AMPed Up E. coli

The Problem
Antibiotic-resistant microbes are a global health concern. Conventional antibiotics continue to lose the fight against troublesome microorganisms, which can quickly develop mutations that confer immunity. An innovative solution is crucial.

Antimicrobial peptides (AMPs) are proteins that are produced by all multicellular organisms as the first line of defense against infection. The nature of their biological structure and unique mechanisms of action enable the ability of microorganisms to develop resistance. Thus, AMPs hold great promise are produced by all multicellular organisms as the mutations that confer immunity.

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Our aim is to create a platform for production and purification of various antimicrobial peptides.

The Plan
- Design and construct new AMP BioBricks
- Create a method of purifying AMPs
- Construct functionalized antimicrobial biomaterials

Applications
Purified AMPs could be used as:
- Supplements to medical sutures and bandages
- Scaffolds for novel antibiotics
- Effective treatment for drug-resistant microbes

Design of AMP constructs
Antimicrobial peptides (AMPs) were codon optimized for expression in E. coli. These AMP sequences were designed as Assembly Standard #23 (Silver Fusion) to allow for protein fusions, and were synthesized with a methionine (atg) residue at the start of the coding region. 10x His-Tag (BBa_K844000) for C-terminal purification was used to recover protein products because it has been proven to be both functional and highly effective.

In addition to the expression of AMPs in E. coli, composite antimicrobial spider silk BioBricks were designed. Spider silk has extraordinary mechanical properties and biological compatibility. Thus, fusing AMPs to spider silk may lead to potential applications in the food (e.g., packaging & storage) and medical (e.g., sutures & implants) industries.

Antimicrobial Biomaterials
Design of AMP production and purification system using BioBricks

Results
N-terminal 10x His-Tag
We successfully designed, built, and tested a 10x His-Tag for N-terminal protein purification. This new His-Tag was fused to GFP in order to test its functionality. The SDS-PAGE gel below shows the purified His-Tag protein fractions from nickel affinity chromatography. The purity in the elution fractions demonstrates that this part (BBa_K1162009) offers an alternative to C-terminal purification using BioBricks.

AMP Expression
GFP/His (BBa_K1162012) was used as a protein fusion partner to monitor AMP production in E. coli. The flow through fractions demonstrate successful AMP expression.

Antimicrobial spider silk
AMP BioBricks were cloned together with 10x His-Tag. After E. coli harboring our LL37 spider silk generator (BBa_K1162306), was cultured and purified. The gel below shows the crude and purified protein fractions after nickel affinity chromatography. The protein band at ~55 kDa demonstrates that we have produced antimicrobial spider silk using BioBricks.

Summary
- Created 47 new BioBricks and submitted 16 to the Parts Registry
- Designed and constructed new AMP BioBricks
- Added to the protein purification tool box through the demonstration of a functional N-terminal 10x His-Tag
- Manufactured AMP-spider silk using BioBricks

Future work
- Optimize scale-up and purification of AMPs
- Test various AMP and AMP-spider silk activities against pathogenic bacteria

Outreach/Human Practices
Our team led two outreach sessions attended by over 140 students from five states to allow the students to learn more about synthetic biology as well as introduce them to the iGEM competition. After these sessions concluded, the students filled out a survey (results below).

Safety Considerations
Discussed safety and ethical implications of our project with a university biological safety committee member Dr. Walsh.

Table: Survey Results

<table>
<thead>
<tr>
<th>Question</th>
<th>Response</th>
<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td>Do you think synthetic biology is safe?</td>
<td>Yes</td>
<td>80%</td>
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<tr>
<td>What is the most useful potential application for synthetic biology?</td>
<td>Healthcare</td>
<td>75%</td>
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We designed an activity to better explain standard operating procedures.