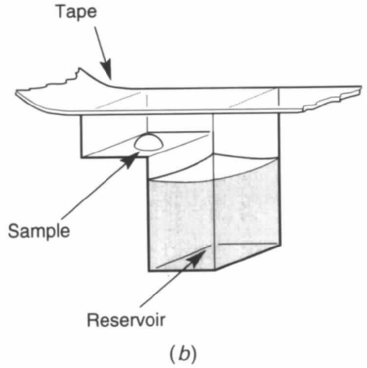
microbatch trials are used for transferring solution from the reservoir to the droplet and also to add protein to create either sitting or hanging drops. The reservoir solutions are dispensed by larger syringes of 10 ml. The trials may be performed in Linbro or Cryschem plates.

Alternatively, the system can use compact plates (Fig. 1) which were developed by Douglas Instruments in consultation with Allan D'Arcy of Hoffman La Roche. The plates were designed to scale down the quantities required for automatic dispensing using the vapour-diffusion method. 96 wells can be fitted into a frame, the size of a microtitre plate; these wells accommodate reservoir volumes of up to 100 μl and drop volumes of 2–15 μl.

In order to test whether crystallization using such small reservoir volumes is comparable to crystallization from larger volumes, a prototype plate was used to crystallize lysozyme in sitting drops of 2, 3 and 5 μl with reservoir volumes of 70 or 100 μl. There was no change in the number, size or the time scale of growth compared with crystals which were grown by the hanging-drop method over reservoirs of 1 ml.



(*a*)



(*b*)

Fig. 1. The compact plate for vapour-diffusion crystallization. (*a*) The plate measures 12.8 × 8.6 cm and is made up of 12 separate strips each containing eight wells. A single strip is also shown. (*b*) Diagramatic illustration of an individual well showing the sample drop sitting on a small shelf above the reservoir. The wells are sealed with polyester tape.

New screening software

A popular way of screening a wide range of crystallization conditions is to use pre-mixed standard stock solutions of precipitating agents and buffers (Jancarik & Kim, 1991). This method is known as sparse-matrix screening. Although this method has proved successful, making up the numerous solutions is time consuming and laborious. New IMPAX software has been developed for (*a*) automatic mixing of solutions for such screens and (*b*) generating unlimited numbers of conditions from selected precipitants, buffers and additives.

A software package called *Pick & Mix* created by Douglas Instruments offers a method of designing and executing crystallization experiments. Trials can be defined by picking ingredients from a list of stock solutions and selecting their concentrations, using a mouse.

Stock solutions of precipitants, buffers, additives, *etc.* are placed in a microtitre plate referred to as the 'stock' plate. The experimenter is allowed to define any selection of stocks which he or she may desire. Solutions from this 'stock' plate are aspirated by the microtip and dispensed into individual crystallization wells of a plate referred to as the 'mixture' plate, thus mixing the specified solution. Protein solution is then added to the wells of the 'mixture' plate. Fig. 2 shows the typical appearance of a PC display for *Pick & Mix*. The programme runs on IBM AT 386/486 and compatibles. (It is written in Borland C++, and uses the TurboVision class library.) *Pick & Mix* is normally used with a two-channel microtip, one channel for transferring solutions from the stock plate to the mixture plate, and the other for adding protein to the wells of the mixture plate. Some of the wells of the stock plate contain a diluent and a few contain water for rinsing the microtip. It is also possible to dispense a series of wells as a gradient of precipitant concentration; this would correspond to scanning a section on a precipitant/protein phase diagram.

The *Pick & Mix* program has been used to test a large number of new combinations of precipitating agents and proteins. In several cases crystals were produced in conditions where crystallization had not occurred previously. One example was lysozyme, which crystallized in the form of rods from 40% Jeffamine 4 K and 50 m*M* HEPES pH 7; another was carboxypeptidase G2, which produced small needles from 1.5 *M* potassium citrate. This protein has previously crystallized under different conditions

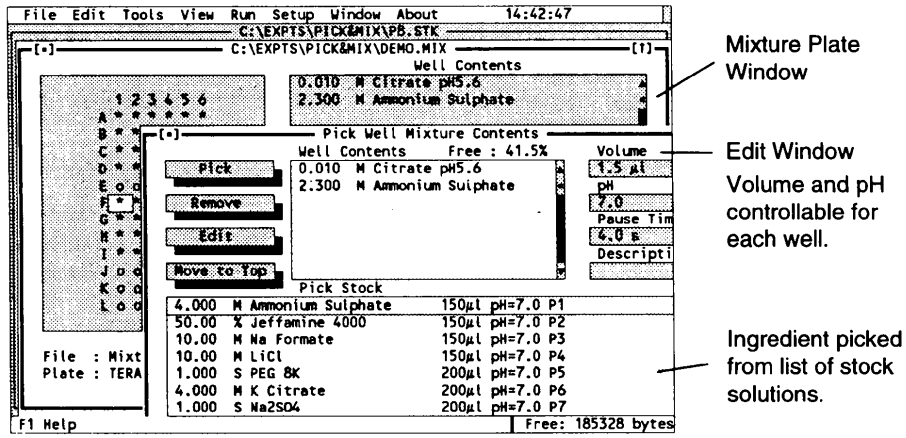


Fig. 2. The typical appearance of a PC display for *Pick & Mix* before dispensing. On the left-hand side, an array of *'s and 0's represent the full and empty wells of a mixture plate. The 'current' well is indicated by a cursor, and the ingredients are shown in the 'well contents' box. The contents of the current well may be edited by moving to a different window, and selecting the ingredients required from a list, as shown in the window on the bottom right of the screen.

but the quality of those crystals requires further improvement. The different crystal form obtained by the use of *Pick & Mix* may provide a new lead for the crystallization of this protein.

Screens using *Pick & Mix* are performed as micro-batch trials under oil where only very small quantities of protein need to be consumed (as little as 1 mg per 100 trials). Once conditions for crystallization have been defined using *Pick & Mix*, optimization to produce X-ray diffraction quality crystals is performed by applying a second program which uses the five-channel microtip as described above and in Chayen *et al.* (1990).

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