Synthetic biology

A new vision of biology

INTERFACE
Synthetic biology

A new vision of biology

AIM: make a bacterium perform a new function

INTERFACE
Synthetic biology

A new vision of biology

AIM: make a bacterium perform a new function

Use of BioBricks
DNA fragments

Assembly
genetic engineering
Introducing the Synthetic Biology Team of iGEM Grenoble 2012 at Midi Minatec, September 14th, 2012.

A multidisciplinary team

Elise, Nicolas, Pierre, Greg, Mathieu, Adeline, Nadia, Julie, Jérôme
The iGEM competition

**International Genetically Engineered Machine**

A ten-months project

**European Jamboree:**
Amsterdam (October 5th to 7th)

**World Championship:**
Boston (November 2nd to 5th)

200 student teams from all over the world
Introduction

Synthetic biology

Team

iGEM

Project

IDEA

design

ethical aspects

safety questions

funds

Experiments and modeling

communication

A « do it yourself » project

iGEM Grenoble 2012
A worldwide problem: nosocomial infections antibiotic resistance
A worldwide problem: nosocomial infections antibiotic resistance

Meeting at the CHU
In the hospital, detection methods are either

EXPENSIVE 15€
or SLOW 20-24 h
Synthetic biology
Team
iGEM
Project

SPECIFICATIONS
Sensitive
Reliable
Fast
Easy to use
Cheap

HOW TO USE IT?

Detection system
Biology

Membrane receptor
Direct detection
Amplification loop
Communication

INPUT

Detection
Amplification
Communication

OUTPUT
Membrane receptor
Direct detection
Amplification loop
Communication

INPUT

Detection
Amplification
Communication

OUTPUT

Biology

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Membrane receptor

Direct detection

Amplification loop

Communication
Membrane receptor

Direct detection

Amplification loop

Communication

Biology

cAMP Production

OmpR

cyA

cAMP

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September, 14th 2012
Check if the circuit has the **desired response** or not

**How sensitive is our receptor?**

\[
\frac{d[\text{OmpR}^*]}{dt} = v [\text{dipeptide}] \frac{[\text{OmpR}]}{K + [\text{OmpR}]} - V' \frac{[\text{OmpR}^*]}{K' + [\text{OmpR}^*]}
\]
OmpR* production versus initial dipeptide concentration

[Graph showing the relationship between OmpR* production rate and dipeptide concentration.]
Direct detection

Membrane receptor

Direct detection

Amplification loop

Communication

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Is the system sensitive enough?

\[
\frac{d[C_ya]}{dt} = V_m \times \frac{[cAMP]^n}{K^n + [cAMP]^n} + p_{cya} - \alpha_z \times [C_ya]
\]

Not sensitive!
The desired evolution of Cya versus initial cAMP concentration

We need to amplify the signal!
Membrane receptor
Direct detection
Amplification loop
Communication

INPUT

Detection

Amplification

OUTPUT

Communication
Amplification loop

- Membrane receptor
- Direct detection
- Amplification loop
- Communication

- cAMP
- cyaA
- GFP
- gfp
Membrane receptor
Direct detection
Amplification loop
Communication
Is the system sensitive enough?

- Membrane receptor
- Direct detection
- Amplification loop
- Communication

**Amplification**

**False positive**

Graph: [Cya] versus [cAMP]
Our system: a threshold detector
A biological « AND gate » ?
A biological « AND gate » ?

- Membrane receptor
- Direct detection
- Amplification loop
- Communication
Answer 2 questions:

1. Has our biosensor a satisfying threshold sensitivity or is it always turned on?

2. How many false positives are there?
Model

\[ [\text{CAMP}] = \frac{k_{\text{cat}} [\text{Cya}]}{\alpha_{\text{CAMP}}} + \frac{\eta}{\alpha_{\text{CAMP}}} [\text{CAMP}_{\text{init}}] \]

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

\[ \frac{d[\text{Cya}]}{dt} = \frac{v_{m\text{Cya}} [(\text{CRP} - \text{cAMP})^{n_1}]}{K_{\text{Cya}}^{n_1} + [(\text{CRP} - \text{cAMP})]^{n_1}} \frac{[(\text{Arac} \ast)^{n_2}]}{K'_{\text{Cya}}^{n_2} + [(\text{Arac} \ast)]^{n_2}} + p_{\text{Cya}} - \alpha_{\text{Cya}} [\text{Cya}] \]
Has our biosensor a satisfying threshold sensitivity or is it always turned on?

Amplification loop

NO amplification loop

10^{-7.5}

10^{-4}
How does it work?

Membrane receptor

Direct detection

Amplification loop

Communication

Steady states

Modeling

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September, 14th 2012

1 kg

[cAMP] < Threshold

Cya

[complex]\n
[5 kg]

[cAMP] ≥ Threshold

5 kg

5 kg
2 How many false positives are there?

- There are stochastic fluctuations in the cell
- Cya may be expressed even if there is nothing to detect

Use of stochastic model
Predictions of stochastic model

% of bacteria turned on after 60 min vs $cAMP_i$

A 4-fold difference after 60 min.
Membrane receptor
Direct detection
amplification loop
Communication

INPUT

Detection
Amplification

OUTPUT

Communication
Membrane receptor
Direct detection
amplification loop
Communication
Bacterial communication

Membrane receptor

Direct detection

amplification loop

Communication

Staphylococcus aureus
Bacterial communication

Membrane receptor
Direct detection
amplification loop
Communication

Staphylococcus aureus
Bacterial communication

Membrane receptor
Direct detection
amplification loop
Communication

Staphylococcus aureus
Bacterial communication

Membrane receptor
Direct detection
amplification loop
Communication
How does the communication work?

- membrane diffusion
- diffusion through the extracellular space
How does the communication work?

- membrane diffusion
- diffusion through the extracellular space
How does the communication work?

- membrane diffusion
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- membrane diffusion
- diffusion through the extracellular space
How does the communication work?

- membrane diffusion
- diffusion through the extracellular space
Membrane receptor

Direct detection

Amplification loop

Communication
Membrane receptor

Direct detection

Amplification loop

Communication

Biology

INPUT

Detection

Amplification

Communication

OUTPUT
Membrane receptor
Direct detection
amplification loop
Communication

Biology

INPUT

Detection
Amplification
Communication

Modular

OUTPUT
Membrane receptor

Direct detection

amplification loop

Communication

**INPUT**

Detection

Amplification

Communication

**OUTPUT**

Modular

Sensitive

Reliable

Biology

iGEM Grenoble 2012

Midi Minatec September, 14th 2012
Membrane receptor
Direct detection
amplification loop
Communication

INPUT

Detection
Modular
Sensitive
Reliable
Fast
Easy to use

Amplification

Communication

OUTPUT

Cheap
1€50

iGEM Grenoble 2012
Midi Minatec
September, 14th 2012
Creation of a Biobrick Safety Sheet:

Safety information storage
Like MSDS
Database
Biobrick Safety Sheet

Risk level: I
Plasmid: pSB4C5
Chassis: *Escherichia coli* (BW25113 strain)

Diagram of the construction

BioBrick code: none for the moment

**Construction method**

- Technic: Gibson Assembly
- BioBricks:
  - plac originates from BBa_I13601
  - fha1 originates from iGEM Grenoble 2011 team work
  - ecfp is extracted from BBa_E0422

**Promoter** : plac 0-1 (BBa_R0011)

**Origin and initial function:**

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 c binding site, there is lacO1 (from *E.coli* Lac operon). LacO1 is an operator from lactose operon, it binds LacI (the lac repressor) which is released upon complexation 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:**

It allows a strong transcription of *ecfp* and which can be induced by IPTG and repressed by LacI.

**Size:**

55 bp
Questions

Collaboration

Partnerships
Achievements

- 6 new genetic constructions
- Start of the test phase
- Validation of the model
Achievements

- 6 new genetic constructions
- Start of the test phase
- Validation of the model

Safety

- Biobrick Safety Sheet design
- 4 collaborations worldwide with other iGEM teams

Project

- Biobrick Safety Sheet design
- 4 collaborations worldwide with other iGEM teams
Our website

http://2012.igem.org/Team:Grenoble
Funding

Achievements

Website

Funding

Budget: 60 500 €

Already obtained
48 500 €
Funding

Budget: 60,500 €

Already found: 48,500 €

Still missing: 12,000 €
Achievements

- 3 more genetic constructions
- Test & validation of the whole system
- Amsterdam Jamboree
- Boston World Championship

Safety

- Biobrick Safety Sheet improvement
- Database creation
- Safety debate
Special thanks

Instructors

- Delphine Ropers
- Franz Bruckert
- Hans Geiselmann
- Hidde de Jong
- Marianne Weidenhaupt
- Robert Baptist

Advisors

- Geoffrey Bouchage
- Jean-Baptiste Lugagne
- Stéphane Pinhal
- Valentin Zulkower
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• Stéphane Pinhal
• Valentin Zulkower

• Alexandre Donzé
• Pr Max Maurin
• Thomas Vialletet
Thanks for your attention.
Do you have any questions?