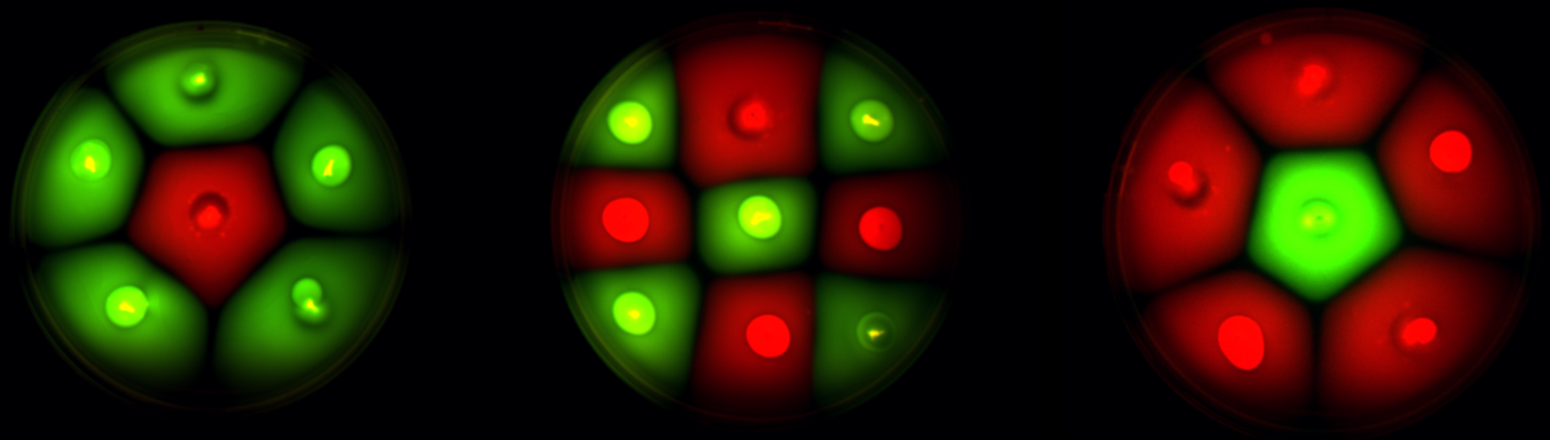
Turn Me On, Lactones!

New Tools For Self-Organized Pattern Formation



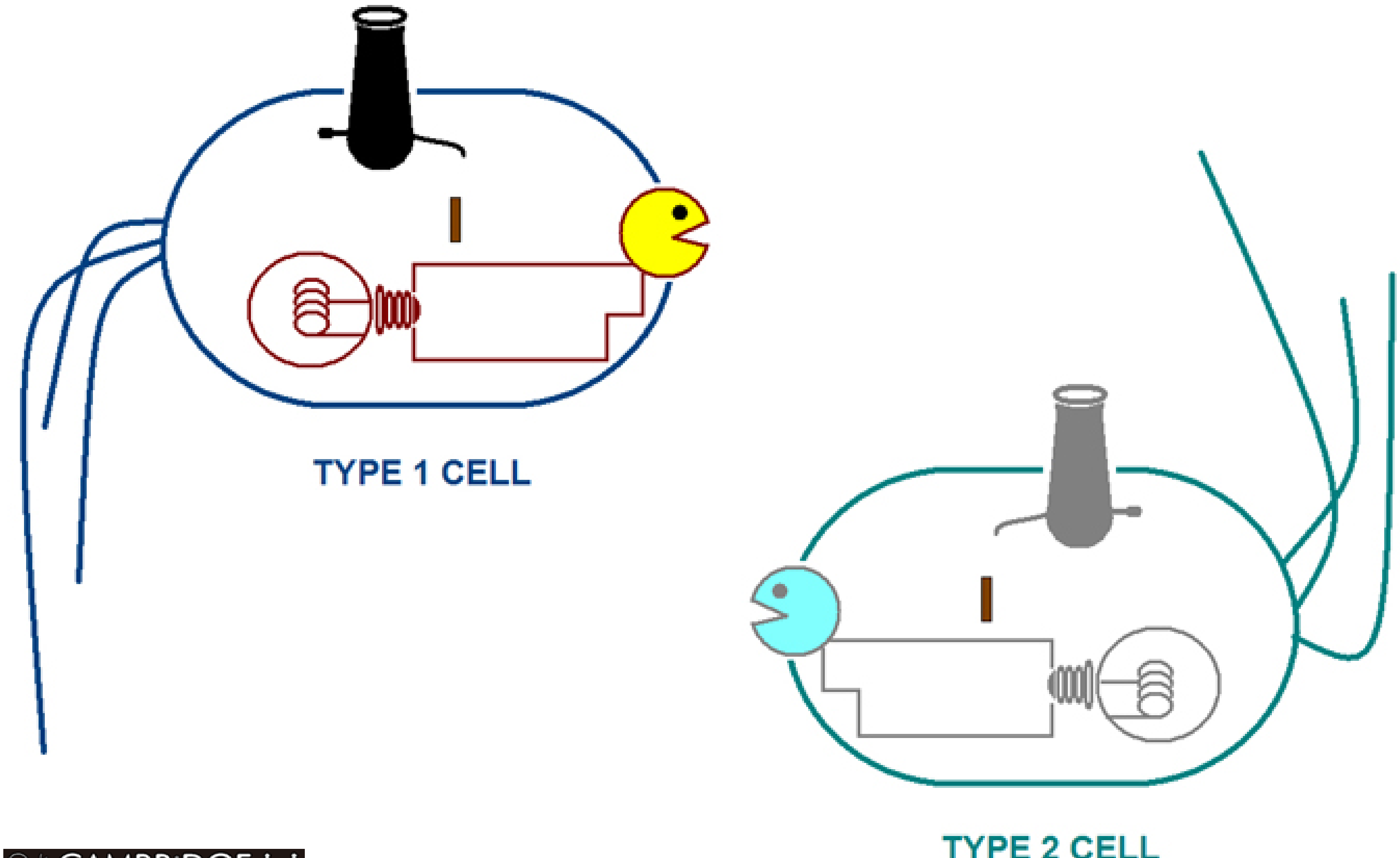
University of Cambridge

iGEM 2006

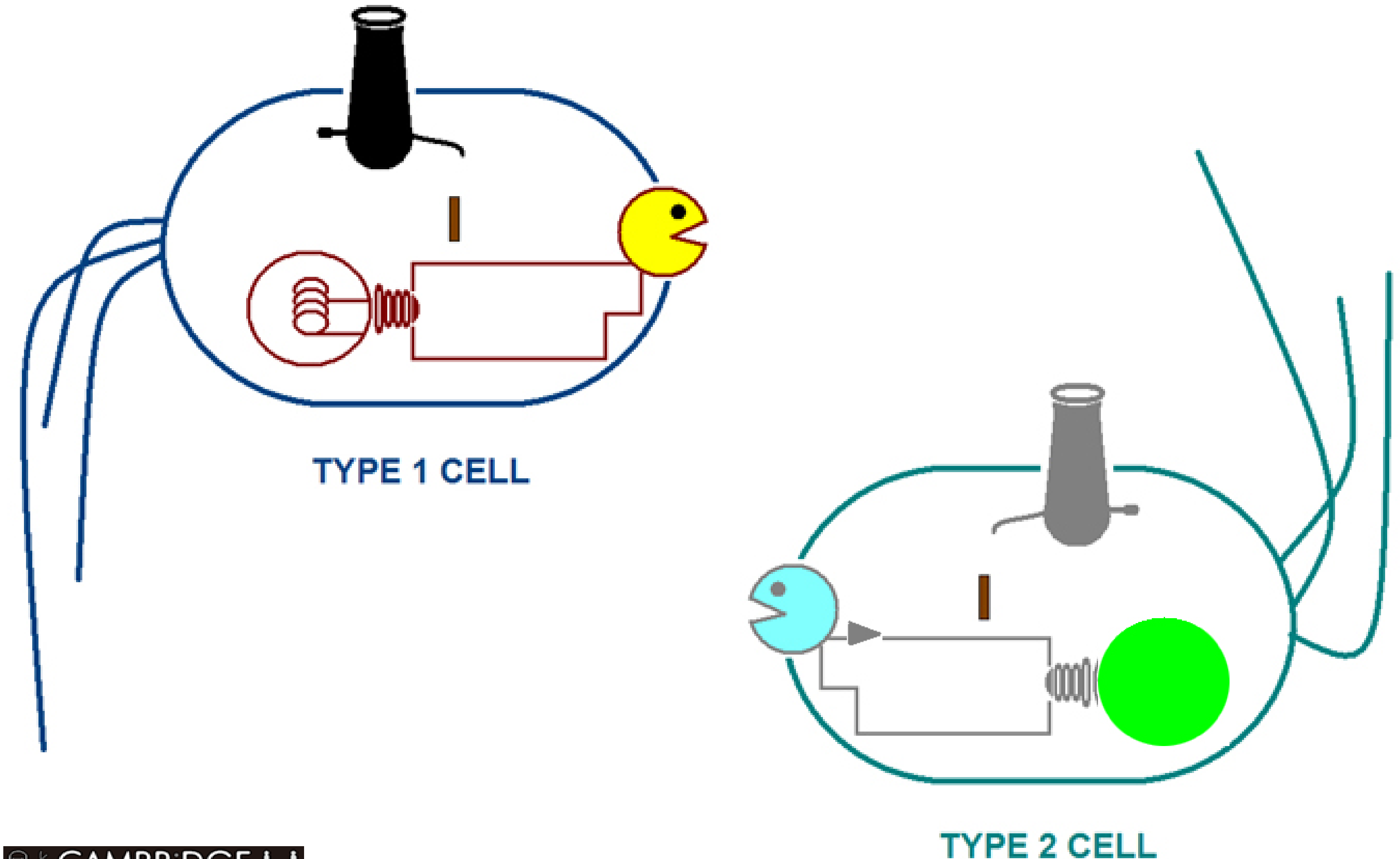
Aims

- **New Tools for Self-organised Pattern Formation**
- Two possible systems the tools could be used for are:
  - Bi-directional signalling
  - A population based bi-stable switch

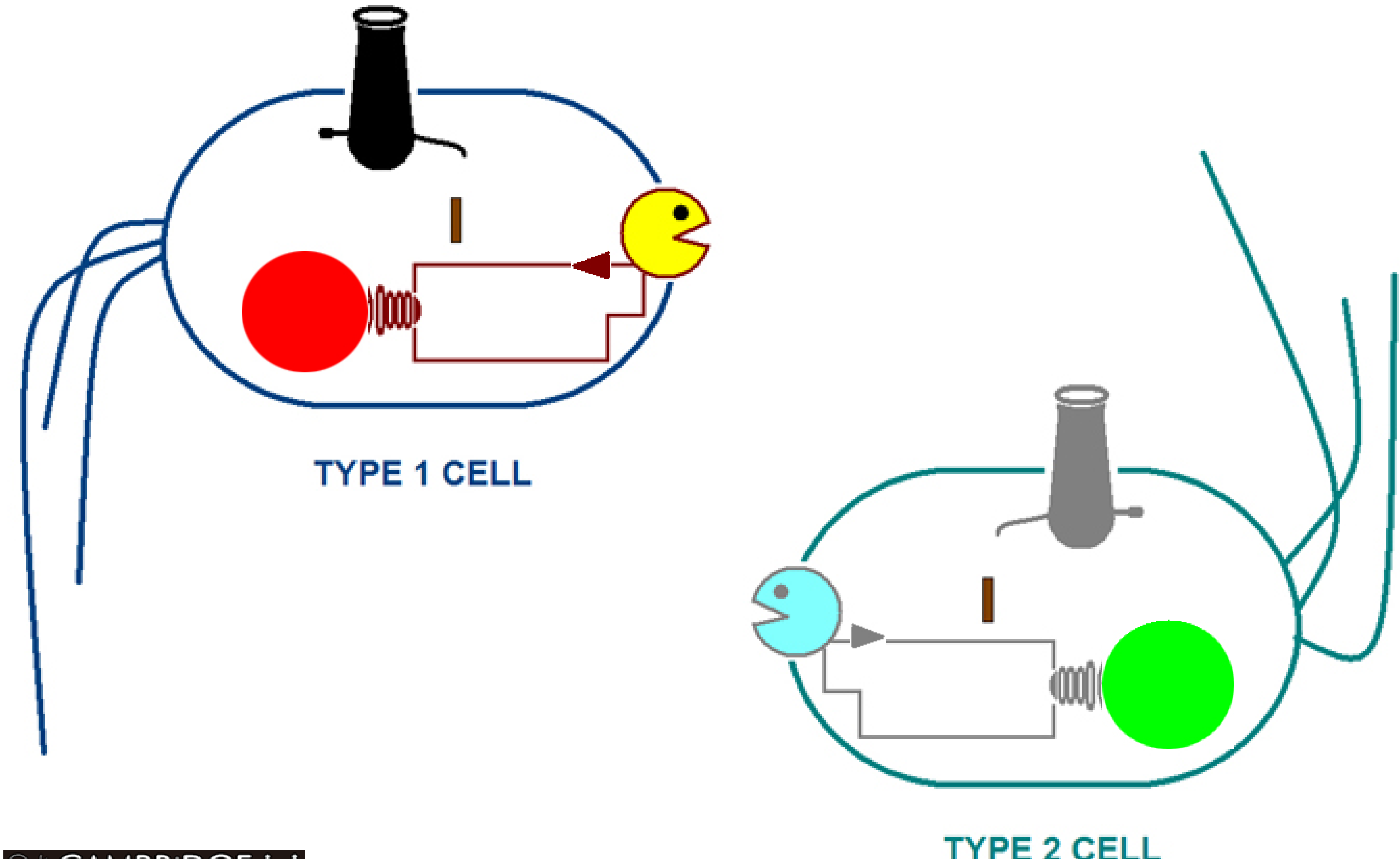
Bi-directional Signalling



Bi-directional Signalling



Bi-directional Signalling



CAMBRiDGE iGEM 2006

Project Overview

New tools for self-organised pattern formation

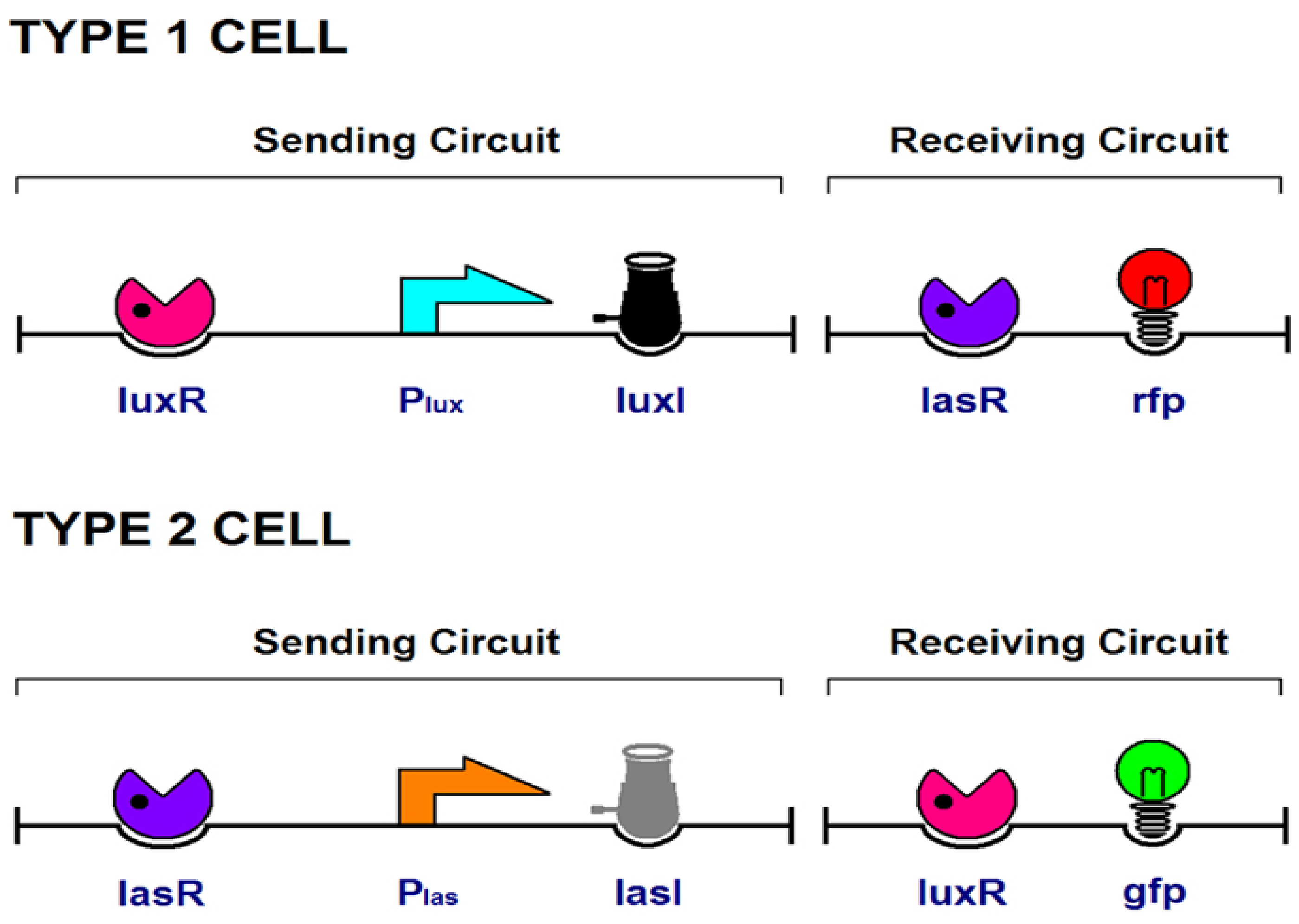
Tools
- Genetic circuit
- Parts

Modelling
- Single-cell dynamics
- Multi-cell interaction

Experiments
- Swimming assay
- AHL bio-assay

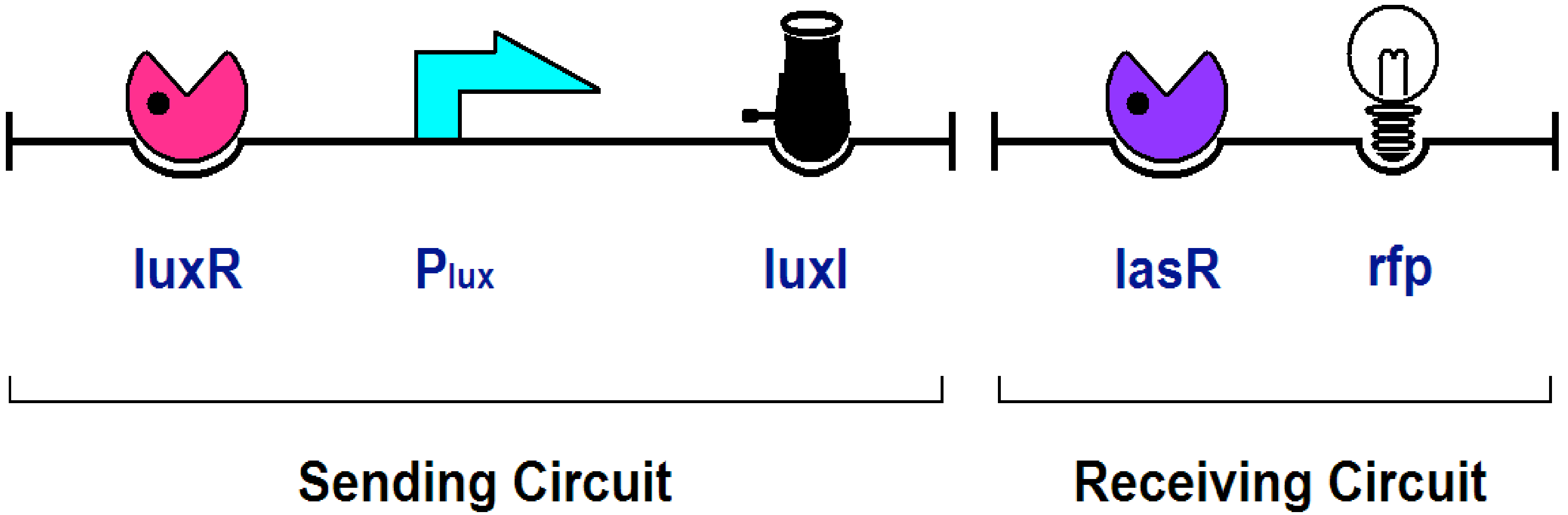
Results – **Autonomous pattern formation**

Genetic Circuit



System Illustration

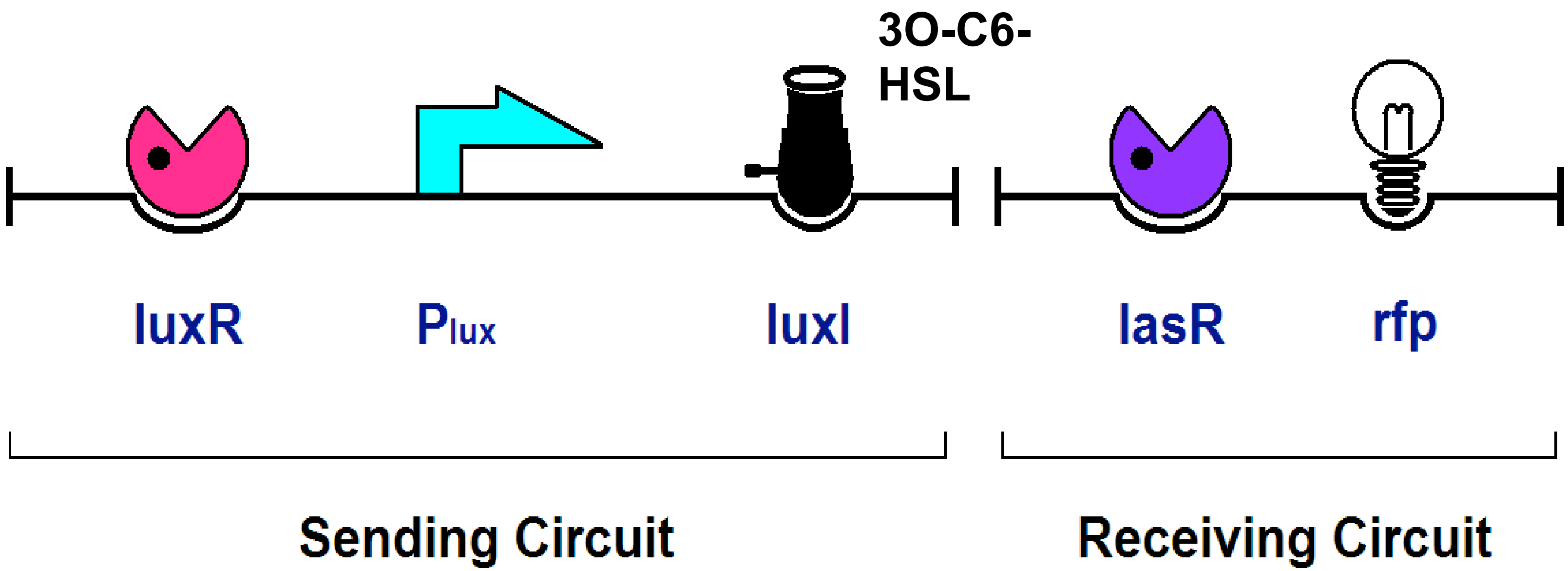
TYPE 1 CELL



Type 2 cells behave in a complementary but analogous manner.

System Illustration

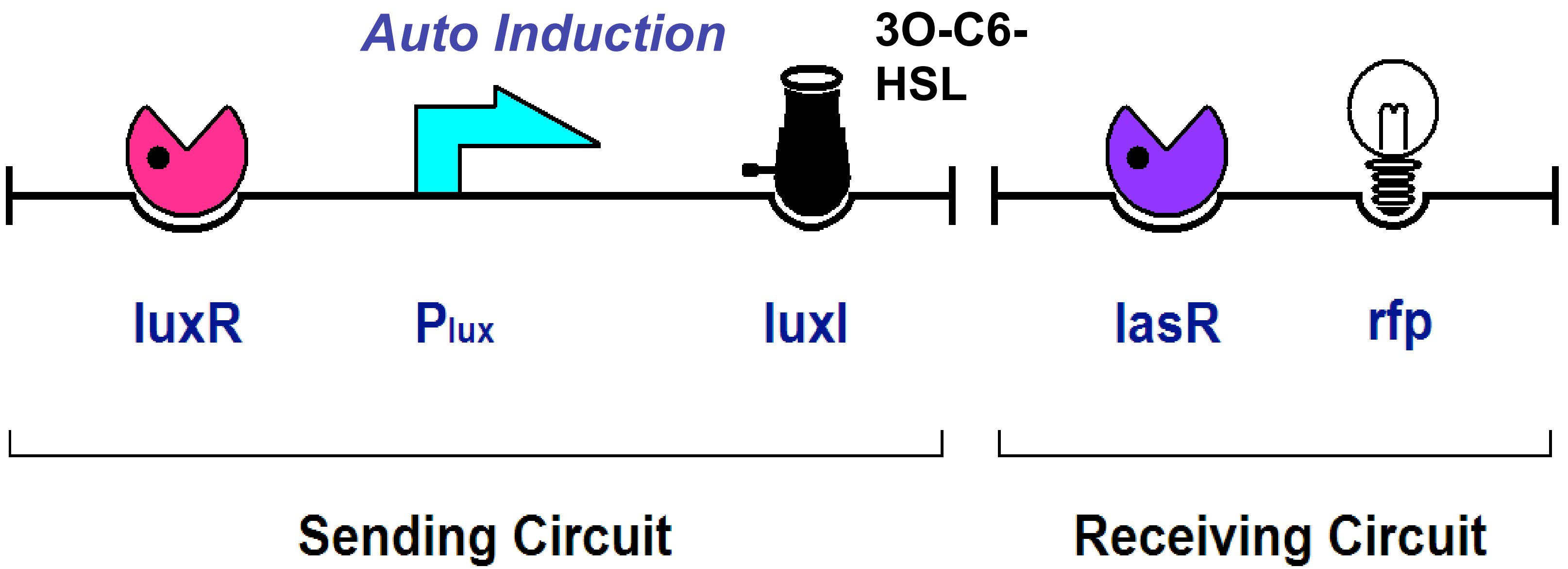
TYPE 1 CELL



Type 2 cells behave in a complementary but analogous manner.

System Illustration

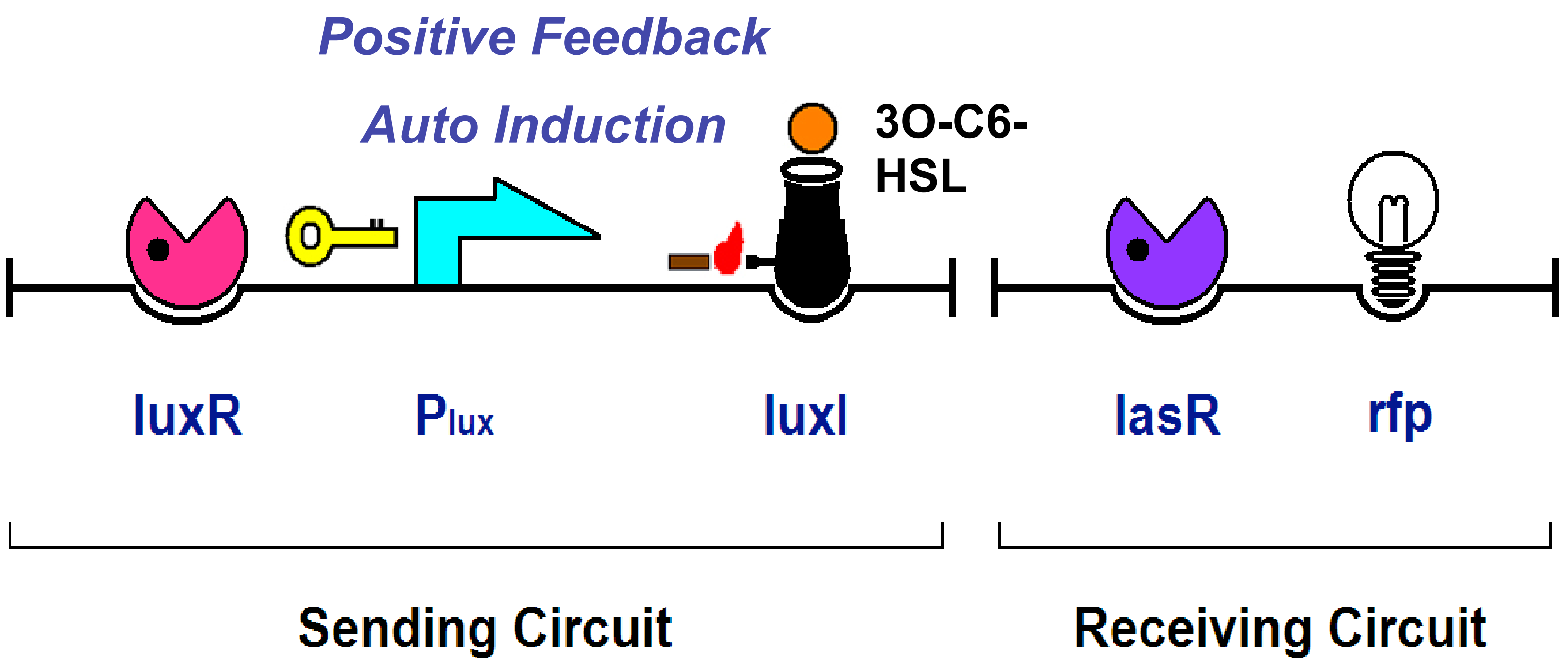
TYPE 1 CELL



Type 2 cells behave in a complementary but analogous manner.

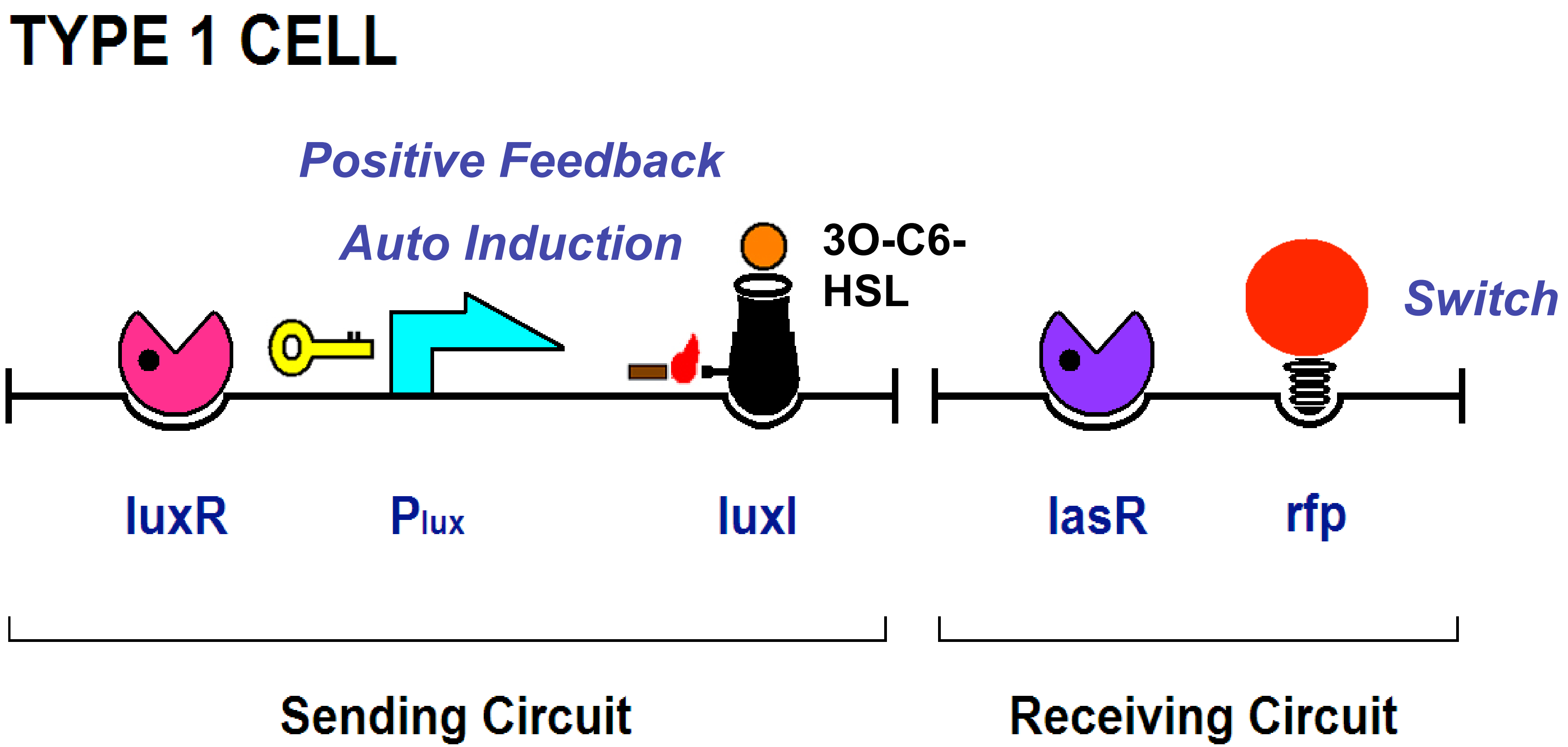
System Illustration

TYPE 1 CELL



Type 2 cells behave in a complementary but analogous manner.

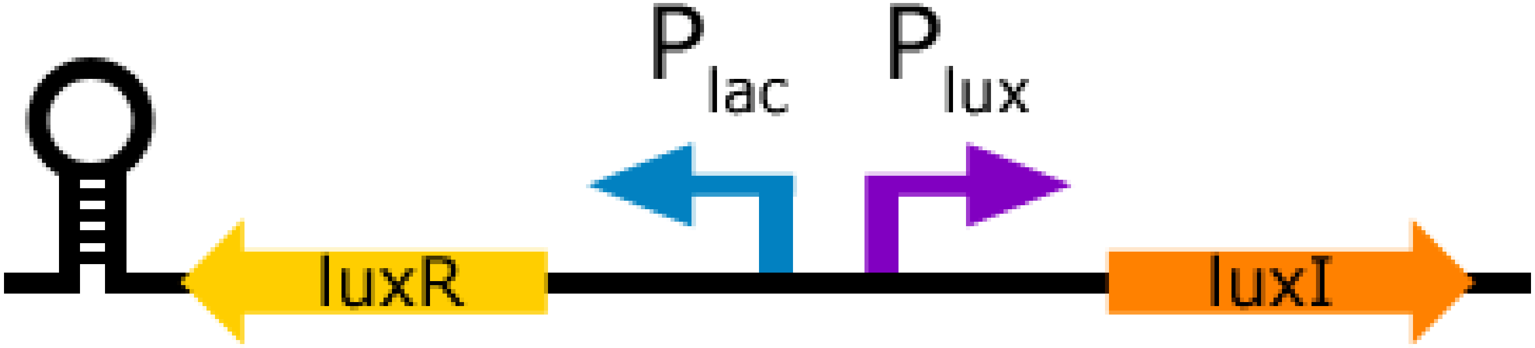
System Illustration



Type 2 cells behave in a complementary but analogous manner.

AHL Sender Cassettes: No. 1

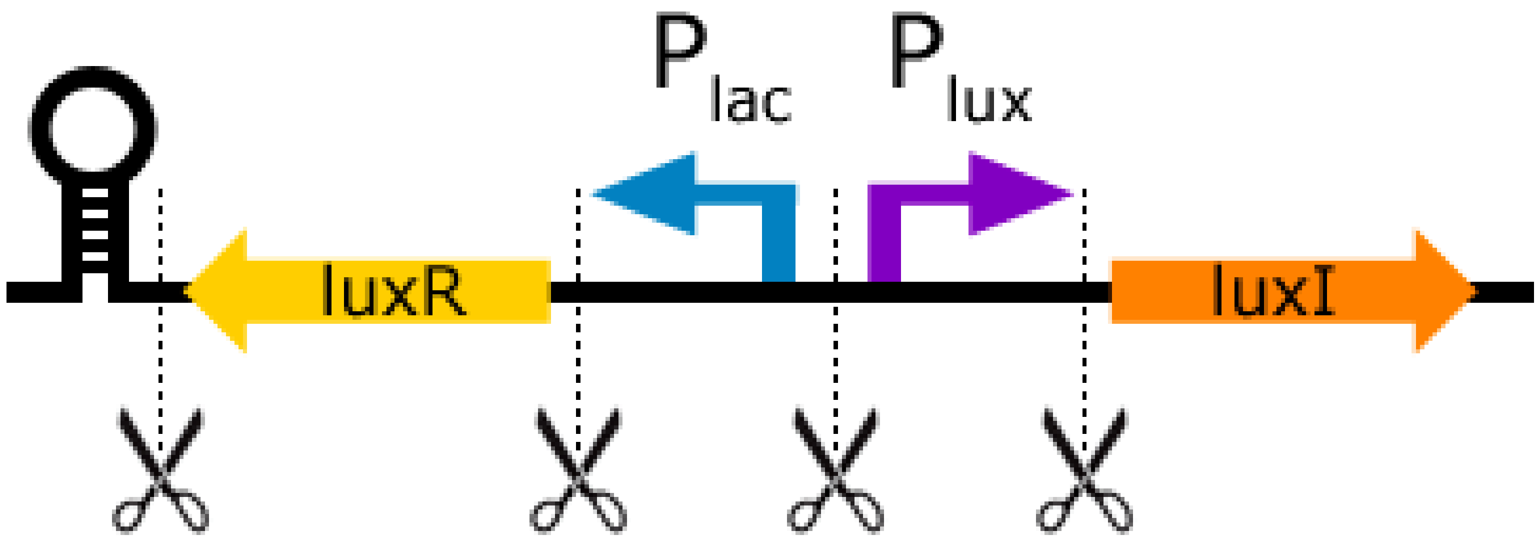
Lux autoinducer: 1681 bp



BBa_J28031

AHL Sender Cassettes: No. 1

Lux autoinducer: 1681 bp

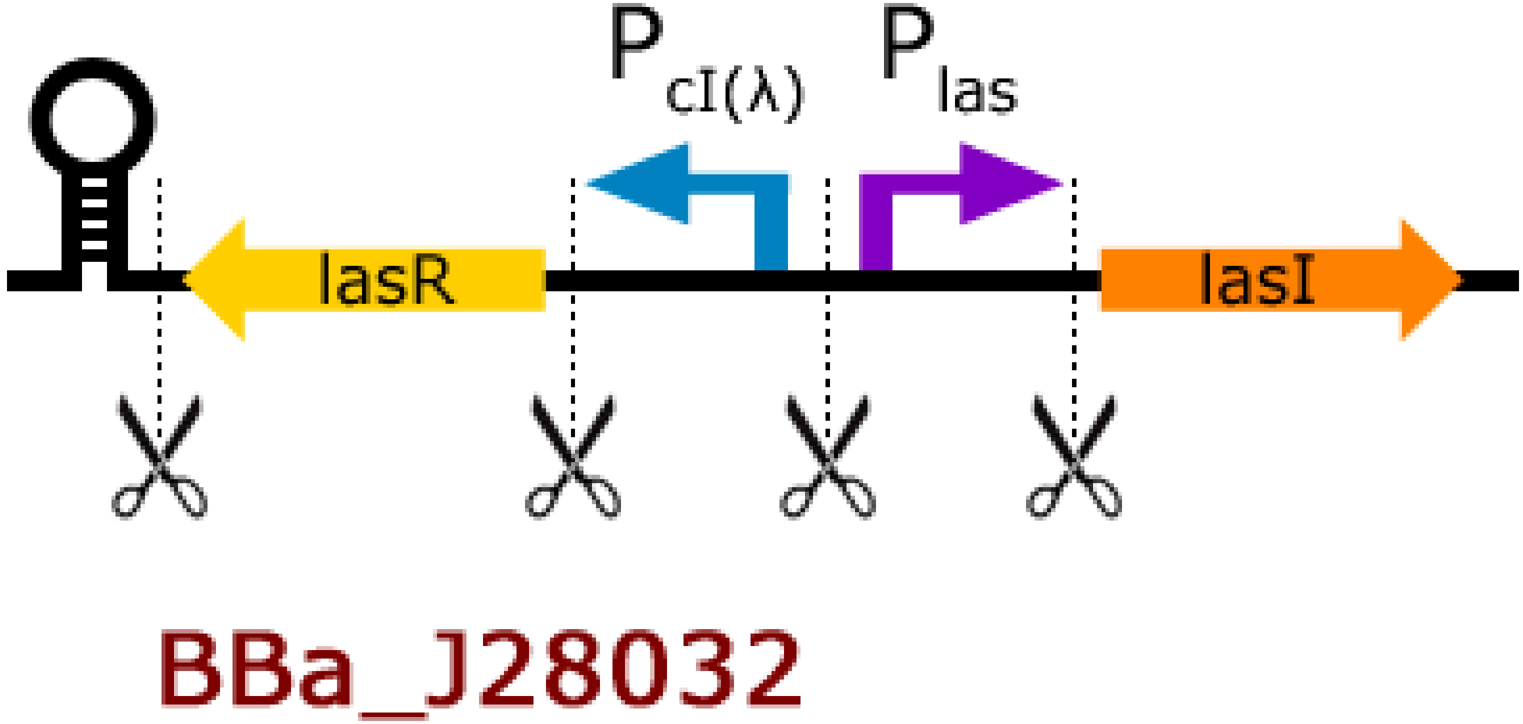


BBa_J28031

CAMBRiDGE iGEM 2006

AHL Sender Cassettes: No. 2

Las autoinducer: 1768 bp



Circuit Properties

Circuit Properties

- ***Non-cell-autonomous*** behaviour
- ***Bi-directionality*** through cross-wiring of lux and las
- ***Equivalence*** of contesting populations
- ***Tunable*** signalling kinetics
- ***Feedback coupling*** of receiver response to sender
- ***Complex signalling dynamics***, directly and vividly visualised

Modelling Single Cell Dynamics

Modelling Single Cell Dynamics

Objectives

Modelling Single Cell Dynamics

Objectives

- Model dynamics of a single cell equipped with the genetic circuit

Modelling Single Cell Dynamics

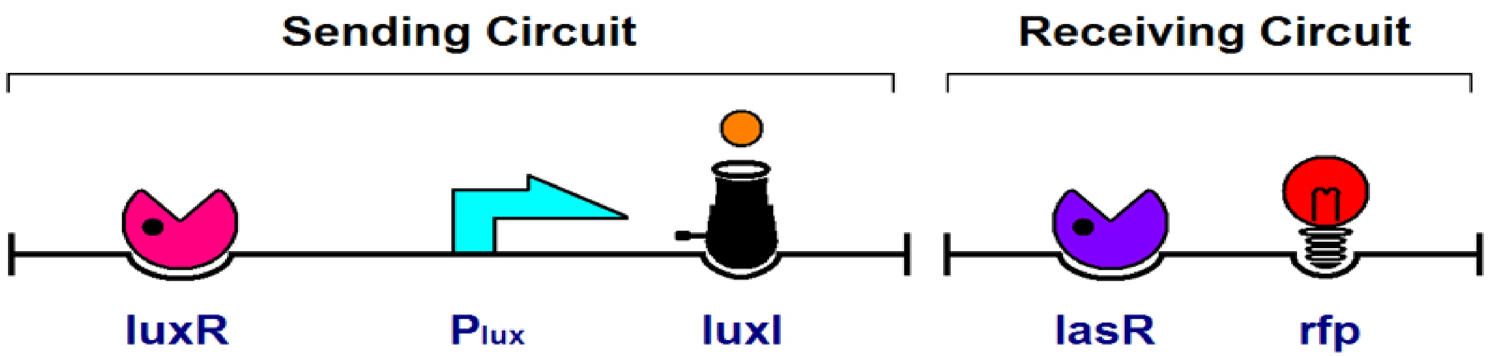
Objectives

- Model dynamics of a single cell equipped with the genetic circuit
- Observe behaviours such as auto-induction and switching

Modelling Single Cell Dynamics

Objectives

- Model dynamics of a single cell equipped with the genetic circuit
- Observe behaviours such as auto-induction and switching



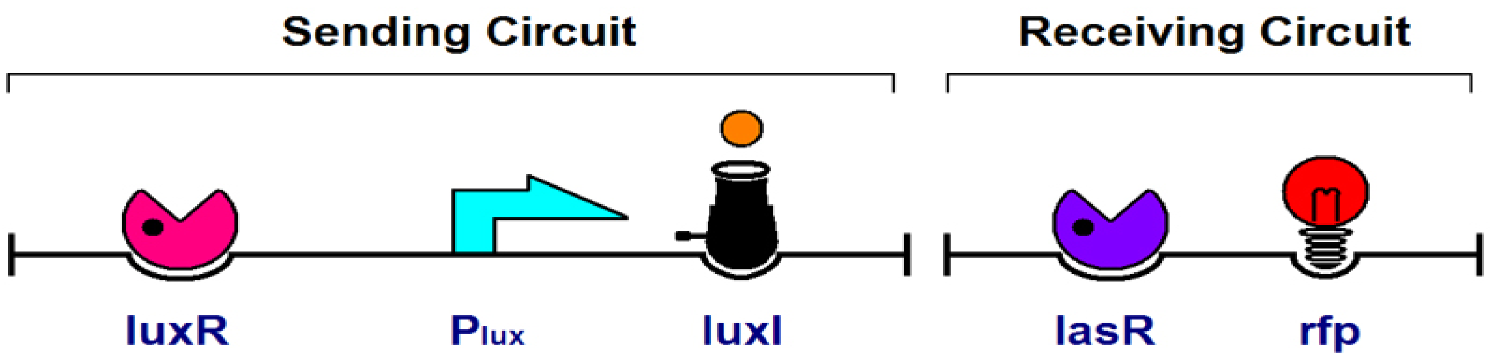
CAMBRiDGE iGEM 2006

Modelling Single Cell Dynamics

Objectives

- Model dynamics of a single cell equipped with the genetic circuit
- Observe behaviours such as auto-induction and switching

Assumptions



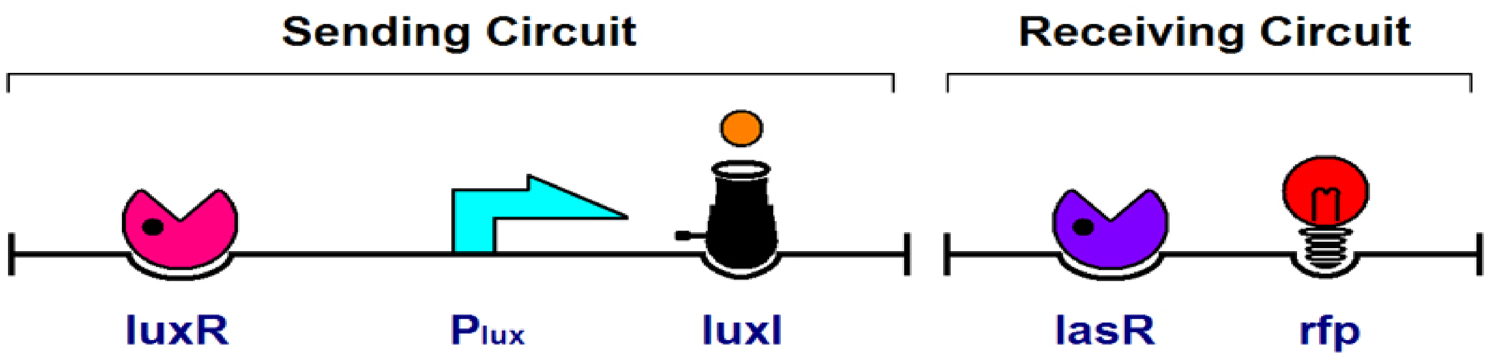
Modelling Single Cell Dynamics

Objectives

- Model dynamics of a single cell equipped with the genetic circuit
- Observe behaviours such as auto-induction and switching

Assumptions

- Type 1 and type 2 cells have identical dynamics



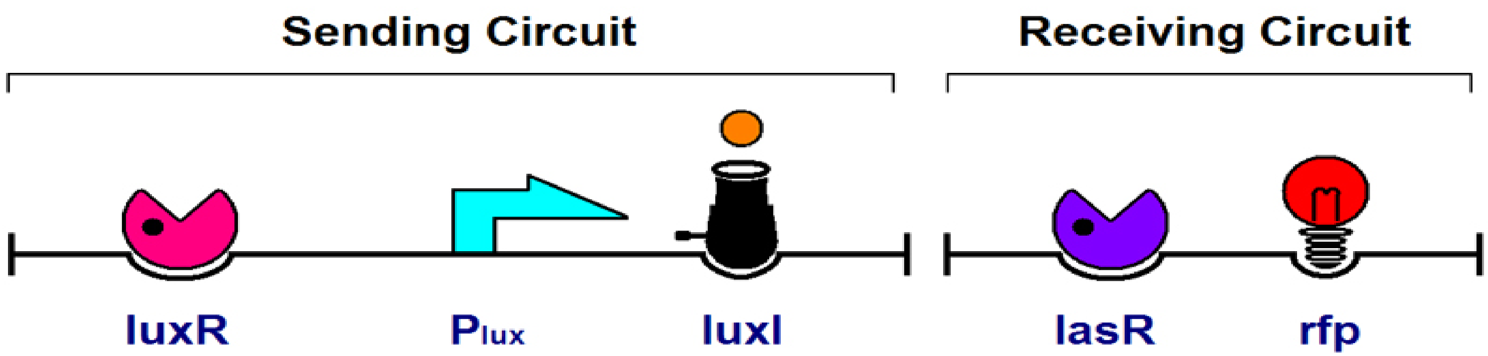
Modelling Single Cell Dynamics

Objectives

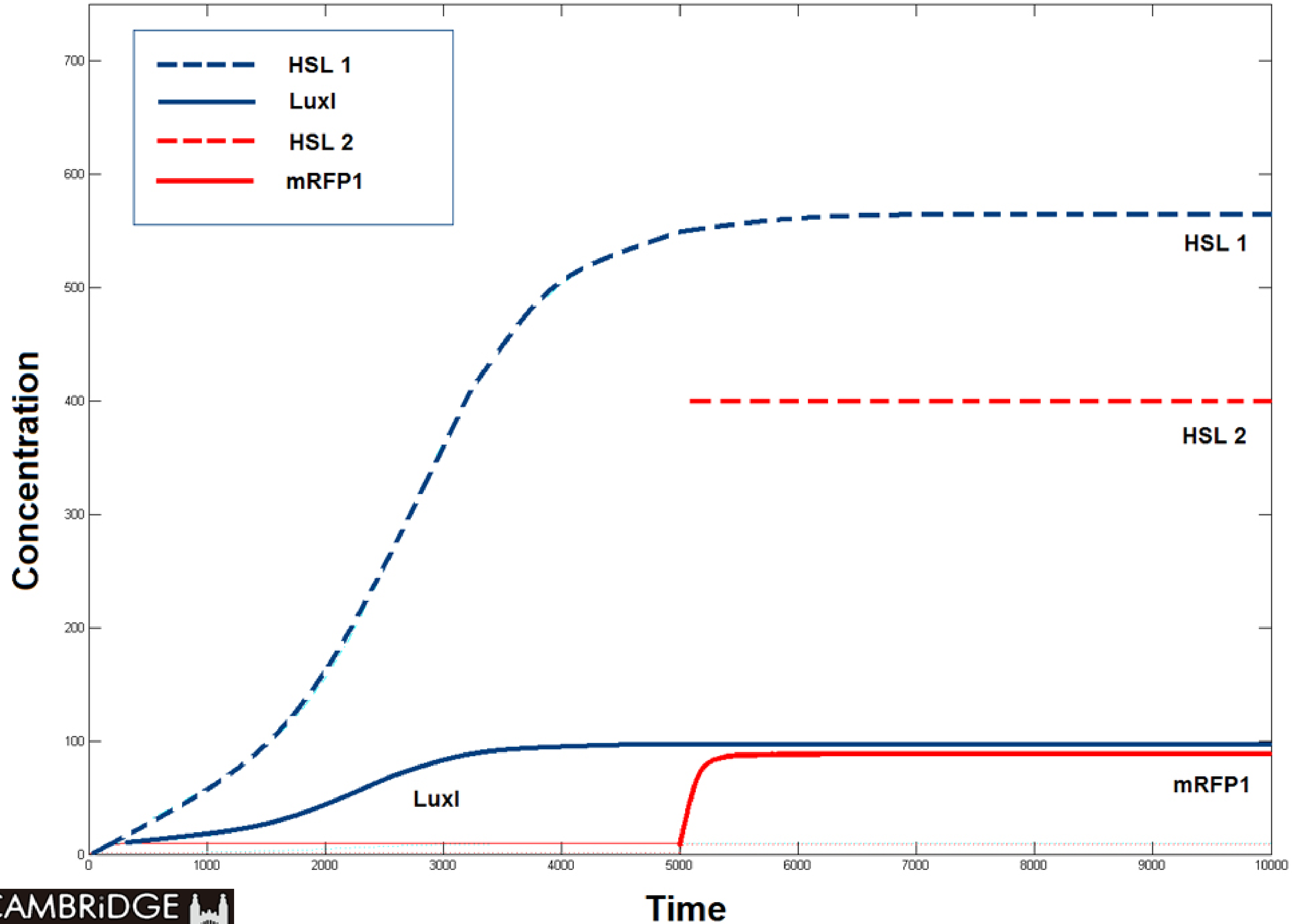
- Model dynamics of a single cell equipped with the genetic circuit
- Observe behaviours such as auto-induction and switching

Assumptions

- Type 1 and type 2 cells have identical dynamics
- Luxr and Lasr genes are constitutively expressed

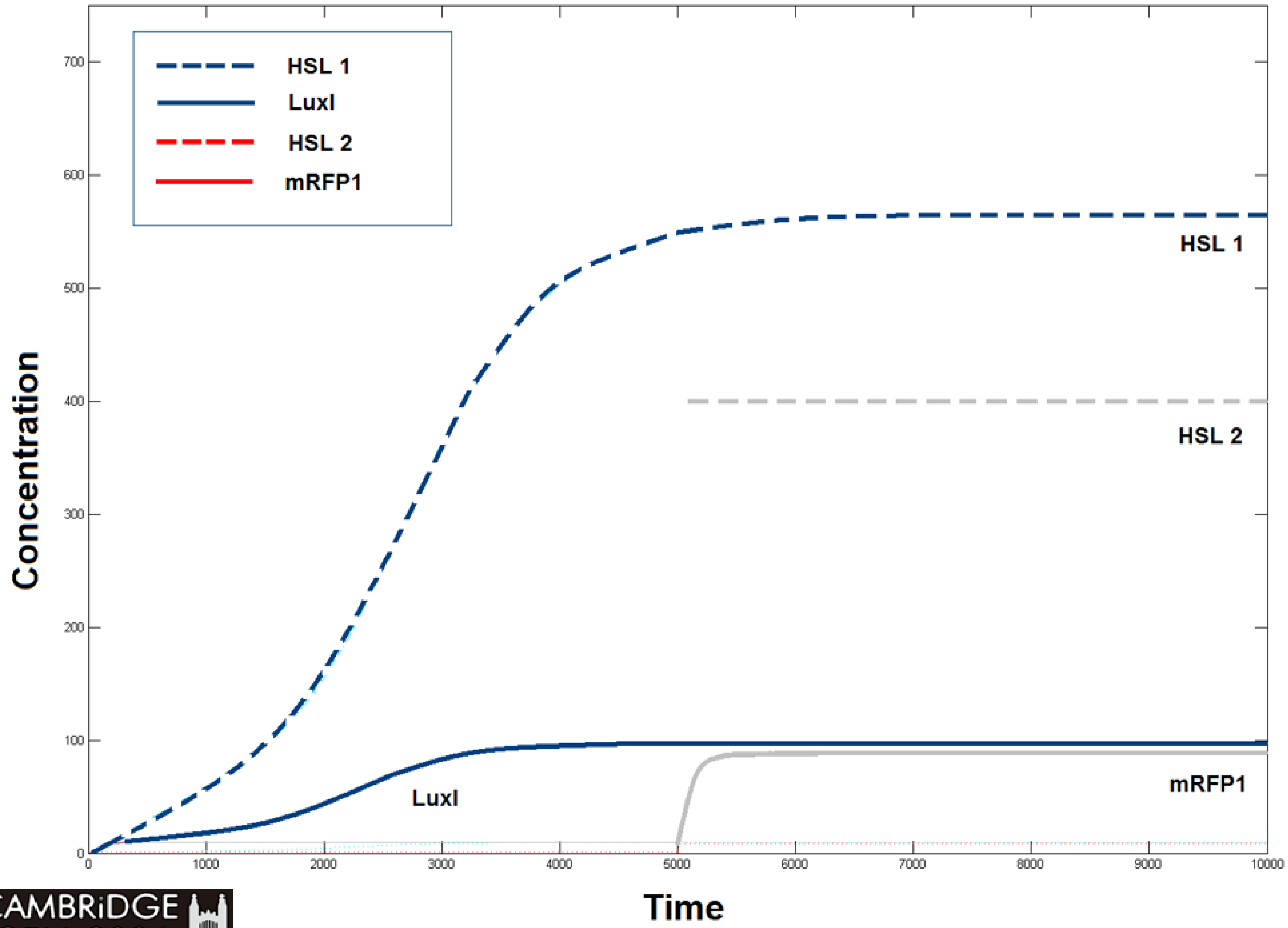


Matlab Plot



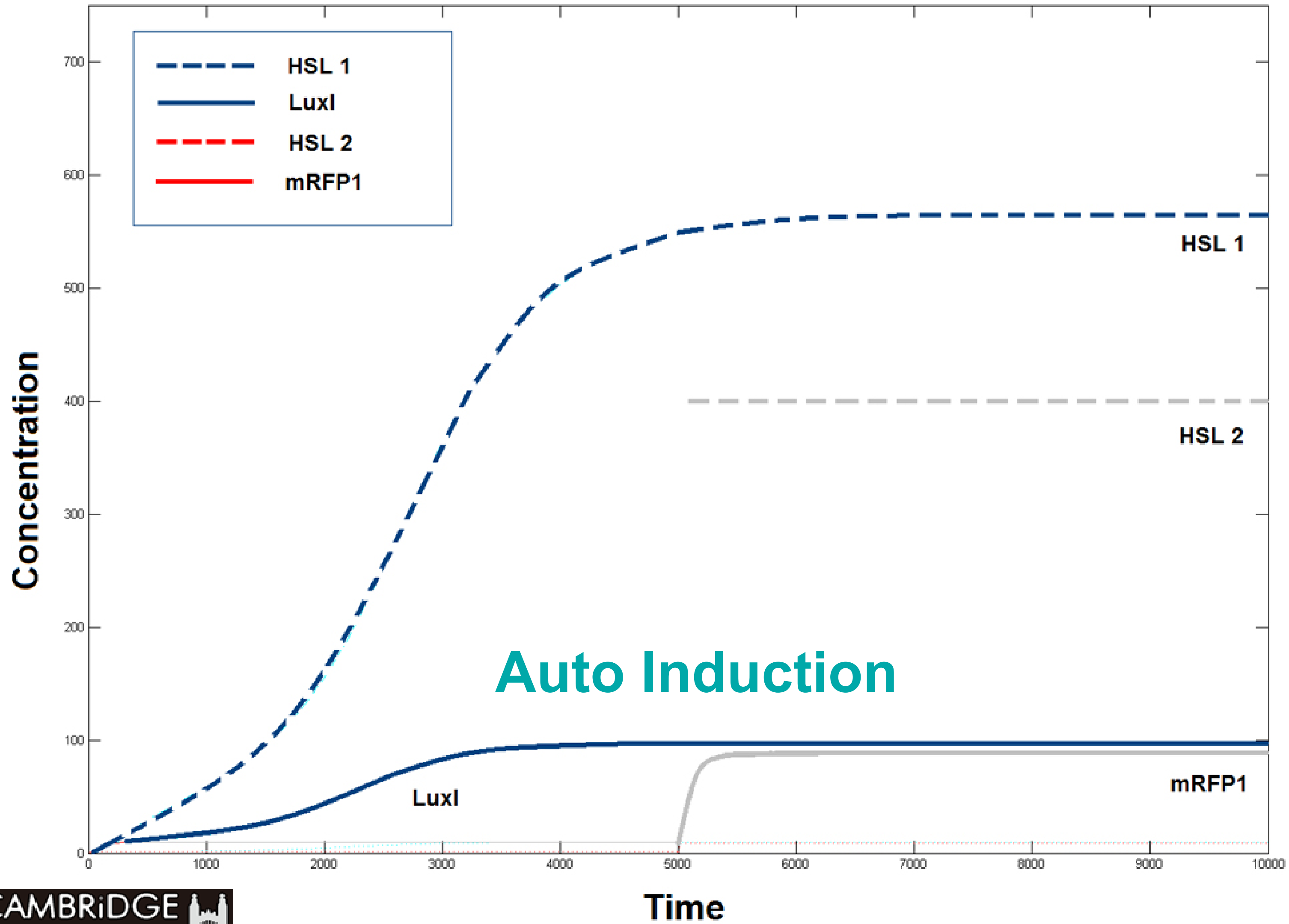
CAMBRiDGE iGEM 2006

Matlab Plot



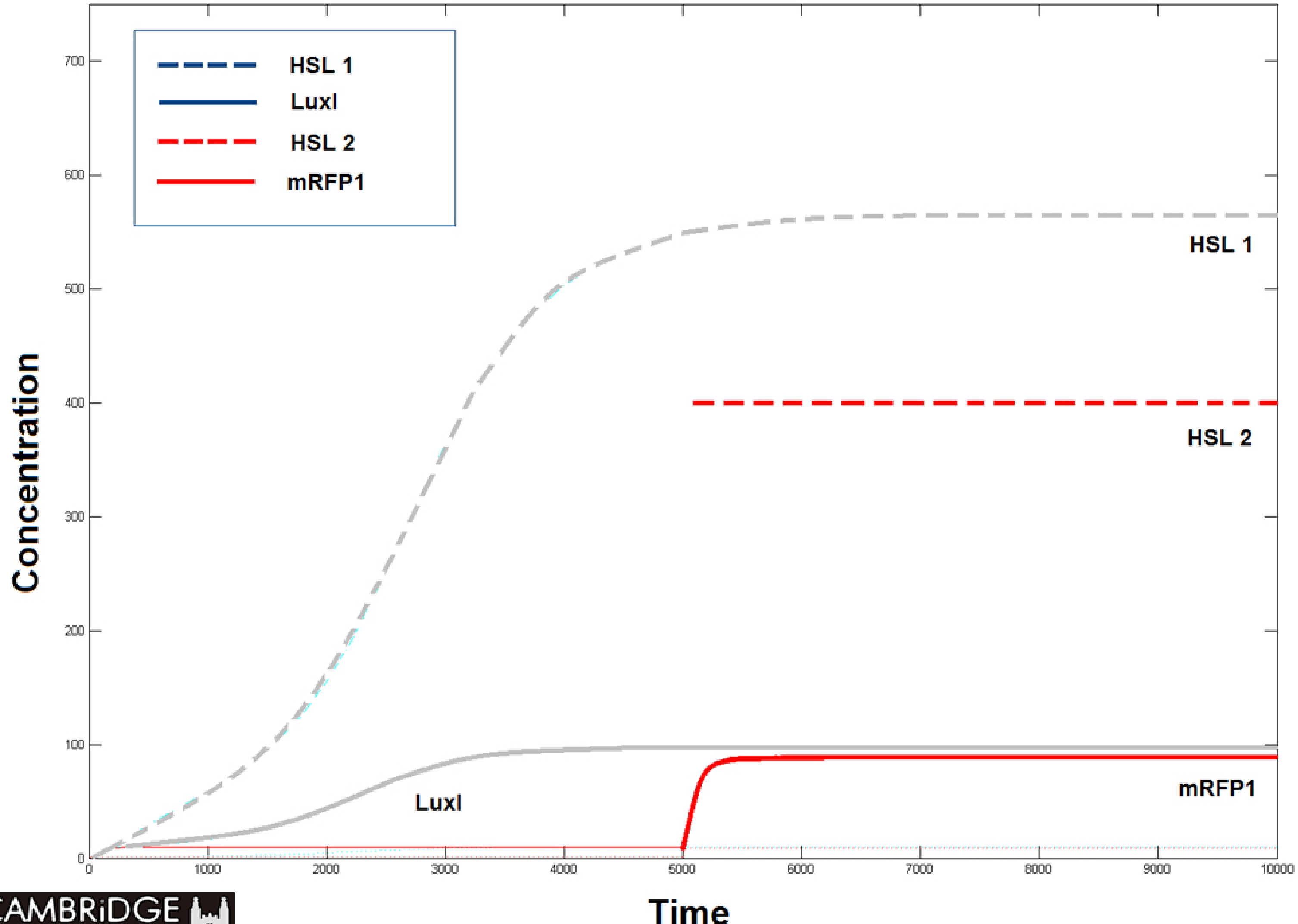
CAMBRiDGE iGEM 2006

Matlab Plot



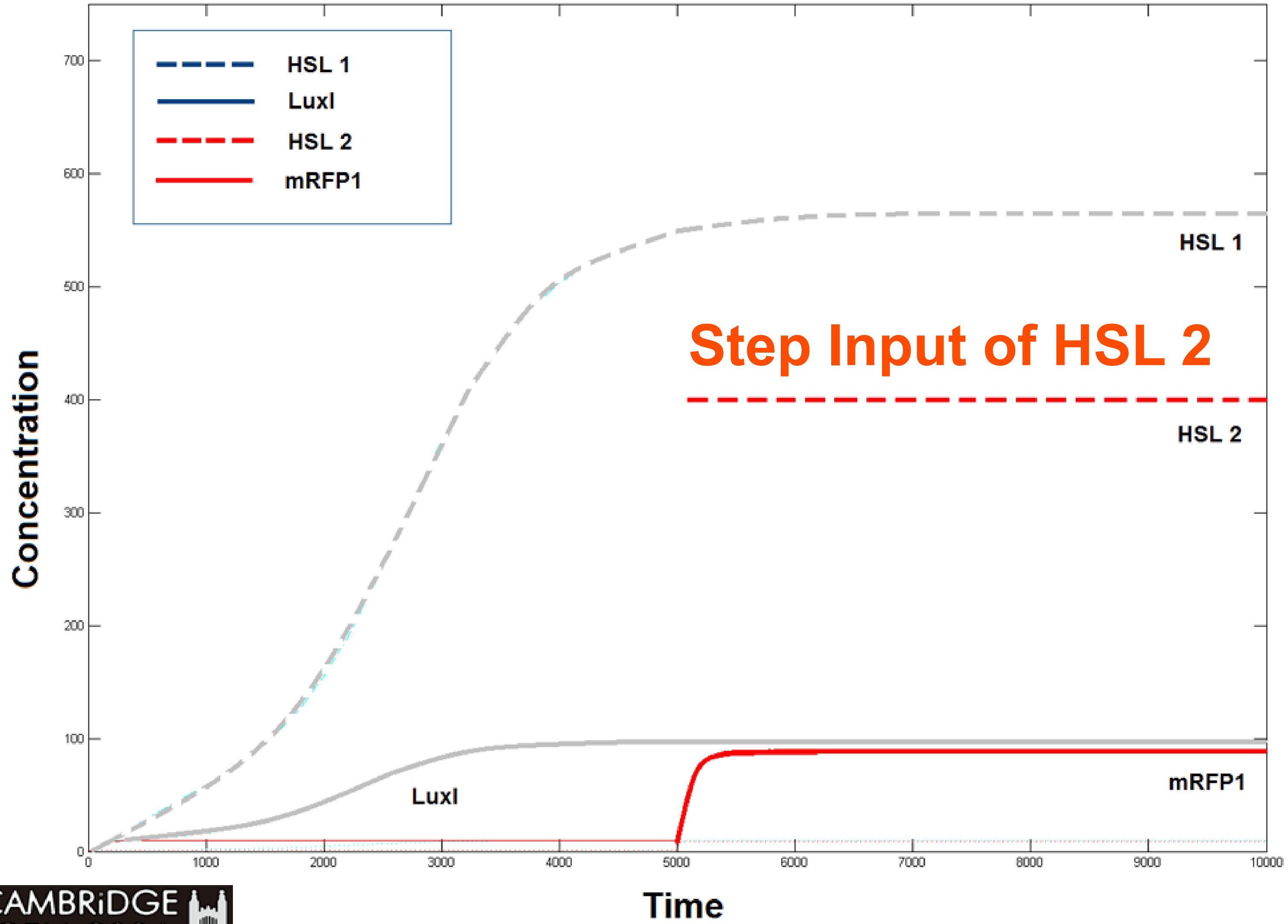
CAMBRiDGE iGEM 2006

Matlab Plot



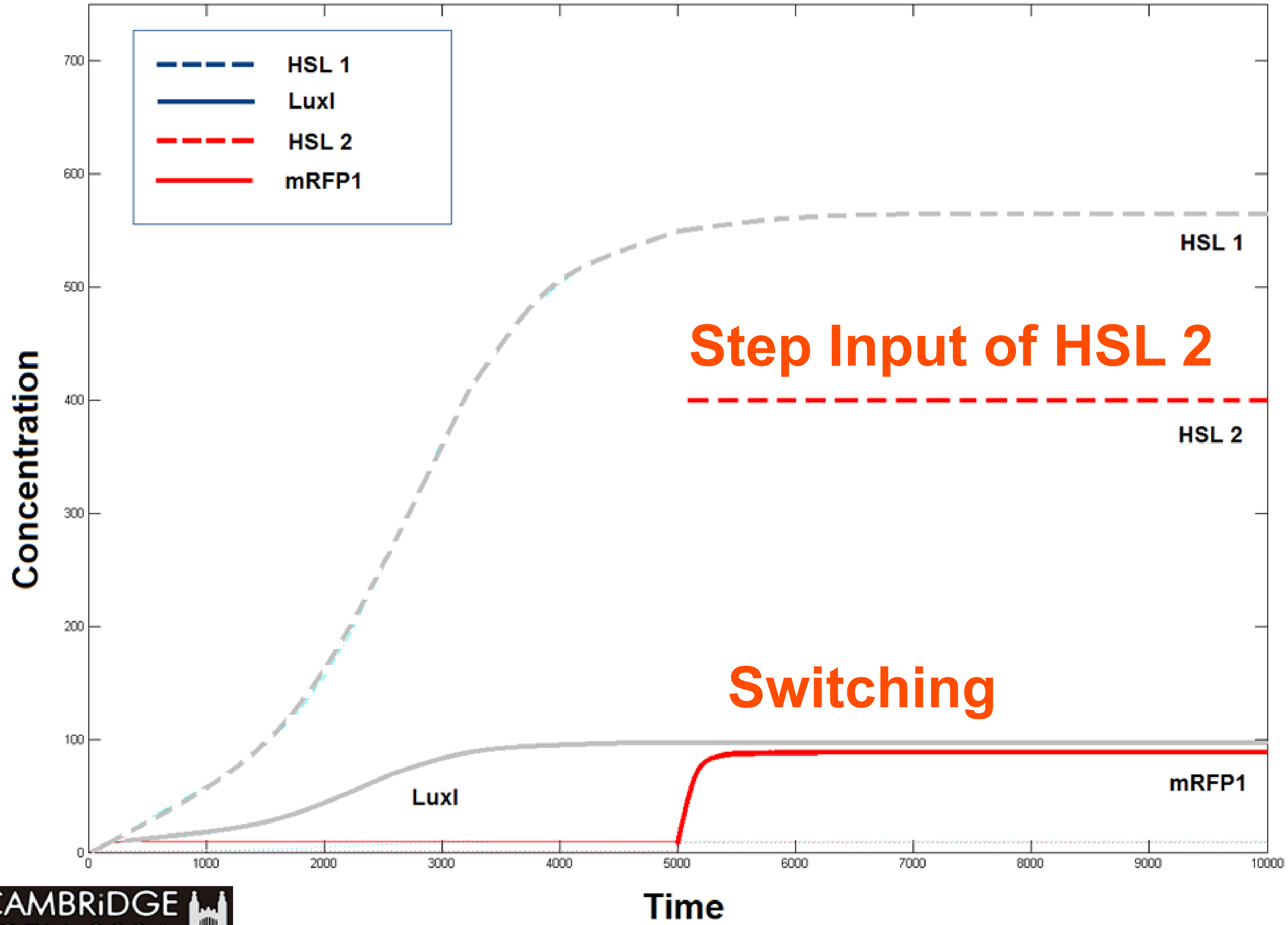
CAMBRiDGE iGEM 2006

Matlab Plot



HSL 1
LuxI
HSL 2
mRFP1
Step Input of HSL 2
HSL 1
HSL 2
LuxI
mRFP1
Concentration
Time
0
100
200
300
400
500
600
700
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000

Matlab Plot



CAMBRiDGE iGEM 2006

Modelling Multi-cell Interaction

Modelling Multi-cell Interaction

<u>Conway's Game of Life</u>

CAMBRiDGE iGEM 2006

Modelling Multi-cell Interaction

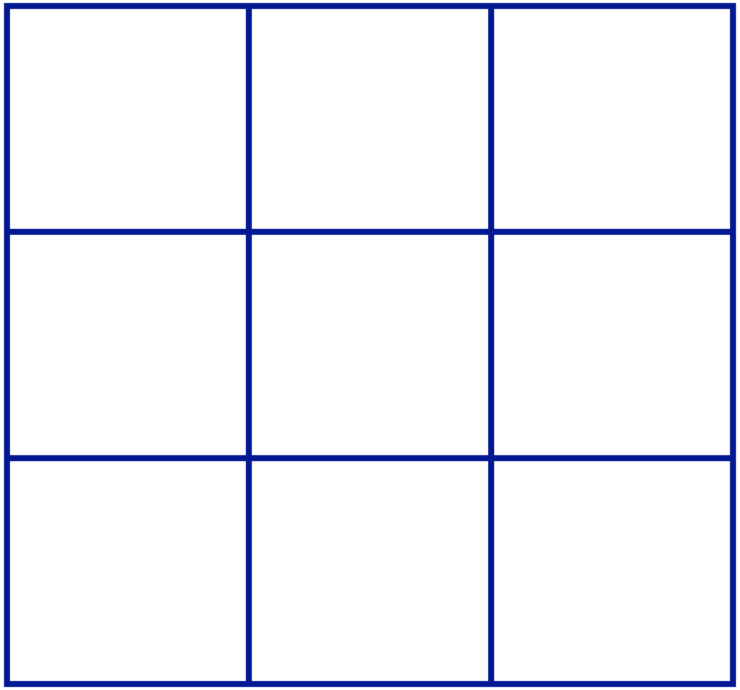
Conway's Game of Life

- Neighbour-dependent => analogous to our system

Modelling Multi-cell Interaction

Conway's Game of Life

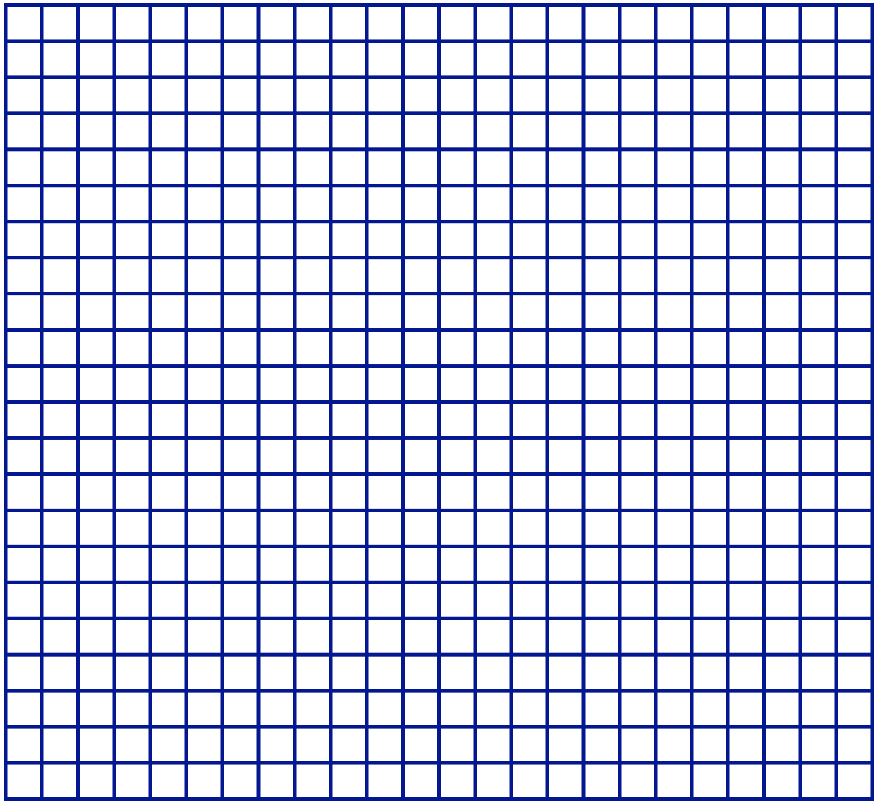
- Neighbour-dependent => analogous to our system



Modelling Multi-cell Interaction

<u>Conway's Game of Life</u>

- Neighbour-dependent => analogous to our system



Modelling Multi-cell Interaction

Conway's Game of Life

- Neighbour-dependent => analogous to our system

Modelling Multi-cell Interaction

Conway's Game of Life

• Neighbour-dependent => analogous to our system

Assumptions

Modelling Multi-cell Interaction

Conway's Game of Life

- Neighbour-dependent => analogous to our system

Assumptions

- No cells die

Modelling Multi-cell Interaction

Conway's Game of Life

- Neighbour-dependent => analogous to our system

Assumptions

- No cells die
- 2 types of cells are identical

Modelling Multi-cell Interaction

Conway's Game of Life

- Neighbour-dependent => analogous to our system

Assumptions

- No cells die
- 2 types of cells are identical
- Colours change at boundary

CAMBRiDGE iGEM 2006

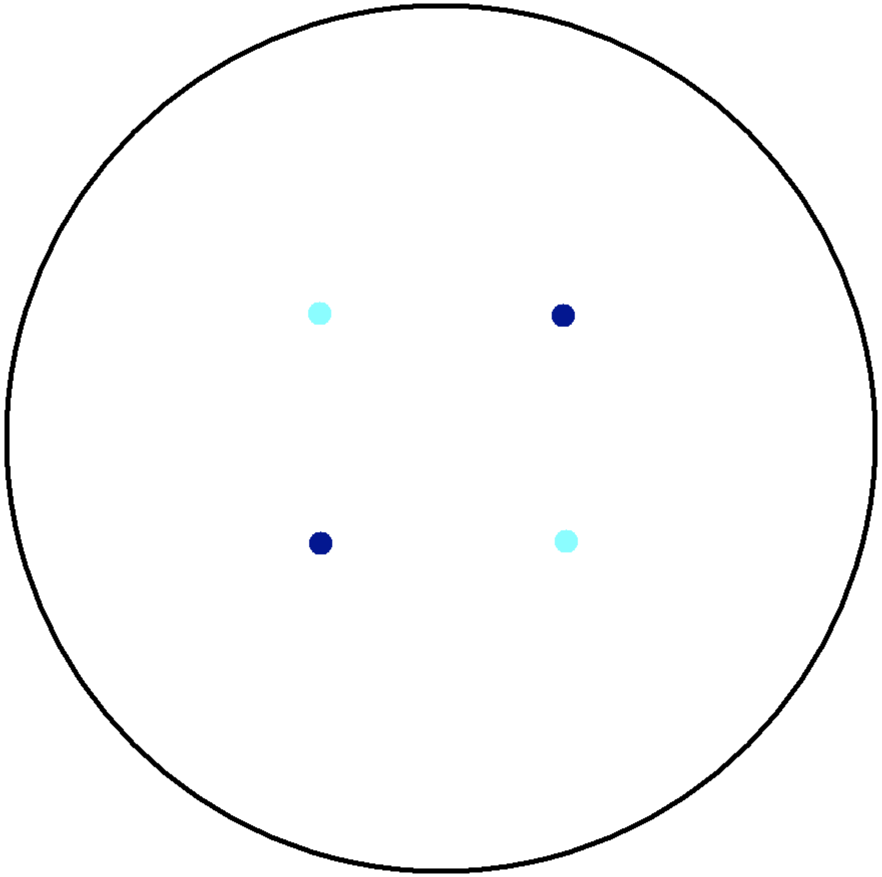
Modelling Multi-cell Interaction

Conway's Game of Life

- Neighbour-dependent => analogous to our system

Assumptions

- No cells die
- 2 types of cells are identical
- Colours change at boundary



CAMBRiDGE iGEM 2006

Modelling Multi-cell Interaction

Conway's Game of Life

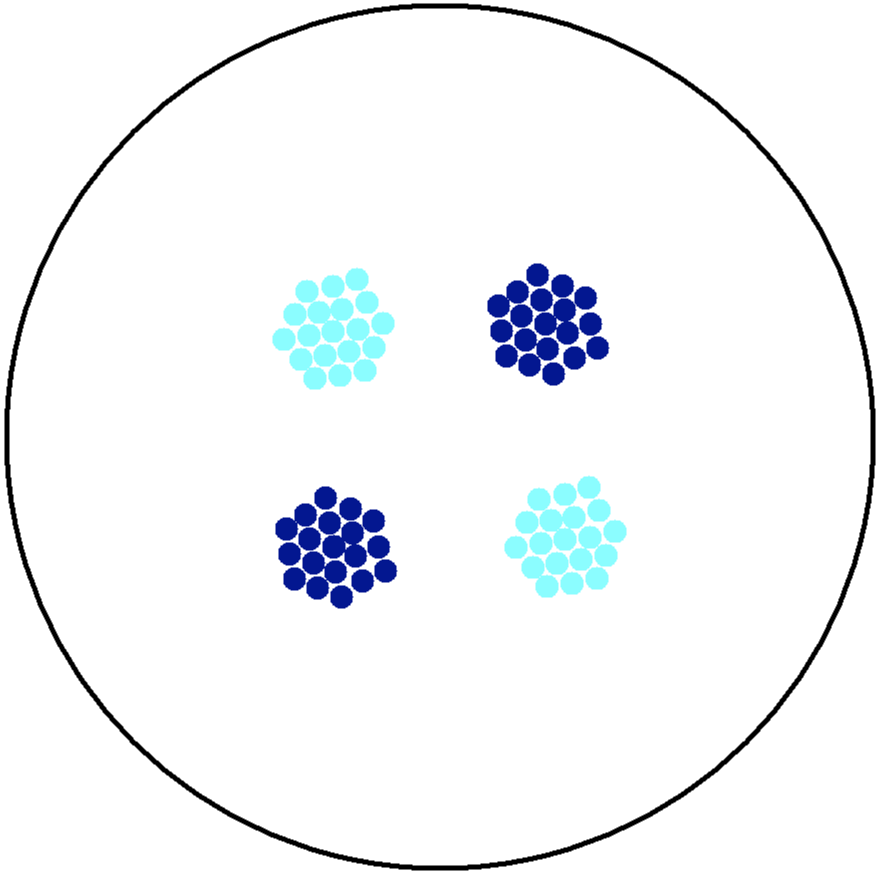
• Neighbour-dependent => analogous to our system

Assumptions

• No cells die

• 2 types of cells are identical

• Colours change at boundary



Modelling Multi-cell Interaction

Conway's Game of Life

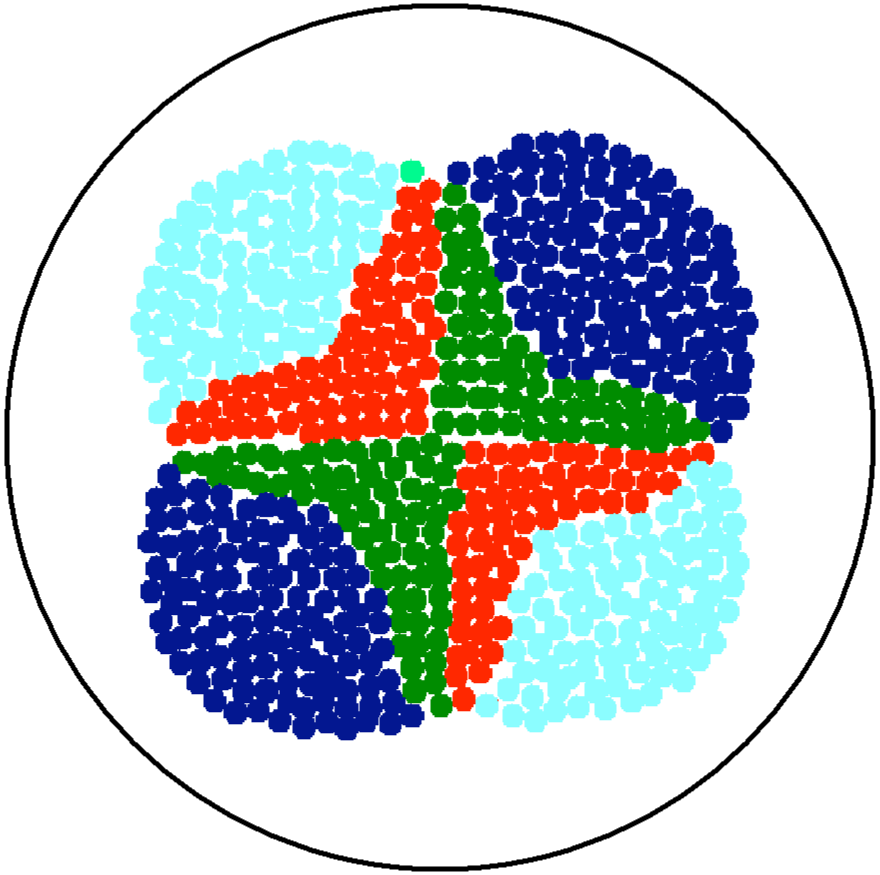
• Neighbour-dependent => analogous to our system

Assumptions

• No cells die

• 2 types of cells are identical

• Colours change at boundary



Simulation Program

Experiments – Swimming Assay

Experiments – Swimming Assay

E. coli MC1000
0.5% Bactoagar

E. coli MG1655
0.3% Bactoagar

E. coli XL-1 Blue
0.3% Bactoagar

All are incubated at 30˚C overnight

CAMBRiDGE iGEM 2006

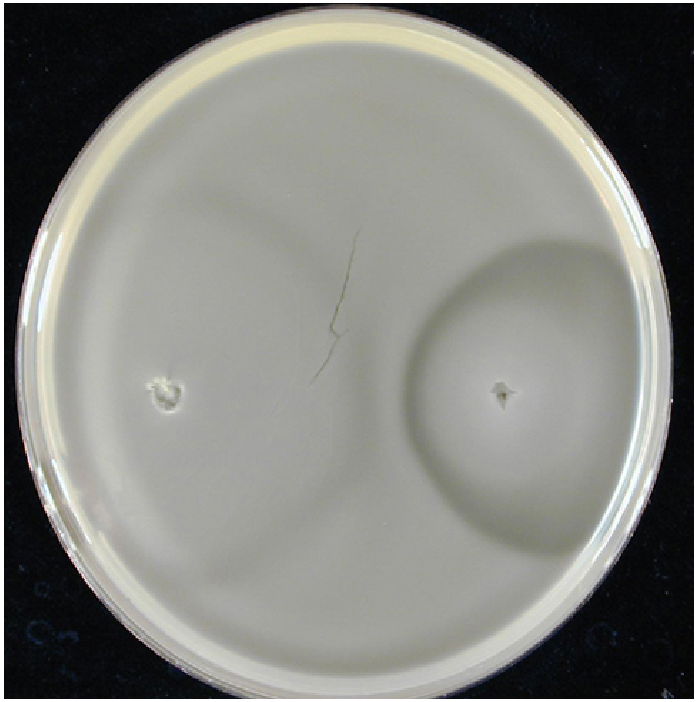
Experiments – Swimming Assay

Experiments – Swimming Assay

Four inoculations of XL-1 Blue Cells

Experiments – Swimming Assay

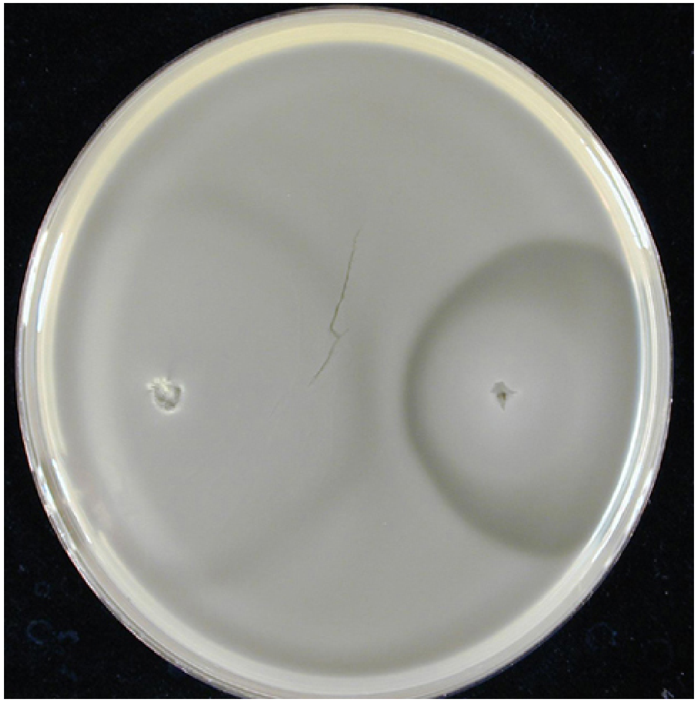
Four inoculations of XL-1 Blue Cells



E. Coli MC-1000 and XL-1 Blue

Experiments – Swimming Assay

Four inoculations of XL-1 Blue Cells

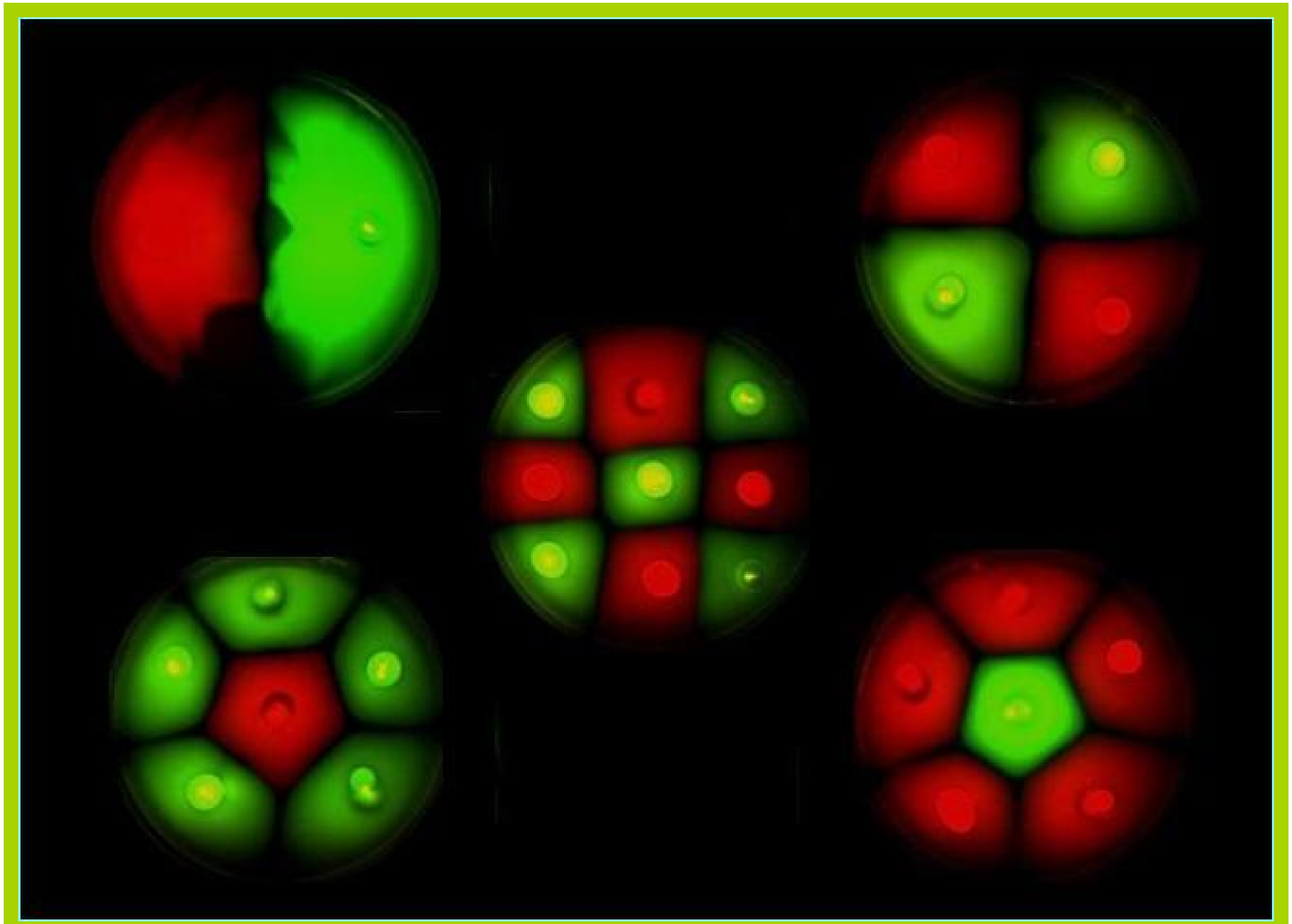


E. Coli MC-1000 and XL-1 Blue

Differential cell motility → Spontaneous pattern formation

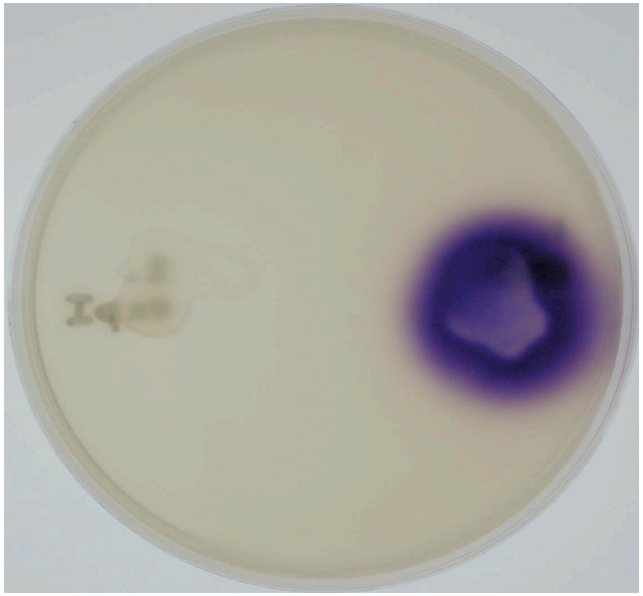
CAMBRiDGE iGEM 2006

Fluorescent Patterns



Bioassay For AHL production

Chromobacterium violaceum CVO26 can be used as a biosensor to detect AHL production

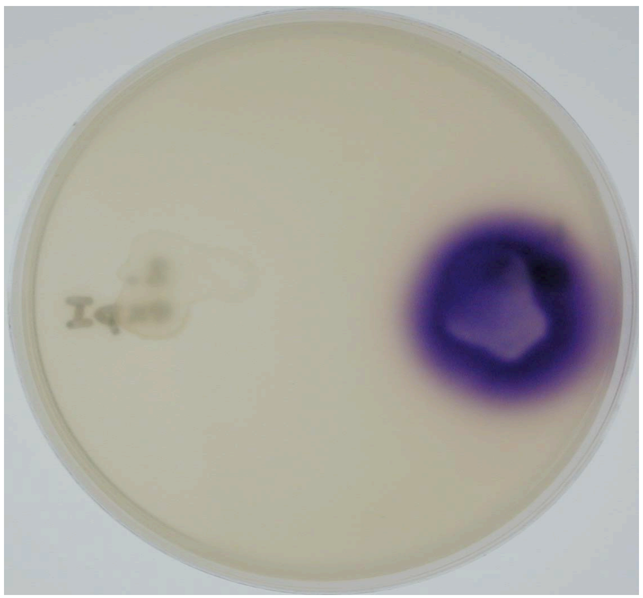


Can distinguish between AHL molecules with N-acyl side-chains from C4-C8 in length and C10-C14 in length

McClean et al, Microbiology143, 1997

Bioassay For AHL production

Chromobacterium violaceum CVO26 can be used as a biosensor to detect AHL production

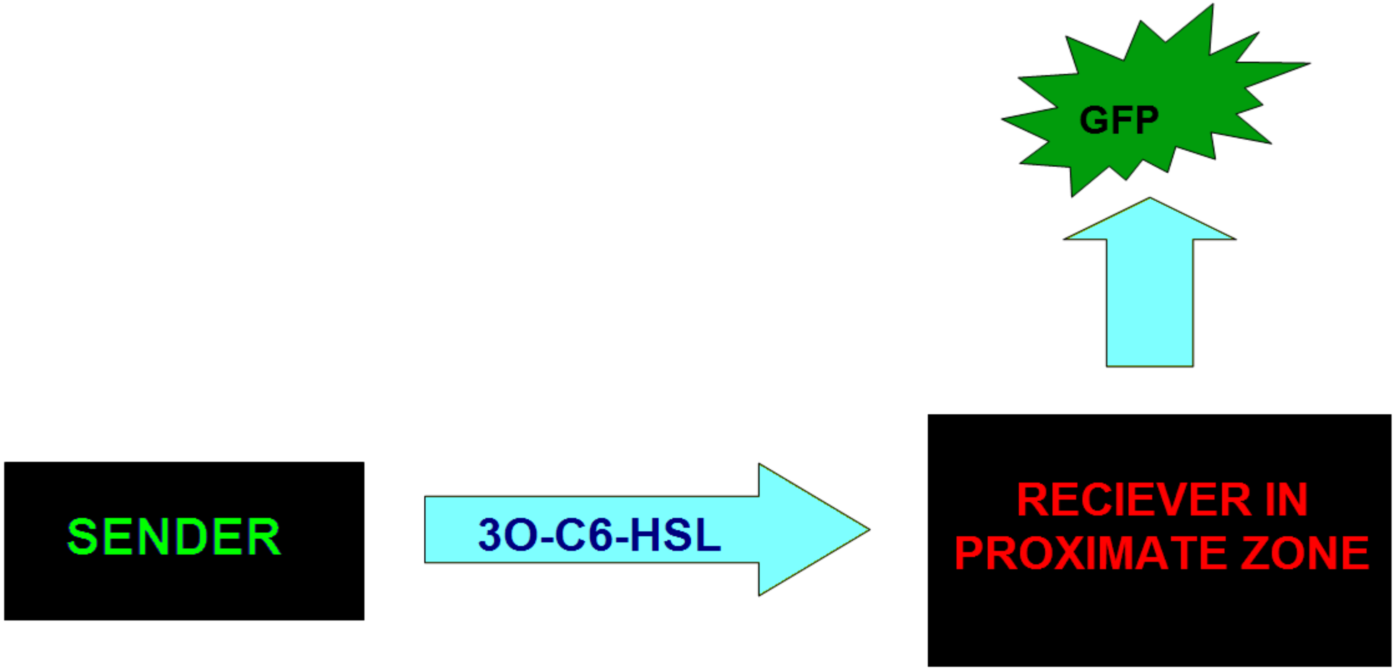


Can distinguish between AHL molecules with N-acyl side-chains from C4-C8 in length and C10-C14 in length

McClean et al, Microbiology143, 1997

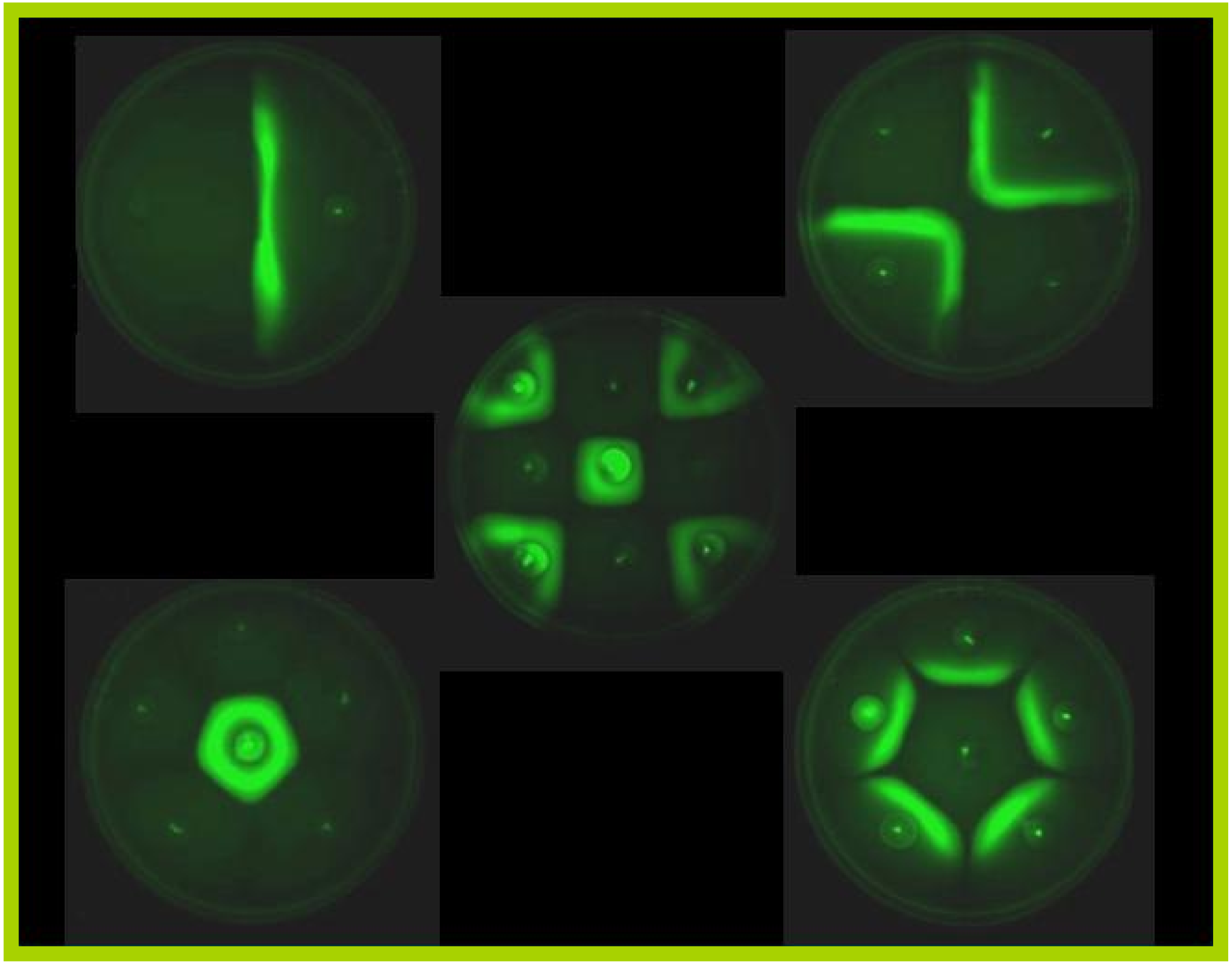
Cell-cell Communication

The <u>interaction</u> of AHL sender cells and AHL receiver cells on a swimming plate; with <u>cell motility</u> defining <u>zones of response</u> leads to ….



CAMBRiDGE iGEM 2006

Patterning Through Communication



Future Work

Population based bi-stable switch

Future Work

Population based bi-stable switch

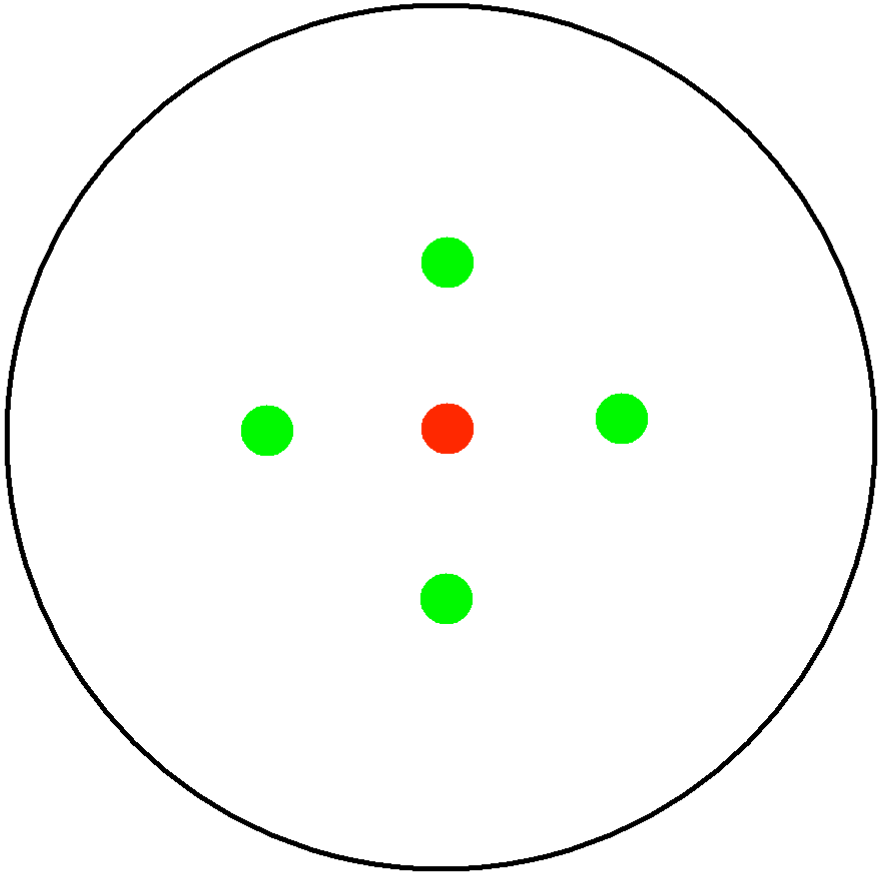
1. Biased inoculations

Future Work

Population based bi-stable switch

1. Biased inoculations

   Greens >> Reds

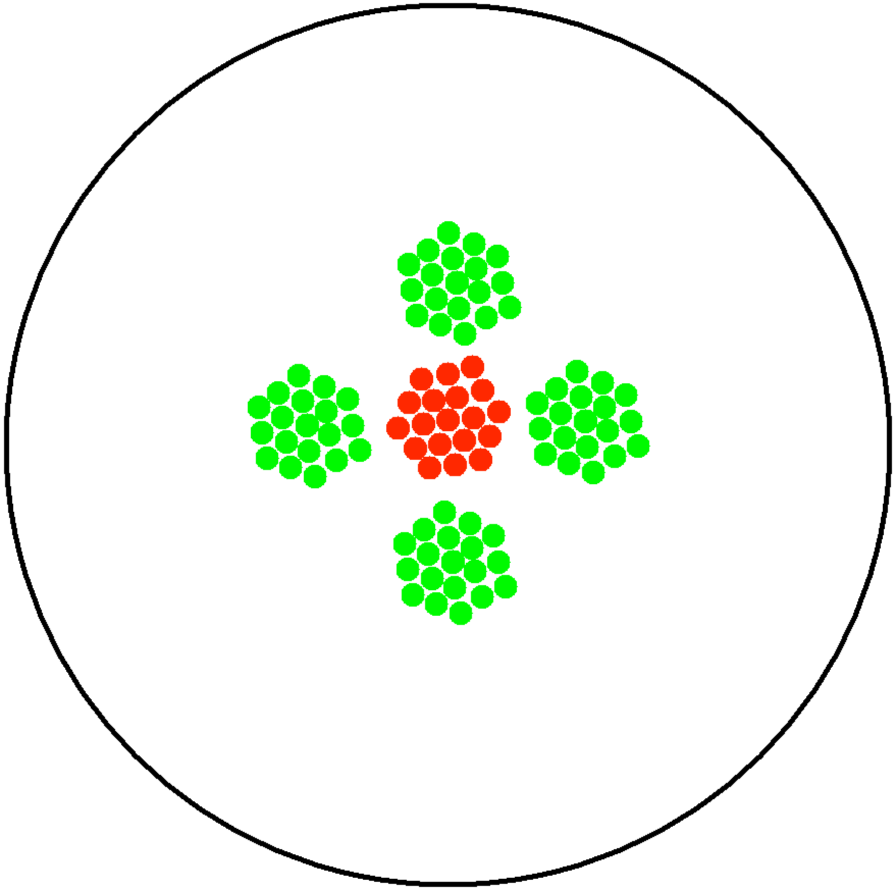


CAMBRiDGE iGEM 2006

Future Work

<u>Population based bi-stable switch</u>

1. Biased inoculations
   Greens >> Reds

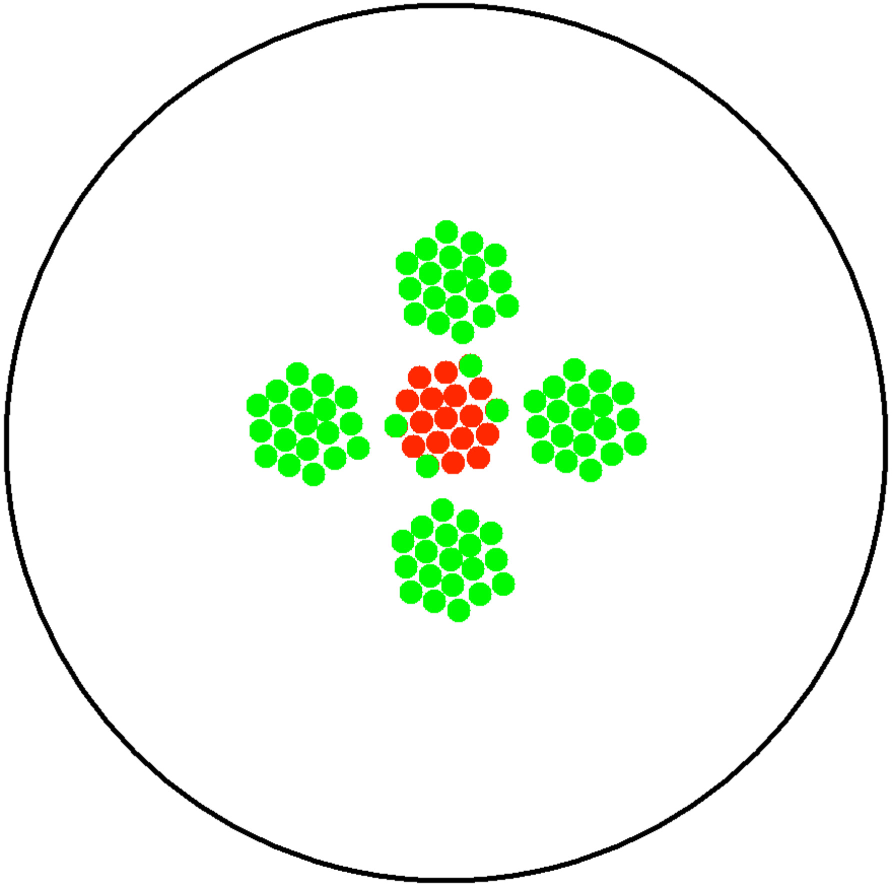


Future Work

Population based bi-stable switch

1. Biased inoculations
   Greens >> Reds

2. Cells replicate over time



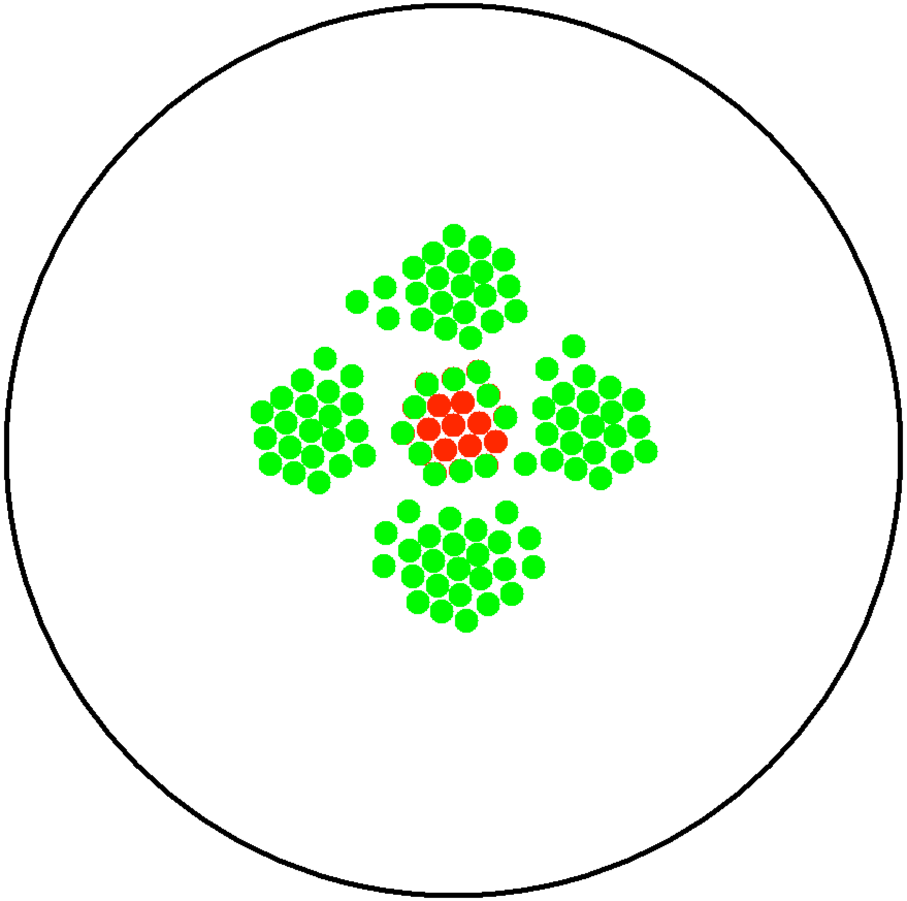
CAMBRiDGE iGEM 2006

Future Work

<u>Population based bi-stable switch</u>

1. Biased inoculations
   Greens >> Reds

2. Cells replicate over time



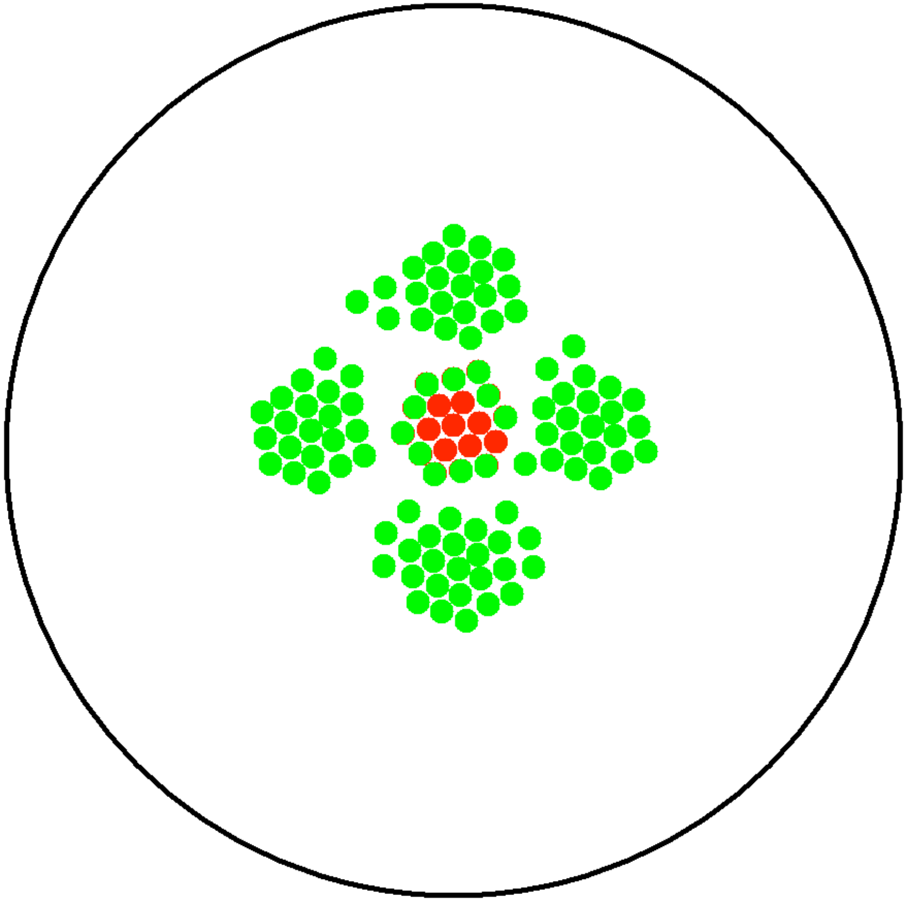
Future Work

Population based bi-stable switch

1. Biased inoculations
   Greens >> Reds

2. Cells replicate over time

3. Green cells outnumber –
   a few red cells convert



CAMBRiDGE iGEM 2006

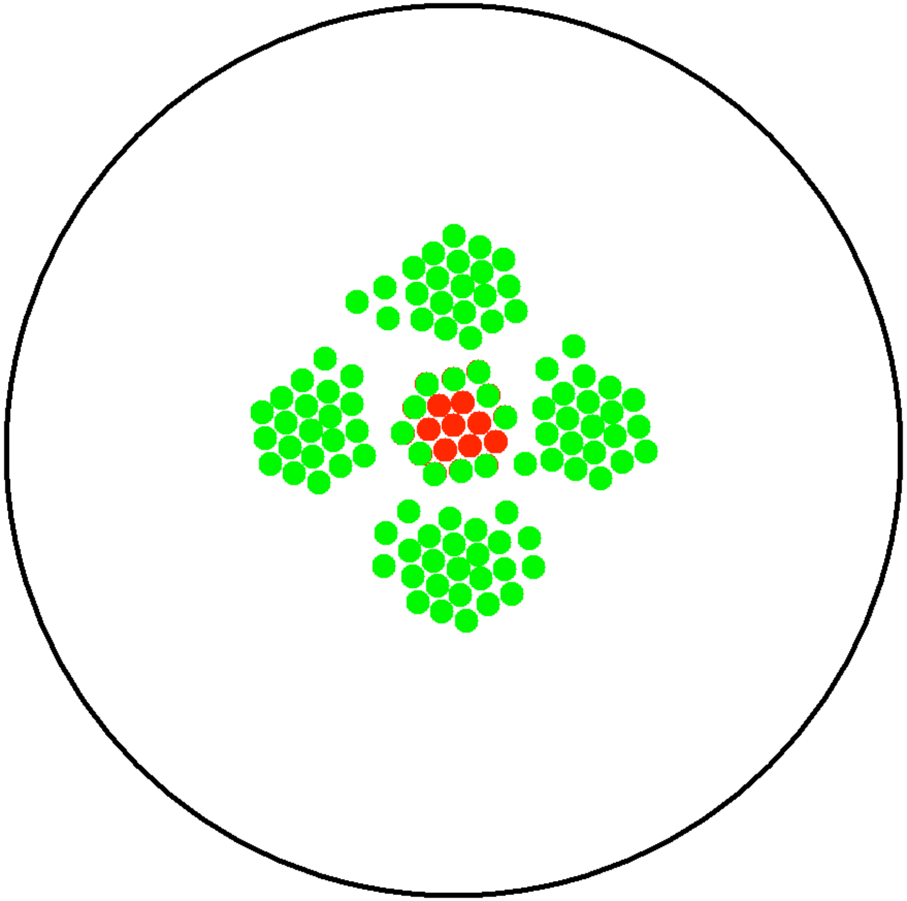
Future Work

Population based bi-stable switch

1. Biased inoculations
   Greens >> Reds

2. Cells replicate over time

3. Green cells outnumber –
   a few red cells convert



CAMBRiDGE iGEM 2006

Future Work

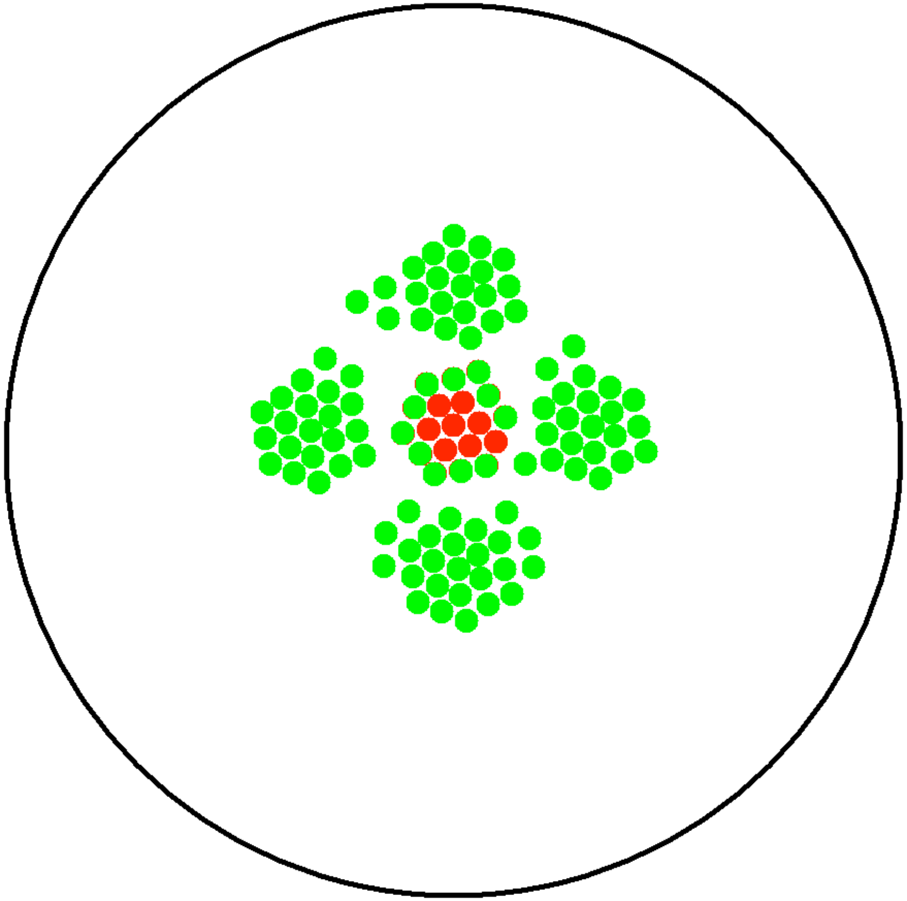
Population based bi-stable switch

1. Biased inoculations
   Greens >> Reds

2. Cells replicate over time

3. Green cells outnumber – a few red cells convert

4. More conversions



CAMBRiDGE iGEM 2006

Future Work

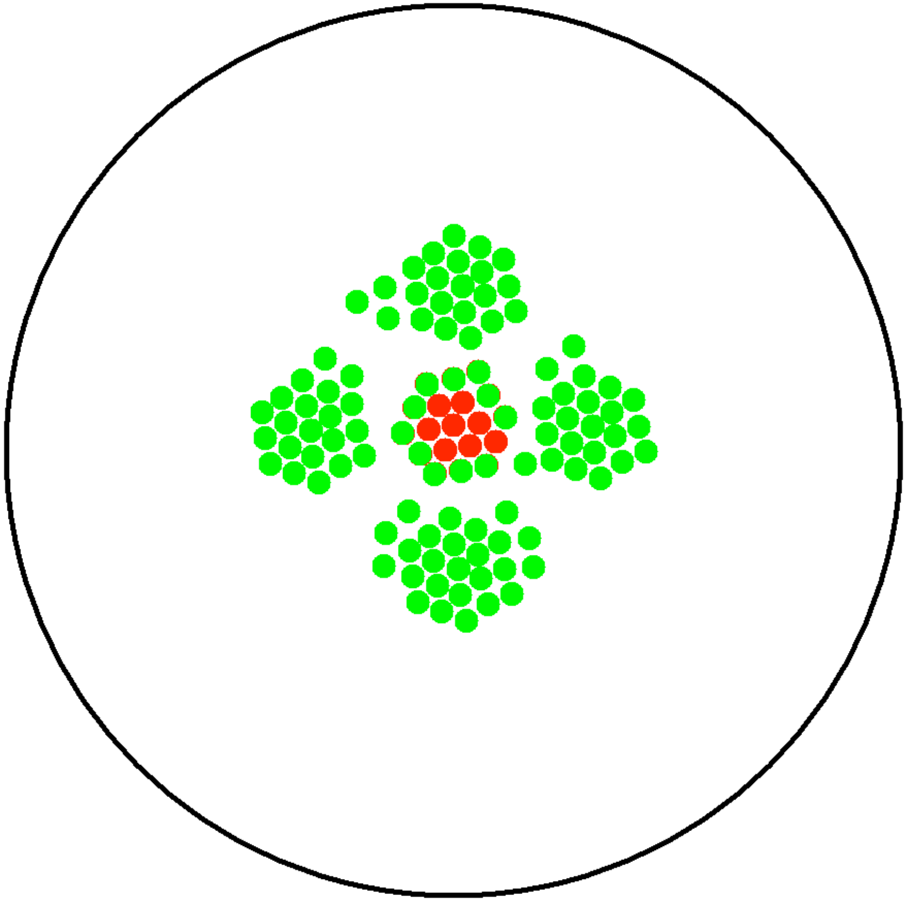
Population based bi-stable switch

1. Biased inoculations
   Greens >> Reds

2. Cells replicate over time

3. Green cells outnumber – a few red cells convert

4. More conversions



CAMBRiDGE iGEM 2006

Future Work

Population based bi-stable switch

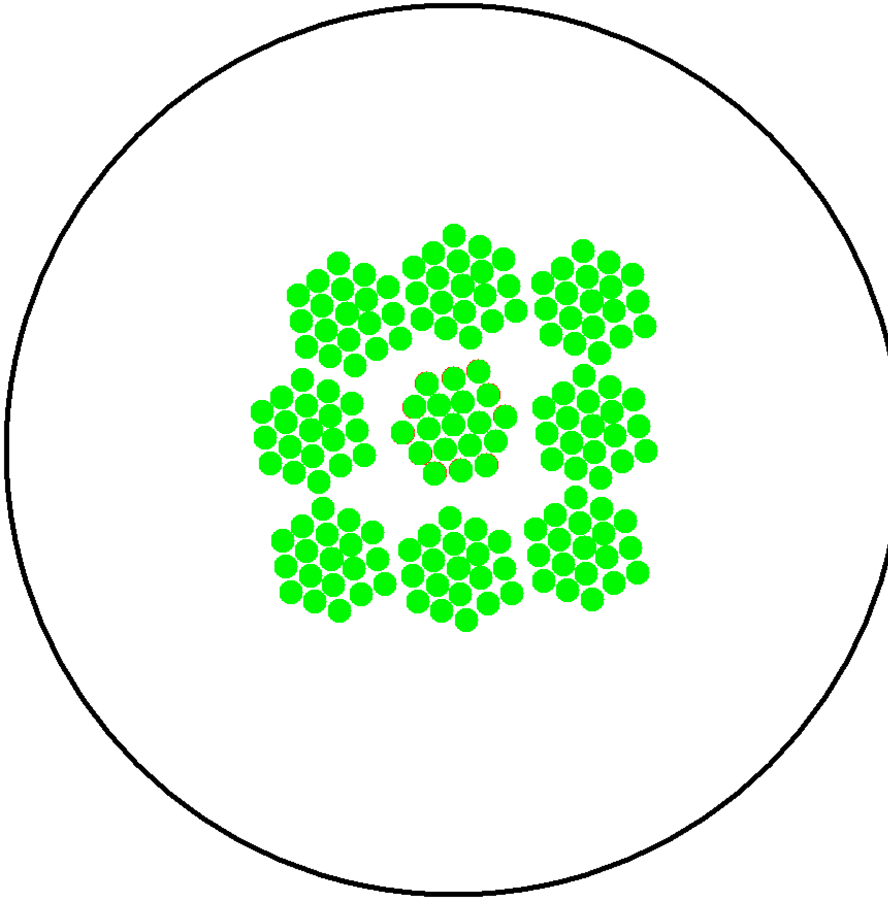
1. Biased inoculations
   Greens >> Reds

2. Cells replicate over time

3. Green cells outnumber –
   a few red cells convert

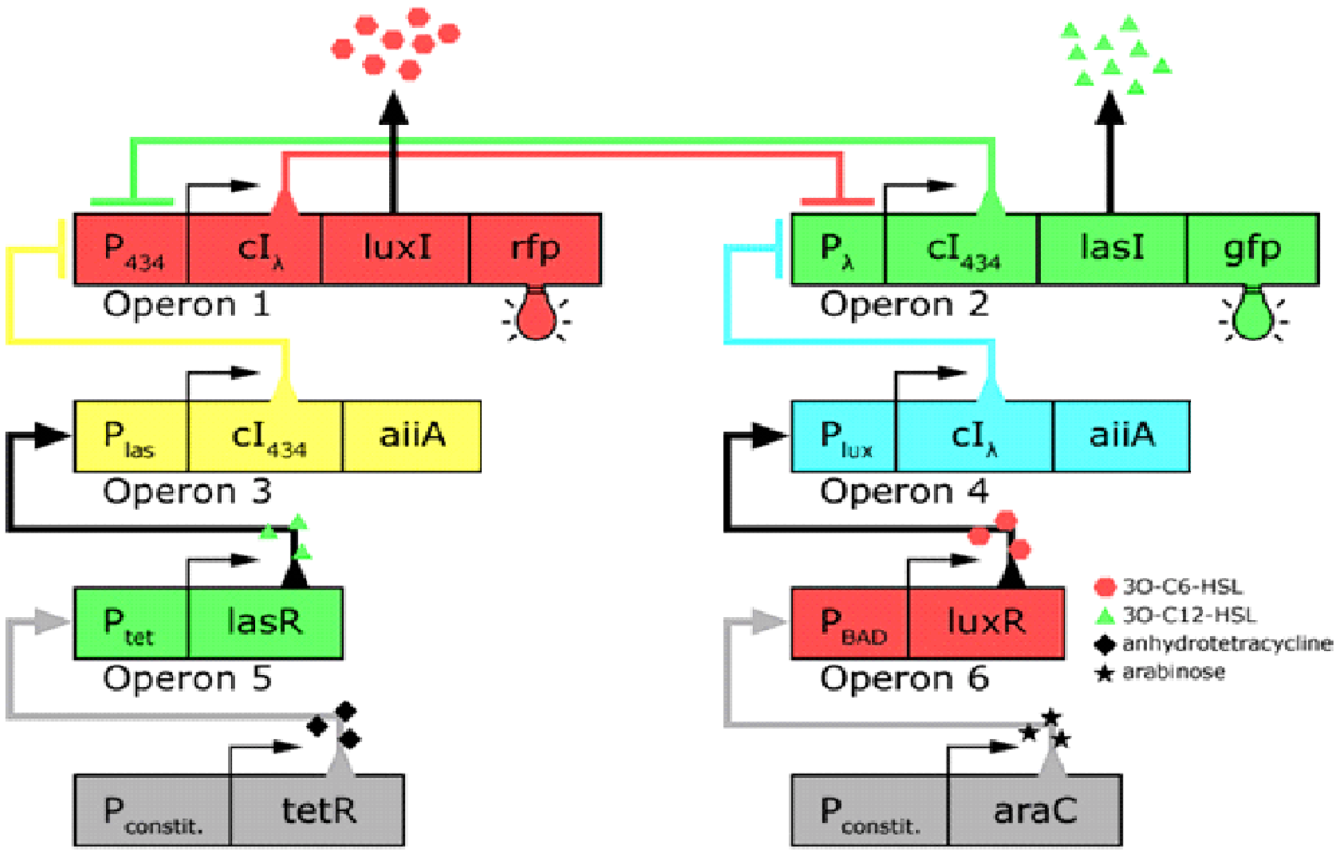
4. More conversions

5. Green cells dominate,



Genetic Circuitry

Population based bi-stable switch

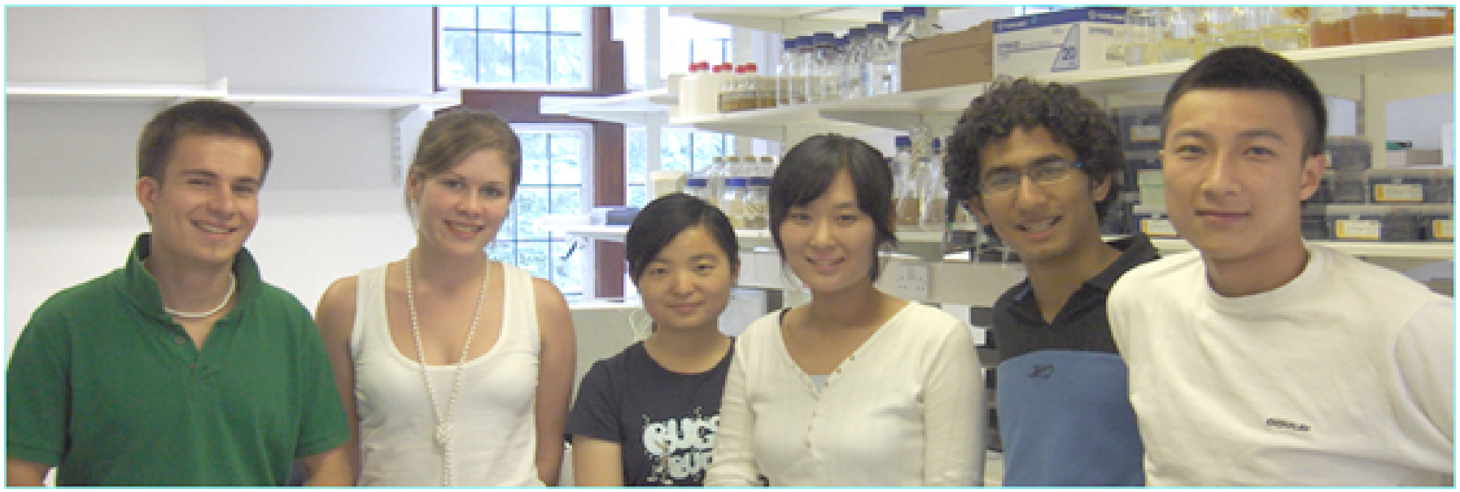


CAMBRiDGE iGEM 2006

Applications

- Understanding development
- Understanding tissue invasion and metastasis
- Understanding bio-films
- Tissue engineering

Cambridge University iGEM 2006



Supervisors

Jim Ajioka | Jim Haseloff | Duncan Rowe | Gos Micklem | Jorge Goncalves

Contributors

James Brown | Jason Chin | Gillian Fraser | Glenn Vinnicombe | Keith Johnstone | Matthew Levin | Pentau Liu | Jan Lowe | Rita Monson

CAMBRiDGE iGEM 2006

Acknowledgements

- The Gatsby Charitable Foundation
- Cambridge University Engineering Department
- European Union (Synbiocomm)
- Cambridge-MIT Institute
- DNA 2.0 Incorporation
- Lucigen Corporation

CAMBRiDGE iGEM 2006