

# MICROCOMPARTMENTALIZED CELL-FREE PROTEIN SYNTHESIS FROM SINGLE MOLECULE TEMPLATE DNA USING SEMI-PERMEABLE ALGINATE MICROCAPSULES

D. Saeki<sup>1,2</sup>, S. Sugiura<sup>3\*</sup>, T. Kanamori<sup>3</sup>, S. Sato<sup>1</sup> and S. Ichikawa<sup>1</sup>

<sup>1</sup>University of Tsukuba, JAPAN, <sup>2</sup>Kobe University, JAPAN and <sup>3</sup>National Institute of Advanced Industrial Science and Technology, JAPAN

## ABSTRACT

This paper presents a novel microcompartmentalized reaction system for cell-free protein synthesis using semi-permeable microcapsules. We used a microfluidic device to encapsulate template DNA and enzymes for cell-free protein synthesis into semi-permeable microcapsules composed of alginate and polyethylene imine (PEI). The template DNA was encapsulated into individual microcapsules at the concentration less than single molecule per microcapsule by limiting dilution. After encapsulation, the essential substrates for cell-free protein synthesis were fed through the semi-permeable membrane of the microcapsules. The proteins synthesized from the single-molecule template DNA in the microcapsules were successfully detected.

**KEYWORDS:** Microcapsule, Cell-free protein synthesis, Single-molecule DNA, Parallelization

## INTRODUCTION

Cell-free protein synthesis is a remarkable system to produce and screen mutant proteins. The system is built up using artificially-mixed essential components for protein synthesis such as enzymes and substrates, and can effectively synthesized native or mutant proteins without living cells. Recently, high throughput screening using cell-free protein synthesis has been developed using microplates [1] or water-in-oil-in-water (W/O/W) droplets [2]. Although these methods partially succeeded in screening of mutant proteins, they had difficulty of further numbering-up and instability of droplets as reaction containers.

In this study, we present a microcompartmentalized cell-free protein synthesis into semi-permeable microcapsules (Figure 1), and demonstrate protein synthesis from the single-molecule DNA in the microcompartment. PEI-coated alginate microcapsules containing template DNA and enzymes for cell-free protein synthesis were prepared by using the previously reported microfluidic device [3]. The microcapsules were incubated in the aqueous solution of substrates for cell-free protein synthesis, and the substrates were fed into the microcapsules through the semi-permeable alginate-PEI polyion complex membrane, resulting to protein synthesis. The presented microcapsules are easier of numbering up than microplates [1] and more stable than W/O/W droplets [2]. Furthermore, protein synthesis from single-molecule template DNA enables preparation of protein library in each microcapsule by limiting dilution of DNA with variation.

## EXPERIMENTAL

Enzymes and substrates in RTS 100 *E. coli* HY kit (5 PRIME, Hamburg, Germany) were used for cell-free protein synthesis. Green fluorescent protein (GFP) coding DNA in RTS 100 *E. coli* HY kit was used as a template DNA.

Semi-permeable alginate microcapsules were prepared by the droplet rupture methods with modification using the previously reported microfluidic device [3]. W/O/W droplets containing sodium alginate, template DNA, and enzymes for cell-free protein synthesis in the internal aqueous phase were prepared using the microfluidic device fabricated out of polydimethylsiloxane (Figure 2). An aqueous solution of 1 wt% sodium alginate, decane solution of 1 wt% tetraglycerin-condensed ricinoleic acid ester, and aqueous solution of 1 wt% dodecyl betaine were used as an internal

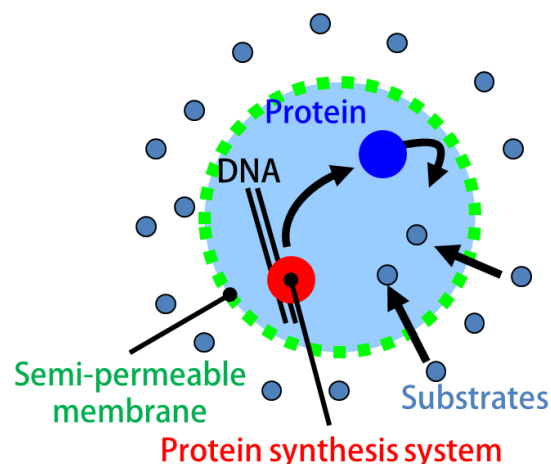


Figure 1: Schematic of cell-free protein synthesis in semi-permeable microcapsules as a model of microcompartmentalized biochemical reaction system.

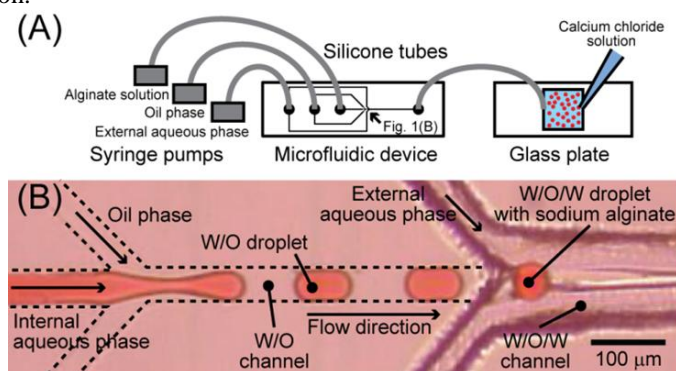


Figure 2: Equipment for formation of calcium alginate microbeads. (A) Schematics of the experimental equipment. (B) Microscope image of formation of W/O/W droplets containing sodium alginate.

aqueous phase, oil phase, and external aqueous phase, respectively. The template DNA and enzymes were added into the internal aqueous phase. Firstly, the microfluidic device was filled with the external aqueous phase. All fluids were injected into the microchannels at constant flow rates controlled by syringe pumps. Flow injection was started in the order of the external aqueous phase, oil phase and internal aqueous phase. The W/O/W droplets were recovered and contacted with an aqueous solution of 0.1 mol/L calcium chloride and 0.1 wt% PEI to rupture the oil phase layer and to form PEI-coated alginate microcapsules.

To initiate the cell-free protein synthesis, the substrates such as nucleic acids and amino acids for the protein synthesis were added into the suspension of microcapsule. The suspension of microcapsule was incubated for 6 h at 30°C for cell-free protein synthesis reaction, and for 24 h at 5°C for protein maturation.

## RESULTS AND DISCUSSION

The cell-free protein synthesis of GFP in the microcapsules was carried out (Figure 3). The fluorescence from the synthesized GFP was detected from the microcapsules and not detected in the case of either the absence of the substrates of cell-free protein synthesis or PEI coating of the microcapsules. By using the confocal laser scanning microscope observation, the fluorescence was detected from the internal space of the microcapsules. The fluorescence was still detectable over several months' storage. These results show that the alginate-PEI polyion complex membranes of the microcapsules behaved as semipermeable membranes and the proteins were only synthesized in the microcapsules. The substrates diffused and transferred into the microcapsules, while the enzymes for cell-free protein synthesis, template DNA, and products were retained in the internal aqueous phase. We confirmed that the prepared microcapsules were not permeable to FITC-dextran, M.W. 10 kDa. The maximum molecular weight of the substrates was less than 0.6 kDa, and that of the enzymes, template DNA, and products were larger than 10 kDa, respectively [4]. Thus, the synthesized GFP were retained in the microcapsules.

The cell-free protein synthesis in the microcapsules with a single-molecule template DNA was demonstrated (Figure 4). While almost all microcapsules had fluorescence from the synthesized GFP using 2.0 ng/μL of the template DNA equivalent to 30 molecules per microcapsule, only a few microcapsules had fluorescence using 0.07 ng/μL of the template DNA equivalent to one molecule per microcapsule. The limiting-diluted template DNA was encapsulated and separated into the individual microcapsules, and the GFP was synthesized from a single-molecule template DNA. A very small volume of the microcapsule enabled to retain the synthesized products derived from the single-molecule DNA in the microcapsules at high concentration.

The appearance ratio of the fluorescent microcapsules to the total number of the microcapsules was lower than 0.63, which is a theoretical value calculated according to Poisson distribution. The low appearance ratio is probably caused by that most of the template DNA were not encapsulated into the microcapsules or their biological function were impaired by the

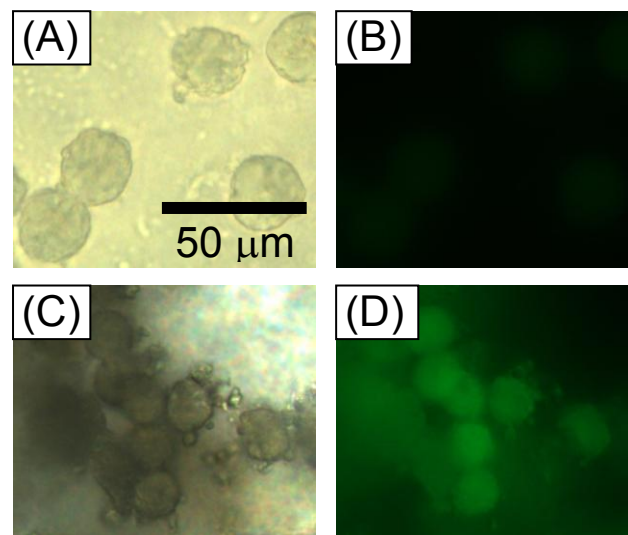


Figure 3: Microcompartmentalization of cell-free protein synthesis system into semi-permeable microcapsules. Microscope images after incubation without substrates (A and B) and with substrates (C and D). (A) and (C) are bright field images, and (B) and (D) are fluorescent images. The concentration of the template DNA was 2.0 ng/μL.

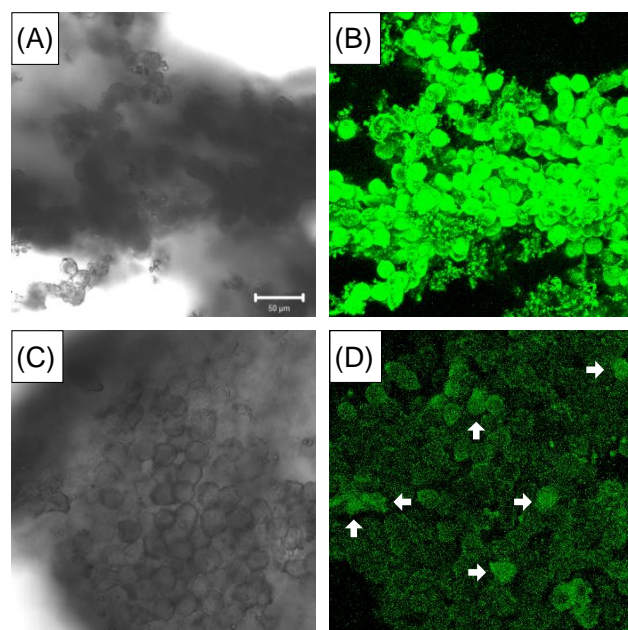


Figure 4: Parallelized cell-free protein synthesis in microcapsules using a single-molecule template DNA. The concentration of the DNA was 2.0 ng/μL equivalent to 30 molecules per microcapsule (A and B) and 0.07 ng/μL equivalent to single molecule per microcapsule (C and D). (A and C) are bright field images, and (B and D) are confocal stack images (50 μm thick). Arrows in (D) indicate the microcapsules in which GFP was synthesized.

absorption of the template DNA to the microchannel surface or the interaction of the template DNA and polycationic PEI.

The reaction volume of the developed system in this study was femto liter order, and extremely smaller than that of conventional microplates [1]. The developed system will be applicable to artificial cells, microbioreactors, and high-throughput screening of functions and activities of single-molecule DNA.

## CONCLUSION

The microcompartmentalized reaction system for cell-free protein synthesis using PEI-coated alginate semipermeable microcapsules was developed. Substrates for the cell-free protein synthesis were fed into PEI-coated alginate microcapsules containing enzymes for the cell-free protein synthesis and template DNA through alginate-PEI polyion complex membranes. The cell-free protein synthesis system in the microcapsules successfully produced the model fluorescent protein, GFP. The produced proteins were detected using fluorescent microscope and retained in the microcapsules. This system was also applied for parallelized protein synthesis on individual microcapsules dispersed in suspension. The GFP were separately synthesized from a single-molecule template DNA limiting-diluted in separated microcapsules.

## REFERENCES

- [1] Y. Koga, K. Kato, H. Nakano, and T. Yamane, "Inverting enantioselectivity of *Burkholderia cepacia* KWI-56 lipase by combinatorial mutation and high-throughput screening using single-molecule PCR and in vitro expression," *Journal of Molecular Biology*, 331, 585 (2003).
- [2] E. Mastrobattista, V. Taly, E. Chanudet, P. Treacy, B. T. Kelly, and A. D. Griffiths, "High-throughput screening of enzyme libraries: In vitro evolution of a beta-galactosidase by fluorescence-activated sorting of double emulsions," *Chemistry & Biology*, 12, 1291 (2005).
- [3] D. Saeki, S. Sugiura, T. Kanamori, S. Sato, and S. Ichikawa, "Formation of monodisperse calcium alginate microbeads by rupture of water-in-oil-in-water droplets with an ultra-thin oil phase layer," *Lab on a Chip*, 10, 2292 (2010).
- [4] Y. Shimizu, A. Inoue, Y. Tomari, T. Suzuki, T. Yokogawa, K. Nishikawa, T. Ueda, "Cell-free translation reconstituted with purified components," *Nature Biotechnology*, 19, 751 (2001)

## ACKNOWLEDGEMENTS

A part of this work was conducted at the AIST Nano-Processing Facility supported by "Nanotechnology Network Japan" of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

## CONTACT

\*S. Sugiura, tel: +81-29-861-6286; shinji.sugiura@aist.go.jp