Cling-*E. coli* : Bacteria on target

Harvard iGEM 2007

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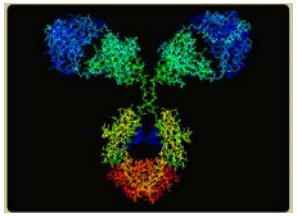
The motivation

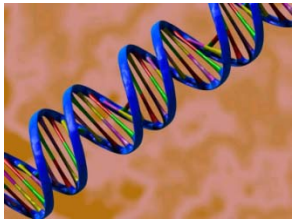
To develop a system for targeting bacteria to a specific substrate and effecting a cellular response

Potential Targets and Applications

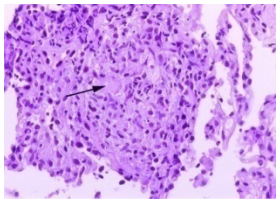
Bind Proteins

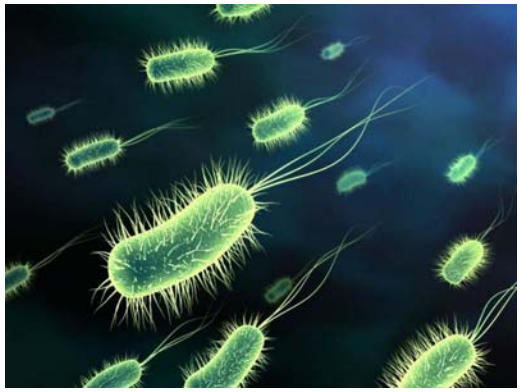


Bind DNA/RNA

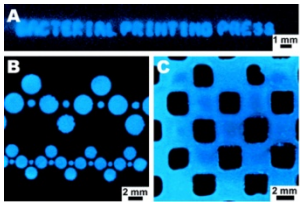


Bind Tissue

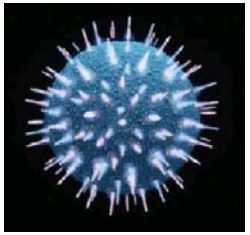




Bind Surface

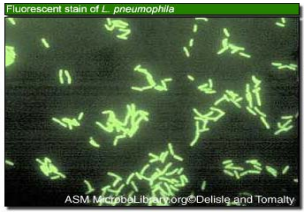


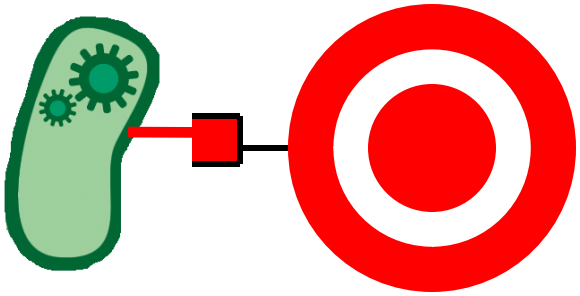
Bind Viruses



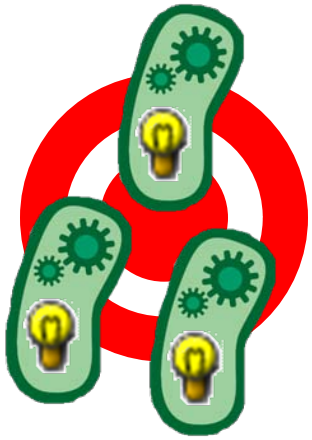
Bind Drugs/Toxins

Bind Other Cells

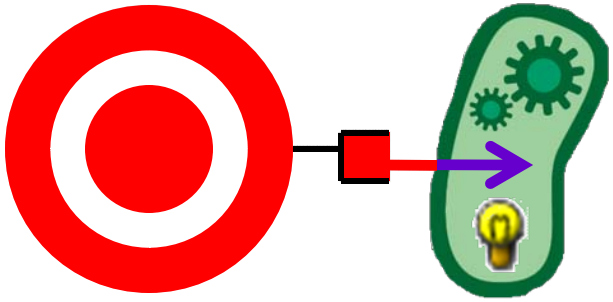




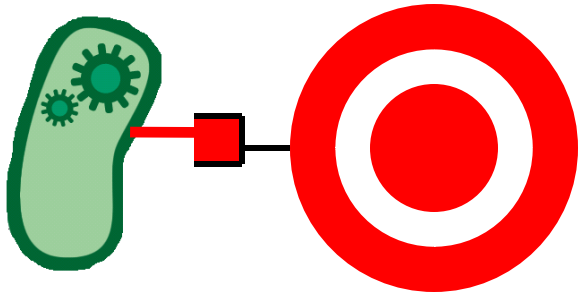
Bacterial targeting



Quorum-sensing

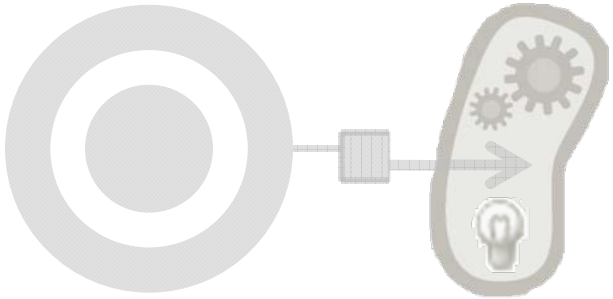


Fec signal transduction



Bacterial targeting

Quorum-sensing



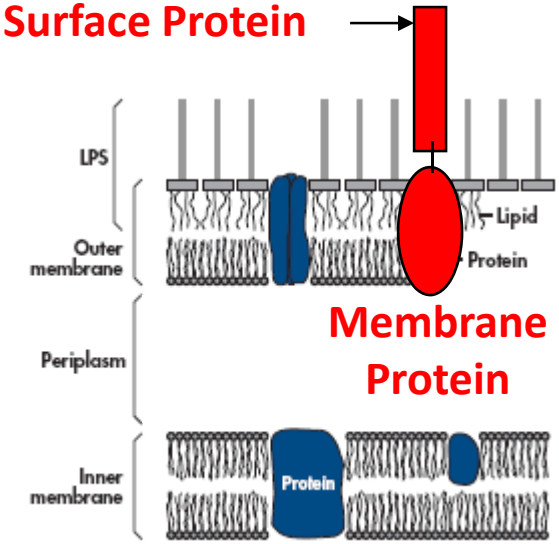
Fec signal transduction

Surface-engineered bacteria

OmpA – C terminal insertion

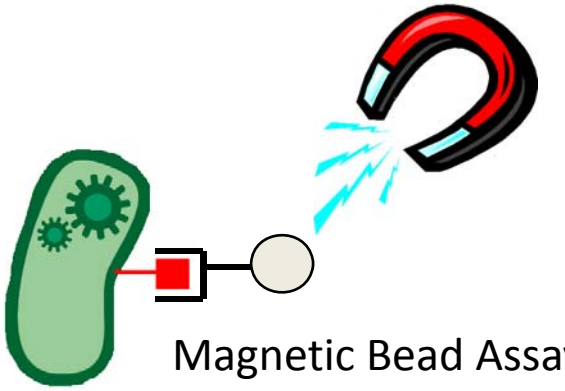
OmpA-Loop1 insertion

AIDA-1 – N terminal insertion



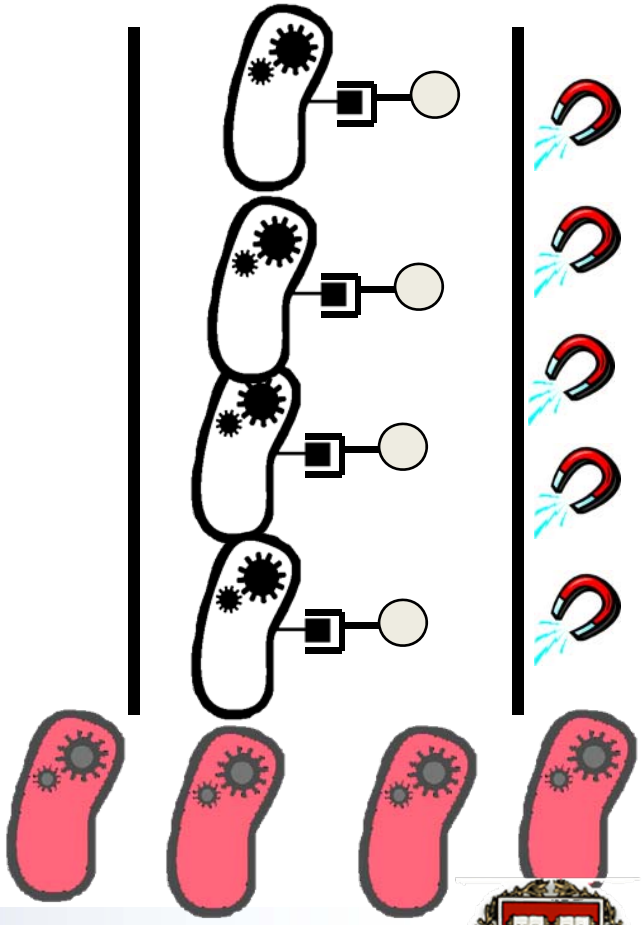
Selecting/enriching for surface-engineered bacteria

- Tags
  - Histidine tag + nickel
  - Strep2 tag + streptavidin
- Assay
  - Magnetic Activated Cell Sorting (MACS)



Magnetic Bead Assays

His and Strep2 tagged bacteria bind to beads



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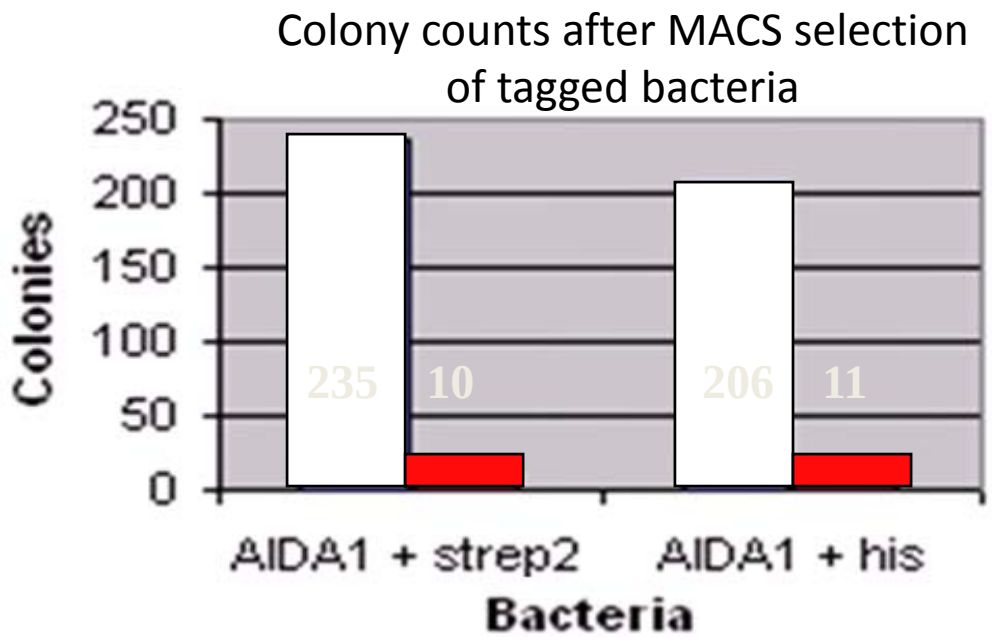
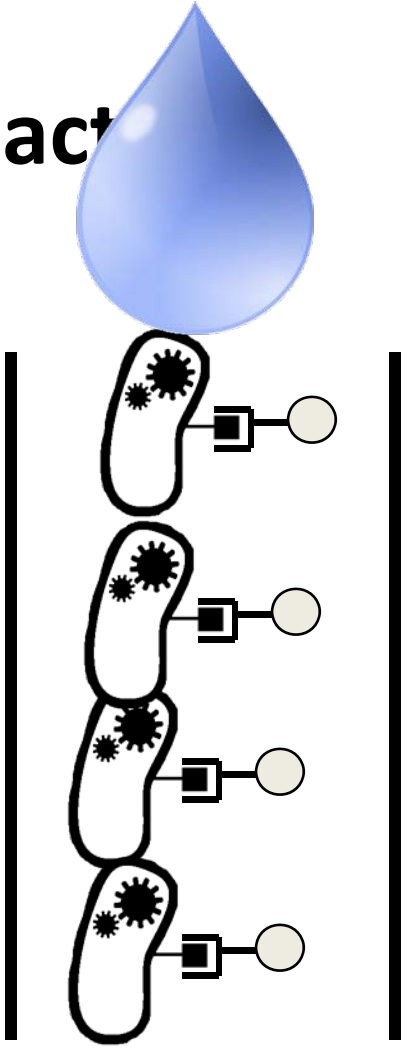
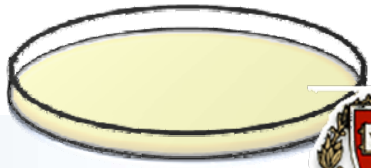
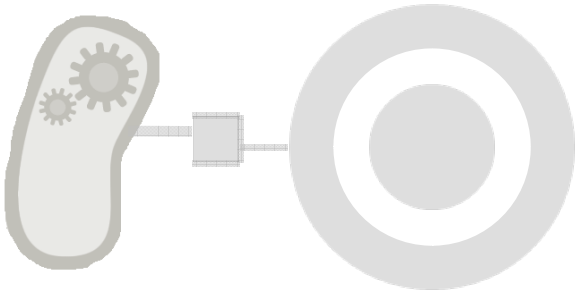




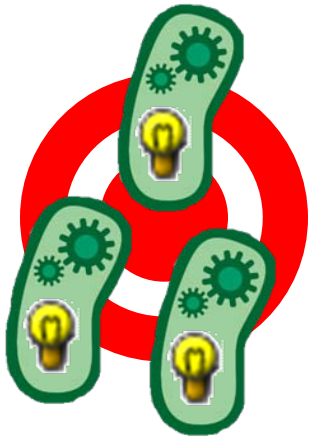


Results

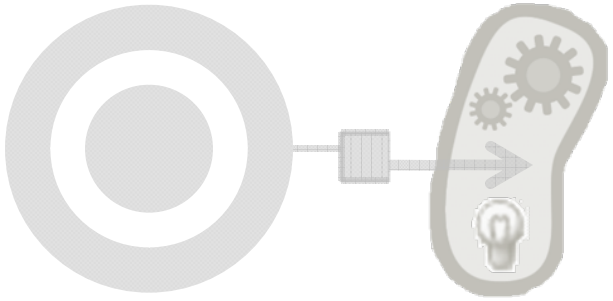
- Surface-expression vehicle – AIDA1
- Engineered surface-displayed histidine tag and strep2 tag
- Demonstrated bacterial targeting to nickel and streptavidin beads through MACS



Bacterial targeting

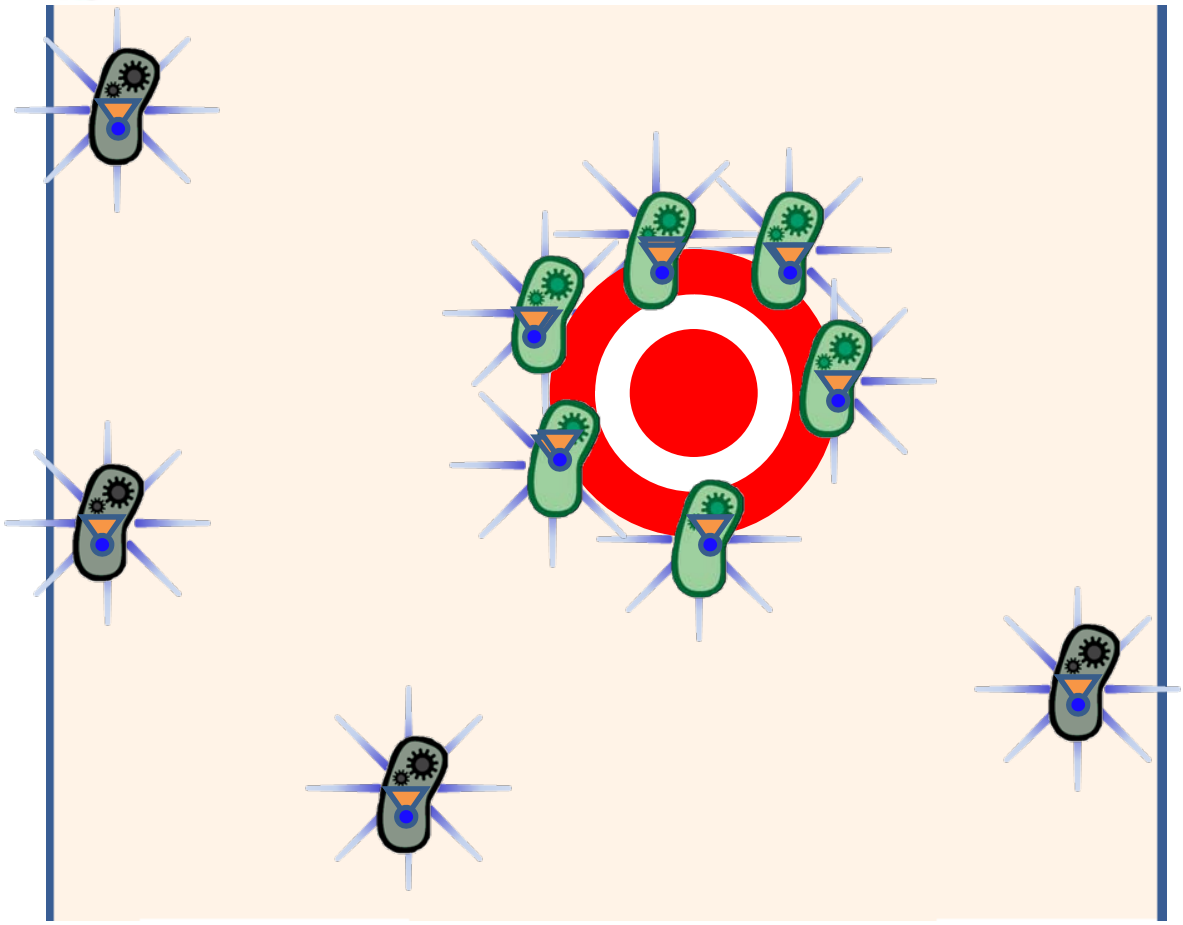


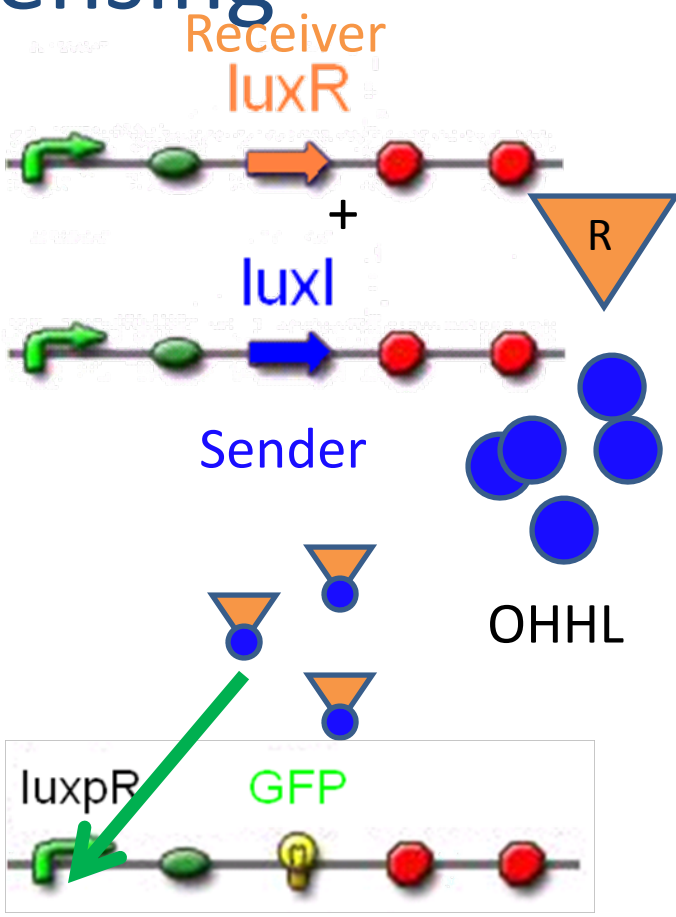
Quorum-sensing



Fec signal transduction

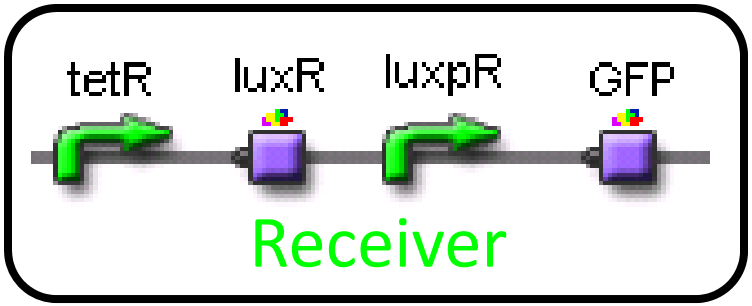
luxI/luxR Quorum Sensing

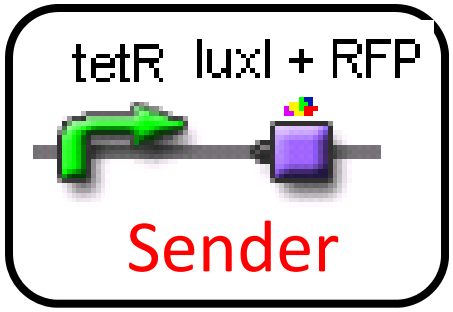




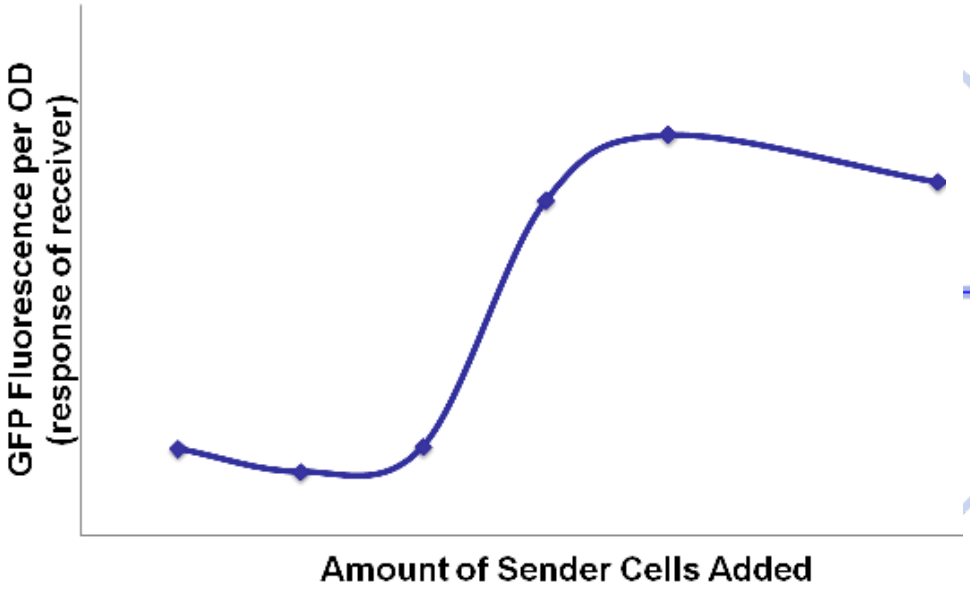
Cell-Cell Signaling Constructs

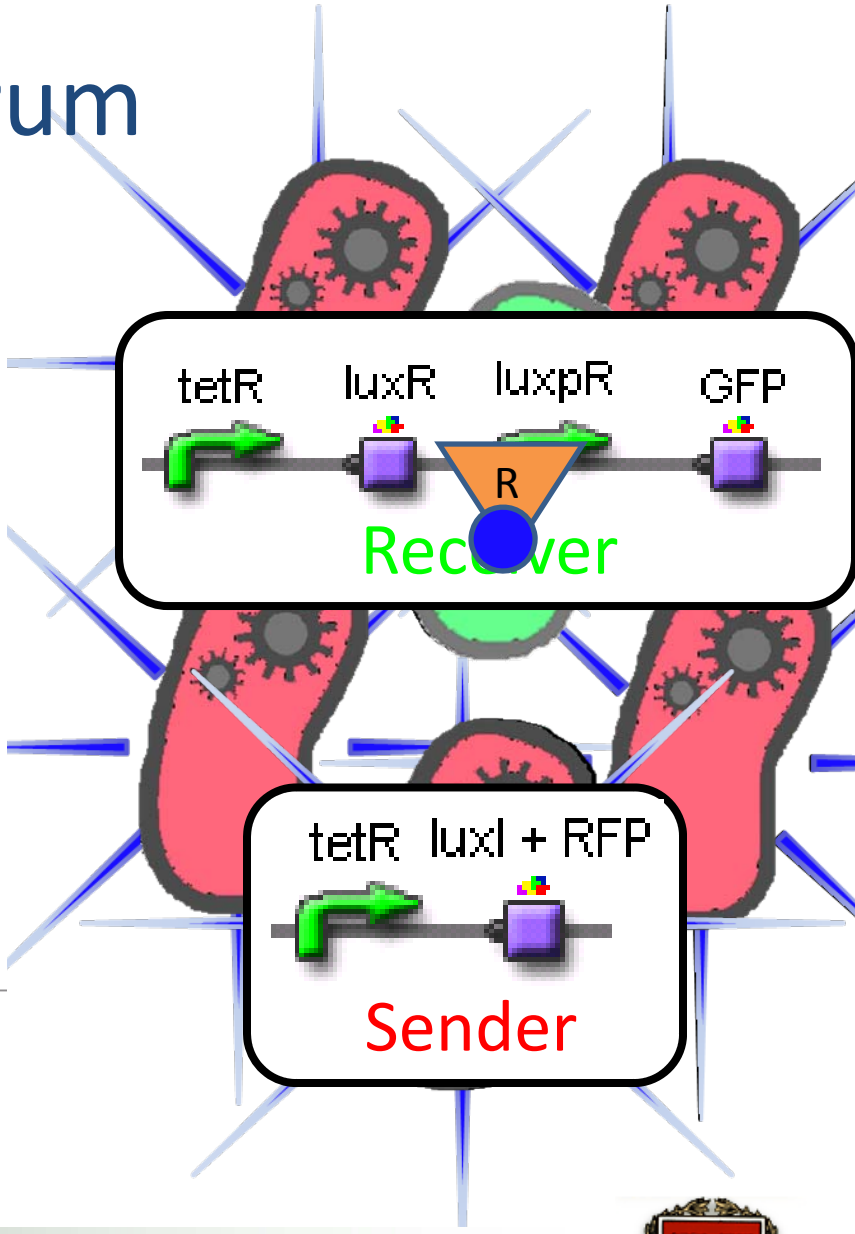
- Receivers (luxR + Reporter)
  - GFP Receivers
    - tetR controlled (Bba_T9002)
    - Quorum controlled (Bba_R0062 + Bba_C0261 + Bba_E0240)
  - mRFP Receivers
    - tetR controlled (Bba_F2620 + Bba_I13507)
    - Quorum controlled (Bba_R0062 + Bba_C0261 + Bba_I13507)
  - mCherry Receivers (Bba_F2620 + 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



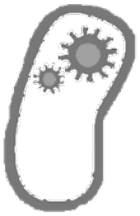


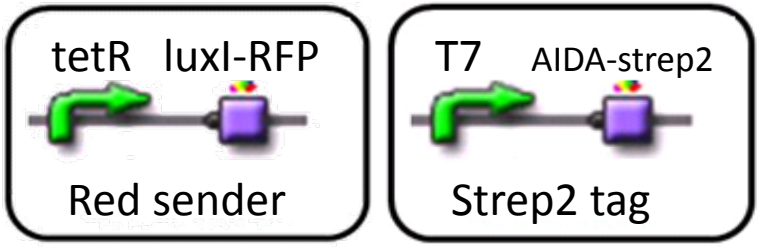
Switch-like Quorum Response





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





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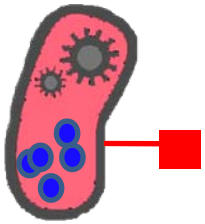

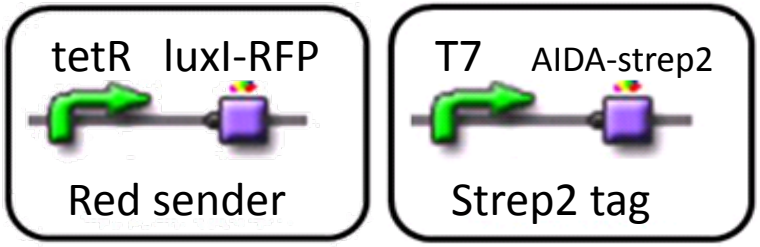

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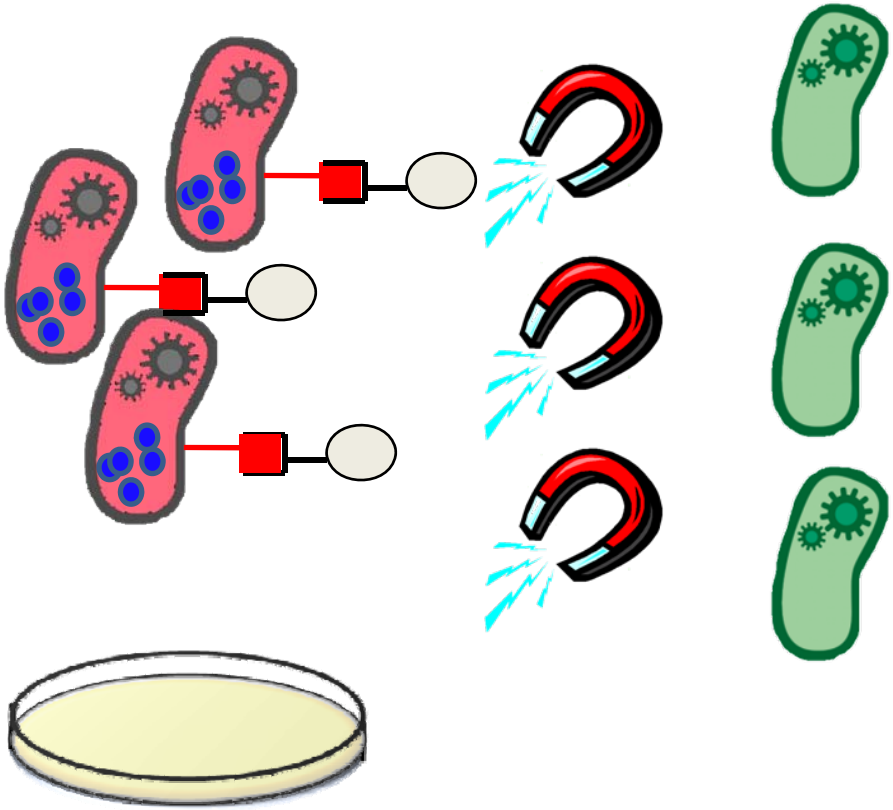
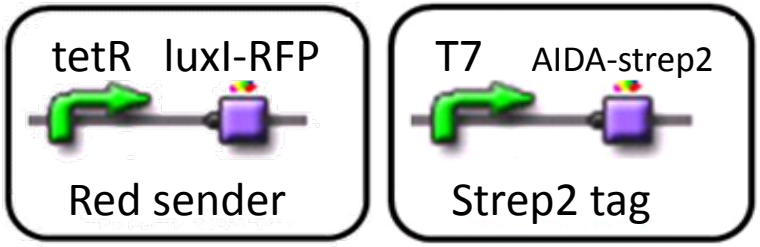
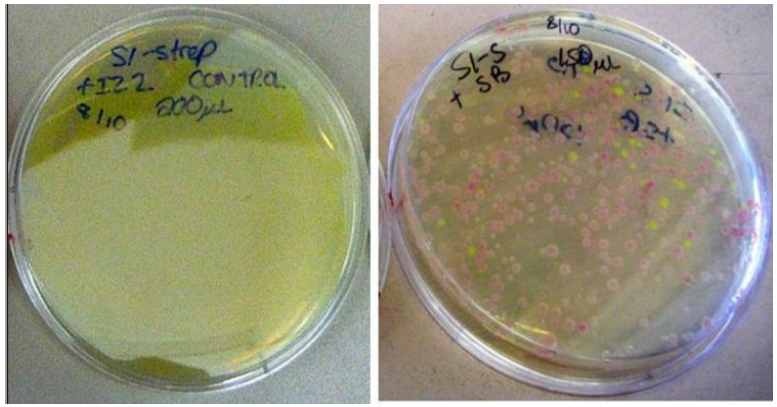


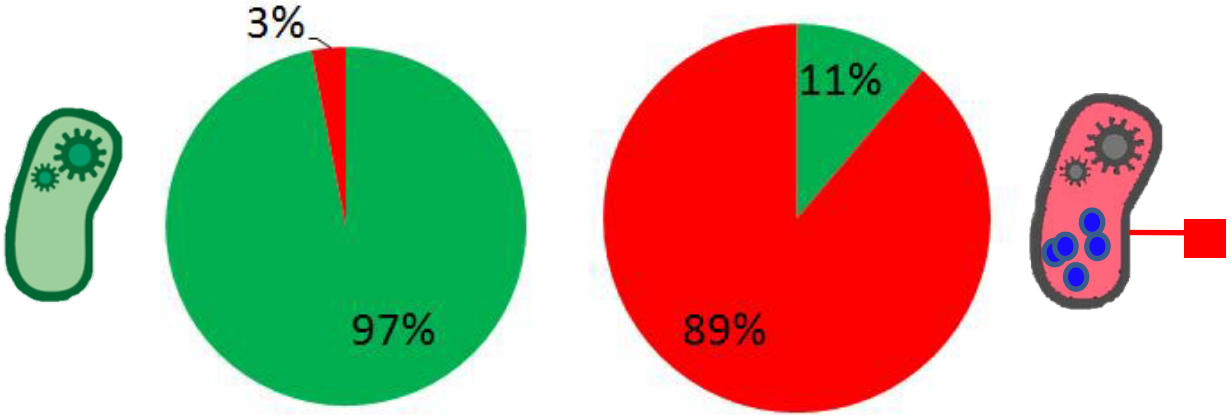


MACS selection of cotransformed luxI-RFP/AIDA-strep2 cells

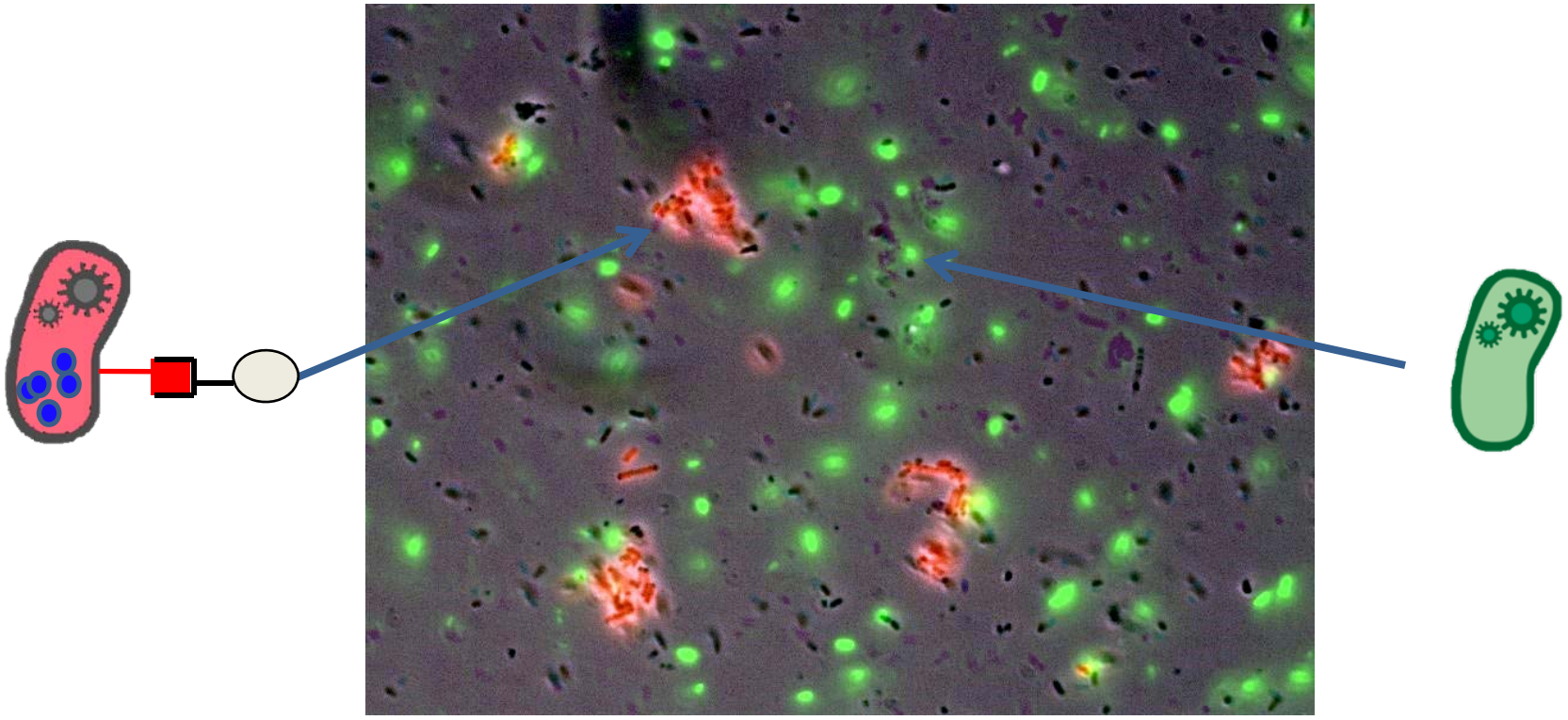
Before

After

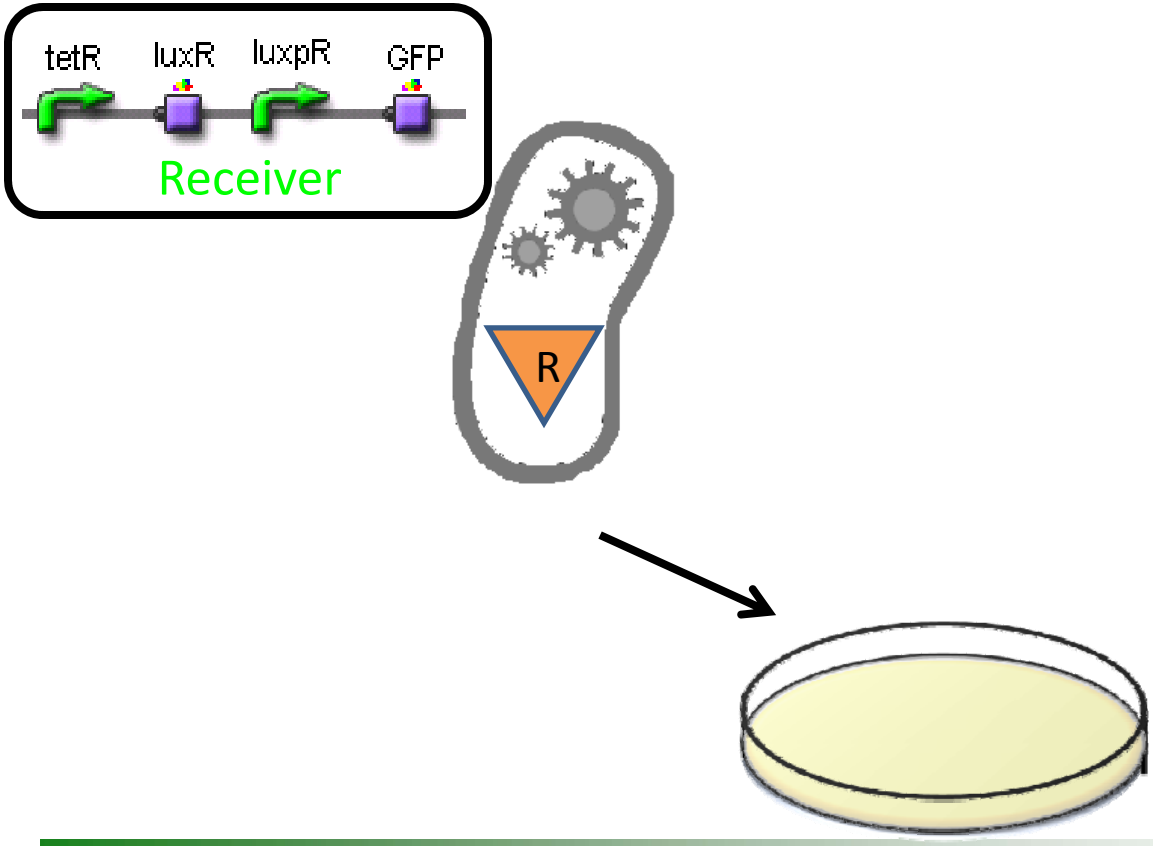




uxI-RFP/AIDA-strep2 cells clump around streptavidin beads



Selected luxI-RFP/AIDA-strep2 cells can send quorum signal



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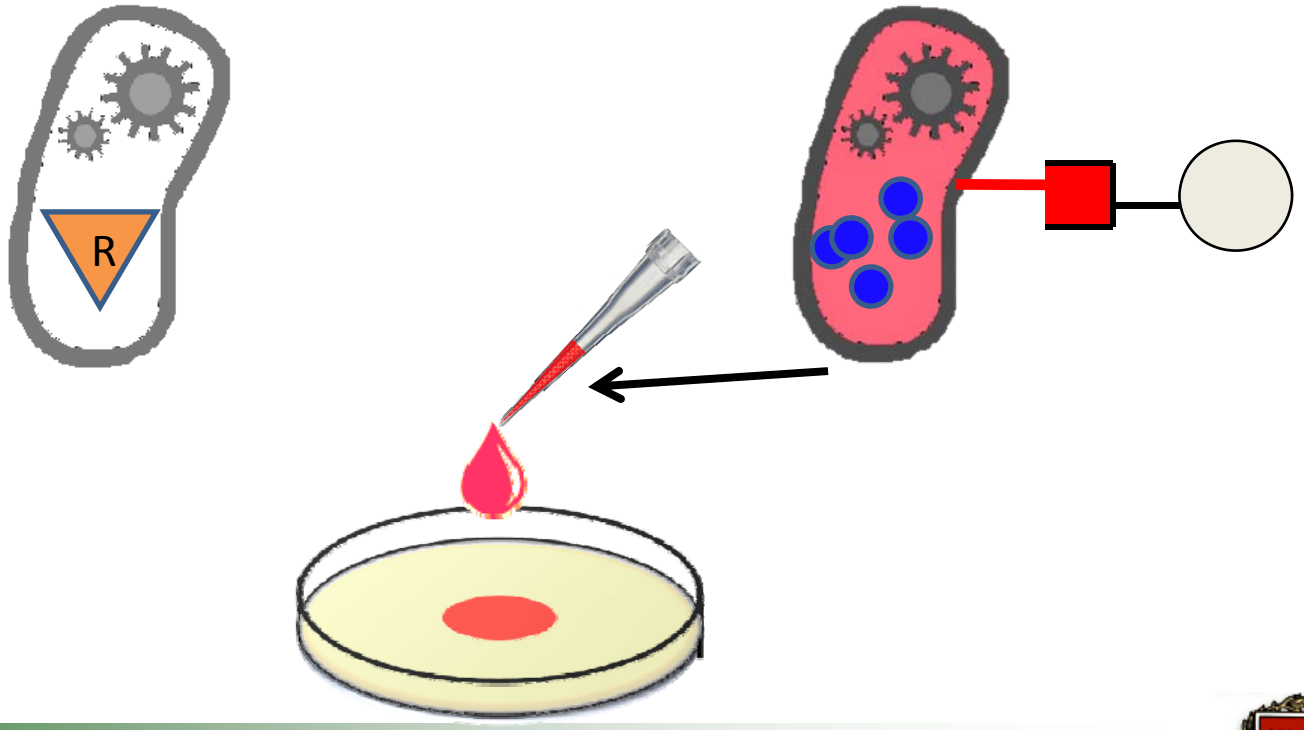

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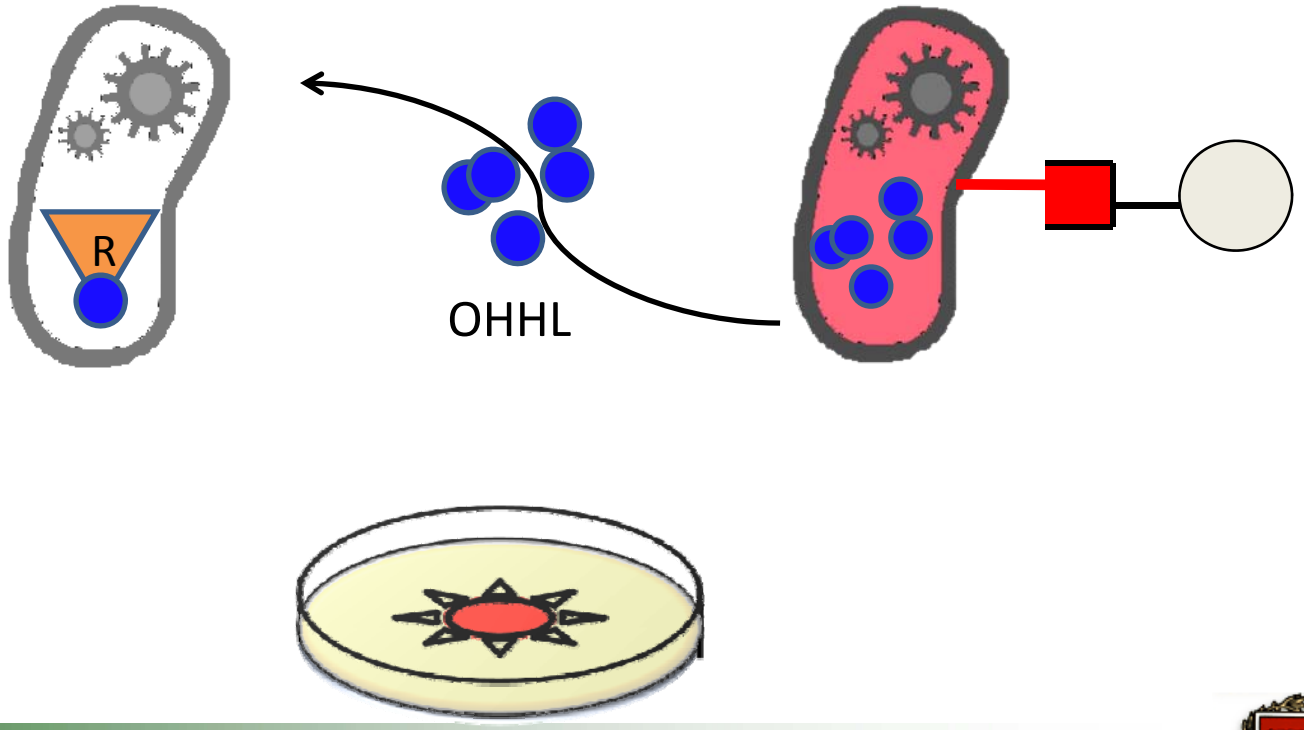

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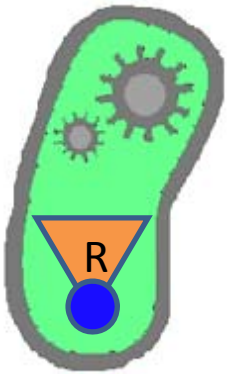
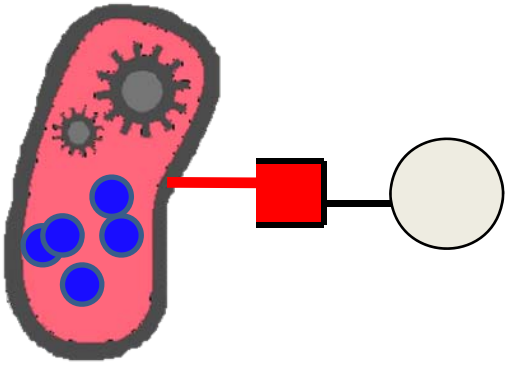
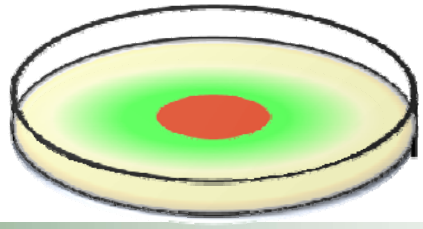
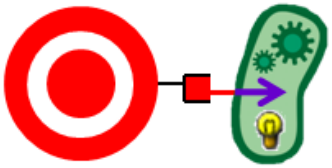
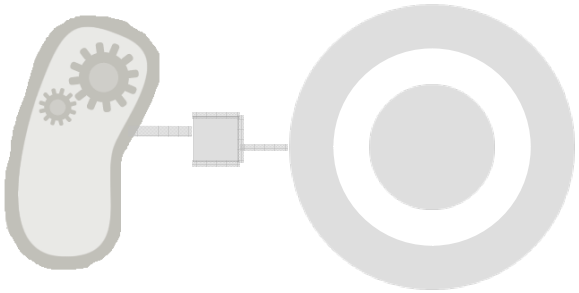


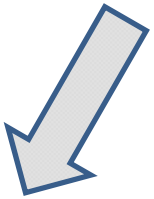






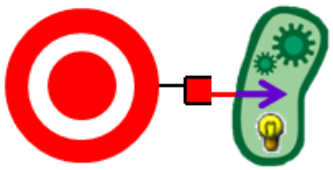


Bacterial targeting



Quorum-sensing

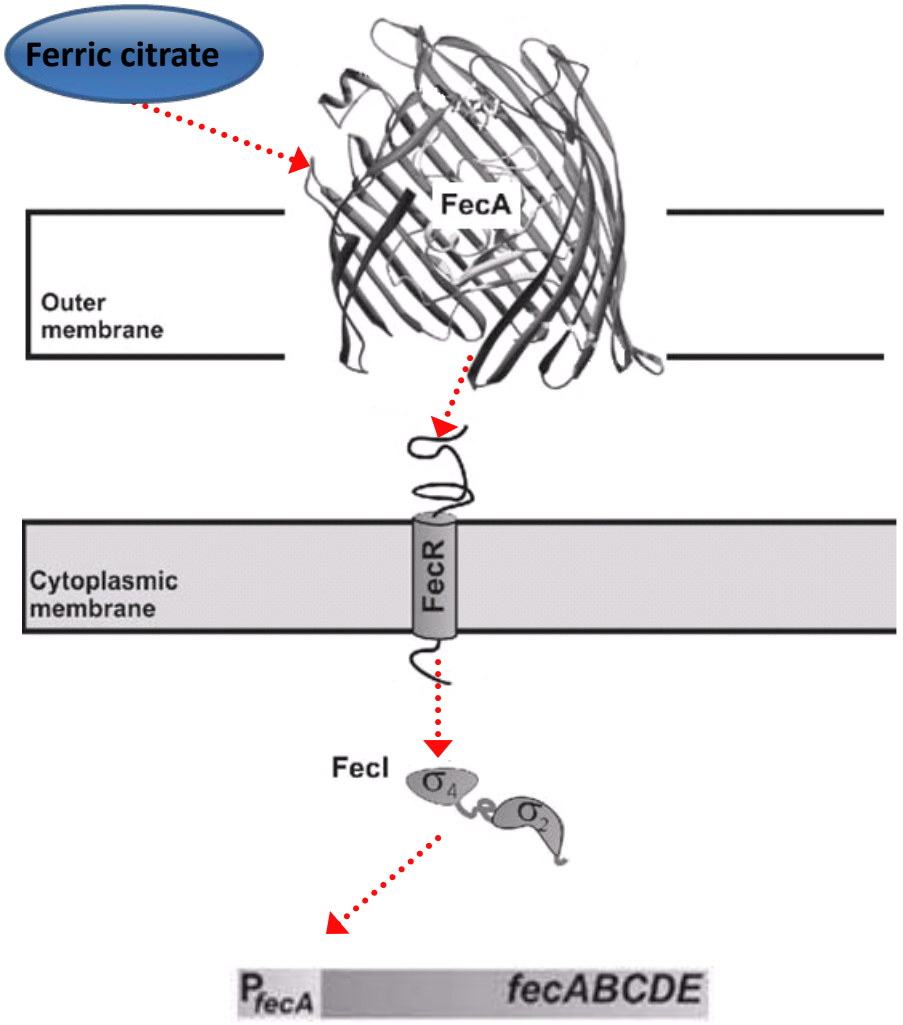
Fec signal transduction



Motivation: Fec System

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

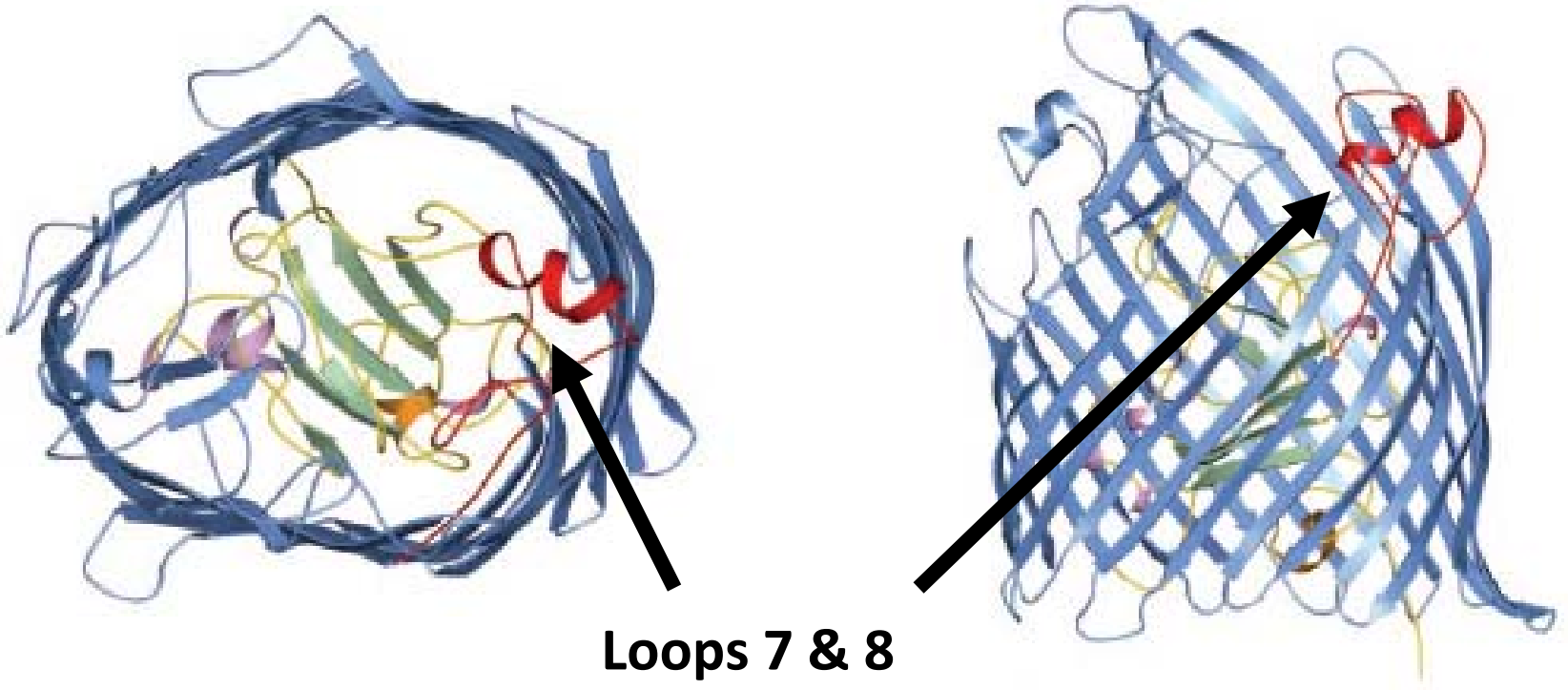
Overview of Fec System



Fec signal transduction

Braun et al. "Gene Regulation by Transmembrane Signaling." Biometals 2006 Apr;19(2):103-13.

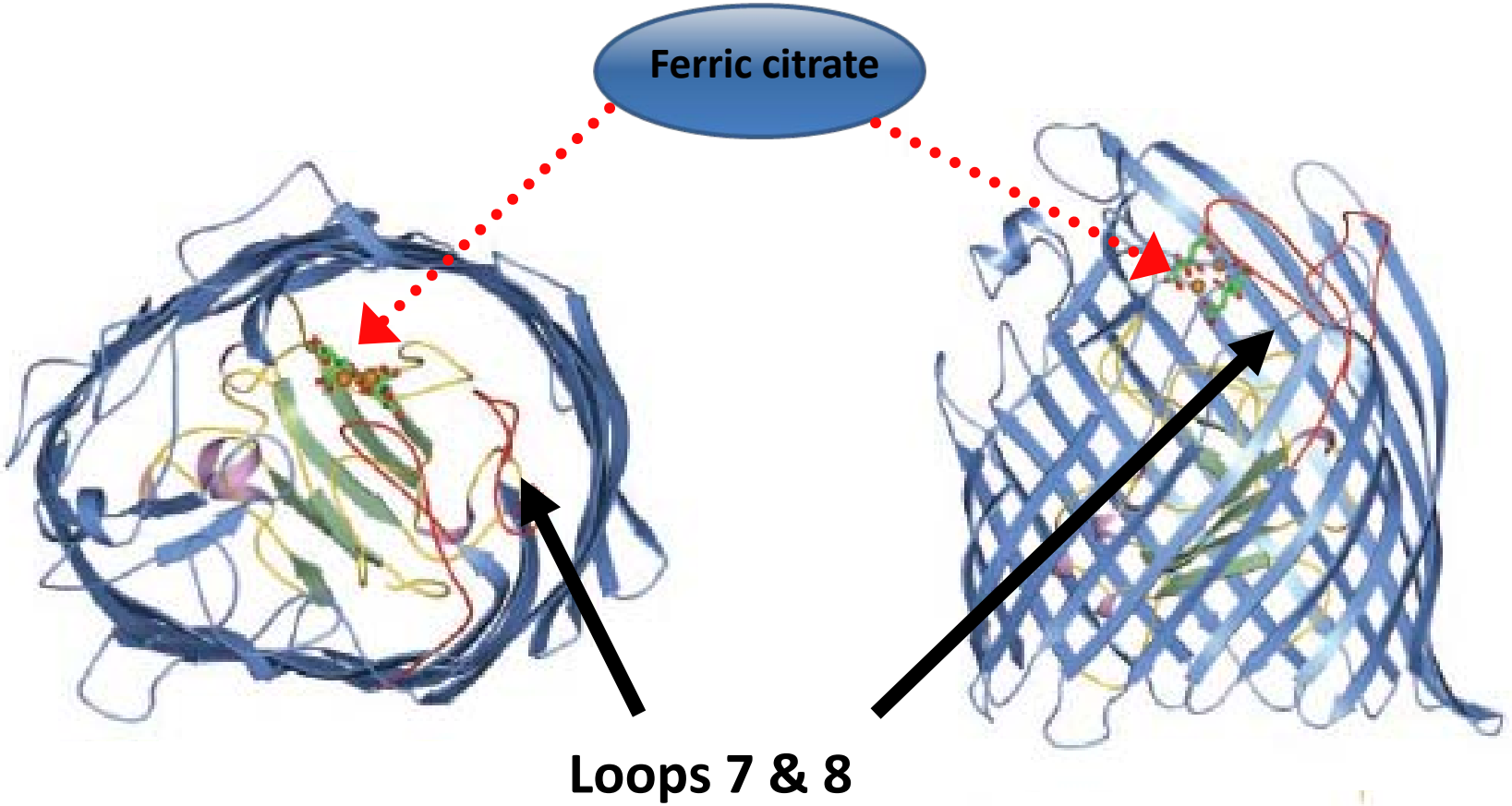
Loops 7 and 8 as potential insertion sites



Loops 7 & 8

Ferguson AD, et al. Structural basis of gating by the outer membrane transporter FecA. Science 2002 Mar 1; 295(5560) 1715-9.

Loops 7 and 8 as potential insertion sites



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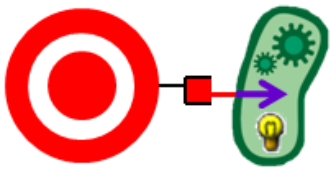
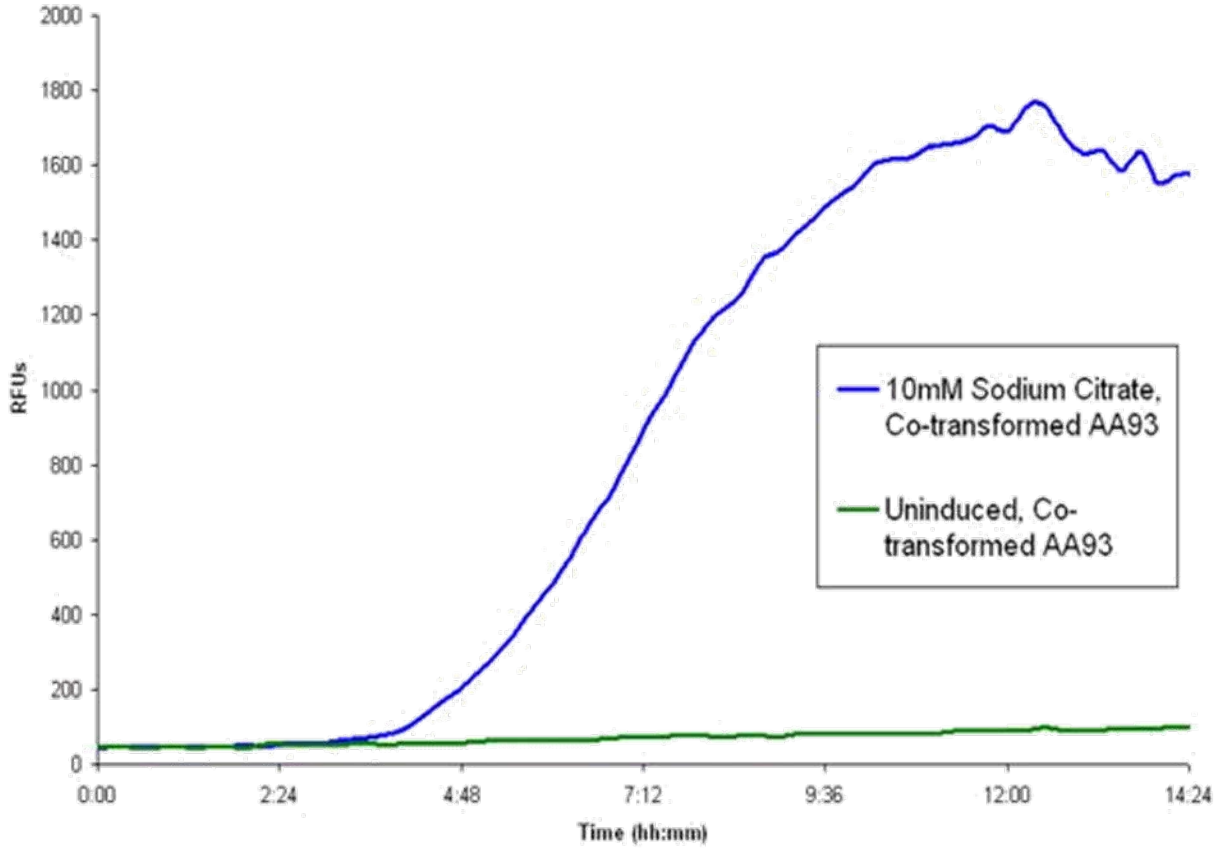


Constructs

- From Braun lab (U. Tuebingen, Germany)
  - Fec knock-out strain, AA93
  - FecIRA plasmid
  - P_{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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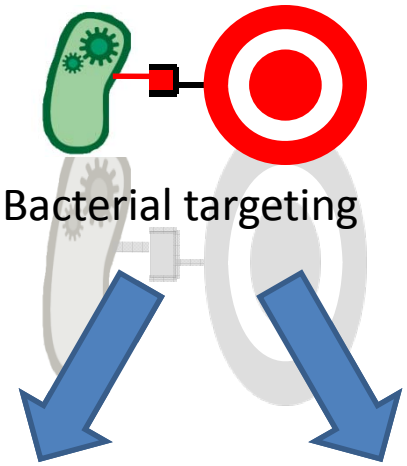
Troubleshooting and Next Steps

- 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



Bacterial targeting

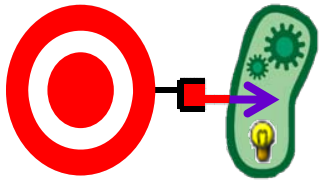
- Surface engineering – AIDA1
- Histidine/Strep2 tags
- MACS

Random peptide libraries

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

Optimize localized quorum response

Quorum-sensing



Fec signal transduction

- Characterized wild-type Fec signaling

Computational design

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