Genetically Engineering
A Heavy Metal Biosensor
using *B. subtilis*

Cornell University
Genetically Engineered Machines
Cadmium Poisoning

• Ingestion of cadmium can cause:
  – Cancer
  – Renal failure
  – Bone weakening and fracture
  – Damage to nervous and immune systems
  – Death

• Danger Level
  – Present in over 60% of hazardous waste sites as identified by the EPA
Sources of contamination

• Mining operations
• Sewage
• Irrigation water

http://farm2.static.flickr.com/1038/861723910_888a1dcdb9.jpg
Cadmium Detection

• Current cadmium measurement techniques
  – Atomic Absorption Spectroscopy

• Advantages of Biosensor
  – More affordable
  – Measures bio-available cadmium

http://meri.njmeadowlands.gov/lab/Images/Varian%20AA220.JPG
Objective
- Engineer *B. subtilis* to sense cadmium

Create Biobrick Parts

Construct Devices

Transform Devices into *B. subtilis*

Characterize Chassis
Chassis

*Bacillus subtilis*

- Gram positive soil bacteria
- Well characterized
- Naturally competent
- Complex metal ion homeostasis system

http://www.nasa.gov/images/content/177389main_POEMS1.jpg
Metal Ion Homeostasis

Influx Protein (MntH)

Efflux Protein (CadA)
Cadmium Influx Regulation

Inactive Repressor (MntR)

Active Repressor (MntR)

Cadmium
Low Intracellular [Cadmium]

- Cadmium
- Influx Protein (MntH)
- Influx Protein Gene (mntH)
- Inactive Repressor (MntR)

Promoter (MntH)
High Intracellular [Cadmium]

Influx Promoter (MntH)

Active Repressor (MntR)

Influx Protein Gene (mntH)
Cadmium Efflux Regulation

Inactive Repressor (czrA)

Active Repressor (czrA)
Low Intracellular [Cadmium]

Cadmium

Active Repressor (CzrA)

Efflux Protein (CadA) Promoter

Efflux Protein Gene (cadA)
High Intracellular [Cadmium]

Efflux Protein Gene (cadA)

Efflux Protein (CadA)

Promoter

Cadmium

Inactive Repressor (CzrA)
Objective

- Engineer *B. subtilis* to sense cadmium

Create Biobrick Parts

- Influx Protein (mntH)
- Efflux Protein (cadA)
- Internal Reference (mrgA, mrgArbs)

Construct Devices

- Cadmium Sensor Module A
- Cadmium Sensor Module B
- Internal Reference Module

Transform Devices into *B. subtilis*

Characterizing Chassis
• Absence of cadmium increases expression of YFP reporter sequence
Module A: Active Form

Inactive Repressor (MntR) + Cd(II) → Active Repressor (MntR)

Repression

Influx Promoter (MntH)

RBS

YFP Coding Sequence

No Expression
Cadmium Sensor Module B

- Absence of cadmium decreases expression of CFP reporter sequence
Module B: Active Form

Active Repressor (CzrA) + Cadmium → No Repression

Inactive Repressor (CzrA)

RBS

CFP Coding Sequence

Efflux Promoter (CadA)

Expression

CFP
# Possible outputs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Module A (influx – mntH)</th>
<th>Module B (efflux – cadA)</th>
</tr>
</thead>
</table>
| Detectable Cadmium   | ![Checkmark](
| Undetectable Cadmium | ![X](x)                  | ![X](x)                  |
| Dead cells           | ![X](x)                  | ![X](x)                  |

**Legend**

- **Active**: Green checkmark
- **Inactive**: Red X

Can’t distinguish between an undetectable level of cadmium and dead cells!
Internal Reference Module

- (Mutated) Metal regulated Gene A (mrgA) reference promoter
- Constitutive
- Similar level of expression between mrgA and cadA promoters
## New possible outputs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Module A (influx – mntH)</th>
<th>Module B (efflux – cadA)</th>
<th>Internal Reference Module</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sufficient Cadmium</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Insufficient Cadmium</td>
<td>✗</td>
<td>✗</td>
<td>✓</td>
</tr>
<tr>
<td>Dead Cells</td>
<td>✗</td>
<td>✗</td>
<td>✗</td>
</tr>
</tbody>
</table>

Can distinguish between conditions!
Integrated System

Module A (Influx protein – mntH)

Module B (Efflux protein – cadA)

Internal Reference Module
Objective

- Engineer *B. subtilis* to sense Cd(II)

Create Biobrick Parts

- Influx protein (mntH)
- Efflux protein (cadA)
- Internal Reference (mrgA, mrgA rbs)

Construct Devices

- Cadmium Sensor Module A
- Cadmium Sensor Module B
- Reference Module

Transform Devices into *B. subtilis*

- Single Homologous Recombination
- Double Homologous Recombination

Identify Appropriate Strain for Chassis

*Intermediate Constructs Completed*
Integration into *B. subtilis* Chromosomal DNA

- Single Homologous Recombination

![Diagram showing integration into B. subtilis chromosomal DNA]

- Efflux Promoter (CadA)
- RBS
- CFP Coding Sequence
- Efflux Protein Gene (cadA)
Integration into *B. subtilis*

Chromosomal DNA

- Double Homologous Recombination

![Diagram showing integration into B. subtilis chromosomal DNA. The diagram illustrates the process of double homologous recombination, where a plasmid containing a reference promoter (mrgA), ribosome binding site (RBS), GFP coding sequence, and amyE sequence integrates into the chromosome.](image-url)
Objective

Engineer *B. subtilis* to sense Cd(II)

Create Biobrick Parts

- Influx protein (*mntH*)
- Efflux protein (*cadA*)
- Internal Reference (*mrgA, mrgA rbs*)

Construct Devices

- Cadmium Sensor Module A
- Cadmium Sensor Module B
- Reference Module

Transform Devices into *B. subtilis*

- Single Homologous Recombination
- Double Homologous Recombination

Characterizing for Chassis

- WT
- *mntH* null
- *mntR* null
Characterizing Chassis: WT

- Need to find appropriate sensing range

Wild Type Growth in Cadmium
Optimizing Sensitivity

• Increase number of healthy cells
• Increase sensitivity of each cell
Characterizing Chassis: mntH null

• mntH null growth normal at high [Cadmium]
Characterizing Chassis: mntR null

- mntR null increases sensitivity to Cadmium
Optimizing Sensitivity

• Increase number of healthy cells
  – Decrease Cadmium influx by reducing mntR expression

• Increase sensitivity of each cell
  – Increase Cadmium influx by reducing mntH
Objective

Engineer *B. subtilis* to sense Cd(II)

Create Biobrick Parts

- Influx protein (mntH)
- Efflux protein (cadA)
- Internal Reference (mrgA, mrgA rbs)

Construct Devices

- Cadmium Sensor Module A
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Transform Devices into *B. subtilis*

- Single Homologous Recombination
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Characterizing for Chassis

- WT
- mntH null
- mntR null
Future Work

- Complete Cadmium Sensor Modules
- Transform constructs in *B. subtilis*
- Compare cadmium sensitivity in different chassis: WT, Influx protein (mntH), Influx protein represssor (mntR) tuned strains
- Additional metal sensing promoters: copper(copZ),arsenic(arsR)
Advisors and Sponsors

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  – Dr. Carl Batt
  – Dr. John D. Helmann
  – Dr. Xiling Shen

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