

Project Title:

Open-Cell: An Open-Source 3D-Printable Device for High-Throughput Cell-Free Protein Synthesis

Report Title:

Progress on fabrication of the 3D-printable device

Summary

The aim of this project was to develop a robust 3D-printable device that could be used to increase the throughput of protein synthesis and purification via CFPS cell-free protein synthesis (CPS) and affinity chromatography, respectively. The device is composed of a CPS reactor that translates an input sequence of DNA into the corresponding protein, which is subsequently purified from the cell-free extract through a downstream affinity chromatography column (Fig. 1). It was designed to fit within a standard 96-well microplate footprint that could integrate into existing instruments and workflows. We have completed a prototype and conducted preliminary tests on liquid flow through the prototype. This has allowed us to identify aspects of the initial design that require improvement, such as the valve system employed to regulate flow through the device. We are currently in the process of implementing these improvements and designing experiments to further test functionality of the device.

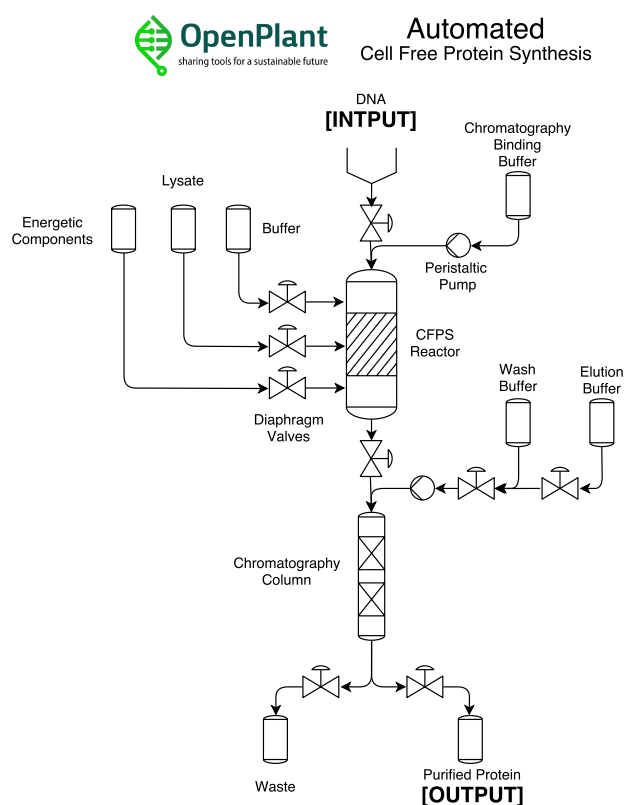


Figure 1: Process flow diagram illustrating basic design of the device. The upper section combines the components required for CFPS in a single reaction chamber. The lower section purifies newly synthesized protein using chromatography. 96 of these are multiplexed within a standard microplate footprint.

Report and outcomes

The initial design specification for our device called for the multiplexing of 96 dual-stage reactors - each of which was composed of a CPS module and a protein purification module – within a 9 mm x 9 mm x 9 mm space (Fig. 2). The system was designed so that the flow of input buffers and reagents required for these modules could be regulated by an external peristaltic pump.

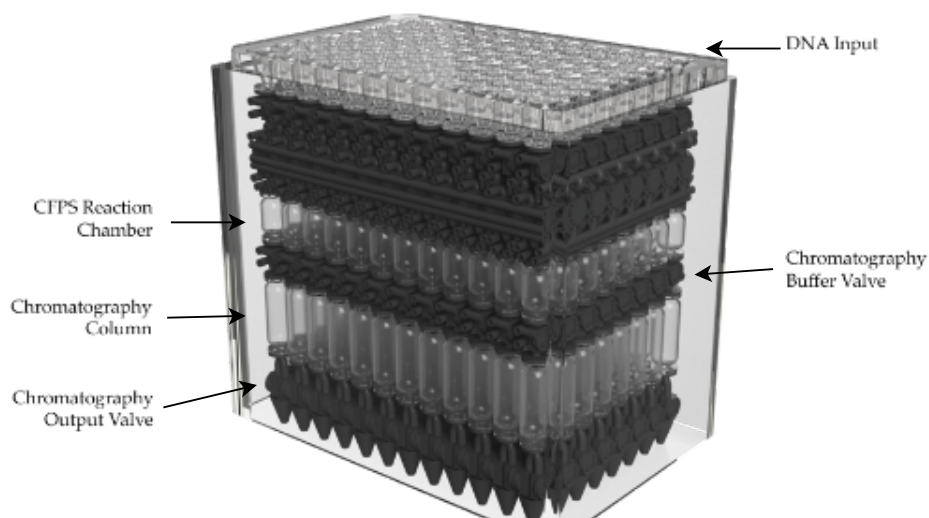


Figure 2: Diagram illustrating 3D design of the device.

The device was printed in several stages using the Formlabs Form 2 UV photolithographic printer. The initial print demonstrated that the diaphragm valve design we employed to control liquid flow worked (Fig. 3A), however, the flexible resin material lacked structural integrity and developed cracks after several days of use (Fig. 3B). As a result, we increased thickness of the tubing, as well as the number and size of support structures. This increased the time until cracks developed but did not completely prevent their occurrence.

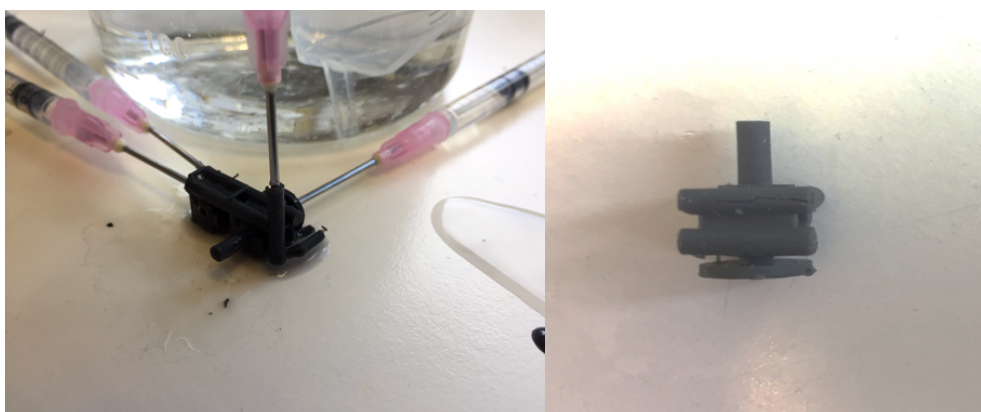


Figure 3: Pressure testing of an individual valve (left) and cracks in the walls of the valve after several days of testing (right).

We are therefore aiming to re-implement the soft valves using a different material. There are several designs reported in the literature that report success using a material similar to the Formlabs Clear Resin – we are currently working towards optimizing the valve using this type of resin. We have also initiated a collaboration with a second OpenPlant team to investigate open-source materials for implementing the chromatography column in the protein purification module of the device. Work on the protein purification module can be decoupled from work on the CFPS and conducted in parallel, so that development of the former will not be affected by the challenges we have faced with the valve design.

Changes to team

There have not been any changes to the team, however, as mentioned above we will be collaborating with another OpenPlant team in the next round of projects. The team in question is composed of Jenny Molloy, Quentin Dudley, and Harry Akligoh.

Expenditure

Since we anticipate that most of the cost associated with this project will be associated with the biological reagents, we have been conservative regarding the supplies for the manufacture and testing of the device. Our expenditure so far is summarised in the table below.

Item	Quantity	Item price (GBP)	Cost (GBP)
Form 2 Resin Tank	2	50.00	100.00
Form 2 Clear Resin Cartridge V4 (1L)	1	120.00	120.00
Form 2 Flexible Resin Cartridge V2 (1L)	1	160.00	160.00
Raspberry Pi 3B+	1	33.77	33.77
Total			413.77

Would you like to claim the £1,000 follow-on fund?

Yes, we would like to claim the £1,000 follow-on fund to be used as described below.

Follow on Plans

Once the new valve design has been optimised and the device is operational with CFPS buffer substitutes like water and glycerol, we will test the device by synthesizing common reporter proteins such as GFP and – in collaboration with our collaborators mentioned above the other team – purify them using a variety of different chromatography column materials. Once this has been carried out, we plan to publish the CAD design files to the DocuBricks site and the code required to operate the pumping mechanism to an open repository on GitHub, both under permissive licenses.