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Research Article

Fabrication optimization of a miniaturized array device for cell-free protein synthesis

Cell-free protein synthesis (CFPS) is an attractive alternative to cell-based protein expression systems because of its advantages including speed, simplicity, and adaptability to various formats. However, two major obstacles exist that have been preventing it from being widely used. One is high cost and the other is low protein synthesis yield. We report here a miniaturized CFPS device that addresses these challenges. The cost saving was achieved by miniaturization, which reduced the reagent consumption by two orders of magnitude. The protein synthesis yield was enhanced by prolonging CFPS reactions through continuous supply of reactants (e.g. nutrients and energy components). The reactants were contained in a feeding solution, which was replenished through a nanoporous membrane and microchannel. The design of the miniaturized device was optimized by running continuous-exchange CFPS in devices with a variation in the type of membrane, the size of the exchange interface, and the volume ratio of the reaction solution to the feeding solution. The effects of these design variations on the protein synthesis yield have been studied. Furthermore, the design was expanded into a 96-unit device that can produce a large number of proteins simultaneously, enabling high-throughput proteomics applications.

Keywords:
Cell-free protein synthesis / Membrane / Microarray / Microplate / Miniaturization
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1 Introduction

Cell-free protein synthesis (CFPS) has become an attractive alternative to the conventional cell-based protein expression systems, especially with the improvement in the protein synthesis yield [1–5]. The advantages of CFPS over the cell-based systems include the ability to add a range of reagents to the expression system and lack of cellular control mechanisms. As a result, they generally do not suffer from the inherent limitations of often-used *Escherichia coli*-based recombinant protein production, including formation of insoluble protein aggregates (inclusion bodies), degradation of proteins by intracellular proteases, and low or no expression for the genes whose products are toxic to the host cell [1–5]. In addition, CFPS are advantageous in speed, simplicity, and adaptability to various formats.

CFPS includes the steps of gene transcription and protein translation [1–5]. In CFPS systems, a cell lysate (without cell membranes) provides protein synthesis machinery (e.g. ribosome) while other components are optimized to achieve an efficient gene transcription and protein translation reactions. One of the CFPS formats that produces high synthesis yield is called continuous-exchange cell-free system, in which the reagents in the reaction solution exchange continuously through a dialysis membrane with a feeding solution containing nutrients (e.g. amino acids) and energy components (e.g. adenosine-5′-triphosphate) [1, 5].

Miniaturization of CFPS has been investigated in several formats, including nano- or pico-liter well array [6, 7], water-in-oil droplets [8–10], and microfluidic devices [11–13]. The objectives of miniaturization were to reduce the reagent consumption and accordingly cost, speed up the reactions (as a result of more efficient diffusion and heat transfer), and enable high-throughput screening.

The continuous-exchange format of miniaturized CFPS has also been explored in the last few years [14–16]. A

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Abbreviation: AP, alkaline phosphatase; **CFPS,** cell-free protein synthesis; **FMG,** fluorescein mono-β-D-galactopyranoside; **GFP,** green fluorescent protein; **GUS,** β-glucoronidase; **β-lac,** β-lactamase; **LAR,** luciferase assay reagent; **MUG,** β-D-glucuronide; **lacZ,** β-galactosidase; **PBA,** *m*-[[(phenylacetyl)glycyl]oxy]benzoic acid; **PPH,** 3-phenylumbelliferone 7-*O*-phosphate hemipyridinium

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nanoporous membrane was integrated in these devices to (i) achieve continuous supply of additional reactants (e.g. nutrients and energy components); (ii) retain proteins produced and large-molecule synthesis machinery; and (iii) dilute the reaction byproducts (e.g. pyrophosphates) and reduce their effects on the reaction equilibrium. Either polydimethylsiloxane (PDMS) or thermoplastics was used to fabricate the devices. Such a device possesses not only the advantages of the miniaturization mentioned above, but also the benefits of the continuous-exchange format (i.e. higher protein synthesis yield).

In this work, we designed and fabricated a number of miniaturized CFPS devices that were integrated with a membrane to achieve the continuous exchange between the reaction and feeding solutions. We optimized the device design by studying the effects of several parameters on the protein synthesis yield. These parameters included the material type of the membrane, the size of the exchange interface, and the volume ratio between the reaction and the feeding solutions. Finally a device with 96 units was fabricated with an optimized design and its performance in protein synthesis was evaluated. The device was found to be compatible with detection instruments (microplate readers) and reagent-dispensing apparatuses, thus it can be used for high-throughput protein synthesis, functional studies, and drug screening [17].

2 Materials and methods

2.1 Materials

Polycarbonate sheets were purchased from McMaster-Carr (Atlanta, GA, USA) whereas polypropylene sheets were from Plastruct Canada (Ontario, Canada). RTS100 Wheat Germ CECF kits and RTS500 E. coli CECF kits were bought from Roche Applied Science (Mannheim, Germany). Luciferase T7 control vector, recombinant luciferase, luciferase assay reagent, and nuclease-free water were purchased from Promega (Madison, Wi, USA). Sylgard 184 silicone elastomer was obtained from Dow Corning (Midland, MI, USA). Flat sheet and disk-shaped dialysis membranes were purchased from Spectrum Laboratories (♯132677 & 132478, Rancho Dominguez, CA, USA). Regenerated cellulose membranes with differentiated filtration and a cutoff of 10 kDa were obtained from Millipore (♯13661AM, Billerica, MA, USA). Polycarbonate membranes with a 20 KDa cutoff were bought from GE Healthcare (♯KN1CP00010, Piscataway, NJ). The thickness of each membrane was measured by using a Dektak 150 profilometer, which scanned through a piece of membrane fixed on a microscope slide.

2.2 Fabrication of 6-unit devices

Design optimization was carried out in a device consisting of 6 units. The structure of the device is very similar to those in the previous effort [16], but the design optimization was not carried out previously. Briefly, the device consists of three layers: a top layer for protein synthesis, a bottom layer for the feeding solution, and a middle membrane layer (Fig. 1A). Both top and bottom layers were machined from polycarbonate sheets. Each unit in the top layer contained a 3-mm-diameter well flanked by two lunar-shaped openings for the access to the feeding chamber, which was a 9-mm-diameter well in the bottom layer.

These three layers were assembled together using microstamping technique [18]. In brief, PDMS mixture was prepared according to the manufacturer's instruction (Sylgard 184, Dow Corning), followed by spinning it (2000 rpm) on a substrate using a spinner (Laurell Technologies). The top layer was then in contact with the PDMS film, transferring a pattern of PDMS onto the bottom of the top layer. In other words, PDMS was not in the area that would function as the reaction chamber. Using a similar approach, a thin PDMS film was transferred on the top contact area of the bottom layer. Three layers were immediately assembled together, fastened using C-clamps, and then cured in an oven at 60°C overnight. The access holes were connected to the feeding chamber by piercing through the local membrane. Note that PDMS was used as an adhesive in three types of membranes tested. The membrane from Millipore did not bind well with polypropylene sheets when PDMS was used, thus an epoxy (353ND-T) from Epoxy Technology (Billerica) was used instead. Using microstamping technique, neither PDMS nor epoxy was in the contact with the center portion of the membrane that allowed transfers of small molecules between two chambers in Fig. 1B.

2.3 Fabrication of 96-unit devices

Using optimum geometry and parameters obtained from testing of various 6-unit devices, we designed and fabricated a device with 96 units. The 96-unit device (Fig. 1C) was designed by following the guidelines of the conventional microtiter plates, which was set by the Society of Biomolecular Screening (SBS) and the American National Standards Institute (ANSI). The height of the plate, the location of wells, and the shape and contour of the plate must be followed so that our device is compatible with reagent-dispensing apparatuses and with commercial microplate readers for detection.

Because the design needed to accommodate both reaction and feeding chambers, our 96-unit device was designed with the size and location corresponding to a 384-well microplate. For each 4 wells in a 384-well microplate, the center of the well in the first quadrant was used as the center of the 4.5-mm-diameter reaction chamber. Three 2-mm-diameter access holes were placed in the rest of the space for accessing the feeding chamber. If only one access hole was made, air could be trapped inside the feeding chamber when dispensing the feeding solution. As a result, two

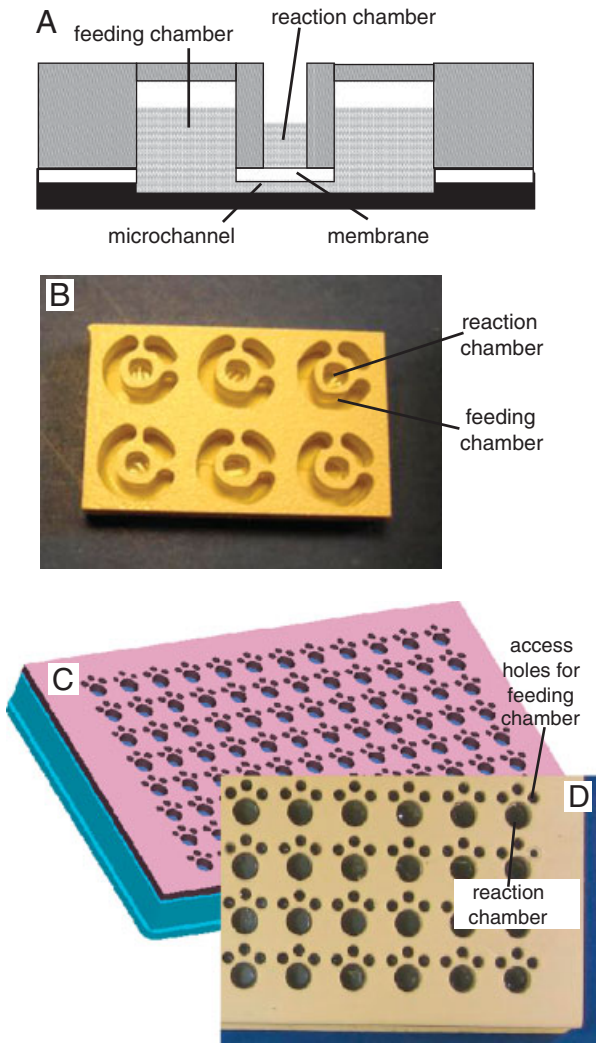

Figure 1. (A) Diagram of the 6-unit device used for the design optimization. Cross-sectional view of one unit consisting of a reaction chamber, feeding chambers, and a microchannel and membrane for connecting two chambers. The drawing is not to scale. (B) Photograph of a 6-unit assembled device. The membrane underneath the reaction chamber can be observed during its reflection of light. (C) CAD drawing of the 96-unit device. (D) Part of photograph of a 96-unit assembled device.

additional access holes were created to allow air to escape. Each feeding chamber in the bottom layer encompassed both the reaction chamber and the three access holes. The feeding chamber was designed to accommodate 200 μL of solution, which would fulfill the optimized 1:20 ratio between the volume of the reaction solution and that of the feeding solution. The volume of the reaction solution was 10 μL, though the total volume of the reaction chamber is 50 μL, which allows the assay reagents to be dispensed into it for studying the function of the produced proteins after the completion of the synthesis reactions.

Both top and bottom layers were machined from polypropylene sheets by TMR engineering (Micanopy, FL, USA). These two layers were assembled with a membrane using the microstamping technique mentioned above. The device was designed to allow either luminescence or fluorescence detection from the reaction chamber of the device. Note that the device was machined due to the fact that we needed only a couple of devices for this work. It would be manufactured using injection molding when it is commercialized and a large number of devices are required, thus the cost should be in a range similar to a 96-well microplate.

2.4 Protein synthesis

Luciferase was expressed using RTS100 wheat germ kit. The reaction solution for protein synthesis and the feeding solution were prepared according to the manufacturer's instructions. The reaction solution was composed of 15 μL of wheat germ lysate, 15 μL of the reaction mix, 4 μL of amino acids, 1 μL of methionine, and 15 μL of an individual DNA vector (2 μg). For negative controls, the DNA vector was replaced with the same volume of nuclease-free water. The feeding solution was prepared by combining 900 μL of the feeding mix, 80 μL of amino acids, and 20 μL of methionine (all of these were provided in the kit and the concentration of each component was fixed by the manufacturer). Typically, 10 μL of the reaction solution was pipetted into the reaction chamber, and 200 μL of the feeding solution was added to the feeding chamber through the access holes. For the design optimization studies, however, the volume of the feeding solution was varied to study its effects on protein synthesis. The device was designed to have the same solution level for the reaction and feeding solutions.

Synthesized luciferase in the 6-unit device was detected by transferring the protein expression product to a 384-well microplate (white color), which was then placed in a Mithras microplate reader (Berthold Technologies, Germany). The reader was programmed to inject 35 μL of the luciferase assay reagent, shake the microplate for 2 s, and measure luminescence over 10 s. For the luciferase synthesized in the 96-unit device, the luciferase assay reagent was directly injected into the device, followed by the luminescence detection using the microplate reader. The device may be reused in some cases after appropriate washing, though we generally used each well only once.

3 Results and discussion

3.1 Membrane

The membrane in the device served two functions. One was to retain proteins synthesized, as well as all protein synthesis machinery including ribosome, polymerases, and the likes. The other function was to allow the transfer of additional reactants from the feeding chamber to the reaction chamber, and the transfer of the reaction byproducts in the reaction chamber to the feeding chamber. The transfers were due primarily to diffusion resulting from the concentration

gradient across the membrane [15]. Both transfers of the additional reactants into the reaction chamber and of the byproducts out of the reaction chamber would shift the equilibrium position of the reactions, prolonging the reaction time and enhancing the synthesis yield. As a result, membrane played a significant role in CFPS.

We fabricated devices using different types of membranes and studied their effects on protein synthesis yield. Table 1 lists the sources of the membranes, the type of materials and molecular cutoff according to the manufactures, their shapes and thickness, and the adhesives we used in microstamping technique [18]. Thickness of each membrane was measured as described in the Section 2.

The effect of the membrane on the synthesis yield of luciferase is shown in Fig. 2. The amount of luciferase synthesized is represented by the luminescence signal that was detected after adding luciferase assay reagents. The optimum membrane cutoff value in CFPS is in the range of 8 kDa–14 kDa according to the literature [5, 19, 20]. All membranes we used were within this range except for the one from GE. As a result, the polycarbonate membrane from GE with a cutoff of 20 kDa was expected to produce lower protein synthesis yield, because its larger pores were not able to maintain the concentrations of various components at an optimum. This also explains that the Millipore membrane (10 kDa cutoff) produced more proteins than the GE membrane although its membrane thickness was $\sim 40\times$ greater. It was surprising to find out that two membranes from Spectrum yielded different protein amounts even though they were the same type of materials with the same molecular cutoff (6–8 kDa). Their difference in the shape (one in sheet, 240 mm × 240 mm, and the other in disk, 33 mm diameter) should not impact protein synthesis. We then measured their membrane thickness and found out that the sheet membrane was about twice thicker than the disk membrane due to the requirement of the sheet to be sturdy. According to Darcy's law, the flux of a chemical across a membrane is inversely proportional to the thickness of the membrane [21], which explains the difference in the synthesis yield. However, the osmotic pressure difference on two sides of the membrane is time-dependent, thus a simple linear relationship between the yield and the membrane thickness was not expected. Among these four membranes tested, we concluded that Spectrum membrane #1 in the disk shape gave highest protein synthesis yield. The experiments discussed below were carried out in the devices using this type of membrane.

3.2 Volume ratio between the reaction and feeding solutions

One of the key advantages of miniaturization is to lower reagent consumption and accordingly achieve significant cost saving. The volume of the reaction solution is generally recommended to be 1 mL in commercially available RTS 500 kit [22]. When the reaction solution is reduced to 10 μL in our device, 2 orders of magnitude reduction in the reagent consumption is achieved. In other words, reagents for one reaction in a conventional container can be used for 100 types of reactions under different conditions. Considering the cost of one RTS 500 kit is more than $2000, the cost saving would be substantial when a high-throughput application is implemented.

The commercial CFPS kits were developed for macroscale protein synthesis. The recommended volume ratio of the reaction solution to the feeding solution is 1:10. It is possible that the ratio of the reaction solution and feeding solution is not at optimum when CFPS is operated in a miniaturized device. In the literature, the volume ratio ranged from 1:5 to 1:100, depending on the system and the setup [23]. Therefore, we varied the amount of the feeding solution while keeping the constant all of the other parameters, including the amount of the reaction solution. When the amount of the feeding solution

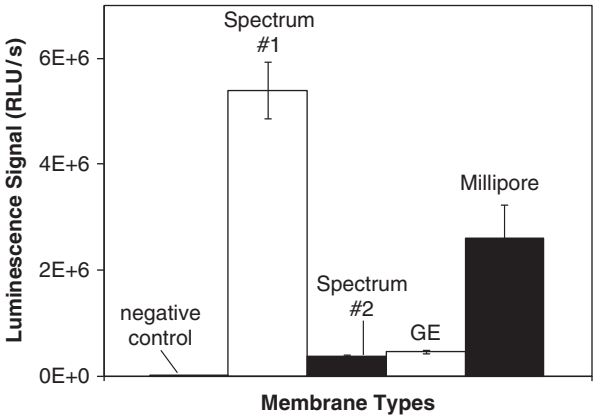

Figure 2. Effects of membrane on protein synthesis yield in the CFPS devices. The properties of each membrane are listed in Table 1. Error bars represent an average obtained from three repeat experiments. Each error bar indicates one standard deviation.

Table 1. Sources and properties of membranes used in this work

Sources	Materials	Cutoff	Shape	Thickness	Adhesives[a)]
Spectrum (#1)	Regenerated cellulose	6–8 kDa	Disk (33 mm D)	42–48 μm	PDMS
Spectrum (#2)	Regenerated cellulose	6–8 kDa	Sheet	76 μm	PDMS
Millipore	Regenerated cellulose with differentiated filtration	10 kDa	Disk (150 mm D)	235 μm	Epoxy
GE	Polycarbonate	20 kDa	Sheet	6 μm	PDMS

D: the diameter of the disk shape membranes.

a) The adhesives refer to what was used for bonding the membrane to the top and bottom layers using microstamping technique.

changed, however, the solution level of the feeding solution would have increased if the same device was used. A higher solution level of the feeding solution would have increased the diffusion rate of the components from the feeding chamber to the reaction chamber due to the hydrodynamic pressure difference between the solutions in two chambers. As a result, the experiment results would have been distorted.

To keep the solution level the same while varying the volume of the feeding solution, we fabricated a variety of devices with the same device design except for the depth of the feeding chamber in the bottom layer. The required feeding chamber depth was calculated by the volume variation when the horizontal cross-sectional areas of the access holes and the feeding chamber were constant.

Figure 3 shows the result obtained when the amount of feeding solution varied while the volume of the reaction solution was fixed at 10 μL. A linear relationship was not expected since (i) more feeding solution would prolong the reaction time but our synthesis time was fixed and (ii) the device geometry was altered due to the requirement to maintain the solution level at both reaction and feeding chambers when a large volume of the feeding solution was used. It is clear that the volume of the feeding solution should be at or greater than 200 μL, which would result in the maximum protein synthesis yield. The result also indicates that the amount of feeding solution more than 200 μL did not have a significant effect on the amount of protein synthesized. As a result, the optimum volume ratio between the reaction solution and the feeding solution for our device is 1:20, which was used in the rest of the experiments.

3.3 Size of exchange interface

The size of the membrane interface between the reaction chamber and the feeding chamber affects the exchange rate between the components in two chambers, thus having a significant effect on protein synthesis yield. The size of the exchange interface is determined by the diameter of the reaction chamber. As a result, we fabricated a variety of devices with different interface sizes and investigated their effects on the protein synthesis yield. While the size of the interface varied, all other parameters remained constant, including the amount of the reaction solution and feeding solution, and the volume of each chamber. However, a change of the interface size (i.e. diameter of the reaction chamber) would result in a variation in the solution level of the reaction solution. Again, we changed the depth of the feeding chamber to maintain the same solution level between the reaction solution and the feeding solution (to avoid any effect due to hydrostatic pressure). The size of the membrane interface size and the corresponding depth of the feeding chamber are listed in Fig. 4A.

Figure 4B shows the luminescence signal from luciferase synthesized as a function of the membrane interface size. The luminescence signal in the *y*-axis is plotted in the log scale. The result indicates larger amount of luciferase was synthesized when the interface size area was increased. As expected, the greater the membrane interface size, the more efficient the reagent transfer, the higher the protein synthesis yield.

As a result, we chose the maximum interface area possibly allowed by the geometric constraints in a 96-unit device. Note that the interface size cannot be infinitely enlarged since the reaction solution must cover all areas of the membrane

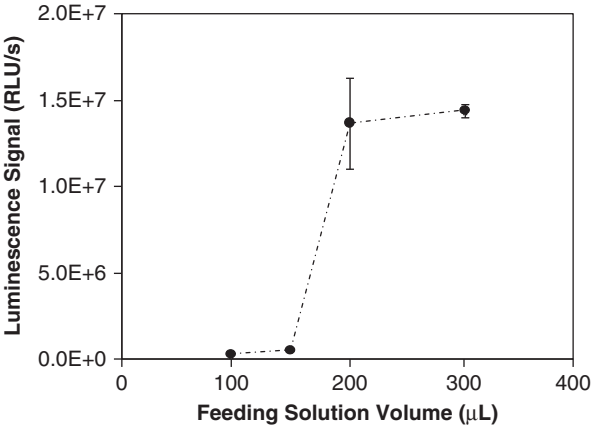

Figure 3. Effects of the volume ratio of the feeding solution to the reaction solution on protein synthesis yield in the CFPS devices. The volume of the feeding solution is indicated in the *x*-axis while the reaction solution was fixed at 10 μL.

A

Interface size (mm^2)	4.95	5.66	6.63	7.78	9.19
Feeding chamber depth (mm)	0.91	1.08	1.26	1.4	1.54

B

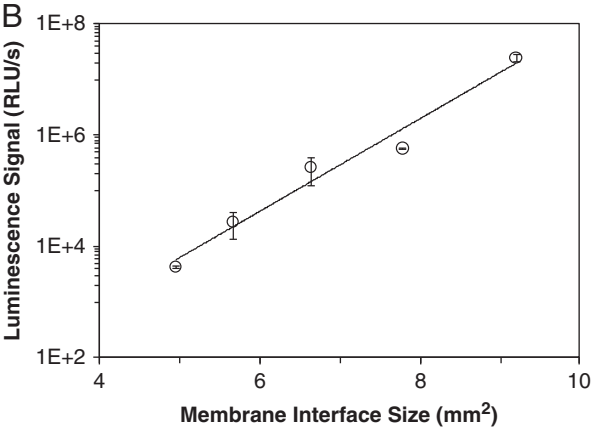

Figure 4. (A) The membrane interface size and the corresponding depth of the feeding chamber in the bottom layer to maintain the same solution level between the reaction and feeding chambers. (B) Effects of the membrane interface size on protein synthesis yield in the CFPS devices. The luminescence signal in the *y*-axis is in log scale. Error bars represent an average obtained from three repeat experiments. Each error bar indicates one standard deviation.

interface in order to have an effect. Also the overly increase in surface area could make the device more susceptible to evaporation, especially in a miniaturized device.

3.4 Protein synthesis

After completing design optimization, a 96-unit device was developed and fabricated (Fig. 1C). The ability of the device for continuous-exchange CFPS was verified by pipetting 10 μL of the reaction solution into the reaction chamber and 200 μL of the feeding solution to the feeding chamber through the access holes. Two control experiments were simultaneously performed. One is to carry out protein synthesis using only 10 μL of the reaction solution in a conventional reaction vessel (e.g. microplate well or microcentrifugal tube). This control will verify the importance of having additional reactants (i.e. nutrients and energy components). The other control is to combine 10 μL of the reaction solution with 200 μL of the feeding solution, and then use the mixture to synthesize luciferase in a conventional reaction vessel. This second control will tell the importance of supplying the feeding solution in a controlled manner through a membrane and microchannel. Simply adding more reactants into the reaction solution does not significantly enhance the protein synthesis yield.

Table 2 lists the luminescence signal obtained from each experimental setup. The net signal for luciferase should be the signal obtained in each setup minus the background signal of the negative control. The results show that the protein synthesis yield was increased 62× in the device when it was compared with that when only the reaction solution was used. When it was compared with the setup in which the feeding solution was added to the reaction solution before the experiment and then protein synthesis was carried out, the production yield was still increased 34×, indicating the necessity of the miniaturized device with a membrane and microchannel. Such a design allowed continuous supply of the reactants and removal of the byproducts, rather than diluting critical components (e.g. polymerase) when the feeding solution was pre-mixed into the reaction solution. Note that the optimized device design showed about 25× improvement over the device reported previously without optimization [16].

Table 2. Comparison between a miniaturized CFPS device and a conventional microplate.

Experimental setups	Luminescence signal (RLU/s)	Signal percentage (%)
Reaction solution+feeding solution in the device	$(1.37 \pm 0.31) \times 10^7$	100
Reaction solution+feeding solution pre-mixed	$(4.05 \pm 0.04) \times 10^4$	3.0
Reaction solution only	$(2.23 \pm 0.40) \times 10^4$	1.6
Negative control	$(4.40 \pm 0.10) \times 10^2$	–

We also evaluated the adaptability of the device into commercially available microplate readers. Although the reader from Berthold Technologies has a ''height adjustment'' option, we did not use it since our device fitted into the reader very well. As a result, the device is expected to be compatible with a wider range of commercial microplate readers. We also tested the reagent dispensing into 96-units of the device. The dispensing unit connected to the microplate reader was used to inject 20 μL of water in the reaction chamber of the device in the same manner when an equal amount of water was dispensed in the same location in a 384-well microplate. The amount of water injected in each of the wells in the device was compared with those in the corresponding location of the commercial 384-well microplate. No statistically significant difference was found between them.

In addition to luciferase, we also synthesized green fluorescent protein (GFP). Furthermore, we purified the synthesized GFP by adding 5 μL of nickel-nitrilotriacetic acid (Ni-NTA) agarose magnetic beads (Qiagen) into the expression product. The six-histidine tag incorporated in GFP could interact with nickel ions hence GFP was extracted out from the solution by magnetic separation [24]. After washing, the retrieved GFP was verified by the observance of its unique fluorescence signal that indicates the synthesized proteins have the correct three-dimensional conformation.

To illustrate high-throughput protein expression, we simultaneously synthesized several proteins and assayed them in the 96-unit device as shown in Fig. 5. Each unit of rows A–H in the device was used for one protein expression condition. Rows A–F was designated for producing GFP, luciferase, β-glucoronidase (GUS), β-galactosidase (lacZ), alkaline phosphatase (AP), and β-lactamase (β-lac), respectively. Row G was used for co-expression of all of these six proteins. Row H functioned as a negative control, which contained no DNA vectors. At the end of protein expression, appropriate assay reagents were added according to columns

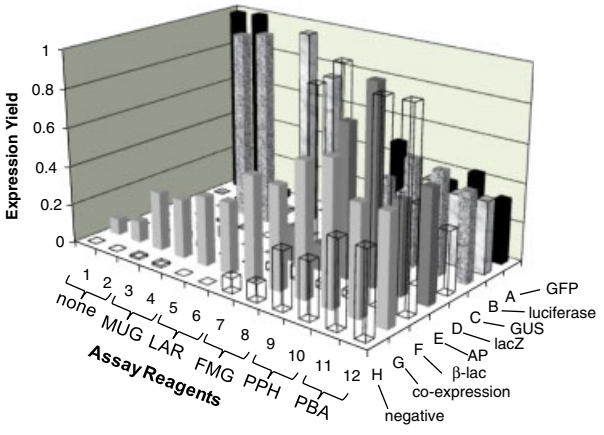

Figure 5. Simultaneous expression and detection of six proteins in a 96-unit device. Proteins were GFP, luciferase, GUS), lacZ, AP, and β-lac. The assay reagents for detecting the proteins expressed were MUG, LAR, FMG, PP, and PBA. The protein expression yields were normalized against the highest signal.

1–12 and the expression yield in each unit was then measured. Adjacent two columns were subjected to the same assay to enhance the precision. Direct fluorescence measurement at 535 nm was performed in the units of columns 1 and 2, designed for measuring GFP. To all units in columns 3 and 4, a solution of 4-methylumbelliferyl β-D-glucuronide (MUG) was added and fluorescence at 460 nm will be detected if GUS is present. Luciferase assay reagent (LAR) was introduced into the units in columns 5 and 6 and luminescence was measured for presence of luciferase. Columns 7 and 8 were designated to assay lacZ by introducing fluorescein mono-β-D-galactopyranoside (FMG) into the reaction chambers. Columns 9 and 10 were used for measuring AP with 3-phenylumbelliferone 7-*O*-phosphate hemipyridinium (PPH) as the assay substrate. The activity of β-lac was measured in the units of columns 11 and 12 using *m*-[[(phenylacetyl)glycyl]oxy]benzoic acid (PBA). The results suggest that the device enables high-throughput cell-free protein synthesis, thus having a potential to be used for high-throughput proteomics applications.

4 Concluding remarks

We have designed, fabricated, and tested a miniaturized array device for cell-free protein synthesis. The CFPS device reduced the reagent consumption by two orders of magnitude, thus significantly reducing the cost when a high-throughput CFPS-based assay is performed. The protein synthesis yield was enhanced by incorporating a nanoporous membrane and a microchannel, which prolonged reactions through replenishment of reactants and removal of byproducts through continuous exchange with a feeding solution.

We experimentally determined the optimal design for the miniaturized CFPS device. We found out that one of four membranes offered the highest protein synthesis yield. Within certain limits, the greater the membrane interface size, the better the protein synthesis yield. We also discovered that the optimum volume ratio between the reaction solution and the feeding solution is 1:20 for the device. At this ratio, the maximum protein expression yield and the minimum reagent consumption could be obtained simultaneously.

We confirmed that the device ameliorated protein synthesis yield over a conventional microplate. An improvement of 62-fold in the amount of synthesized luciferase was obtained in the device due to the incorporation of a nanoporous membrane and microchannel. We also verified its compatibility with commercial dispensing apparatuses and detection systems. Therefore, the device has a potential to be used in high-throughput applications such as drug screening, toxin detection, and enzyme screening.

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The authors have declared no conflict of interest.

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