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Chapter 12

An efficient nanoliter-volume multi-channel device for highly viscous materials used in membrane protein crystallization

Jinghui Luo¹, Raphaël Zwier², Jan Pieter Abrahams¹*
1, Department of Biophysical and Structural Chemistry, Leiden Insitute of Chemistry, Leiden University, 2300 RA Leiden, The Netherlands.
2, Fine Mechanical Department, Leiden Institute of Physics, Leiden University, Leiden, The Netherlands.
* Corresponding author: abrahams@chem.leidenuniv.nl

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12.1 Abstract

The crystal structures of various important membrane proteins could not have been solved without lipidic cubic phase (LCP) crystallization. Yet, compared to traditional in surfo crystallization, LCP crystallization is not widely used because its extreme viscosity makes the cubic phase difficult to handle. Robots that can dispense LCPs are very specialized and therefore very expensive. Here, an accurate multi-channel device is described. It dispenses LCP on glass plates down to volumes of 20 nl accuracy and that has an accuracy of 10% when dispensing 200 nl – the lower bound of LCP volumes dispensed for crystallization trials. Because of its multi-channel tips, operation speed goes up by a factor of four compared to simpler devices. It can be operated by hand, but its design also allows it to be built into a basic dispensing robot. Thus, the device lowers the threshold for LCP crystallization of membrane proteins/peptides.

12.2 Introduction

Lipidic cubic phase (LCP) is a structured and transparent membrane-mimetic matrix, consisting of lipid and solution (water/protein) in appropriate proportions. According to literature, LCPs provide membrane proteins nucleation sites and support crystal growth by stabilizing proteins(1–3). Membrane proteins are important targets for drug development and for understanding fundamental biological phenomena, and several membrane proteins, such as GPCRs, photosynthetic proteins, antibiotic peptides and ion channels, have been crystallized using LCPs(4–7) . However, LCPs are not yet broadly used in the membrane protein crystallization community due to the difficulties of handling these viscous materials. A straightforward mechanical mixer for small volumes of viscous material was developed and it allows preparing LCPs easily and quickly(8) . A single channel nanoliter-volume dispenser was designed to dispense the extraordinarily viscous LCPs on glass slides(9, 10). These technologies were integrated into a dispensing robot to set up screens(11).

The LCP dispensing robot was a breakthrough for high-throughput work. However, not all labs have access to such devices or the means to purchase and maintain them. Manual dispensing is laborious and therefore an easy, affordable and efficient device for dispensing cubic phases more quickly is urgently needed to further LCP technologies. Here, we describe such a device.
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12.3 Materials & Designs

12.3.1 Materials

Monoolein (1-oleoyl-rac-glycerol; lot M239-MA26-N) was purchased from Nu Chek Prep Inc. The lipidic cubic phase was prepared at room temperature using a lipid-mixing device as described(8). PEG-600 (lot 39927-08-7) was from Fluka Analytical and was used fresh without further purification. Dispenser (PB600) was purchased from Hamilton.

12.3.2 Design of multi-channels dispenser and dispenser stands

The multi-channel LCP dispenser (figure 1 and 2) has the following parts: syringe (1), mounting frame (2), seal (3), capillary connector (4), stainless steel tips (5), and a mounting nut (6). The seal (3) makes a gastight connection between the capillary connector (4) and the head of the syringe (1). It is a compression seal: it protrudes a distance of 0.2mm from the connecting face plane of the capillary connector and is made of Teflon. The outside dimensions of the seal are based on the existing (standard) Hamilton gas-tight syringe seal. The body of the capillary connector (4) is made of stainless steel and has four drilled holes with laser welded needles (5). The upper part of the mounting frame connects directly to the head of a Hamilton gas-tight syringe,
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Figure 2: Schematics for the various parts of multi-channel tips device. All dimensions are in millimeters.

secured by means of a setscrew, and the lower part of the frame holds the 4 needles in a configuration that matches the standard spacing of a well-plate or grid. The ends of the needles are aligned horizontally to each other and in the current design protrude 1.7 mm from the frame, preventing the dispensed material from sticking to the frame through capillary forces. A mounting nut is used to fix the capillary connector with the Teflon seal, to the head of the syringe. The syringe can be placed in a holder that allows accurate Z alignment using a micrometer, combined with manual flipping up of the device as is required for re-positioning. The dead volume is about 1 µl per needle, so with four needles, it is about 4 µl in total.

Dispensing is effectuated by pushing the plunger of the syringe. The pressure applied results in extrusion of the viscous LCP from the four capillaries. The pressure can be applied manually, by means of a mechanical tool like a repeating dispenser or ratchet bar (the syringe in fig. 4a is fitted with such a device) or even a dispensing robot. If the back-pressure of all four capillaries is equal, the volume dispensed by each capillary should also be equal. We therefore took great care in accurately machining the connection between capillaries and syringe, and ensured that the four capillaries had identical dimensions and shapes. Reproducibility tests described below indicate our performance in this respect.

12.4 Results & Discussions

In order to test if the device was gas tight and did not drip, we first fitted our multi-channel tips with a 50 µl syringe and dispensed water. Then viscous materials (PEG 600 and LCP) were tested. Drops came out equally from the tips and were dispensed on glass efficiently as shown in fig 3(a-b). The device could dispense extremely viscous samples into equal amounts down to 184nl – with an overall standard deviation of 21 nl – as shown in fig 3(c-d,g-h). The graph in fig. 3g shows very high dispensing reproducibility
Figure 3: (a-b) Dispensing of PEG 600 from multi-channel tips and delivery on a glass plate (c-d) dispensing of lipidic cubic phase: the viscous LCP can be seen protruding from the multi-channel tips (c-d). Each drop was 184nl+/−21nl and we used a 50 µl volume syringe. The diameter size of needle used here is 0.26 mm. (e-f) Dispensing of smaller volumes (22nl) using our multi-channel tips dispenser. (g-h) Reproducibility of our dispensing device (using a 50 µl syringe).

of each individual needle, with standard deviations between 1% and 4% of the dispensed volume, yet also systematic differences between the four needles. The stingiest needle 4 systematically dispensed 25% less than the most generous needle 1. These systematic differences are the result of imperfect engineering of the device: despite considerable efforts, there are still very small differences in capillary length and curvature between each of the needles. The systematic variability is not an absolute volume: if half the volume is dispensed, the difference in volumes dispensed by the four needles is also halved. Slow dispensing by applying less pressure also reduces inter-needle variability. The systematic differences do not invalidate the use of the dispensing device, but do need to be taken into account when comparing the outcomes of crystallization trials.

Cubic phase crystallization requires the volumes to be as small as possible. The dispensed volumes were reduced in several ways. First of all, a syringe with a smaller volume was used to decrease the output volume of the multi-channel tips. Secondly, use of a ratchet bar with 0.4 mm steps – replacing a 1.2 mm step commercially available ratchet bar(10) – decreased the dispensed volume by a factor of three. To compare: with a 1.2 mm ratchet bar, using a 10 µl syringe, our multi-channel dispenser could dispense
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Figure 4: Dispenser stands. (a) multi-channel tips microsyringe dispenser and stand assembly. (b) stand (c) holder of microsyringe LCP multi-dispenser.

4 drops which can be calculated to be about 22 nl (fig 3(e-f)). This volume was too low to measure accurately enough with our digital caliper. Under the microscope, the droplets appeared to be about equal in size (cf. fig. 3f). To compare: the commercial dispenser PB600 cannot dispense volumes smaller than 200nl using a 10 µl syringe.

In order to accurately and reproducibly control dispensing LCPs on glass plates, we engineered a stand for our dispenser (figure 4). The stand comprised of a coarse manually operated Z-movement and a separate accurate Z-movement made by a micrometer head provides accurate Z-axis translation, giving semi-automatic operation for setting up plates for LCP crystallization. And a base plate is to provide a stable platform for the dispensing grids.

In summary, a straightforward, low-cost device with a specially engineered multi-channel tip, but otherwise assembled from standard components, allows dispensing very small volumes of viscous LCPs dispersions more efficiently. The developed multi-channel tip is able to reproducibly deliver 20-200 nl volumes of extremely viscous material for membrane protein crystallization. There may be systematic differences between channels, but the reproducibility of any single channel is high, with a standard deviation of just a few percent when dispensing 200 nl. Although not requiring a dispensing robot, it could be fitted to such a robot. We hope this new device will lower the threshold of LCP crystallization for labs that do not have ready access to LCP dispensing robots.

12.5 References
