In-vivo implementation of bioengineering circuits

ICOS seminars

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Outline

Bioengineering

Design

Modelling

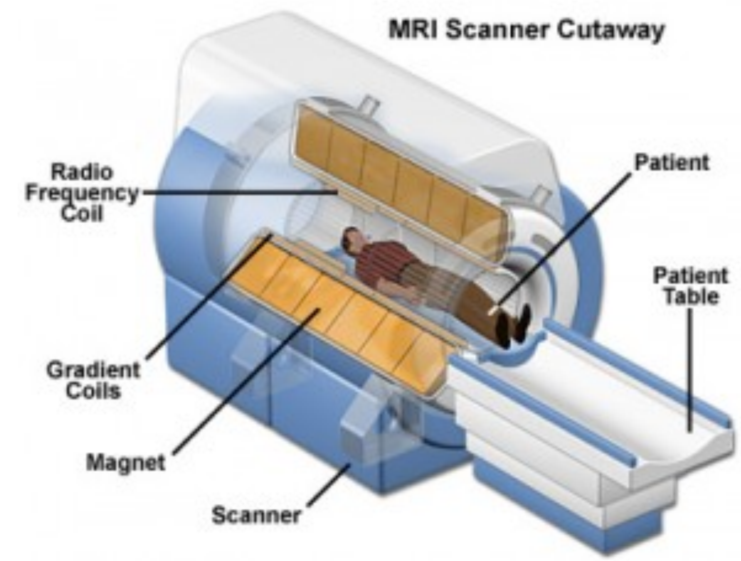
Characterisation

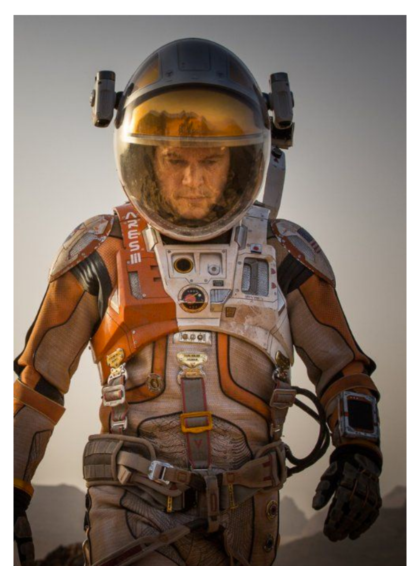
Applications

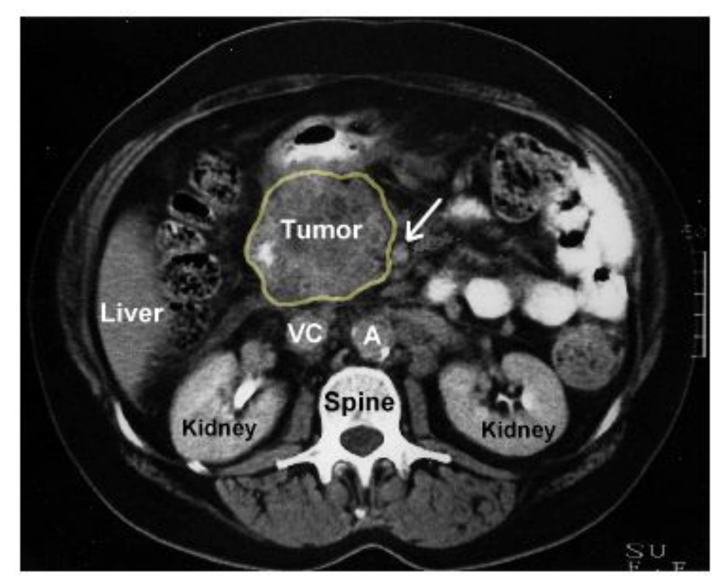
What is bioengineering?

- Taking a bit of biology and appending it some physics, electronics, computer science…

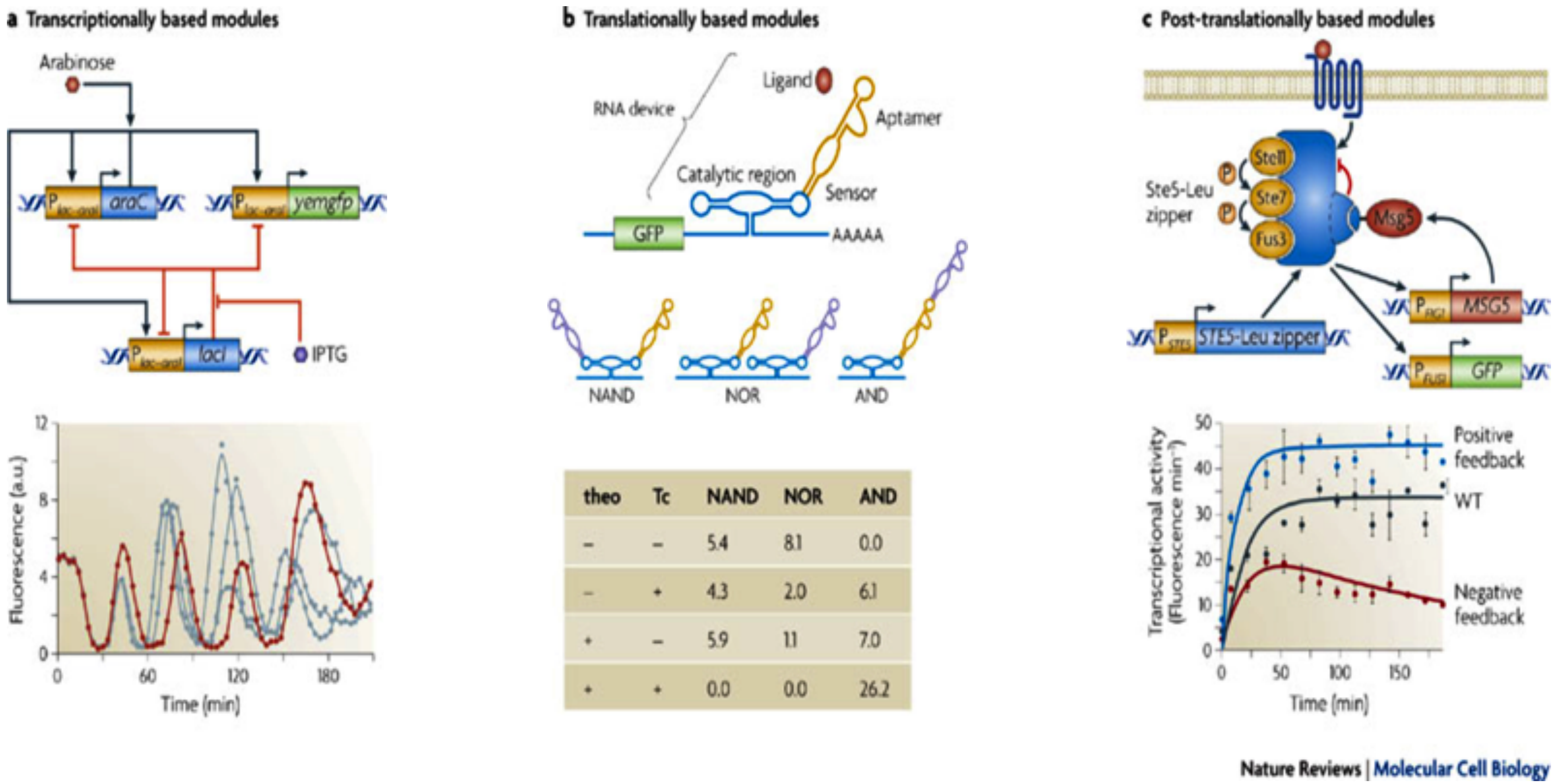
- Examples:
  - biomedical equipment (MRI/CT scan)
  - prosthetic limbs
  - astronaut suits







Genetic circuits in synthetic biology



theo	Tc	NAND	NOR	AND
–	–	5.4	8.1	0.0
–	+	4.3	2.0	6.1
+	–	5.9	1.1	7.0
+	+	0.0	0.0	26.2

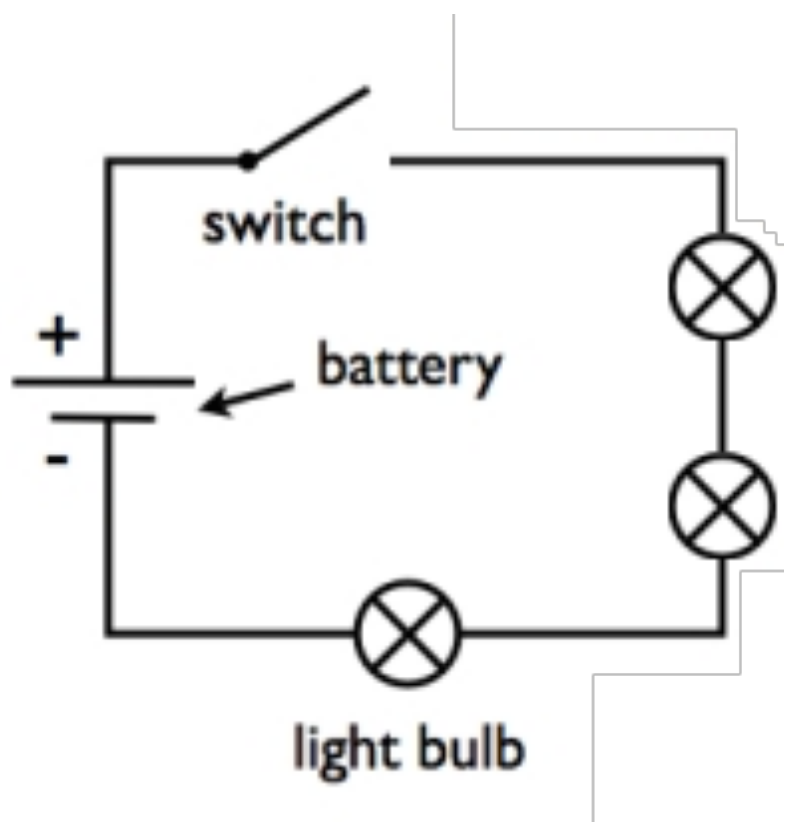
Oscillatory behaviour

Protein expression or degradation

Positive/negative feedback loops

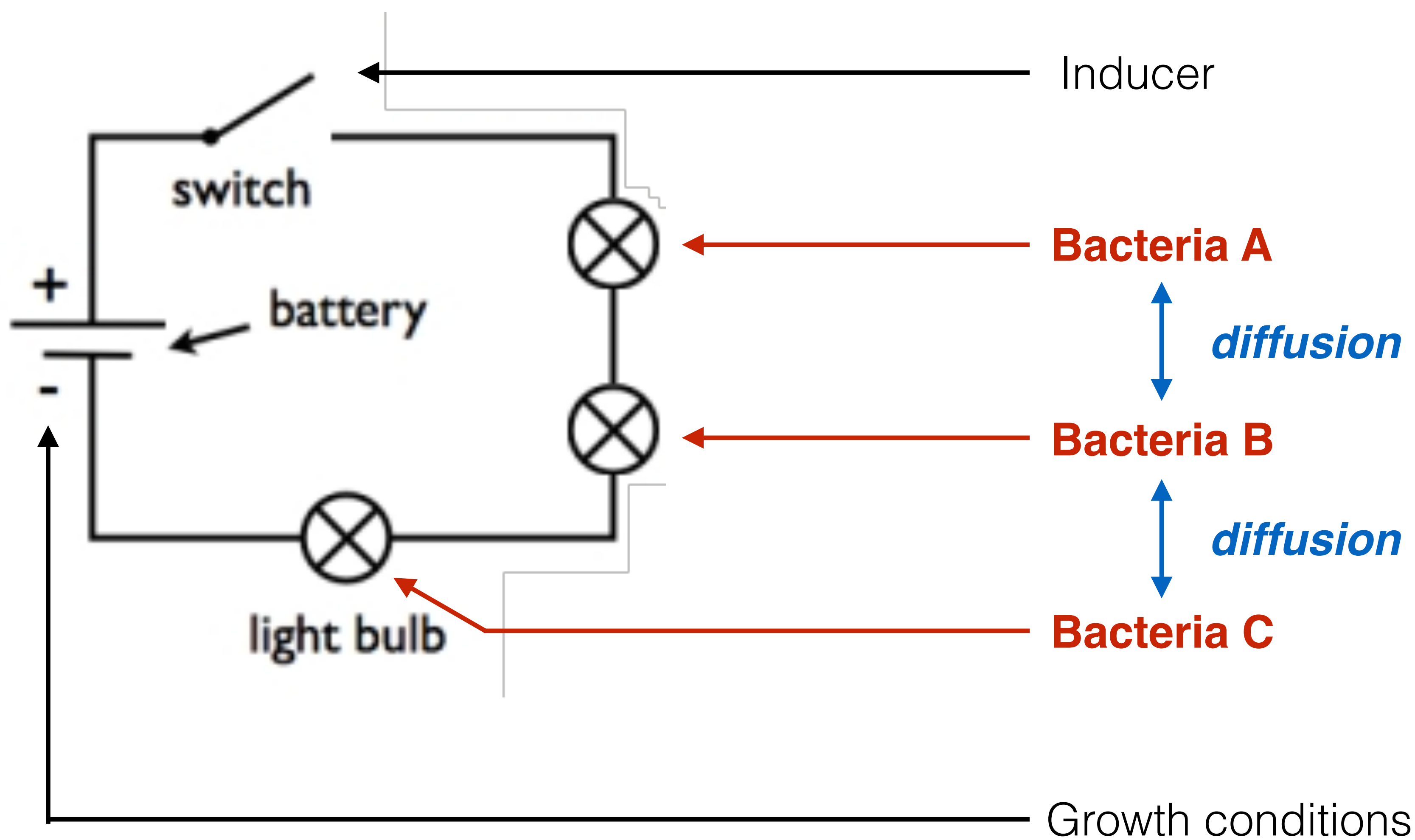
Cell-to-cell communication

Electrical
circuit diagrams



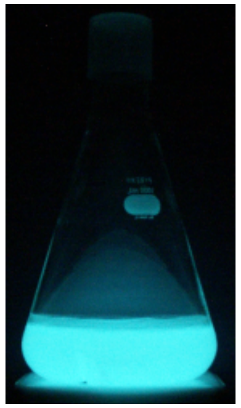
Cell-to-cell communication

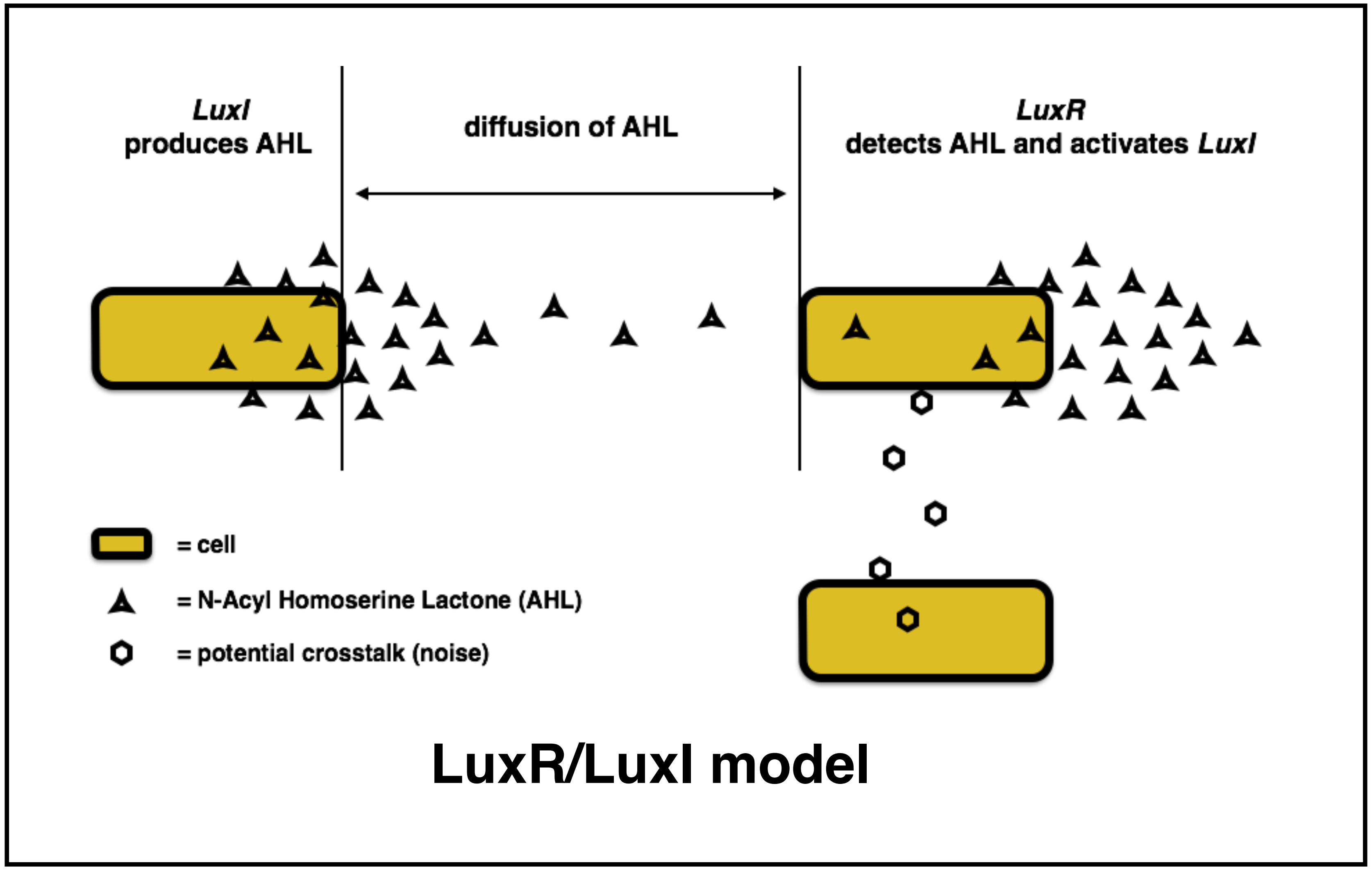
Electrical circuit diagrams → Biological circuit diagrams



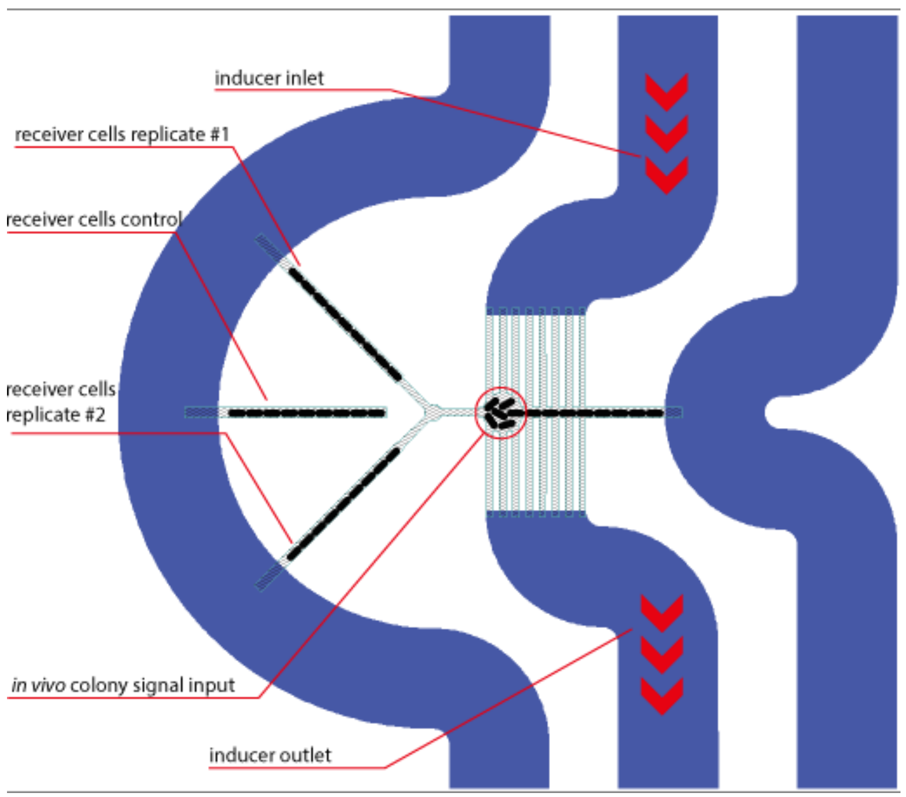
Quorum-sensing as molecular wire

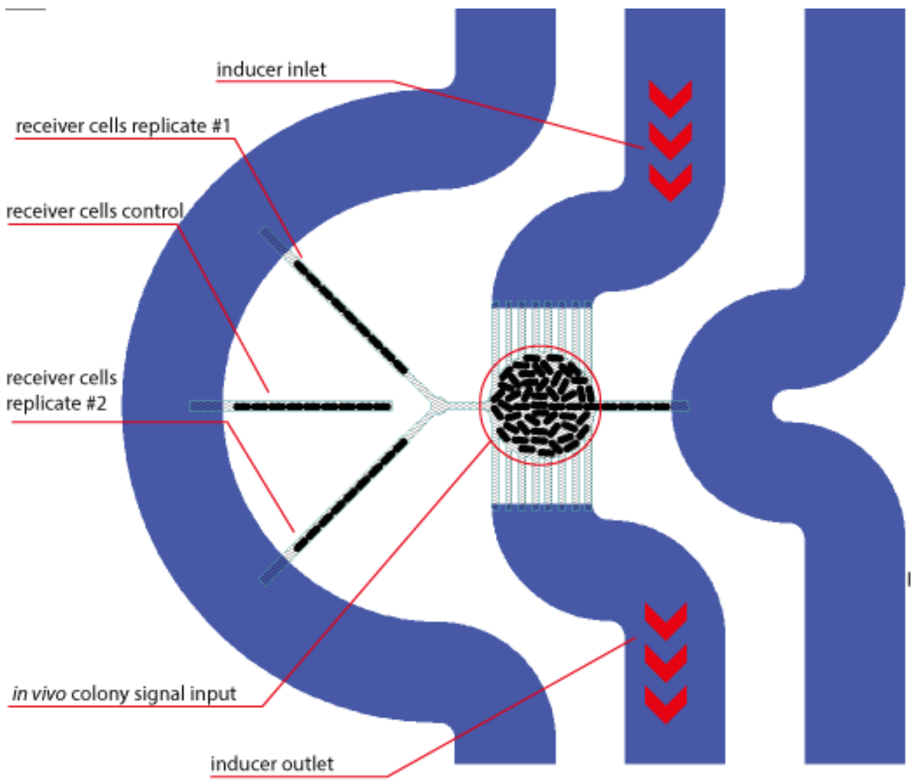
- Quorum-sensing is used to modulate multicellular synchronisation in *V. fischeri*
- Small molecules diffusion through bacterial membranes





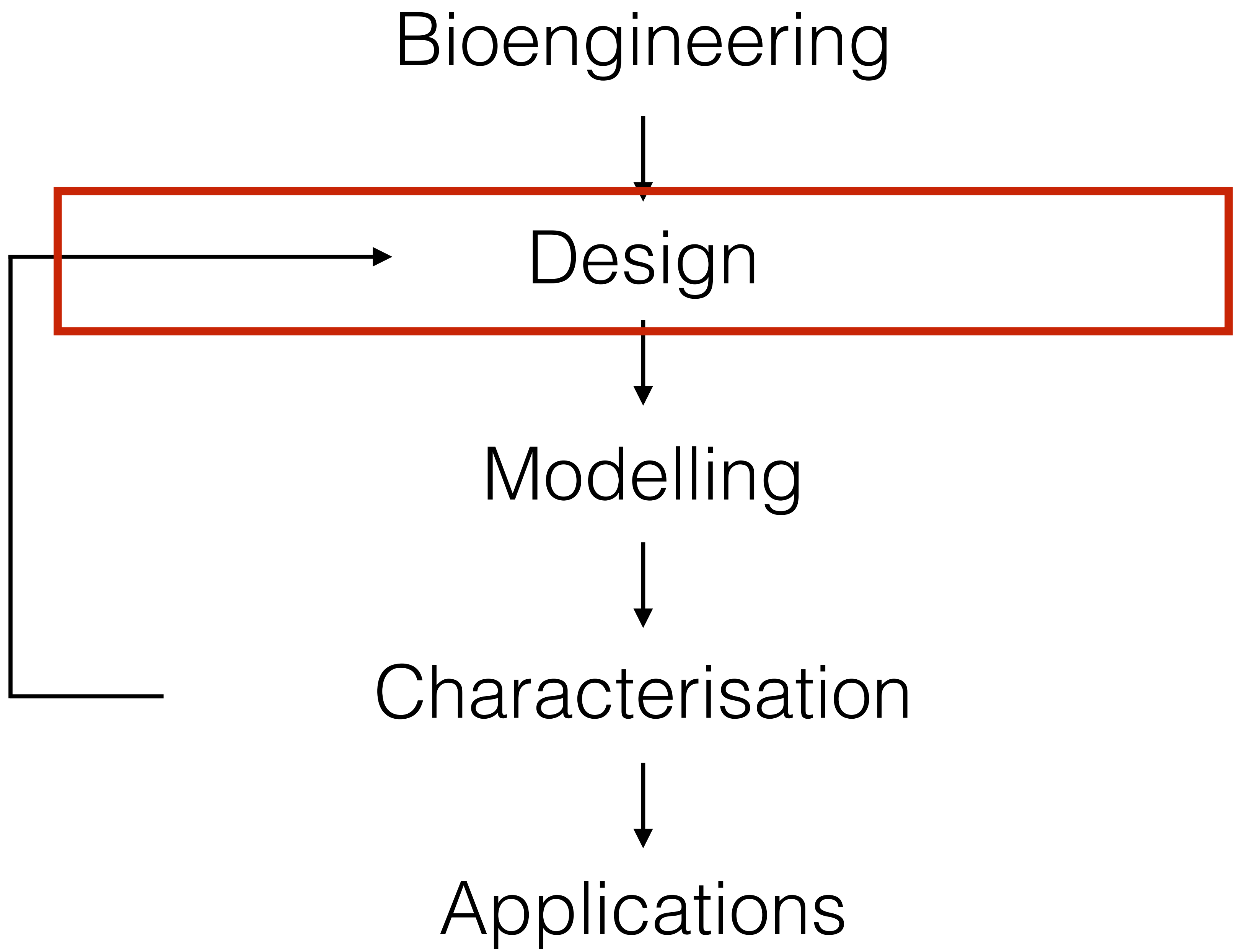
Microfluidics rhymes with microdistances





Study of bacterial communication via chemical wires

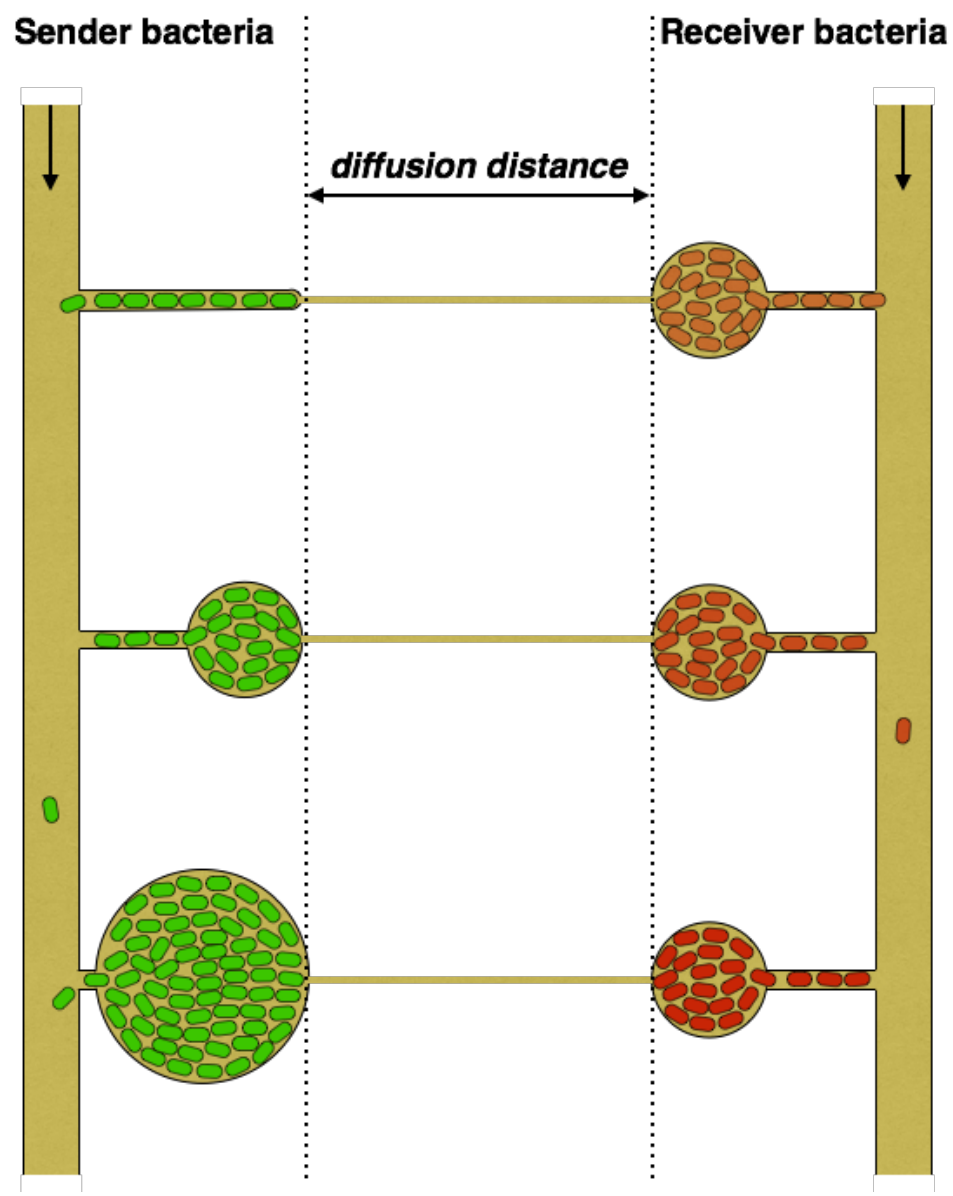
1. **Restricted space in growth chambers: fixed number of cells**
2. **Diffusion speed/response between distant bacterial colonies**


Bioengineering
Design
Modelling
Characterisation
Applications

Sender-receiver system

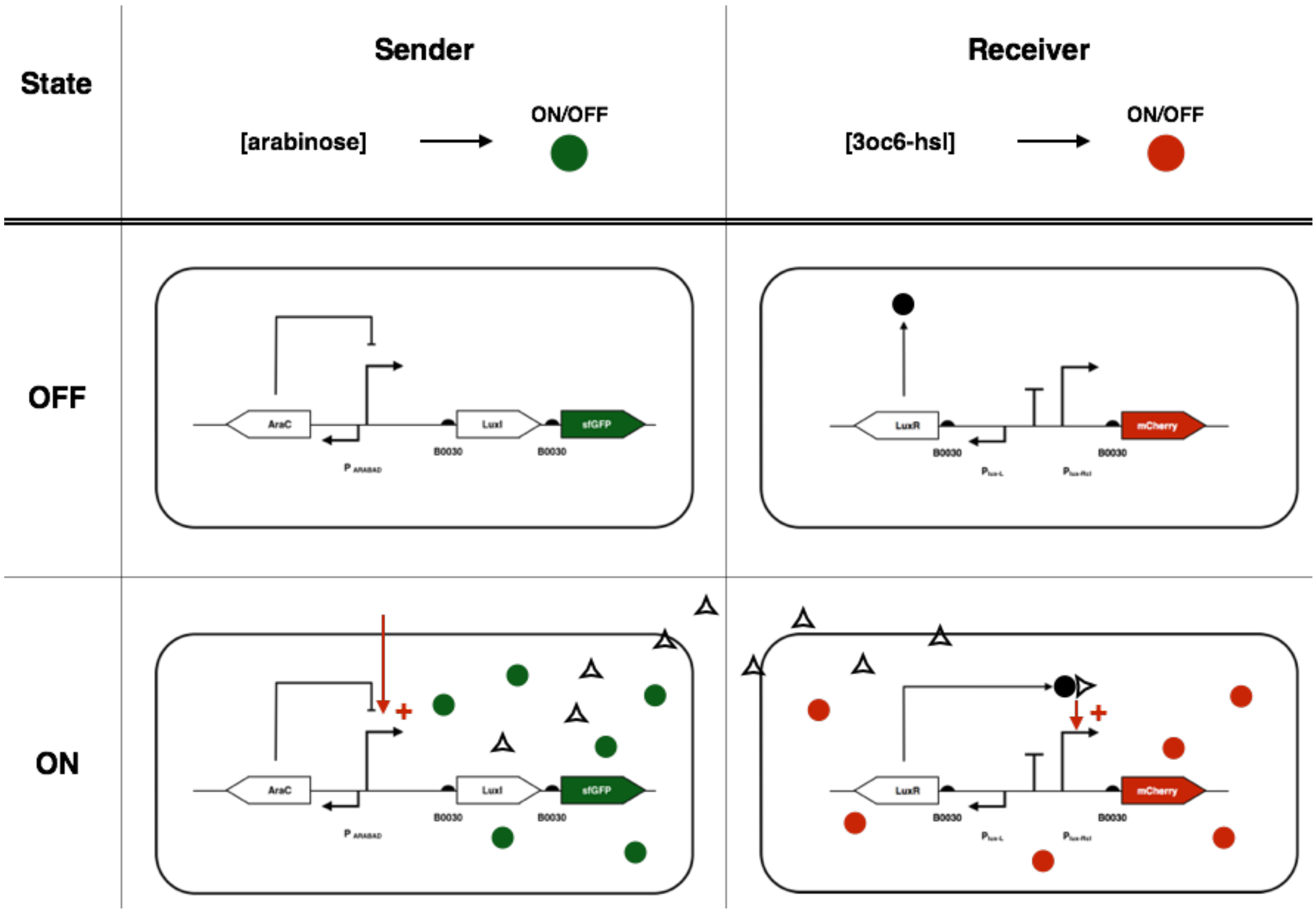
From the *lux* operon of *V. fischeri*

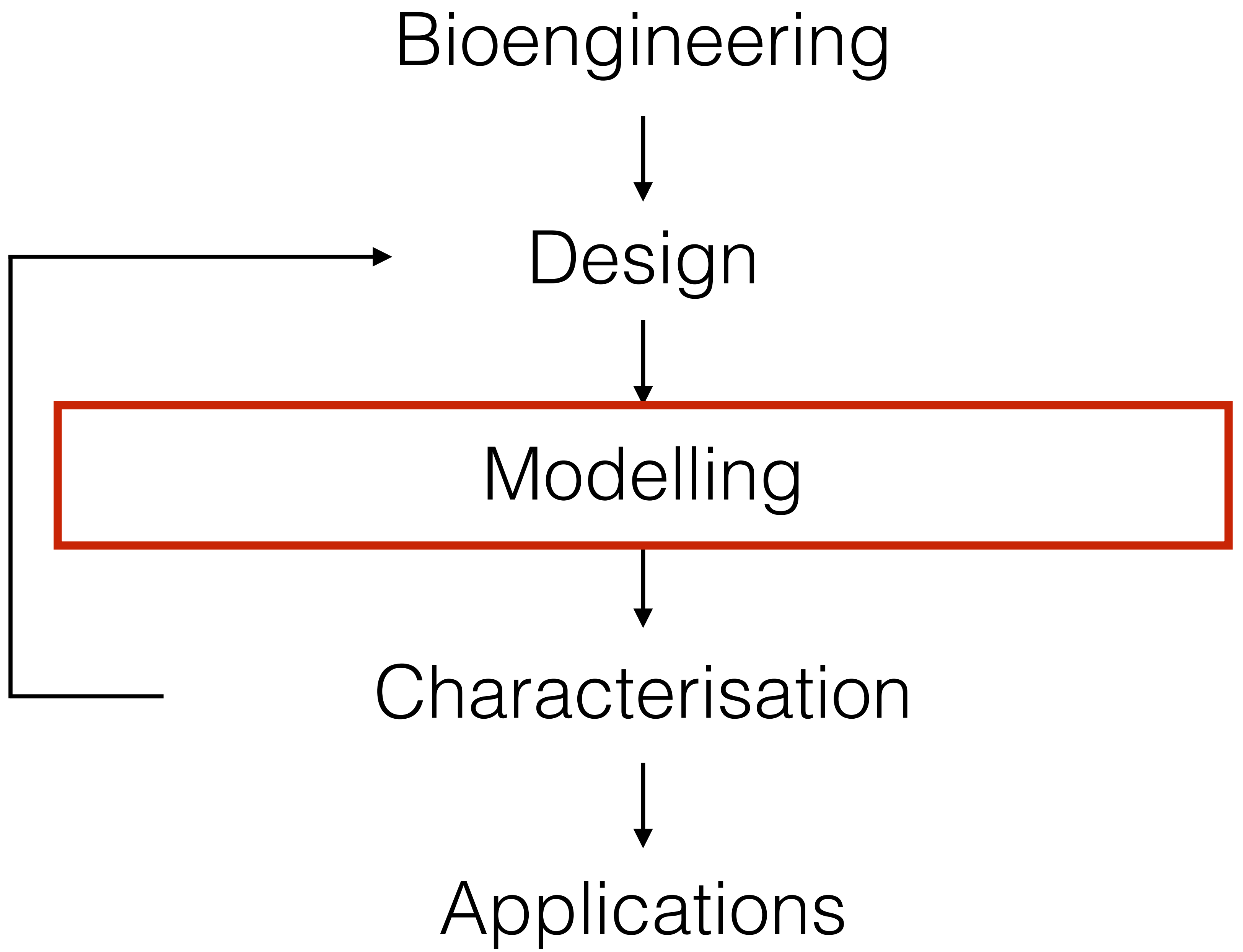
Produces the quorum-sensing signal N-(3-oxohexanoyl) homoserine lactone (**AHL**) and green fluorescence (**sfGFP**)



Detects **AHL** and complexes with **LuxR** to activate red fluorescence (**mCherry**)

States behaviour

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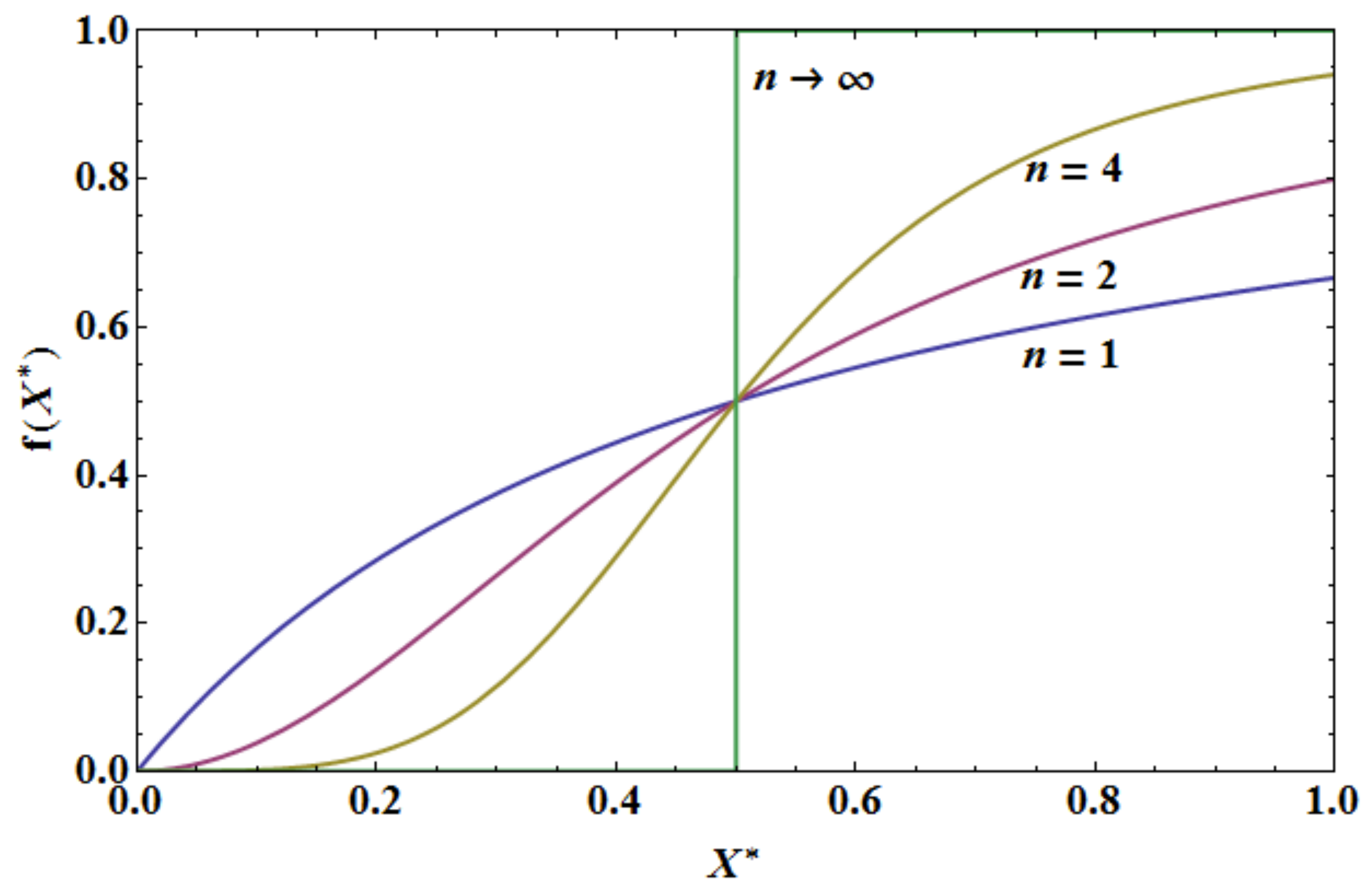
Easy maths for biologists

How to figure out the production rate of the sender?

Activable promoters can be described as Hill functions:

$$f(X^*) = \frac{\beta_{max} \cdot {X^*}^n}{K^n + {X^*}^n}$$



Sender mathematical model

Activity of the pBad promoter

$$P_{BAD} = \frac{k_1 + k_2 C}{1 + k_1 + k_2 C + k_3 C_F}$$

Expression of autoinducer protein

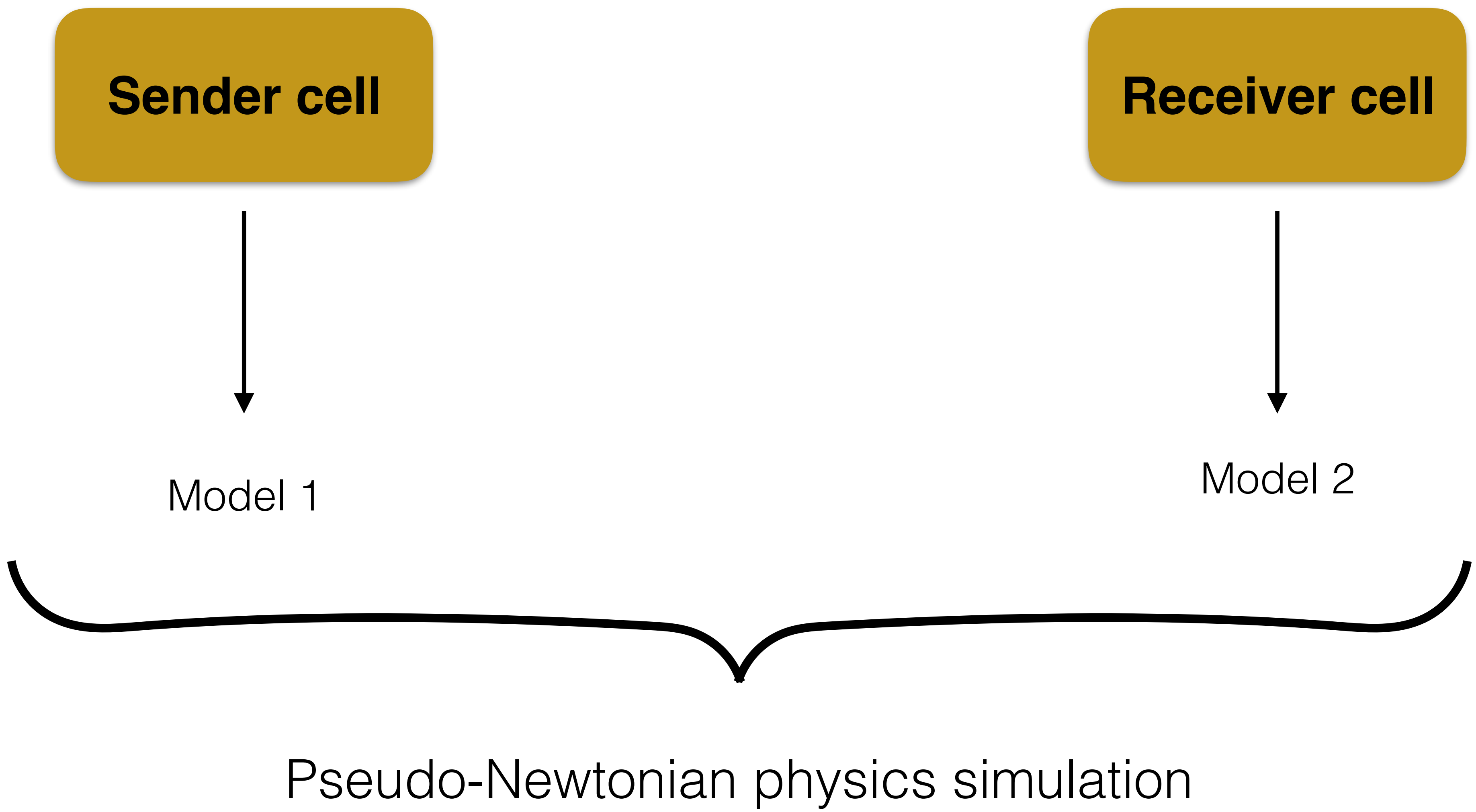
$$\frac{\delta S}{\delta t} = \rho P_{BAD} - \Delta S$$

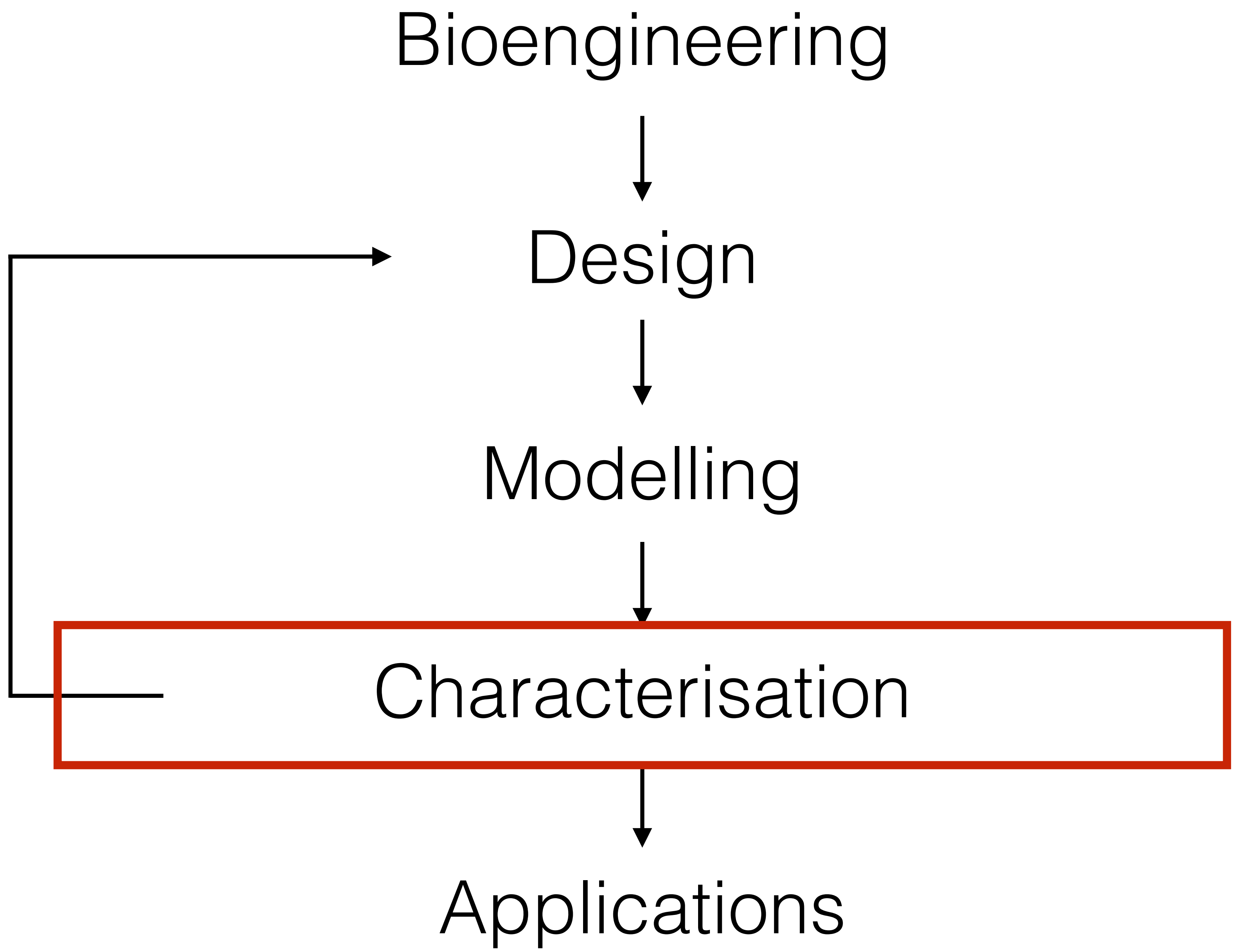
Production/diffusion of wiring molecule

$$\frac{\delta L}{\delta t} = D \times \frac{d^2 L}{dx^2} + \lambda_S - \Delta_L$$

Physical model

Bioform, a 3D physical modelling environment (Jonathan Naylor)

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Sender induction: green fluorescence detection

Flow-cytometer (3rd floor, CBCB)

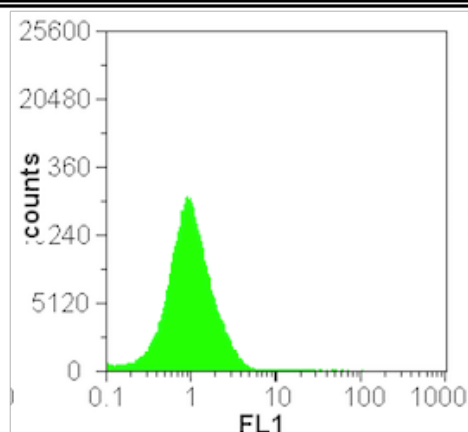
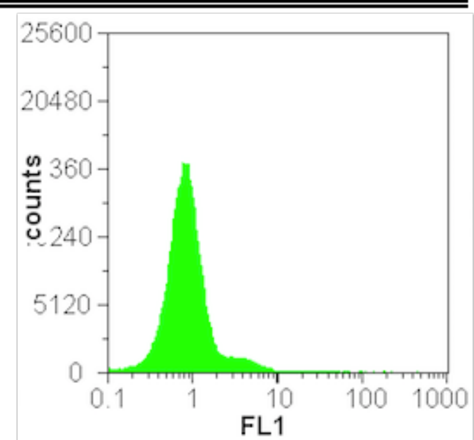
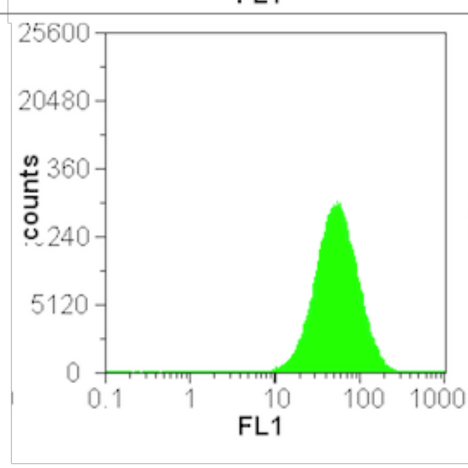
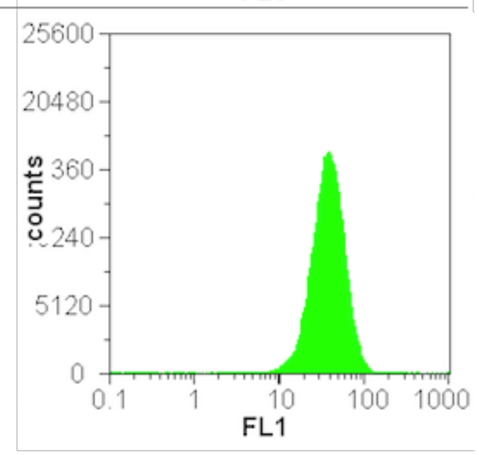
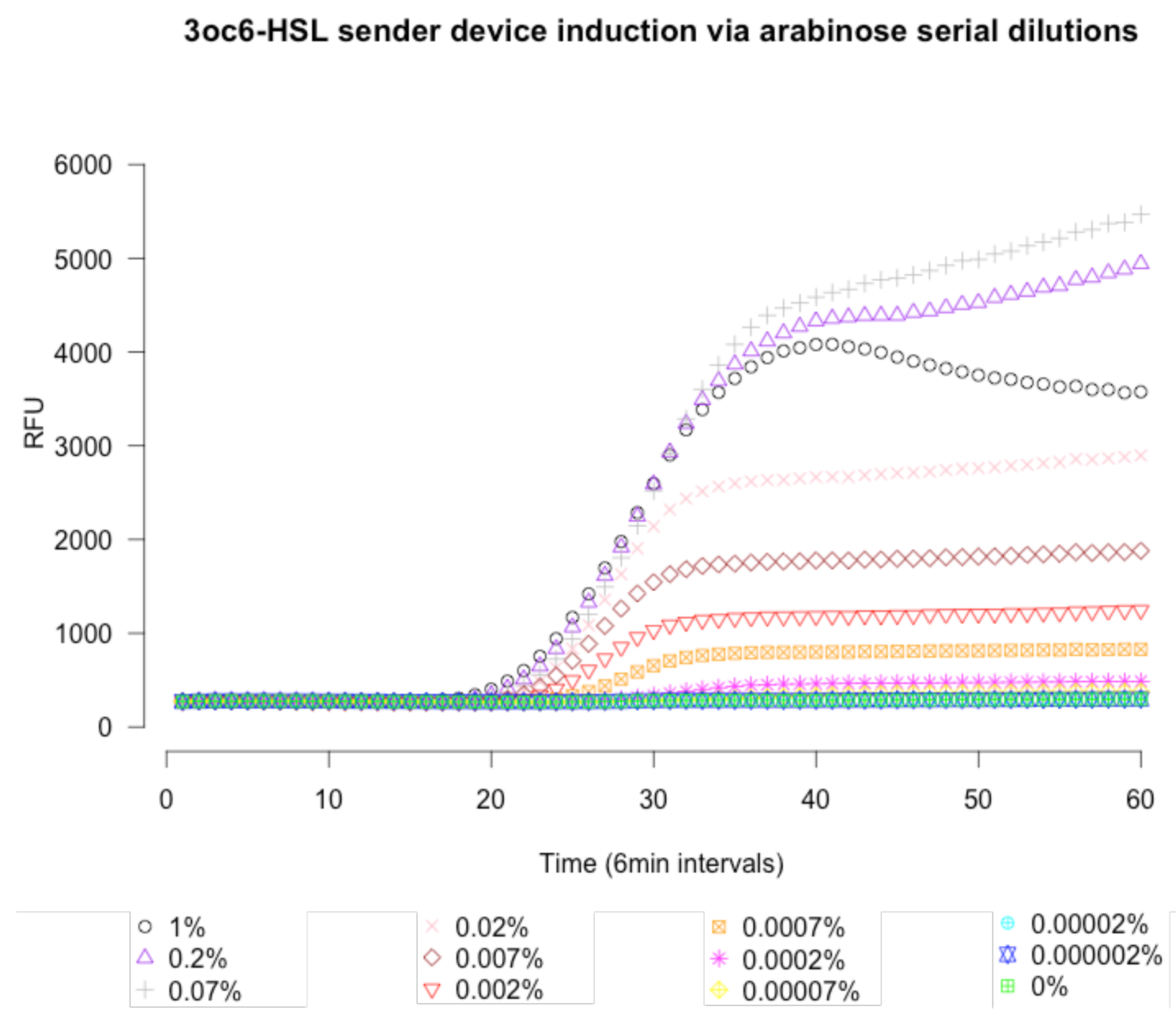
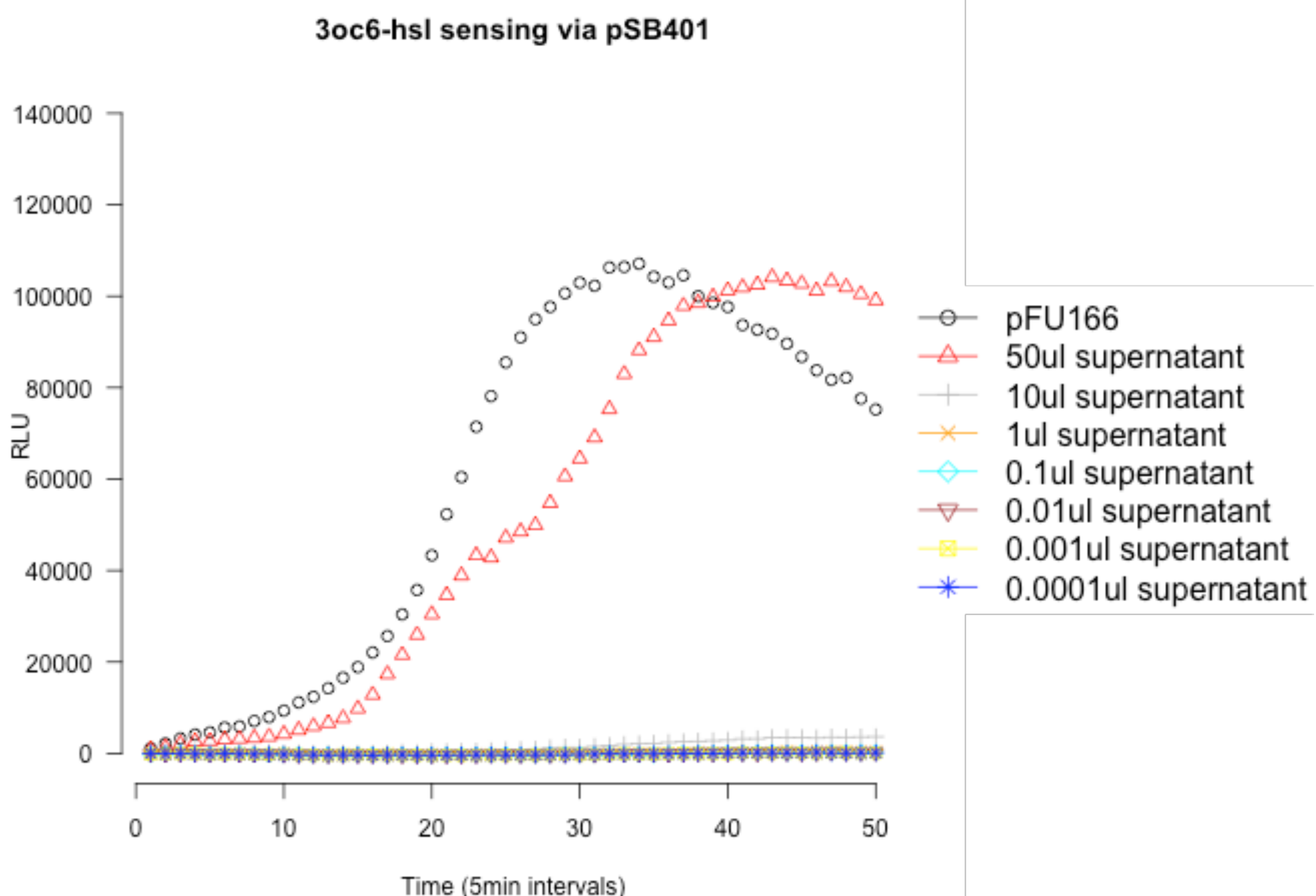
Induction	dh5a	MG1655
Uninduced		
0.2% arabinose		

Plate reader (4th floor, CBCB)

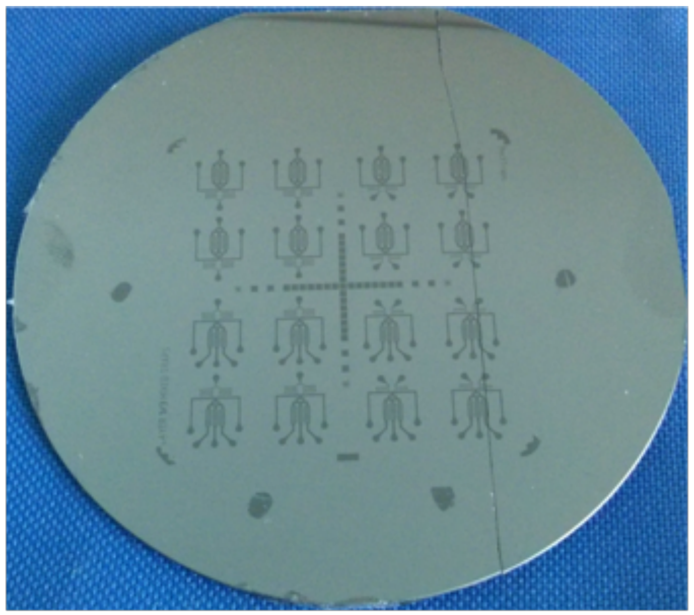


Receiver induction: luciferase activity detection

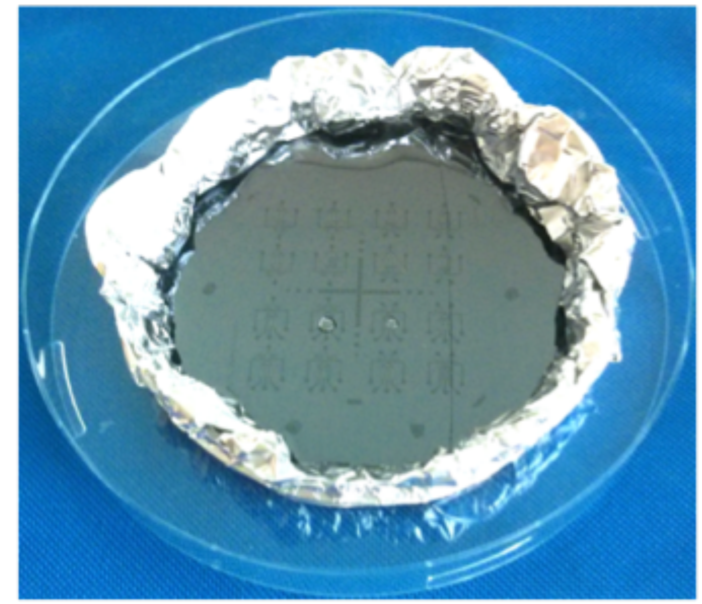
Plate reader (4th floor, CBCB)



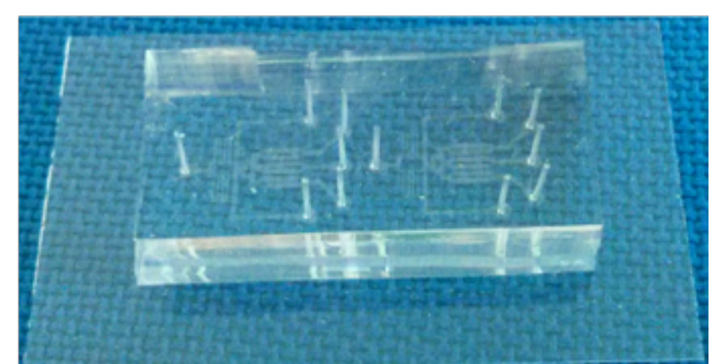
Microfluidics chips fabrication



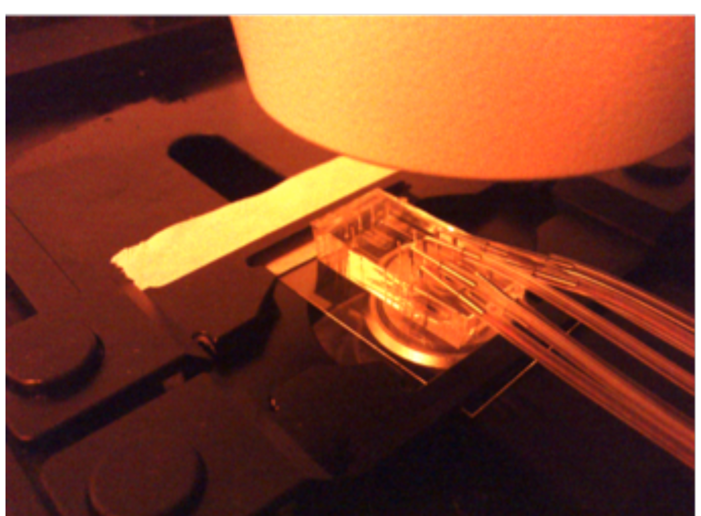
Polymer preparation
Air removal



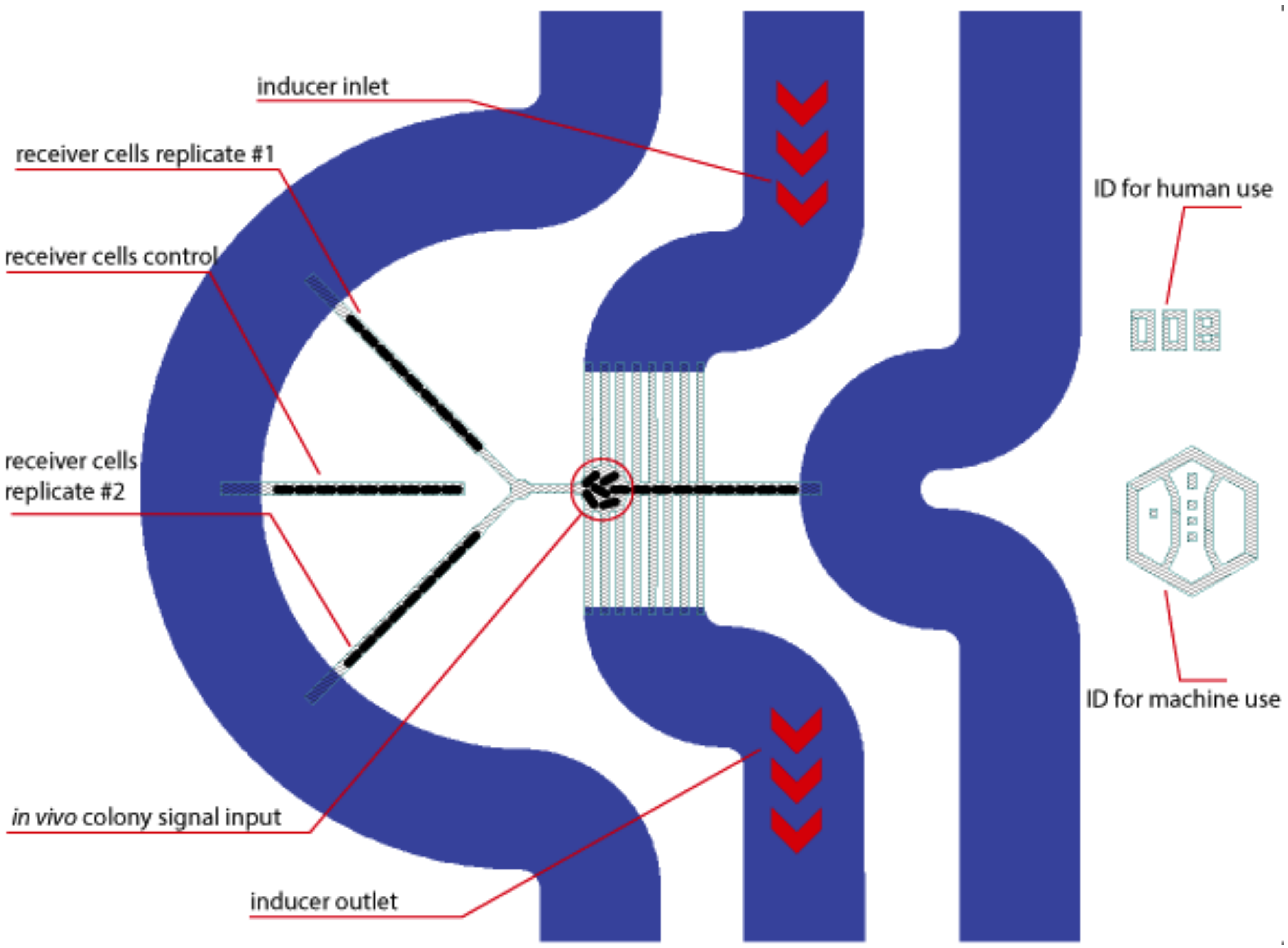
Crosslinking
Cut chips / ports
Plasma activation
Bonding

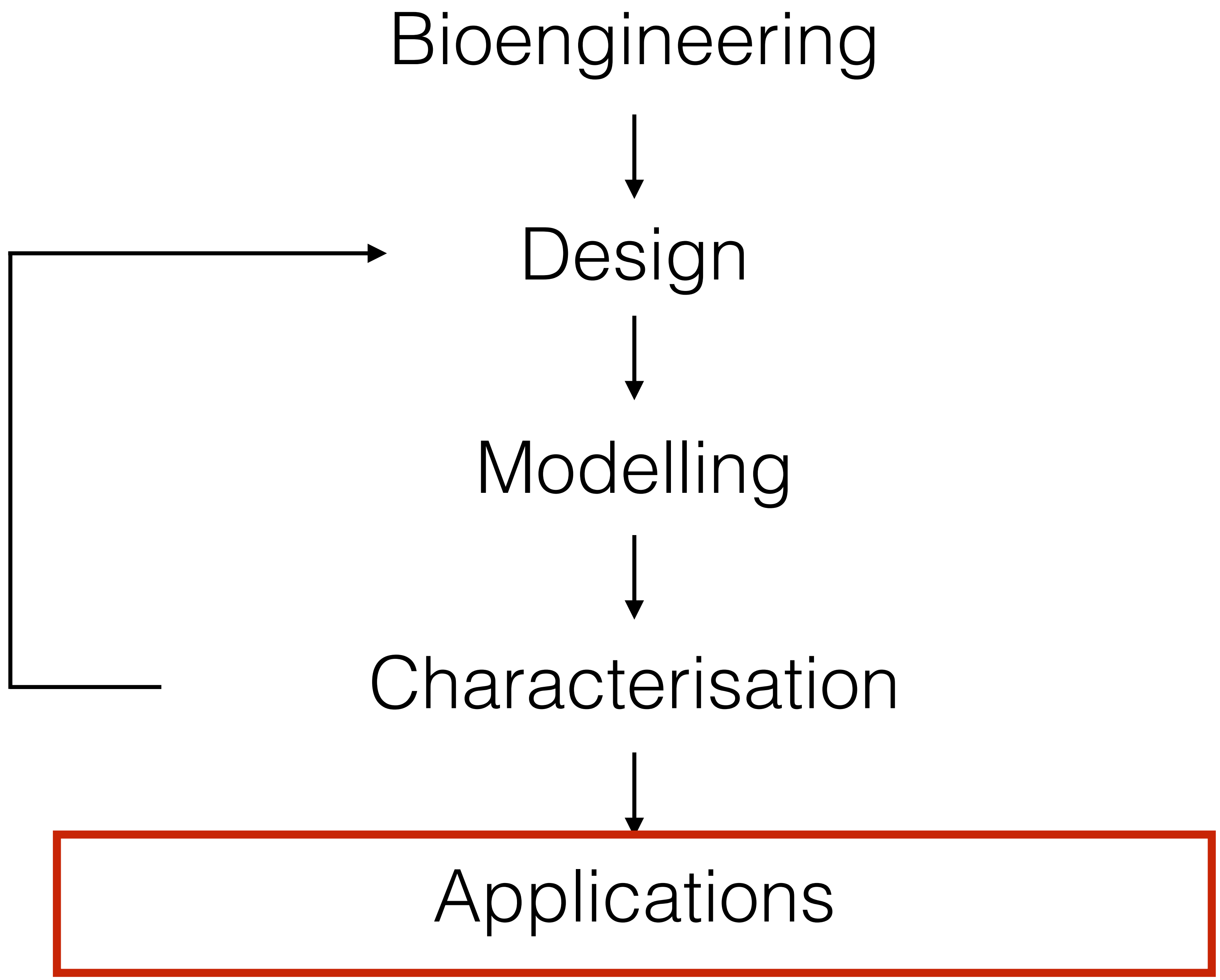


live-imaging



Diffusion distance / cell copy number

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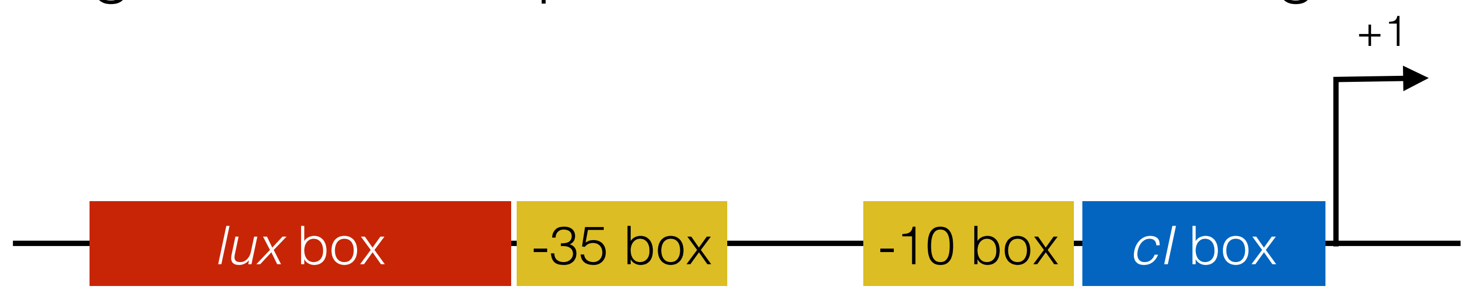
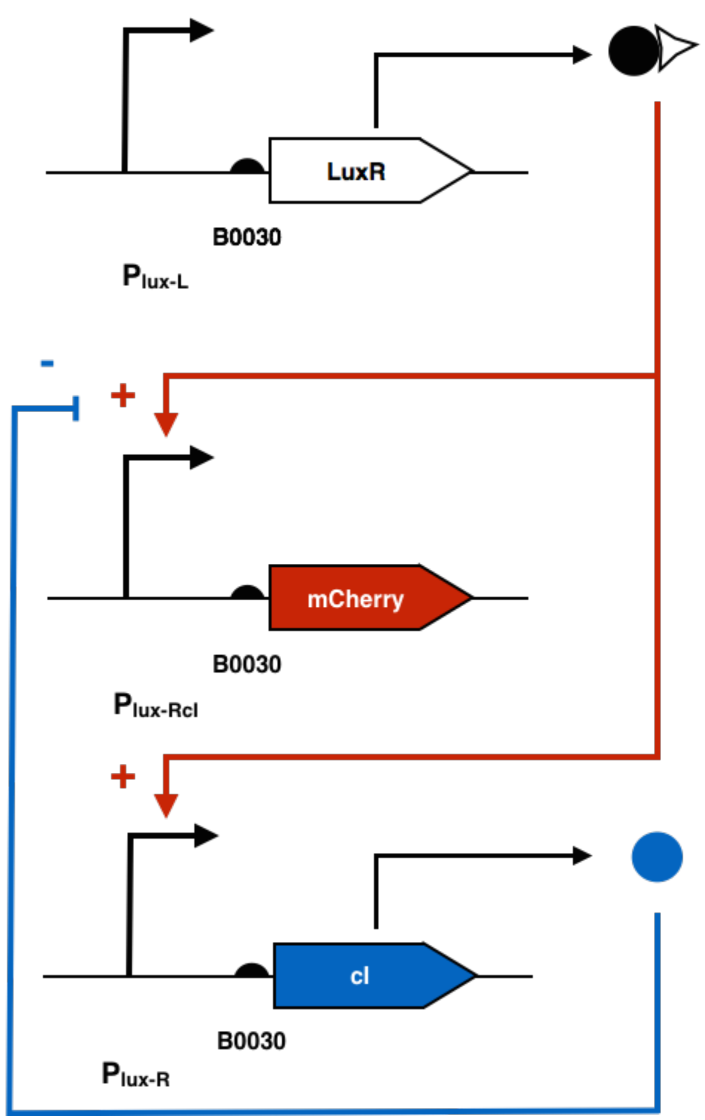
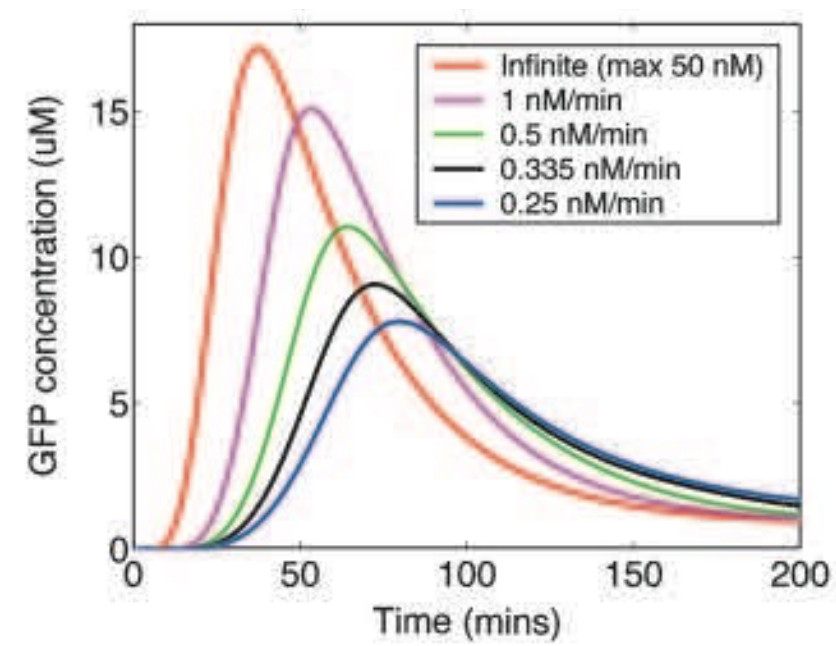
Pulse generator: the $P_{lux\text{-}RcI}$ promoter

Engineered $P_{lux\text{-}R}$ promoter with a cI binding site:







Pulse generator simulation

How it works in large-scale microfluidics

nature Vol 463|21 January 2010|doi:10.1038/nature08753

ARTICLES

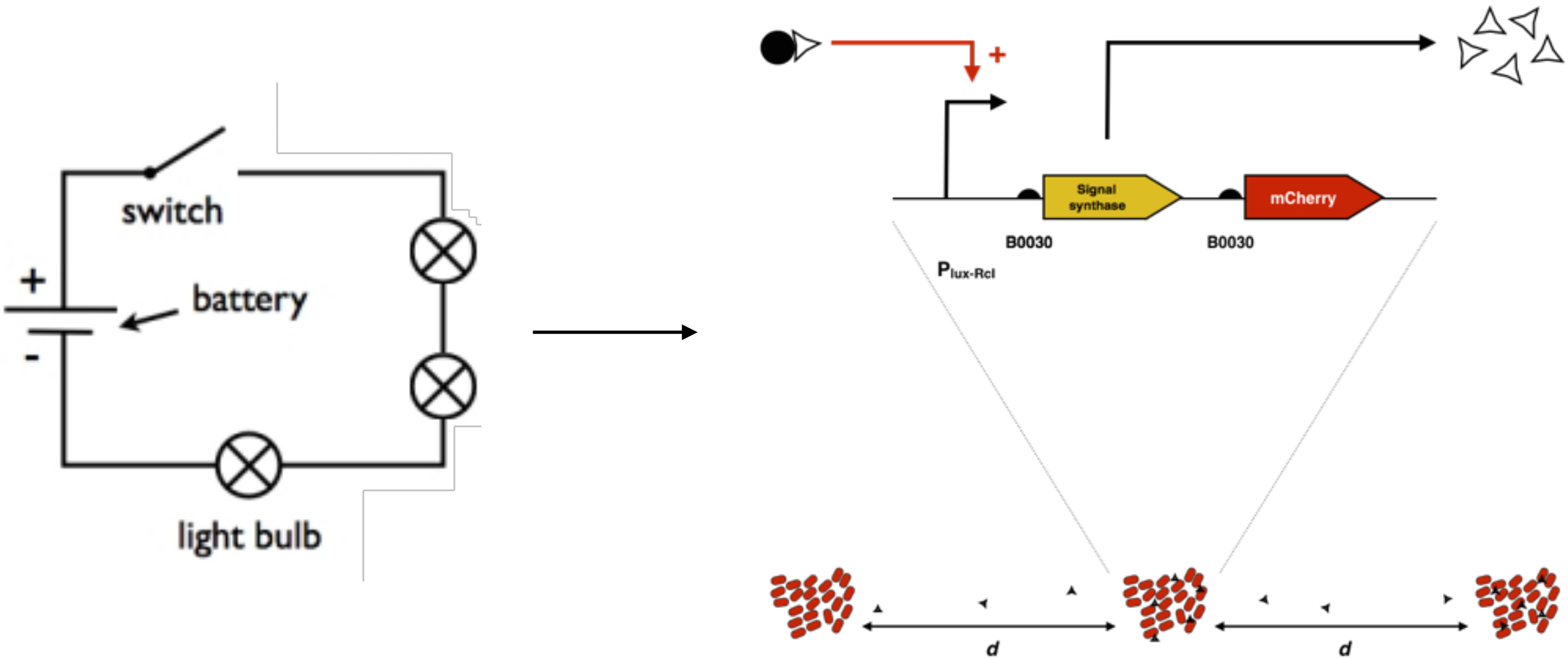
A synchronized quorum of genetic clocks

Tal Danino[1]*, Octavio Mondragón-Palomino[1]*, Lev Tsimring[2] & Jeff Hasty[1,2,3]

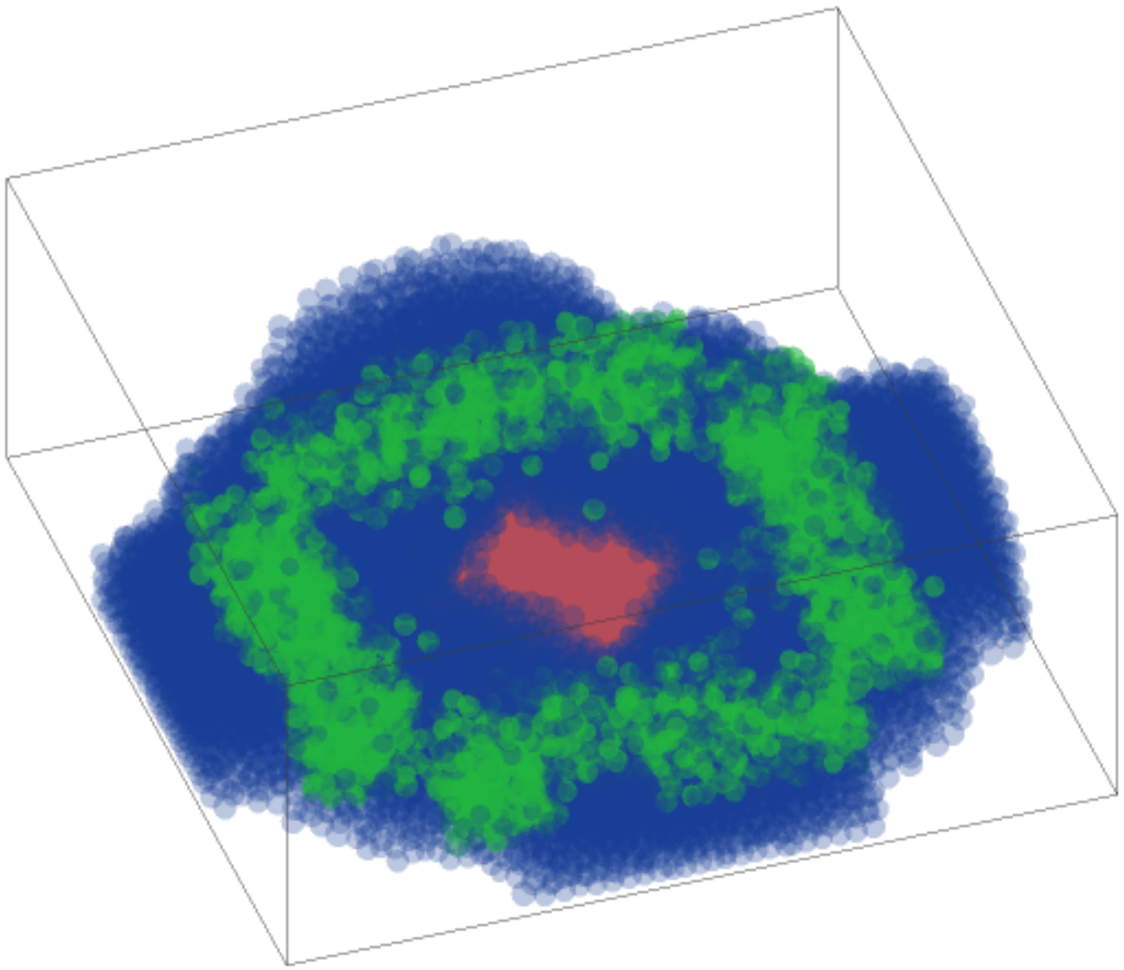
A really nice genetic clock synchronisation

Band detector

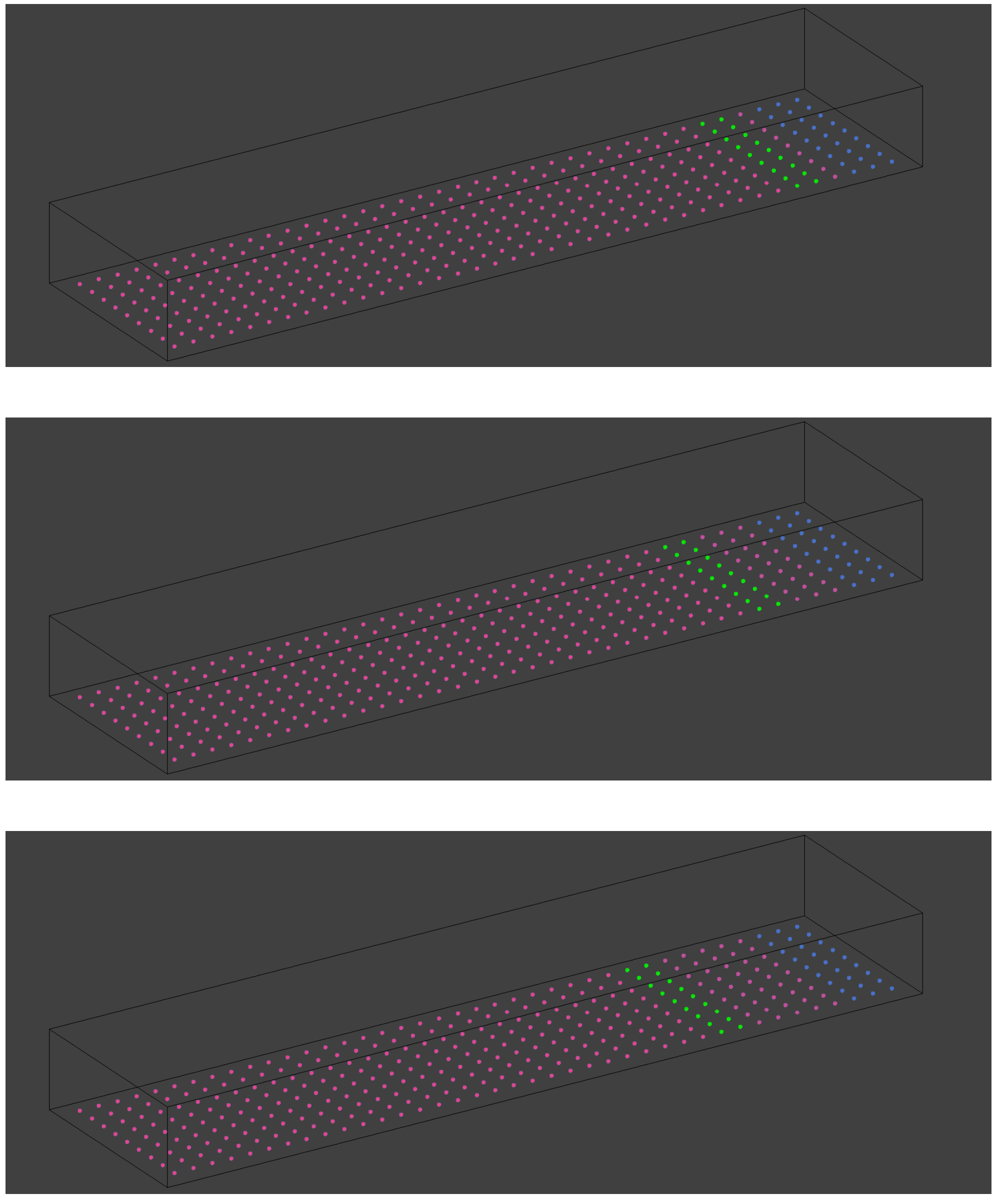
How to wire circuits of sender-receiver systems?



Down to the physics with Bioform



Band detector

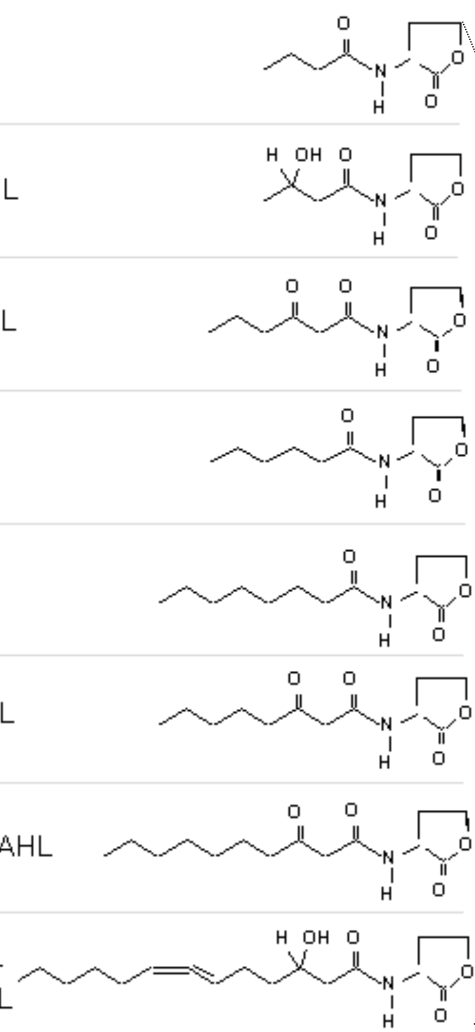


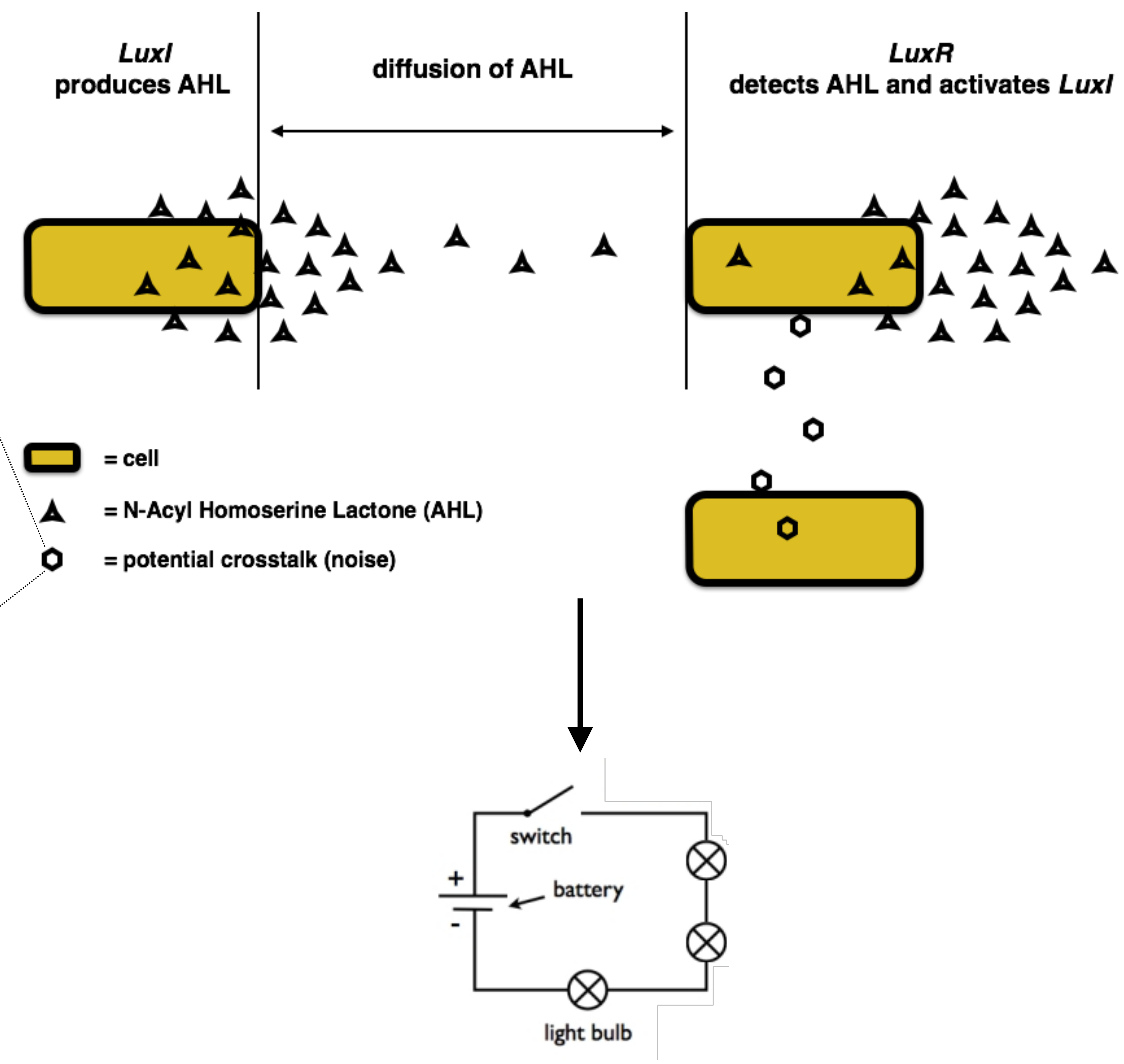
Propagating pulse generator

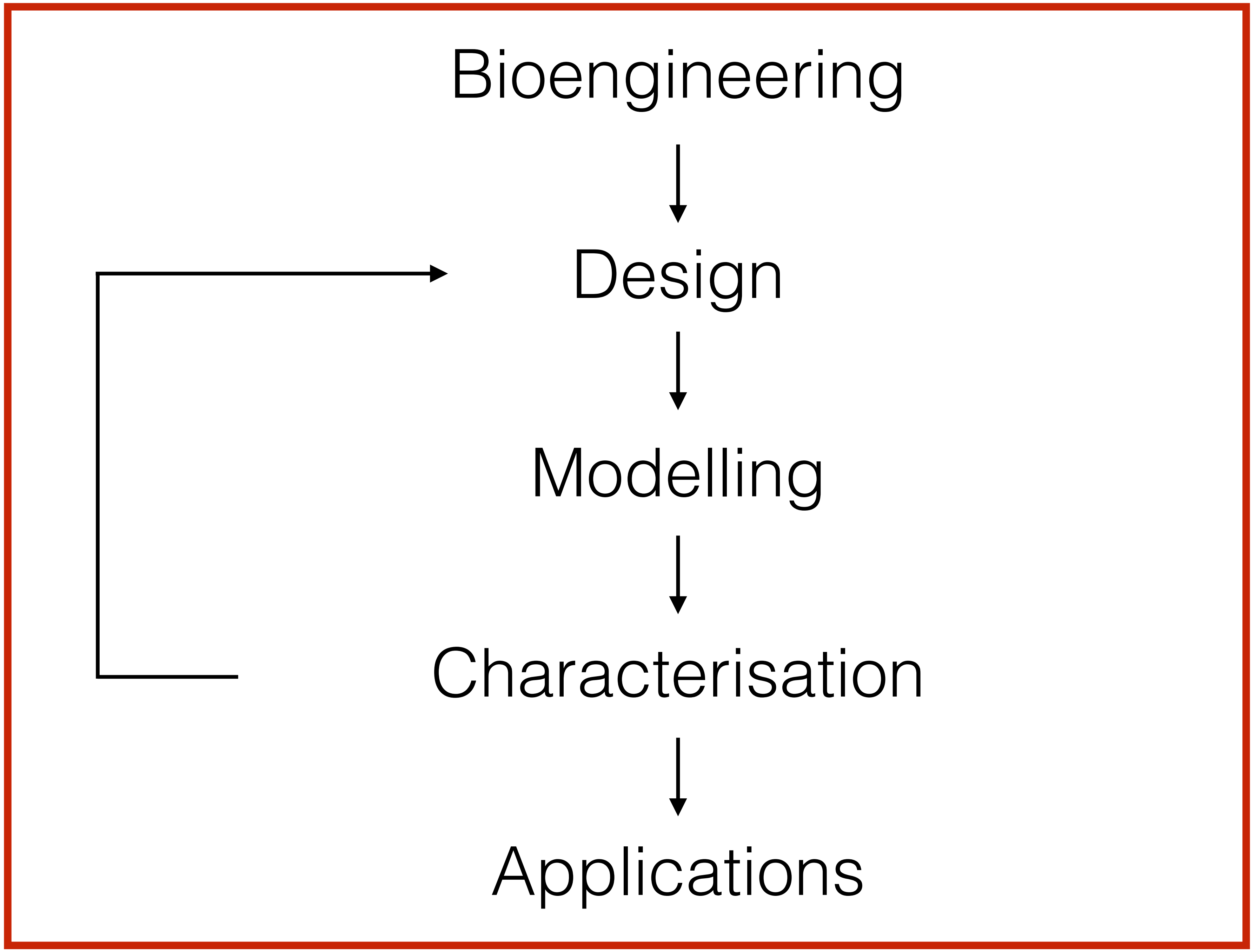
Quorum-sensing crosstalk

A list of AHLs can create potential crosstalk in LuxR/LuxI -like systems

Pseudomonas aeruginosa	Butyryl AHL
Vibrio harveyi	Hydroxybutyryl AHL
Erwinia caratovora *Pantoea stewartii*	3-oxohexanoyl AHL
Pseudomonas aureofaciens	Hexanoyl AHL
Ralstonia solanacearum	Octanoyl AHL
Agrobacterium tumefaciens	3-oxooctanoyl AHL
Pseudomonas aeruginosa	3-oxododecanoyl AHL
Rhizobium leguminosarum	3R-hydroxy-7-*cis*-tetradecenoyl AHL



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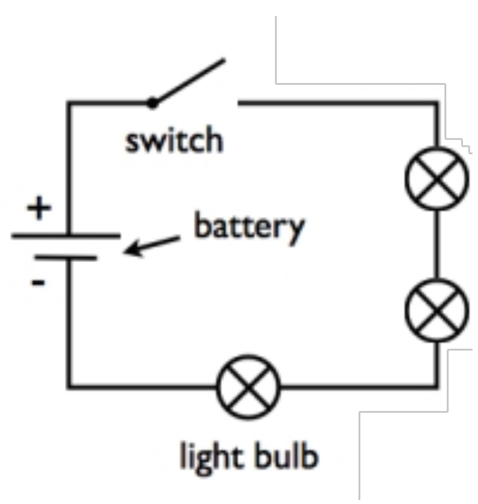
Summary

- Integration / characterisation of *V. fischeri* LuxR/LuxI system into *E. coli* as a sender-receiver system

- Assess AHL diffusion distances between independent bacterial colonies via microflulidics

- Based on the collected data:
    - Generate a library of quorum-sensing sender-receiver systems
    - Use soft-photolithography to design new circuits that wire multiple sender-receiver systems



Can we build such circuits with bacteria?

Acknowledgements

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