How can Synthetic Biology Improve Medicine?
Current Medical Therapies

Specific Effects
Non-specific effects

Parameters:
1. Targeting Specificity
2. Dosage Control
How can we use Synthetic Biology to improve Targeting Specificity and Dose Control? By Engineering Bacteria to act as the Therapeutic.
Cancer As a Case Study

Current Cancer Therapies

Targeting Specificity
- Spatial – Radiation Therapy
  or
- Cellular – mAb, Chemotherapy

Dose Control
- Passive Diffusion
- Dose Scheduling
# Cancer As a Case Study

<table>
<thead>
<tr>
<th>Current Cancer Therapies</th>
<th>Proposed Bacterial Therapy</th>
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<tbody>
<tr>
<td><strong>Targeting Specificity</strong></td>
<td><strong>Targeting Specificity</strong></td>
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<tr>
<td>Spatial – Radiation Therapy</td>
<td>Combined Spatial and Cellular Targeting</td>
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<tr>
<td>or</td>
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<tr>
<td>Cellular – mAb, Chemotherapy</td>
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<tr>
<td><strong>Dose Control</strong></td>
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<tr>
<td>Passive Diffusion</td>
<td>Active Diffusion</td>
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<tr>
<td>Dose Scheduling</td>
<td>Tunable Transgene Expression System</td>
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**Introduction**

**Light-Based Drug Delivery**

**Surface Display & Targeting**

**Human Practices**

**Conclusion**
Achieving Light-Activated Cell Lysis

Goal is to demonstrate that:

• YF1/FixJ (pDawn) blue light sensor can be activated for downstream transgene expression

• YF1/FixJ blue light sensor allows for light dependent lysis of mammalian cells
### YF1/FixJ BL Sensor Allows For Light-Dependent Transgene Expression

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Image of Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>0h</td>
<td>![Image](YF1/FixJ BL Sensor)</td>
</tr>
<tr>
<td>1h</td>
<td>![Image](YF1/FixJ BL Sensor)</td>
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<tr>
<td>2h</td>
<td>![Image](YF1/FixJ BL Sensor)</td>
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<tr>
<td>3h</td>
<td>![Image](YF1/FixJ BL Sensor)</td>
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<td>4h</td>
<td>![Image](YF1/FixJ BL Sensor)</td>
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<td>6h</td>
<td>![Image](YF1/FixJ BL Sensor)</td>
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<tr>
<td>8h</td>
<td>![Image](YF1/FixJ BL Sensor)</td>
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<tr>
<td>22h</td>
<td>![Image](YF1/FixJ BL Sensor)</td>
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</tbody>
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**pDawn-mCherry Fluorescence**

<table>
<thead>
<tr>
<th>Time in Light (h)</th>
<th>Fluorescence/OD600 (a.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
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<tr>
<td>10</td>
<td>1.0</td>
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<tr>
<td>15</td>
<td>1.5</td>
</tr>
<tr>
<td>20</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
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</tbody>
</table>
Cytolysin A (ClyA) (Wallace et. al, 2000)
Light-triggered lysis of mammalian cells by pDawn-ClyA bacteria
Spatial control of cell lysis

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Characterizing ClyA-mediated lysis of SKBR3 cancer cells

ClyA Cytotoxicity in SKBR3 cells (High HER2 Level Expression)

ClyA Cytotoxicity in HEK293T cells (Basal HER2 Level Expression)

**** p<0.0001
Ice Nucleation Protein, NC Domain

- Outer membrane protein
  Discovered in *P. syringae*
- Promotes ice crystallization
- Can remove internal repeats and display proteins on the surface of *E. coli*
Displaying DARPin H10-2-G3
Assaying Display of DARPin H10-2-G3

- HA tag allowed detection of surface proteins

![Image](image-url)

INPNC-HA (-IPTG)  INPNC-HA (+IPTG)
DARPin was Successfully Displayed!

DARPin-HA (-IPTG)

DARPin-HA (+IPTG)

INPNC-DARPin-HA (-IPTG)

INPNC-DARPin-HA (+IPTG)
Can our Bacteria Bind to Cancer Cells?

• SKBR3 Cells are derived from breast tumors
• Overexpress HER2

DARPin-displaying Bacteria Bind to SKBR3 Cells Preferentially

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HEK293T (Basal HER2)

SKBR3 (High HER2)

+E. coli (-IPTG)

+E. coli (+IPTG)

DAPI

HER2

eGFP

+IPTG

-IPTG
DARPin-displaying Bacteria Bind to SKBR3 Cells Preferentially
Submitted BioBricks

- ClyA BioBricks: BBa_K811000-K811002
- INPNC BioBricks: BBa_K811003-K811004
- INPNC-MCS: BBa_K811005
- General surface display vector for any iGEM team
- Only one ligation needed!
- Regional “Best BioBrick, Engineered”
Human Practices
VerifiGEM is a program that matches iGEM teams with one another to check each other's biobricks. Our goal is to utilize the magnitude of teams competing in iGEM each year to work together to verify each other's parts through transformation, sequencing, and other functional protocols. Together, we as teams can maximize the accuracy of biobricks submitted to the registry.

Don't have an account? Register here

Get Started Now!

Click on register, submit all your team and biobrick information, and wait for an email informing you of your match!

Conditions

VerifiGEM depends on you! Please be honest about how many biobricks you anticipate being able to send as well as test. Most importantly, have fun!
VerifiGEM User Interface

Biobrick information

- Expected number of biobrick parts you will submit: 3
- Expected number of parts you will be able to test: 5

Note: This must be at least as many as you will submit.

- Expected date your biobricks will be ready (please estimate): 10/25/2012

Competencies (please check off the following protocols your team is able to perform for biobrick verification)

- Transformation: No
- Sequencing and Restriction Digest: No
- Protein Purification: Yes
Check out the teams participating!

000028 Registered Teams
Many people have tried to apply synthetic biology to treat disease.

- Over 75 Health/Medicine teams since 2009
- Many, many papers since 1995!

Where are they now?

Why?
Perception Barriers to Bacterial Therapeutics

Perception Barriers

• Negative portrayal of E. coli in the media
• Public unfamiliar with synthetic biology

Public perception of E. coli is negative
Perception Barriers: The Hype Cycle

Education & Outreach

- Presentation/Q&A session with high school students
- Clark Park Science Discovery Day
Biological Barriers to Bacterial Therapeutics

Biological Barriers

• E. coli produces compounds that are immunogenic
• Lab strains are poor candidates for use in the human body
Addressing the Biological Barrier

- **E. coli Nissle 1917**
  - Nonpathogenic
  - Used as a probiotic supplement in Canada and Europe
  - Low immunogenicity

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The pDawn Expression System is Functional in Nissle 1917

- Chemically competent Nissle 1917 achieved light controlled ClyA hemolysis
Recommendation for Future H&M iGEM Teams

• When designing a project, keep clinical endpoints in mind
• Utilize strains of bacteria like Nissle 1917
  – Scientific benefits
  – Perception benefits
• Use outreach events as an opportunity to smooth out the hype curve
Accomplishments

A Novel, Modular Platform for a Targeted Bacterial Therapeutic

**Light-Activated Drug Delivery**
- Functionalized bacteria as a light-activated drug delivery platform
- Demonstrated light-dependent hemolysis in a spatially controlled manner

**Surface Display and Targeting**
- Created an easily adapted surface display BioBrick
- First to display DARPin on the surface of *E. coli*
- Showed HER-2 dependent binding of bacteria to human cells

**Human Practices**
- VerifiGEM
- Analyzed barriers to bacterial therapeutics
- Nissle 1917 – future chassis for bacterial therapeutics
Future Directions

- Use other wavelengths of light (such as red) with more clinical relevance
- Decrease non-specific binding of our engineered therapeutic through modification of *E. coli* surface

- Port the entire system into Nissle 1917
- Test our system in a mouse model *in vitro*
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- Penn Engineering
- CORNING
- IDT Integrated DNA Technologies
- GENEWIZ
- mt Management & Technology
- BioLabs
Questions and Answers

Penn iGEM 2012
Targeted Bacterial Therapeutics
Supplementary Slides
Testing the System with mCherry
Testing the System with mCherry

- Fused INPNC to mCherry with 12aa GS Linker
- Sonicated INPNC-mCherry expressing *E. coli* and separated lysate and membrane fraction
DARPin Binding

HEK293T + 3nM DARPin

SKBR3 + 3nM DARPin
Coomassie Gel
Selective binding of SKBR3 cells in HEK293T/SKBR3 Co-Culture
Light-Activated Cytotoxicity

SKBR3 Light-Activated Cytotoxicity

% Cytotoxicity

Dark | Light
--- | ---
mCherry | ClyA