

# MICROFLUIDIC CHIPS FOR MEMBRANE PROTEIN CRYSTALLIZATION

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## ABSTRACT

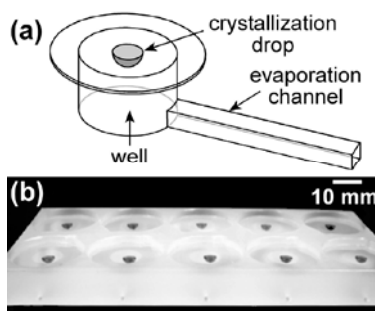
This paper reports novel microfluidic chips designed to screen for protein crystallization conditions with control over the kinetics of supersaturation. Using preliminary designs, we demonstrated that kinetic control can influence both the size and morphology of crystals of a model membrane protein.

**Keywords:** microfluidic, kinetic control, protein, crystallization

## 1. INTRODUCTION

Analysis indicates that nearly 30% of the proteins encoded for in the genomes of *E. coli*, *S. cerevisiae*, and *H. sapiens* reside in cellular membranes [1]. Membrane proteins act as mediators for signal, energy, and material transduction into and out of the cell. As such, their malfunction has been implicated in a number of serious diseases including autism, epilepsy, migraines, depression, drug abuse, and cystic fibrosis [2]. However, of the more than 44,000 protein structures deposited in the Protein Databank [3], only 256 are of membrane proteins [4]. Because of the limited quantities resulting from difficulties in expression, extraction, and purification, the development of microscale screening strategies to determine crystallization conditions is critical for further protein structural studies.

In prior work we have demonstrated control of the evaporation rate of solvent from a microliter-sized hanging drop in a small compartment and the resulting kinetic control over crystal nucleation and growth (Figure 1) [5]. The evaporation rate is determined by the cross-sectional area and length of a microfluidic channel connecting the compartment to the surrounding environment. In this study, we used this evaporation-based method to crystallize the model membrane protein bacteriorhodopsin while demonstrating kinetic control over the size of the resulting crystals. We also present work miniaturizing the crystallization platform to reduce sample consumption, increase throughput, and automate crystallization trials. Preliminary microfluidic trials on the nanoliter-scale demonstrated control over both the size and morphology of bacteriorhodopsin crystals.



**Figure 1:** (a) Schematic of the evaporation-based platform. (b) Optical micrograph of a multi-well evaporative crystallization platform for  $\mu\text{L}$ -sized droplets.

## 2. EXPERIMENTAL

Using the previously reported platforms [5], crystals were grown from microliter-sized droplets containing bacteriorhodopsin (solubilized in 25 mM  $\text{NaH}_2\text{PO}_4$  with 1.2 w/v% octyl  $\beta$ -D-glucopyranoside, pH 5.6) at different initial concentrations ( $C_{p0}$ ). The wells were vented to the outside environment through channels 0.6 mm in diameter and 7 mm long.

To miniaturize the evaporation-based crystallization process, we first created a simple microfluidic device consisting of only a microfluidic channel (100  $\mu\text{m}$  wide and 10  $\mu\text{m}$  high) molded in polydimethylsiloxane (PDMS) and sealed to a glass substrate after brief exposure to oxygen plasma. An inlet hole, approximately 0.5 mm in diameter and 5 mm long, was punched with a needle to the channel. We infused a solution of bacteriorhodopsin (8 mg/mL) into the channel. The device was left under ambient conditions, allowing water vapor to diffuse out both through the inlet hole and the bulk of the PDMS, although we believe water is lost more rapidly through the inlet.

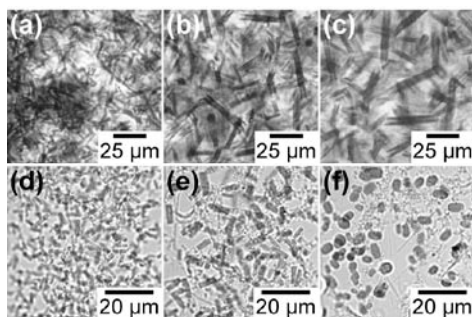
We have also completed a more exact microfluidic replication of the original evaporation-based crystallization device. A microfluidic network with integrated valves was used to direct, meter, and dispense discrete quantities of reagents. The network was fabricated in PDMS by multilayer soft lithography [6]. Through holes were fabricated in a separate layer and used to connect the network to an array of wells underneath. To fabricate the through holes, PDMS (5:1 w/w prepolymer/cross-linker) was spun onto a wafer containing an array of posts at 4000 rpm, >30 seconds, such that the posts protruded above the PDMS [7]. To prevent evaporation through the PDMS, the underside of the PDMS assembly was first exposed to oxygen plasma in a reactive ion etcher (300W, 200 mTorr, 20 sccm  $\text{O}_2$ , 10 min.), and then 20 nm of  $\text{HfO}_2$  was deposited via an e-beam evaporator at 0.5  $\text{\AA}/\text{s}$ . Wells and microfluidic channels used to control evaporation were fabricated by molding Norland optical adhesive 73 on a glass slide using a PDMS stamp containing inverse relief structures of the wells and channels. The optical adhesive was exposed to a long wavelength UV light source through the PDMS stamp until almost fully cured. The PDMS components were aligned with the crystallization wells, and then the whole assembly was exposed to the UV light source to complete the fabrication.

### 3. RESULTS AND DISCUSSION

Using the microliter-scale device reported previously [5], we showed that the rate of supersaturation at the solubility boundary was important in influencing the size of the membrane protein crystals (Figure 2a-c). At a constant evaporation rate and equal volumes, droplets with greater bacteriorhodopsin concentrations crossed the solubility boundary at slower rates of concentration compared to droplets with smaller initial concentrations of protein. This lower rate allowed for the nucleation and growth of fewer, larger crystals.

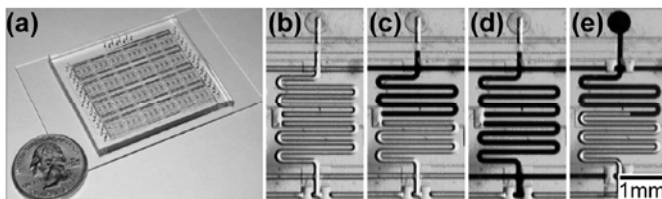
In the single channel microfluidic device, we demonstrated that crystals grown closer to the inlet (higher rate of solvent evaporation) formed small bricks, while further from the inlet, larger crystals of different morphologies were seen (Figure 2d-f).

To interrogate the rate of evaporation more rigorously on the nanoliter-scale, we designed a more sophisticated microfluidic chip that retained the main concepts of the



**Figure 2:** Optical micrographs of bacteriorhodopsin crystals obtained on microliter-scale (a-c) and nanoliter-scale (d-f) evaporation platforms. (a)  $C_0 = 2$  mg/mL, (b)  $C_{P0} = 4$  mg/mL, (c)  $C_{P0} = 6$  mg/mL at constant rate of evaporation. (d-f)  $C_{P0} = 8$  mg/mL with decreasing rate of evaporation (d) to (f).

larger evaporation-based devices (Figure 3). In addition to evaporation wells and channels, it also included microfluidic circuitry to perform the intricate fluid handling needed for crystallization screens. First, reagents were metered into a



**Figure 3:** (a) Photograph of the miniaturized evaporation-based crystallization device. (b-e) Micrographs of metering and dispensing of reagents on-chip to an individual well.

serpentine channel, where the ratio of the reagents was controlled by a pneumatic valve (Figure 3b-d). The individual chambers were then isolated from the main distribution channels, and reagents were extruded through vias into 30 nL crystallization wells (Figure 3e). A thin HfO<sub>2</sub> film inhibited evaporation through the PDMS components of the device so that the rate of evaporation was controlled by the dimensions of channels that vent the wells to the outside environment.

Currently, the chip is configured to screen one protein solution against 48 different precipitating reagents at a time. For each precipitant, the device formulates three different protein/precipitant concentration ratios, leading to a total of 144 trials per. The device could also be configured so that one protein/precipitant concentration ratio is tested at multiple evaporation rates, etc.

#### 4. CONCLUSIONS

We have shown that kinetic control over crystallization conditions is crucial to determining the size and morphology of membrane protein crystals. We have designed a device that will reduce sample consumption, increase throughput and enable automation while retaining control over kinetics of the crystallization process.

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