LCG–UNAM–Mexico

Singing bacteria!
Overview

- Introduction & Objective
- Inspiration & Justification
- System components
- Wet Lab
- Model
- Concluding remarks & Further Work
The aim of this project is to make bacteria sing!

How? By engineering a cell such that it imports nickel given an inducing signal, which will cause a change in the medium’s resistivity. A computer will read this and emit a sound accordingly.
How did we come up with this idea?

- **Brainstorming** – A lot of crazy ideas!

  **Singing bacteria?** – Seemed like a joke… but then, one of us thought it was feasible if we used a metal efflux pump and a small regulatory cascade…

  So, these were the origins of the design!
And all this… for what?

- Gain insight into a fundamental aspect of ecological dynamics which is currently not well understood.

- Show that measuring changes in resistivity is an effective way to determine the activity of the efflux pump, and that this can become an efficient indicator of real time transcription for *in vivo* experiments.

- The real parameters are unknown; first time describing a metal efflux pump.
System components

- **Unk** – unknown *E. coli* nickel import channel
- **RcnA** – *E. coli*’s nickel efflux pump
- **Nickel** – Intracellular and extracellular
System components

- **AHL (Acyl Homoserine Lactone)** – Free diffusion molecule, inducing signal

- **AiiA (Acyl Homoserine Lactone Lactonase)** – Efficiently degrades AHL, ensures the signal fades

- **LuxR (V. fischeri’s quorum sensing component)** – Binds to AHL to form a transcriptional activator
System components

- Dimer of complexes – Transcriptional activator
- CI – λ Phage repressor
- Promoters – Inducible by AHL and LuxR, repressible by CI
System components

- Dimer of CI – Active form of the repressor

  Change in extracellular and intracellular nickel, which translates into different pitches!
RcnR is RcnA’s natural repressor. When there is nickel, it no longer represses RcnA (That’s why it is not taken into account in the system components)

It is the simplest design and model given cell requirements and known information
Integrating System Components

Introduction & Objective | Inspiration & Justification | System components | Wet Lab | Model | Concluding remarks & Further Work
Assembly of biological devices which will carry the system components

Conventional techniques to assemble all parts

Measurements were carried out in different conditions
Device BBa_K119009 can be coupled with a different regulatory cascade
Device BBa_K119009 (Efflux pump):
- Completed, sent physically to the Registry
- Used in measurement experiments

Device BBa_K119010/9011 (Regulatory cascade)
- Wild type LacZ promoter / mutated LacZ promoter
- We have already assembled two parts
- Some problems with cloning
Wet Lab: Electronic device

- Device to sense changes in the medium’s resistivity, needed to be sensitive enough

\[ I = \frac{V}{R} \]

\[ \text{Conc} = 640 \times I \]
Noise filtering

![Graph showing resistance over time](image1.png)

![Graph showing resistance over time](image2.png)
Linear regression to relate nickel concentration to resistivity

[Ni] VS Resistivity in LB medium: Linear Regression
Wet Lab: Results

Measurements comparison: Internalization parameter

RcnA - OD=0.5 - No nickel vs nickel

![Graph showing measurements comparison: Internalization parameter](image-url)
Wet Lab: Conclusion

- We can detect changes in resistivity with different nickel concentrations even in complex media (LB + cells)

- Good results were obtained with homemade electrodes, but nickel-specific could be useful to increase sensitivity and reduce noise

- We need more replicates in other strains (RcnA+, RcnA–+PBB1RMCS5, RcnA–+PBB1RMCS5+ BBa_K119009)

- Once BBa_K119010/9011 is ready we’ll be able to evaluate the system’s performance
We aim to better know and understand the system through the identification of critical parameters and species, and thus be able to obtain the desired dynamics.

Our system is composed of 13 species and 11 coupled biochemical reactions that completely describe it. All simulations were done with MATLAB’s Simbiology.
Once there is nickel in the medium, RcnR no longer participates in the pump’s regulation.

Cell membrane permeability to AHL is not considered inside the model.

All decrease in AHL concentration is due to AiiA.

The change in the transcription of cI* is dependent only on AHL concentration.
Model: Assumptions

- It is a **homogeneous system**
- The quantity of nickel used by the cell is **negligible** compared to the concentrations in and out of the cell
- The production of RcnR, LuxR and AiiA is constitutive and their concentrations have reached the **steady state** at the beginning of the experiment
- **NikABCDE** will not play a role in our model
# Model: Biochemical Reactions

<table>
<thead>
<tr>
<th>Name</th>
<th>Reaction</th>
<th>Kinetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Degradation of AHL by AiiA</td>
<td>AiiA + AHL → AiiA</td>
<td>Michaelis–Menten</td>
</tr>
<tr>
<td>2. Complex formation and dissociation between AHL and LuxR</td>
<td>AHL + LuxR ↔ AHL:LuxR</td>
<td>Mass Action</td>
</tr>
<tr>
<td>2.1 Dimer formation and dissociation between AHL:LuxR complexes</td>
<td>2 AHL:LuxR ↔ (AHL:LuxR):(AHL:LuxR)</td>
<td>Mass Action</td>
</tr>
<tr>
<td>3.1 CI synthesis induced by AHL and LuxR complexes dimer</td>
<td>ρcI + (AHL:LuxR):(AHL:LuxR) → ρcI + (AHL:LuxR):(AHL:LuxR) + CI</td>
<td>Mass Action</td>
</tr>
<tr>
<td>3.2 Constitutive CI synthesis</td>
<td>ρcI → ρcI + CI</td>
<td>Mass Action</td>
</tr>
<tr>
<td>4. Natural degradation of CI</td>
<td>CI → Ø</td>
<td>Mass Action</td>
</tr>
<tr>
<td>4.1 Dimer formation and dissociation between CI molecules</td>
<td>2 CI ↔ CI:CI</td>
<td>Mass Action</td>
</tr>
<tr>
<td>6. RcnA production</td>
<td>ρ → ρ + RcnA</td>
<td>Hill Kinetics</td>
</tr>
<tr>
<td>7. Nickel efflux by RcnA</td>
<td>RcnA + Niint → RcnA + Niext</td>
<td>Mass Action</td>
</tr>
<tr>
<td>8. Natural degradation of RcnA</td>
<td>RcnA → Ø</td>
<td>Mass Action</td>
</tr>
<tr>
<td>9. Nickel import by unknown channel</td>
<td>Unk + Niext → Unk + Niint</td>
<td>Mass Action</td>
</tr>
</tbody>
</table>
To describe the flux of these reactions, 18 kinetic constants are needed, of which we could find 13 researching through literature.

We could find a biologically plausible range for 2 of the remaining 5, and the rest we adjusted accordingly to the desired results.
Model: Set of ODEs

1. \[ \frac{d[AiiA]}{dt} = 0 \]

2. \[ \frac{d[AHL]}{dt} = k'_2[C] - \frac{k_{AHL}[AHL]}{K_m + [AHL]} - k_2[AHL][LuxR] \]

3. \[ \frac{d[LuxR]}{dt} = k'_2[C] - k_2[AHL][LuxR] \]

4. \[ \frac{d[C]}{dt} = k_2[AHL][LuxR] + 2k'_2[C] - k'_2[C] - k_{2,1}[C]^2 \]

5. \[ \frac{d[C : C]}{dt} = k_{2,1}[C]^2 - k'_2[C : C] \]

6. \[ \frac{d[\rho_{cl}]}{dt} = 0 \]

7. \[ \frac{d[CI]}{dt} = k_{3_{on}}[\rho_{cl}][C : C] + k_{3_{off}}[\rho_{cl}] + 2k'_1[CI : CI] - k_4[CI] - k_{4,1}[CI]^2 \]

8. \[ \frac{d[CI : CI]}{dt} = k_{4,1}[CI]^2 - k'_{4,1}[CI : CI] \]

9. \[ \frac{d[\rho]}{dt} = 0 \]

10. \[ \frac{d[RcnA]}{dt} = \frac{k_4[\rho]}{1 + \frac{k_{5,1}}{k_{5,1}} + \frac{k_{5,2}}{k_{5,2}} + \frac{k_{5,3}}{k_{5,3}}} - k_8[RcnA] \]

11. \[ \frac{d[Ni_{in}]}{dt} = k_7[Unk][Ni_{ex}] - k_7[RcnA][Ni_{in}] \]

12. \[ \frac{d[Ni_{ex}]}{dt} = k_7[RcnA][Ni_{in}] - k_7[Unk][Ni_{ex}] \]

13. \[ \frac{d[Unk]}{dt} = 0 \]
Model: Defining the initial state

constitutive proteins (AiiA, LuxR, CI –constitutive synthesis–, CI:CI –due to constitutive synthesis), based on
- efficiency rate of their promoters
- number of promoters per cell
- degradation rate of their mRNAs
- translation efficiency
- degradation rate of the proteins

AHL:LuxR complex, dimer of complexes, CI and CI:CI due to complex activation
- set to 0, given these are all due to the action of AHL
Model: Defining the initial state

- *cl* and *rcnA* promoters,
  - set to 10 based on plasmid copy number

- RcnA and Unk,
  - set consistent to the desired rate and within biologically possible bounds

- AHL and nickel,
  - determined by us to obtain the desired results
Ci dimerization constant
Set to $0.00001 \text{(molecules s}^{-1}) / 0.01 \text{s}^{-1}$

Different $k_{4.1}/k_{-4.1}$ ratios (1/50:1/1000)
Nickel internalization and efflux

Set to 500 (molecules·s)^{-1}/500 (molecules·s)^{-1}

K7 & k9 Scan; ki=(0:1)
Model: Analyses

- **Sensitivity Analysis**: Nickel is sensitive to almost all kinetic constants at the time of highest response.

- **Stoichiometric Matrix**: Three conditions must be met to reach the steady state:
  - \( v_4 = v_{3.1} + v_{3.2} \), which means that the total synthesis of CI should be equal to its degradation;
  - \( v_6 = v_8 \), the synthesis of RcnA equal to its degradation; and
  - \( v_7 = v_9 \), the rate of nickel uptake equal to its efflux rate.

- **St**: Only one steady state, reached after \(~75,000\) seconds.

- **Jacobian & Modal**: Fast nickel response.
- 20,000 seconds, ODE15s solver
- AHL, AHL:LuxR complex and dimer of complexes
CI and CI dimer
Model: Simulation

RcnA

![Graph showing RcnA molecule concentration over time](image)
Intracellular and extracellular nickel

Model: Simulation

SINGING BACTERIA!
Scan with different AHL concentrations

Different AHL concentrations; [AHL]=(0:1000)
Scan with different nickel concentrations

![Graph showing Nickel scan (10,000:150,000)]
There is a range of nickel concentrations that permit the experiment (they block RcnR, but do not kill the cells)

We estimated the range for nickel uptake and efflux to get the desired response, and demonstrated they fall within biologically possible values

The adjusted values (CI dimerization and dissociation constants and RcnA degradation) are comparable to other typical biochemical constants
We showed the idea is plausible, although more rigorous experiments are needed to find the real parameters.

More work is needed to standardize the protocols and to investigate the effect of other variables such as temperature.

We gathered all available knowledge on the system, which is integrated in the model.
Given the quick nickel response, we have demonstrated that measuring resistivity can be a novel in vivo transcriptional indicator.
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