BioBots: The Next Generation of Biosensors

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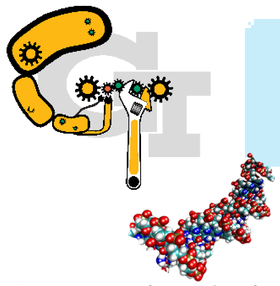


GOAL

Our team goal is to develop novel bacterial BioBots that can not only sense but also interact with the extracellular environment.

Mammalian cells communicate with the extracellular matrix (ECM) using heterodimeric cell surface receptors, integrins. They can signal in a bi-directional manner between the cell interior and ECM.

We aimed to express the αIIbβ3 integrin in E. coli cells. To promote dimerization of the integrin subunits, we attempted to optimize bimolecular fluorescence complementation of split GFP using surface display technologies.

To verify αIIbβ3 function, we developed an integrin activity sensor consisting of the ligand derived from fibrinogen (KQAGDV) coupled to GFP.

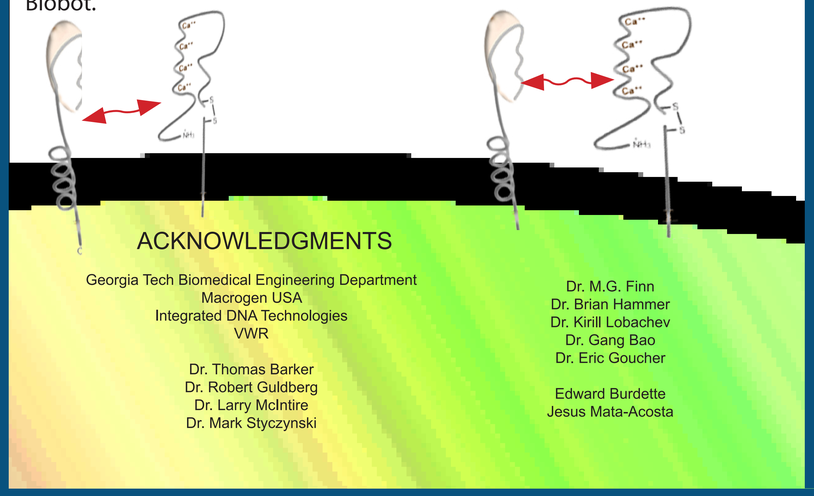
Finally, we created a new standard for RBS addition that inserts a strong RBS (BBa_B0034) in front of any standard BioBrick part beginning with ATG. This method for addition is efficient and more successful than the standard procedure 3A/standard assembly.

INTRODUCTION

Cells perform their intended functions not individually but collectively. They do that by forming temporally evolving, 3D structures comprised of clusters of cells, and by forming active or passive cell to cell and cell-ECM interactions. Thus, the development of engineered, integrative cellular systems that self-heal and adapt to a variety of stimuli in the surrounding micro environment will revolutionize the way bioengineers design. Multi-cellular biological machines can be engineered to have desired functionality and perform prescribed tasks.

Fortunately, nature provides plenty of inspiration. One of the tools that eukaryotic cells have to detect their surroundings are heterodimeric sensor molecules, known as integrins.

Not only do integrins receive and facilitate cellular integration of extracellular cues, but they also support inside-out signaling; therefore they can dynamically impact their micro environment. Integrin's critical role in this cell-ECM "dynamic reciprocity" represent the ideal sensor to be built into a Biobot.



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PET-mCHERRY

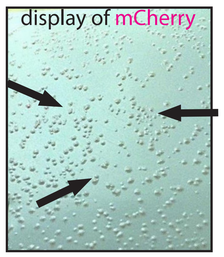
In 2012 the Georgia Tech iGEM team developed a novel biosensor ---Split-GFP. From this project, we began to think how we could develop more complex sensing technology in bacteria. Taking into consideration how mammalian cells sense and react to their environment, we asked the question:

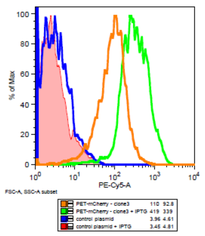
Can bacteria express human integrins?

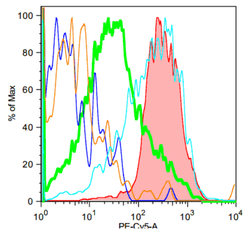
To start answering this question, we needed to find a way to transport large proteins and anchor them to the outside of the bacteria cell.

Autodisplay technology seemed like one possible solution to this problem. The PET β-barrel that's native in E.Coli, was looked at as a candidate that could successfully transport and translocate the RFP, mCherry.

We wanted to design and test this construct to determine whether or not PET β-barrel would be a good candidate for integrin transport.

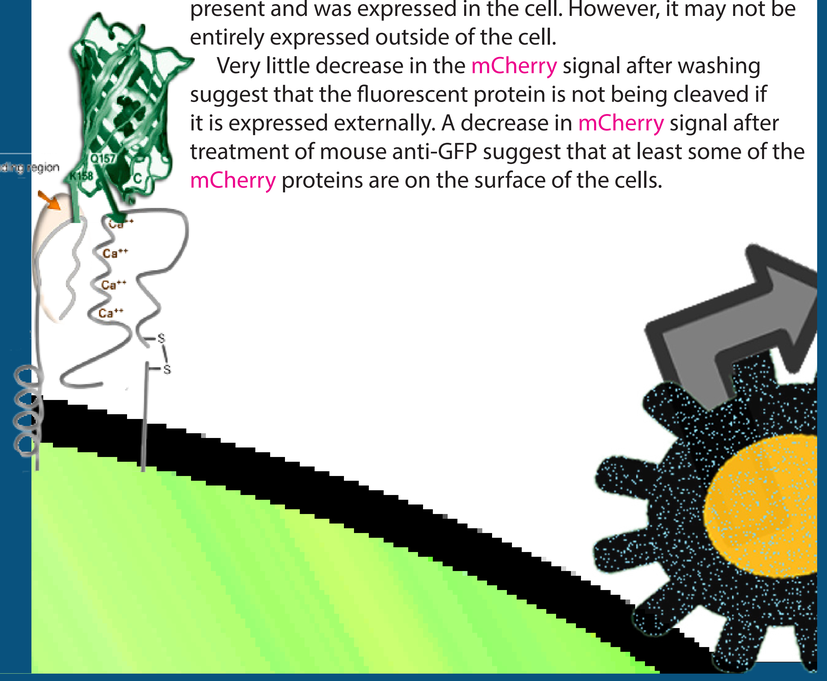




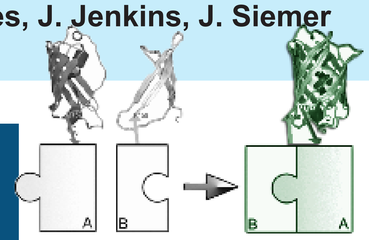


The flow cytometry results conclude that mCherry was present and was expressed in the cell. However, it may not be entirely expressed outside of the cell.

Very little decrease in the mCherry signal after washing suggest that the fluorescent protein is not being cleaved if it is expressed externally. A decrease in mCherry signal after treatment of mouse anti-GFP suggest that at least some of the mCherry proteins are on the surface of the cells.



SPLIT-GFP OPTIMIZATION



We optimized the split GFP project that was left behind by the last year's Georgia Tech iGEM team.

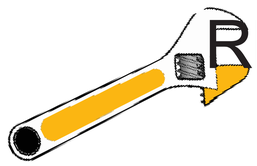
Split-GFP is a green fluorescent protein being split into two subunits. When close enough, the subunits will interact to produce GFP. The two subunits can be attached to the subunits of another part. Then, the fusing of GFP subunits makes it possible to quantify the successful dimerization of the new part.

According to other iGEM teams, split-GFP, however, wasn't functioning the way it's supposed to. As a result, we decided to restore this part into functioning order.

From sequencing result, the part was not in standard 10 format. It contained an EcoR I restriction site in the middle of the part sequence, and it did not contain a start codon.

We were able to resolve the problems with the sequence and add a promoter, operator, and RBS to the construct for other team's convenience. The final construct: T7+LacI+RBS+N-Terminus Split GFP+C-Terminus Split GFP.

RBS PRIMER



Small BioBrick parts, such as RBS and promoters, are problematic when they are being inserted into the front of another BioBrick part. The most common result is an empty vector.

Our approach to this problem revolutionized the way these small parts are inserted into a vector. The creation of this oligo primer with a strong RBS (BBa_B0034), makes the addition of small parts easier and with a **HIGH** (87.5%) **SUCCESS** rate . Our procedure only requires one PCR reaction, which is less costly and labor intensive compared to a standard procedure.

BioBrick Standard Prefix for Parts Starting with ATG

EcoRI XbaI Start of Part
GAATTCGCGGCCGCTTCTAGATG

The primer should preserve the XbaI site and the scar between the RBS and the Part.

EcoRI XbaI Start of Part
GAATTCGCGGCCGCTTCTAGAG-RBS-TACTAGATG
Scar

In order to create a melting temperature of around 60 degrees Celcius, the beginning needs to have two nucleotides added. Luckily there where two nucleotides in the standard backbones that were the same across all backbones.

EcoRI XbaI Start of Part
TGGAATTCGCGGCCGCTTCTAGAG-RBS-TACTAGATG
Scar

The final design is here:

RBS Start of Part
TGGAATTCGCGGCCGCTTCTAGAGAAAGAGGAGAAATACTAGATG