BioBots
Developing the Next Generation of Biosensors
## 2013 Georgia Institute of Technology Wetware Team

Abstract

Our team goal is to develop novel bacterial BioBots that respond to the extracellular tissue environment. Mammalian cells communicate with the extracellular matrix (ECM) using heterodimeric cell surface receptors, called integrins, which can signal in a bidirectional manner between the cell interior and ECM. We aimed to express the integrin αIIbβ3 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. We cloned split GFP parts, assembling the T7-promoter, LacI-operator, and ribosomal binding site (RBS) upstream of the protein-coding region. To verify αIIbβ3 function, we developed an integrin activity sensor consisting of the ligand derived from fibrinogen (KQAGDV) coupled to GFP. Finally, we successfully created a new standard for RBS addition that inputs the strong RBS (BBa_B0034) in front of any standard BioBrick part, which is efficient and more successful than the usual 3A/standard assembly.

Introduction

Cells perform their intended functions not individually but collectively by forming temporally evolving, three dimensional structures comprised of clusters of cells, and through active or passive cell-cell and cell-extracellular matrix (ECM) interactions. Thus the development of engineered, integrative cellular systems that self-heal and adapt their microstructure to a variety of stimuli in the surrounding microenvironment will revolutionize the way bioengineers design. Multi-cellular biological machines can be engineered to have desired functionalities and perform prescribed tasks. These machines consist of sensing, information processing, actuation, protein expression, and transport elements that can be effectively combined to create functional units. Fortunately, nature provides plenty of inspiration through similarly functioning organisms and systems. It is therefore feasible to assume that artificial materials and organisms of the future will incorporate such naturally inspired designs. The imitation of the aforementioned functions will require development of systems similar to specialized cells in the various human tissues.

The GT iGEM team worked toward the lofty goal of developing of cells and/or extracellular vesicles (e.g. platelets) that display designer sensory-response behaviors. In particular, small 'smart' biobots are an interesting avenue to duplicate the function of cells responsible for repair and adaptation. One of the tools that eukaryotic cells have at their disposal to detect their surroundings are heterodimeric sensor molecules present on the cell surface, known as integrins. Not only do integrins receive and facilitate cellular integration of extracellular cues, but they also support inside-out signaling and thus can dynamically impact their microenvironment. As a consequence of integrin's critical role in this cell-ECM "dynamic reciprocity", they represent the ideal sensor to build into a biobot. Synthetic biology applications are traditionally performed in the bacterial systems due to their availability, convenience, and speed.

Pet-mCherry

In 2012 the Georgia Tech iGEM team developed a novel biosensor based off of green fluorescent protein. The sensor consisted of two subunits of the protein that separately were inactive but once dimerized expressed fluorescence. From this project, we began thinking about how we could develop more complex sensing technology in bacteria. Taking into consideration how mammalian cells sense and react to their environment, we started asking the question: Can bacteria express human integrins?
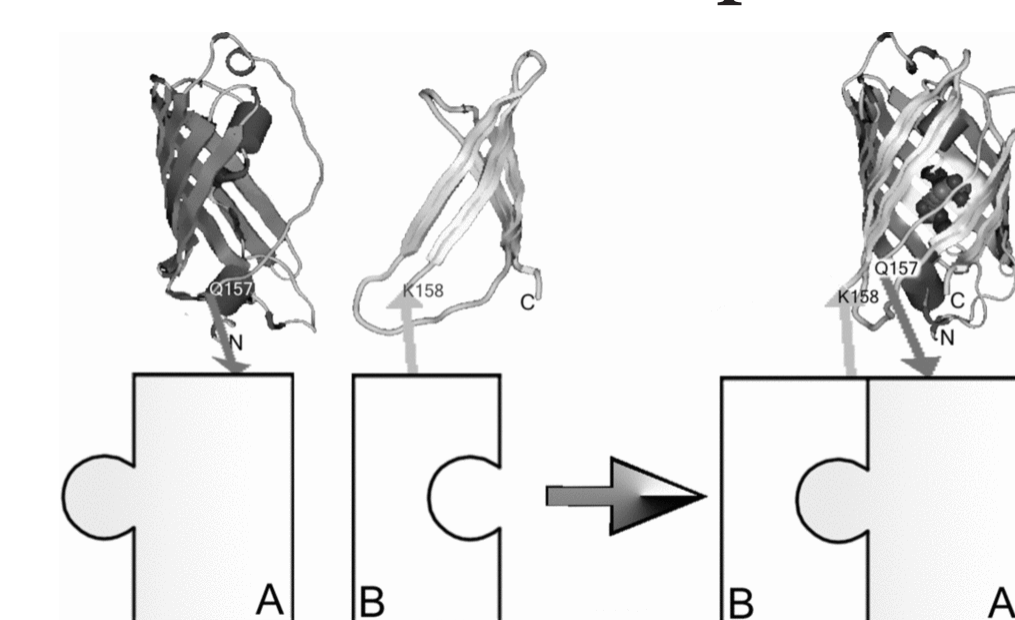
To start answering this question, we needed to find a way to transport large proteins and anchor them to the outside of the cell. Autodisplay technology seemed like one possible solution to this problem. The Pet-mCherry β-barrel was looked at as a candidate that could successfully transport and translocate the RFP, mCherry. We wanted to design and test this construct to determine whether or not PET would be a good candidate for integrin transport.
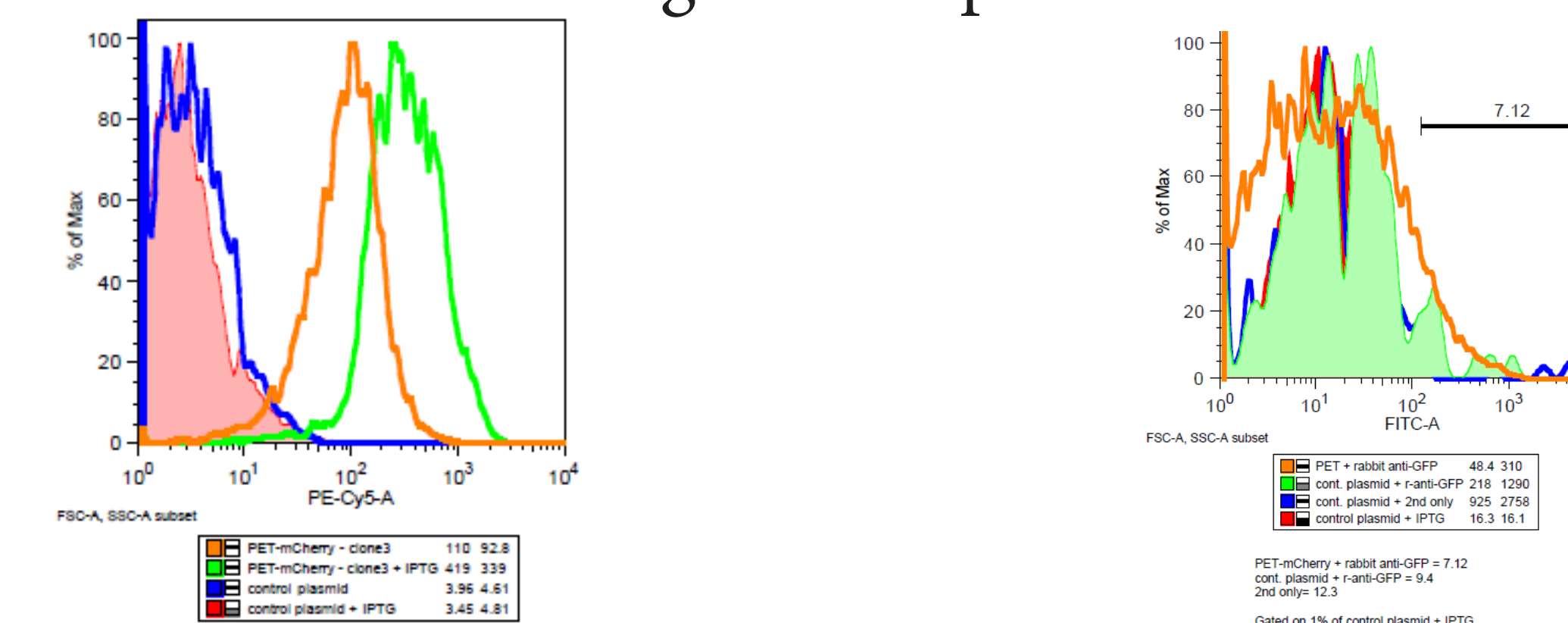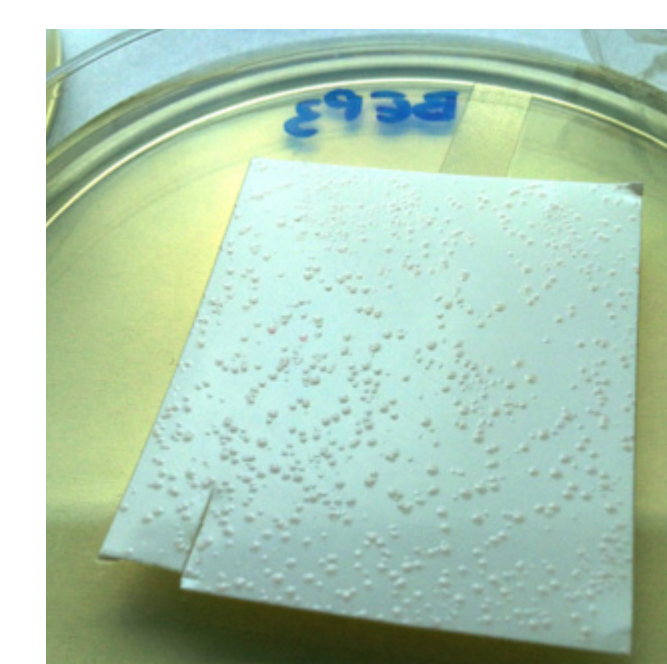
The flow cytometry results conclude that mCherry is present and was expressed in the cell. However, it may not be enitirely expressed outside of the cell. Very little decrease in the mCherry signal after washing suggest that the protein is not being cleaved if it is expressed externally. A decrease in mCherry signal after treatment of mouse anti-GFP does suggest that at least some of the proteins are on the surface of the cells.

Optimized split-GFP

The split GFP project was one left behind by the last year's Georgia Tech iGEM team. The idea is to take the green fluorescent protein genes and split it into two subunits (the N and C terminus), that when close enough would interact to produce GFP. This would be useful in any situation where two subunits must be close enough to interact and to determine how much interaction was needed. For this part, we began by sequencing the parts to see what might have gone wrong. One part was not BioBrick formatted and contained in EcoRI restriction site in the middle of the sequencing. Also, both needed an ATG start codon for the sequence to work. Then, the parts had to be assembled with a promoter, operator, and RBS. We used standard assembly to assemble the RBS part BBa_B0034 onto the each GFP part. We found that 3A assembly did not work for small parts such as the strong ribosomal binding site, so we had to use the standard assembly instead. Simultaneously, we assembled the strong T7 promoter (BBa_I719005) with a LacI, IPTG induced operator (BBa_R0010) using 3A assembly. After, we worked with 3A assembly to combine the T7+LacI+RBS+N-Terminus Split GFP+C-Terminus Split GFP.

RBS Primer

Small BioBrick parts, such as ribosomal binding sites and promoters, can prove problematic when an attempt is made to insert them in front of another BioBrick part. The most common result is an empty vector, missing both parts since the less than 20 basepair ribosomal binding site was too small to be attached to the downstream part. In order to remedy this problem, the team decided on a new approach to revolutionize the way these small parts are inserted into a vector.
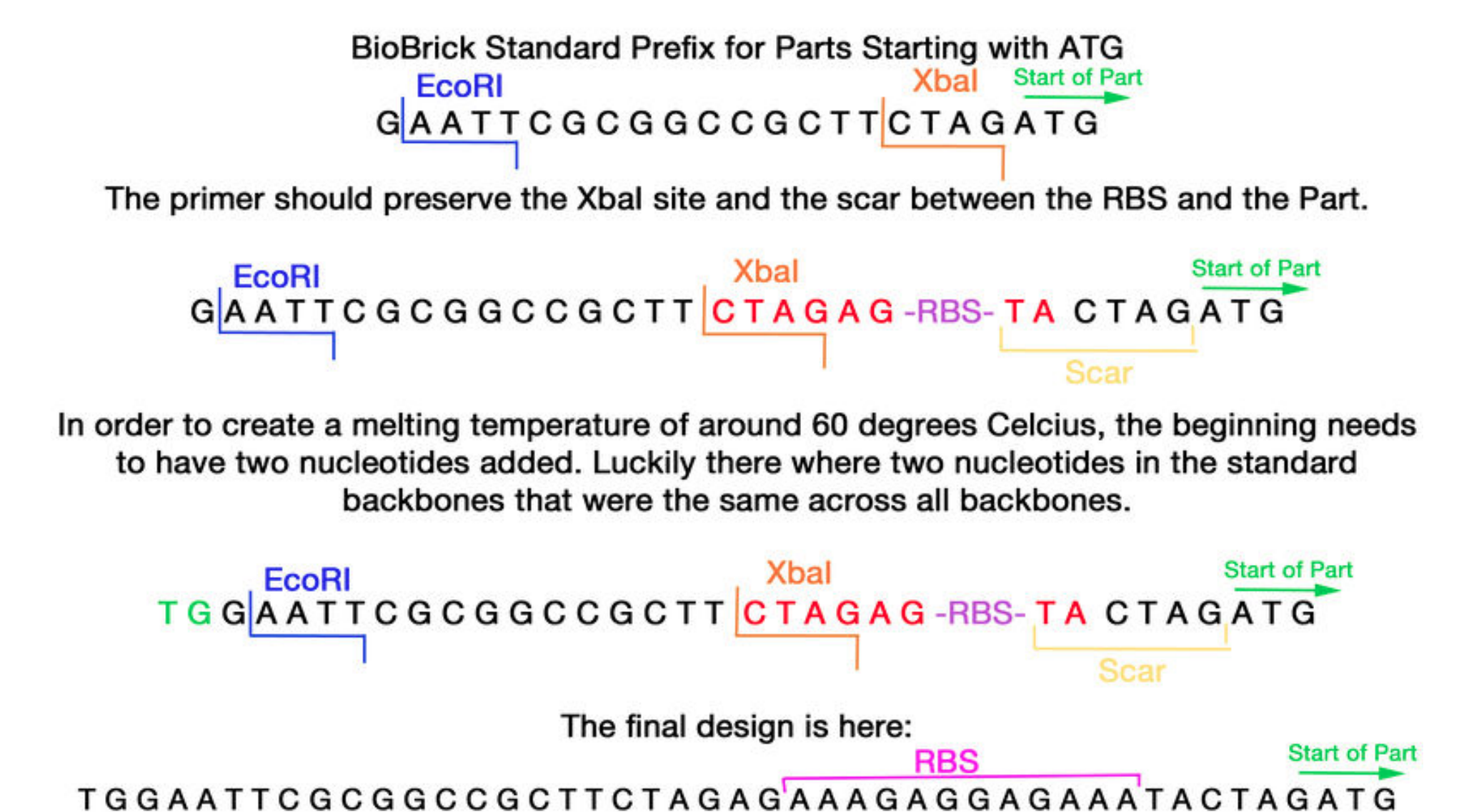
The creation of an oligo primer with a strong Ribosomal Binding Site, BBa_B0034, is easier and more efficient to place this part in front of a protein. Then, there is only a PCR reaction to be run, and the product can easily and efficiently be digested and ligated onto a standard backbone. The RBS primer has been optimized for use with BioBricks compatible with BioBrick standard 10. It also can only be used with parts that start with ATG, the parts with the most common need for an RBS.

Future Research

As synthetic biology grows as a field, so will the applications of this research. While our team was unable to successfully assemble an integrin to implement into a cell, much of the footwork necessary for this is now set. Autotransporters (such as pet_mCherry) are a necessity as larger proteins are formed to make it to the cell's surface. Also, if signaling units can be placed in the membrane of bacterial cells, there is no reason why the signal cascades and underlying units cannot also be implemented into E. coli cells. With this, a new age of simple bacteria would be created by allowing them to sense and interact with their environments in a way that they never would have before. Another area would be that of the RBS primer, which while successfully amplified, had issues in being cloned. Looking into a perfection of this process would allow for a streamlined process of placing small sequences into vectors, without all of the restriction enzymes, saving scientist precious time.