Adapting Cell-Free Protein Synthesis as a Platform Technology for Education

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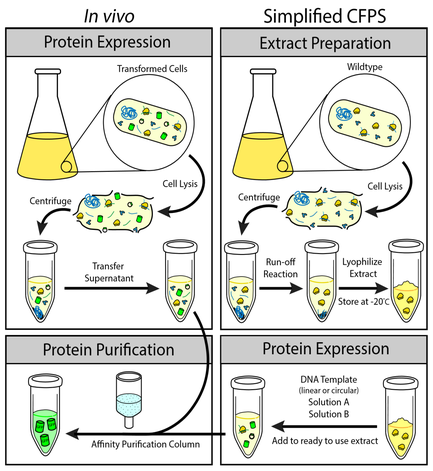
The Biochemistry of Life

The processes of transcription and translation are essential to all living organisms as they convert information stored in DNA into functions executed by proteins. Most biotechnologies that leverage protein synthesis rely on living cells to biosynthesize proteins of interest for industrial and medical applications. Here we present a method for harnessing protein synthesis in a test tube without a living cell, through an emergent technology called Cell-Free Protein Synthesis (CFPS). We also present our efforts to use this platform technology for biochemical education.

Why Cell-Free Protein Synthesis?



Graphic developed by Wesley Kao and Nicole Gregorio

The advantages of CFPS technology include:

1. Direct manipulation of the environment of protein production
2. Removes the need to keep the cell alive
3. Total energy of the system is solely used for the production of a single protein product.

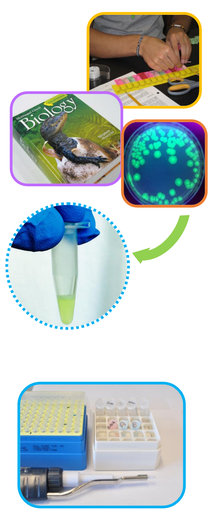
Our work aims to contribute additional advantages of CFPS including

1. Improving access for classroom use through addressing the cost of reaction components.

Approach: The energy source, PEP, is the most expensive reagent, contributing more than 16% of total costs per CFPS reaction. This project aims to lower costs by reformulating and optimizing the energy system to decrease the cost per protein yield.

Protein Synthesis Classroom Kit

While chemistry and physics often have hands-on science kits, there are few biology kits due to the cost of expensive equipment to keep living organisms viable, potential hazards, complexity of reaction set-up, and expenses of reagents. Options for teaching protein synthesis include animations, interactive computer or paper-based games and models. However, these do not allow for direct manipulation of transcription and translation.



Adapting CFPS for the classroom provides students with the opportunity to access these cellular processes directly for hands-on experimentation. Students may also engage in experimental design through a biochemical engineering approach.

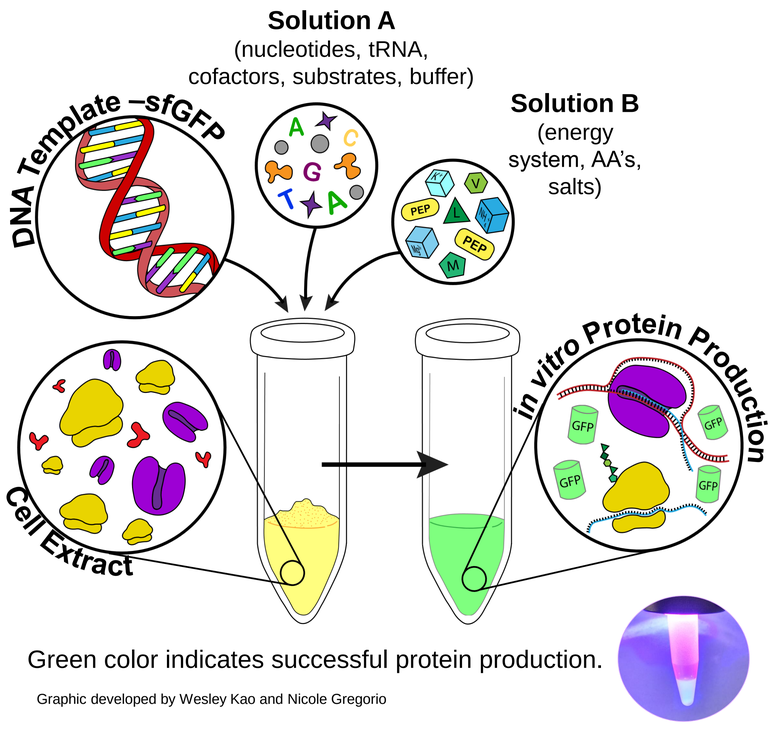
Method

Cell Growth Day 1	• Streak a plate with E. coli BL21*(DE3) • Grow one colony in LB broth overnight • Prepare growth media: 2xYTPG
Cell Growth Day 2	• Inoculate media with overnight culture • Monitor cell growth • Centrifuge, wash, and pellet
Cell lysis	• Deliver ~850 J of energy via sonication to effectively lyse 1.4 mL of cell culture
Purification of cell extract	• Centrifuge and remove pellet • Run-off reaction • Flash-freeze and store in -80C freezer
CFPS Reaction	• Add reaction components (below) • Reactions are set up in quadruplicates and incubated at 37℃ for at least 3 hours
Quantification and analysis	• Quantify green fluorescent protein (GFP) using computer software and standard curve. Samples are run in triplicates

Growth Media: 2xYTP

Add additive Replace PEP with 3-PGA

Reaction Setup



Green color indicates successful protein production.

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References & Acknowledgements

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Results

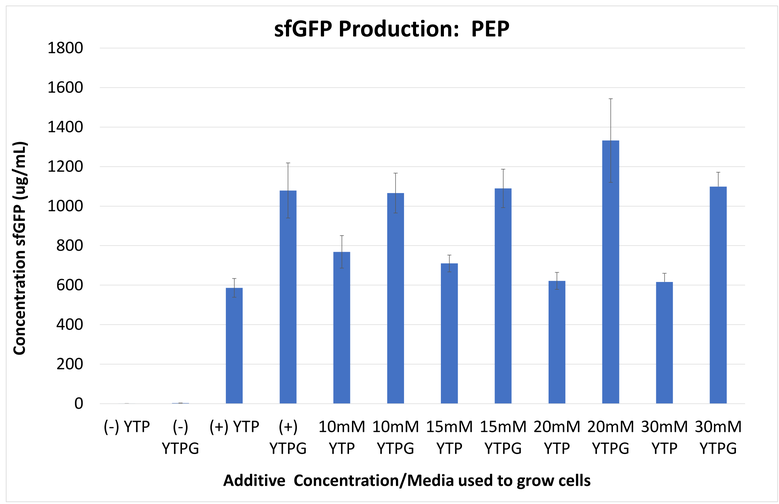

Figure 1: Protein yields of CFPS reactions in the PEP energy system. An additive was added to CFPS reactions with extract grown in two media, 2xYTP and 2xYTPG. Additive concentrations of 10-30mM does not have a significant effect on 2xYTPG for high-performing extracts. For 2xYTP, the optimal additive concentration appears to be 10mM. Future experiments will need to be done to confirm these findings.

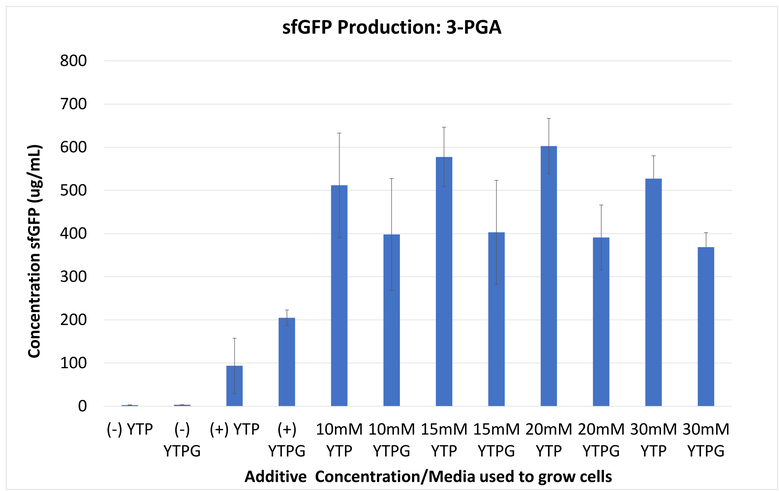

Figure 2: Protein yields of CFPS reactions. The energy source, PEP, was replaced with 3-PGA. The same additive from the previous experiment was added to the CFPS reaction with extract grown in two media, 2xYTP and 2xYTPG. The additive coupled with 3-PGA boosted protein yields. There may be an optimal additive concentration of 20mM for 2xYTP. Future experiments will need to be done with greater accuracy to confirm these findings.

Cost Breakdown

One 15µL reaction of the traditional CFPS (PEP + glucose) costs 26¢
One 15µL reaction of reformulated CFPS (3-PGA + additive) costs 22.897¢
Reformulated reaction
(-) PEP at a concentration of 33mM: 3.09¢
(-) Glucose: 0.2175¢
(+) 3-PGA at a concentration 2.43mM: 0.244¢
(+) Additive at 20mM: 0.000000381¢

Cost per µL of reaction:
- PEP + glucose system : 1.728¢
- 3-PGA + additive system: 1.524¢

Cost per µg sfGFP produced:
- PEP + glucose (1079 µg/mL): 1.604¢
- 3-PGA + 20mM additive (600 µg/mL): 2.54¢

A lower cost per protein yield is ideal for reducing costs without sacrificing efficiency.

Conclusions

I was able to achieve a working reaction in reformulating the CFPS reaction to replace PEP with 3-PGA coupled with an additive. Although protein yield of the 3-PGA + additive system was less than the PEP + additive system, the additive played a larger role in increasing protein yield in the 3-PGA system. 3-PGA coupled with the additive worked better for the cell extract grown on 2xYTP compared to 2xYTPG. The reformulated CFPS reaction costs less than the traditional CFPS reaction; however, the cost per protein yield was less than the traditional CFPS. It is important to note that protein yield was remarkably high for the traditional reaction, which contributed to lower cost per protein yield than the otherwise comparable 3-PGA + additive system. To be more useful for CFPS, further optimization is needed to produce higher GFP a at lower cost.