Cling-\textit{E. coli} : Bacteria on target

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The motivation
To develop a system for targeting bacteria to a specific substrate and effecting a cellular response
Potential Targets and Applications

Bind Proteins

Bind DNA/RNA

Bind Tissue

Bind Surface

Bind Viruses

Bind Other Cells

Bind Drugs/Toxins

Introduction

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Introduction

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Quorum-sensing

Bacterial targeting

Fec signal transduction
Bacterial targeting

Quorum-sensing

Fec signal transduction
Surface-engineered bacteria

- OmpA – C terminal insertion
- OmpA-Loop1 insertion
- AIDA-1 – N terminal insertion

Bacterial Targeting
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Selecting/enriching for surface-engineered bacteria

- **Tags**
  - Histidine tag + nickel
  - Strep2 tag + streptavidin

- **Assay**
  - Magnetic Activated Cell Sorting (MACS)
His and Strep2 tagged bacteria bind to beads
His and Strep2 tagged bacteria bind to beads

Colony counts after MACS selection of tagged bacteria

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDA1 + strep2</td>
<td>235</td>
</tr>
<tr>
<td>AIDA1 + his</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>11</td>
</tr>
</tbody>
</table>
Results

• Surface-expression vehicle – AIDA1
• Engineered surface-displayed histidine tag and strep2 tag
• Demonstrated bacterial targeting to nickel and streptavidin beads through MACS
Bacterial targeting

Quorum-sensing

Fec signal transduction

Quorum Sensing

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luxI/luxR Quorum Sensing

Quorum Sensing
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Cell-Cell Signaling Constructs

- Receivers (luxR + Reporter)
  - GFP Receivers
    - tetR controlled (Bba_T9002)
    - Quorum controlled (Bba_R0062 + Bba_C0261 + Bba_E0240)
  - mRFP Receivers
    - tetR controlled (Bba_F2620 + Bba_I13507)
    - Quorum controlled (Bba_R0062 + Bba_C0261 + Bba_I13507)
  - mCherry Receivers (Bba_F2620 + Bba_J06702)
- Senders (bicistronic luxI + Reporter)
  - mRFP Sender
    - tetR controlled (Bba_S03623 + Bba_I13507)
    - lacI controlled (Bba_S03608 + Bba_I13507)
    - Quorum controlled (Bba_R0062 + Bba_A340620 + Bba_I13507)
  - GFP Sender
    - tetR controlled (Bba_S03623 + Bba_E0240)
    - lacI controlled (Bba_S03608 + Bba_E0240)
    - Quorum controlled (Bba_R0062 + Bba_A340620 + Bba_E0240)
  - mCherry Sender
    - tetR controlled (Bba_S03623 + Bba_J06702)
- Single Cell
  - Constitutive (Bba_J23039 + Bba_T9002)
  - Quorum Controlled (Bba_R0062 + Bba_A340620 + Bba_C0261 + Bba_E0240)
- Construction Intermediates

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Switch-like Quorum Response

GFP Fluorescence per OD (response of receiver) vs. Amount of Sender Cells Added

Quorum Sensing
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MACS selection of cotransformed luxI-RFP/AIDA-strep2 cells
MACS selection of cotransformed luxI-RFP/AIDA-strep2 cells
MACS selection of cotransformed luxI-RFP/AIDA-strep2 cells
MACS selection of cotransformed luxI-RFP/AIDA-strep2 cells

Before

After

3%  97%

11%  89%
uxl-RFP/AIDA-strep2 cells clump around streptavidin beads
Selected luxI-RFP/AIDA-strep2 cells can send quorum signal
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Bacterial targeting

Quorum-sensing

Fec signal transduction

Fec signal transduction

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Motivation: Fec System

- Goal: Direct cell signaling
- Method: Re-engineer an existing signal transduction pathway
- Fec system:
  - well-characterized
  - only outer membrane signal transducer
Overview of Fec System

Ferric citrate

Outer membrane

Cytoplasmic membrane

Fec signal transduction

Loops 7 and 8 as potential insertion sites

Fec signal transduction
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Loops 7 and 8 as potential insertion sites

Fec signal transduction

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Constructs

• From Braun lab (U. Tuebingen, Germany)
  – Fec knock-out strain, AA93
  – FecIRA plasmid
  – $P_{\text{Fec}}$-GFP plasmid
• pColA Duet Vector
  – Allows regulated expression of Fec genes under T7 promoter
Fec-induced GFP expression

Fec signal transduction
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Troubleshooting and Next Steps

- **Problems:**
  - Growth media: WL vs. LB?
  - Toxicity: membrane disruption?

- **Goals:**
  - Nickel and Streptavidin Binding
  - Finding new targets with signaling
    - Random library
    - Computational Approach
Conclusions and Future Directions

- Surface engineering – AIDA1
- Histidine/Strep2 tags
- MACS

Random peptide libraries

- One-cell/two-cell quorum systems
- Characterized
- Targeted quorum senders

Optimize localized quorum response

Quorum-sensing

- Characterized wild-type Fec signaling

Computational design

Fec signal transduction

Bacterial targeting
Acknowledgements

Advisors
George Church
Debra Auguste
Jagesh V. Shah
William Shih
Pamela Silver
Alain Viel
Tamara Brenner

Special thanks to...
Volkmar Braun (U of Tuebingen)
Costas Maranas (Penn State U)

Teaching Fellows
Nicholas Guido
Bill Senapedis
Mike Strong
Harris Wang

Funding
HHMI
Harvard Provost
Harvard Life Sciences Division
Harvard School of Engineering and Applied Sciences

Conclusion
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