Yale iGEM 2014 Sponsor Update

26 November 2014

iGEM 2014 Giant Jamboree at the Hynes Convention Center in Boston, MA

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Project Abstract:

One aim of synthetic biology is to produce environmentally safe industrial goods. To this effect, we recognized a problem with biofilm formation and its potentially damaging effects in medicine and industry. We chose to address the problem by designing an environmentally-friendly, adhesive antimicrobial coating that could prevent biofilm formation on surfaces and an improved T7 system by which we could synthesize antimicrobial molecules in E. coli.

Sponsor Update Contents:

Project Overview:

Why Address Biofilm Formation?

Biofilm formation is an important issue for industry that has yet to be resolved with typical synthetic methods. Biofilms can grow on a variety of surfaces ranging from medical implants to the metal hulls of ships. They are difficult and costly to remove through mechanical means and often display increased resistance to antibiotics. Below are just some figures that demonstrate the impact of biofilms in current fields of industry.

- **Medicine:** Protein adsorption, cell adhesion, and subsequent biofilm formation can lead to the failure of medical implants and septic infections in patients.[1]

- **Shipping:** Government and industry spend upwards of $5.7 billion annually on controlling marine biofouling. High levels of biofouling can result in increased drag and subsequent loss of hydrodynamic performance.[2]

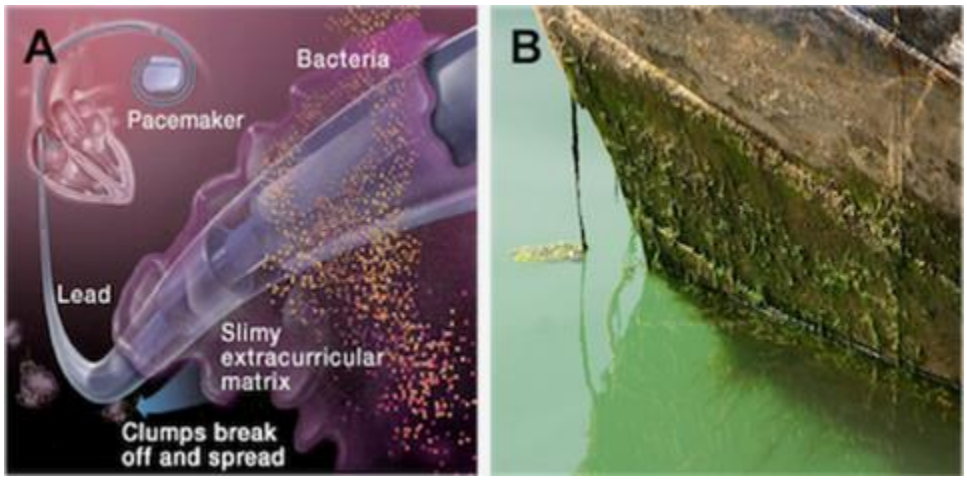


Figure 1: A) *Biofilm formation leads to failure of implanted cardiac devices and heart valve infection.* ***B)*** *Biofilm formation on the hulls of ships gives larger organisms an organic platform to anchor to. This leads to the loss of optimal hydrodynamic performance and structural failures.*

Our Approach:

Design a peptide that could be synthesized in genetically-recoded *E. coli* and that would be able to adhere to surfaces and prevent microbes from initially settling and forming a biofilm.

1. We chose to link an adhesive protein domain to an antimicrobial protein domain through a long, flexible amino acid chain. Expressing the protein in genetically recoded *E. coli* allowed us to use a nonstandard amino acid, L-DOPA, in the primary structure of our protein, to achieve adhesive functionality.

2. Additionally, we modified the *E. coli* ribo-regulation system designed by Farren Isaacs with the T7 RNA Polymerase, to develop a plasmid system with low basal expression, which can be used to grow up large quantities of bacteria before initiation of gene expression and collection of the anti-bacterial peptides.

1. A Modular Anti-Microbial Peptide Coating based on Mussel Foot Proteins

As our adhesive domain, we selected the mussel foot protein (mfp) consensus sequence mefp 1-mgfp 5-mefp-1, which was shown to have strong adhesive properties (Lee *et al.*, 2008). The LL-37 antimicrobial peptide, which is short enough to be inserted via primer overhang, is linked to the adhesive domain via a 36 residue linker.

At the N-terminus, we included a twin Strep-FLAG tag, used in the purification and isolation of our construct and that can be readily cleaved. On C-terminus we included an sfGFP connected by a shorter linker, which can be used to assay for the presence and yield of our peptide. The sfGFP will be removed from the final product. Using targeted primers, the construct can be amplified in its entirety, or only with the anti-microbial or GFP segment. Note that the entire construct was designed so that a variety of functional peptide domains can be substituted for LL-37. The construct was ordered through Genscript.

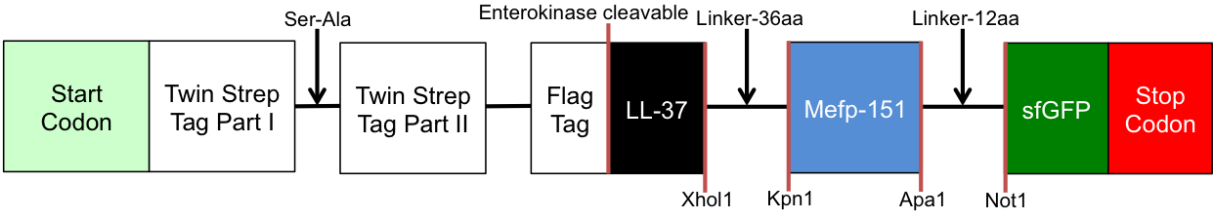


Figure 2: *A diagram illustrating the components in our final construct. The black domain is our anti-microbial peptide, LL-37, while the blue domain represents the recombinant mussel foot protein adhesive component. All other components are labeled accordingly and restriction sites are highlighted to emphasize the modularity of each separate region.*

2. Control Expression of Anti-Microbial Peptides Using an Improved T7 Riboregulation System

Since we intend to synthesize an anti-microbial peptide, it is likely that the peptide will be toxic to the *E. coli* used in our synthetic route.

To improve our yield, we designed a plasmid system involving a riboregulated T7 RNA Polymerase that would minimize basal expression of our product. This was accomplished by placing a riboregulatory cis-repressing RNA sequence (crRNA) upstream of the gene for T7 RNA Polymerase, commonly used for protein overexpression, driven by an inducible promoter. A trans-activating RNA sequence (taRNA) was placed on the same plasmid under a separate inducible promoter. The toxic gene would be driven by the T7 Promoter which is orthogonal to normal E. coli polymerases and is only transcribed by T7 RNA Polymerase.

This is an improvement over the standard BL21(DE3) strain which has T7 RNA Polymerase genomically integrated under a leaky promoter.

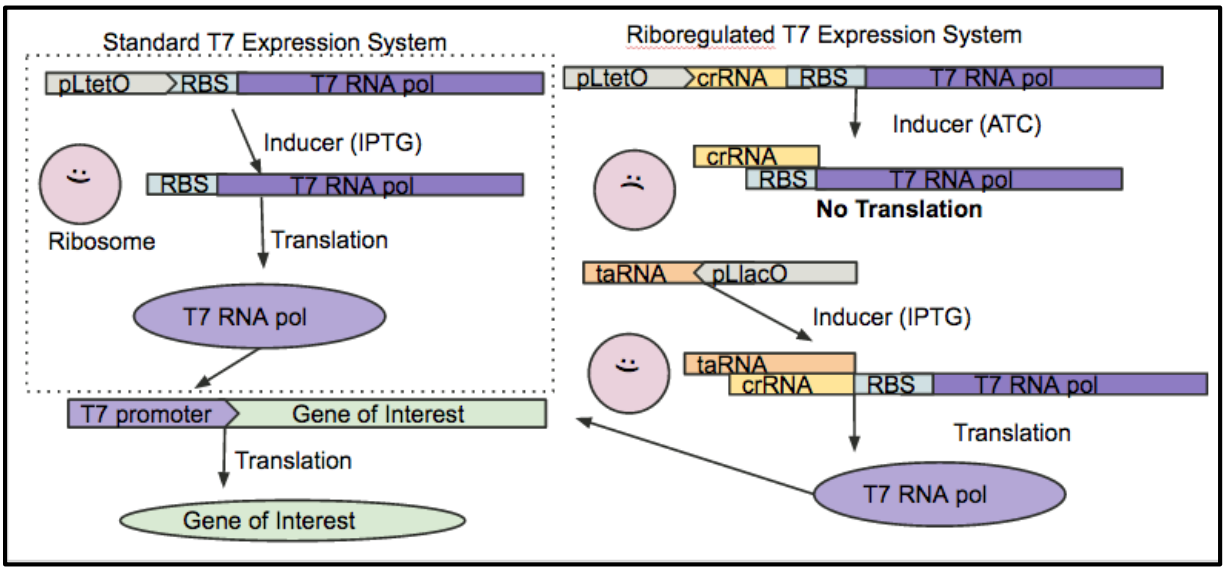


Figure 3: *A comparison between standard and improved T7 expression systems. Here we illustrate the integration of the riboregulation system to control expression of our protein. We regulate T7 polymerase, which targets the T7 promoter with high specificity, giving the third lock on our expression system.*

3. **Characterize our peptide's adhesion and anti-microbial properties:**

We developed a number of assays to test the erosion resistance of our adhesive coating using an original apparatus designed to introduce erosion by laminar flow through a liquid bath.

To assess the efficacy of our peptide in inhibiting biofilm formation, we intend to perform a minimum biofilm eradication concentration (MBEC) assay (Innovotech).

Further information on the assays that we investigated for adhesion and antimicrobial properties are detailed in the Materials and Methods section of our website.

Results: Antimicrobial Coating Peptide (Ampersand)

1. Synthesis and Assembly

The gene construct for the antimicrobial peptide coating (discussed above) was synthesized by Genscript. In place of L-DOPA amino acids we had TAG codons. TAG is the rarest stop codon, appearing in 321 genes throughout the *E. coli* genome. In recoded E. coli all instances of a TAG stop codon are replaced with a TAA codon, allowing TAG to be reassigned to a different amino acid, including novel, nonstandard amino acids. We transferred the gene sequence into an expression plasmid, pZE21, and transformed the plasmid into recoded E. coli.

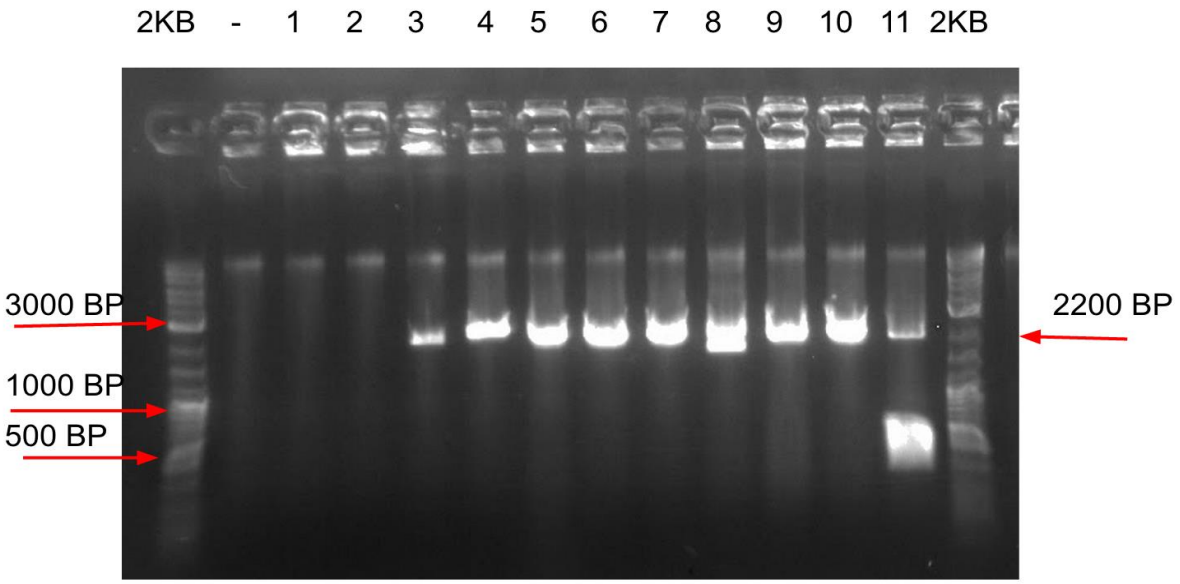


Figure 4: *PCR screening results of the transformation of the Gibson assembly product of the construct into pZE21 plasmid backbone. Universal pZE21 primers were used. Lanes with bands at 2200 bp signify presence of construct in clone.*

2. Characterization with Recoded E. Coli

The plasmid containing the antimicrobial adhesive DNA construct was transformed into two separate strains of ECNR2, one containing an L-DOPA aminoacyl-tRNA synthetase and tRNA sequence, and a similar plasmid containing a Tyrosine suppressor. The first strain would read through TAG codons as L-DOPA and the second would read through TAG codons as Tyrosine, producing the mussel foot protein in its standard form before L-DOPA modifications. We then performed a growth assay over a period of 16 hours to compare fluorescence as a measure of protein production. We saw little growth in most cultures, but one culture demonstrated a dampening sinusoidal growth pattern that suggested toxic expression of the peptide. Results of the experiment are shown below.

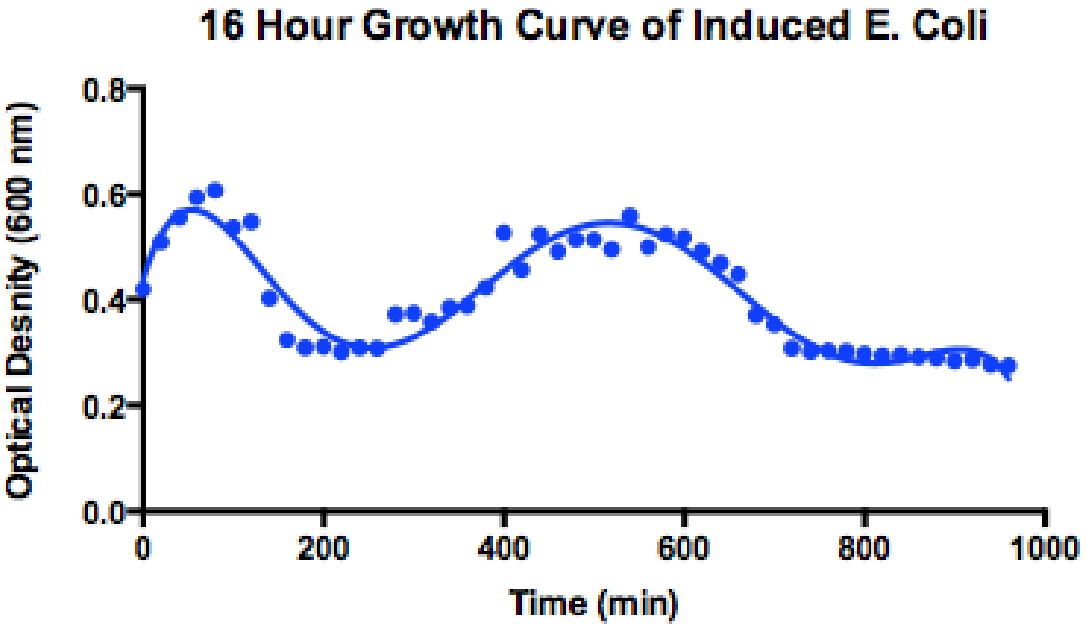


Figure 5*: 16-hour growth assay of culture expressing anti-microbial coating peptide without regulation. A damped sinusoidal growth pattern is observed, indicating toxic effects of antimicrobial peptide in a strain expressing the peptide with a tyrosine suppressor.*

Results: T7 Riboregulation System

1. T7 RNA polymerase design and assembly

The T7 Riboregulation System works by a “three-lock system.” The first lock is the cis-repressing RNA (crRNA), which is induced by isopropyl β-D-1-thiogalactopyranoside (IPTG). The second lock is the trans-activating RNA (taRNA), which is induced by anhydrotetracycline (ATc). If the taRNA is unlocked, it will bind to the crRNA, removing the hairpin and making the ribosomal binding site accessible for ribosomal binding, leading to translation of a specific protein, in this case, T7 RNA Polymerase. This riboregulation system was initially developed by Dr. Farren Isaacs, and has been shown to work with chloramphenicol resistance (chloramphenical acetyl transferase gene) in place of the T7 RNA Polymerase gene. The plasmid was synthesized using Gibson assembly, and confirmed by sequencing.

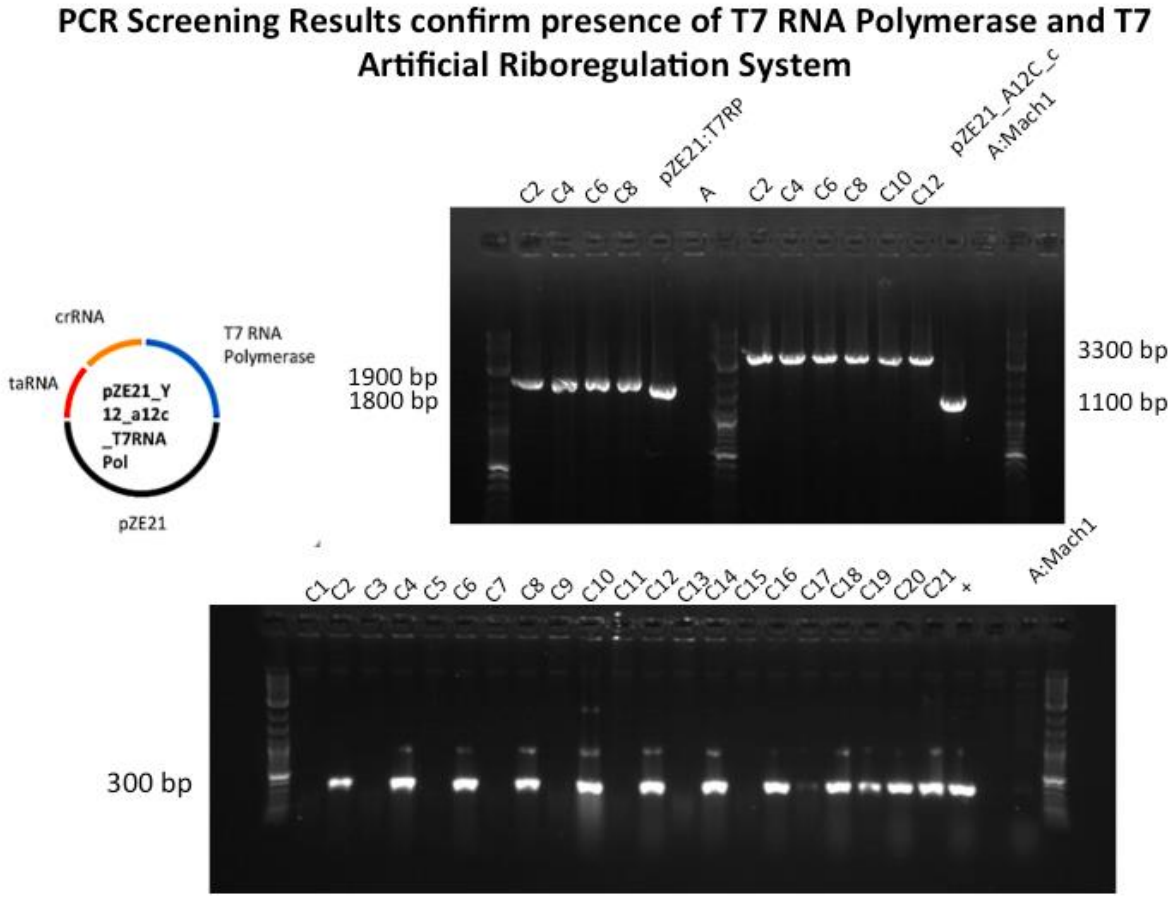


Figure 6: *Preliminary gel screening of Mach 1 strains containing transformed pZE21_A12C_T7RNA plasmids created via Gibson Assembly.*

2. **GFP Fluorescence Assay for the Riboregulated T7 system**
   Experimental plan for the GFP fluorescence assay testing the efficacy of the T7 riboregulation system. The T7 riboregulation system, pZE21_A12C_T7RNA, would express sfGFP behind a T7 promoter, in the plasmid pZA21. Either plasmid, and both plasmids together, were transformed into ECNR2 and induced with either IPTG and ATC. ECNR2 is the ancestral strain. A positive control was the same pZA21_T7sfGFP plasmid in ECNR2, and the same T7 RNA polymerase gene inserted in a regular pZE21 plasmid with a pLtetO promoter, and a negative control with the pZA21_T7sfGFP in ECNR2 without any plasmid that contains T7 RNA.

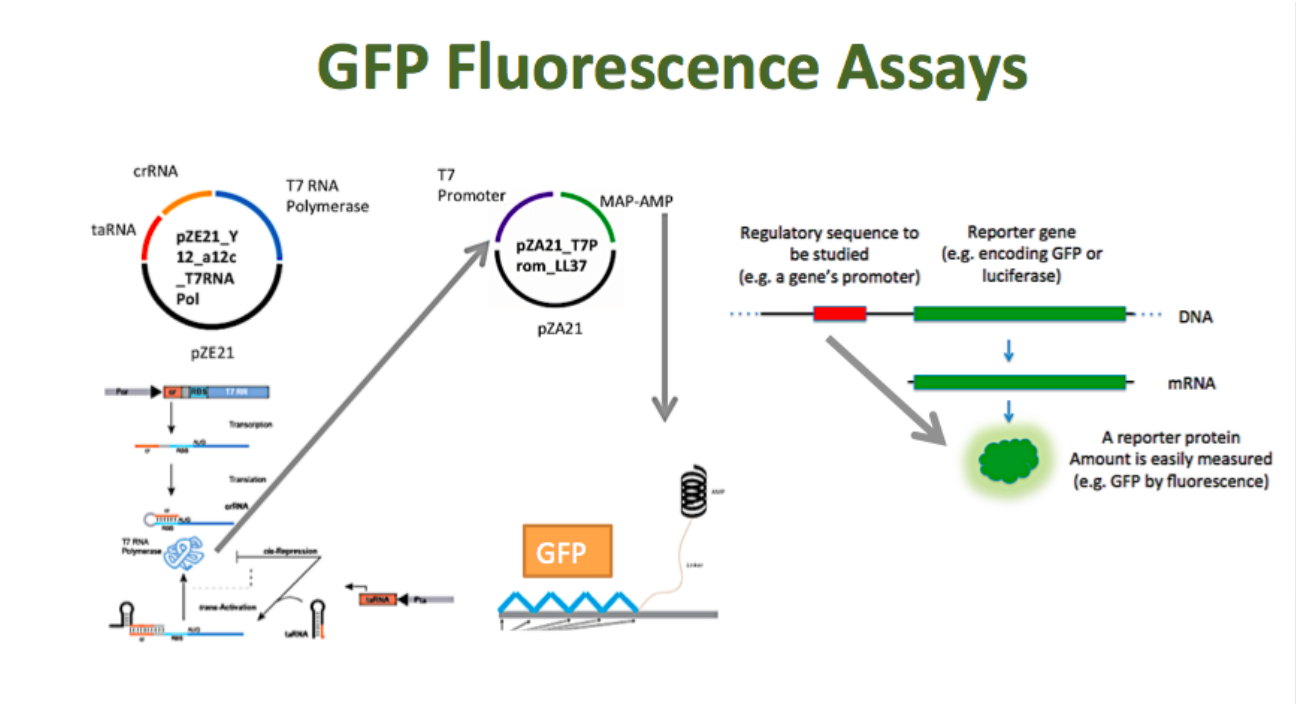


Figure 7. *Schematic detailing the GFP assay as described above.*

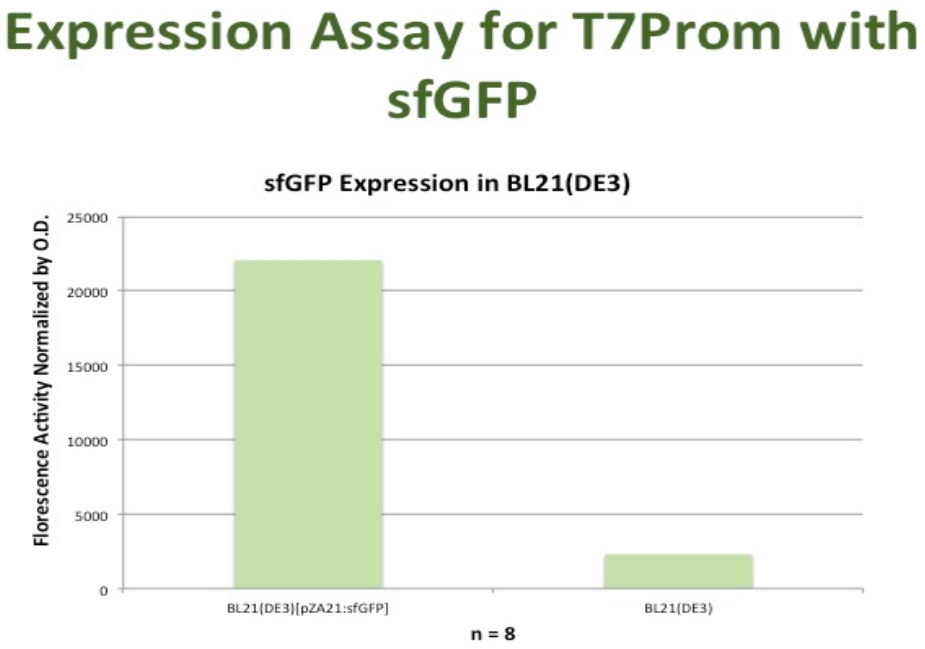


Figure 7: *Conformational assay to test the functionality of the pZA21_T7sfGFP, which is sfGFP placed behind the T7 promoter. The plasmid was transformed into a BL21(DE3) strain, which constitutively expresses T7 RNA polymerase. The strain, as well as untransformed BL21(DE3), were grown overnight and assayed using a Synergy H1 Biotek Platereader. Fluorescence measurement was taken by exciting the cells at 485 nm and detecting at 528 nm, with a bandpass of 4 nm on each side. The optical density was also taken at 600 nm, and the fluorescence data was normalized by dividing fluorescence by optical density. What is shown is the average of 4 replicates each.*

Plasmid	Antibiotic Marker	Inducer Condition 1	Inducer Condition 2	Inducer Condition 3	Inducer Condition 4
pZE21_A12C_T7RNA, pZA21_T7sfGFP	Kanamycin, Spectinomycin	ATC, IPTG	ATC	IPTG	No Inducer
pZE21_A12C_T7RNA	Kanamycin	ATC, IPTG	ATC	IPTG	No Inducer
pZA21_T7sfGFP	Spectinomycin	ATC, IPTG	ATC	IPTG	No Inducer
ECNR2, no plasmids	None	ATC, IPTG	ATC	IPTG	No Inducer

Table 1. *Experimental conditions for the GFP assay. Each plasmid combination was paired with each inducer combination, and the conditions were made in six replicates.*

Adhesion Characterization

The development of a robust assay to quantify erosion of protein-coated surfaces saw direct collaborations with a number of Yale faculty including the labs of Dr. Yongli Zhang, Dr. Eric Dufresne, Dr. Michael Rooks at YINQE, and Dr. Helmut Ernstberger. In addition to these labs, numerous Yale faculty across departments in biology and engineering provided valuable advice to this aspect of our project. We also engaged in an inter-university collaboration with the lab of Dr. James Rusling and Dr. Islam Mosa at the University of Connecticut for quartz crystal microbalance experiments. Specifically, this work was focused on assembling a number of tests that could be applied to our protein once it had been synthesized:

1. **Development of a Robust System for Introducing Erosion on Coated Surfaces**

A number of ASTM assays used in industrial coating testing were investigated, but none offered the level of quantitation desired for our applications. Therefore, an original rig was designed and built to introduce liquid based erosion by laminar flow through a bath (see Figure 8). This system directly mimics the drag that a coated surface might experience on a ship's hull.

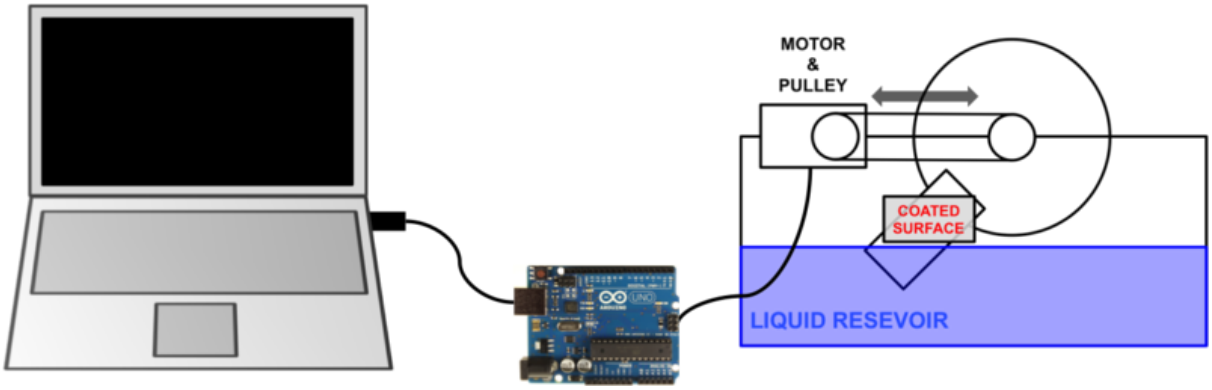


Figure 8. *A diagram illustrating the configuration of the erosion rig developed to introduce coated surfaces to liquid erosion.*

2. Mass Retention of Mussel Adhesion Proteins (MAPs) Under Stress

Preliminary proof of concept testing was conducted on a commercially available MAP-based product known as Cell-Tak™ (see Figure 9). Cell-Tak™ is designed to facilitate cell adhesion to normally non-biocompatible surfaces such as microscope slides and petri dishes. We deposited ~20 µg films of Cell-Tak onto borosilicate substrates under conditions of 0.2 M $NaHCO_3$ (pH 8-9) and proceeded to erode them under deionized H_2O and 5% acetic acid. The results from this experiment are presented below and illustrate the design of our assay to test a variety of solvent and erosion conditions on MAP films. A microbalance (Mettler Toledo MX5) that can read to uncertainties of 1 µg was used to determine the mass of protein remaining after subjecting the substrate to erosion. An exponential decay curve was fitted to these experiments giving decay rates of 0.002 µg/pass and 0.049 µg/pass for deionized H_2O and 5% acetic acid, respectively.

As lower pH reverses the coordination of L-DOPA, it is expected that the acidic conditions engender the higher rate of decay. This experiment presents a preliminary result that validates our ability to apply erosion onto MAP-coated surfaces. We intend to apply a similar protocol to metal and plastic surfaces as well as erode surfaces under different pH conditions to provide a more comprehensive picture of the optimal conditions for mussel foot protein adhesion.

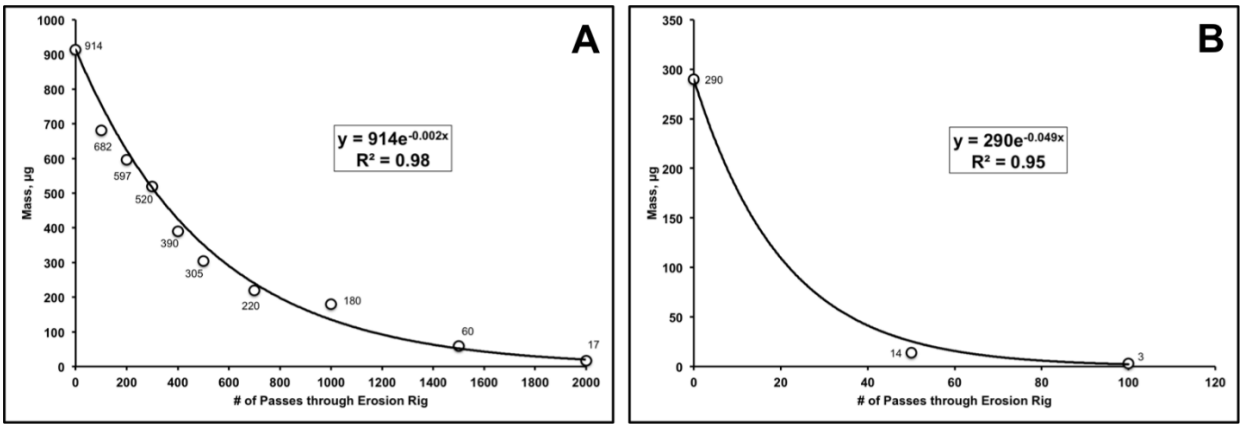


Figure 9. (A) *The erosion of Cell-Tak ™ under conditions of DI water erosion.* ***(B)*** *The erosion of Cell-Tak ™ under conditions of 5% acetic acid erosion.*

3. Determination of Surface Energies of MAP Films

A contact angle measurement of a Cell-Tak™ coated surface was recorded and served as an indication for presence of peptide on surfaces. The contact angle is measured between the surface of the drop and the table-top. Larger contact angles are indicative of more hydrophobic surfaces while shallower contact angles correspond to more wettable surfaces. A contact angle of 25.053º was obtained between an untreated silica substrate and a 2 µL drop of DI H_2O. However, when surfaces were treated with the MAP, the contact angle increased to 62.007º (see Figure 10), indicative of an increase in the hydrophobicity of our substrate. This result validates the evolutionary need for mussels to secrete proteins that are resistant to water in order to survive and anchor themselves in constantly wet environments.

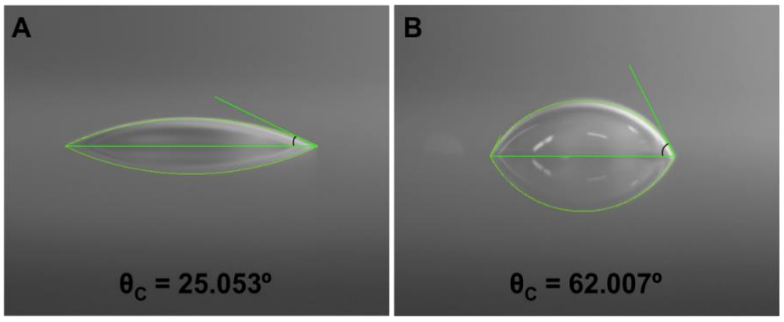


Figure 10. (A) *The profile photograph of a drop on an untreated silica substrate used for contact angle determination.* ***(B)*** *The profile photograph of a Cell-Tak ™ treated surface used for contact angle determination.*

4. Erosion of Cell-Tak™ Coated Surfaces Under EQCM Flow

Hwang et al. in their 2007 manuscript detailing the recombinant synthesis of mfp-151, described subjecting their films to a washing procedure followed by measuring mass retained with a quartz crystal microbalance (QCM). In collaboration with Dr. Islam Mosa in the lab of Dr. James Rusling in the University of Connecticut, we tested a different type of QCM known as an EQCM (added E= electrochemical) with a flow cell built for in-situ measurement of mass while erosion is occuring (see Figure 11 center). This type of setup would have been the first measurement of in-situ erosion of mussel foot proteins.

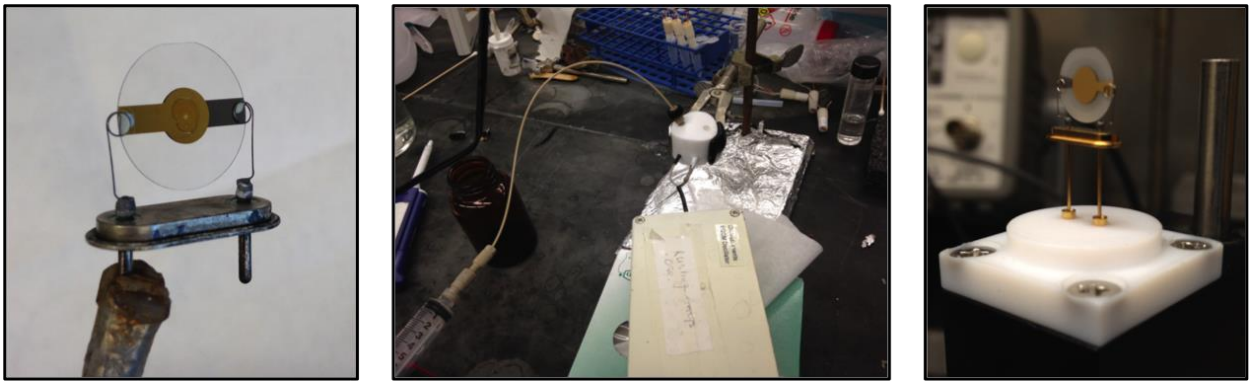


Figure 11. *(left) A QCM resonator coated in a Cell-Tak™ film. (center) An EQCM equipped with a flow-cell and syringe pump in which real-time protein film mass can be monitored as a function of flow-rate. (right) A static QCM resonator used in conventional QCM analyses.*

QCM's measure mass by monitoring change in the resonant frequency of the quartz crystal resonator. Higher ΔF indicates loss of mass due to an increase in resonant frequency. Higher resonant frequencies indicate the the mass on the resonator is lower because it is easier for the crystal to resonate at these higher frequencies. We optimized the protocol needed for EQCM erosion with a BSA film as a control (see Figure 12B). As DI H_2O was passed over our resonator at a 1 mL/min flow rate, real-time mass was monitored. The ΔF showed consistent increase due to the loss of protein mass as a function of time. The decay rate can then be calculated from the slope of the curve before constant ΔF is observed. We hope to apply this technique to our mfp films once these adhesive proteins have been synthesized.

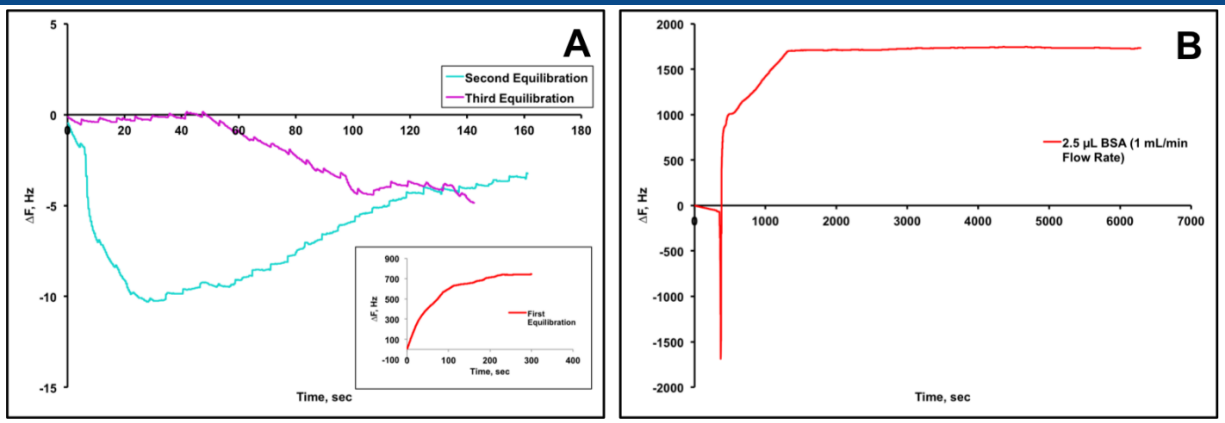


Figure 12. ***(A)*** *Equilibration of a blank EQCM resonator. Inset shows first equilibration resulted in removal of some residual coating as ΔF increases.* ***(B)*** *Erosion of a BSA film showing loss of mass by increase in ΔF. Rate of decay can be calculated from slope of curve before constant mass is observed.*

5. Future Work

We hope to conduct colloidal probe atomic force microscopy on our protein films as a more absolute measure for the pull-off force of our protein films (conducted with YINQE). We have been in contact with the lab of Dr. Yongli Zhang to conduct a force measurement using his optical tweezer setup as the first single-protein measurement of mussel foot protein adhesion. Lastly, we intend to work with our collaborators at UCONN to apply our flow EQCM technique to our protein films.

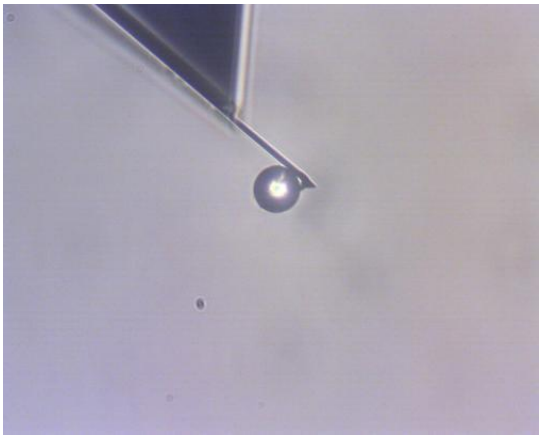


Figure 13. *AFM cantilever with a 20 µm silica bead fixed to the tip. By functionalizing the tip, we can control the adhesion interface for which we test our MAP adhesives. In this case, we intend to use a silica bead to measure the adhesion of our coating to a silica interface.*

Industry Outreach:

In addition to wet-lab work, our team sought ways to educate the general population about our research and engage them in the practice and utility of synthetic biology.

Applications in Shipping:

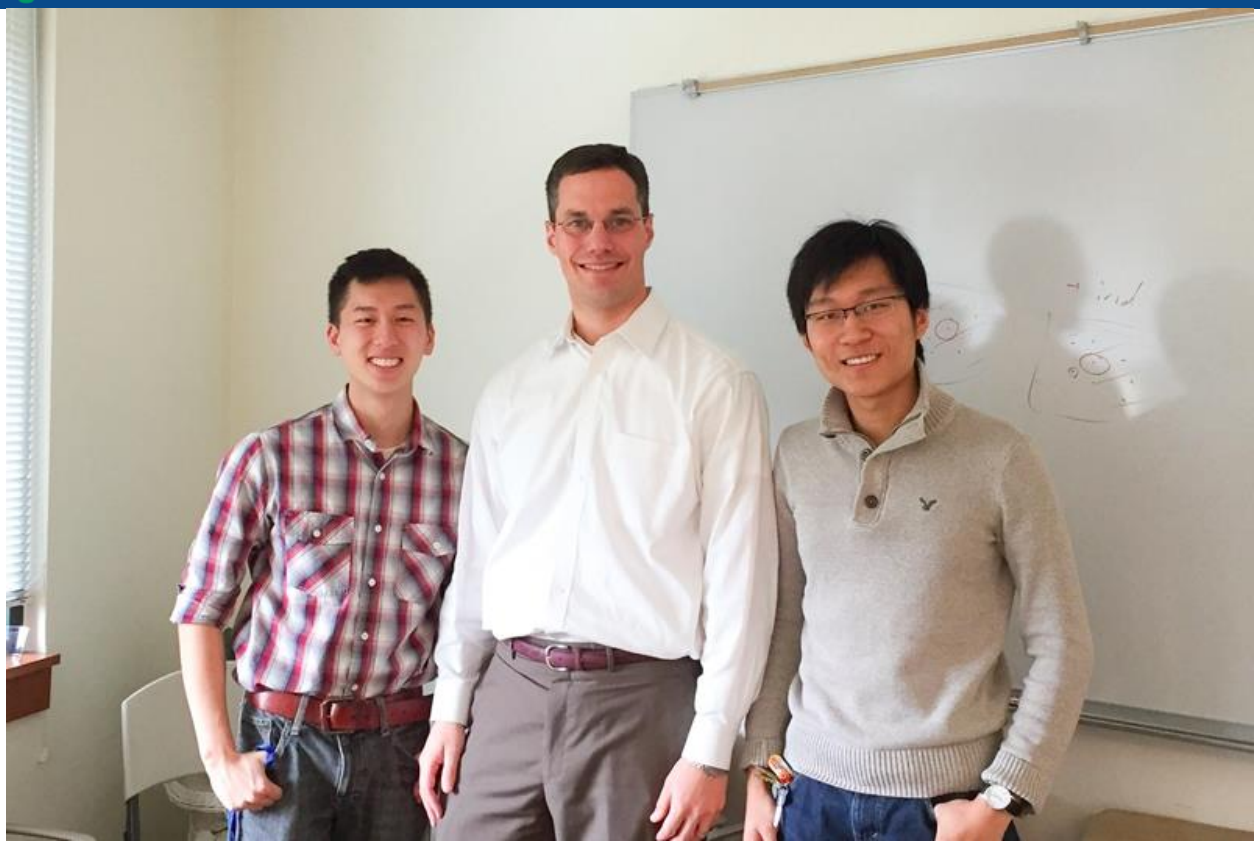
Over the summer, Yale iGEM member Cathy Ren interviewed Mr. James S.C. Tai, Technical Director and General Manager of Fleet Management Department, Orion Overseas Container Line Limited (OOCL). We asked about the importance of biofouling in the shipping industry and the possible application of our antimicrobial coating.

iGEM member Cathy Ren with Mr. Tai of Orion Overseas Container Line Limited

Applications in Medical Devices:

Yale iGEM also interviewed Dr. Christopher Loose, co-founder of Semprus Biosciences, on entrepreneurship and using research to address unmet needs in medicine.

Dr. Loose co-founded Semprus BioSciences, a company focused on anti-fouling on medical devices, with MIT professor Robert Langer and David Lucchino in 2006. He served as CTO until the company was acquired by Teleflex Incorporated in 2012. He currently serves as Executive Director of the Yale Center for Biomedical and Interventional Technology (CBIT) and as Assistant Professor Adjunct, Urology and Lecturer, Biomedical Engineering and Lecturer School of Management.



Board Members Ming Chen and Ed Kong with Dr. Loose

Local Outreach:

Over the summer, Yale iGEM member Ariel Hernandez-Leyva delivered a class for local high school students via Yale Splash entitled: **New Pills for Old Ills: Antimicrobial Peptides and a Possible New Approach to Molecular Medicine**.

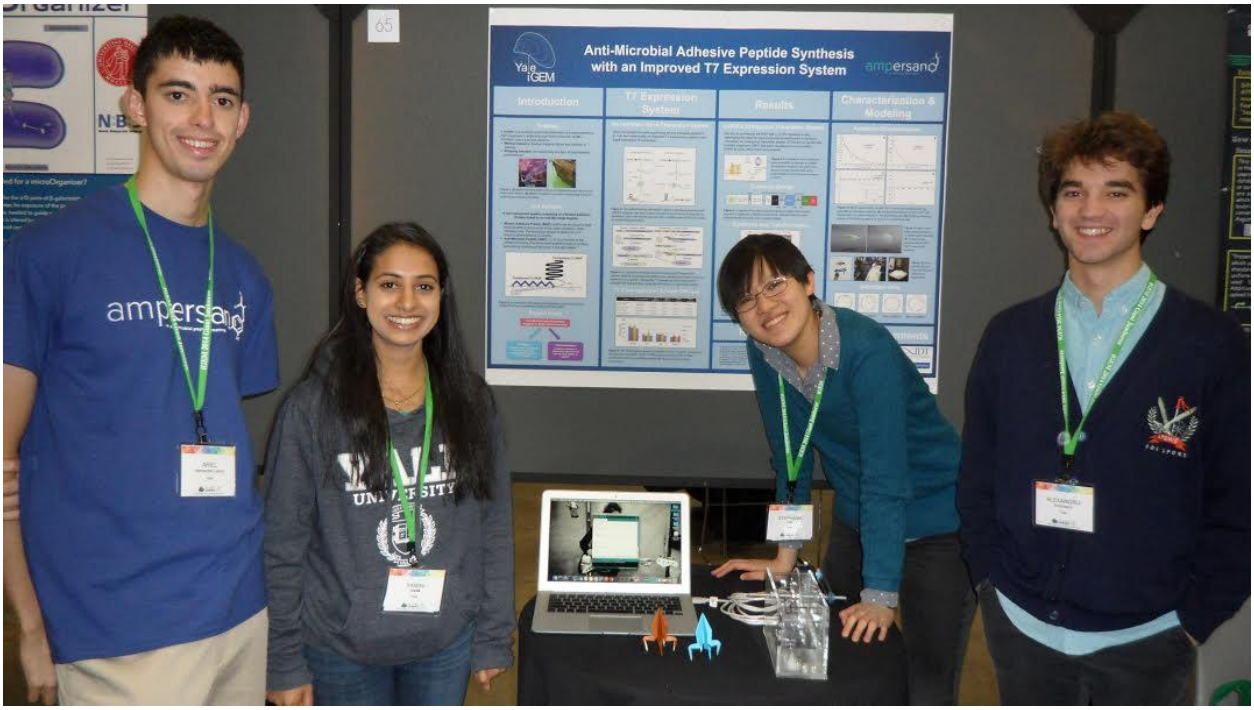
The course started by focusing on the history of antibiotics and anti-microbials and moved on to discuss the current issues with antimicrobial resistance. A possible solution to this process, anti-microbial peptides, was discussed and the biochemistry behind the peptides was explained. To conclude, we talked about how the science of synthetic biology could fit into an era of molecular medicine through a discussion of the production of anti-microbial peptides in bacteria for medical use.

Competition Outcomes

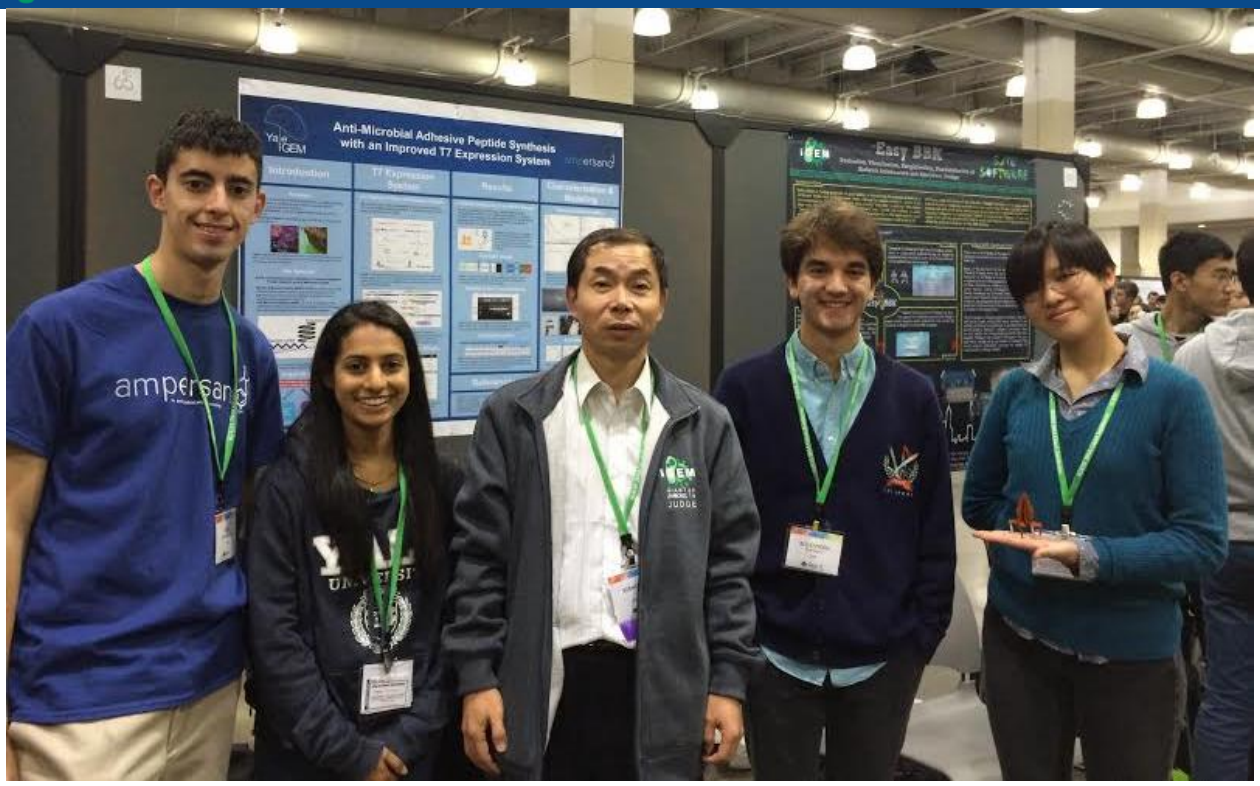
This year, iGEM changed their competition format so there would no longer be a regional competition. Instead, all the iGEM teams came together in the Hynes Convention Center in Boston from October 30 to November 3. Yale iGEM team members Ariel Leyva-Hernandez, Yamini Naidu, Alexandru Buhimschi, Stephanie Mao, and advisor Natalie Ma attended the conference, along with 244 other teams from across the world.

On Halloween, Friday the 31st, Yale members attended a variety of team presentations and workshops, before preparing for their presentation on November 1st. For the undergraduate division, prizes went to Heidelberg (for developing circularized, heat-resistant peptides), Imperial (for synthesizing and harvesting 'custom' cellulose fibers), and NCTU Formosa (for creating a library of moth pheromone precursors for pest control).

Yale's iGEM team finished the competition with a Bronze medal designation. Inspired by the wide range of possibilities synthetic biology holds, the team returned to New Haven already planning for the next year.



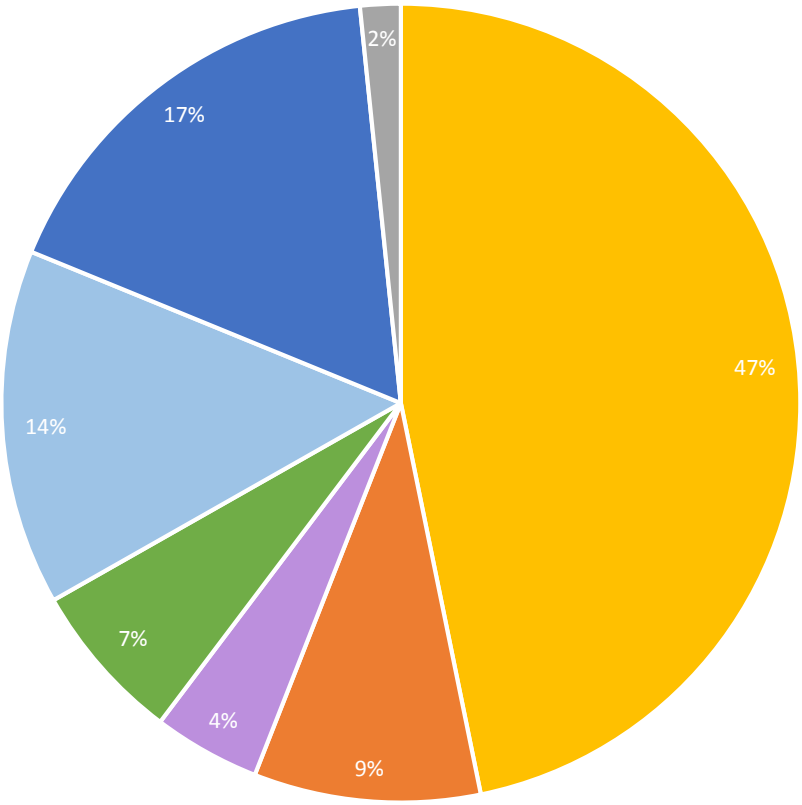
Members of the Yale iGEM team, from left to right: Ariel Hernandez-Leyva, Yamini Naidu, Stephanie Mao, and Alexandru Buhimschi, pose in front of their poster and demonstration



The iGEM team takes a photo with one of their judges, from Anhui University of Technology

Alexandru Buhimschi presents his results on contact angle measurements during the presentation

Report of Expenditures



Cost By Category

Category	Cost
Competition	$7,250.00
Lodging	$1,420.80
Other	$671.51
Services	$1,006.50
Reagents	$2,230.83
Supplies	$2,661.40
Travel	$254.00
Total:	$15,495.04

Itemized List of Expenditures

Date	Description	Category	Vendor	Amount
3/28/14	iGEM 2014 Team Registration Fee	Competition	iGEM HQ	$3,500.00
7/2/14	Rnase A 100mg	Reagents	Medical School Stockroom at Yale	$30.09
7/2/14	Hifi Hotstart Readymix 500rxns	Reagents	Fisher Scientific	$367.50
7/2/14	Kapa 2g 500x25 uL	Reagents	Fisher Scientific	$137.90
7/2/14	2-Log DNA ladder 1.25ml	Reagents	Kline Stockroom at Yale	$46.92
7/2/14	Sybr Safe DNA Gel Stain 400uL	Reagents	Kline Stockroom at Yale	$53.10
7/2/14	Glycerol Anhydrous 500mL	Reagents	Kline Stockroom at Yale	$14.52
7/2/14	Tryptone 500g	Reagents	Kline Stockroom at Yale	$47.96
7/2/14	Yeast Extract 1kg x2	Reagents	Kline Stockroom at Yale	$78.00
7/2/14	Sodium Chloride 1kg	Reagents	Kline Stockroom at Yale	$8.29
7/2/14	TBE Buffer 10x solution 4L	Reagents	Kline Stockroom at Yale	$30.52
7/2/14	Pipette Hydrophobic Filters 0.45um	Supplies	Thermo Scientific	$22.83
7/2/14	S1 Pipette Filler	Supplies	Thermo Scientific	$391.95
7/2/14	Gene Pulser/ Micropulser Cuvettes	Supplies	Kline Stockroom at Yale	$104.80
7/2/14	Falcon Petri Dishes 100 x 15mm x500	Supplies	Fisher Scientific	$72.25
7/2/14	Serological Pipette 10ml x 50 x 4	Supplies	Fisher Scientific	$26.24
7/2/14	Serological Pipette 25ml x 50 x 4	Supplies	Fisher Scientific	$68.20
7/2/14	Serological Pipette 5ml x 50 x 4	Supplies	Fisher Scientific	$33.20
7/2/14	Strip Tubes and Caps x 80 x 2	Supplies	Fisher Scientific	$214.00
7/2/14	Spinsmart Plasmid Columns x50	Supplies	Kline Stockroom at Yale	$41.00
7/2/14	pcr Purification columns	Supplies	Kline Stockroom at Yale	$46.00

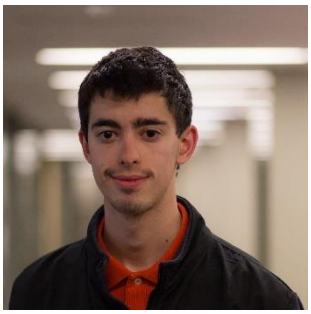
7/9/14	Ethanol 100% 500ml	Reagents	Kline Stockroom at Yale	$31.32
7/9/14	Bel-Art Autoclave Gloves	Supplies	Fisher Scientific	$31.45
7/9/14	Bel-Art Polypropylene Sterilizing Trays	Supplies	Fisher Scientific	$52.43
7/13/14	Gibson Assembly Master Mix 50rxns	Reagents	New England Biolabs	$502.40
7/16/14	Keck Gene Sequencing (x20 sequences)	Services	Keck	$58.00
8/1/14	Pipette Calibration x15	Supplies	Pipette Calibration Services	$395.00
8/5/14	2G Fast Hot Start Readymix 500rxns	Reagents	Medical School Stockroom at Yale	$131.25
8/5/14	DpnI Restriction Enzyme x5000	Reagents	New England Biolabs	$192.00
8/5/14	Sterile Reagent Reservoirs, 25ml, x100	Supplies	Fisher Scientific	$58.53
8/5/14	Corning 96 well culture plate x50	Supplies	Fisher Scientific	$69.52
8/11/14	Jamboree Registration for 5 members	Competition	iGEM HQ	$3,750.00
8/11/14	Hotel Reservations (2 rooms, 4 nights)	Lodging	Holiday Inn	$1,420.80
8/26/14	Gene Construct Synthesis	Services	Genscript	$598.50
9/8/14	Corning Cell-Tak 1mg	Reagents	Fisher Scientific	$225.03
9/9/14	iGEM 2014 Wiki Page and Logo	Services	Design at Yale	$50.00
9/24/14	3-(3,4-Dihydroxyphenyl)-L-alanine, 25g	Reagents	Fisher Scientific	$249.30
9/24/14	Twin-Strep Purification Kit, E. coli	Supplies	IBA GMBH	$1,034.00
10/9/14	Same Day Delivery of Biobricks	Services	Ground Transport	$300.00
10/17/14	D-Biotin 1g	Reagents	Fisher Scientific	$84.73
10/26/14	Yale iGEM Team Shirts	Other	CustomInk	$296.78
10/26/14	Transportation to Jamboree	Travel	Amtrak	$146.00
10/30/14	Team Dinner at Jamboree	Other	Douzo Modern Japanese	$77.27
10/30/14	Transportation to Jamboree	Travel	Amtrak	$54.00
10/30/14	Transportation to Jamboree	Travel	Amtrak	$54.00
10/31/14	Team Breakfast at Jamboree	Other	Paris Creperie	$27.71

11/1/14	Team Dinner 2 at Jamboree	Other	Paris Creperie	$77.40
11/1/14	Team Lunch at Jamboree	Other	Atrium Café	$73.28
11/1/14	Team Breakfast 2 at Jamboree	Other	Paris Creperie	$28.04
11/2/14	Team Lunch 2 at Jamboree	Other	Paris Creperie	$42.77
11/2/14	Team Breakfast 3 at Jamboree	Other	Paris Creperie	$13.80
11/3/14	Team Lunch 3 at Jamboree	Other	Paris Creperie	$42.91
11/3/14	Breakfast at Jamboree	Other	Au Bon Pain	$5.35

Undergraduate Team Members

Researchers

Alex Buhimschi ‘17

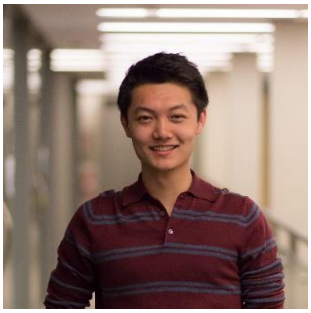


Ariel Hernandez-Leyva ‘16

Stephanie Mao ‘17

Yamini Naidu ‘17

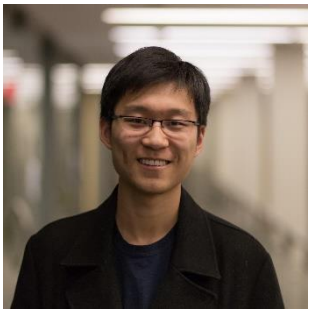
Board Members



Jiahe (Ben) Gu ‘16

Ming Chen ‘15

Joel Sher ‘16



Ed Kong ‘16

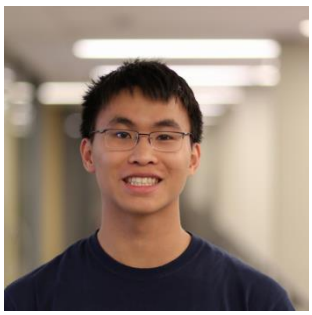
Advisors

Natalie Ma

Farren Isaacs (PI)

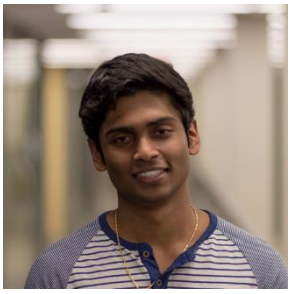
Web Design

Ananth Punyala ‘16



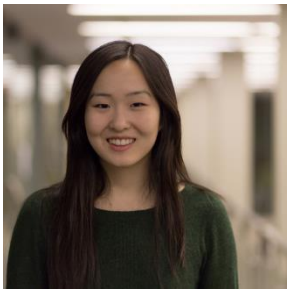
Cameron Yick ‘17

Additional Team Members

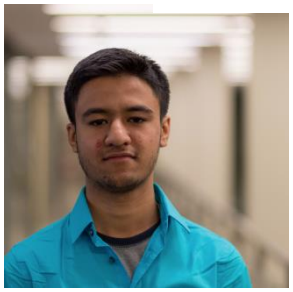


Anoj Ilanges ‘16

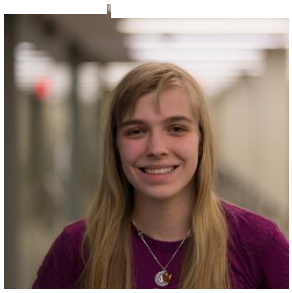
Nolan Maloney ‘16



Cathy Ren ‘16



Akarsh Sharma ‘17



Olga Wroblewski ‘16

Additional Advisors (not pictured):

Alexis Rovner, Ryan Gallagher, Jaymin Patel, Edward Barbieri, Miriam Amiram

Administrative Support: Amy Mulholland

We are extremely grateful for the support of our research advisors who have it made possible for us to design, execute and present such a project. We are also incredibly indebted to our sponsors both at Yale and outside of Yale for providing us the necessary space, finances and resources to operate our research lab, purchase reagents, and register and travel to compete. We look forward to an even better 2015 competition year and hope to have your continued support. Thank you!

For additional information on our project please see our project wiki (http://2014.igem.org/Team:Yale/Team), or reach out to us directly at igem@yale.edu.