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An apparatus for electric-field-induced protein crystallization

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A new device has been fabricated for electric field-induced protein crystallization with the microbatch method. In conjunction with this, a specialized tray using inexpensive plastic film has been developed for economical set-up of the experiments. Crystallization can be conducted using AC or DC voltage.

1. Introduction

Electric field (EF)-induced protein crystallization experiments can be classified into two main categories, depending upon whether electrodes are directly in contact with the protein solution (called internal EF) or if the solution is isolated from the electrodes (called external EF).

A number of devices have been developed to set-up experiments under electric field, *e.g.* by Taleb *et al.* (1999, 2001), Nanev & Penkova (2001), Charron *et al.* (2003), Mirkin *et al.* (2003), and Sazaki *et al.* (2004). We have developed a new device for electric-field-induced protein crystallization, in which the experiments are set up using a microbatch under oil method. Either AC or DC voltage can be employed.

2. A new device (electrode panel)

An electrode panel (without insulation layer) has been fabricated on a 1 mm thick substrate (polyimide) with copper as the electrode material. The panel is composed of multiple pairs of straight electrodes. The colour of the electrode panel is white (Fig. 1), which facilitates imaging and observation. The insulator thickness, electrode width (w) and electrode gap (g) are described in Table 1 and shown in Fig. 2. Voltage is applied between each pair of electrodes by a power supply. There are only two connections as all positive electrodes are connected together, and similarly all negative electrodes are connected together. When a voltage is applied between the two electrodes, an EF is generated. The field is concentrated mostly in the insulator, between the droplet and the electrodes, because of the relatively good conducting property of the aqueous droplet. Inside the droplet, the EF appears only in a thin layer close to the bottom part of the droplet overlaying the electrodes.

Table 1
Basic set of parameters for the electrode panel, model 0512.

Parameter	Value
Electrode gap (g)	300 µm
Electrode width (w)	700 µm
Insulator thickness (t)	10 µm

A specially designed plastic film tray (PFT), Fig. 3, is placed over the electrode panel and the plastic film (polyvinylidene chloride, PVDC) acts as an insulator. The PFT has two parts: a holder (made from polycarbonate) that tightly holds the plastic film, and the plastic film itself. A schematic of a droplet on the electrode panel is shown in Fig. 4, while Fig. 5 shows a PFT containing oil and protein–crystallant (precipitant) droplets on an electrode panel.

3. Experiments

Experiments were carried out both with an EF and without applying an external electric field (NEF). The experiments are set up using the

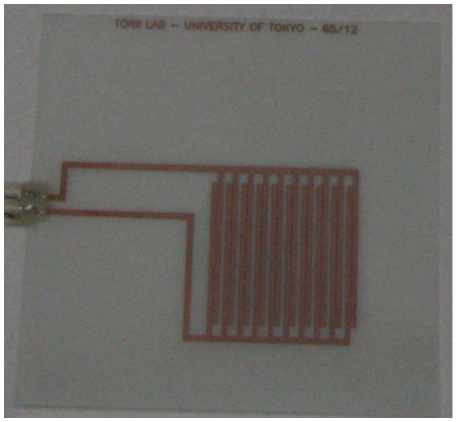

Figure 1
A bare electrode panel (model 0512).

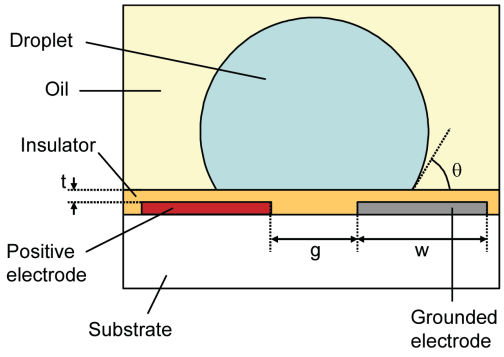

Figure 2
Schematic of the droplet on the electrode panel.

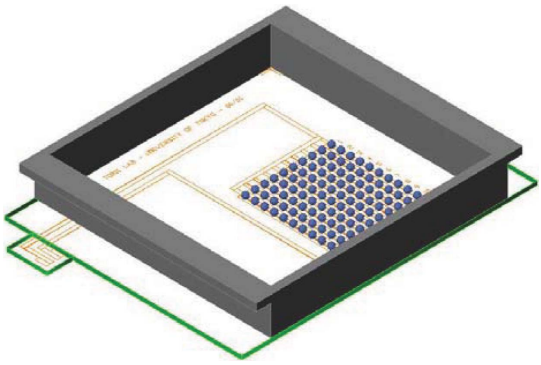

Figure 3
Schematic of a plastic film tray (PFT) placed over an electrode panel, model 0512.

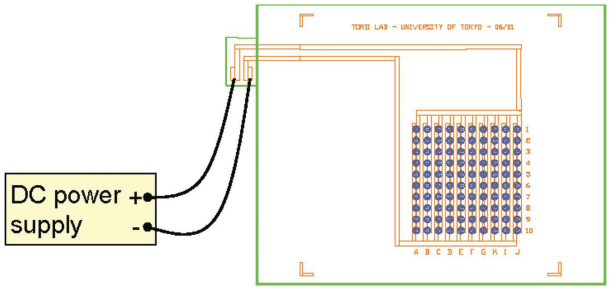

Figure 4
A schematic of the microdroplet array formed on the electrode panel.

microbatch under oil (MB) method of protein crystallization. The oil is poured into the PFT. Then, protein and crystallant droplets are dispensed into the oil on the PFT above the centre of the electrode where they merge. An array of microdroplets is hence formed on the electrode panel (Fig. 5). The micro-droplet array is made in such a way that the droplets stand between positive and negative electrodes. After microdroplet-array formation, the card is plugged into the power supply (model PK-80H of Matsusada Co. Ltd, Tokyo). The potential can be varied from 0 to 320 V to deliver a high DC output voltage. Then, this assembly is placed inside an incubator at 293 K. The resulting crystals are observed under a microscope and images of the crystals recorded at periodic intervals.

4. Results

Comparative analyses on chicken egg white lysozyme (CEWL) crystals have demonstrated that application of an external EF has positive effects. Dozens of experiments have so far been conducted on CEWL. It has a tendency to decrease the number of crystals in a drop. In the NEF samples, the mean number of crystals in a drop is eight, whereas for the samples in EF (30~270 V), the mean number of crystals ranges from one to six (Fig. 6). Using the apparatus, we have also conducted experiments on electric-field-induced protein crystallization of thaumatin and bovine-serum albumin (Al-Haq *et al.*, 2005*a*,*b*, 2006).

5. Discussion and conclusion

A new device has been developed that can be used to study the effect of an EF on crystallization. The 'separable plastic film tray', developed for electrostatic actuation, is inexpensive, easy to use and a

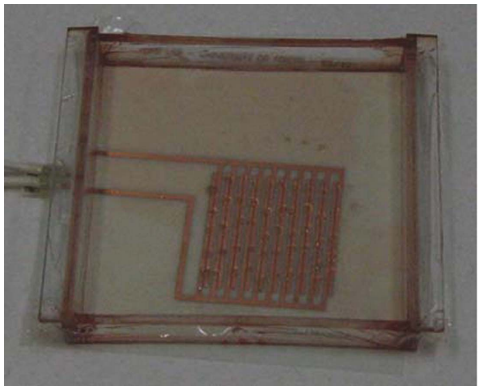

Figure 5
A PFT containing oil and protein–precipitant droplet mixture placed on an electrode panel.

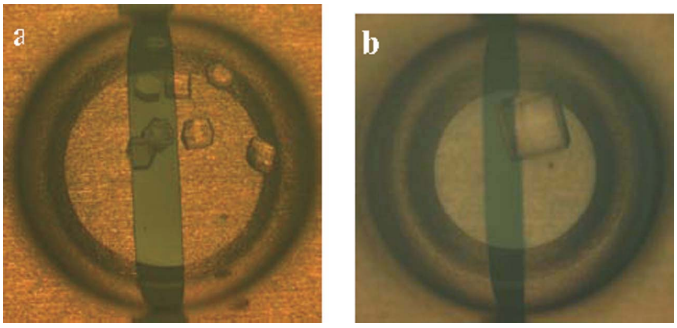

Figure 6
CEWL crystals in (*a*) NEF, showing eight crystals, and (*b*) EF, showing only one crystal. Nucleation was reduced under EF.

flexible tool that has several potential applications, *e.g.* any application that can be performed by electrostatic actuation, like biological assays, chemical reactions (bioreactors), *etc.* The EF-induced crystallization appears to have a positive effect in our preliminary studies. An external EF results in large protein concentration gradients inside the mother liquor, leading to local supersaturation regions of the crystallization solution. Protein molecules are charged since the pH solution is far from the protein's isoelectric point. When a small direct and constant current is imposed on the system, a potential difference is established between the electrodes, provoking an orientation effect over the macro-ions (protein molecules). The electrolyte solution in contact with an electrode feels asymmetric forces and orders itself, forming an electric double layer. The first layer is composed of water molecules with their dipoles oriented, and the second layer is composed of counterions (Bard & Faulkner, 2001). Positively charged protein molecules need a negative ion for favourable protein–protein interactions [*e.g.* Cl^- for CEWL (Vaney *et al.*, 2001), tartrate for thaumatin]. The potential difference established in the cell allows the migration of anions of the precipitating agent into the capillary tube, encouraging initial interactions with protein molecules in solutions. Then the counterions of the electric double layer will act as a support for positively charged protein molecules or nuclei, letting the crystal grow over the anode. Under appropriate conditions of temperature, pH and precipitating agent composition, crystals of larger volume result. The device may also offer some potential for the crystallization of samples that have not been crystallized with current methods. Comparative analyses on CEWL have demonstrated that an external

EF applied during protein crystallization experiments had positive effects, namely (*a*) less nucleation resulting in a reduction in the number of crystals per drop and (*b*) a larger size of CEWL crystals.

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