biOrigami

Stanford-Brown iGEM 2015

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ABSTRACT

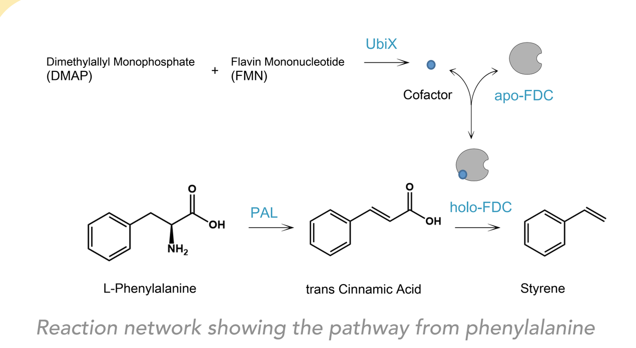
Space exploration lies at the inquisitive core of human nature, yet high costs hinder the advancement of this frontier. We are harnessing the replicative properties of biology to create biOrigami—biological, self-folding origami—to reduce the mass, volume, and assembly time of materials for space missions. Our project consists of three main components: (1) producing biologically-based substrate materials, (2) engineering folding mechanisms, and (3) designing functional products. In addition to biOrigami, we are creating a novel method to efficiently transform bacteria by using the CRISPR/Cas9 system. Our project integrates and improves manufacturing processes for space exploration on both the micro and macro levels.

1 2 3

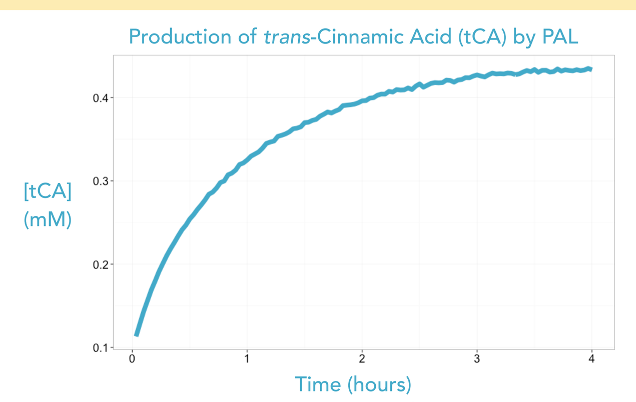
SUBSTRATES WHAT ARE WE FOLDING?

Polystyrene

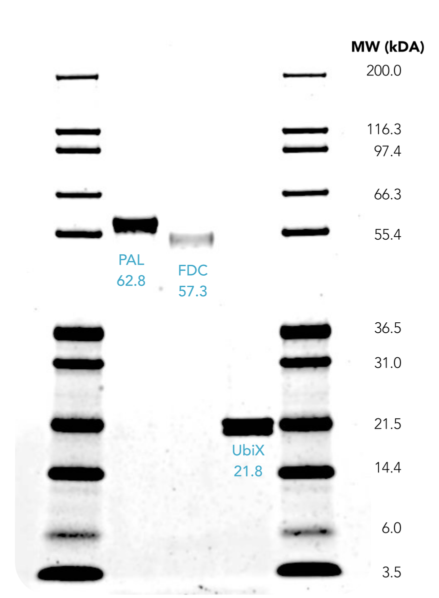
Polystyrene is a plastic that folds upon heating. It is composed of a repeating chain of styrene monomers. We have cloned the genes that will enable the first documented *in vitro* biosynthesis of styrene. We have successfully purified the enzymes needed to produce styrene from phenylalanine—PAL, FDC, and UbiX—and are analyzing their activity. We used the data from these experiments to inform our mathematical models of the reaction network. In turn, we used a model of operon configuration and translational efficiency to intelligently design a construct that comprises all three of our styrene production genes. We are currently testing our operon *in vivo*. Finally, we refined procedures for extracting styrene as it is being produced and polymerizing this styrene into polystyrene for folding.



Reaction network showing the pathway from phenylalanine to styrene.

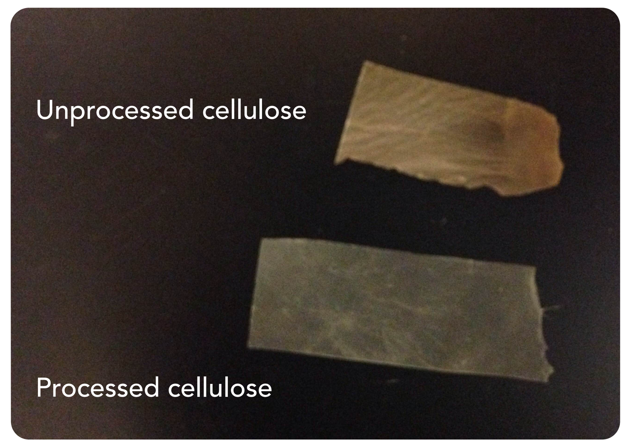


Kinetic time course of the conversion of phenylalanine to tCA by PAL. tCA was measured at 270 nm in a spectrophotometer.



SDS polyacrylamide gel electrophoresis confirming the successful purification of both PAL and FDC. The proteins were extracted using the anti-FLAG magnetic bead protocol.

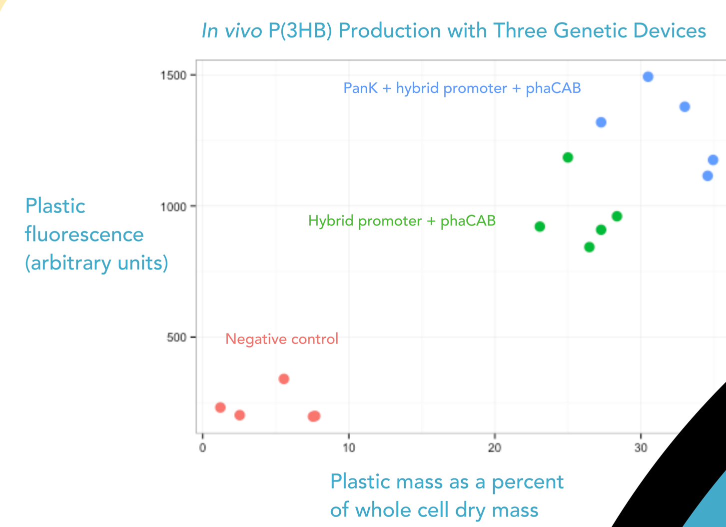
Cellulose



We used the acetic acid bacterium *Gluconacetobacter hansenii* to produce bacterial cellulose. Because of its fibrous, tough, water-insoluble properties, bacterial cellulose is the perfect substrate for biOrigami. After making the cellulose, we processed it into a flat paper-like sheet using a paper-making protocol. We also made use of a class of proteins known as cellulose binding domains (CBDs) that bind cellulose sheets. We designed a universal CBD (uCBD) that allows for the attachment of any protein onto a cellulose sheet. Specifically, we used our uCBD to attach spores to cellulose, resulting in functional self-folding strips (see *Folding Mechanisms* below).

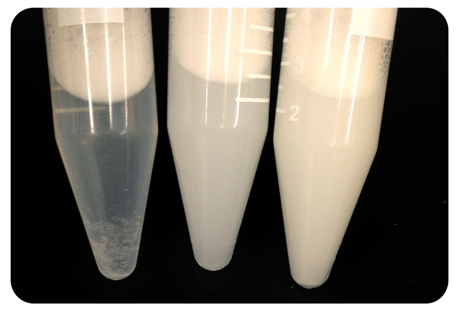
Poly-(3-hydroxybutyrate)

P(3HB) is a biodegradable polymer with thermoplastic properties, meaning that it contracts in heat. Building on previous iGEM teams' work, we have contributed BioBricks that increase P(3HB) production and facilitate its extraction from *Escherichia coli*. By adding the gene for pantothenate kinase (PanK), we were able to effect a 23% increase in the mass of plastic produced by *E. coli*. We have also processed the plastic that our bacteria produced into useful sheets.



By adding the PanK gene, we were able to increase plastic production as measured by percent cell mass and fluorescence of a plastic-binding dye.

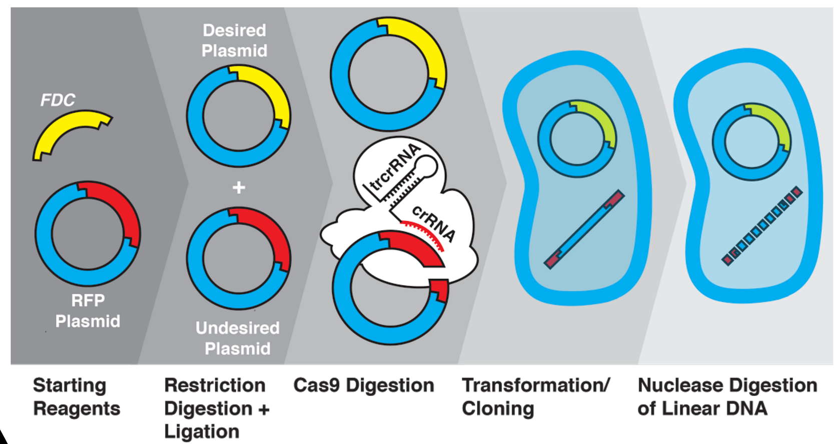
In vivo P(3HB) production. Left: negative control. Middle: phaCAB operon with hybrid promoter. Right: phaCAB operon with both hybrid promoter and panK gene.

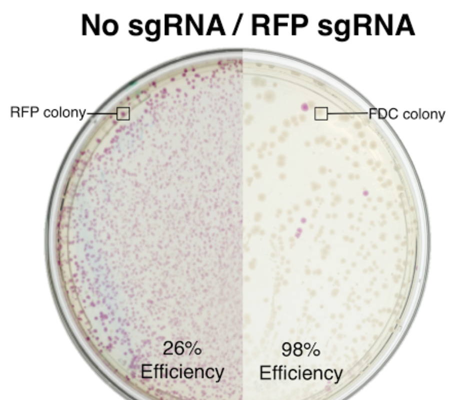


THE CRATER METHOD TRANSFORMING MOLECULAR BIOLOGY

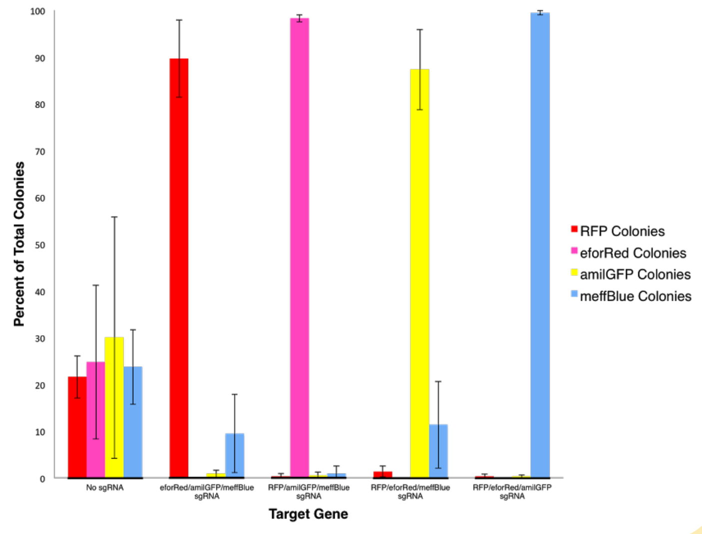
We have successfully developed a novel technique to increase bacterial transformation efficiency. Transformation—the insertion of foreign DNA into chassis—is integral to synthetic biology. By utilizing the CRISPR/Cas9 cleavage system, we are able to select for plasmids containing our gene insert by cleaving unwanted plasmid byproducts. We have proven the efficacy of our technique via a series of transformations involving bacteria expressing a variety of chromogenic proteins. We call our method **CR**ISPR/Cas9-**A**ssisted **T**ransformation-**E**fficient **R**eaction, or CRATER.

CRATER works by selectively digesting unwanted plasmids following transformation





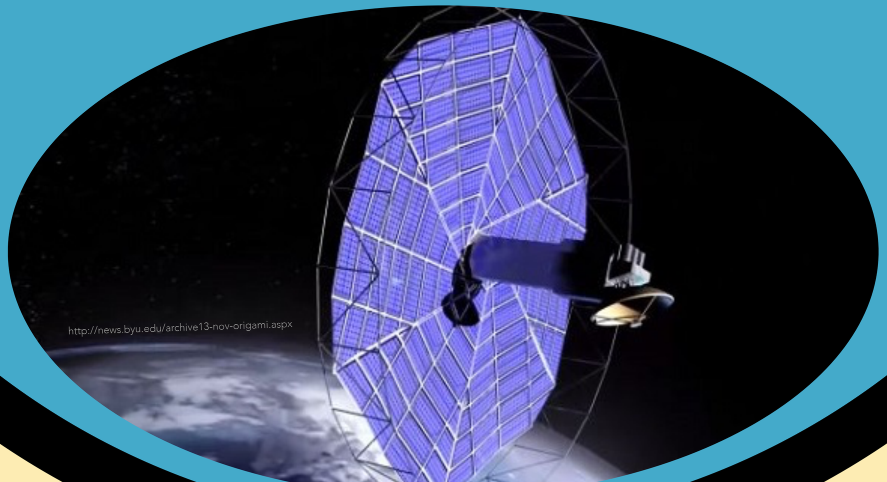
Transformation efficiency increased from 26% in the absence of our targeted sgRNA to 98% in the presence of the sgRNA. This difference is visibly apparent in this plat.



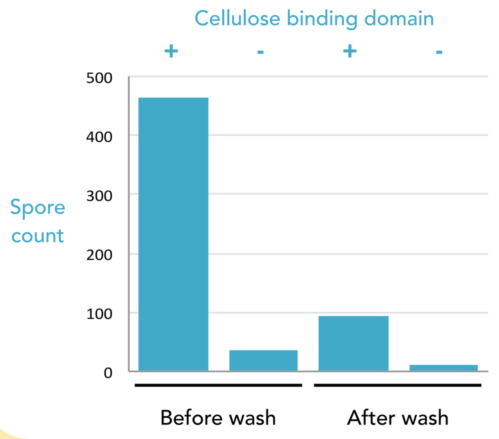
CRATER allows us to selectively transform a single chromogenic protein out of a mixture of four.

OUR VISION

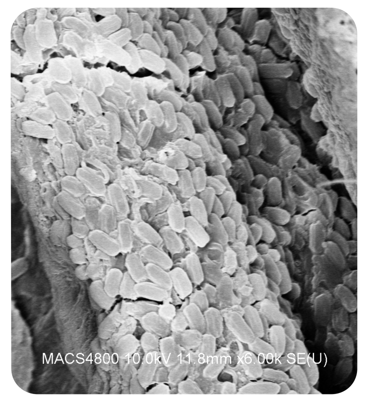
biOrigami seeks to become a widespread platform for manufacturing materials for space travel. The products that we envision must be able to be transported in the small volume of a space ship's payload bay and be assembled quickly and easily. Experts in the fields of space exploration and planetary science have imagined the usefulness of biOrigami in precursor missions: for creating a fleet of inexpensive weather-monitoring robots, for unfolding tents that harvest lunar water, for forming boxes for sample collection, and for facilitating solar panel deployment as depicted below.



FOLDING MECHANISMS HOW ARE WE FOLDING?



Spores equipped with our cellulose binding domain adhere to cellulose much better than do wild type spores, even after a PBS wash.



Scanning electron micrograph of the spores generated in our lab.

BioHYDRA

BioHYDRA is a project to create biological artificial muscles that respond to changes in humidity. This past year, a new technique was devised (Chen et al., 2015) that uses the fact that *Bacillus subtilis* spores expand and contract depending on changes in ambient humidity to create contractile structures coined as "HYDRA" (hygroscopy driven artificial muscles). We wanted to improve on this technology by creating fully biological hydras, using cellulose instead of polyimide and incorporating cellulose binding sites (uCBDs; see above) on the spore coats instead of using artificial glue. In this way we can create a fully biological folding mechanism that not only responds to humidity but is also reversible.

Bioplastic Folding

Plastic is a versatile material that can be designed to contract along a desired axis when heated to its glass transition temperature. When a sheet of plastic that has been marked with a pigment is exposed to light, the pigmented segment heats up more quickly than the rest of the sheet. The resulting selective contraction causes folding.

Upon heating, the strips of polystyrene fold at the joints.

For more information visit our website at http://2015.igem.org/Team:Stanford-Brown

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