iGEM 2012

The bacterial Eyespot

Bordeaux Team (France)

Denis Dupuy

Sophie Vaud

Antoine Ribeiro

Marie Beurthon-Aimar

Julie Di Martino

The 2012 Bordeaux Team

Arnaud Frèche

Cécile Quéré

Jonathan Millet

Mysterious fungus

Christophe Diemiel

Plan

- Introduction : The idea
- Chapter 1 :The project
- Chapter 2 : The simulation
- Chapter 3 : The labwork
- Conclusion : The prospect

INTRODUCTION
THE IDEA

Where our project came from ?

When I went to school, they asked me what I wanted to be when I grew up. I wrote down 'happy'. They told me I didn't understand the assignment, and I told them they didn't understand life. " John Lennon
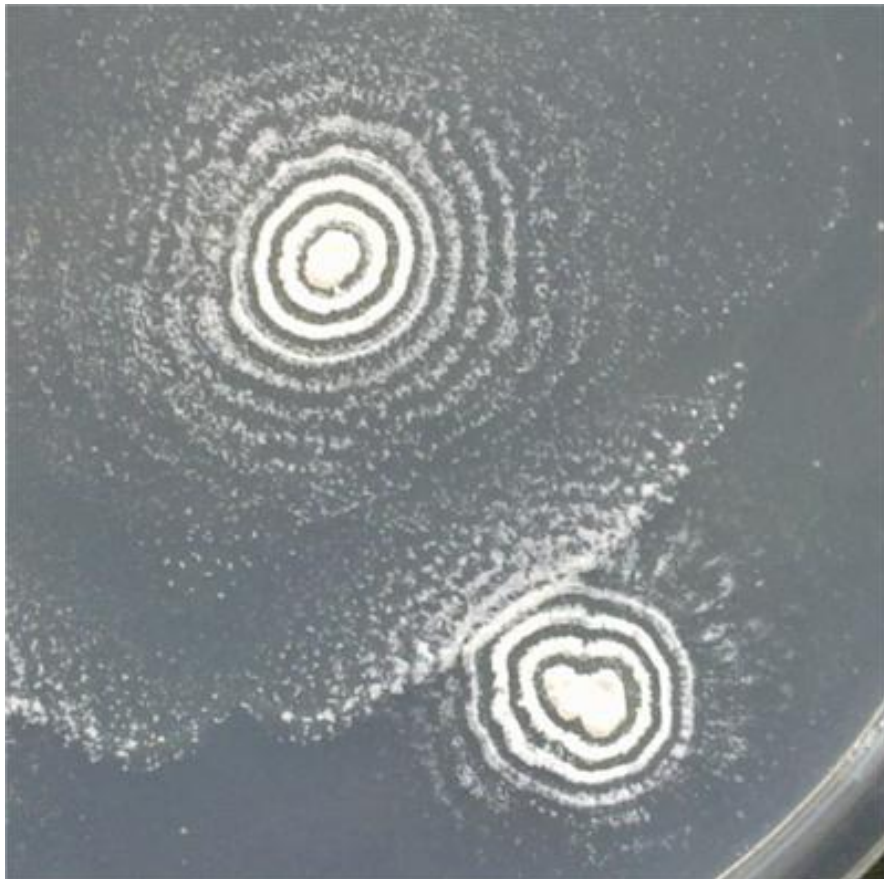
Introduction

Introduction

Zebra (Equus quagga)

Various pattern can be observed in nature
Leopard (Panthera pardus)
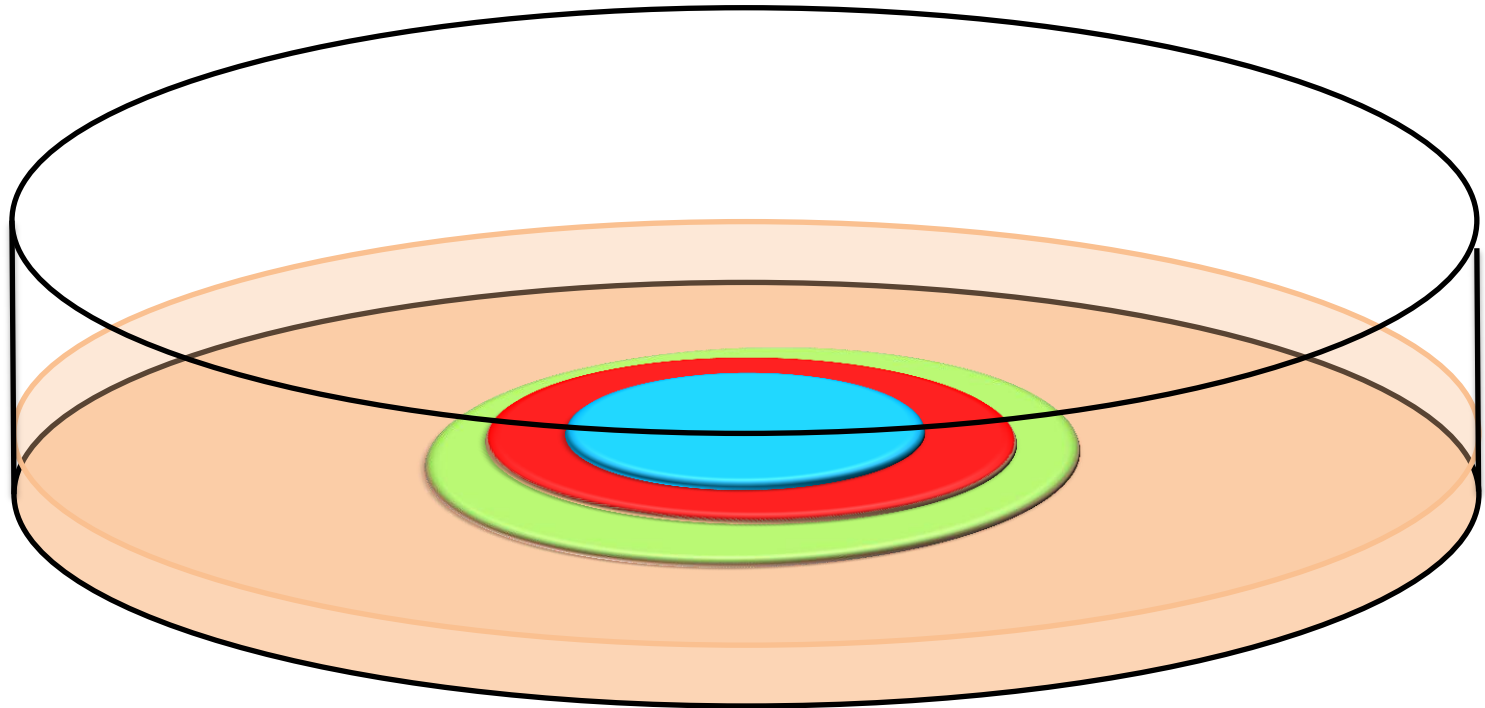
1

Introduction

Junonia coenia

Inachis io

Eyespots can be observed on some butterflies wings

The idea

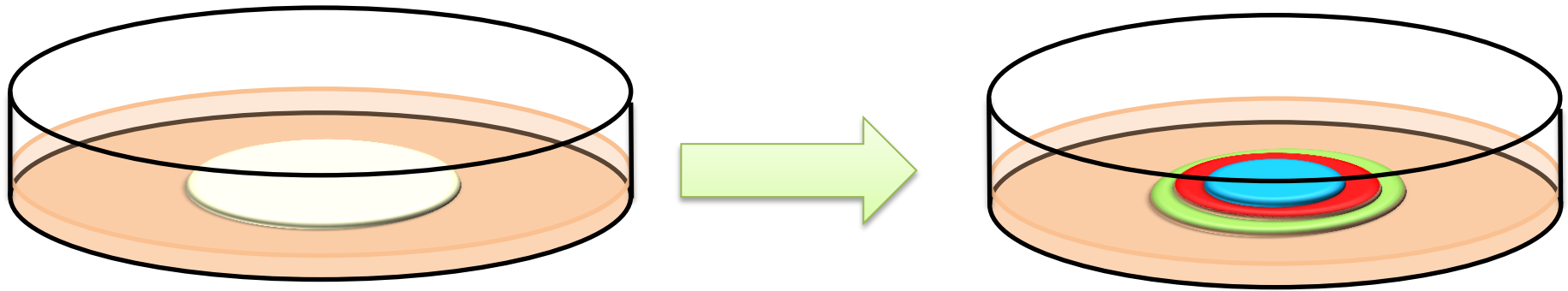A bacteria strain drawing concentric circles on a Petri dish

CHAPTER 1
THE PROJECT

How to make it real ?

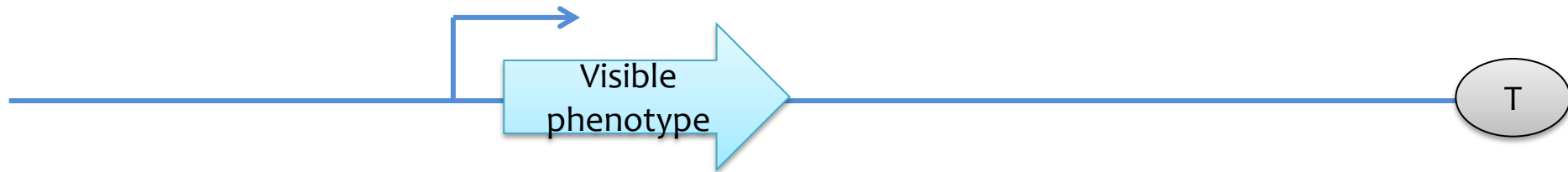*" A man who is no longer able to marvel at practically stopped living"* **Albert Einstein**

The project

- Bacterial Lawn (One enginereed strain)

- 3 colored states (Operon-based differenciation)
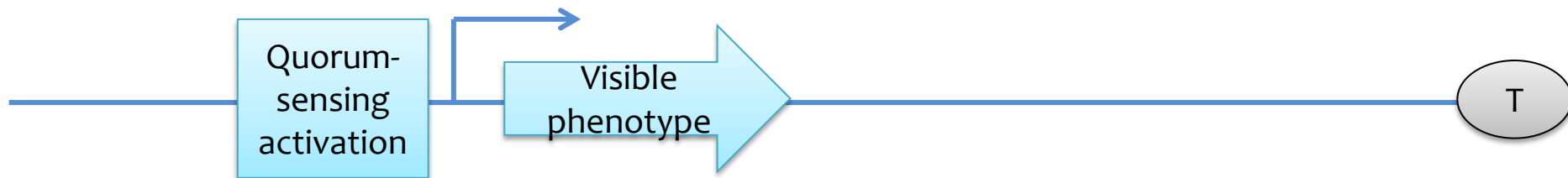
- Quorum-sensing signalisation

Operon-based cell Differenciation

3 operons with :

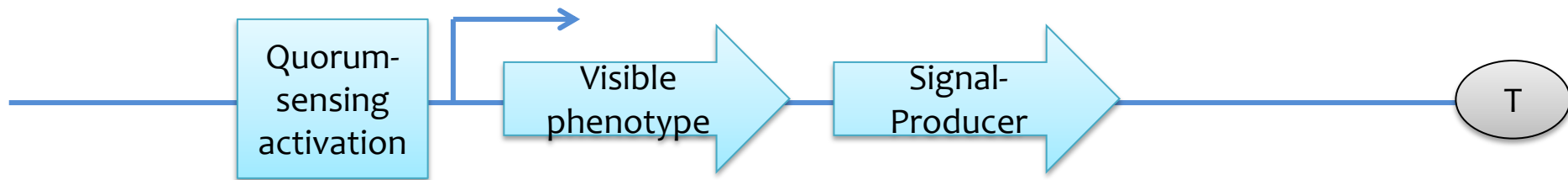- A visible phenotype (LacZ/mCherry/GFP)

Operon-based cell Differenciation

Quorum-sensing activation

Visible phenotype

T
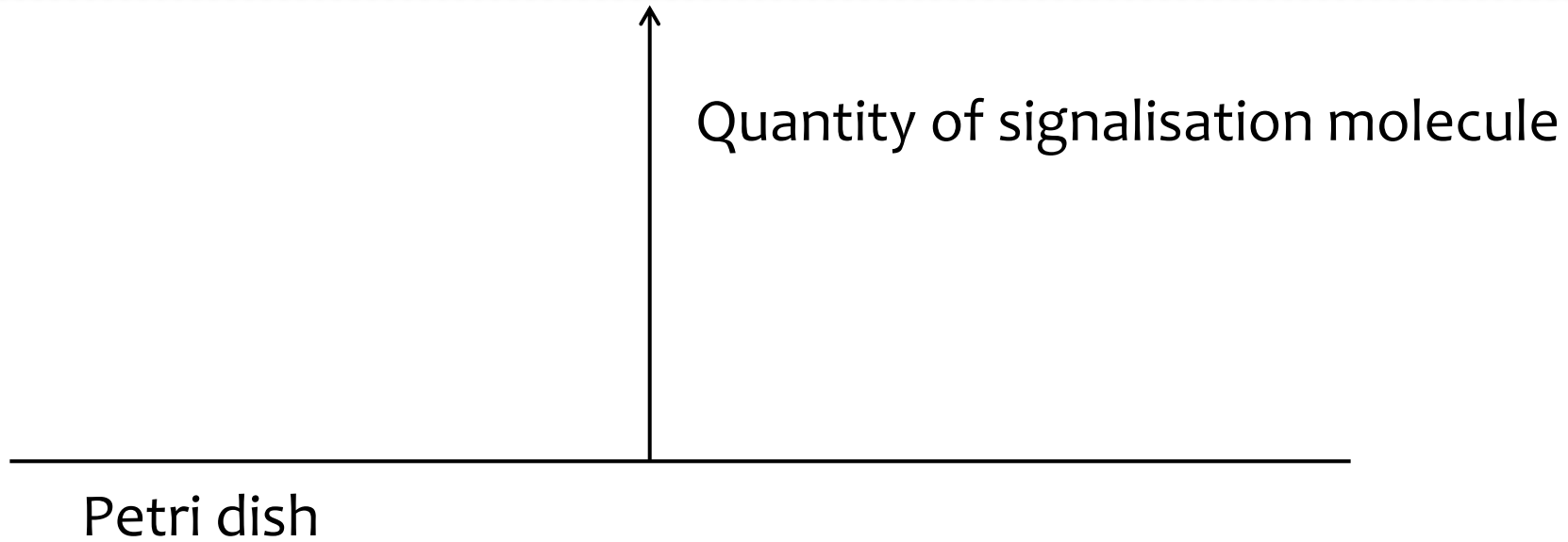
3 operons with :

- A quorum-sensing activated Promoter

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Operon-based cell Differenciation

| Quorum-sensing activation | Visible phenotype | Signal-Producer | T |

3 operons with :

- A quorum-sensing signalling molecule producer

Cell-to-cell communication

Quantity of signalisation molecule

Petri dish

- 3 different types of quorum-sensing

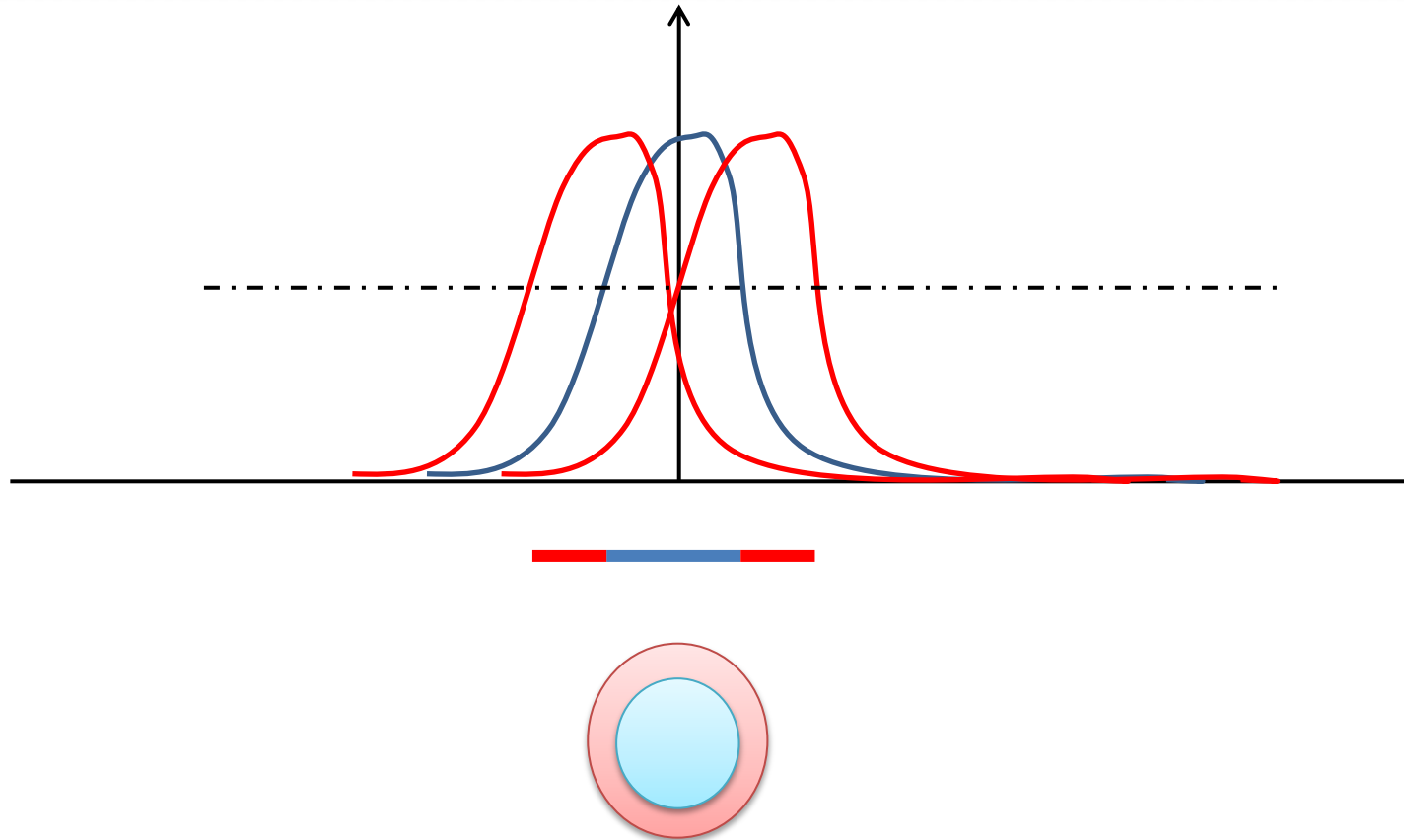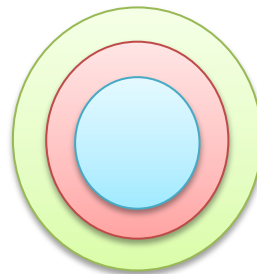# Cell-to-cell communication
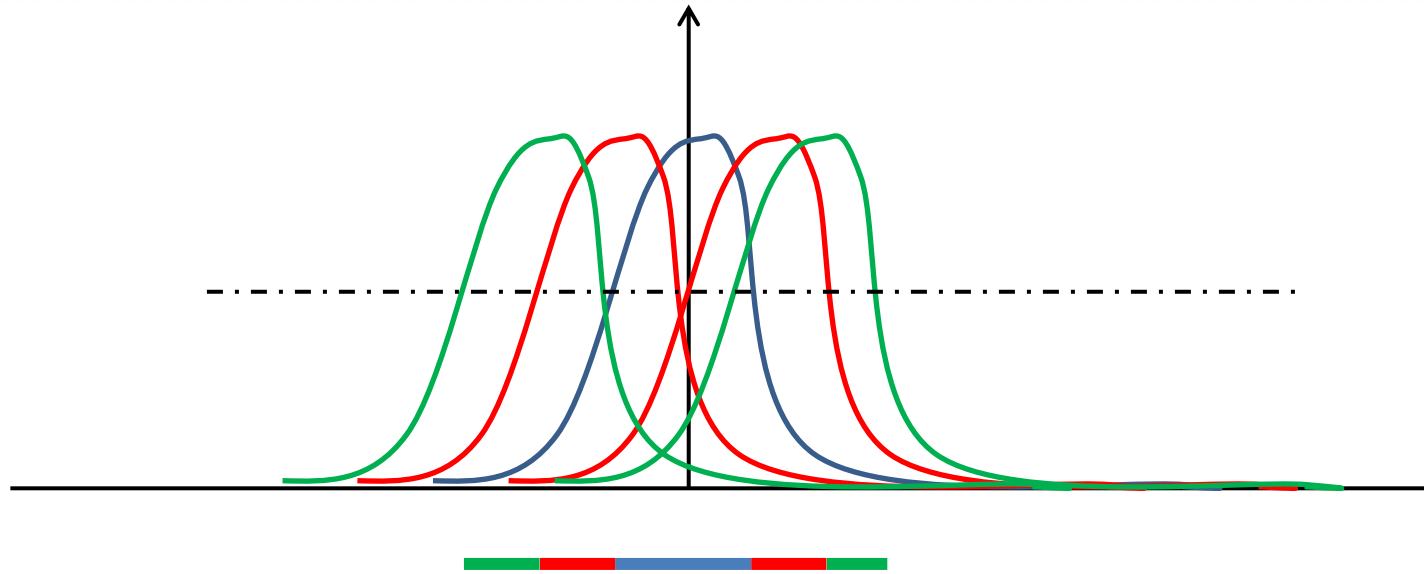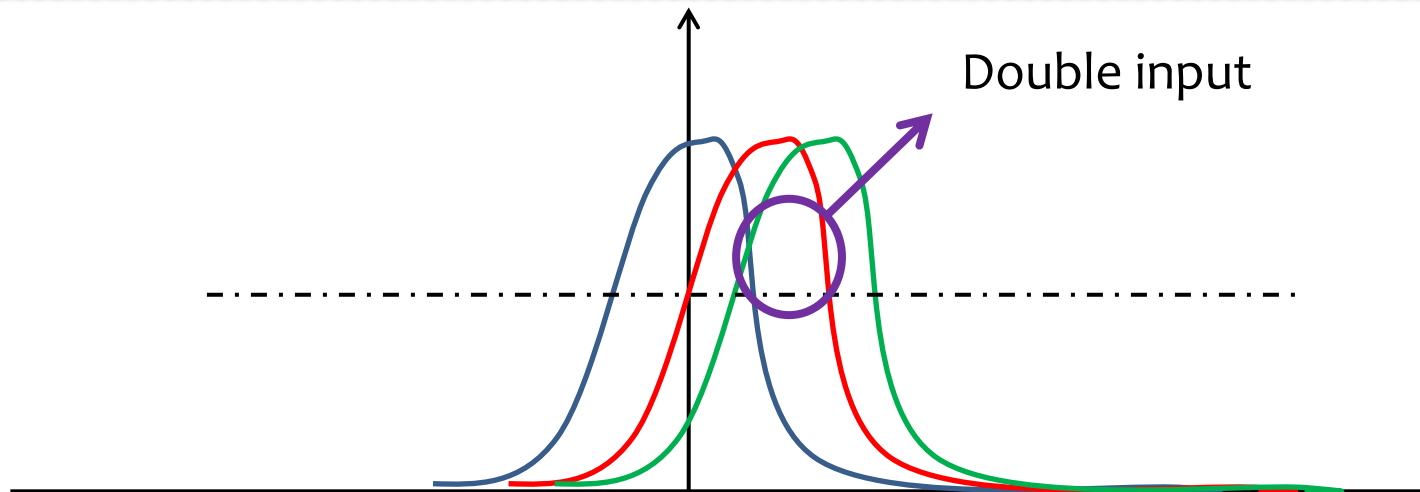

Activation
Threshold

• 3 different types of quorum-sensing

6

Cell-to-cell communication

- 3 different types of quorum-sensing

Cell-to-cell communication

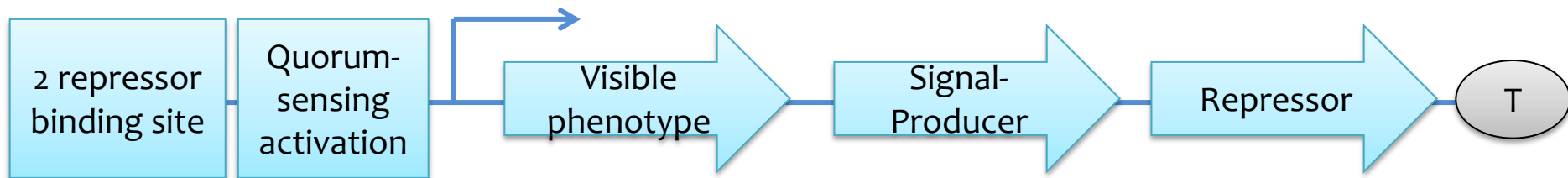- 3 different types of quorum-sensing
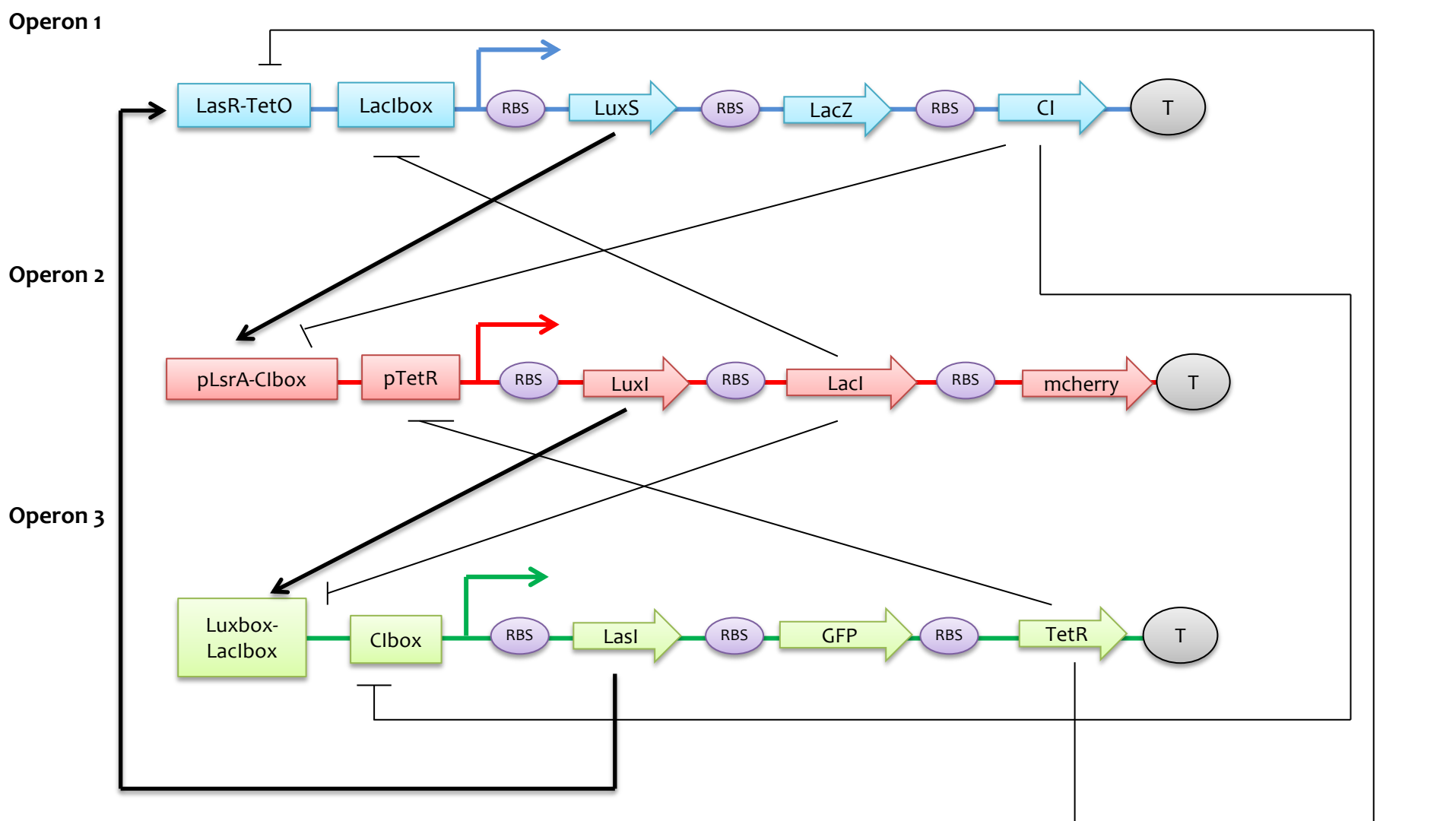
6

Cell-to-cell communication

Double input
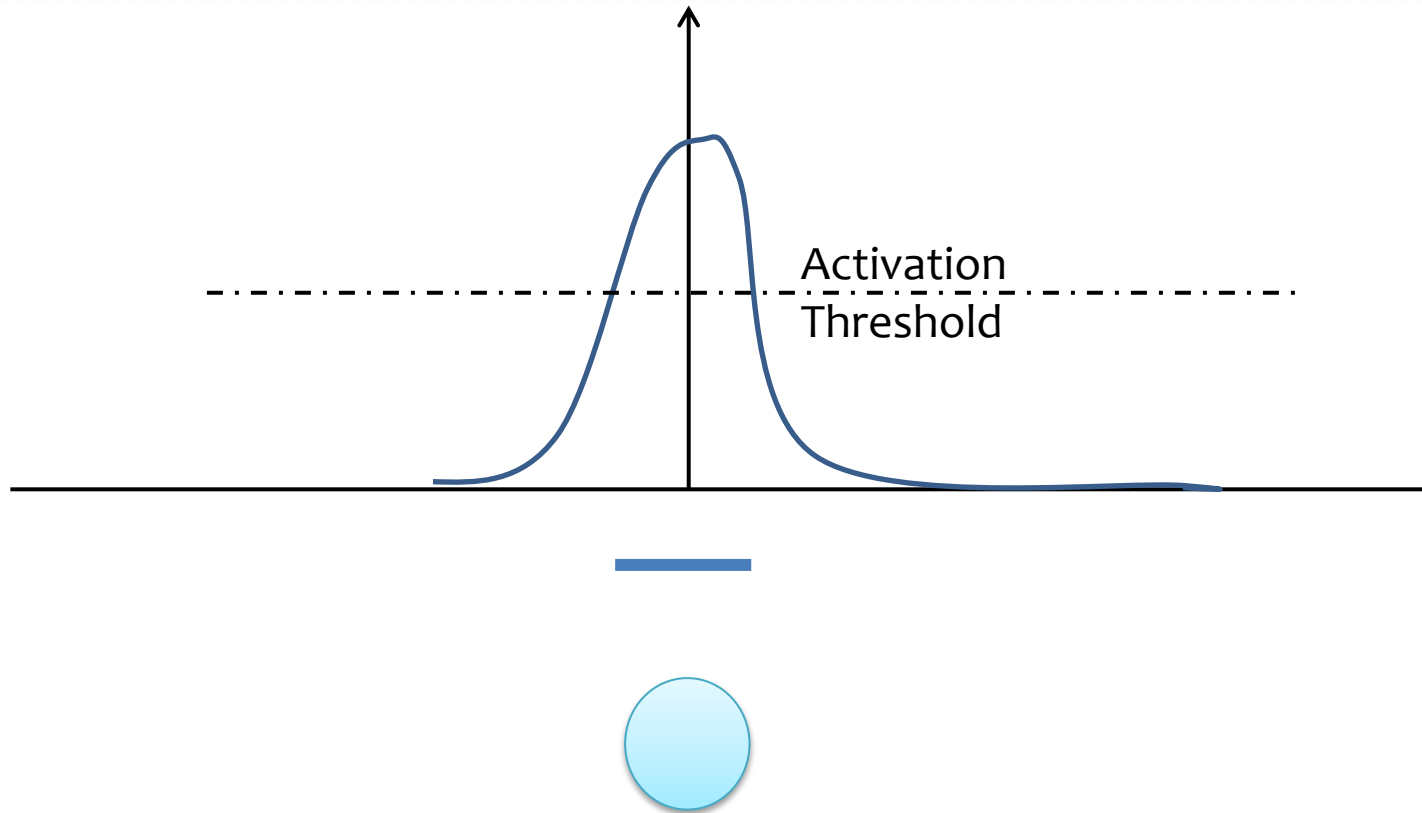
- The necessity to use repressors to avoid signalisation conflict

Operon-based cell Differenciation

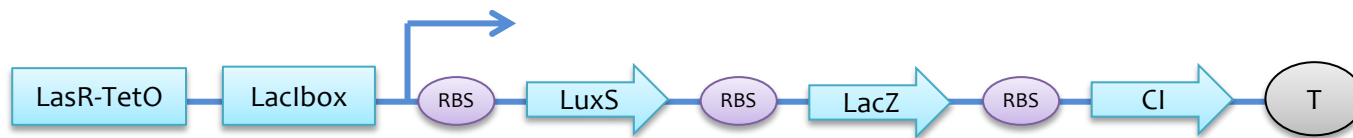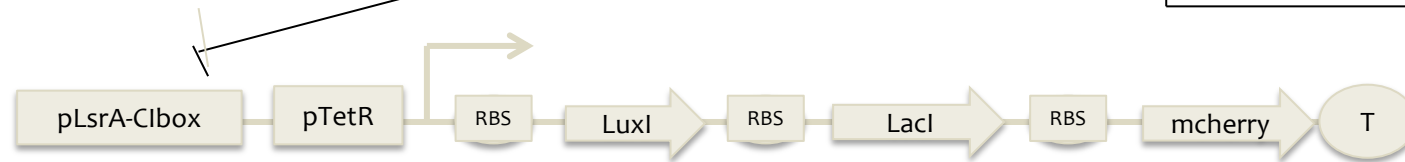| 2 repressor binding site | Quorum-sensing activation | | Visible phenotype | Signal-Producer | Repressor | T |

3 operons with :

- A repressor of the 2 other operons
- Two repressing sites in the promoter

Operon 1

LasR-TetO | LacIbox | RBS | LuxS | RBS | LacZ | RBS | CI | T

Operon 2

pLsrA-CIbox | pTetR | RBS | LuxI | RBS | LacI | RBS | mcherry | T

Operon 3

Luxbox-LacIbox | CIbox | RBS | LasI | RBS | GFP | RBS | TetR | T

Operon 4

Pc_Op4 | RBS | LsrR | RBS | LsrK | RBS | LuxR | RBS | LasR | T

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Operon 1

LasR-TetO | LacIbox | RBS | LuxS | RBS | LacZ | RBS | CI | T

Operon 2

pLsrA-CIbox | pTetR | RBS | LuxI | RBS | LacI | RBS | mcherry | T

Operon 3

Luxbox-LacIbox | CIbox | RBS | LasI | RBS | GFP | RBS | TetR | T
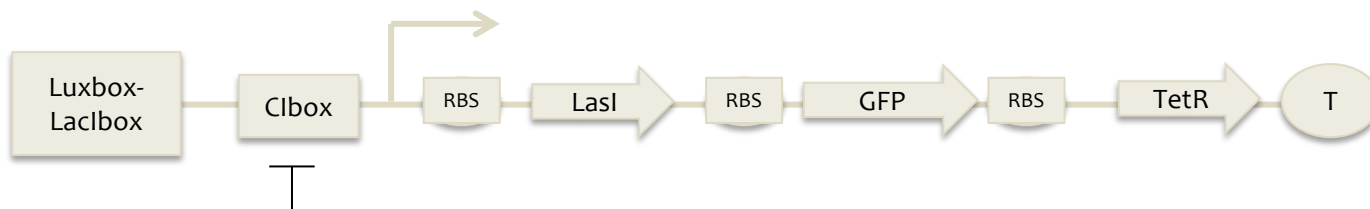
Regulation within the bacteria
Operon I inhibits operons II and III

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Operon 1

| LasR-TetO | LacIbox | RBS | LuxS | RBS | LacZ | RBS | CI | T |

Operon 2

| pLsrA-CIbox | pTetR | RBS | LuxI | RBS | LacI | RBS | mcherry | T |

Operon 3

| Luxbox-LacIbox | CIbox | RBS | LasI | RBS | GFP | RBS | TetR | T |
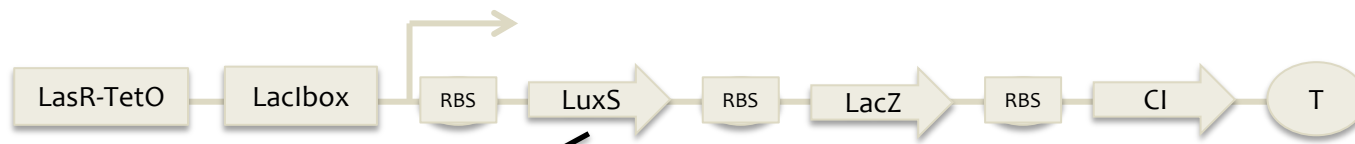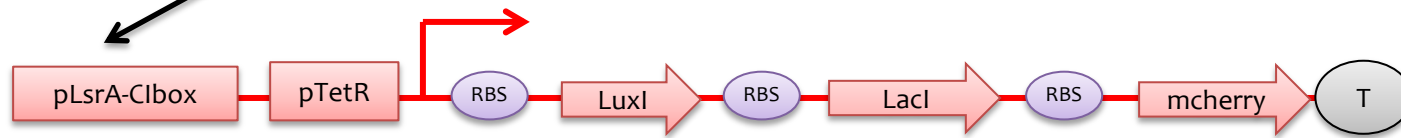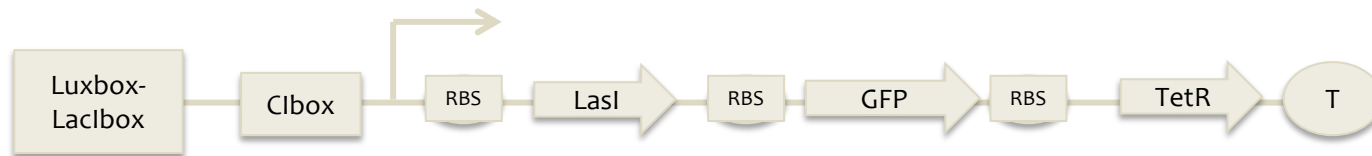
Operon I activates operon II in the neighboring bacteria
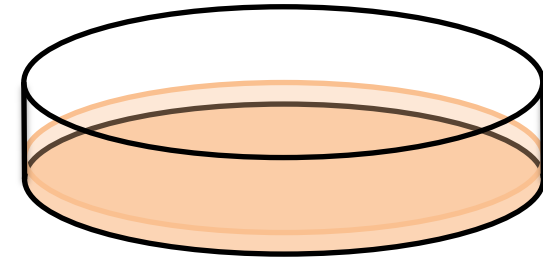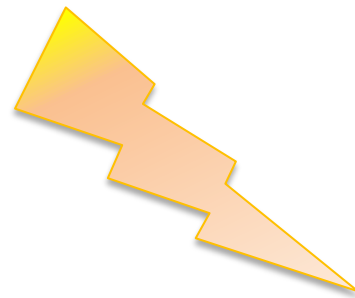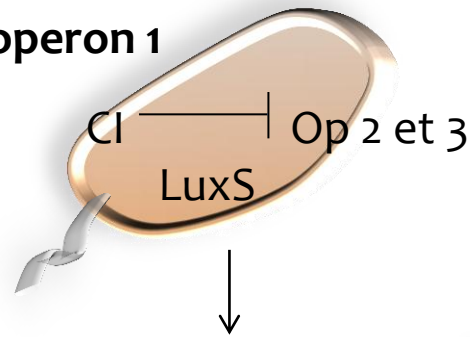
8
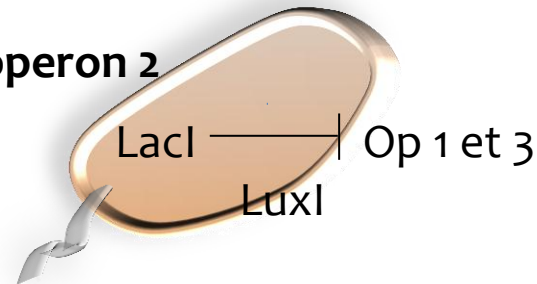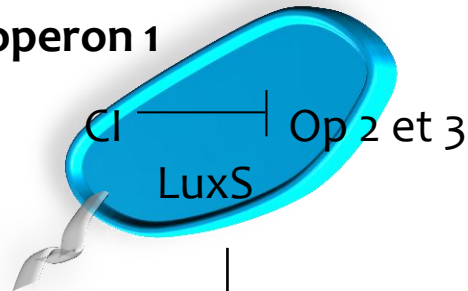
Operon-based cell Differenciation

Active operon 1

CI ⎯⎯⎯⎯⊣ Op 2 et 3

LuxS

Active operon 2

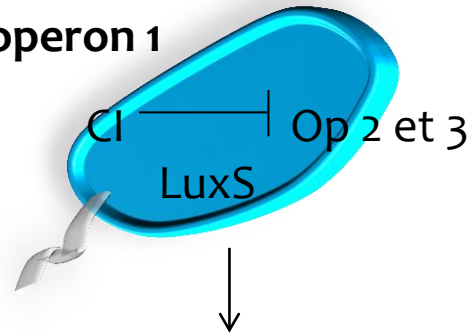LacI ⎯⎯⎯⊣ Op 1 et 3

LuxI

Operon-based cell Differenciation

Active operon 1

CI ———| Op 2 et 3
LuxS

Active operon 2

LacI ———| Op 1 et 3
LuxI

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Operon-based cell Differenciation

Active operon 1

CI ——————| Op 2 et 3

LuxS

Active operon 2

LacI ——————| Op 1 et 3

LuxI

Operon-based cell Differenciation

Active operon 3

Tet R ⊣ Op 1 et 2

LasI

Active operon 1

CI ⊣ Op 2 et 3

LuxS

Active operon 2
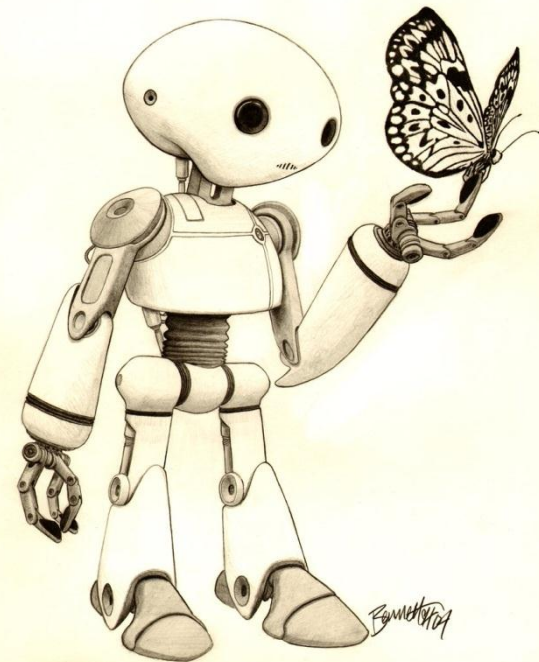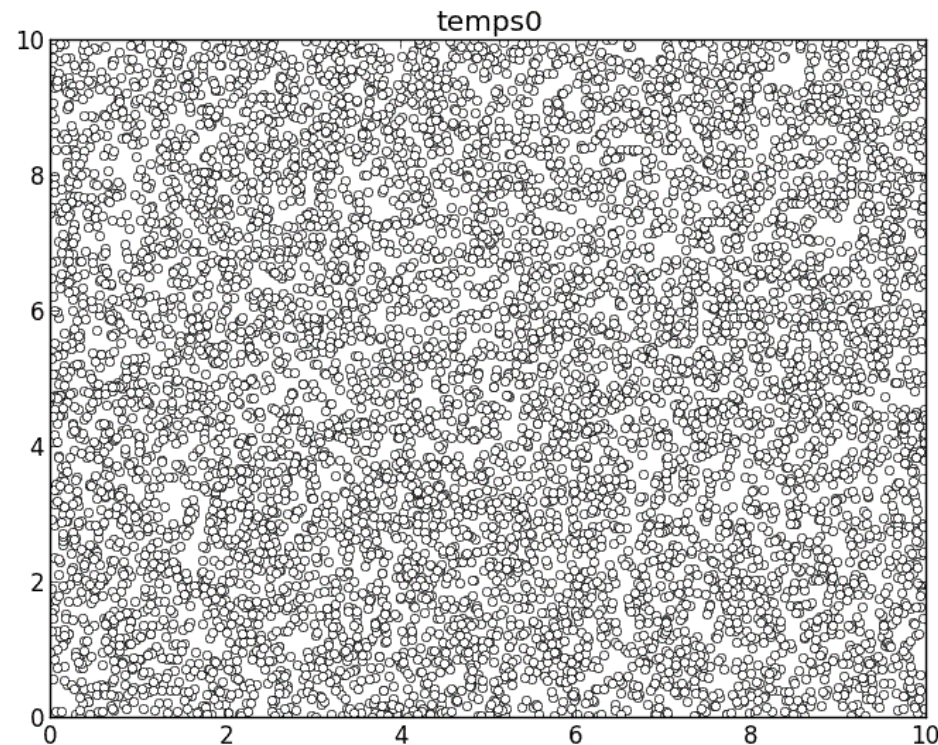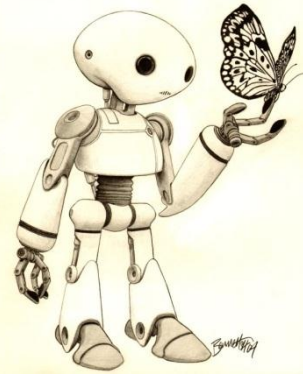
LacI ⊣ Op 1 et 3

LuxI
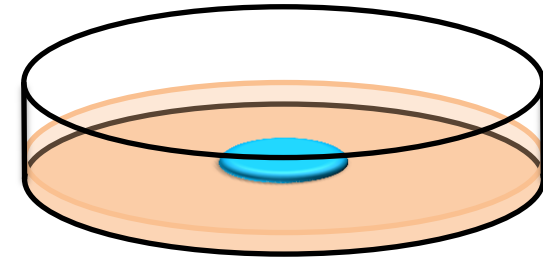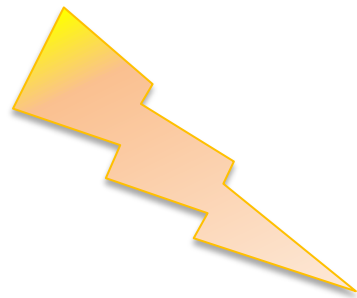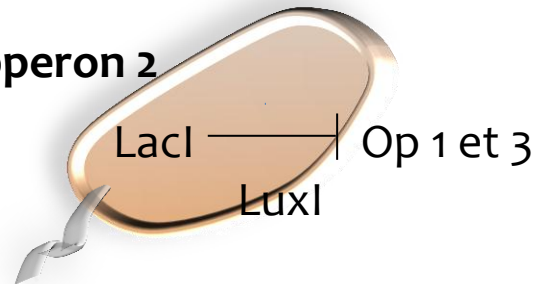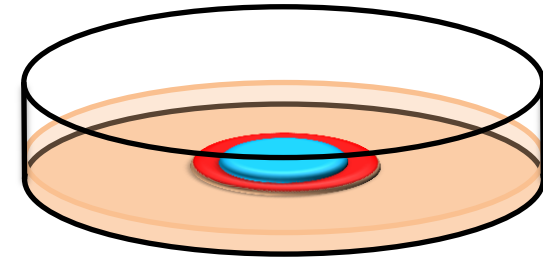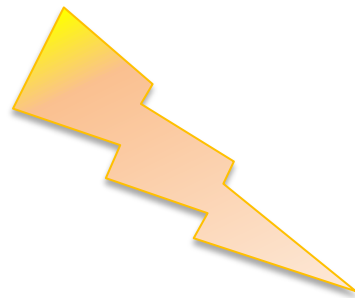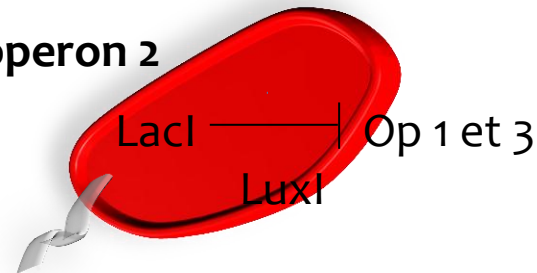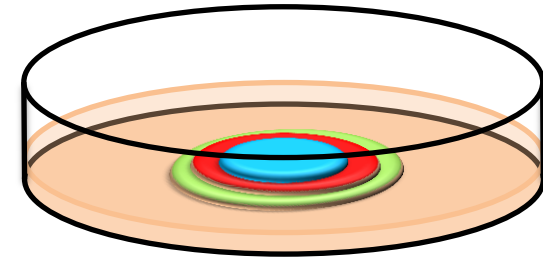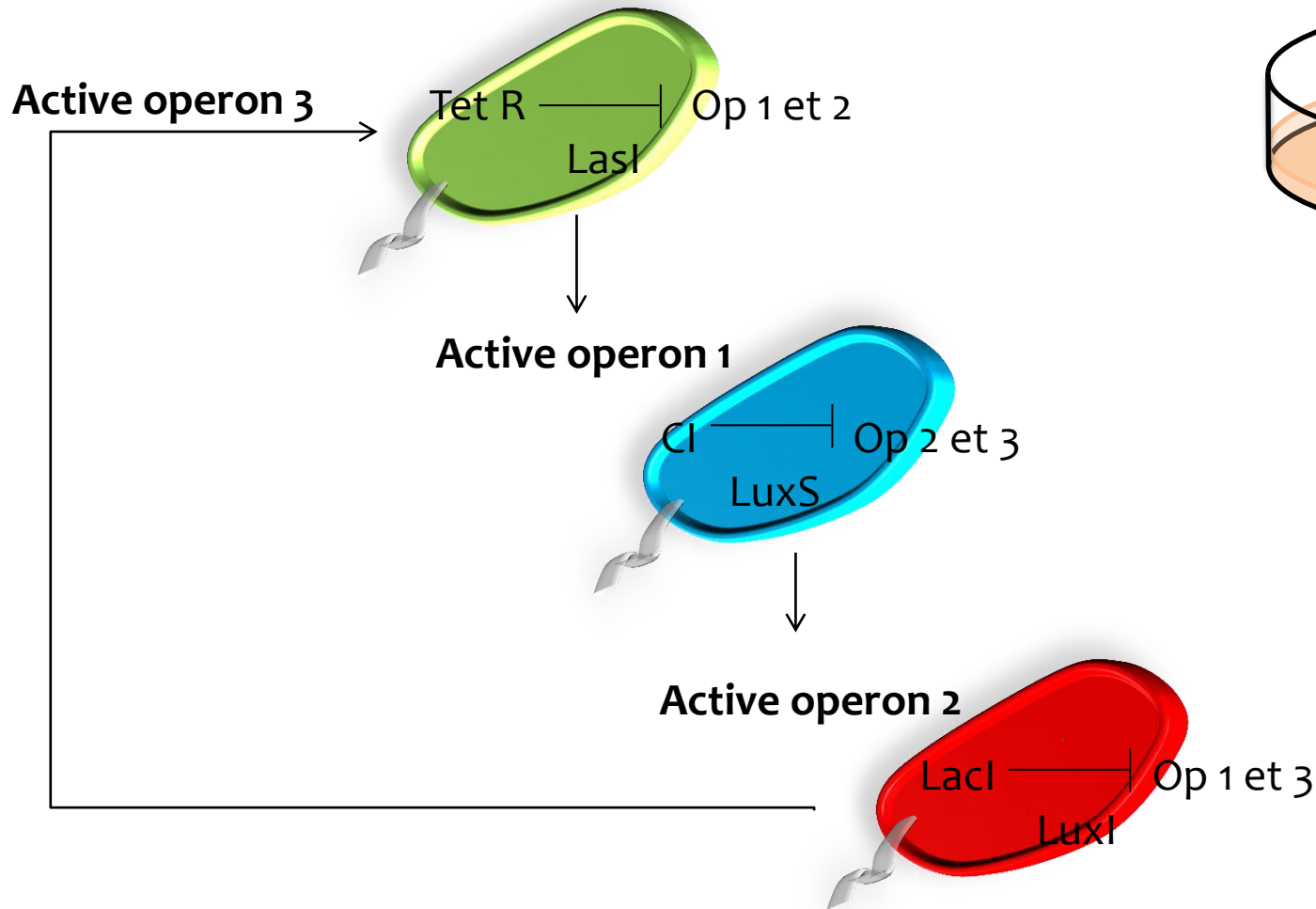
CHAPTER 2
THE SIMULATION

What can computer teach us ?

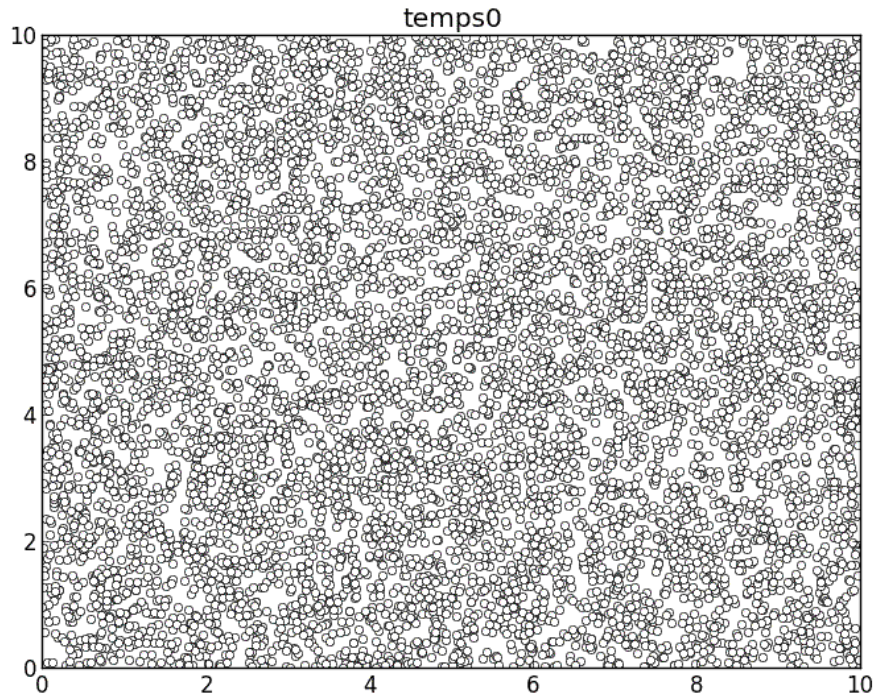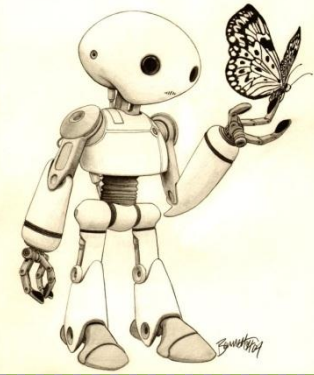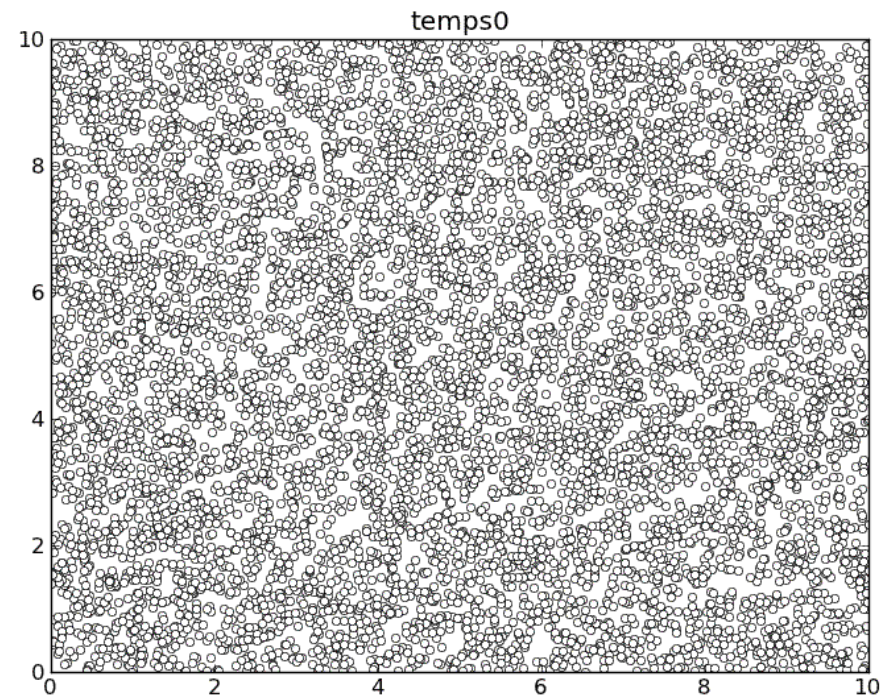*"They didn't know it was impossible, so they did it."* Mark Twain

The simulation

temps0

Models our genetic regulatory network
Includes eventual promoter leakage, mutation, etc…
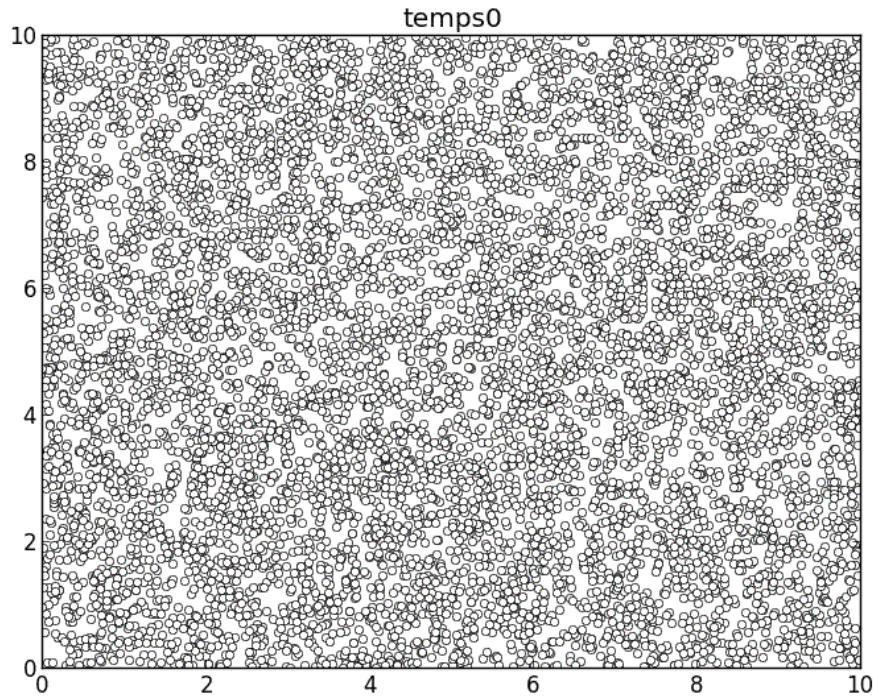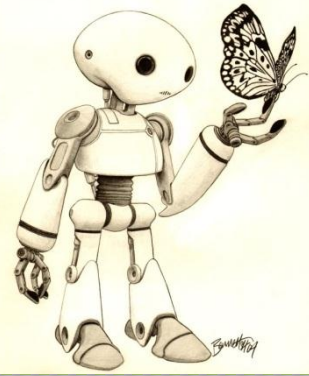Python programming language

The simulation

temps0

temps0
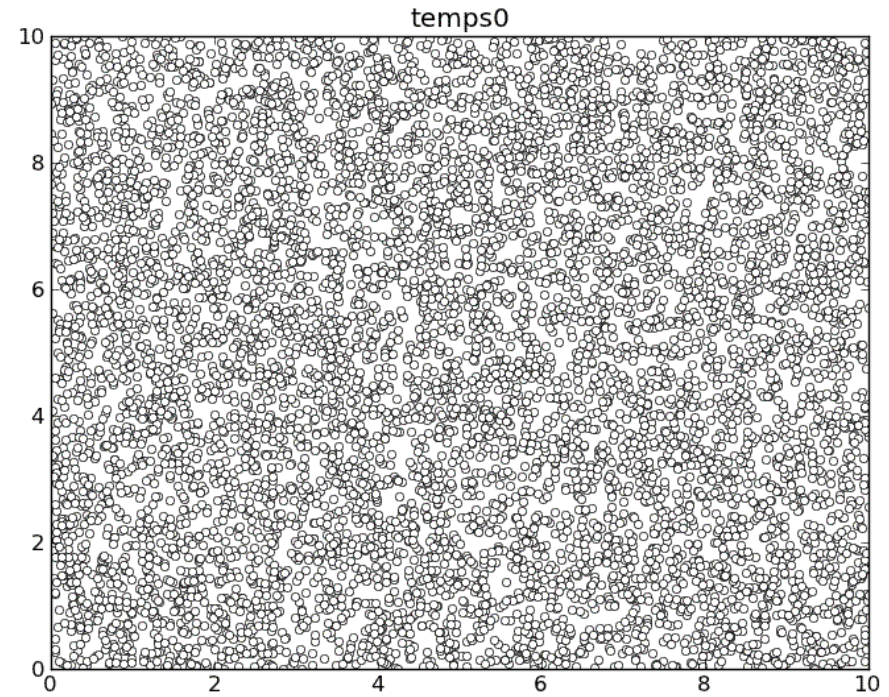
Everything is fine

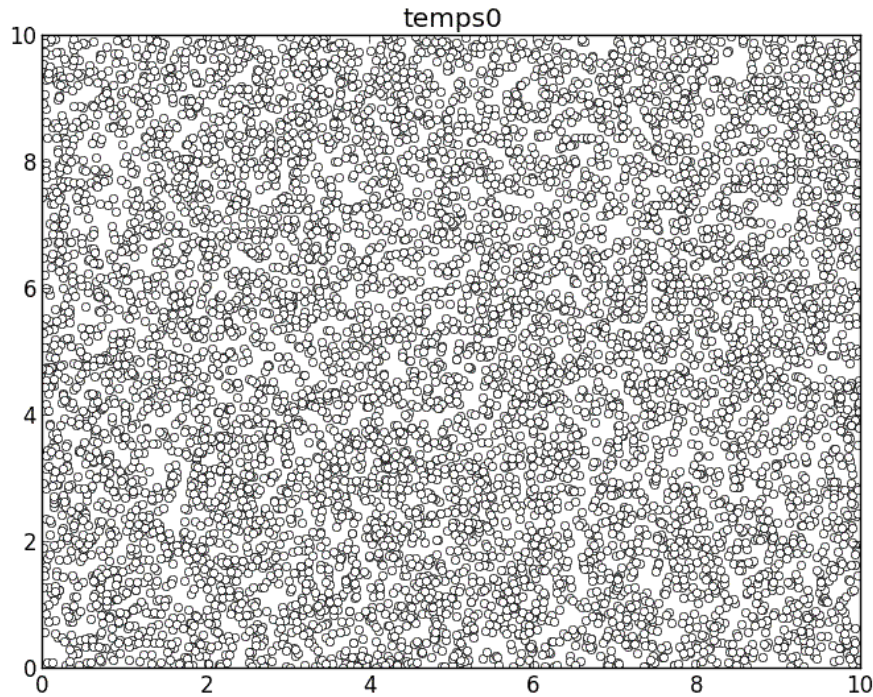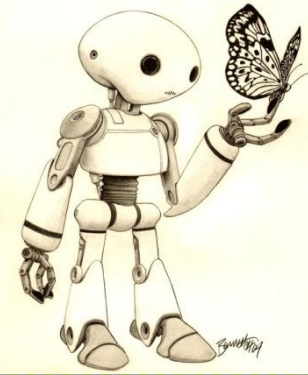Operon I not signaling

The simulation

temps0
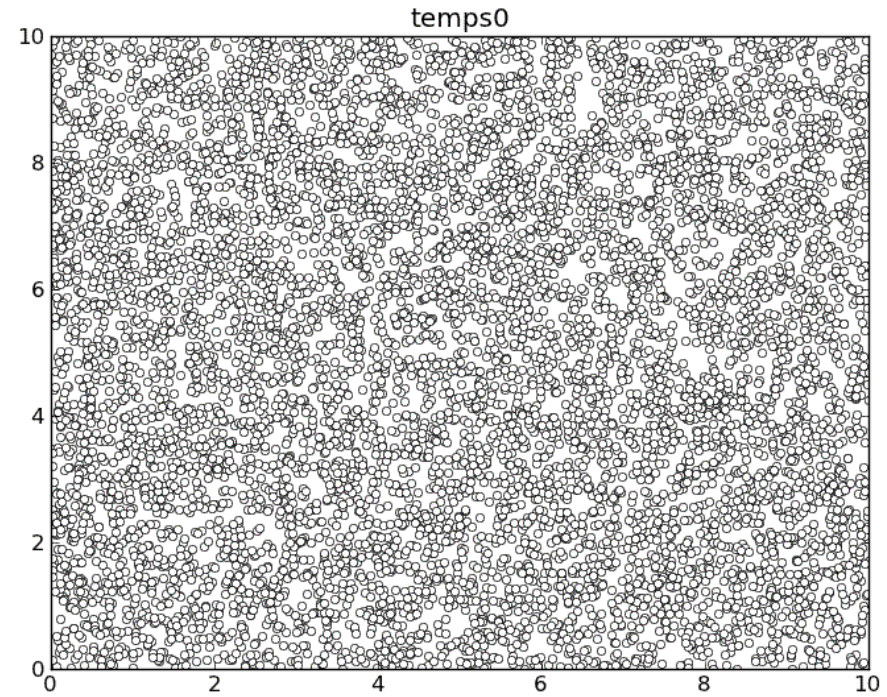

temps0

Everything is fine

Operon II not signaling

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The simulation

Everything is fine

Operon III not signaling
And promoter leakage

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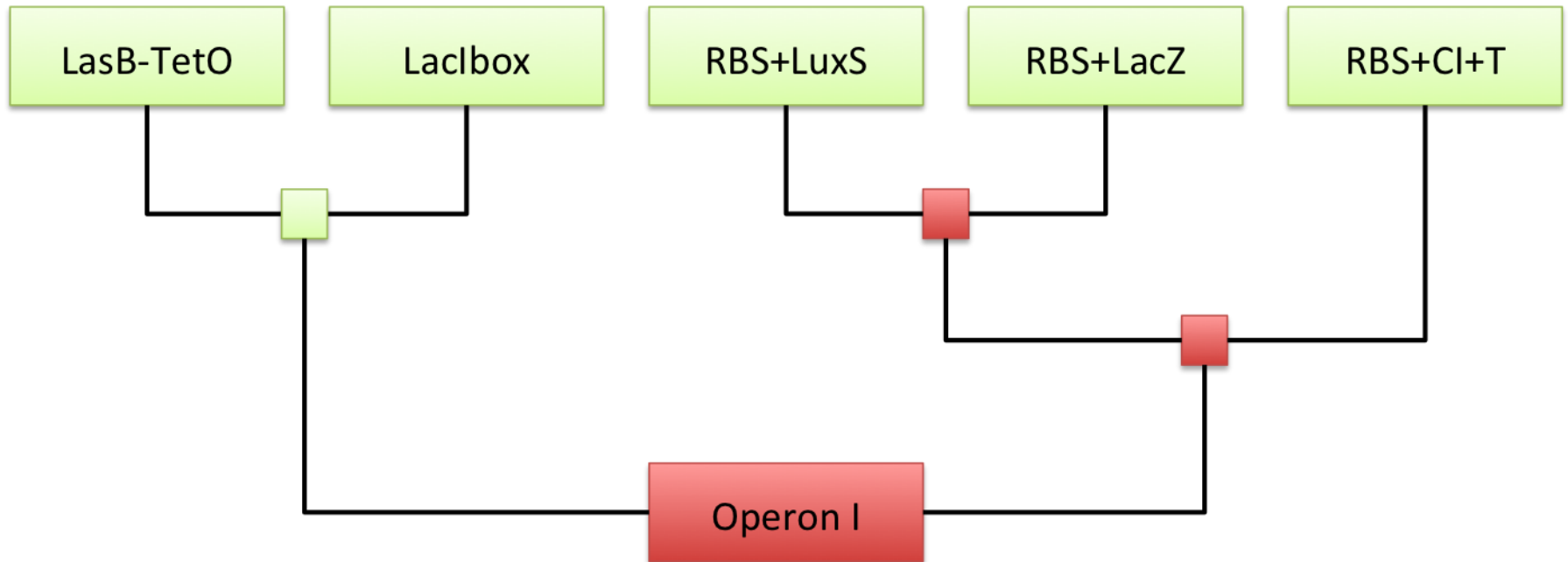# CHAPTER 3
THE LABWORK

Where are we now ?

" Science, my lad, is made up of mistakes, but they are mistakes which it is useful to make, because they lead little by little to the truth " **Jules Verne, Journey to the Center of the Earth**

Assembly

| LasB-TetO | LacIbox | RBS+LuxS | RBS+LacZ | RBS+CI+T |

Operon I

- Operon I : 3 assemblies left

Assembly

- Operon II complete

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Assembly

| Luxbox-LacIbox | CIbox | RBS | LasI | RBS+GFP | RBS+TetR+T |
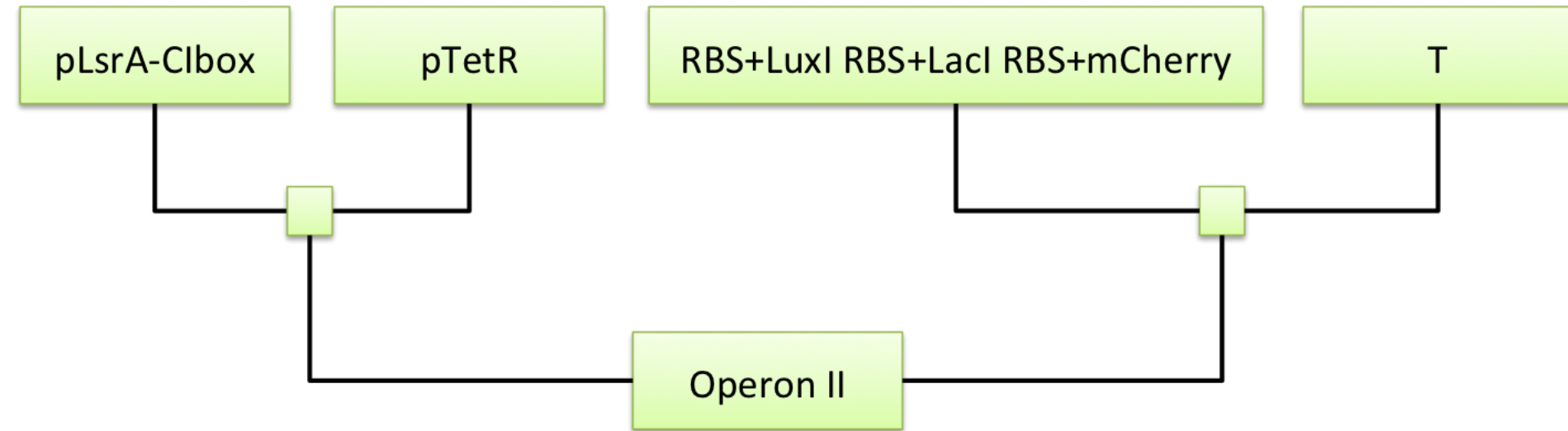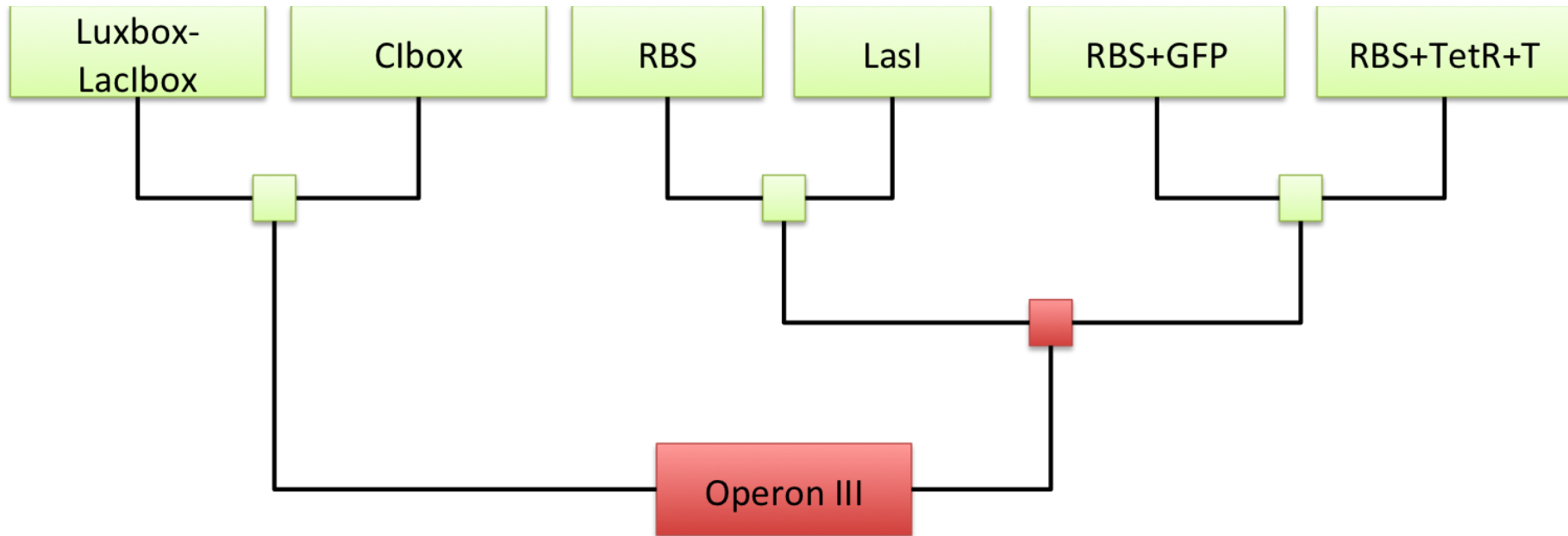
Operon III

- Operon III : 2 assemblies left

16

Assembly

| Pc_Op4 | RBS | LsrR | RBS | LsrK | RBS+LuxR | RBS+LasR+T |
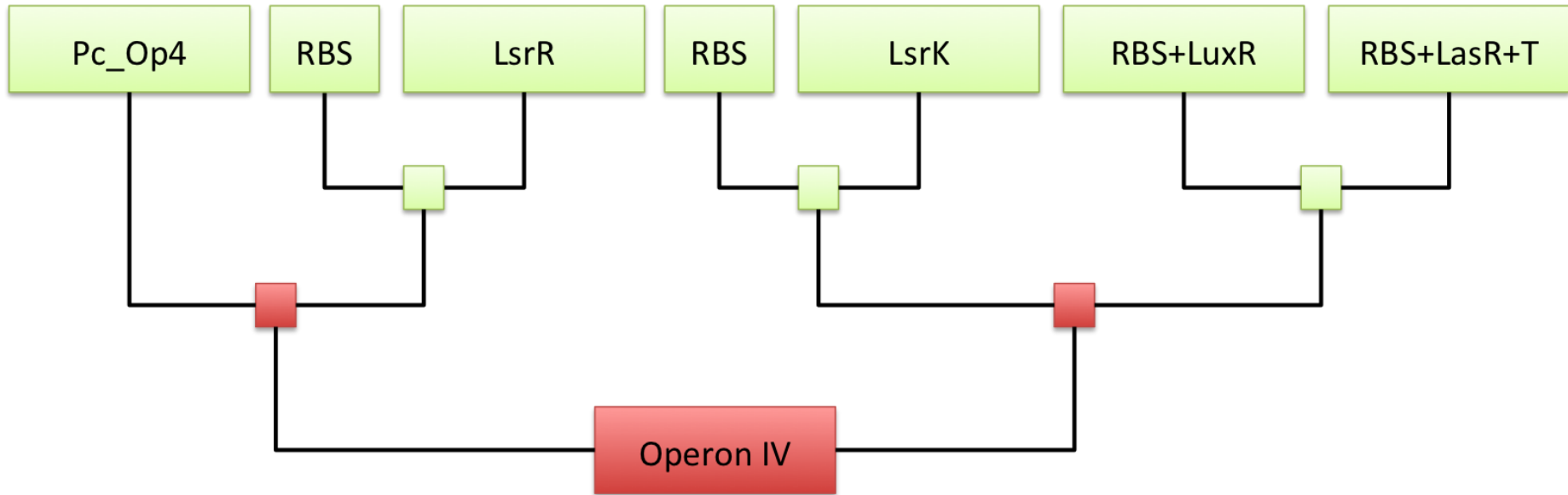
Operon IV

- Operon IV : 3 assemblies left

Assembly

- What is left ?

10/20 assemblies completed.

18

Assembly

- Moving to a simpler system :

2 colored state (3 operons)

Operon II

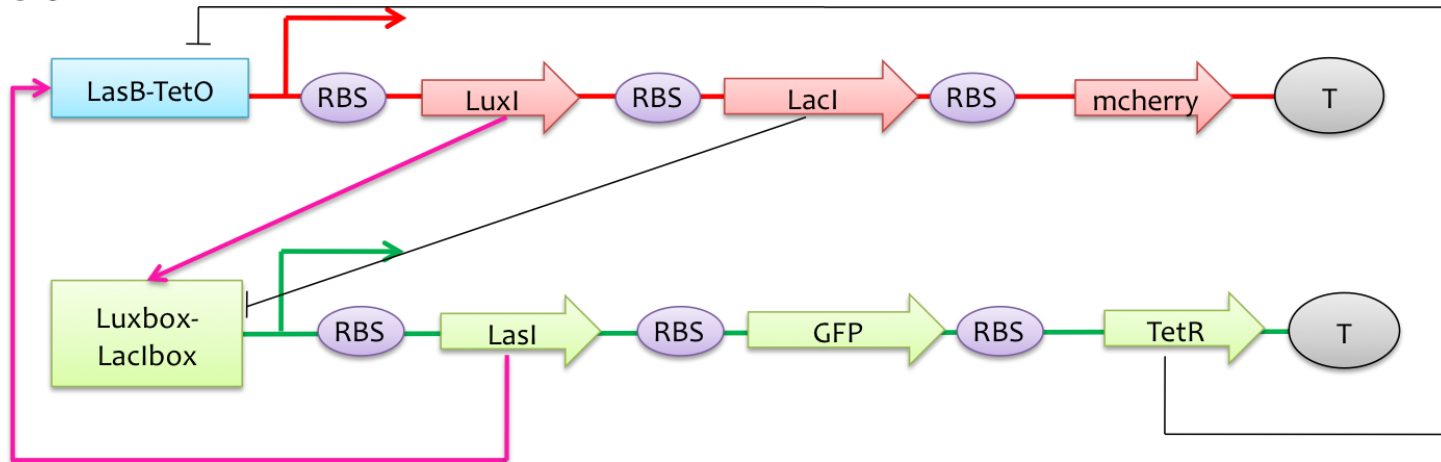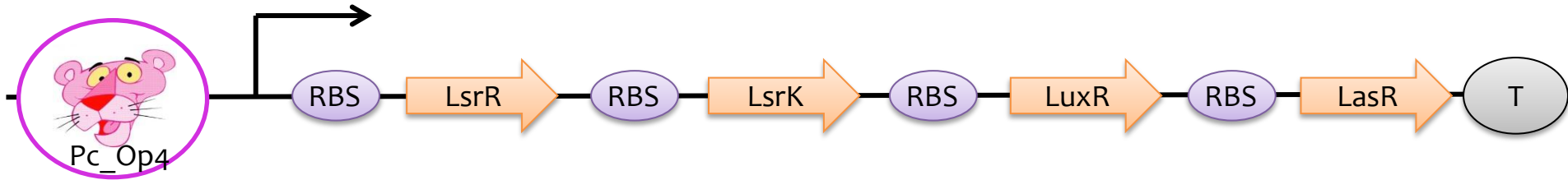**Operon III**

CONCLUSION
THE PROSPECT

What could came out of this project?

" You should aim higher with your fantasies " **Lem, Veridian Dynamics**

The prospect

Pc_Op4 — RBS — LsrR — RBS — LsrK — RBS — LuxR — RBS — LasR — T

Constitutive expression driven by biobrick J23100 *aka* « Pink Promoter »

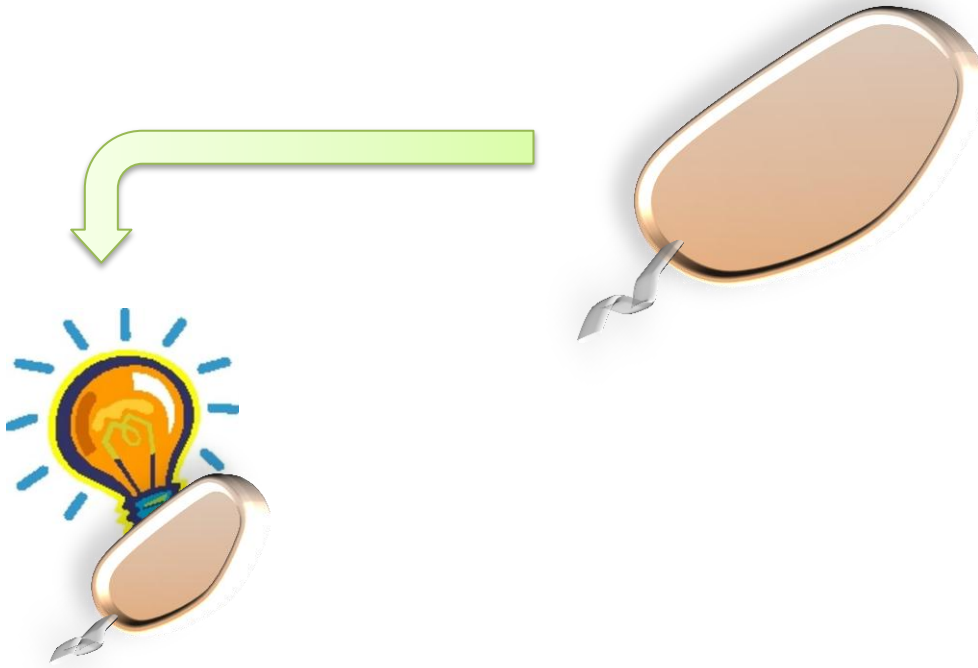- Multiple Quorum-sensing Responsive bacteria

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The prospect

Naive state

- Enabling bacteria to have multiple functionnality
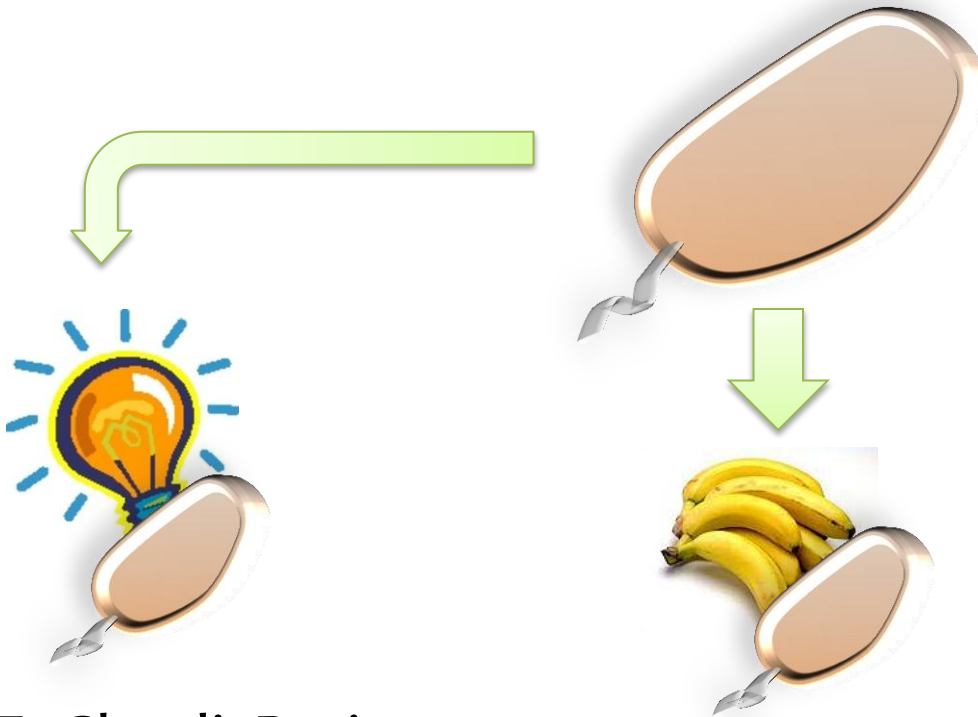
The prospect

E. Glowli Project
(2010 Cambridge)

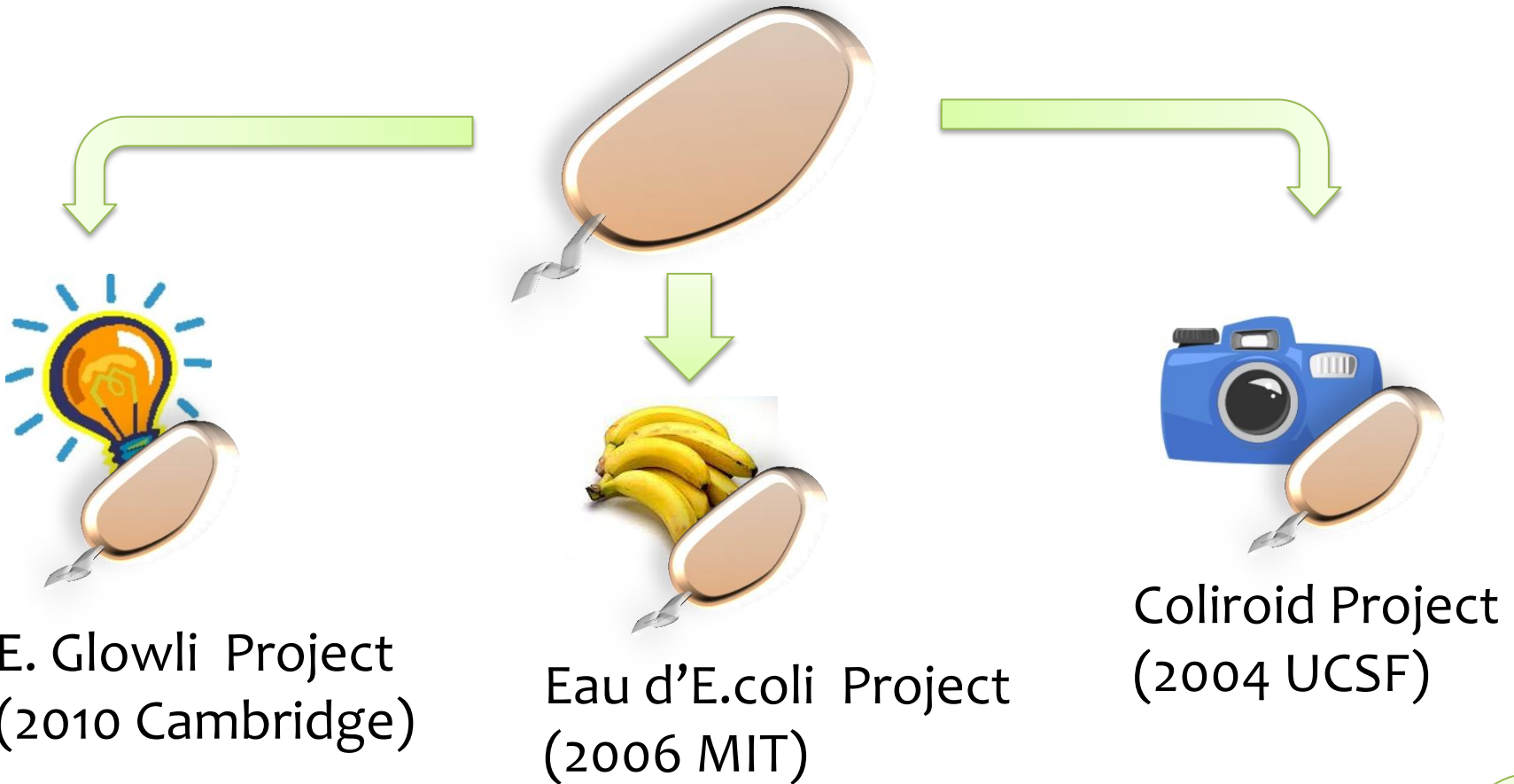- Enabling bacteria to have multiple functionnality

The prospect

E. Glowli Project
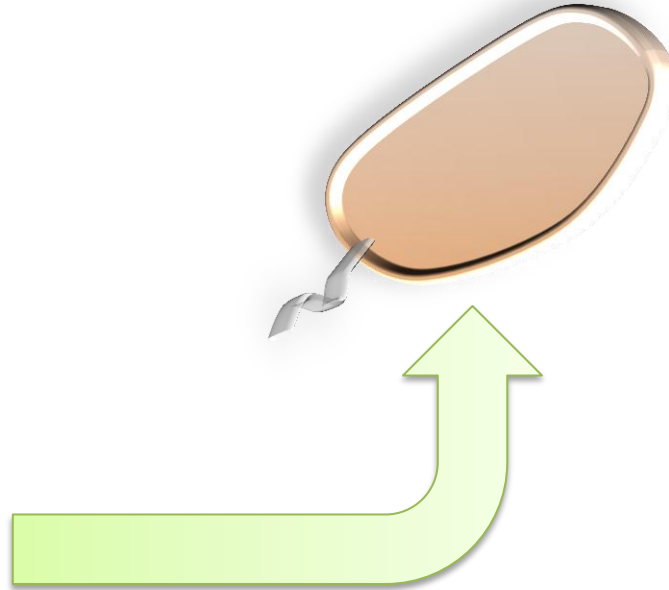(2010 Cambridge)

Eau d'E.coli Project
(2006 MIT)

- Enabling bacteria to have multiple functionnality

The prospect

E. Glowli Project
(2010 Cambridge)

Eau d'E.coli Project
(2006 MIT)

Coliroid Project
(2004 UCSF)

- Enabling bacteria to have multiple functionnality
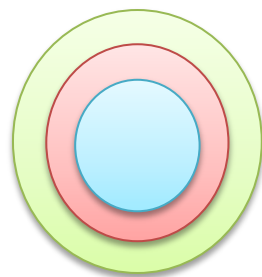
The prospect

Naive state

« Reboot » function :
Allow dedifferentiation

E. Glowli Project
(2010 Cambridge)

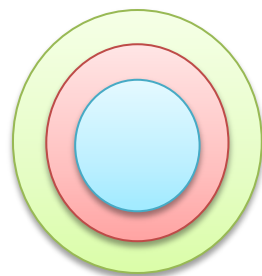- Enabling bacteria to have multiple functionality

The prospect

1X signal

If number of ring/circles dependant of the amount of initial signal :

- Easy-to-read visible readout for chemical input

The prospect

1X signal   2X signal
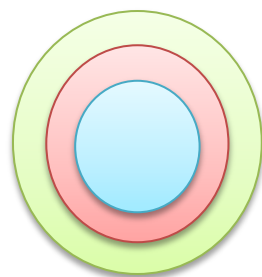
If number of ring/circles dependant of the amount of initial signal :

- Easy-to-read visible readout for chemical input

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The prospect

1X signal   2X signal   3X signal
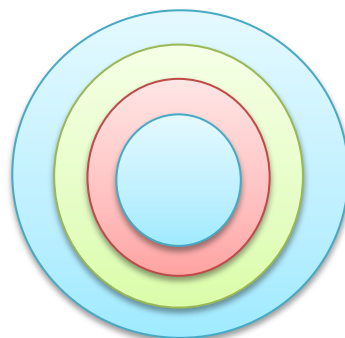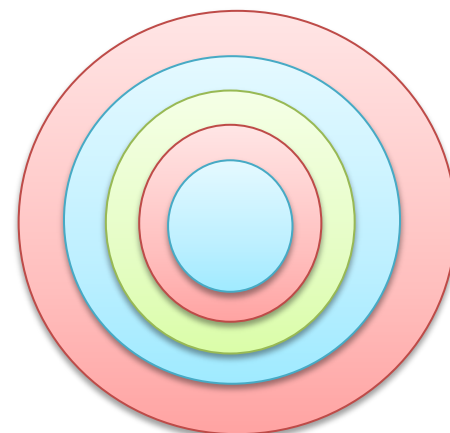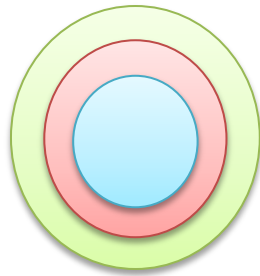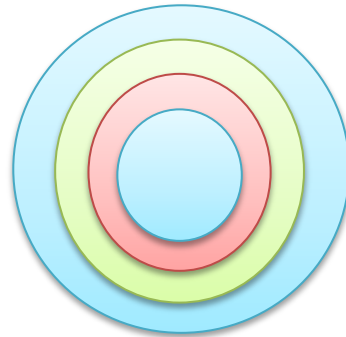
If number of ring/circles dependant of the amount of initial signal :
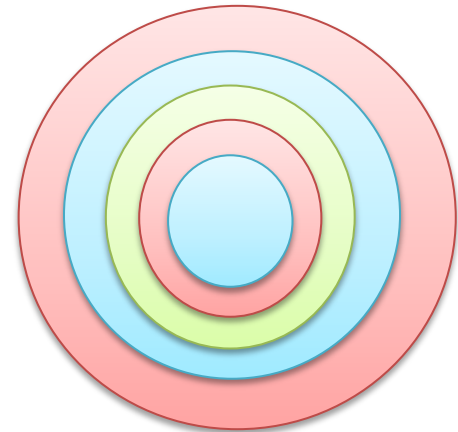
- Easy-to-read visible readout for chemical input

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The prospect

1X signal   2X signal   3X signal

Coupling with other iGEM project :

Arsenic biosensor project (2006 Edinburgh)

Annexe

Image source :

http://www.thelensflare.com/imgs/eyespot-butterfly_47484.html
http://artistjerrybennett.deviantart.com/art/Robot-and-Butterfly-215933149
http://en.wikipedia.org/wiki/File:Zebra_in_Mikumi.JPG
http://en.wikipedia.org/wiki/File:Slleo1.jpg
http://fr.wikipedia.org/wiki/Fichier:Inachis_io_LC0131.jpg
http://fr.wikipedia.org/wiki/Fichier:Junonia_coeniaPCCA20051015-1147B.jpg

The sponsors

Thank you for your time !

The world is my country, science is my religion.' – **Christiaan Huygens, Dutch Physicist (1629-1695)**