# KINETICS OF PROTEIN EXPRESSION IN SINGLE CELLS USING MICROFLUIDICS

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## ABSTRACT

We have coexpressed two target proteins, alkaline phosphatase(AP)[1] and Red Fluorescent Protein(mRFP1)[3], in cells while they have been encapsulated in microdroplets. The picoliter sized droplets are manipulated in a PDMS (polydimethylsiloxane) based microfluidic device specially designed for droplet storage[2]. We interrogate the enzymatic activity of AP and the expression of mRFP1 by following the fluorescence of stored droplets.

**KEYWORDS:** microfluidics, droplet, protein expression, kinetics

## INTRODUCTION

Assays based isolating bacterial colonies on agar or on microtiter plates, or FACS (fluorescence-activated cell sorting) analysis, are end-point assays yielding a single data point per experiment. Therefore, no resolved information about the time course of an enzymatic transformation is obtained. Even with *IVC (in vitro compartmentalisation)[4]*, time resolved phenomenon have been hardly studied and there is an increasing need to obtain several data points over time to distinguish different mutants in more detail by their kinetic parameters.

## THEORY

Microdroplets can provide a promising methodology to study kinetics of ezymatic activities. A microfluidic device was constructed using multi layer soft lithography[5], in which aqueous droplets were formed in oils[6], stored in wells[2] and monitored with time-series measurement. After droplets were formed they were flowed through a flow channel, which has a rectangular cross section of typically 100 μm width and 30 μm height. The device was designed such that the channels flatten and elongate the droplets. The droplets can be stored in wells, which are located above the flow channels, with typical dimensions of 50 μm width, 40 μm depth and spaced 15 μm apart. A droplet in a well has a more spherical shape, minimizing its surface area and thus surface tensional energy with the resulting force acting to drive and store the droplet into the well.

In addition, the bottom of the wells were constructed from a thin PDMS membrane (15 μm thick) that is slightly permeable to water[7]. The other side of the membrane contained a reservoir, through which flowed either water or an aqueous salt solution, which produced a chemical potential between the droplet stored in the well and the reservoir. This is required to maintain the droplet size by flowing a solution in the reservoir, which is osmotically equivalent to the droplet, otherwise, the volume significantly shrinks in a few minutes. The ability to store droplets and to maintain the concentration allowed us to study kinetics of protein expression in cells.



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## EXPERIMENTAL

As described in figure 1, droplets were generated at the nozzle of the microfluidic device, which encapsulated transformed *Escherichia coli* cells having single plasmid DNA that contained two promoter expression vectors, and a mixture of a fluorogenic substrate(fluorescein diphosphate) and inducer(isopropyl β-D-thiogalactopyranoside, IPTG). Monolithic valves, embedded in the microfluidic device[5], enabled droplets to flow into the storage region, in which the wells were constructed as shown in figure1-(b). After storing droplets into wells by closing the vavles, water was flowed through the reservoir to maintain the droplet concentration.

IPTG triggers the gene expression to produce proteins in cells while encapsulated in the microdroplets. The cell concentration was adjusted to enclose a few cells in each droplet so that we can address the production of proteins in specific individual cells. The fluorescence emitted from the stored droplets at two different wavelengths was periodically (typically every 20 min) measured to report accumulations of fluorescent products resulting from the enzymatic reaction of AP and expression levels of mRFP1 using an EMCCD camera (iXon, Andor Tech.) coupled to an inverted microscope (IX71, Olympus). In order to determine the dynamic range of expression level and activity of the enzyme, a coexpression plasmid of wild type (WT) AP-mRFP1 was prepared.

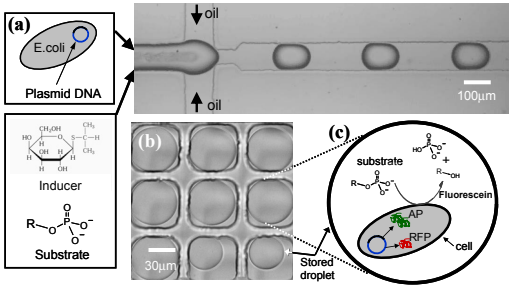


*Figure 1: Schematic processes of protein expressions in vivo. (a)Droplet formation at nozzle. One syringe has cell, the other has inducer and substrate. (b)A photograph showing stored droplets in the device. Squares are wells and circles are stored droplets surrounded by oil. (c)Enzymatic reactions occured in a stored droplet. AP hydrolyze substrate into fluorescent product. mRFP1 is directly detectible.*

## RESULTS AND DISCUSSION

As shown in figure 2-(a), fluorescein concentrations in droplets where AP was expressed slowly increased for first few hours, which is mainly due to auto-hydrolysis of substrate. Five hours after the droplets were formed, the concentrations began rising significantly for the next 4~5 hours and reached a plateau at around 10 hour. Interestingly, a time lag was observed between droplet formations and the observation of fluorescence. It could be due to the time taken for enzyme synthesis, export to periplasm and protein maturation such as disulfide bond formation and dimerization. Each droplet has different time lag between 4 hour to 6 hour.

mRFP1 is detected in cells around 2 hours after the droplet formation. The amount increases significantly over next few hours and plateaus after 10 hours as shown in figure 2-(b). The time lag is a few hours shorter than was observed for the enzyme activity. As two genes of AP and mRFP1 are constructed on a same plasmid, the gene expression for two target proteins is simulataneously triggered, therefore, we attribute the earlier appearance of mRFP1 to the fact that it is neither ex-

ported to periplasm, nor does it require diffusion of the fluorogenic substrate through cell membranes to generate fluorescent product from the enzymatic reaction.

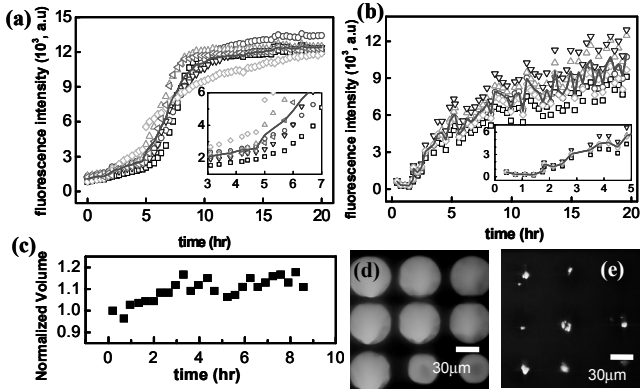


*Figure 2. (a)-(b) Each symbol represents reaction occurred in different droplets that have identical aqueous condition. There are a few cells per each droplet at most. The solid line is an average of intensities at each measurement. The insertions show the time lag in detail. (a) Fluorescence intensities showing accumulations of fluorescein produced by AP. The time lag is about 5 hours. (b) Kinetics of mRFP1 expression. The insertion shows mRFP1 is observable at 2 hour. (c) Droplet volume change to time. The droplet volume stored in wells changes about 15% of its initial size. The volume after 20 hour stays in this range. Without reservoir in a PDMS microfluidic device, the droplet volume shrinks to less than half in one hour[2]. (d) Fluorescence photograph (520 nm) showing AP activity in droplets. Circles are droplets stored in the well. The hydrolyzed substrate, fluorescein, is distributed uniformly over droplets. (e) Fluorescence photograph (607 nm) showing mRFP1 expression. The bright spots are cells enclosing mRFP1 encapsulated in microdroplets.*

## CONCLUSIONS

We have studied the kinetics of protein expression in individual cells based on microfluidic device designed specifically for long term experiments. We are currently extending this approach to measure the enzymatic activities of various mutants of AP and to quantify the enzyme expression simultaneously, through which we can differentiate specific mutations of enzyme that have a promising activity.

## ACKNOWLEDGEMENTS

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