Multicellular feedback control for microbial consortia

Davide Fiore

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Department of Mathematics and Applications "R. Caccioppoli"

Padua, May 4th, 2023

Background and research experiences

- Educational background:
  - Laurea and Laurea Magistrale (December 2013) in “Ingegneria dell’Automazione”

  - **Dottorato di Ricerca** (4th May 2017) – 3 years in “Information Technology and Electrical Engineering”
    Supervisor: Prof. Mario di Bernardo
    - **Visiting student** – 2 months
      Supervisor: Prof. S. John Hogan

University of BRISTOL

    - **Visiting student** – 4 months
      Supervisors: Prof. Rodolphe Sepulchre & Dr. Fulvio Forni

UNIVERSITY OF CAMBRIDGE

Background and research experiences

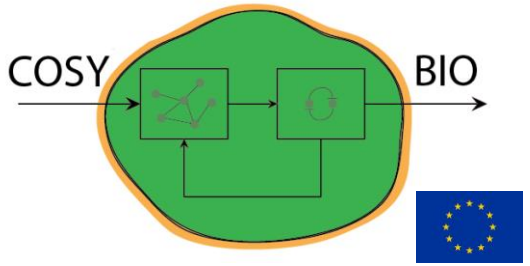
- Post-doctoral experiences:
  - **Research Intern** – 4 months

    Supervisor: Prof. Giovanni Russo

  - **Assegnista di Ricerca** – 2 years

    Principal Investigator: Prof. Mario di Bernardo

IBM Research Ireland



- Ricercatore RTD-A (started on 4th May 2019)
  at the Department of Mathematics and Applications “R. Caccioppoli”
  - Currently co-supervising 1 Postdoc and 3 PhD students working on synthetic biology

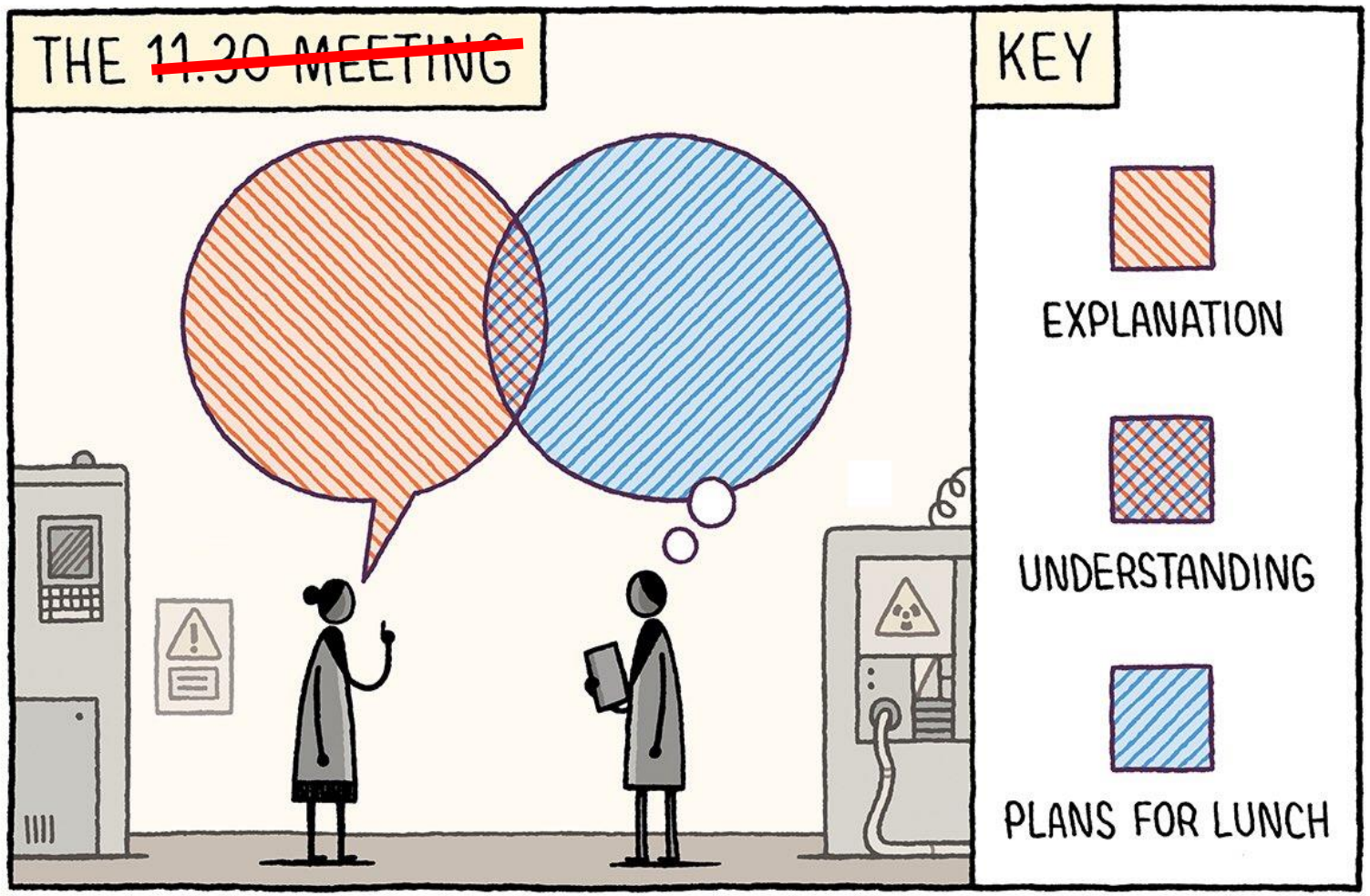
Research activity

- During these years I (mainly) worked on:
  - Stability and convergence analysis of non-smooth systems
  - Synchronization and consensus in complex networks
  - **Control in Synthetic Biology**
- And some other “side-quests”:
  - Pattern formation
  - Herding
  - Control of ecological systems

Another side project

Another side project

12:15 SEMINAR

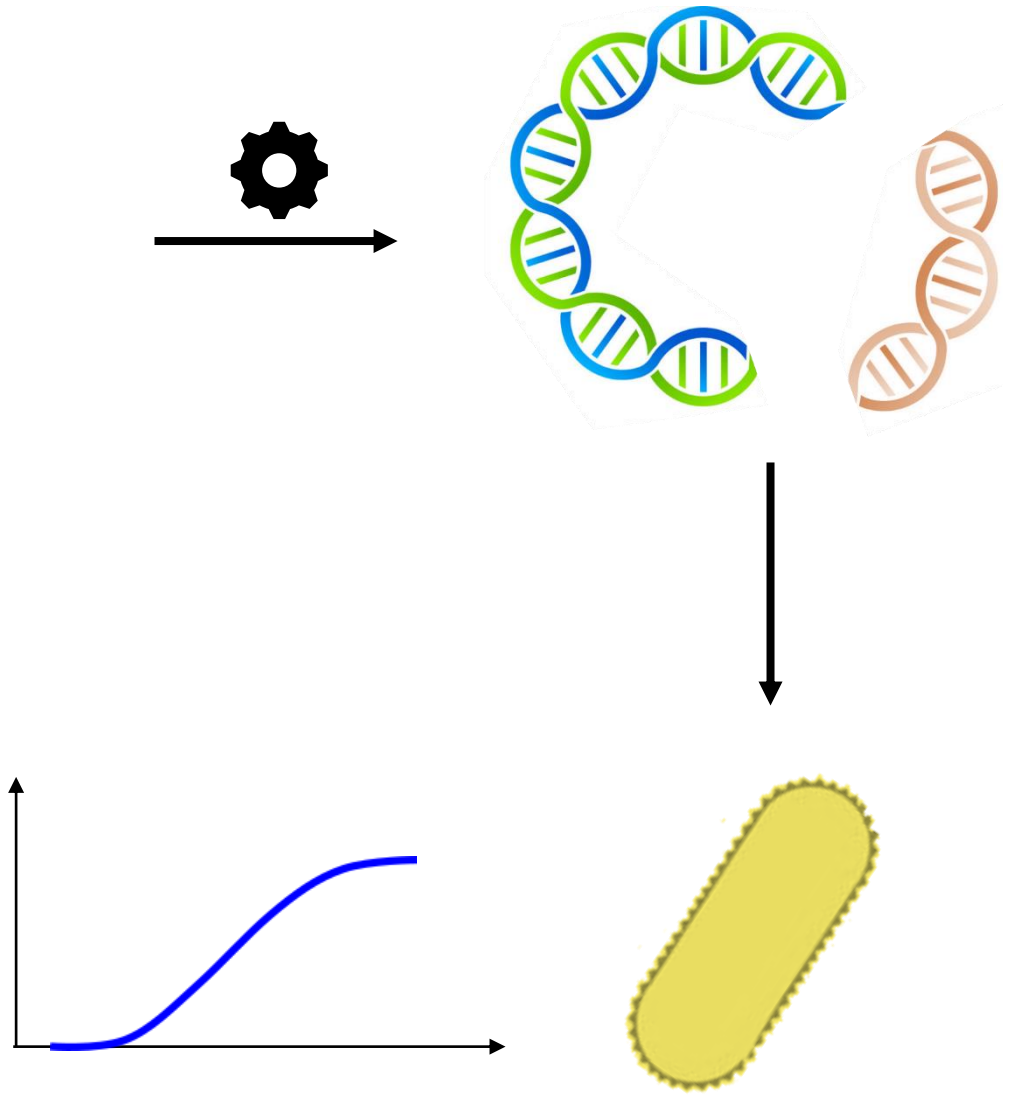


Multicellular control

(but before a brief introduction to Synthetic Biology)

Synthetic Biology

- Synthetic Biology is an interdisciplinary research area whose aim is to engineer new functionalities in living cells.
- Key in this field is the ability to design new genetic circuits.
- Lots of ongoing research since the first examples were implemented in the early 2000s.



[1] Gardner et al., Nature (2000)
[2] Shou et al., PNAS (2007)
[3] Din et al., Nature (2016)

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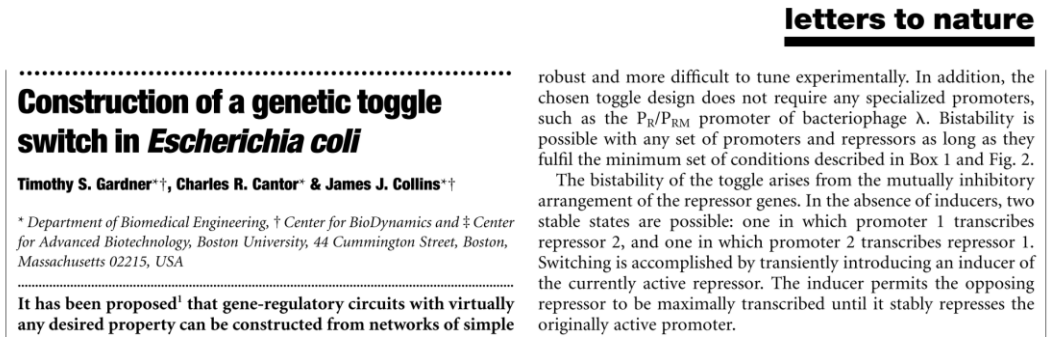


letters to nature

Construction of a genetic toggle switch in *Escherichia coli*

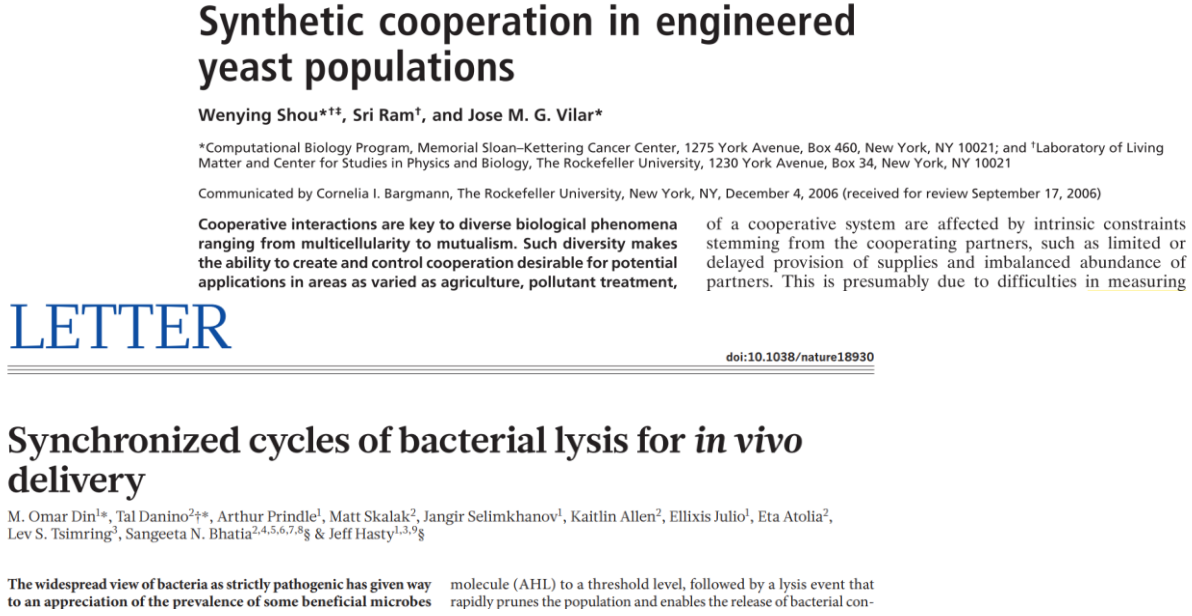
Timothy S. Gardner*†, Charles R. Cantor* & James J. Collins*†

** Department of Biomedical Engineering, † Center for BioDynamics and ‡ Center for Advanced Biotechnology, Boston University, 44 Cummington Street, Boston, Massachusetts 02215, USA*

It has been proposed[1] that gene-regulatory circuits with virtually any desired property can be constructed from networks of simple

robust and more difficult to tune experimentally. In addition, the chosen toggle design does not require any specialized promoters, such as the P_R/P_{RM} promoter of bacteriophage λ. Bistability is possible with any set of promoters and repressors as long as they fulfil the minimum set of conditions described in Box 1 and Fig. 2.

The bistability of the toggle arises from the mutually inhibitory arrangement of the repressor genes. In the absence of inducers, two stable states are possible: one in which promoter 1 transcribes repressor 2, and one in which promoter 2 transcribes repressor 1. Switching is accomplished by transiently introducing an inducer of the currently active repressor. The inducer permits the opposing repressor to be maximally transcribed until it stably represses the originally active promoter.



Synthetic cooperation in engineered yeast populations

Wenying Shou*†‡, Sri Ram†, and Jose M. G. Vilar*

*Computational Biology Program, Memorial Sloan–Kettering Cancer Center, 1275 York Avenue, Box 460, New York, NY 10021; and †Laboratory of Living Matter and Center for Studies in Physics and Biology, The Rockefeller University, 1230 York Avenue, Box 34, New York, NY 10021

Communicated by Cornelia I. Bargmann, The Rockefeller University, New York, NY, December 4, 2006 (received for review September 17, 2006)

Cooperative interactions are key to diverse biological phenomena ranging from multicellularity to mutualism. Such diversity makes the ability to create and control cooperation desirable for potential applications in areas as varied as agriculture, pollutant treatment,

of a cooperative system are affected by intrinsic constraints stemming from the cooperating partners, such as limited or delayed provision of supplies and imbalanced abundance of partners. This is presumably due to difficulties in measuring

LETTER

doi:10.1038/nature18930

Synchronized cycles of bacterial lysis for *in vivo* delivery

M. Omar Din[1]*, Tal Danino[2]†*, Arthur Prindle[1], Matt Skalak[2], Jangir Selimkhanov[1], Kaitlin Allen[2], Ellixis Julio[1], Eta Atolia[2], Lev S. Tsimring[3], Sangeeta N. Bhatia[2,4,5,6,7,8]§ & Jeff Hasty[1,3,9]§

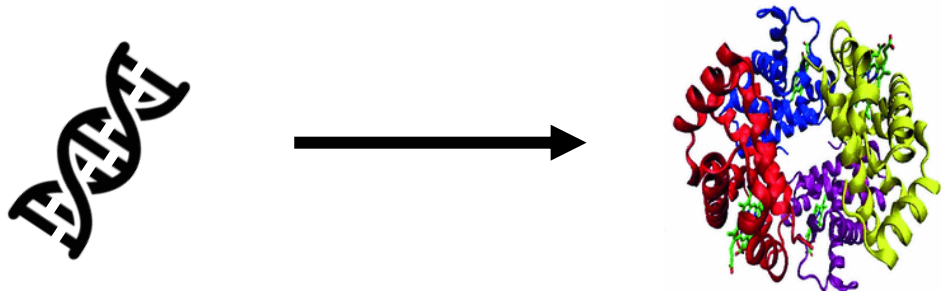
The widespread view of bacteria as strictly pathogenic has given way to an appreciation of the prevalence of some beneficial microbes

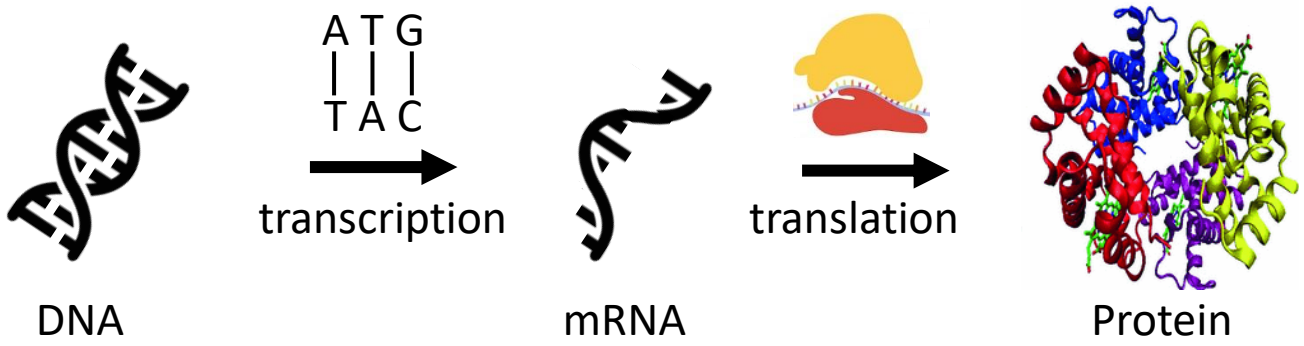
molecule (AHL) to a threshold level, followed by a lysis event that rapidly prunes the population and enables the release of bacterial con-

[1] Gardner et al., Nature (2000)
[2] Shou et al., PNAS (2007)
[3] Din et al., Nature (2016)

The central dogma of molecular biology

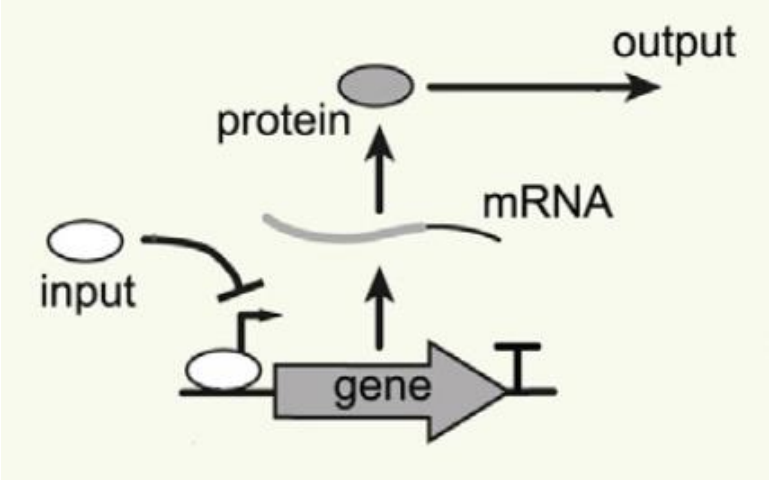
- All synthetic biology systems rely on the production of proteins starting from the information coded in the DNA.
- The process is carried in two steps:
  - DNA information are copied in a mRNA during the transcription.
  - Ribosomes translate the information coded in the mRNA to build proteins.

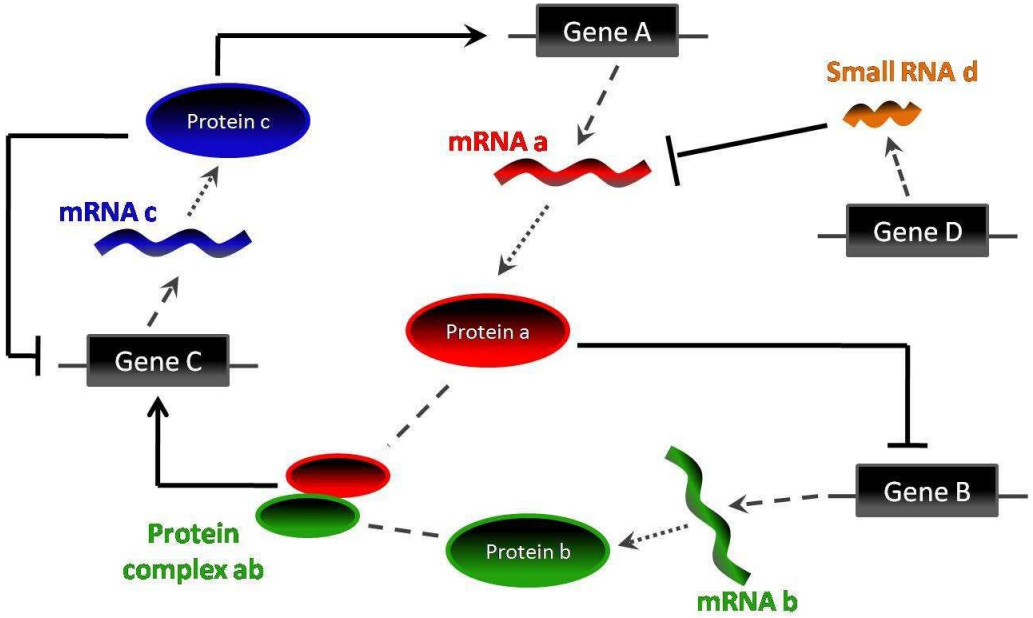




What is a genetic circuit?

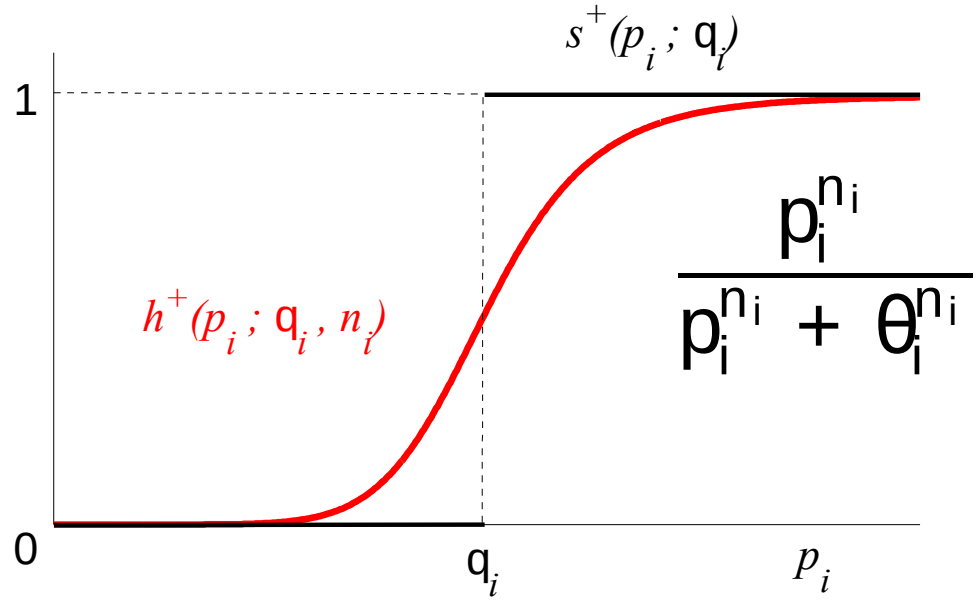
- A genetic circuit is composed by genes connected in networks or *gene regulatory networks.*
- The network relationships between genes are mainly of two kinds: activation and repression.



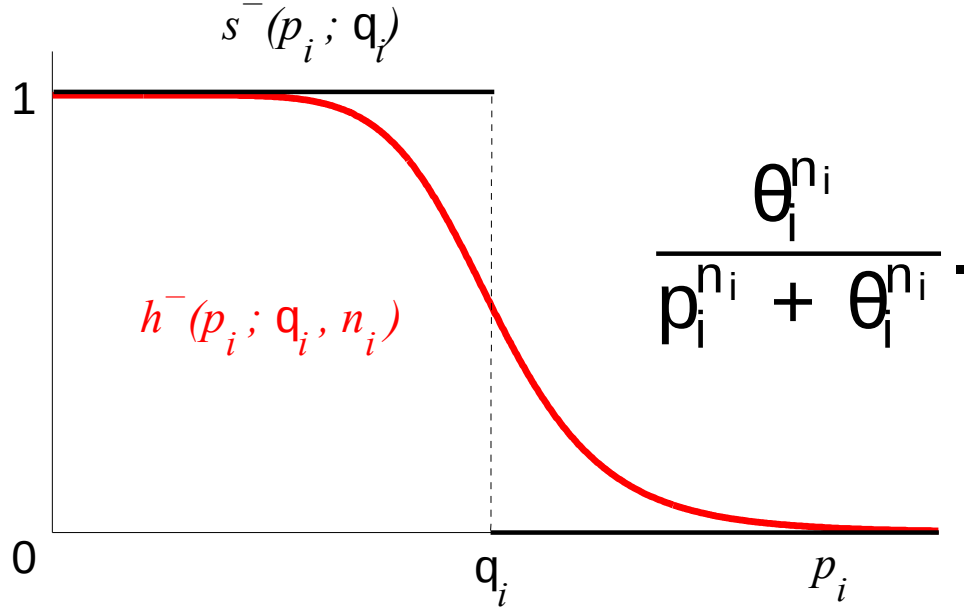


Activation and repression

- Activation and repression are described by Hill kinetics.
- These are strongly nonlinear terms taking into account saturation effects in the links between genes in a circuit.



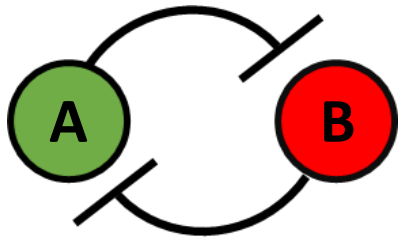
(a) Activation function



(b) Inhibition function

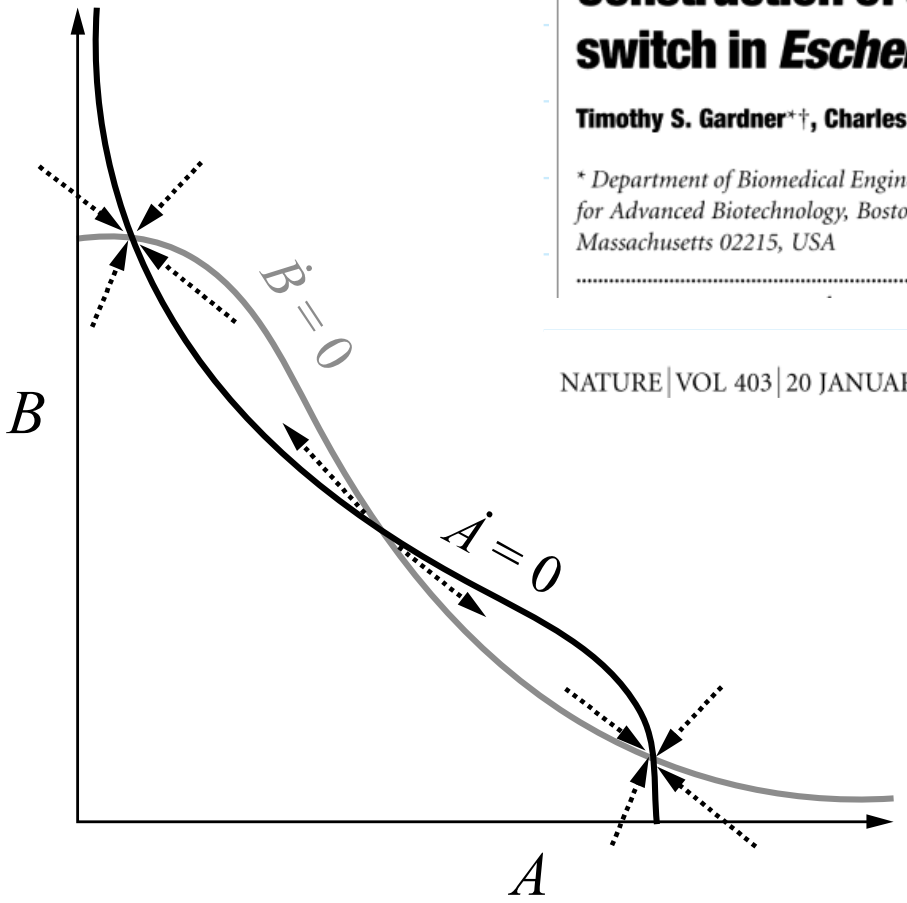
A simple example: the Genetic Toggle Switch

- A genetic toggle switch is the simplest example of synthetic memory mechanism.



$$\frac{dA}{dt} = \frac{\beta}{1+(B/K)^n} - \gamma A,$$

$$\frac{dB}{dt} = \frac{\beta}{1+(A/K)^n} - \gamma B,$$



Construction of a genetic toggle switch in *Escherichia coli*

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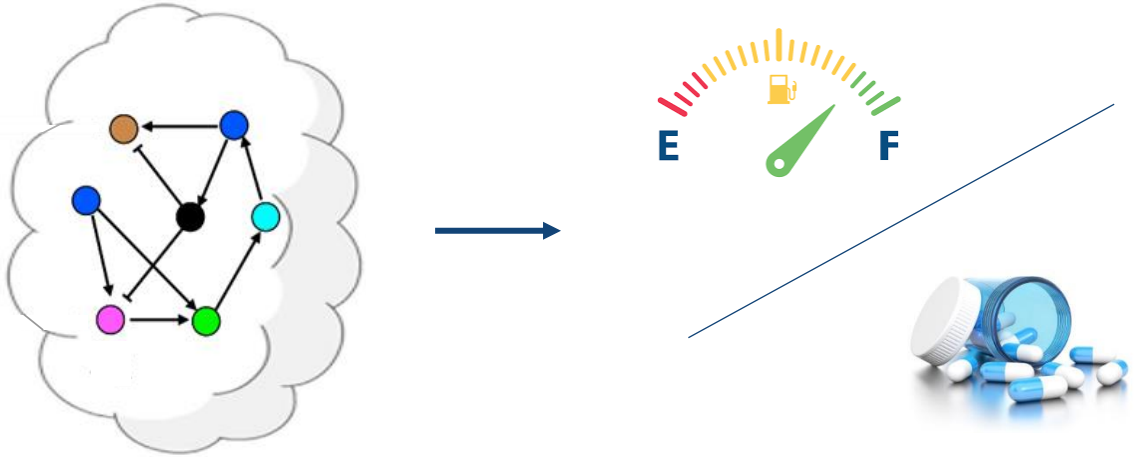
NATURE | VOL 403 | 20 JANUARY 2000 | www.nature.com

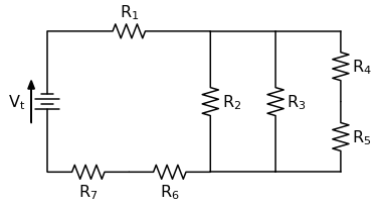
The importance of designing gene networks

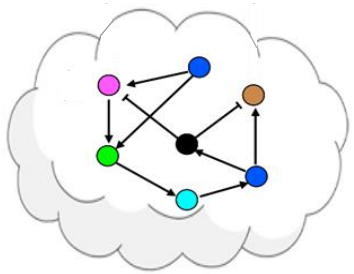
- Synthetically engineered organisms can be invaluable in many application fields, such as:
  - Growth control in bacterial populations
  - Drug or Fuel production in bioreactors
  - Personalized cell therapies

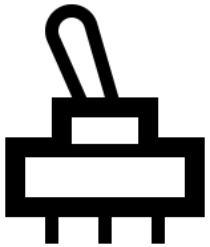


Crucial difference with Circuits

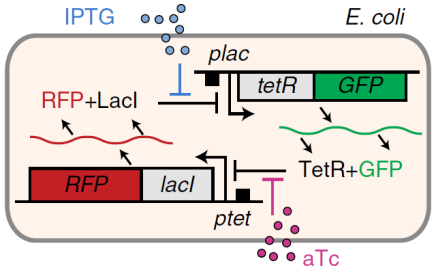
- Despite some similarities, genetic and electrical circuits are very different.
- While the response of electric circuit is often reliable and robust, their genetic counterparts are not, due to **the high levels of noise.**
- The genetic circuits are also influenced from downstream processes (**retroactivity**).

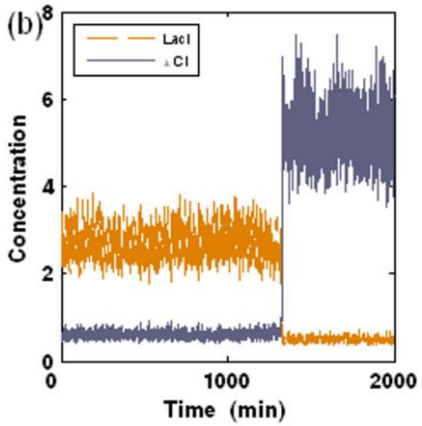


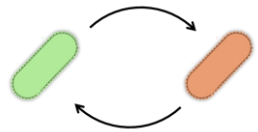




Electric toggle-switch



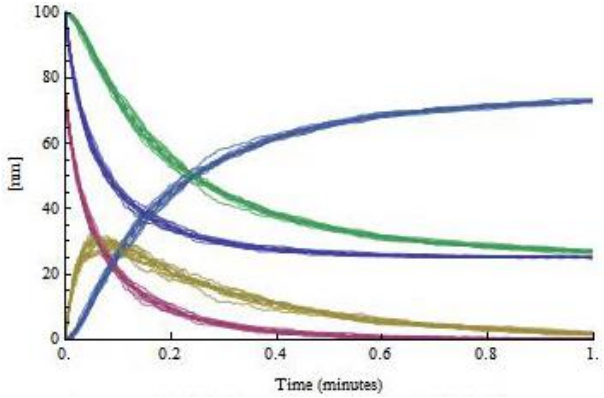


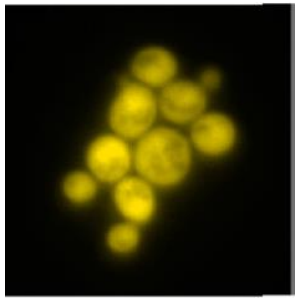


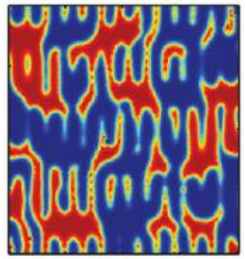
Genetic toggle-switch

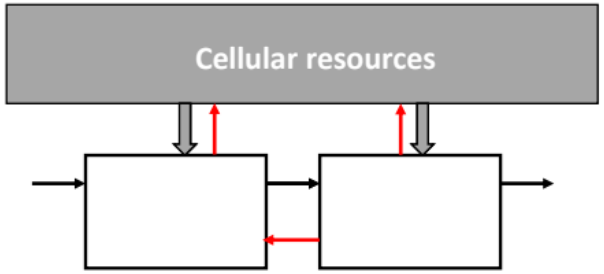
Challenging problems

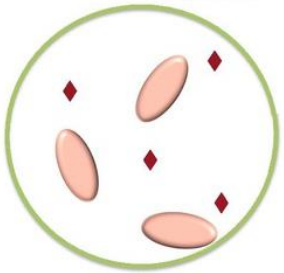
- New methodologies are required to model and control these systems due to additional problems, such as:
  - Context-dependence
  - Stochastic effects
  - Cell-to-cell variability
  - Spatially distributed dynamics
  - Intercellular communication
  - Isolation of modules
  - Cellular growth, division, mutation





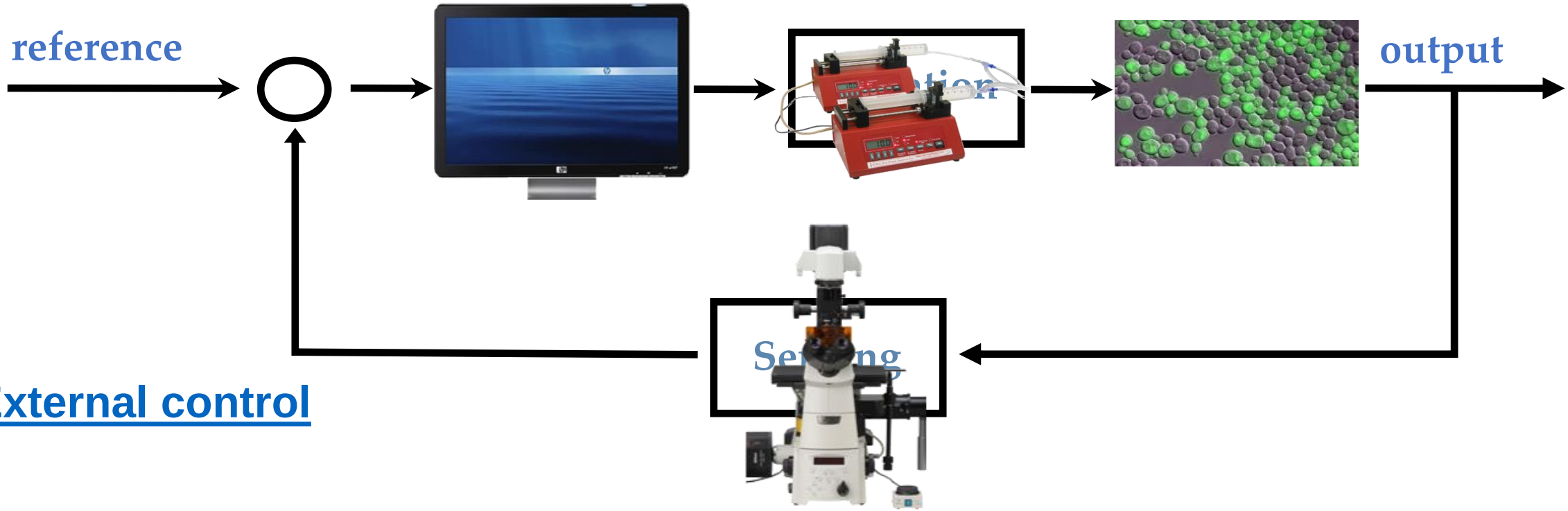






The need for feedback

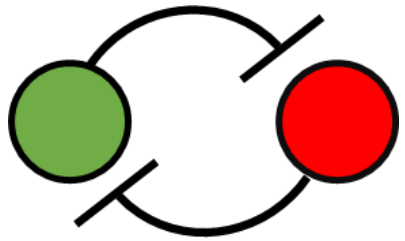
- These features of genetic circuits strongly indicate that to make synthetic biological devices operate correctly one needs to introduce FEEDBACK.
- Feedback control can be used to manipulate in real-time the behaviour of living cells.



[1] Milias-Argeitis et al., "*In silico feedback for in vivo regulation of a gene expression circuit*", Nature (2011)
[2] Menolascina et al. "*In-vivo real-time control of protein expression from endogenous and synthetic gene networks*", PLoS Comp Bio (2015)

External control of the genetic toggle-switch

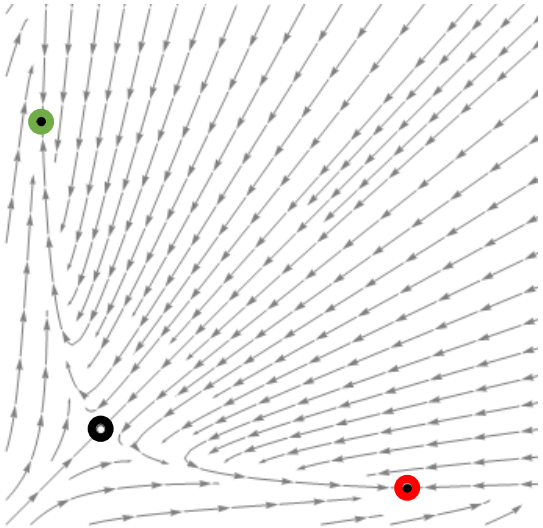
- An example of application of the external control in synthetic biology is the control of a toggle-switch on its unstable equilibrium.

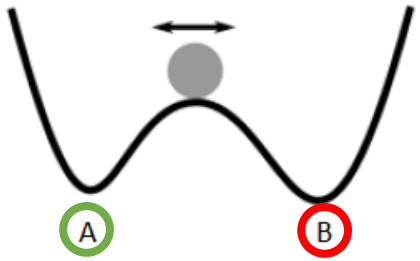


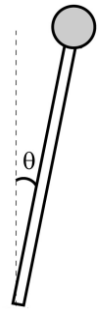
- The toggle-switch [1] is a bistable dynamical system:

$$\dot{x} = \kappa_1^0 + \frac{\kappa_1}{1 + \left(\frac{y}{\theta_y}\right)^2 \cdot u_x} - \gamma\, x$$

$$\dot{y} = \kappa_2^0 + \frac{\kappa_2}{1 + \left(\frac{x}{\theta_x}\right)^2 \cdot u_y} - \gamma\, y$$





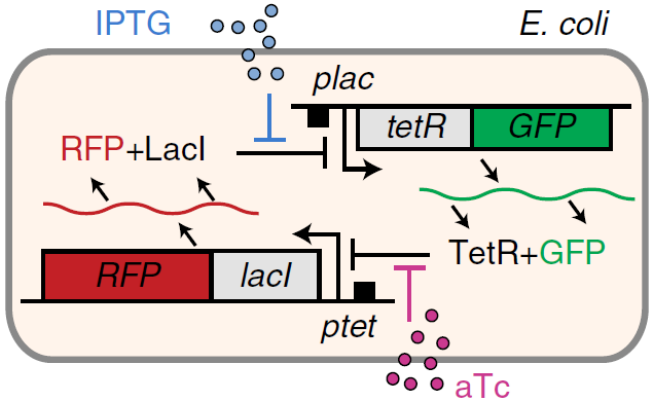


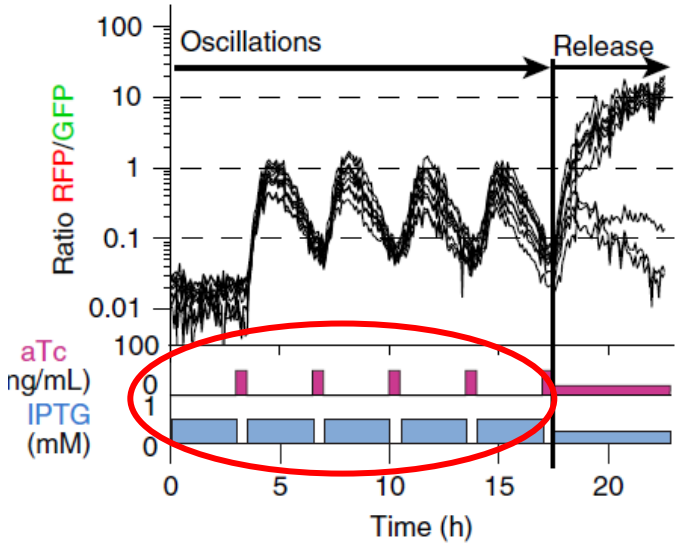
It works as a “reversible memory”

[1] Gardner, Cantor, Collins – “*Construction of a genetic toggle switch in Escherichia coli*”, Nature (2000)

External control of the genetic toggle-switch

- Various strategies were proposed [1], the most relevant was an open-loop control signal.
- However, they did not provide analytical justifications of what they observed.
- The questions that moved our initial research were:
  - Can we give an analytical description of what they observed in experiments?
  - Can we exploit this description to design more robust and reliable controllers?





[1] Lugagne et al. – "*Balancing a genetic toggle switch by real-time feedback control and periodic forcing*", Nature Communications (2017)

External control of the genetic toggle-switch

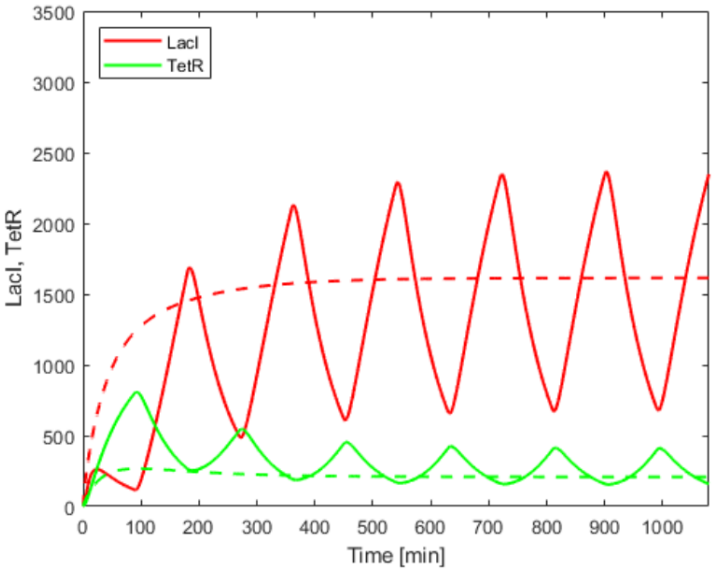
- By exploiting the timescale separation between system and inputs, we applied <u>averaging theory</u> to get the autonomous ***average system***

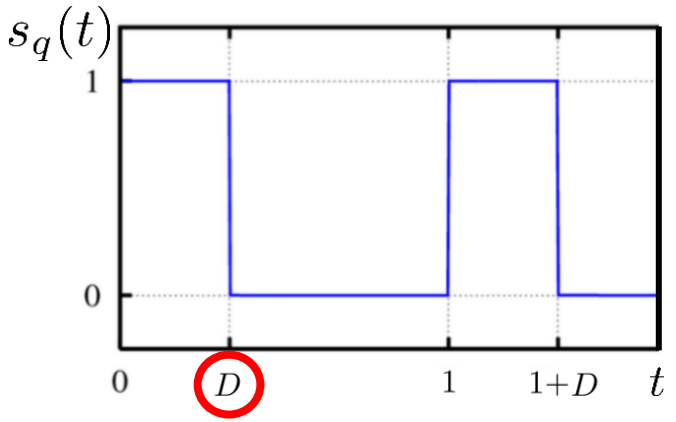
$$\dot{x} = \varepsilon \begin{bmatrix} k_1^0 + k_1 \left(\dfrac{D}{1+x_2^2} + \dfrac{1-D}{1+x_2^2 \cdot \bar{w}_{\mathrm{aTc}}} \right) - x_1 \\ k_2^0 + k_2 \left(\dfrac{D}{1+x_1^2 \cdot \bar{w}_{\mathrm{IPTG}}} + \dfrac{1-D}{1+x_1^2} \right) - x_2 \end{bmatrix}$$

where

- $\varepsilon = T/\tau_p$, and τ_p is the translation time-constant
- $D \in [0,1]$ is the duty cycle of the input PWM signals
- $\bar{w}_{\mathrm{aTc}}$ and $\bar{w}_{\mathrm{IPTG}}$ depend on the amplitudes $\bar{u}_{\mathrm{aTc}}$ and $\bar{u}_{\mathrm{IPTG}}$ of the input signals:

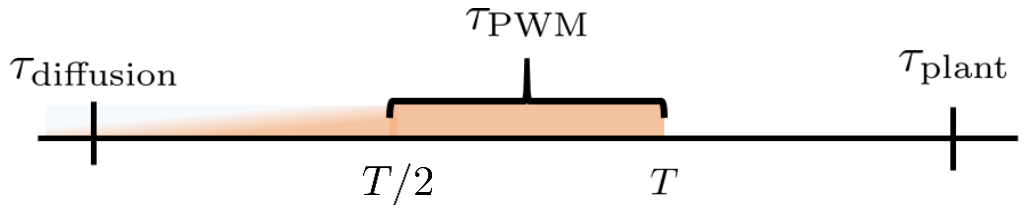
$$\begin{cases} u_{\mathrm{aTc}}(t) = \bar{u}_{\mathrm{aTc}} \cdot (1 - s_q\,(t/T)) \\ u_{\mathrm{IPTG}}(t) = \bar{u}_{\mathrm{IPTG}} \cdot s_q\,(t/T) \end{cases}$$

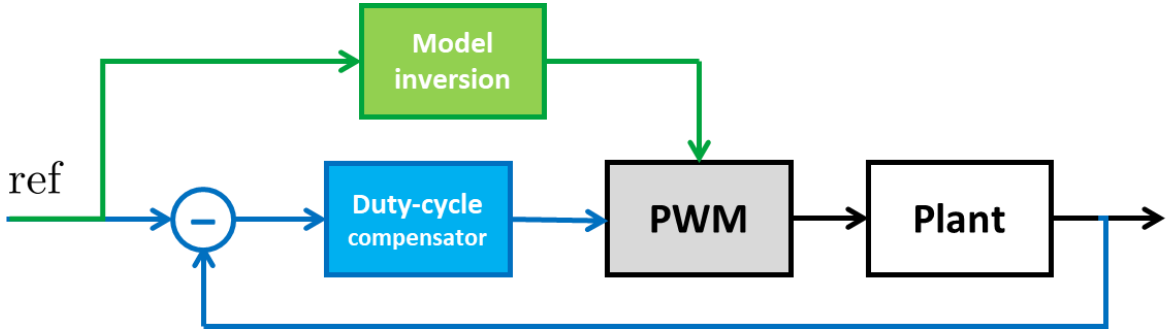


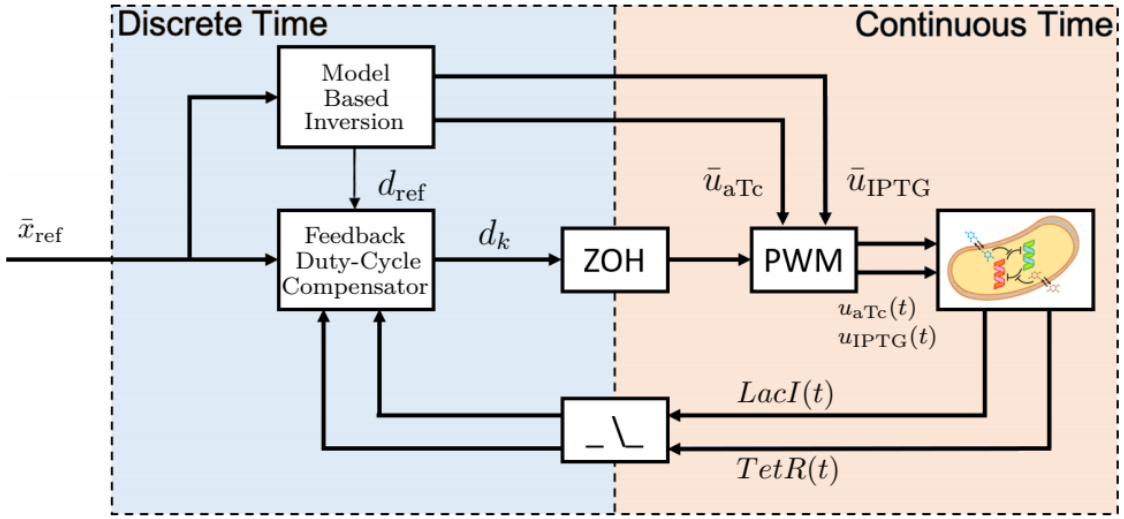


A) Research outputs

- The average model allowed us to get insights into how the parameters of the system and of the control signal must be chosen.
- We exploited these results to design **more reliable and robust** feedback controllers (PI/PWM, MPC).





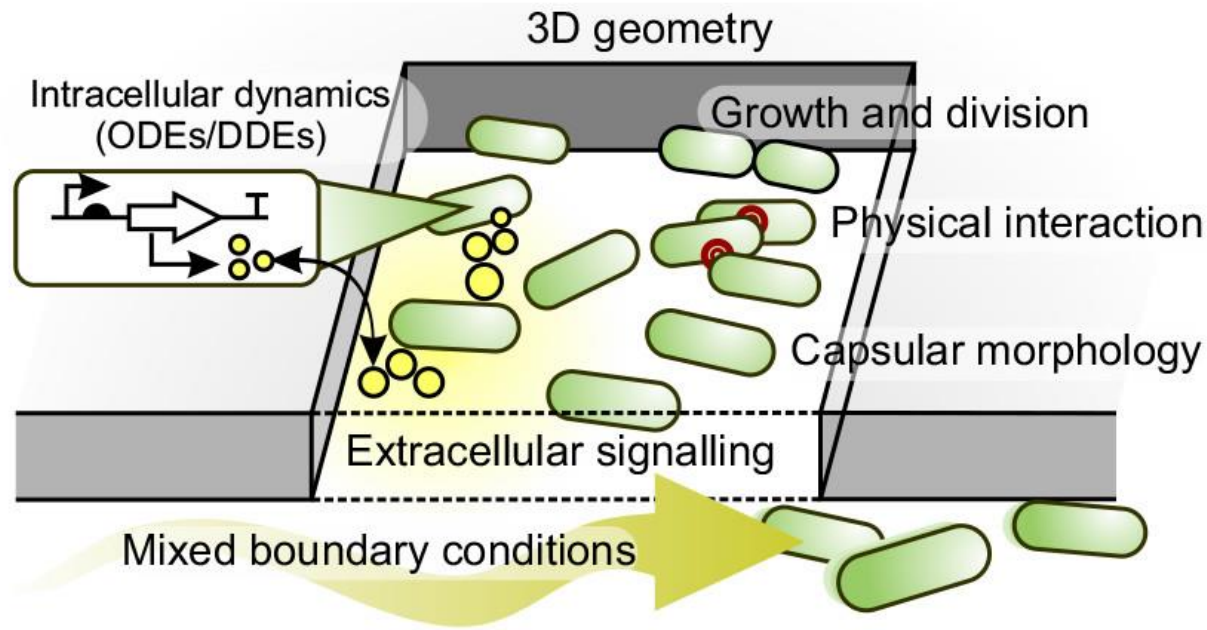


[1] Fiore et al. – "*Analysis and control of genetic toggle switches subject to periodic multi-input stimulation*", IEEE Control Systems Letters (2018)

[2] Guarino et al. – *"Balancing cell populations endowed with a synthetic toggle switch via adaptive pulsatile feedback control",* ACS Synthetic Biology (2020)

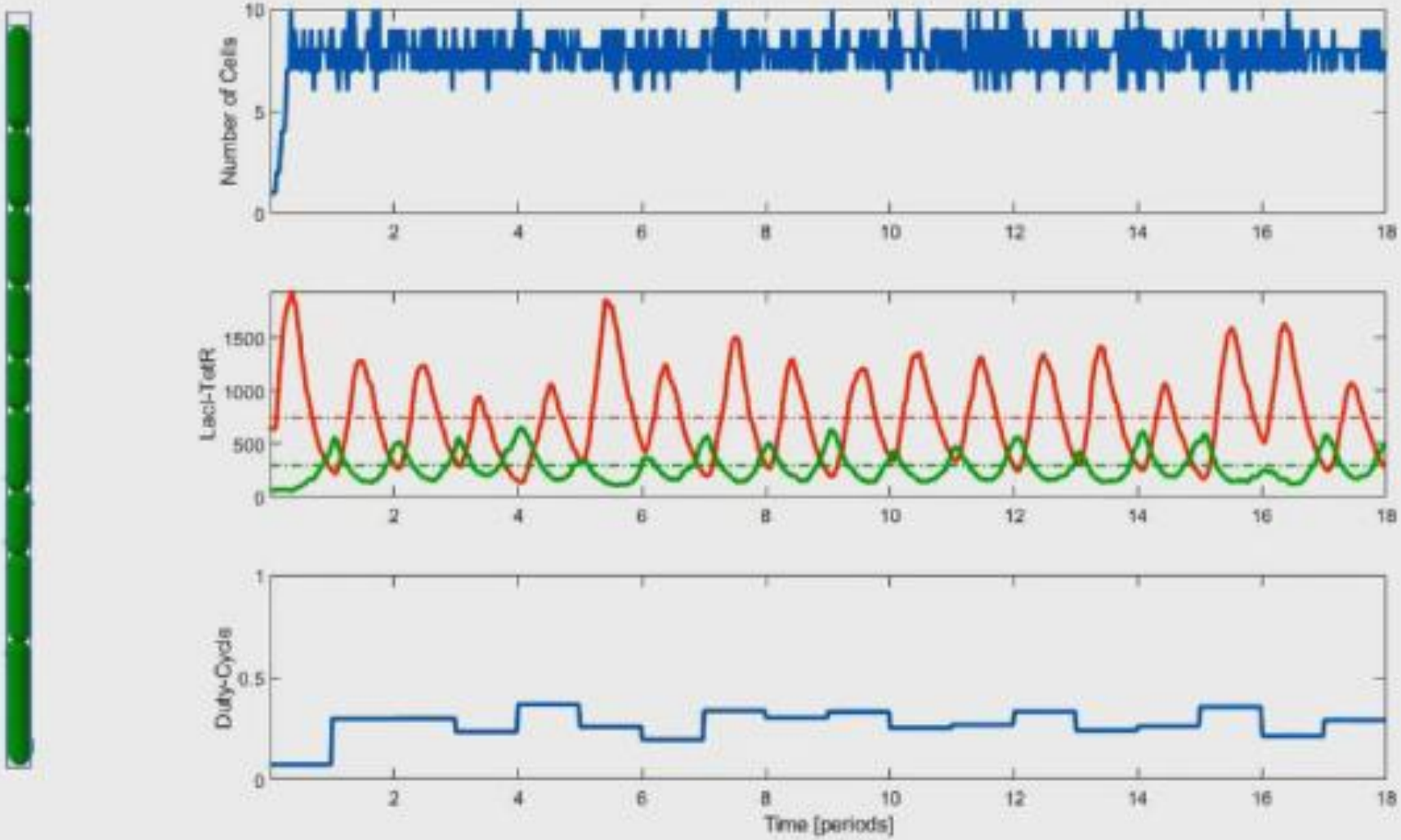
In-silico validation

- We validated their performance and robustness by means of realistic agent-based simulations in BSim.



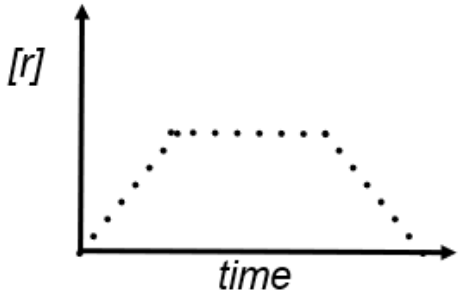
[1] A. Matyjaszkiewicz et al., "BSim 2.0: An advanced agent-based cell simulator," ACS Synth. Biol., (2017)

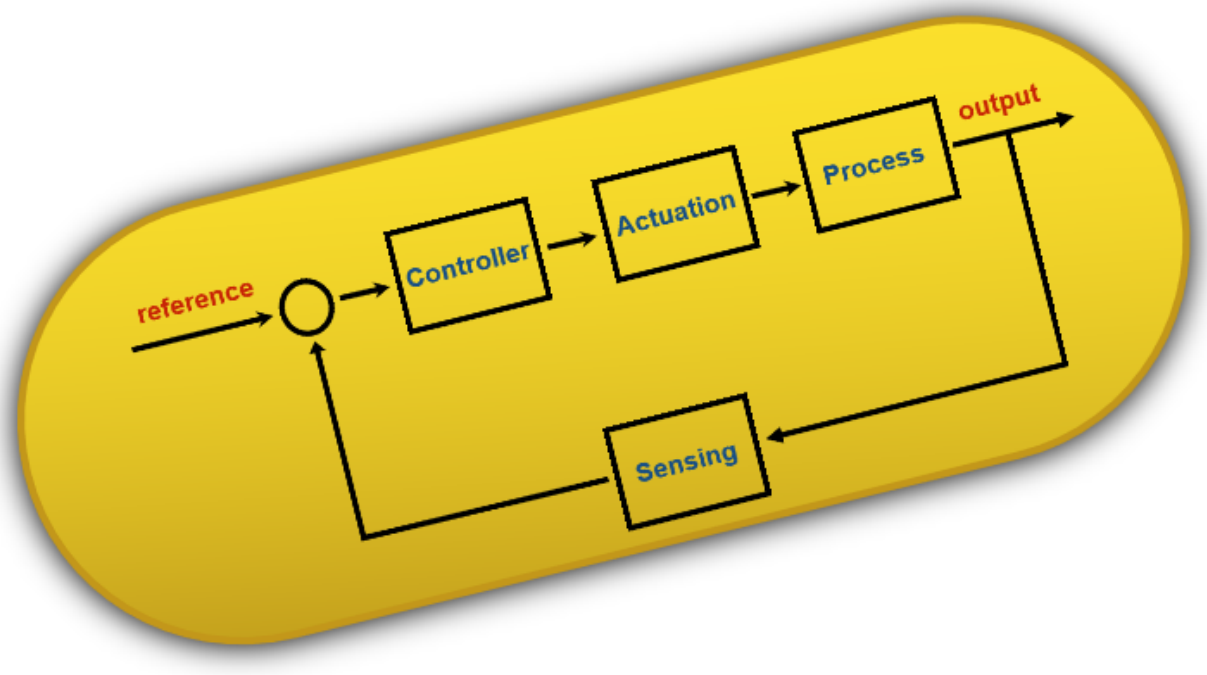
In-silico validation

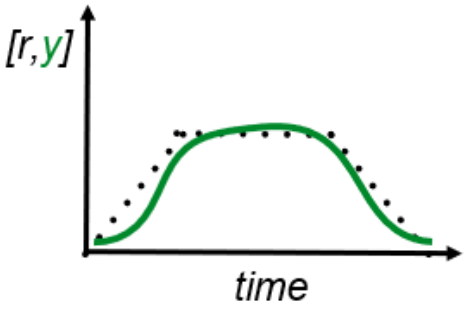


Internal cellular control

- Another approach is to embed all functions required for control via gene regulatory networks:

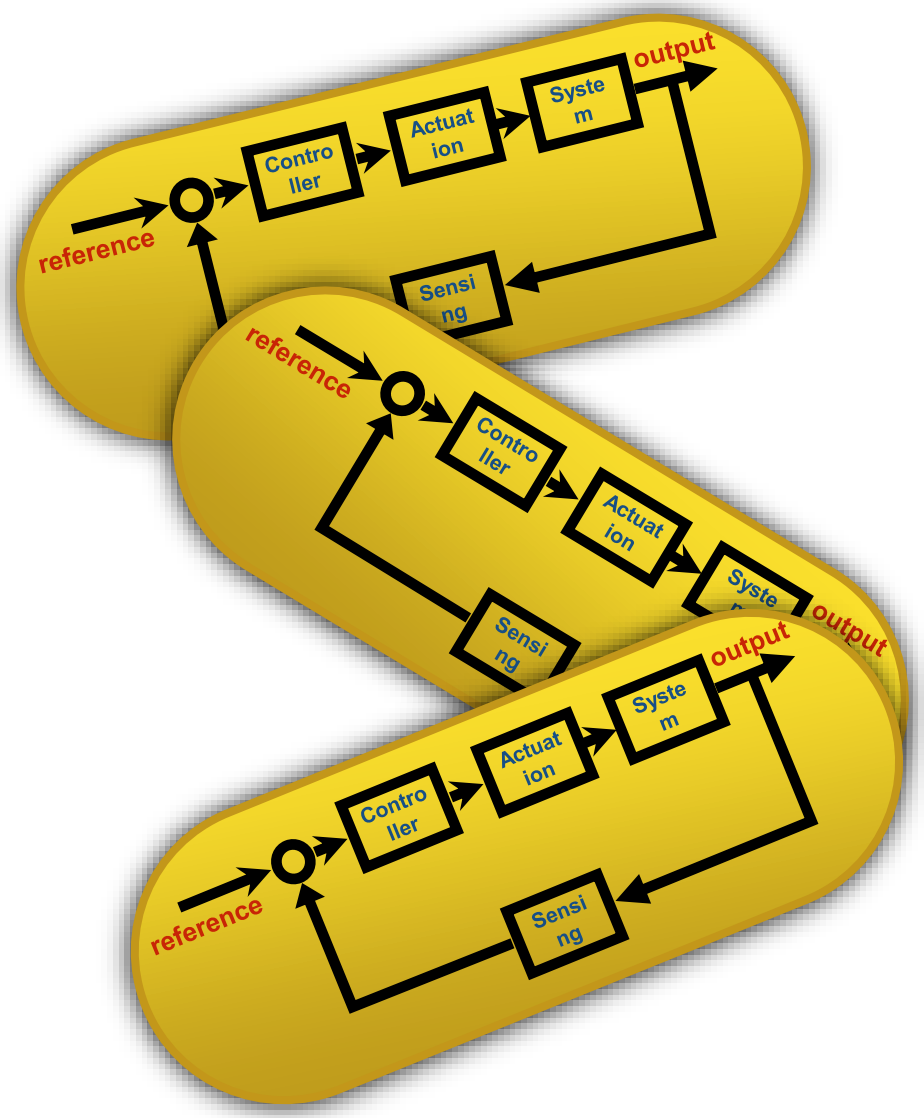






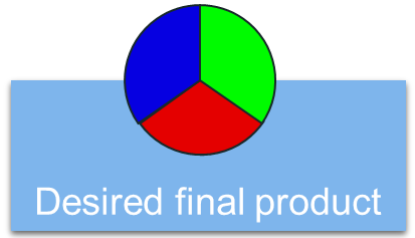
Wish list

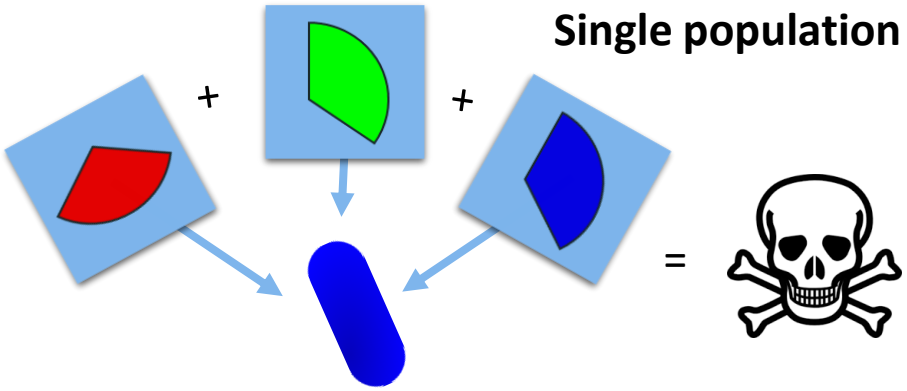
- **feasible**
  - *available parts*
  - *metabolic burden in host cells*
- **reliable**
  - *reliable dynamics*
- **robust**
  - *environmental fluctuations*
  - *intrinsic stochasticity*
- **modular**
  - *reuse of parts/modules*

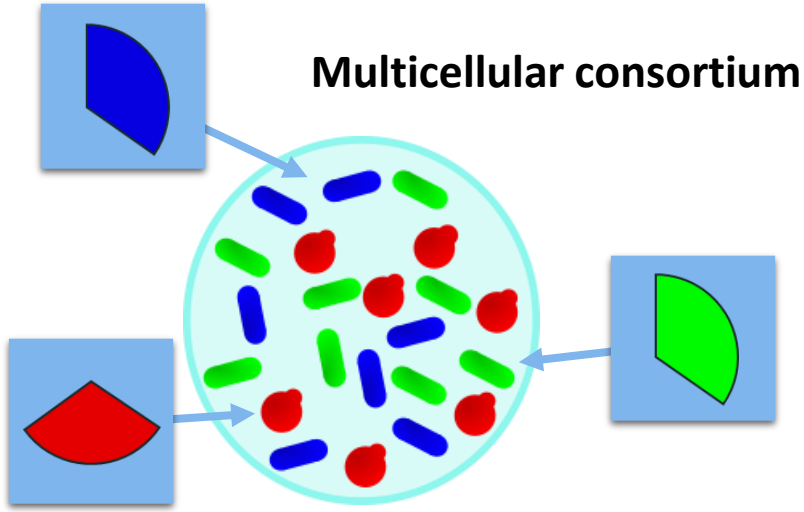


Designing synthetic Multicellular Consortia

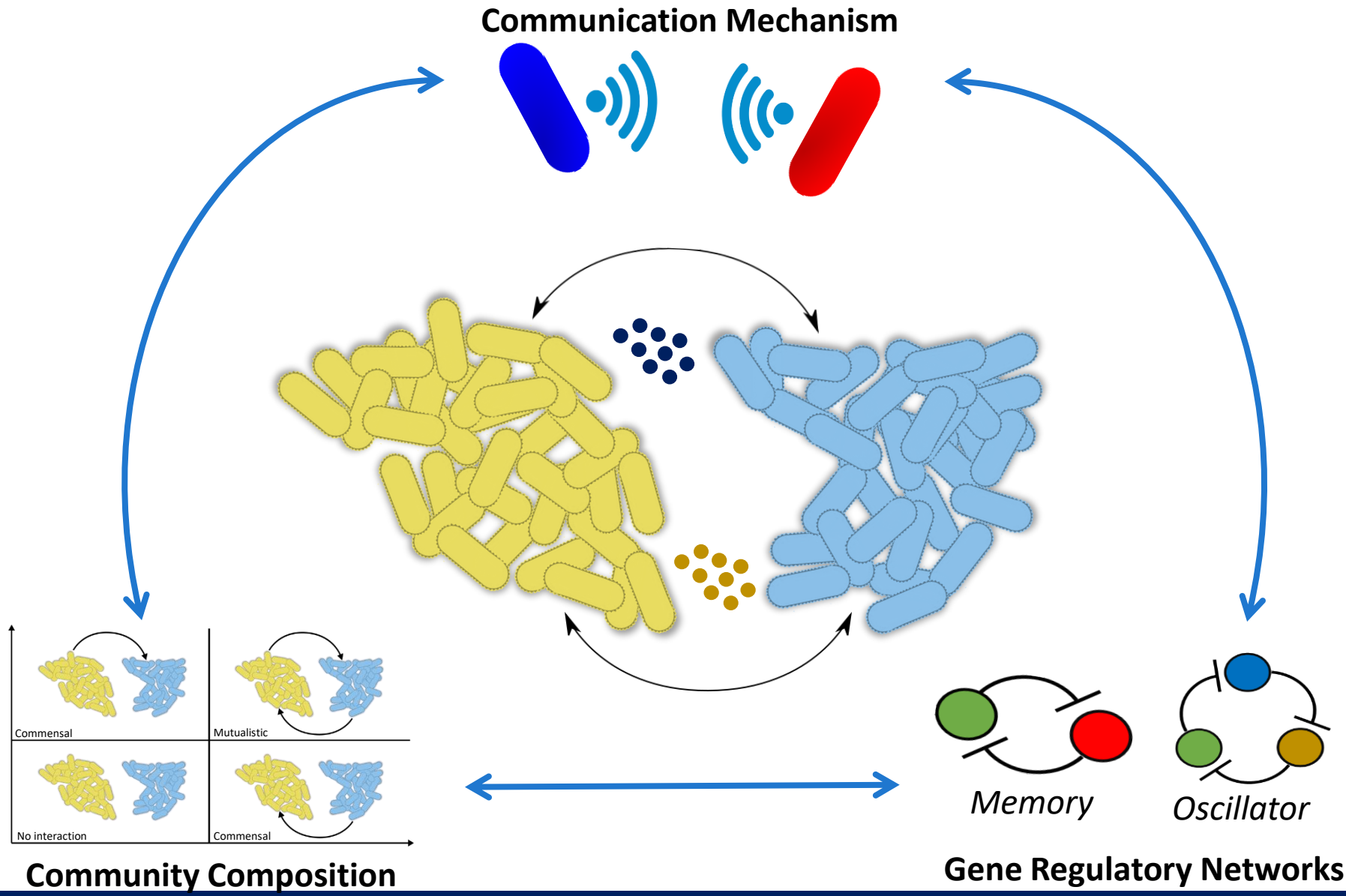
- Communities of interacting cellular populations can aid the realization of some points from the wish list.
- Assembling cellular consortia can be instrumental to achieve modularity…
- …and avoid or mitigate undesired effects, such as
  - excessive metabolic burden
  - incompatible reactions
  - competition for limited resources
  - pathways interactions







Key ingredients of synthetic consortia

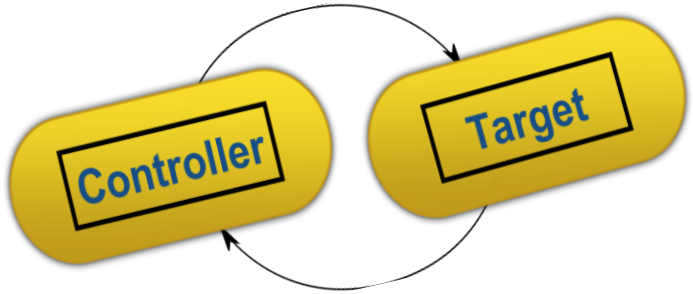


Design approach

- There are two possible paradigms:

1. **Engineered consortia** where the functions of each population are designed ad hoc off-line so that a specific desired behaviour emerges when they are mixed

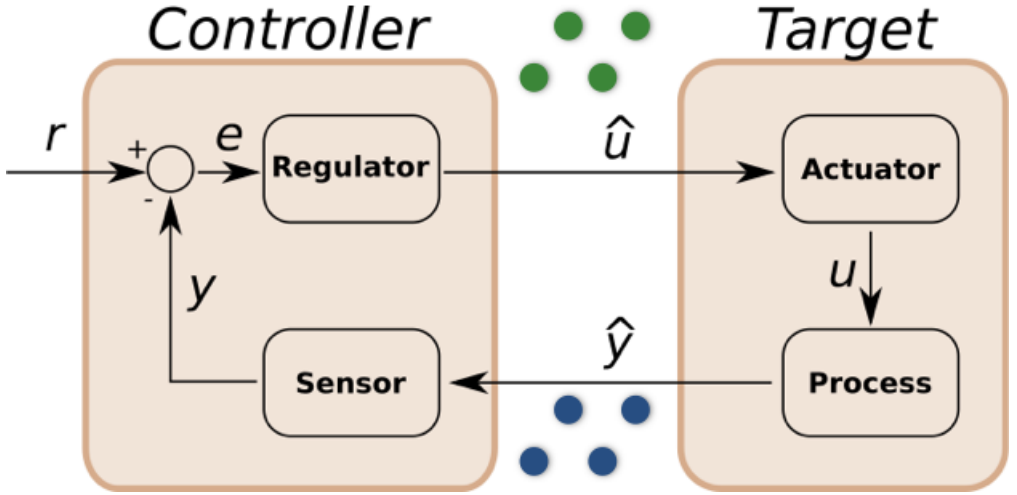
2. **Multicellular control architectures** where sensing, actuation and computation functions are split across populations and the desired behaviour can be set by varying a reference



Multicellular control

- **Goal:** Engineer a consortium of two (or more) cell populations where the *Controllers* can sense and regulate some phenotype of the *Targets*

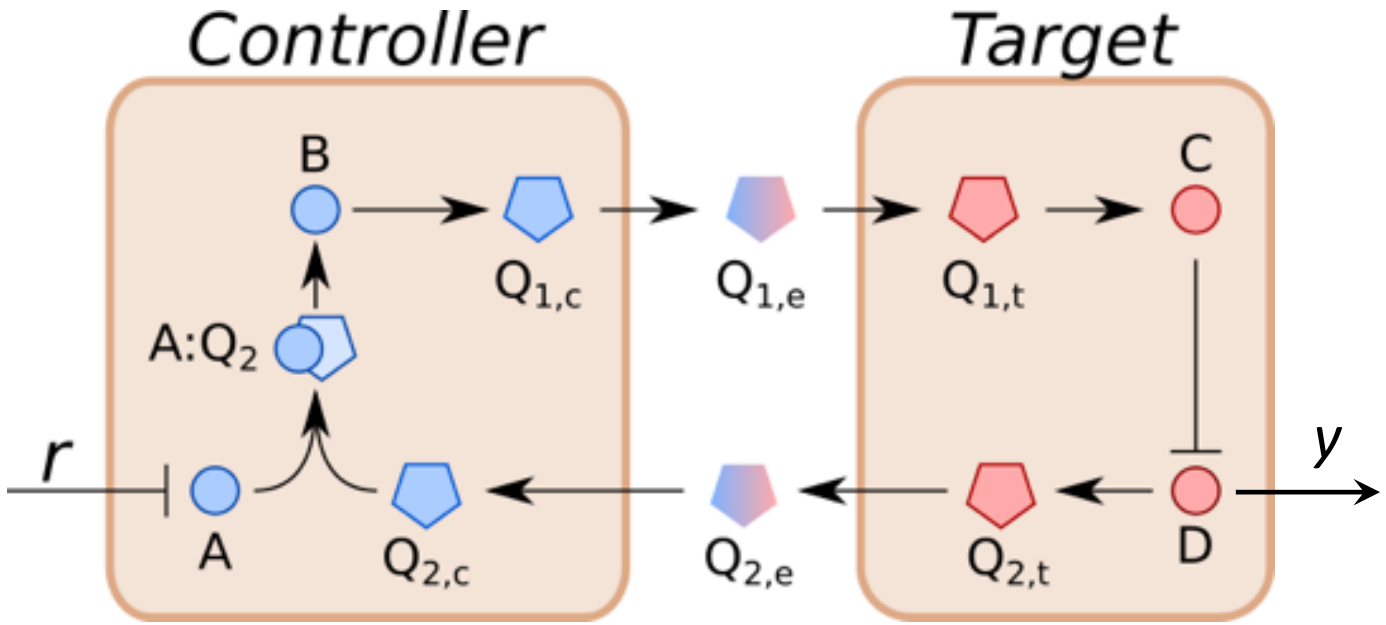
- Cooperative microbial consortia
  - Allow to achieve more advanced functionalities
  - Give additional modularity and flexibility



University of BRISTOL

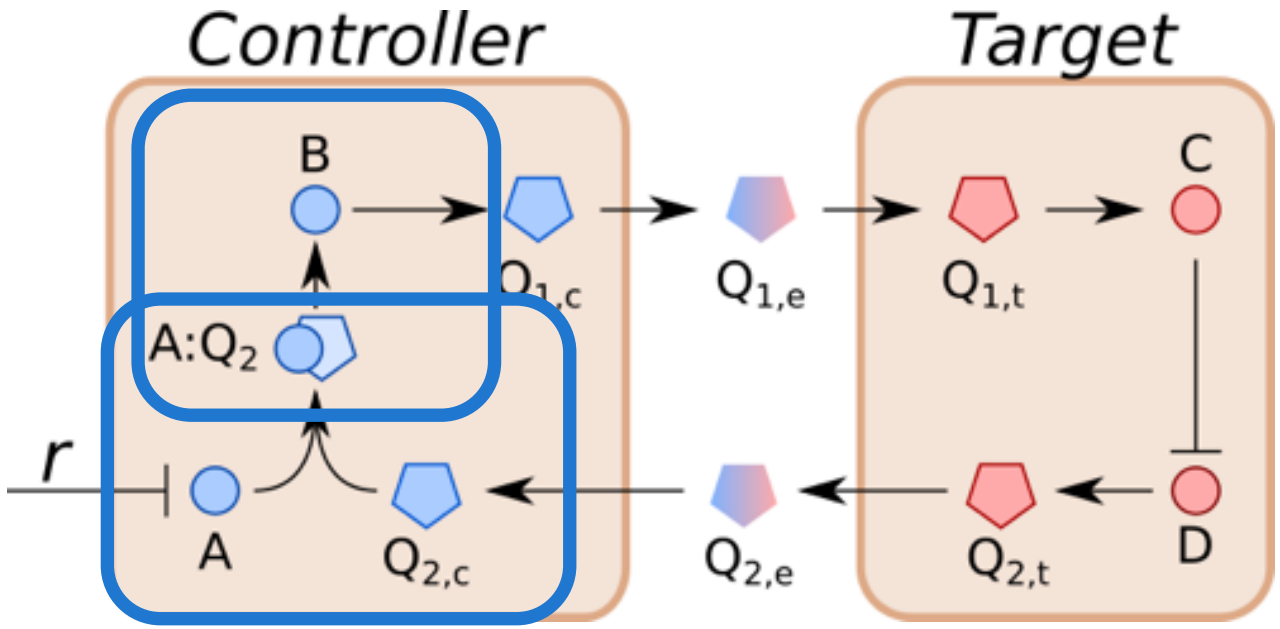
[1] G. Fiore, A. Matyjaszkiewicz, F. Annunziata, C. Grierson, N. Savery, L. Marucci, M. di Bernardo – *"In-silico analysis and implementation of a multicellular feedback control strategy in a synthetic bacterial consortium",* ACS Synthetic Biology (2017)
[2] B. Shannon, C. Zamora-Chimal, L. Postiglione, D. Salzano, C. Grierson, L. Marucci, N. Savery, M. di Bernardo – *"In vivo Feedback Control of an Antithetic Molecular-Titration Motif in Escherichia coli using Microfluidics",* ACS Synthetic Biology (2020)

Muticellular Control strategy



1. A reference signal is compared with a signal *Q*2 *from* the targets
2. The computed error generates via *B* a signal *Q*1 *for* the targets
3. The control input is then delivered to the target cell
4. The input via C represses the target gene D
5. *D* generates the signal *Q*2 that is sent to the controller cells

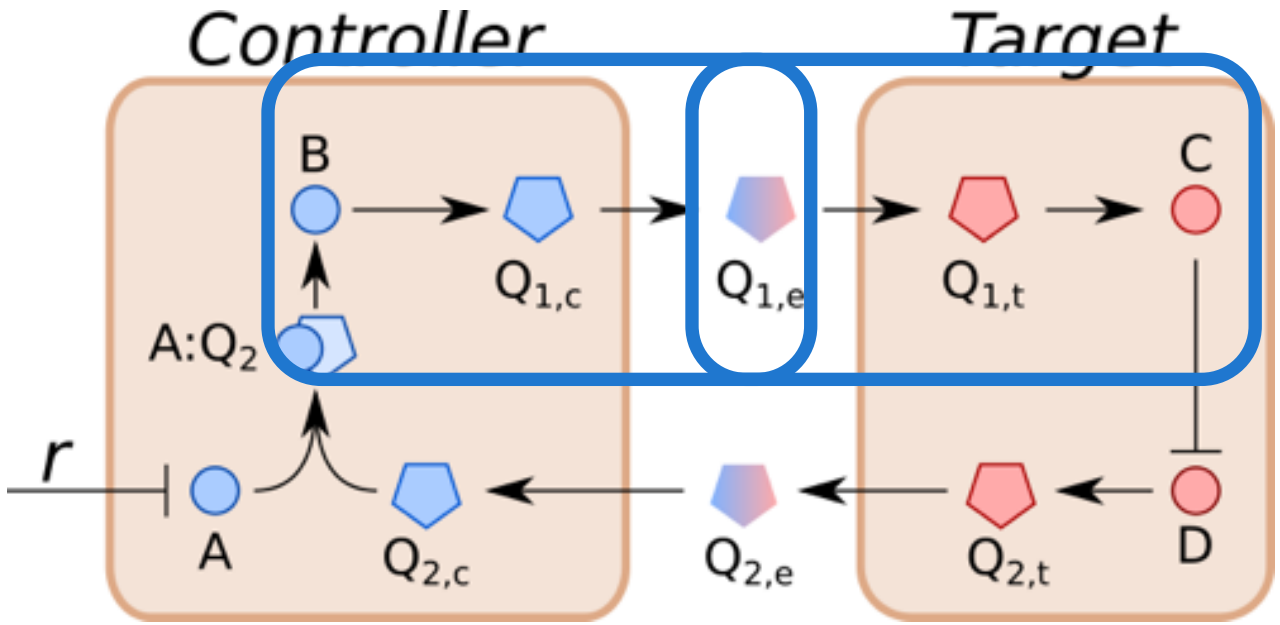
Modelling of Controllers



$$\frac{d[A:Q_2]}{dt} = \left(\chi_{A:Q,r,0} + \chi_{A:Q,r}\frac{K_r^{n_r}}{K_r^{n_r} + [r]^{n_r}}\right) \cdot \left(\chi_{A:Q,a,0} + \chi_{A:Q,a}\frac{[Q_{2,c}]_r^{n_r}}{K_q^{n_r} + [Q_{2,c}]^{n_r}}\right) - \gamma_{A:Q}[A:Q_2]$$

$$\frac{d[B]}{dt} = \chi_{B,0} + \chi_B\frac{[A:Q_{2,c}]^{n_r}}{K_r^{n_r} + [A:Q_{2,c}]^{n_r}} - \gamma_B[B]$$

Modelling the Signalling To Targets

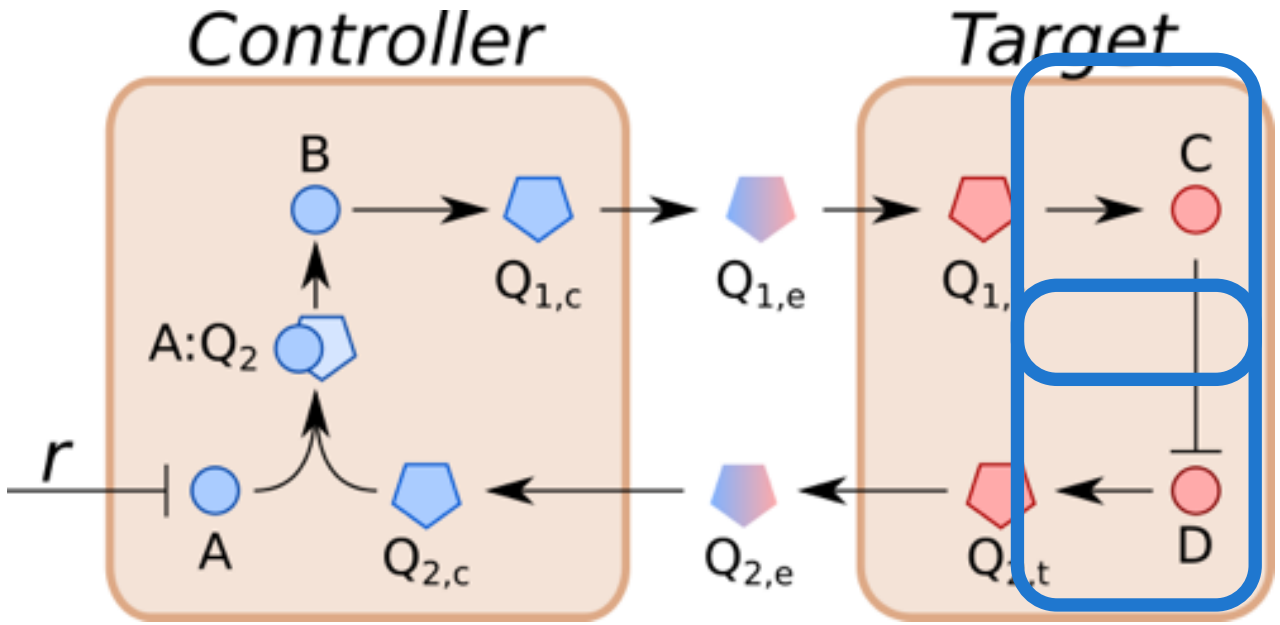


$$\frac{d[Q_{1,c}]}{dt} = K_{Q_1}[B] + \eta([Q_{1,e}] - [Q_{1,c}]) - \gamma_i[Q_{1,c}]$$

$$\frac{\partial[Q_{1,e}]}{\partial t} = \eta([Q_{1,c}] - [Q_{1,e}]) + \eta([Q_{1,t}] - [Q_{1,e}]) - \gamma_e[Q_{1,e}] + \Theta\nabla^2[Q_{1,e}]$$

$$\frac{d[Q_{1,t}]}{dt} = \eta([Q_{1,e}] - [Q_{1,t}]) - \gamma_i[Q_{1,t}]$$

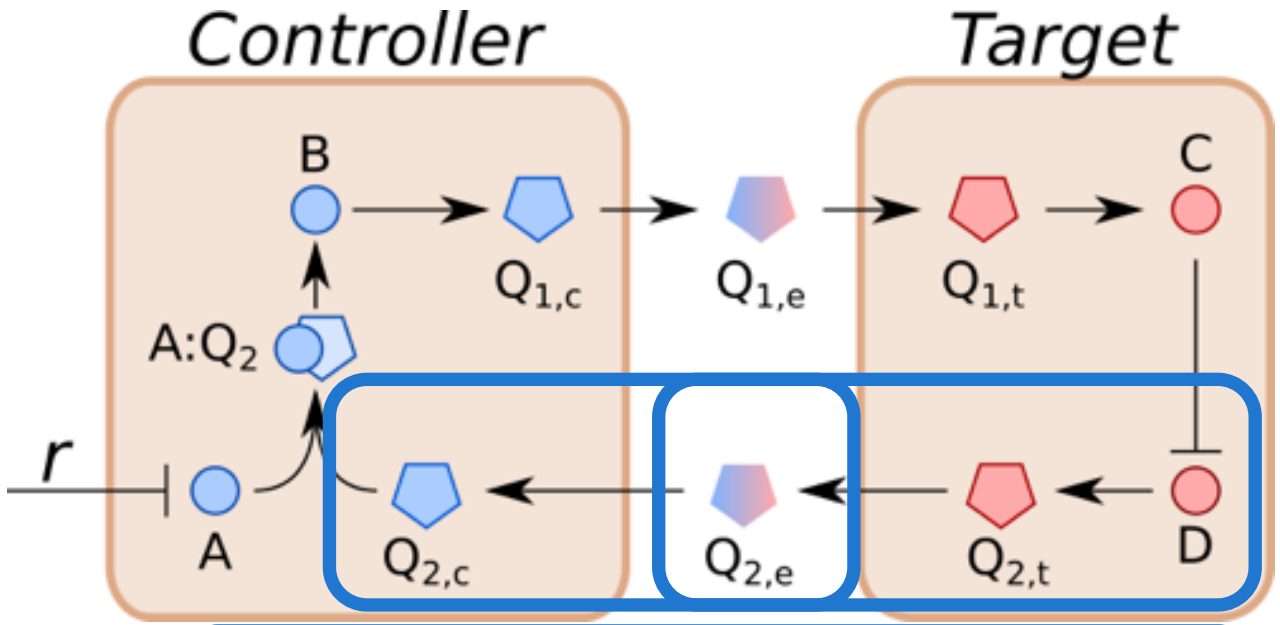
Modelling of Target cells



$$\frac{d[C]}{dt} = \chi_{c,0} + \chi_c \frac{[Q_{1,t}]^{n_c}}{K_c{}^{n_c} + [Q_{1,t}]^{n_c}} - \gamma_C[C]$$

$$\frac{d[D]}{dt} = \chi_{d,0} + \chi_d \frac{K_d{}^{n_d}}{K_d{}^{n_d} + [C]^{n_d}} - \gamma_D[D]$$

Modelling the Signalling To Controllers

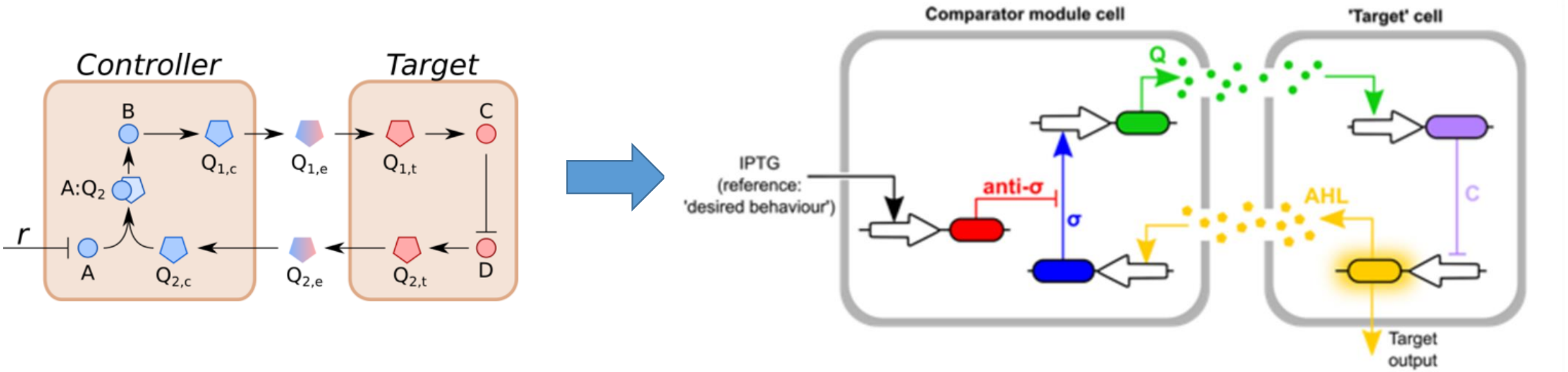


$$\frac{d[Q_{2,t}]}{dt} = K_{Q_2}[D] + \eta([Q_{2,e}] - [Q_{2,t}]) - \gamma_i[Q_{2,t}]$$

$$\frac{\partial[Q_{2,e}]}{\partial t} = \eta([Q_{2,c}] - [Q_{2,e}]) + \eta([Q_{2,t}] - [Q_{2,e}]) - \gamma_e[Q_{2,e}] + \Theta\nabla^2[Q_{2,e}]$$

$$\frac{d[Q_{2,c}]}{dt} = \eta([Q_{2,e}] - [Q_{2,c}]) - \gamma_i[Q_{2,c}]$$

Testing multicellular control *in-vivo*



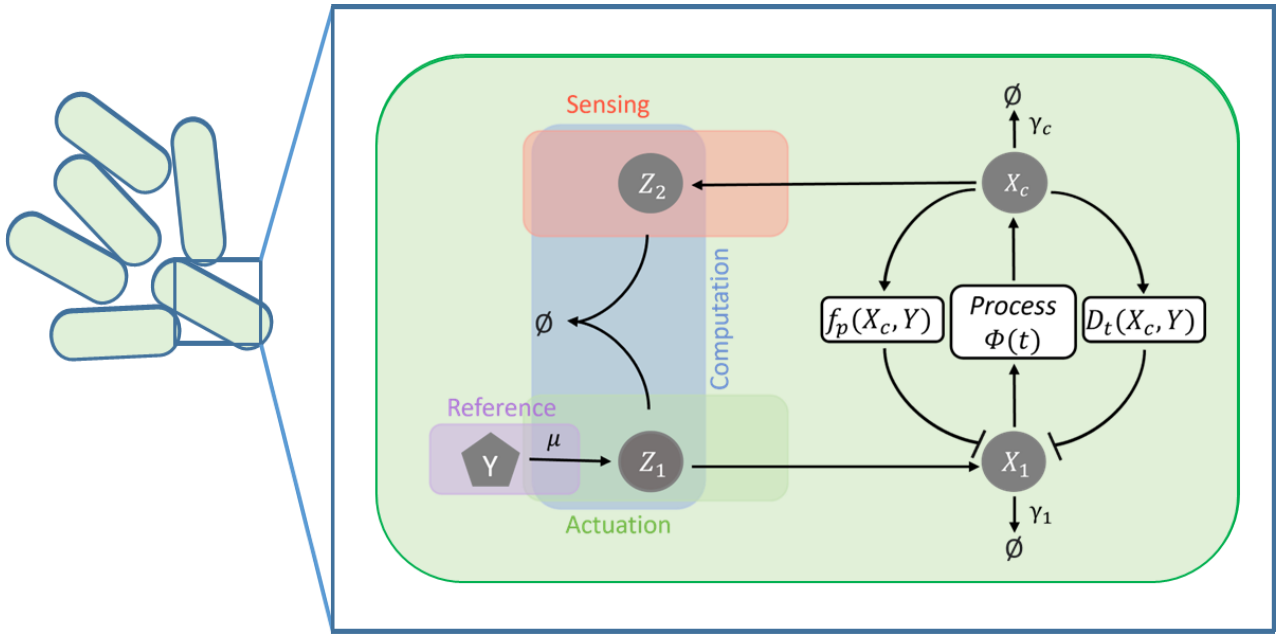
- The scheme has been recently validated through in vivo experiments at the University of Bristol (and a paper will soon be out).
- It is essential to mantain **stable coexistence** between cell populations (hard to keep also in controlled experiments).

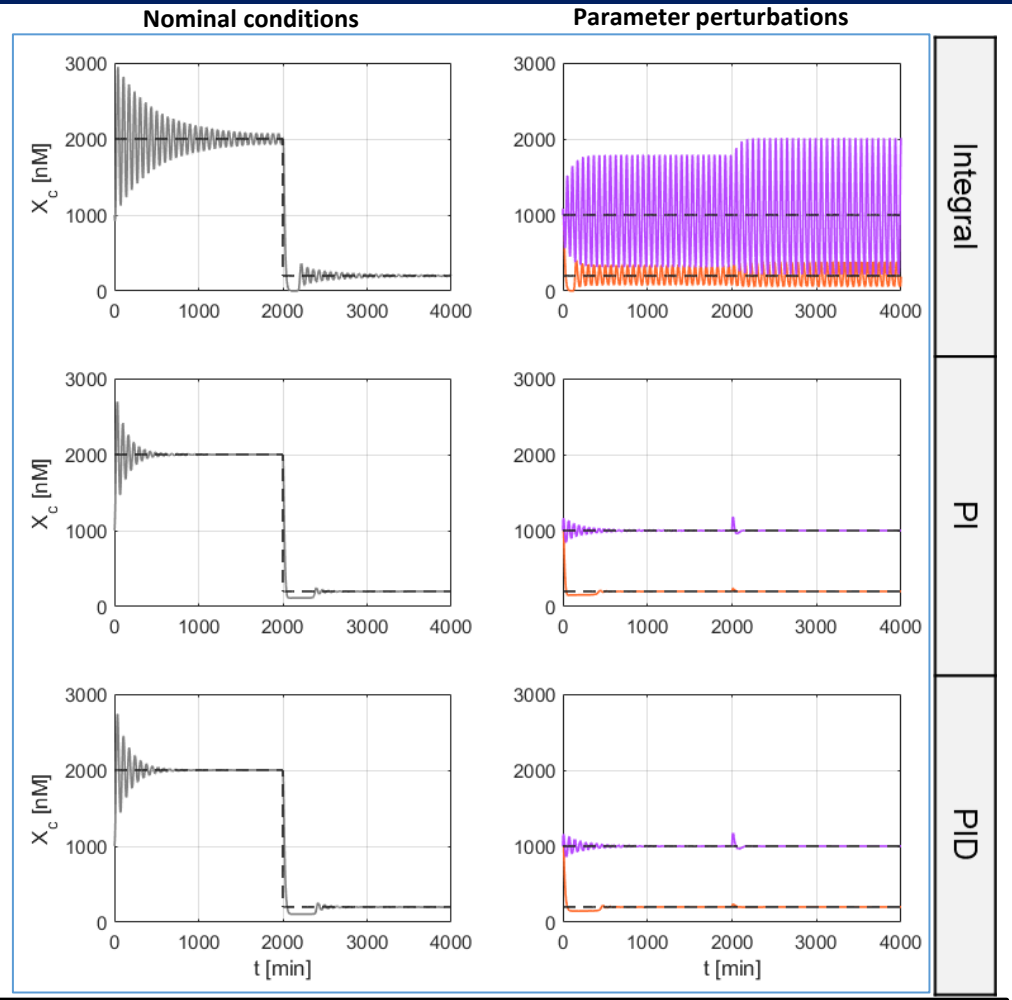
[1] F. Annunziata et al. – "*An orthogonal multi-input integration system to control gene expression in Escherichia coli*", ACS Synthetic Biol. (2017)

[2] N. Kylilis et al. – "*Tools for engineering coordinated system behaviour in synthetic microbial consortia*", Nature communications (2018)

Embedded PID controller

- Recently it has been proposed a genetic circuit implementing an integral action guaranteeing perfect robust adaptation.

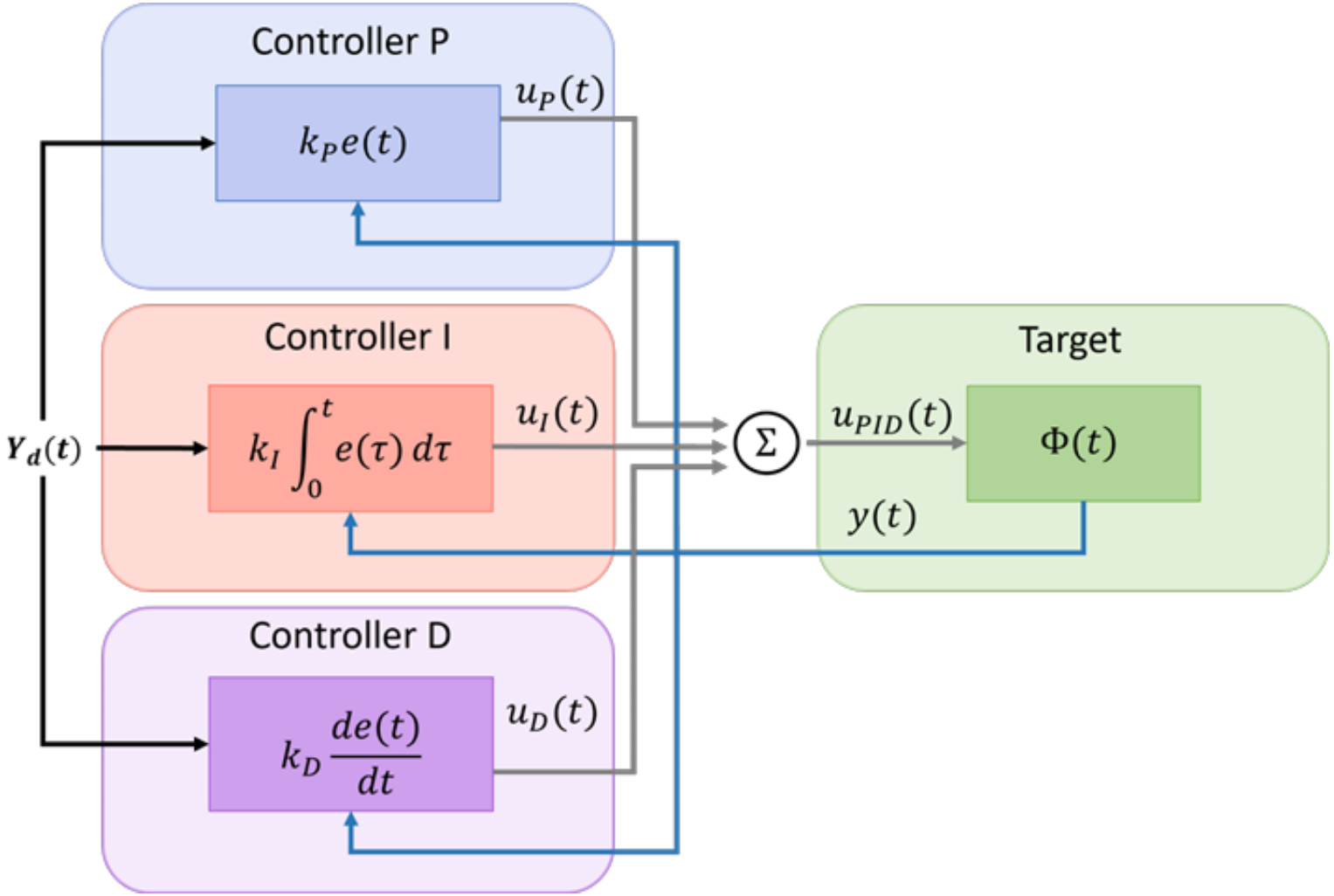




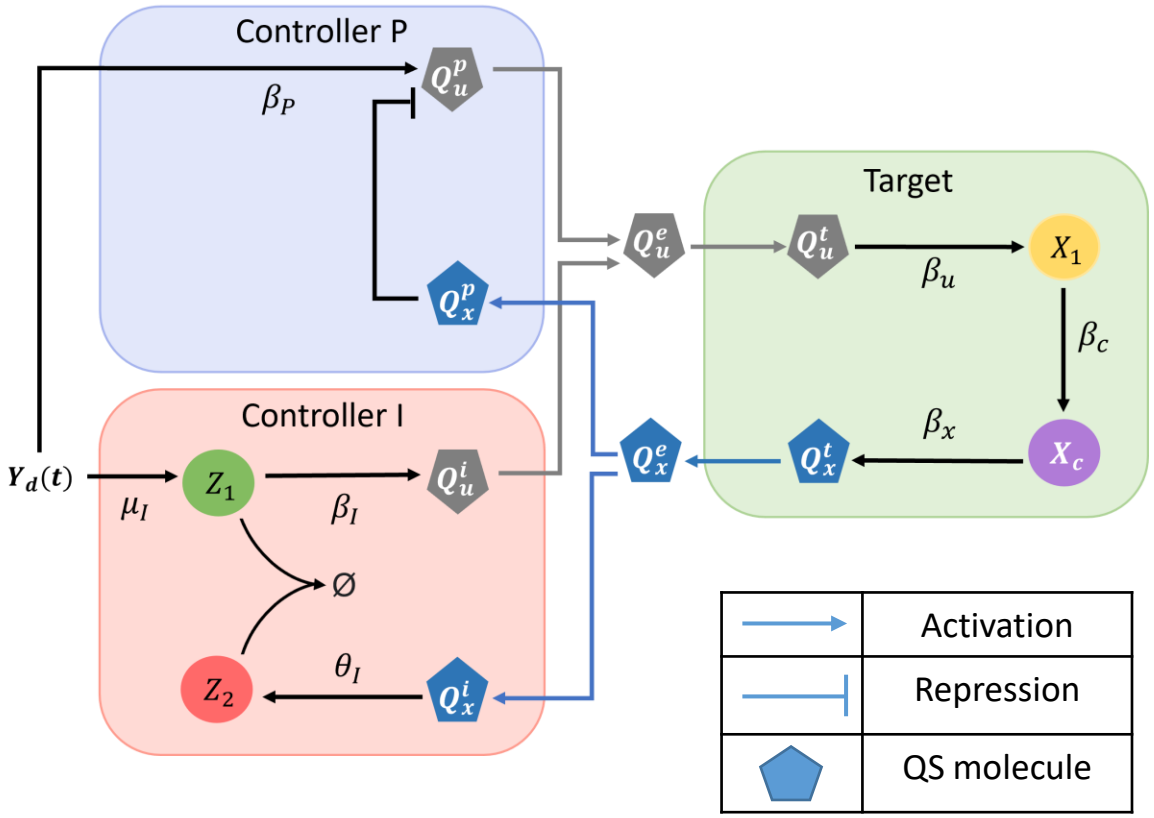
The addition of the Proportional and Derivative actions improves the system robustness and performance.

[1] C. Briat, A. Gupta, and M. Khammash, *"Antithetic integral feedback ensures robust perfect adaptation in noisy biomolecular networks,"* Cell Systems (2016)
[2] M. Chevalier, M. Gómez-Schiavon, A. H. Ng, and H. El-Samad, *"Design and analysis of a proportional-integral-derivative controller with biological molecules,"* Cell Systems (2019)

Multicellular PID Controller



Multicellular PI Controller



$Q_{u,0}^p = \beta_P Y_d \frac{\mu Y_d}{\mu Y_d + \theta Q_x^p}$	$Q_{u,0}^i = \beta_I Z_1$	$Q_{x,0}^t = \beta_x X_c$

Target process dynamics:

Control action

$$\dot{X}_1 = -\gamma_1 X_1 + \beta_u Q_u^t$$

$$\dot{X}_c = \beta_c X_1 - \gamma_c X_c$$

Integral population dynamics:

$$\dot{Z}_1 = \mu Y_d - \gamma_z Z_1 Z_2$$

$$\dot{Z}_2 = \theta Q_x^i - \gamma_z Z_1 Z_2$$

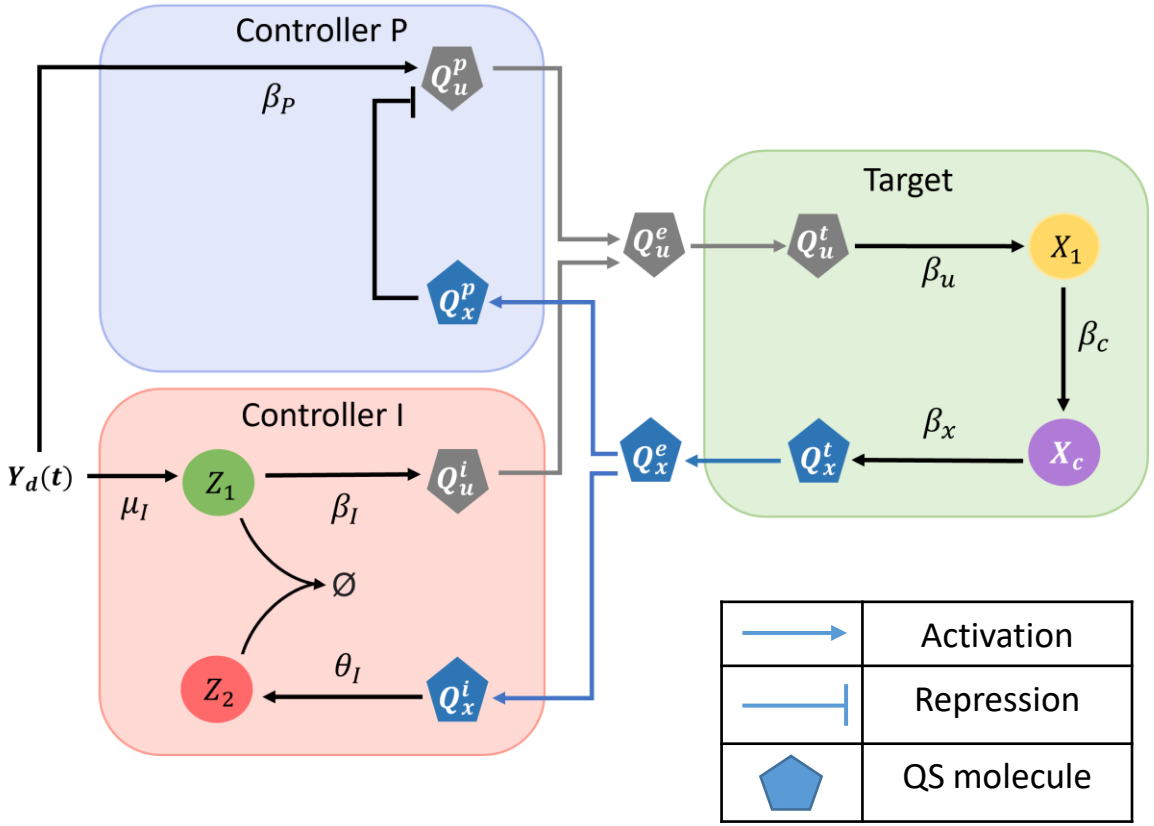
Dynamics of the QS:

$$\dot{Q}_k^j = Q_{k,0}^j + \eta\left(Q_k^e - Q_k^j\right) - \gamma_j Q_k^j$$

$$\dot{Q}_k^e = M\eta \sum_{j \in S} \left(Q_k^j - Q_k^e\right) - \gamma_e Q_k^e$$

$$j \in S, \qquad S = \{p, i, t\}, \qquad k \in \{u, x\}$$

Multicellular PI Controller: circuit design



- Under some mild assumptions the system has **a non-negative** equilibrium point if:

$$\beta_P^* \leq \frac{8\mu\gamma^4}{\beta_c\beta_x\beta_u\theta}$$

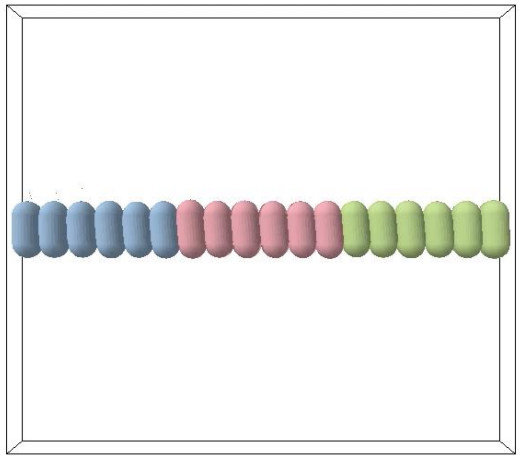
- The equilibrium point is **locally asymptotically stable** if the value of the Integral gain β_I does not exceed a threshold:

$$\beta_I < \frac{\beta_P\gamma}{2\mu} + \frac{18\gamma^5}{\beta_c\beta_x\beta_u\theta}$$

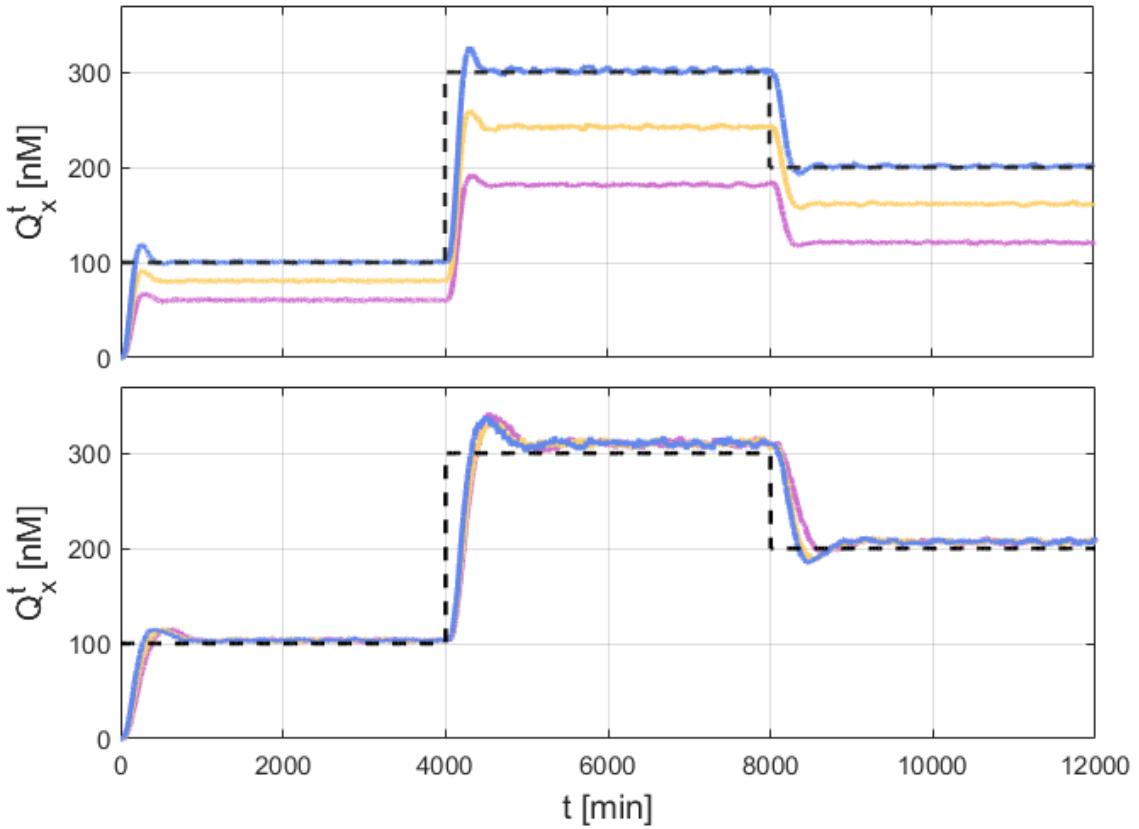
- The range of β_I can be widen:
  - Choosing fast dividing cells
  - Reducing of the strength of the promoters

BSim simulations: nominal conditions

Numerical simulations are performed in Bsim to take into account cells growth and division, and the diffusion of quorum sensing molecules.



	Target
	Controller P
	Controller I



PROPORTIONAL

$\beta_P = 0.0414$

$\beta_P = 0.0300$

$\beta_P = 0.0200$

PI

$\beta_P = 0.0414, \beta_I = 0.0002$

$\beta_P = 0.0300, \beta_I = 0.0002$

$\beta_P = 0.0200, \beta_I = 0.0002$

[1] A. Matyjaszkiewicz et al., "BSim 2.0: An advanced agent-based cell simulator," ACS Synth. Biol. (2017)

Robustness to cell-to-cell variability

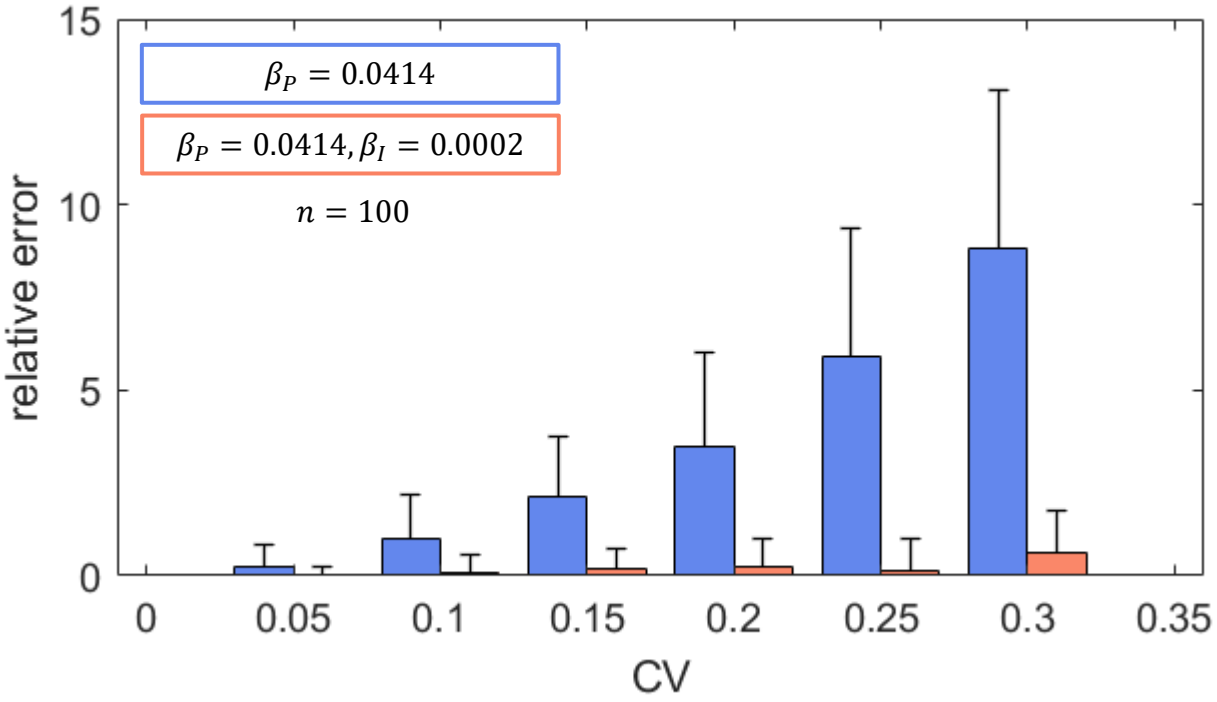
- Cell-to-cell variability is tested assigning different values of parameters to the daughter cells each time a cell splits into them
- The parameters are drawn from a normal distribution centered in their nominal value μ with standard deviation

$$\sigma = CV \cdot \mu$$

- The relative error is computed as:

$$e_{\%} = \frac{1}{n}\sum_{j=1}^{n}\left|\frac{\bar{Q}_x^{t,j} - Q_{x,d}^t}{Q_{x,d}^t}\right|$$

$$n = number\ of\ simulations$$
$$\bar{Q}_x^{t,i} = mean\ value\ over\ a\ time\ interval$$
$$Q_{x,d}^t = desired\ value$$

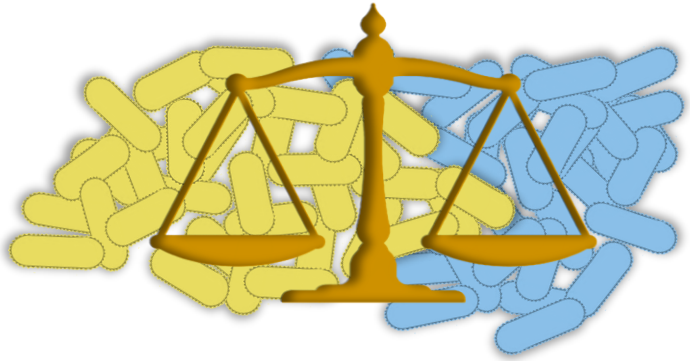


- We have recently finished the analysis and design of a PD multicellular control scheme and we are currently investigating the full multicellular PID controller.

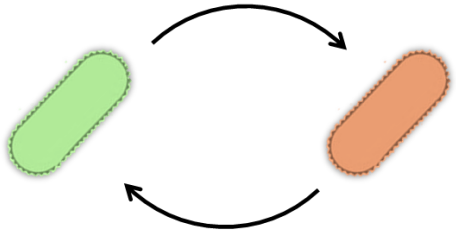
[1] Martinelli et al. - “Multicellular PI control for gene regulation in microbial consortia", IEEE Control Systems Letters (2022)
[2] Martinelli et al. – "Multicellular PD Control in Microbial Consortia", bioRxiv (2023)

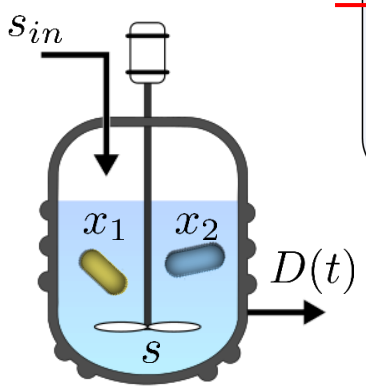
Ratiometric and Growth Control

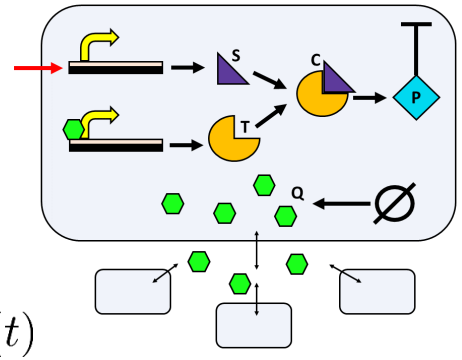
- A crucial issue for multicellular control is to **guarantee stable coexistence** of the cell populations and to mantain their **ratio** to some desired value.
- **Goal:** Develop strategies (external or embedded) to control the ratio between two cell populations or their growth rates.



- We proposed and tested 3 different approaches:
  1. Use reversible differentiation by endowing cells with toggle-switches that can determine their functions
  2. Develop GRNs to allow self-regulation of cell growth in a population
  3. Synthesize control strategies for the dilution rate to allow co-existence of two populations in a chemostat

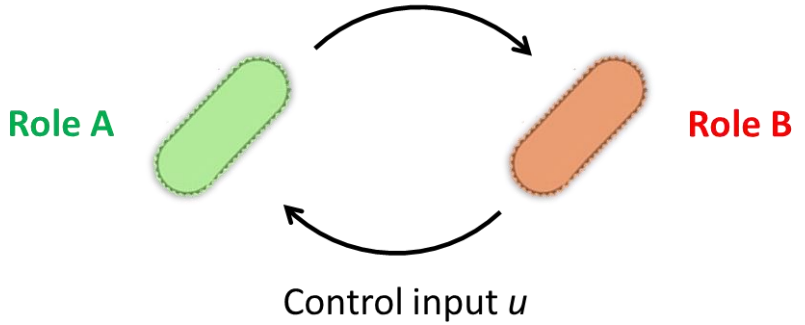




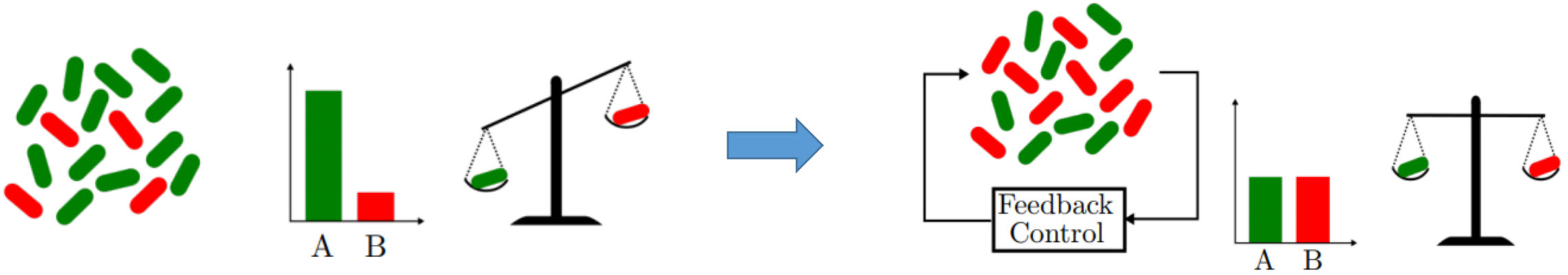


Ratiometric Control

- In this scenario we assumed that:
  - All cells belong to the **same strain**
  - The cells embed a **reversible bi-stable memory** mechanism
  - Its active state encodes the current **role** played by the cell
  - This role can be switched in response to **external events/inputs**



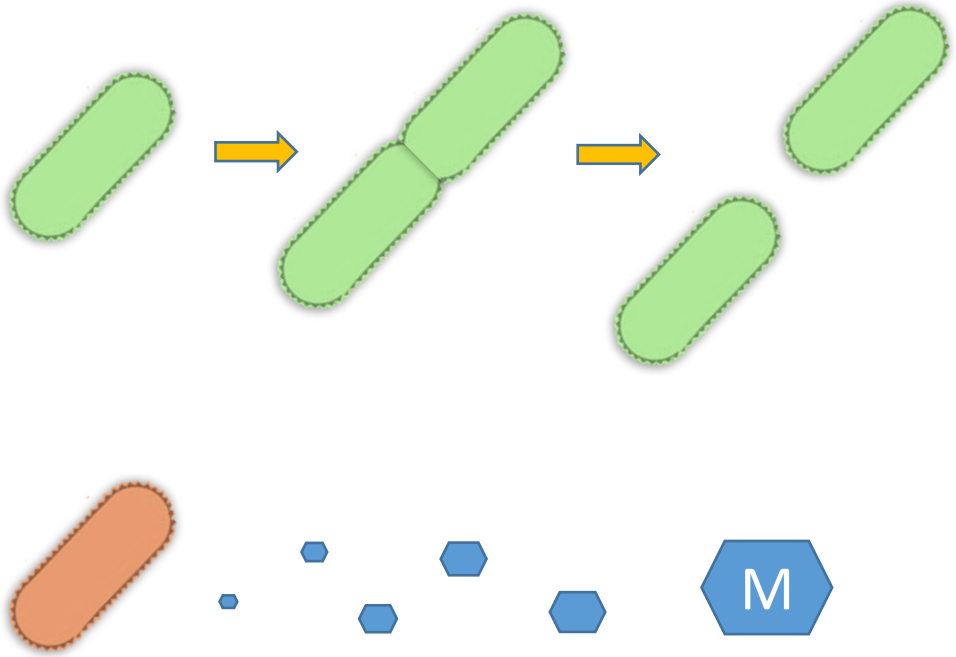
- Objective:



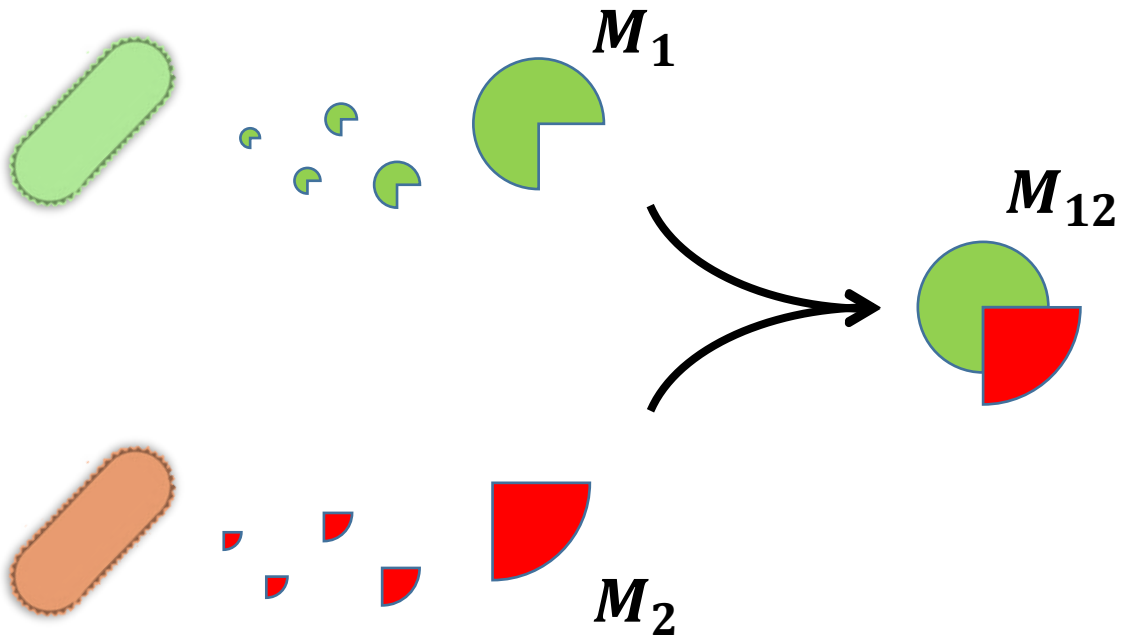
Possible example of applications

- Producers/Growers [3]:

[3] Weill, Andreani, Aditya, Martinon, Batt, Bonnans, Ruess, *Proc. of European Control Conference*, 2019



- Cooperative bioproduction (e.g. dimers):



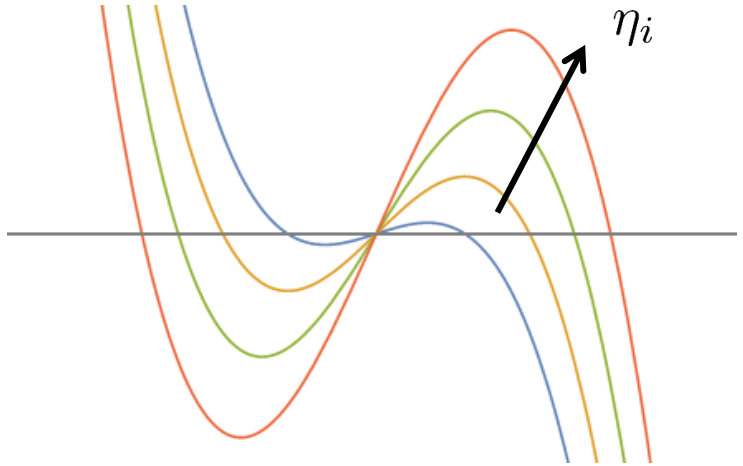
- We want to <u>regulate the ratio to different set-points</u>, depending on desired working loads.

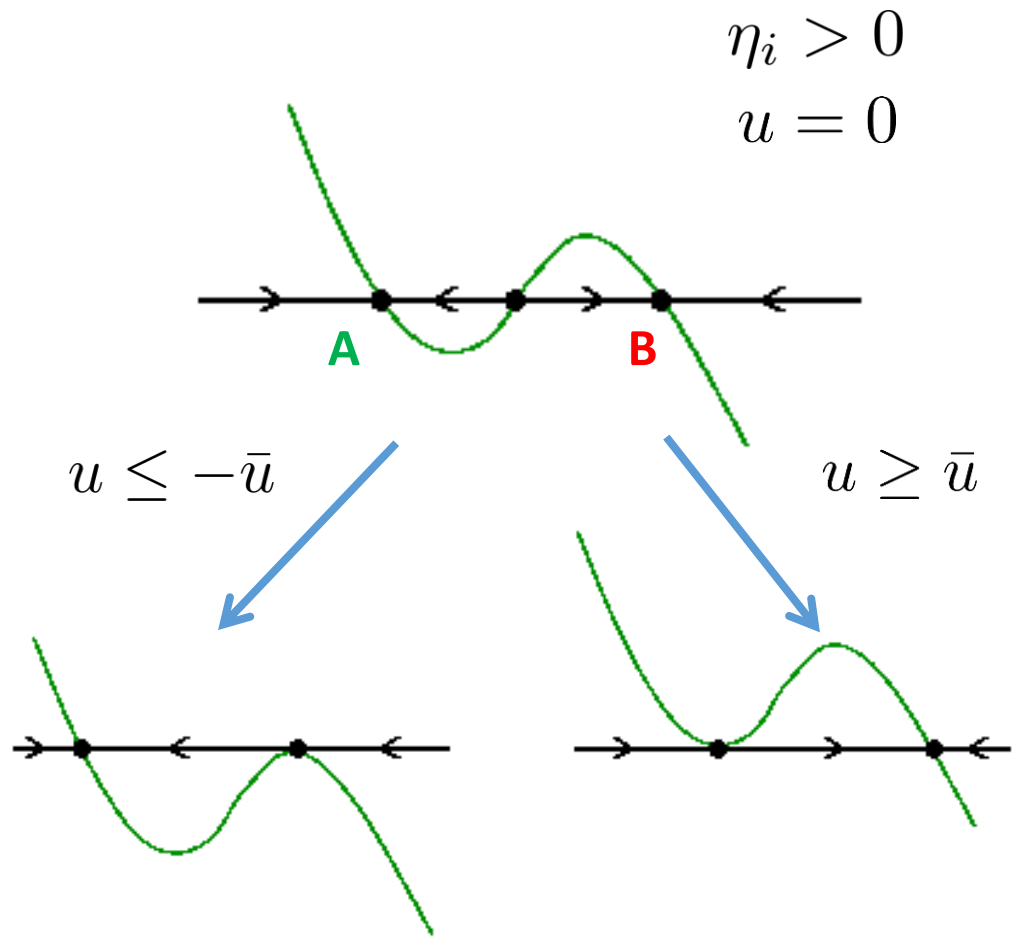
Reversible differentiation

- To prove convergence of the closed-loop system, we considered the simplified model

$$\dot{x}_i = \eta_i x_i - x_i^3 + u$$

To model cell-to-cell variability





[1] D. Salzano, D. Fiore, M. di Bernardo – *"Ratiometric control for differentiation of cell populations endowed with synthetic toggle switches"*, CDC 2019

[2] D. Salzano, D. Fiore, M. di Bernardo – *"Ratiometric control of cell phenotypes in monostrain microbial consortia"*, J. of the Royal Society Interface (2022)

Ratiometric control problem

- **Objective**: Find a feedback control law $u(t,x)$ such that at steady-state two populations emerge in the consortium

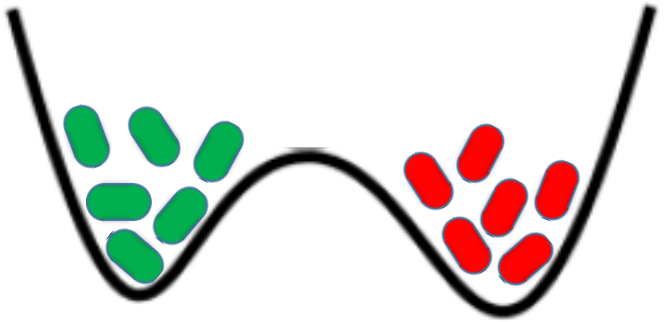
  (whose cells' dynamics can be described as $\dot{x}_i = \eta_i x_i - x_i^3 + u$)

  and their number converge to the ***desired ratio*** $r_d \in [0,1]$, that is

$$r(t) \to r_d, \text{ as } t \to \infty.$$

Example: $N = 10$

$r_d = r_B(t \to \infty) = 60\%$



A

$r_A = 0.5$

B

$r_B = 0.5$

- We considered two simple controllers: (i) a bang-bang controller, and (ii) a PI controller

Populations' ratio:

$$r_A(t) = \frac{n_A(t)}{N(t)} \qquad r_B(t) = \frac{n_B(t)}{N(t)}$$

- then we
  i. used event-driven modelling for the error system, and
  ii. exploited a Lyapunov-like analysis to the resulting map.

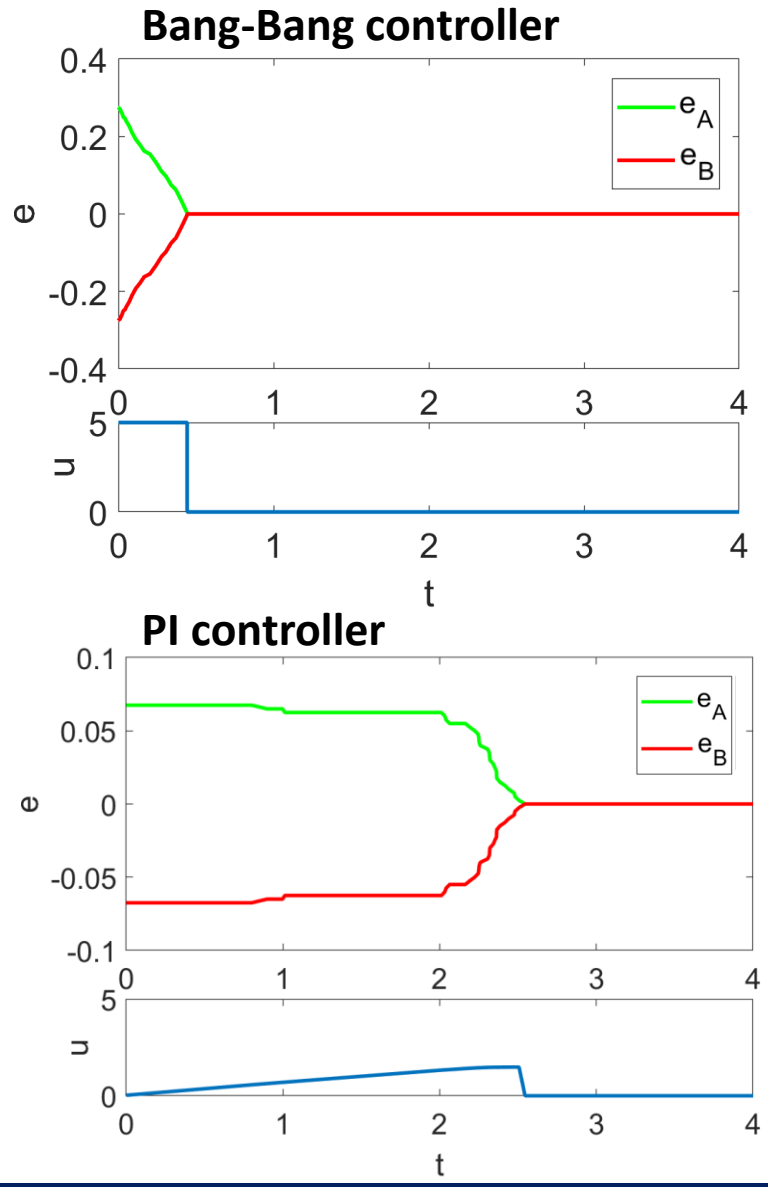
Ideal case

- Specifically, we proved that bang-bang and PI controllers can ***exactly*** solve the previous problem <u>if all cells are controllable.</u>

- A cell $\dot{x}_i = \eta_i\, x_i - x_i^3 + u$ is ***controllable*** if by varying $u \in U$ it can be moved from one group to the other and vice versa.

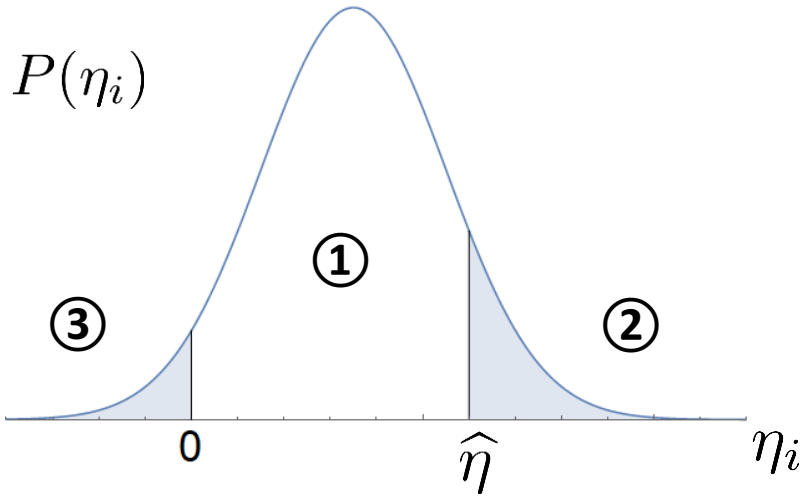
Control errors:

$$e_A(t) = (1 - r_d) - r_A(t)$$
$$e_B(t) = r_d - r_B(t)$$

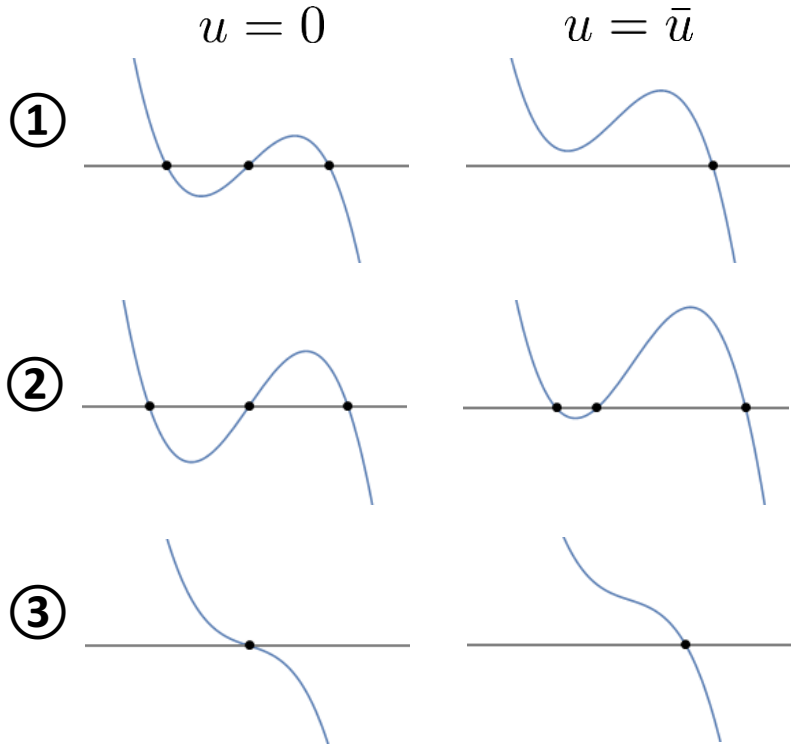


Effects of uncontrollable cells

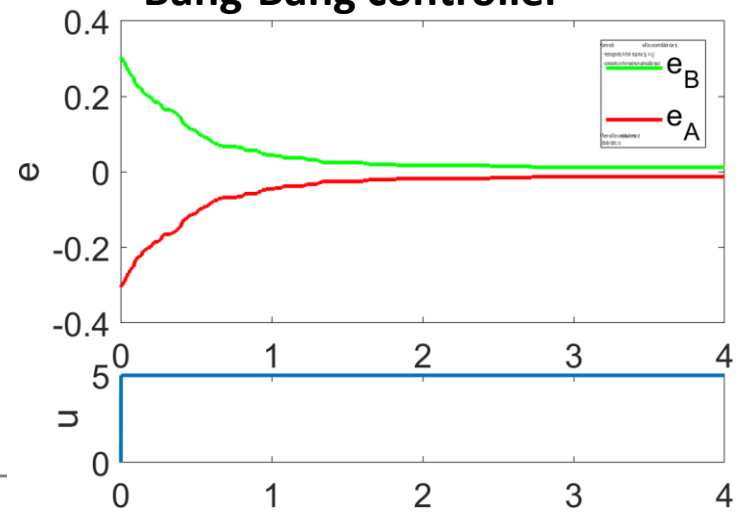
- Some cells $\dot{x}_i = \eta_i\, x_i - x_i^3 + u$ will be uncontrollable due to
  - heterogeneity in their response ($\eta_i \neq \eta_j$) $\Rightarrow P(\eta_i)$
  - constraints on the maximum admissible input $\bar{u} \Rightarrow \widehat{\eta}$



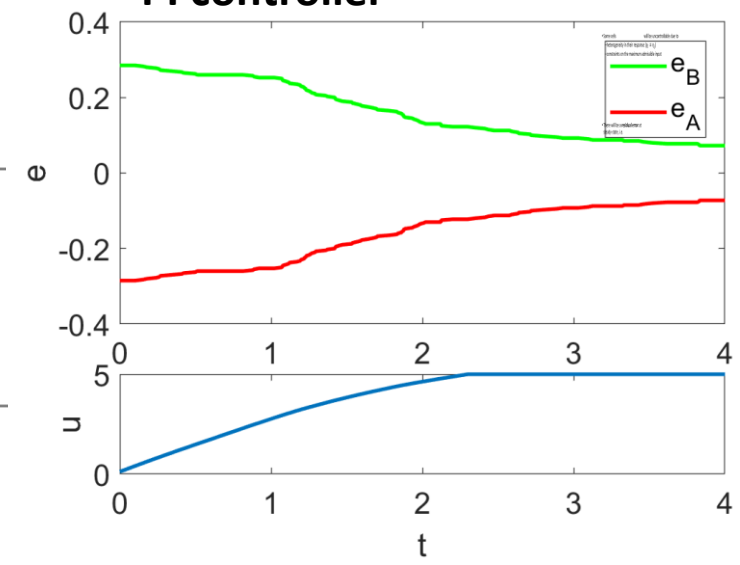
- There will be a **residual error** at steady-state, i.e. $|e_\infty| \geq e_r > 0$



Bang-Bang controller



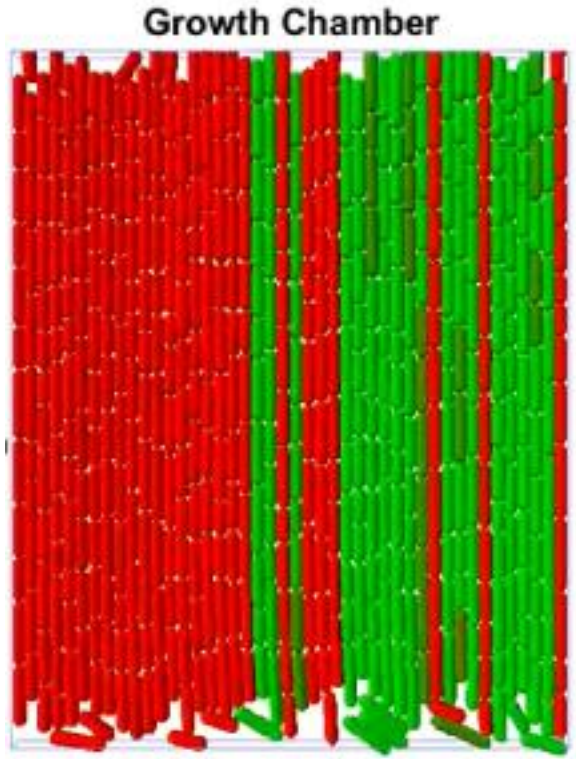
PI controller

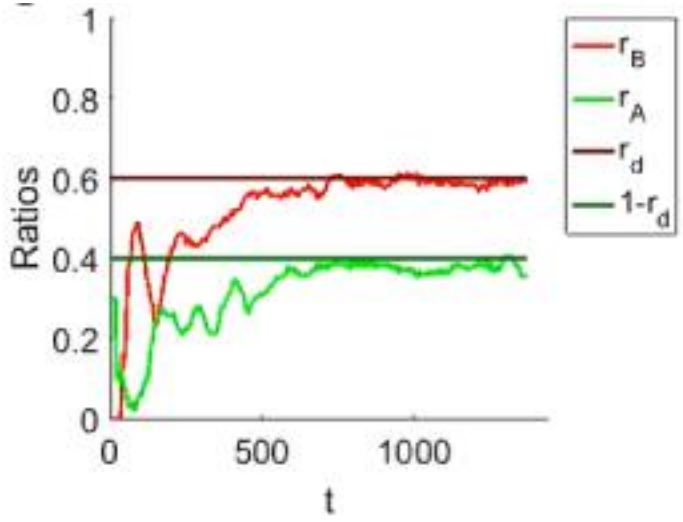


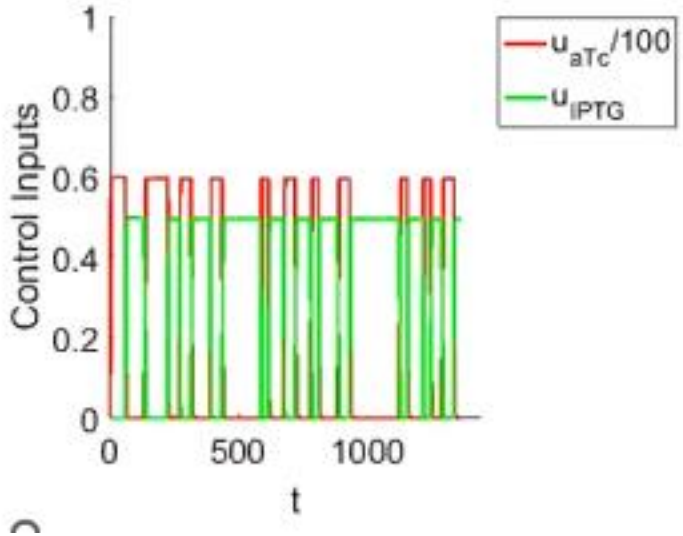
A realistic example in BSim simulations

- We used BSim [1-2] to simulate
  - cell division, geometry, spatial diffusion, flush-out
  - and other physical constraints in **microfluidic devices**.

- Next step: in vivo experiments



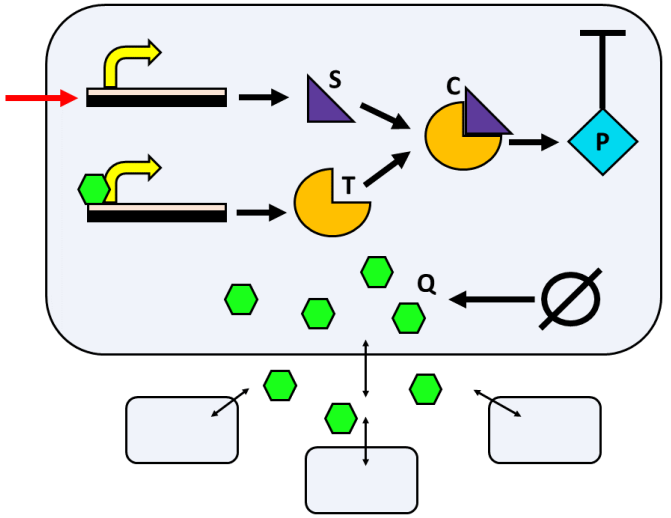


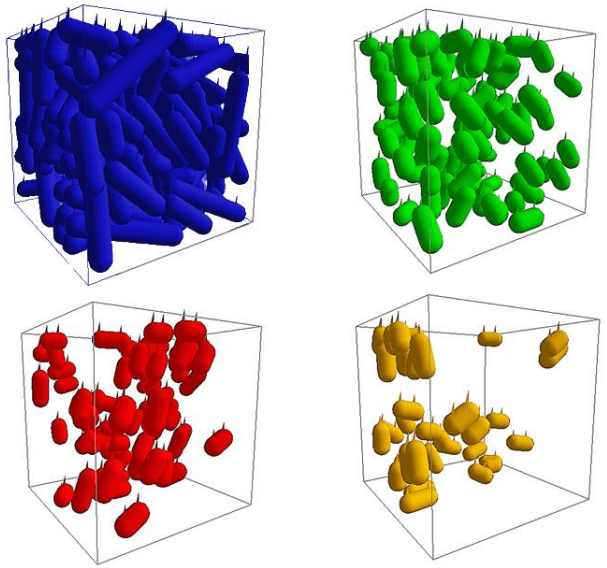


[1] Gorochowski et al., *PloS One* (2012)
[2] Matyjaszkiewicz et al., *ACS Synthetic Biology* (2017)

2 - Self-regulation of growth rate

- Inspired by [1-2], we investigated the use of a Tunable Expression System to implement a **population growth control system** [3].
- The production rate of the toxin P is regulated by the quorum sensing molecule Q.
- The TES provides flexibility and fine tunability to the system.
- The proposed design showed good performance and robustness via in-silico simulations in BSim.





- Currently, we are developing an extension for the case of two co-existing populations (which shows very rich dynamics).

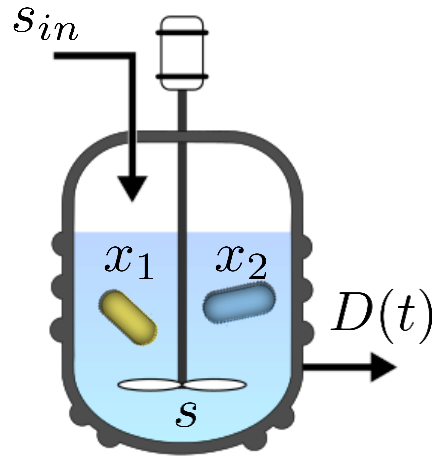
[1] L. You, R.S. Cox, R. Weiss, F.H. Arnold, *"Programmed population control by cell–cell communication and regulated killing",* Nature (2004)
[2] V. Bartoli, G.A. Meaker, M. di Bernardo, T. Gorochowski, *"Tunable genetic devices through simultaneous control of transcription and translation",* Nature Communication (2020)
[3] V. Fusco, D. Salzano, D. Fiore, M. di Bernardo – *"Embedded control of cell growth using tunable genetic systems",* International Journal of Robust and Nonlinear Control (2022)

3 - Control of dilution rate in chemostats

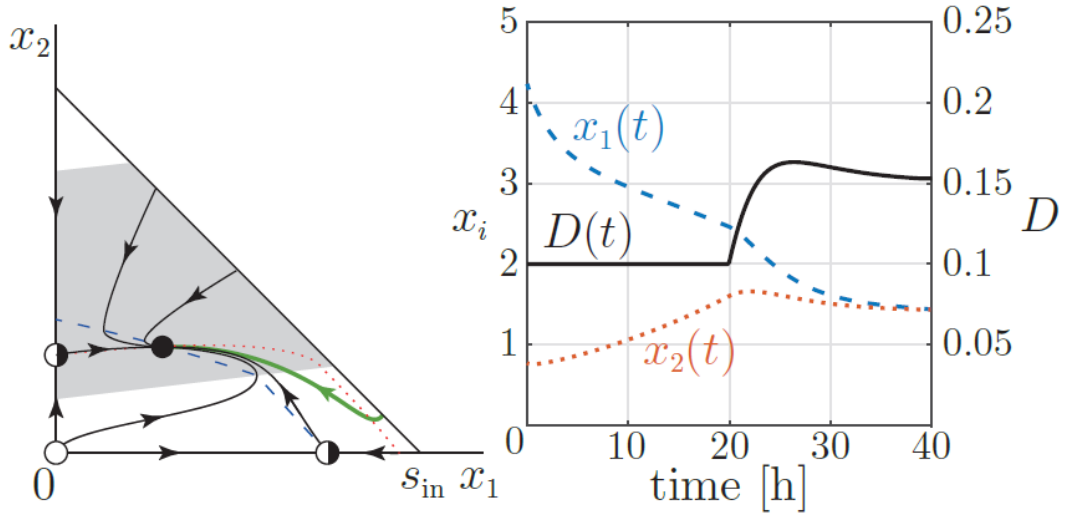
- We developed (i) a gain-scheduled state feedback controller, and (ii) a switching controller with sliding to regulate the **ratio** of two independent populations with dynamics

$$\begin{aligned}\dot{x}_1 &= \big(\mu_1(s) - D(t)\big)\, x_1 \\ \dot{x}_2 &= \big(\mu_2(s) - D(t)\big)\, x_2\end{aligned}$$

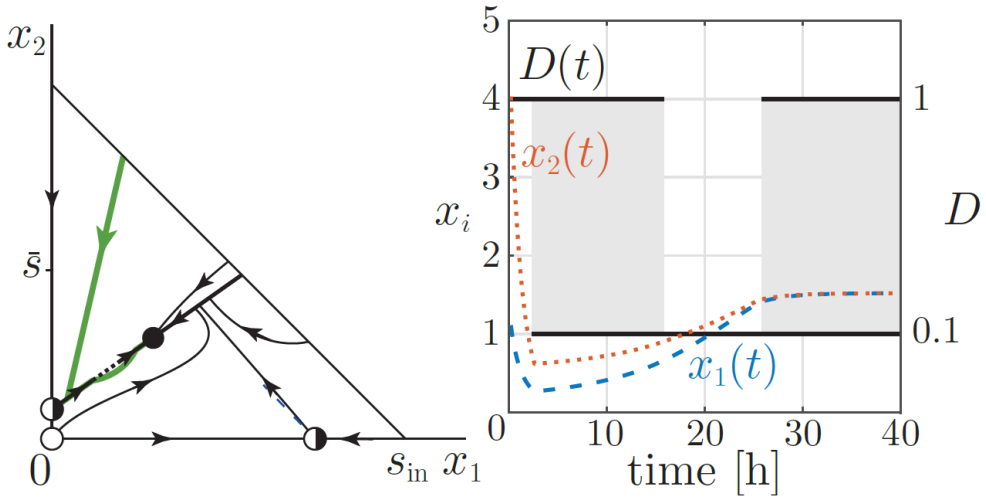
, with μ_i being of Monod's type.



(i)



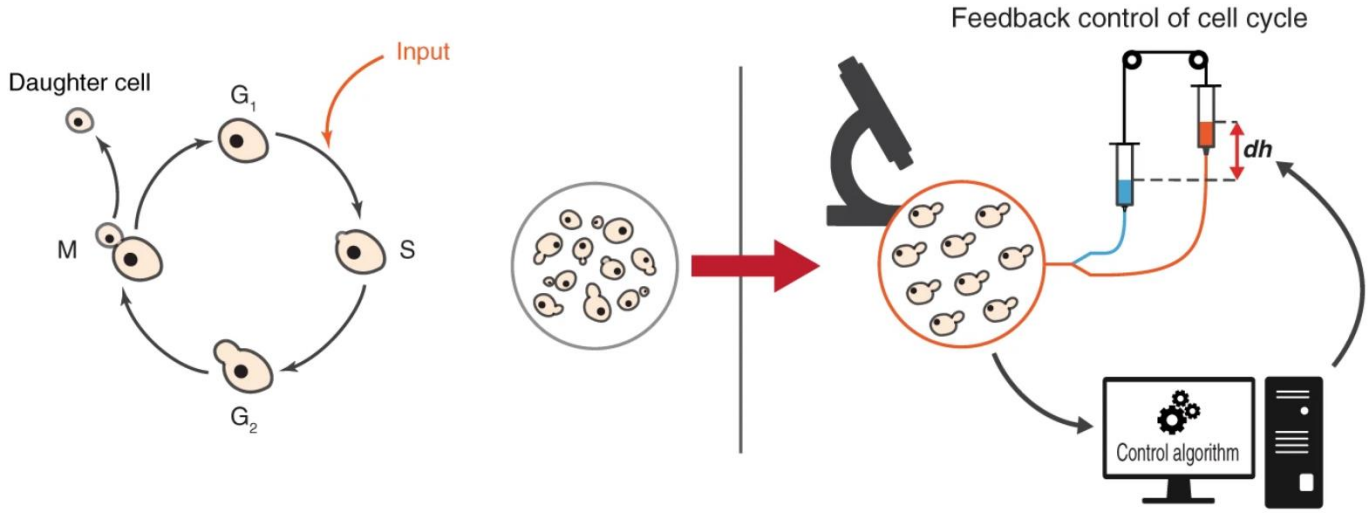
(ii)

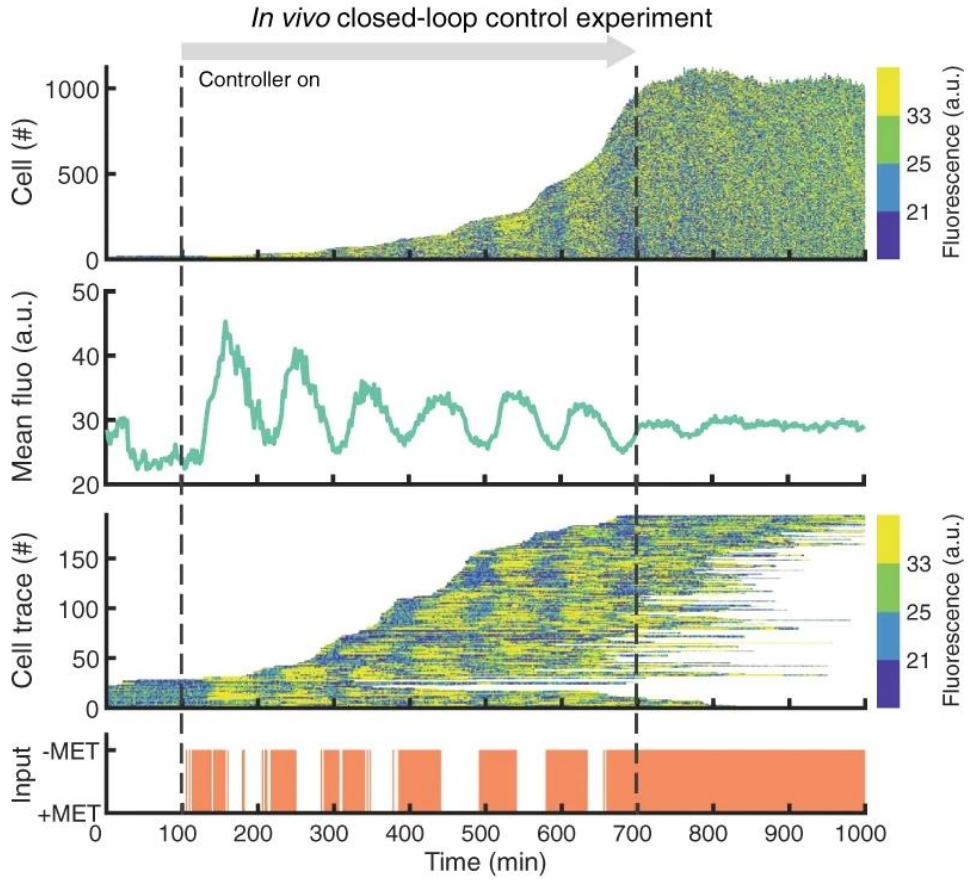


[1] D. Fiore, F. Della Rossa, A. Guarino, M. di Bernardo – *"Feedback ratiometric control of two microbial populations in a single chemostat"*, IEEE Control Systems Letters (2021)

Cell cycle synchronization

- We developed feedback control strategies to achieve **long-term synchronization** of the cell cycle in budding yeast.



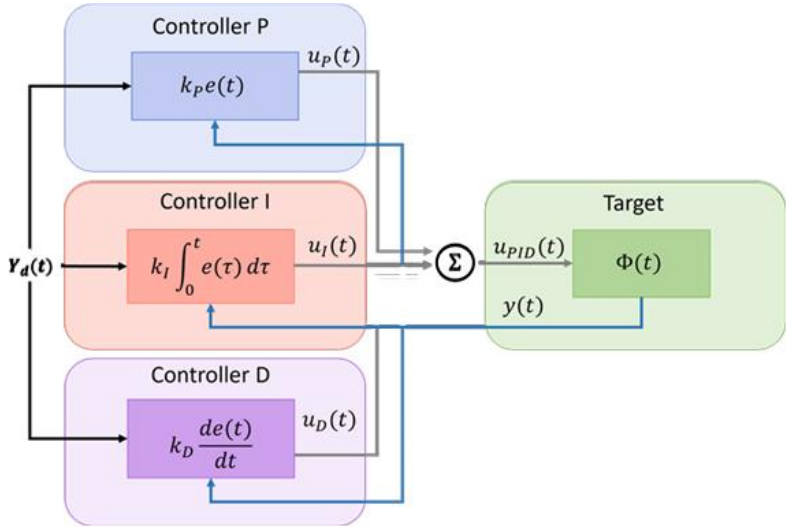


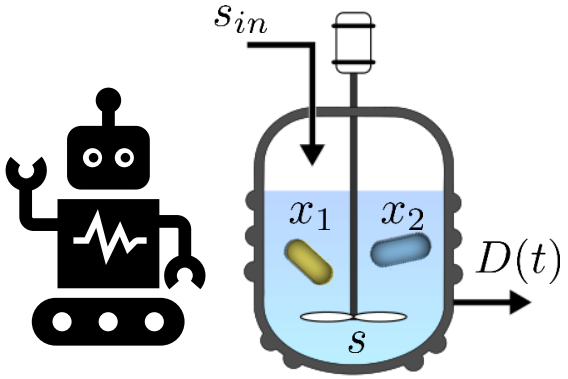
- We successfully validated the proposed approach by means of in-vivo experiments in microfluidics.

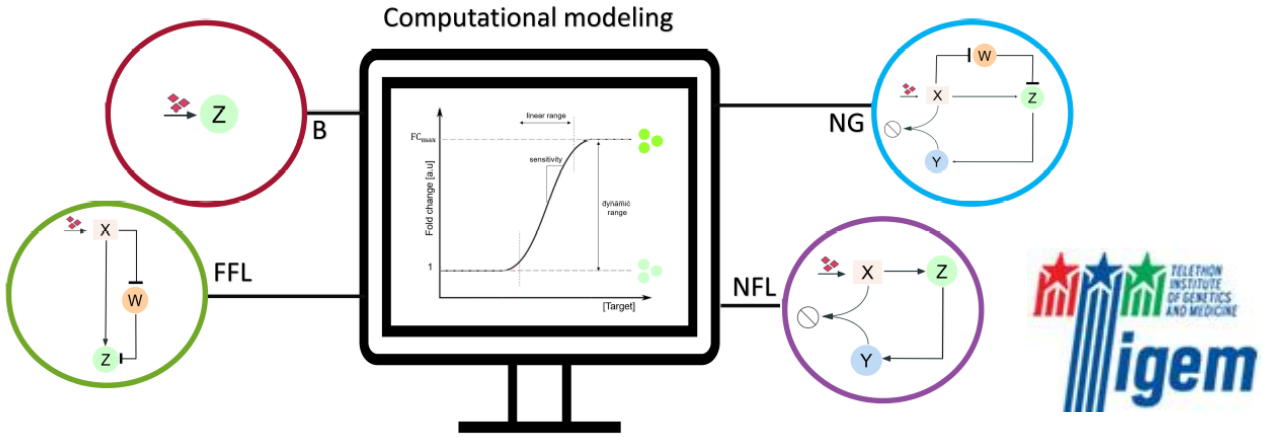
[1] G. Perrino, S. Napolitano, F. Galdi, A. La Regina, D. Fiore, T. Giuliano, M. di Bernardo, D. di Bernardo – *"Automatic synchronisation of the cell cycle in budding yeast through closed-loop feedback control"*, Nature Communications (2021)

Ongoing research

- We are investigating the following topics:
  - Multicellular PID controller
  - Ratiometric control via reinforcement learning
  - Novel designs for whole-cell biosensors



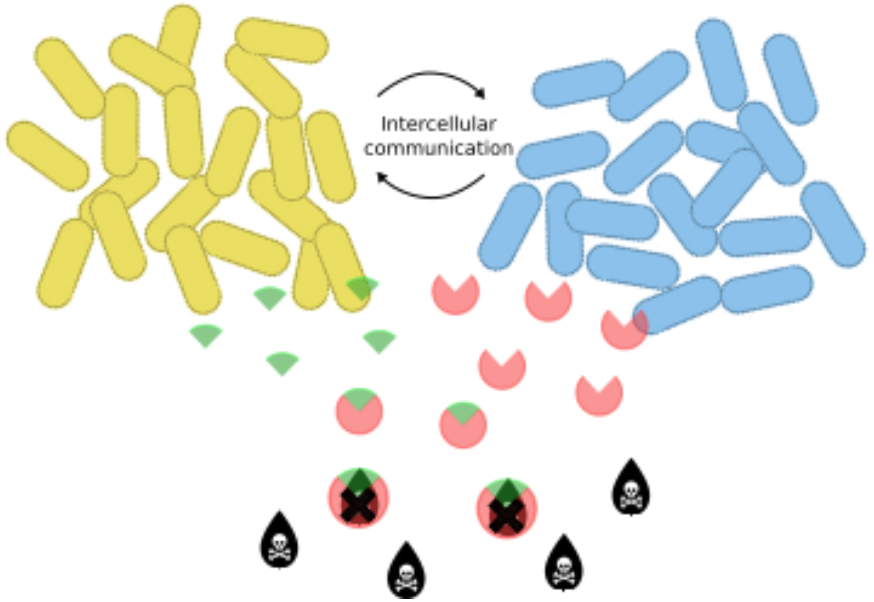


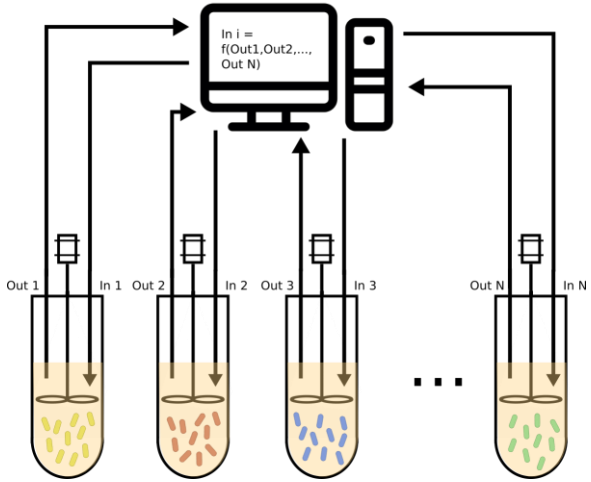


Future project (if funded)

- Title: “Control of smart microbial communities for wastewater treatment”
- Call PRIN 2022 PNRR – Budget € 300.000
- Objectives:

1. Develop new methodologies to accelerate the engineering and the deployment of **microbial communities** for more efficient and reliable wastewater treatment
2. Develop new **data-driven feedback control** strategies to enhance throughput in bioreactors
3. Design and build an innovative multi-chamber bioreactor for **fast prototyping** of microbial communities





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M. di Bernardo

D. Salzano

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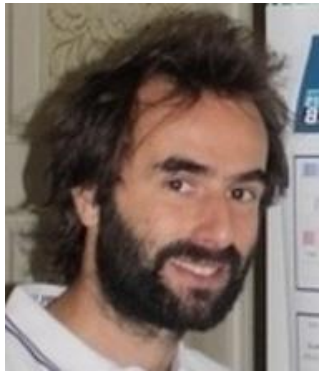
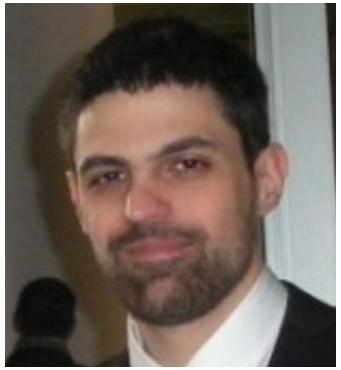


F. Della Rossa

A. Guarino

D. di Bernardo

V. Fusco



G. Perrino

S. Napolitano

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- D. Salzano, D. Fiore, M. di Bernardo – *“Ratiometric control of cell phenotypes in monostrain microbial consortia”,* J. of the Royal Society Interface (2022)
- D. Fiore, F. Della Rossa, A. Guarino, M. di Bernardo – *"Feedback ratiometric control of two microbial populations in a single chemostat"*, IEEE Control Systems Letters (2021)
- V. Fusco, D. Salzano, D. Fiore, M. di Bernardo – *"Embedded control of cell growth using tunable genetic systems",* International Journal of Robust and Nonlinear Control (2022)
- G. Perrino, S. Napolitano, F. Galdi, A. La Regina, D. Fiore, T. Giuliano, M. di Bernardo, D. di Bernardo – *"Automatic synchronisation of the cell cycle in budding yeast through closed-loop feedback control"*, Nature Communications (2021)
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- V. Martinelli, D. Salzano, D. Fiore, M. di Bernardo, *"Multicellular PD control in microbial consortia"*, bioRxiv (2023)