Engineering Recombinant Proteins for Surface Presentation in Synthetic Cells

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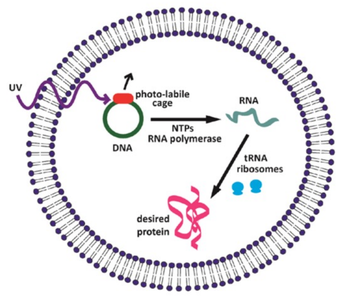
Introduction

Cells are a valuable tool for medical use because of their ability to detect changes in their environment and produce specific molecules. However, their range of functions is specific to their survival needs and thus is often not useful for our medical needs. Synthetic cells, on the other hand, can be modified in numerous ways and thus pose as an attractive alternative to native cells.

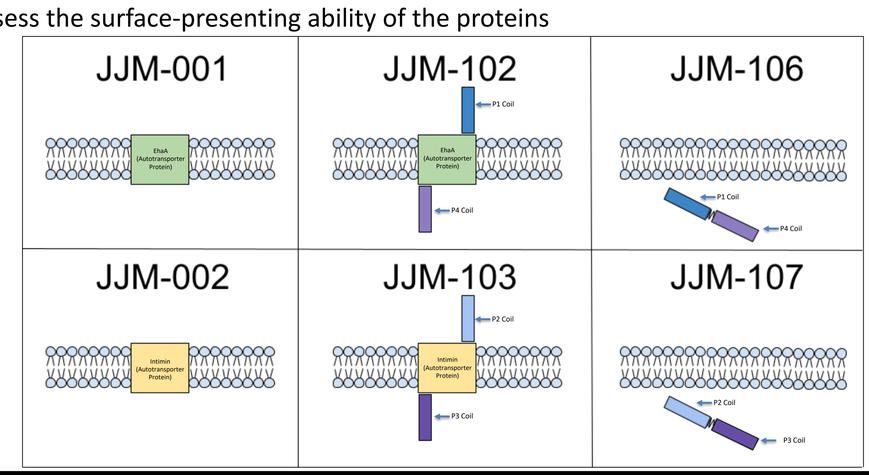


Synthetic Cell Model
[1] Schroeder A, et al. 2012

Protein expression in response to a stimulus, such as light, has been demonstrated in synthetic cells already[1], however for therapeutic applications, environment-responsive protein synthesis is desirable. We hypothesize that a synthetic cell decorated with surface proteins can be used to monitor and respond to a cell's surroundings. Our experiments were performed in live *E. coli* cells, which can act as a proxy for synthetic cells.

Objectives

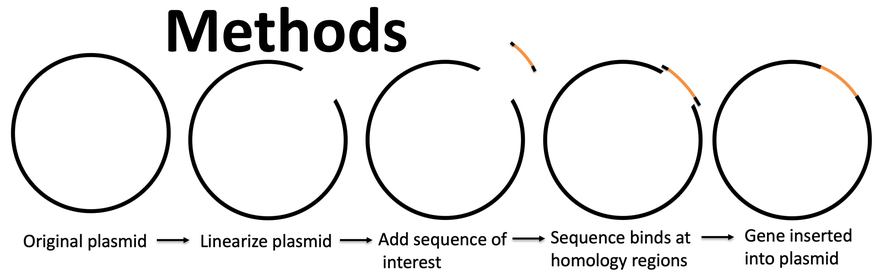
1) Engineer recombinant proteins for the display of docking coils outside the cell membrane
2) Assess the surface-presenting ability of the proteins



Methods

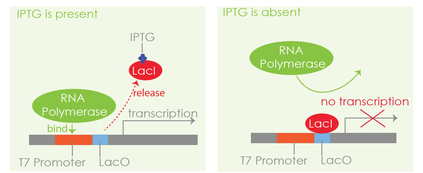
Gibson Cloning

Method of inserting a DNA sequence into a plasmid



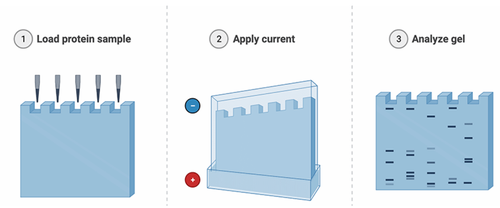
Protein Induction

Isopropyl ß-D-1-thiogalactopyranoside (IPTG) releases LacI repressors from the DNA, which allows RNA polymerase to bind to the promoter region and begin transcription. **This is our stimulus.**



Characterization

SDS-PAGE
Assesses proteins by their **molecular weight**.



Antibody tagging, flow cytometry & plate reader
The cells were exposed to Anti-His tag Alexa Fluor 488 antibodies, which would attach uniquely to specific sites on our protein (His-tags).

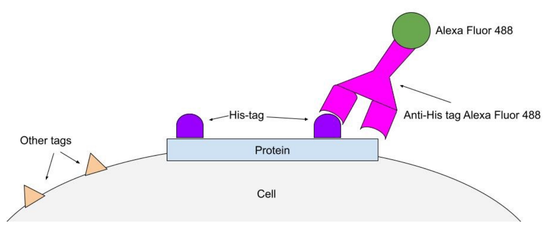
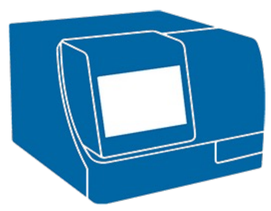
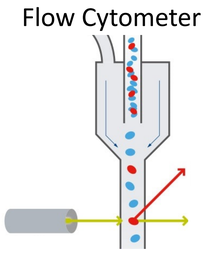


Plate Reader



Measures a population at once

Flow Cytometer



Measures a population of cells individually

The plate reader and flow cytometer measure the fluorescence of the cells which indicates whether the antibodies have docked, and thus if the protein is present. This assesses the protein's **function**.

By assessing two orthogonal properties – molecular weight and function – we can reduce the chances of a false positive result.

Results

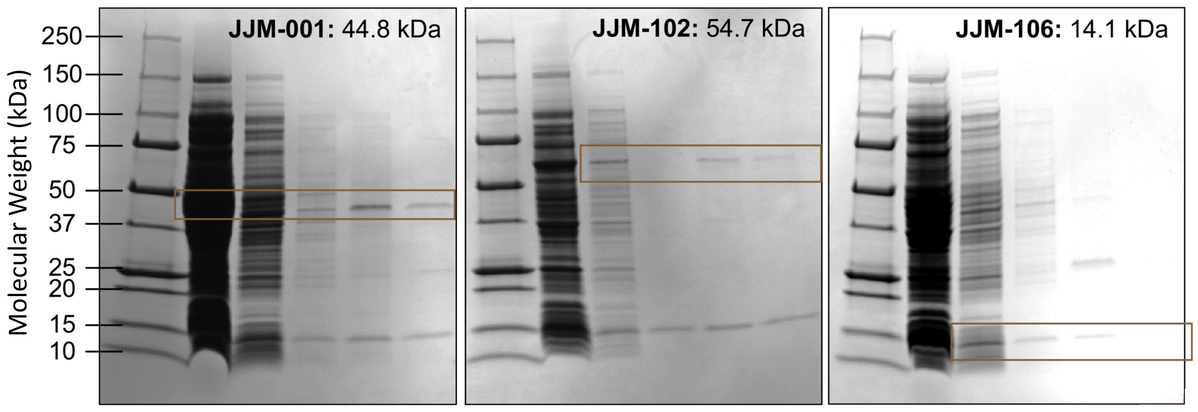


Figure 1: SDS-PAGE gels. The leftmost well contains a ladder, which produces bands at known molecular weights in the gel, and the other wells contain protein samples.

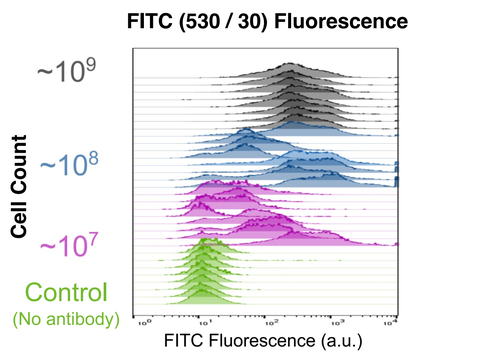


Figure 2: Method development for flow cytometer induction assay. Each sample was induced and had varying cell counts. Quantified 10^9 cells per sample as a lower bound for the cell density required for a reproducible result.

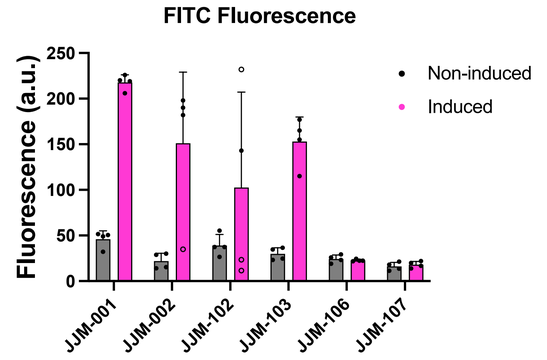


Figure 3: Flow cytometry data of the induced and non-induced samples using the FITC laser. Samples with less than 10^9 cells are marked with an open circle.

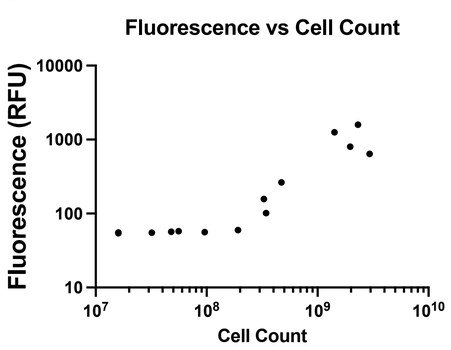


Figure 4: Method development for plate reader induction assay. Each sample was induced and fluorescence measurements came from the plate reader. Quantified $2*10^8$ cells per sample as a lower bound for the cell density required for a reading in the curve's linear region.

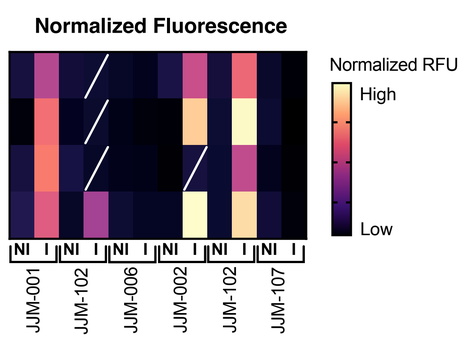


Figure 5: Plate reader fluorescence measurements of induced (I) and non-induced (NI) samples, normalized by cell count. Each column contains the same types of cells; the experiment was designed in quadruplicate. Samples with less than $2*10^8$ cells are slashed out.

Discussion

Since we know the DNA sequence that encodes the proteins, we can predict the molecular weights of our proteins:

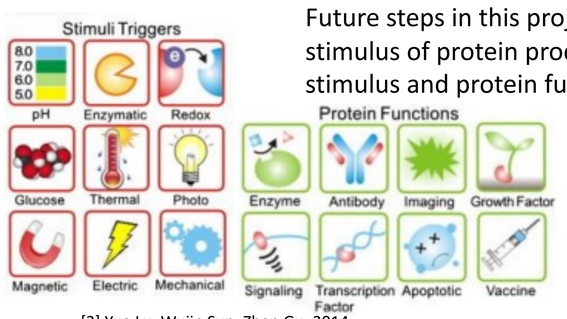
JJM-001 – 44.8 kDa
JJM-102 – 54.7 kDa
JJM-106 – 14.1 kDa

In Figure 1, bands are visible at all of these locations in the gel, which confirms that there is a protein in the cell that matches the weight of our proteins.

In Figure 3, there is an increase in the fluorescence of the induced samples versus non-induced samples for plasmids JJM-001, -002, -102, and -103, which demonstrates that our protein is present on the outer membrane. Plasmids JJM-106 and -107 were negative controls because the proteins were not equipped with an autotransporter, so they were not surface presenting. As a result, the antibodies could not attach.

Figure 5 corroborates the data from Figure 3.

These results demonstrate that our recombinant proteins were expressed and shuttled to the cell's surface in response to the IPTG. Additionally, the variety of proteins we were able to produce reflects that we can modify our proteins for different applications while still maintaining their surface presentation.



[2] Yue Lu, Wujin Sun, Zhen Gu, 2014

Future steps in this project could come from varying either the stimulus of protein production or the protein's function. The stimulus and protein function in our plasmids are currently not very applicable to use in medicine, however, using cell specific markers as a stimulus, for example, or making the protein enzymatically active will have more applications in medicine.

References

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Multi-Mode Microplate Reader [Infographic]. (n.d.). https://www.moleculardevices.com/themes/moldev/images/multi-mode-microplate-reader.png

SDS-PAGE workflow [Infographic]. (n.d.). https://www.rockland.com/globalassets/globalpics/resources-pics/sds-page.png

Acknowledgments

I would like to thank my mentor, Juan Jaramillo Montezco, for the guidance and insight he has provided me, as well as Professor Grinstaff and Professor Green for giving me the opportunity to work in their labs and for the support they have provided.