MOTIVATION

A worldwide problem: nosocomial infections antibiotic resistance

Meeting at the hospital

Detection methods

PCR: FAST & EXPENSIVE (21.7 €)
PLATES: SLOW (20-48 h) & CHEAP
SPECIFICATIONS

sEnsiColi, a biosensor:

- **Sensitive**
- **Reliable** no false negatives
- **Fast** few hours

**Cheap**
**Easy to use**
1. Detection
2. Amplification
3. Communication
Need of an $\Delta envZ \Delta cyaA$ strain
SENSITIVITY OF THE DIPEPTIDE RECEPTOR

\[
\frac{d[OmpR\_P]}{dt} = v [\text{dipeptide}] \frac{[OmpR]}{K + [OmpR]} - V' \frac{[OmpR]}{K' + [OmpR]}
\]

\[
\frac{d[AC]}{dt} = Vm \cdot \frac{[OmpR^*]^n}{k^n + [OmpR^*]^n} + p_{AC} - \alpha_{AC} \cdot [AC]
\]
For low dipeptide concentration, can we amplify the signal?

**SENSITIVITY OF THE DIPEPTIDE RECEPTOR**

[Graph showing the sensitivity of the dipeptide receptor with [AC] versus initial [dipeptide].]
AMPLIFICATION LOOP

Detection
Amplification
Communication
A BIOLOGICAL « AND » GATE?
A BIOLOGICAL « AND » GATE?
A BIOLOGICAL « AND » GATE?

Detection

Amplification

Communication
Analysis of the amplificator’s behavior

\[
\frac{d[Arac]}{dt} = \frac{v_{mArac} [(CRP - cAMP)]^n}{K_{Arac}^n + [(CRP - cAMP)]^n} + P_{Arac} - \alpha_{Arac} [Arac]
\]

\[
[Arac^*] = \frac{Arac_{total}}{K_{c1} + \frac{1}{\text{arabinose}}}
\]

\[
\frac{d[AC]}{dt} = \frac{v_{mAC} (CRP - cAMP)^n}{K_{AC}^{n_1} + (CRP - cAMP)^n_1 - \alpha_{AC} [AC]}
\]

\[
[cAMP] = \frac{k_{cat}}{\alpha_{cAMP}} [AC] + \frac{\eta}{\alpha_{cAMP}} [cAMP]
\]

- 5 equations
- 20 parameters found in the literature
- Equilibria and stabilities computed with matlab.
Equilibrium without AND Gate

Because of basal production AC is always expressed.
Equilibria with and without AND Gate

The AND Gate enables an ON / OFF system
Our amplification system improves the sensit
CELL TO CELL COMMUNICATION

Detection
Amplification
Communication
CELL TO CELL COMMUNICATION

Detection

Amplification

Communication

Staphylococcus aureus
CELL TO CELL COMMUNICATION

Detection

Amplification

Communication

Staphylococcus aureus
CELL TO CELL COMMUNICATION

Detection

Amplification

Communication
## CELL-TO-CELL COMMUNICATION

<table>
<thead>
<tr>
<th>( i, j-1 )</th>
<th>( cAMPout(xi,yj) )</th>
<th>( i, j+1 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( xi-1, yj )</td>
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**Detection**

**Amplification**

**Communication**
Signal visible 100mn after the first bacterium is turned on.
WHOLE SYSTEM RESPONSE TIME

- Detection: 20mn
- Amplification: 200mn
- Communication: 100mn

**TOTAL** 320 mn ≈ 5h20mn

COMPARISON TO CURRENT METHODS

**sEnsiColi**:
- Time: 5h
- Cost: 0.23 €

**PCR**:
- Time: 2-3h
- Cost: 21.70 €

**Plates**:
- Time: 20-48h
- Cost: 2 €
1. Detection
2. Amplification
3. Communication

Sensitive
Reliable
Fast
Easy to use
SAFETY ISSUES

Related to our device

User guide
Safety precautions

Synthetic Biology

Increasing number of teams ➔ biobricks
No standardized safety database
Biobrick Safety Sheet

Risk level:

Chassis: *Escherichia coli* (BW25113 strain)

This promoter is a hybrid one made up of two natural promoters. It consists of the phage lambda promoter $P(L)$ which activates the pathogenicity by increasing the transcription. The phage lambda destroys *E. coli* using a toxin which destroys the membrane. In this regulatory region, instead of the cl binding site, there is lacO$_1$ (from *E. coli* LacI operon). LacO$_1$ is an operator from lactose operon, it binds LacI (the lac repressor) which is released upon complexion with IPTG, the inducer.

*E. coli*: are bacteria commonly used in laboratories. Some strains are dangerous but most of them are harmless.

Phage lambda: is an *E. coli* virus without any pathogenicity towards humans.

Purposes in the system:

55 bp

Technic:

Gibson Assembly

BioBricks:

- *pLAC* - *rbs* comes from BBa I13601

Diagram of the construction

BioBrick code: none for the moment

Plasmid: pSB3C5

It can let the production of RsmA, which binds to the fha1 RBS. This prevents the production of mCherry.

Construction method:

- Technic: Gibson Assembly
- BioBricks:
  - *pLAC* - *rbs* comes from BBa I13601
ACHIEVEMENTS

- Design of a Biobrick Safety Sheet
- Collaborations to test Biobrick Safety Sheet

- Ultra-sensitive pathogene detector
  → application in the medical field
- 3 modules → 3 models
- New application & proof of concept: paraBAD « AND » gate
- Double KO: ΔcyA A envZ strain
- A new hybrid receptor designed and assembled
- A new cell-cell communication based on cAMP
10 new BioBricks registered
POST JAMBOREEE

- **Test**: the amplification module
- **Build**: the *link between detection and amplification*
- **Improvement** of Biobrick Safety Sheet
SPECIAL THANKS

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Thanks for your attention.
Do you have any questions?
Is the given solution the only possible one? The isoclines can give the answer

Before the threshold: $[\text{cAMPi}]=10^{-7} \text{ Mol.L}^{-1}$

• It seems like there a low steady state.
• We need to zoom to be sure.
Is the given solution the only possible one? The isoclines can give the answer

Before the threshold: \([\text{cAMP}_i]=10^{-7} \text{ Mol.L}^{-1}\)

- There’s a low steady state
- The system stops at the low steady state because it’s stable.