Synthetic biology:
from modules to systems

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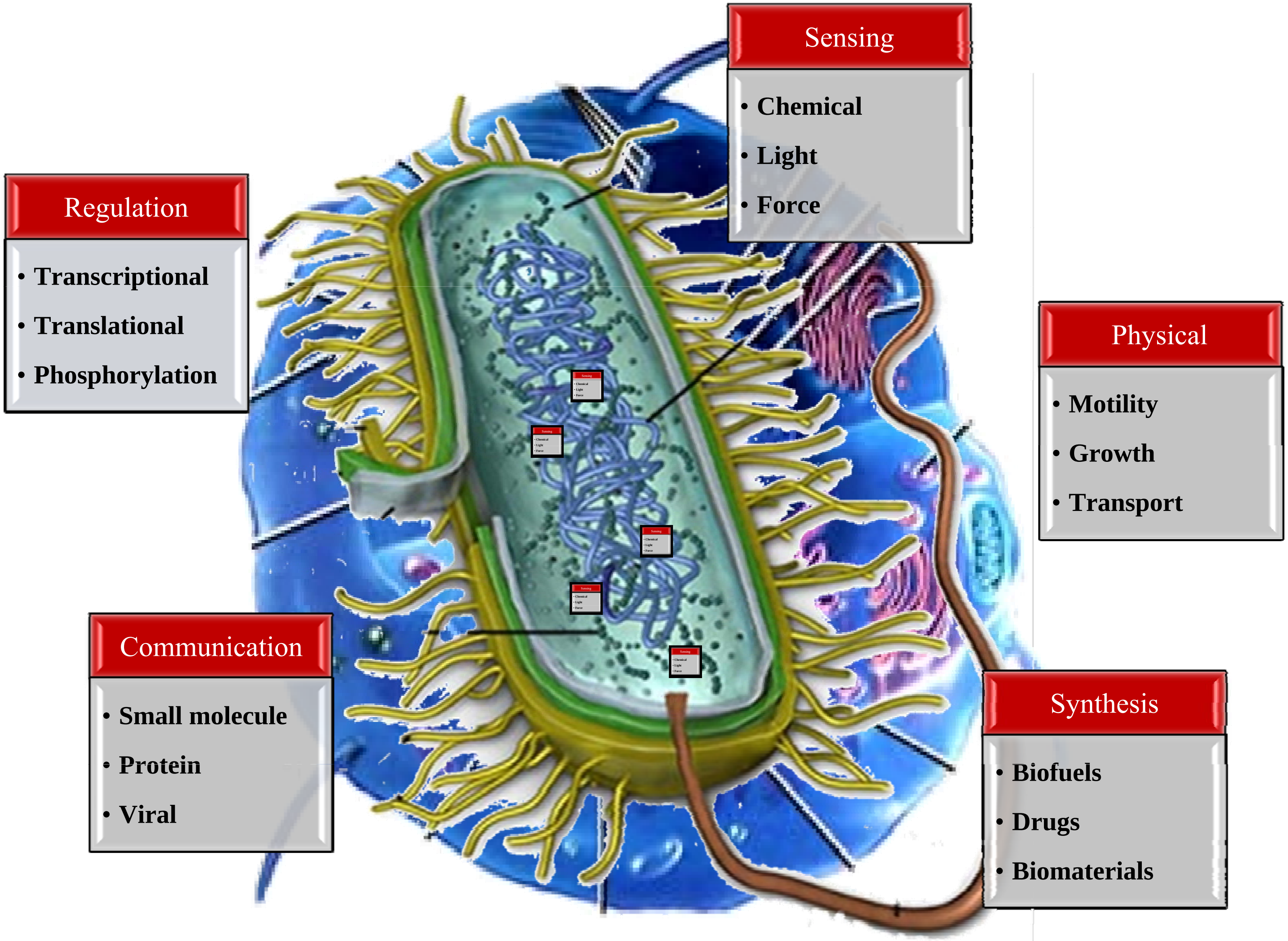
CSAIL

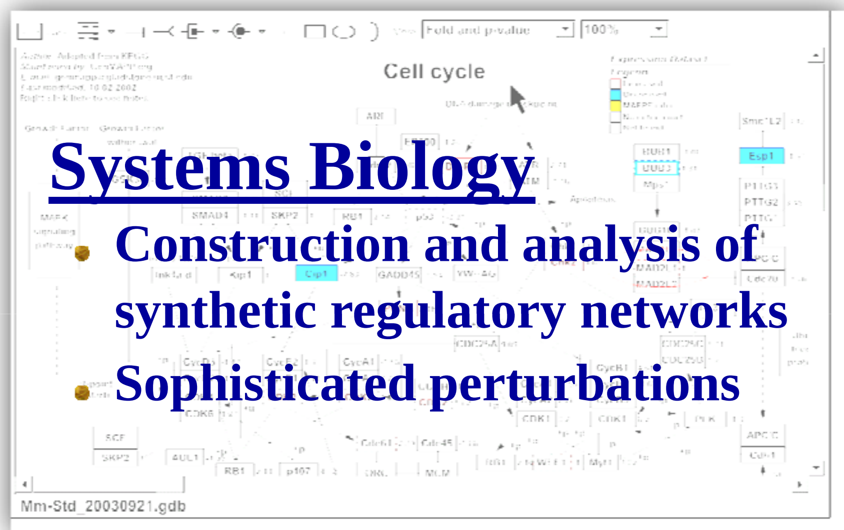
Systems-level bioengineering



- Program cell populations to perform various tasks reliably

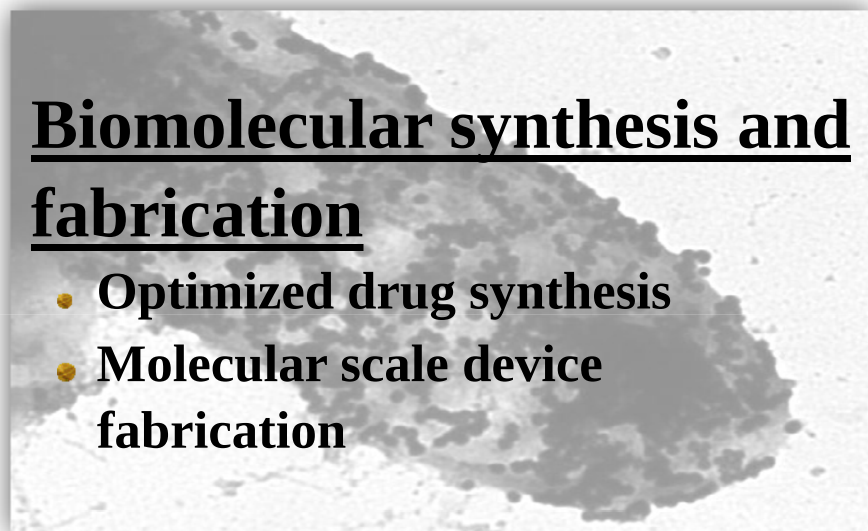
http://www.ebi.ac.uk/microarray/biology_intro_files/cell.jpe

Applications



Systems Biology

- Construction and analysis of synthetic regulatory networks
- Sophisticated perturbations

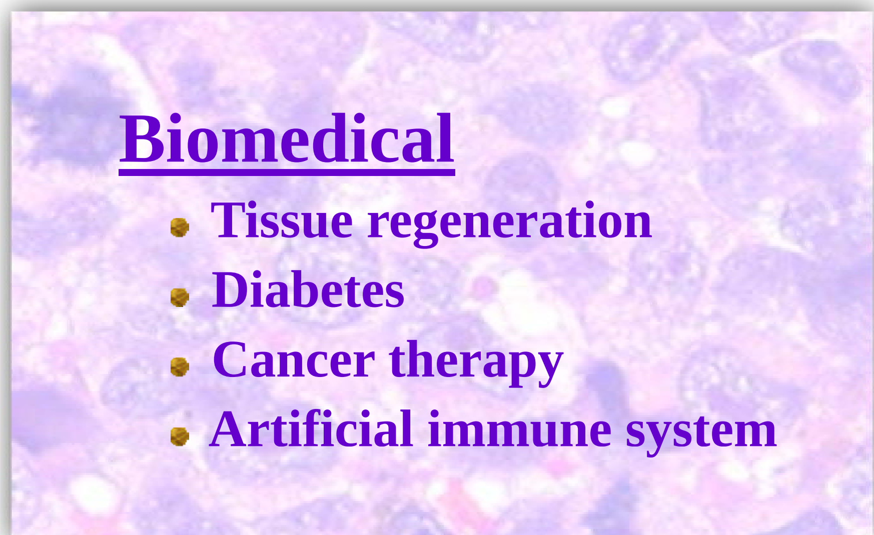


Biomolecular synthesis and fabrication

- Optimized drug synthesis
- Molecular scale device fabrication

Environmental

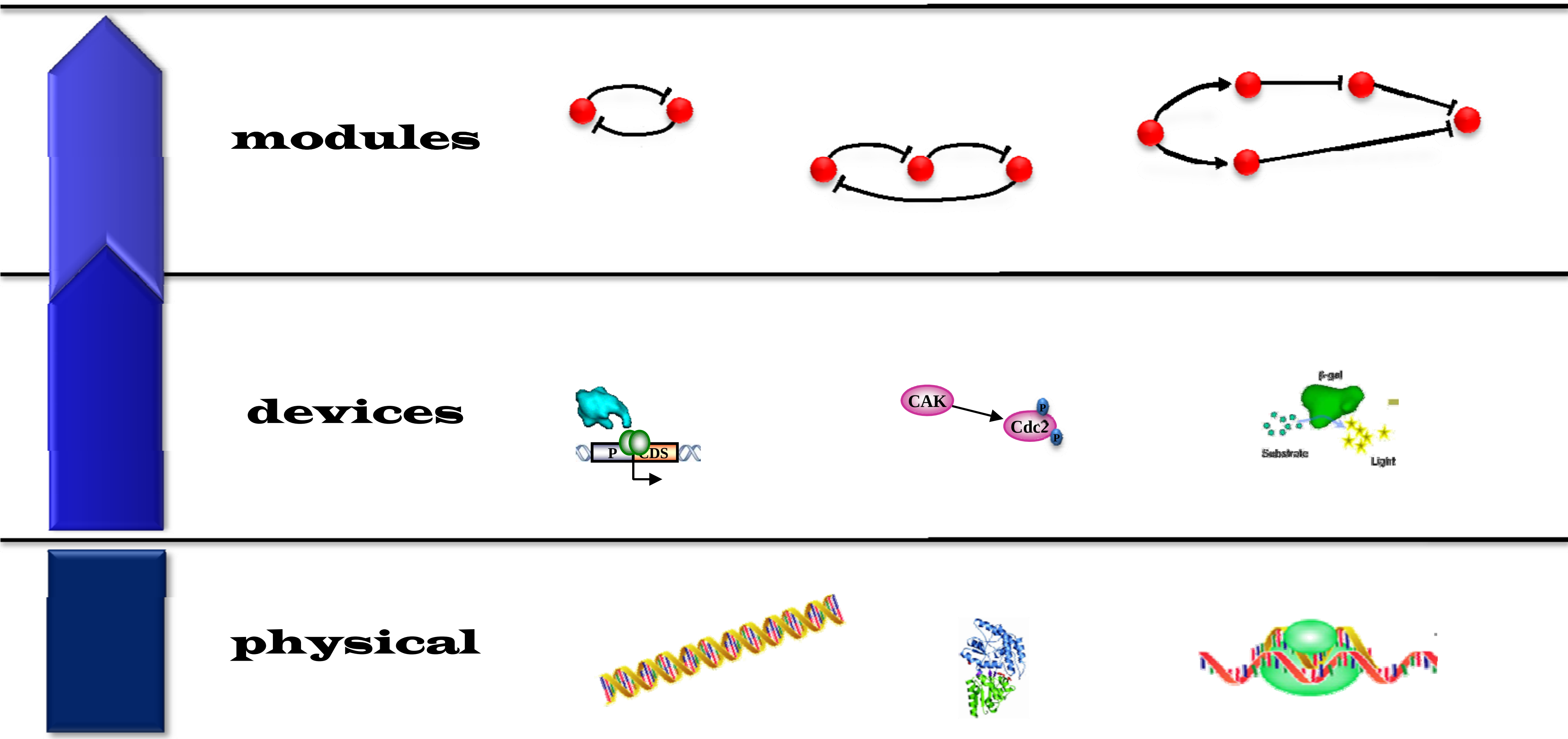
- Biosensing
- Environmental remediation
- Energy production
- Reducing CO_2 levels



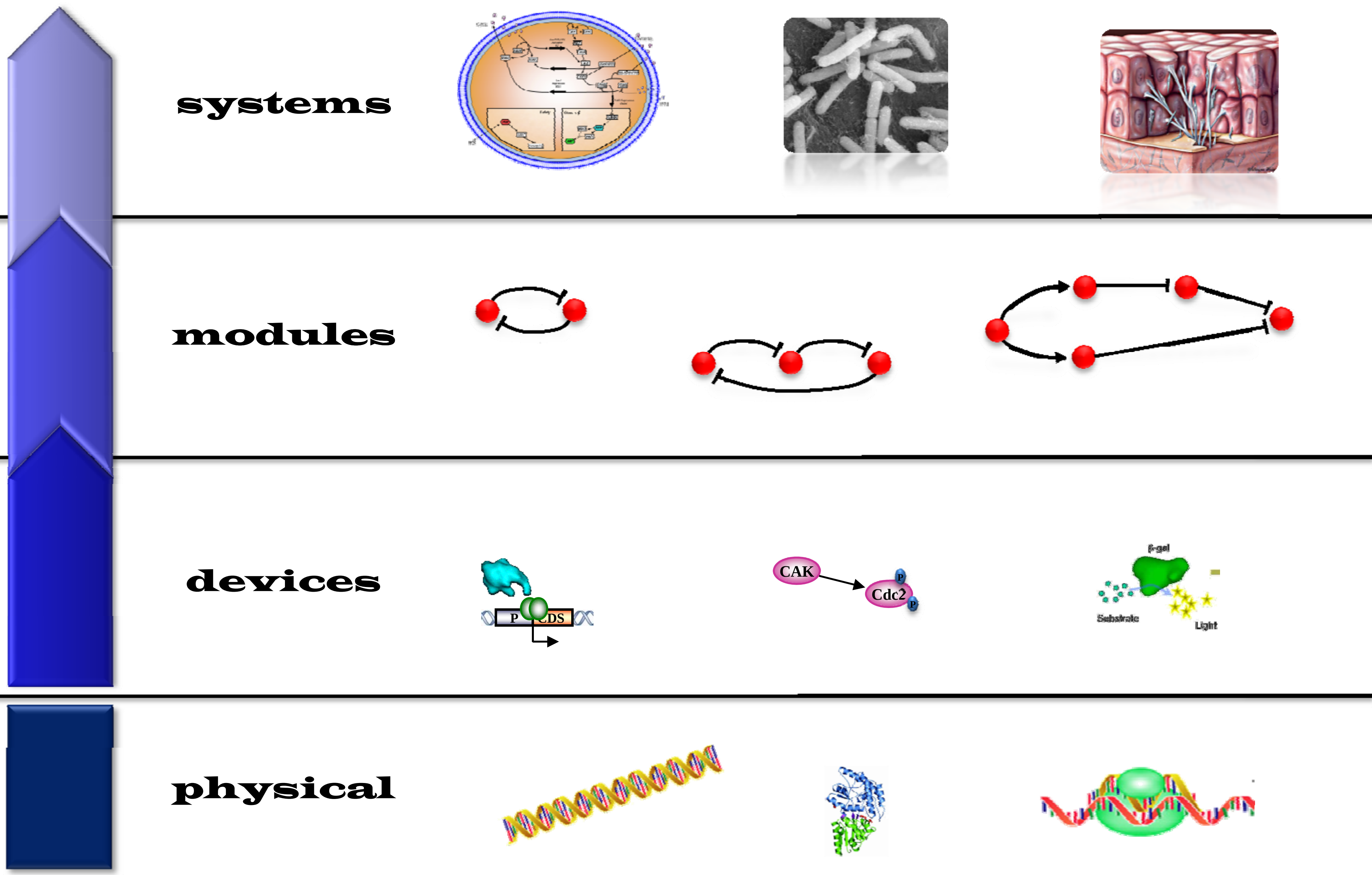
Biomedical

- Tissue regeneration
- Diabetes
- Cancer therapy
- Artificial immune system

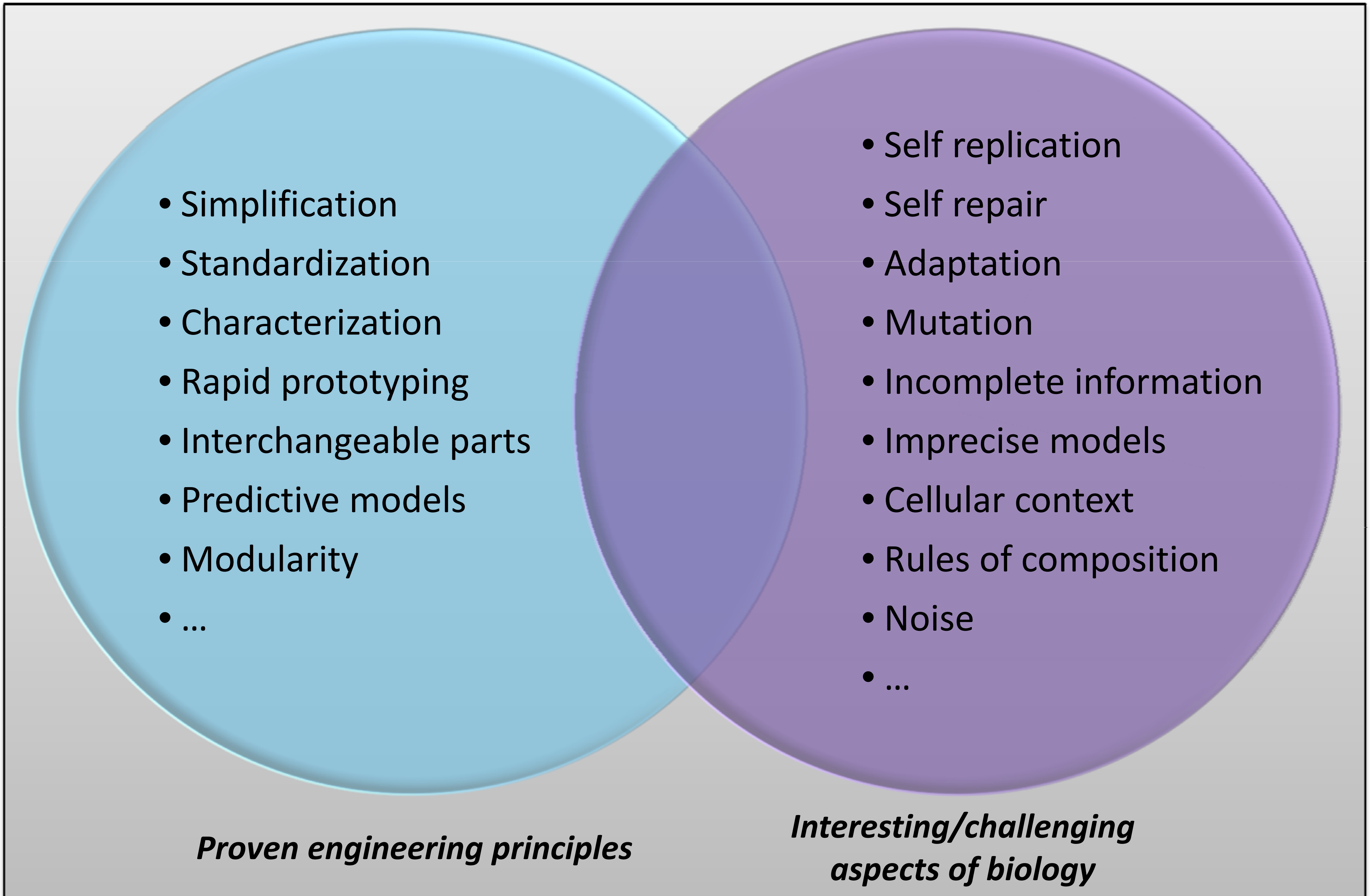
The 1st wave of synthetic biology



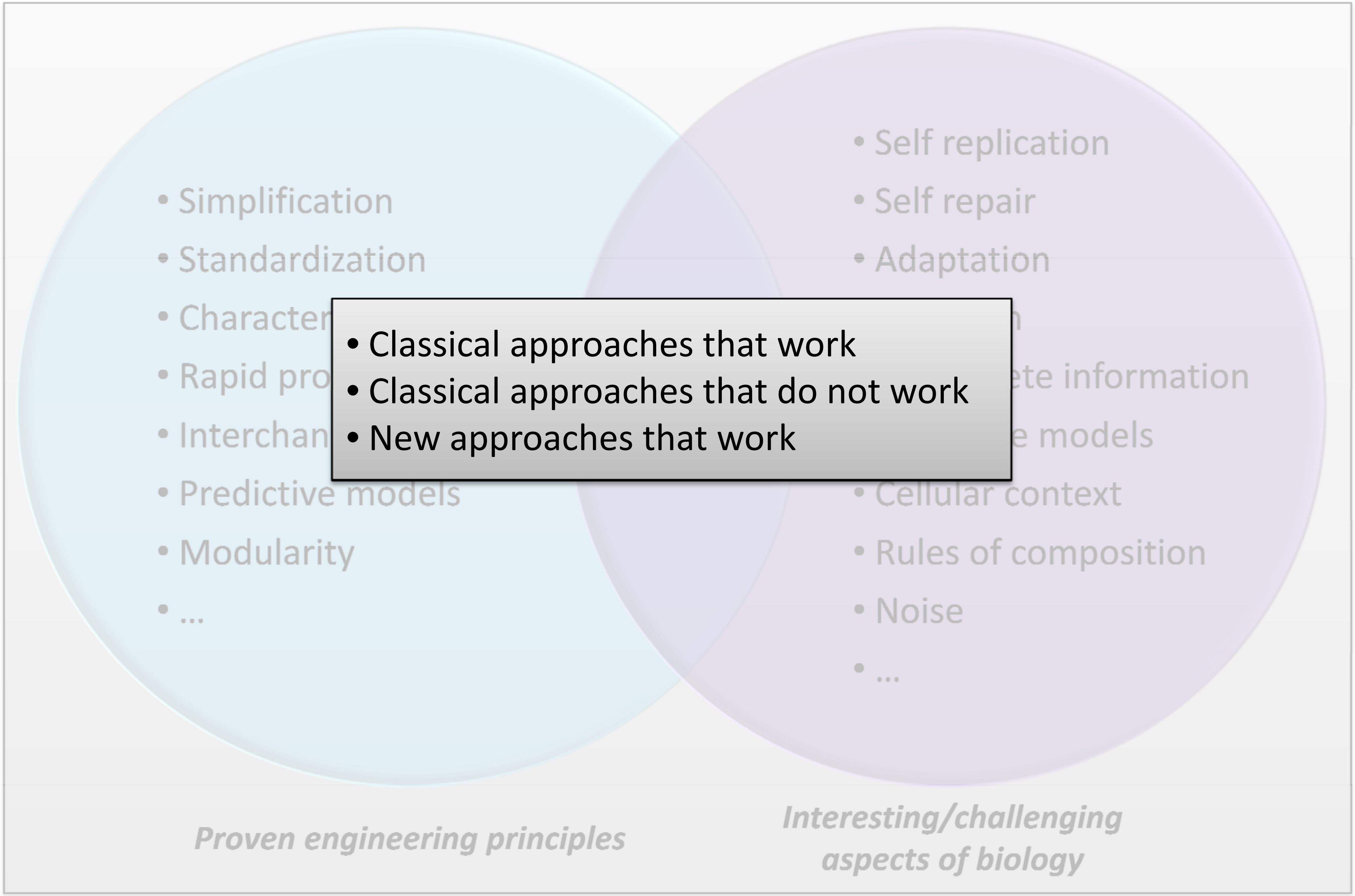
The 2nd wave of synthetic biology



Synthetic biology: A new engineering discipline



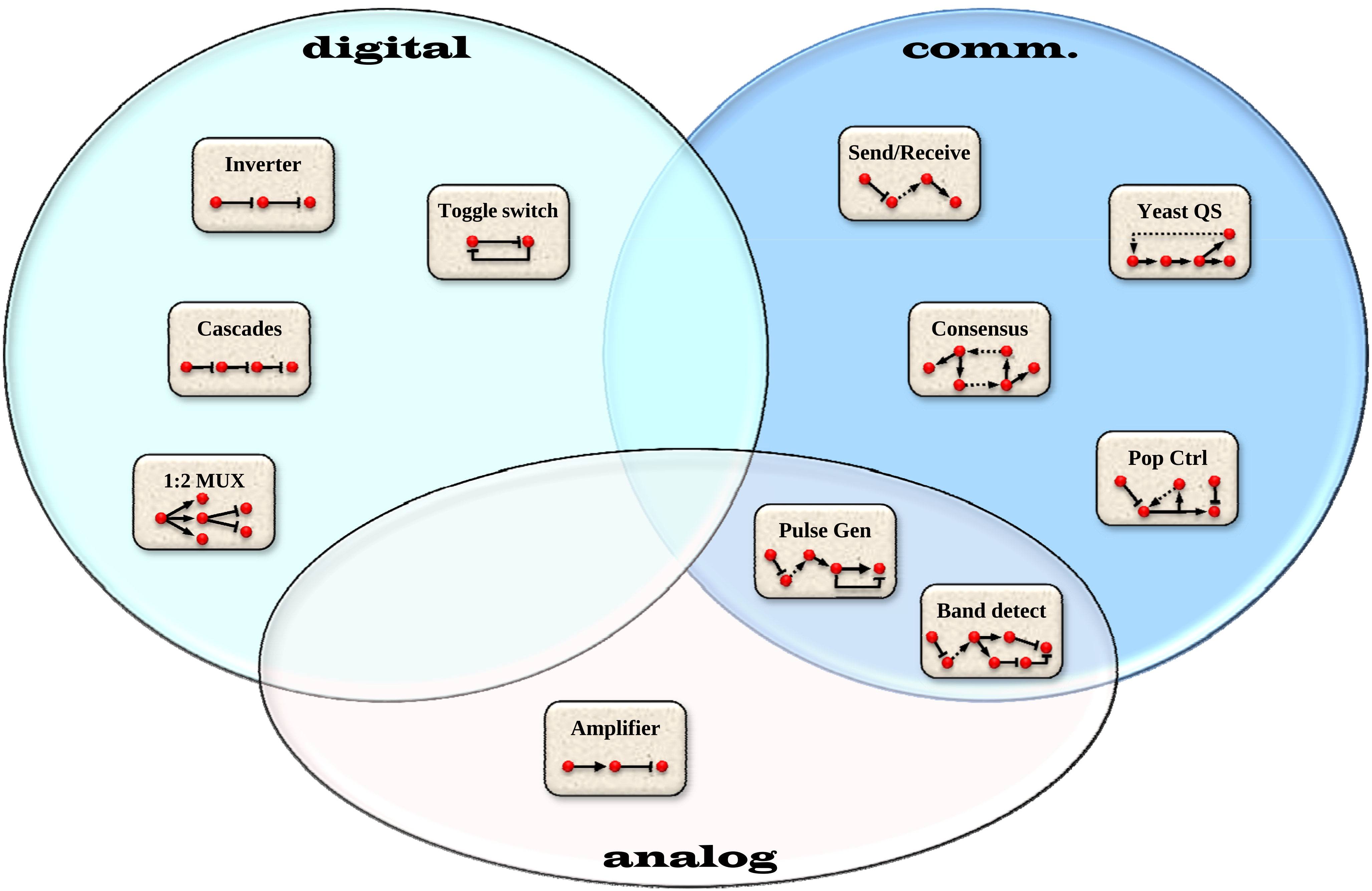
Synthetic biology: A new engineering discipline



Overview

- Basic modules
  - Digital, analog, multicellular
- Local rules / global behavior
  - Turing pattern formation (Ting Lu)
  - Conway’s Game of Life (Jingjing Sun)
- Larger scale system design
  - Artificial tissue homeostasis

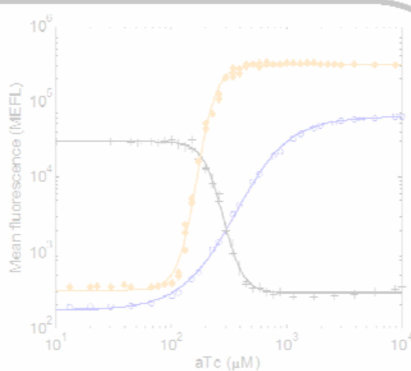
Modules implemented in Weiss lab

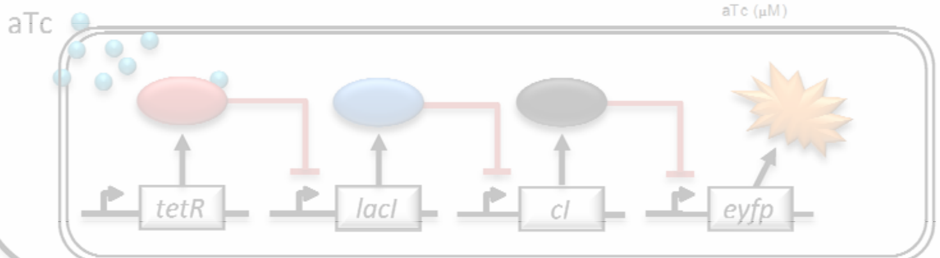


Modules implemented in Weiss lab

Ultrasensitive Switch

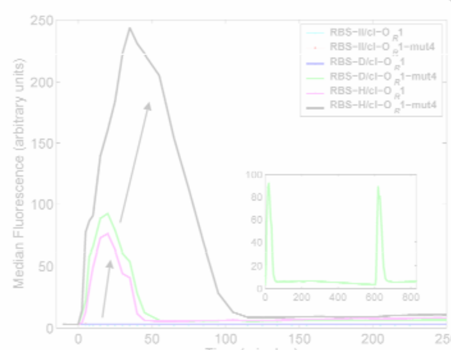
EYFP = NOT(NOT(aTc))

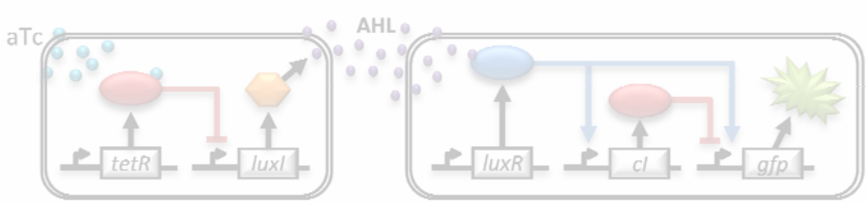




Pulse Generator

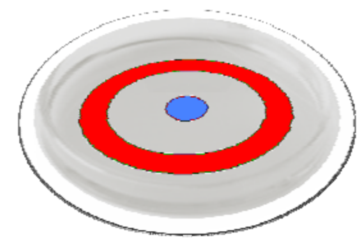
- Sender: send(AHL)
- Receiver: Transient GFP expression

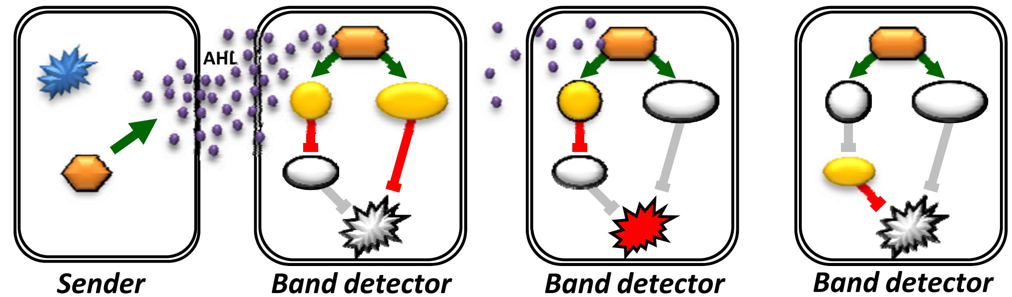


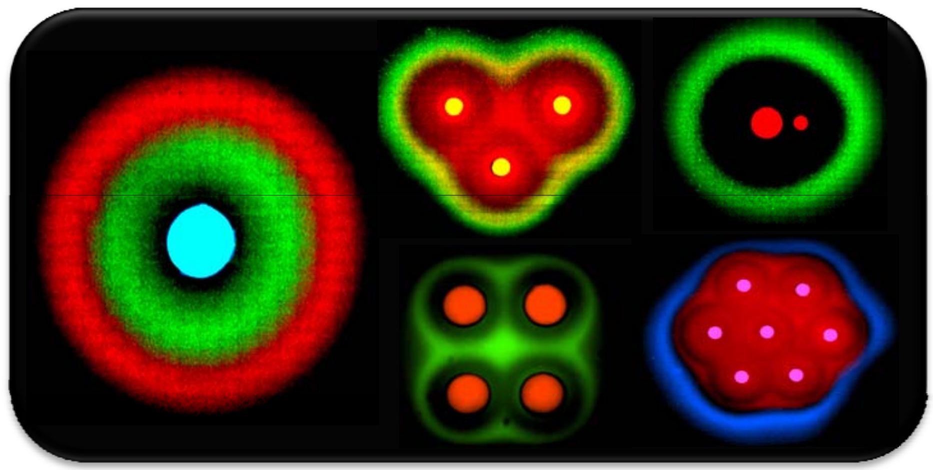


Ring-like spatial patterns

- Sender: send(AHL)
- Receiver:
  If (AHL = medium)
  then express (RFP)

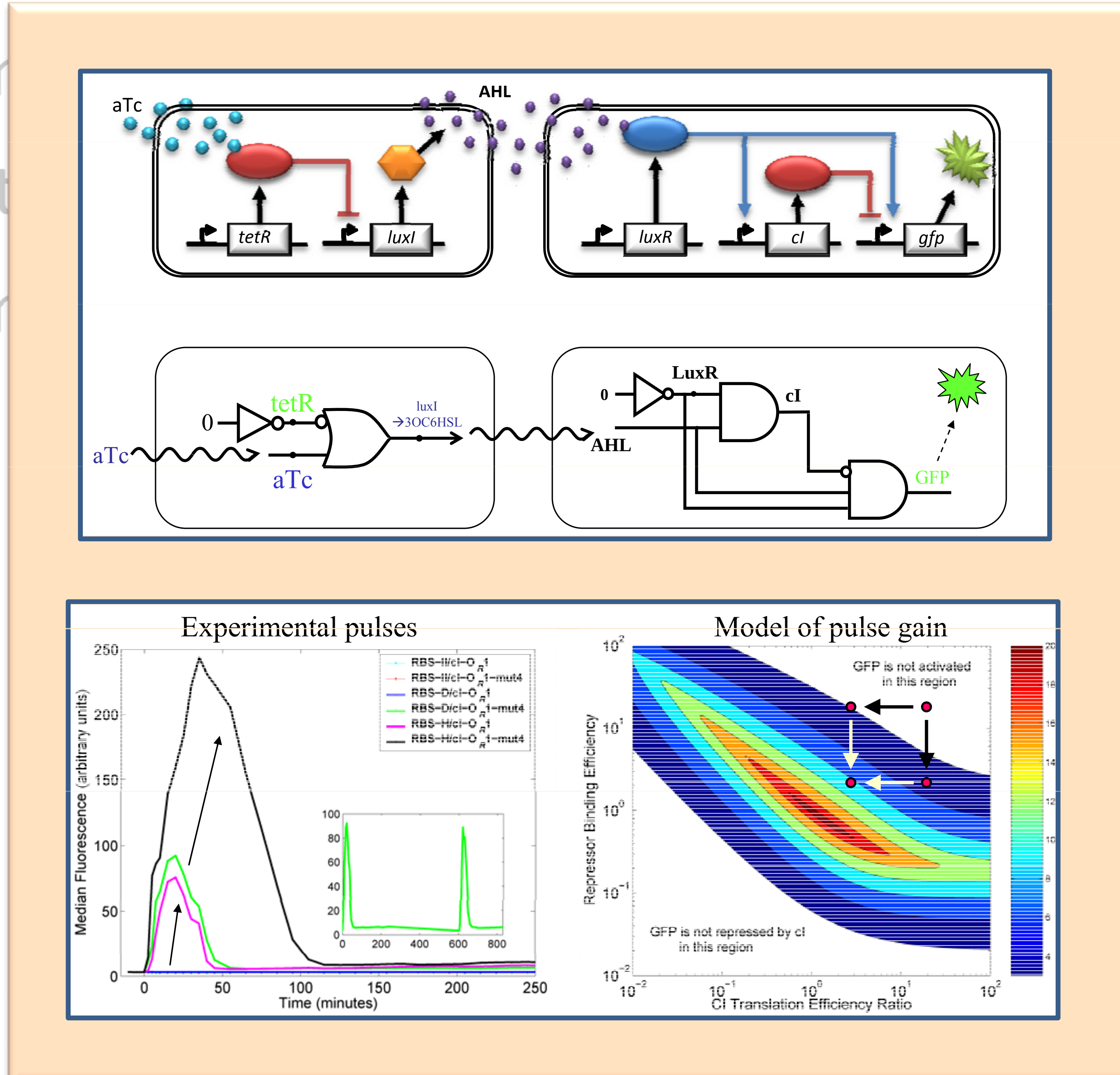




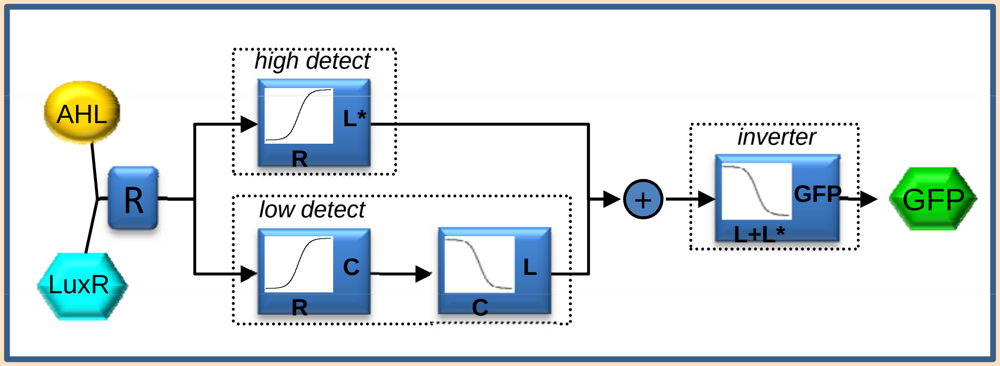


Useful CAD tools & mechanisms

- Param
- Sensit
- Param



Useful CAD tools & mechanisms



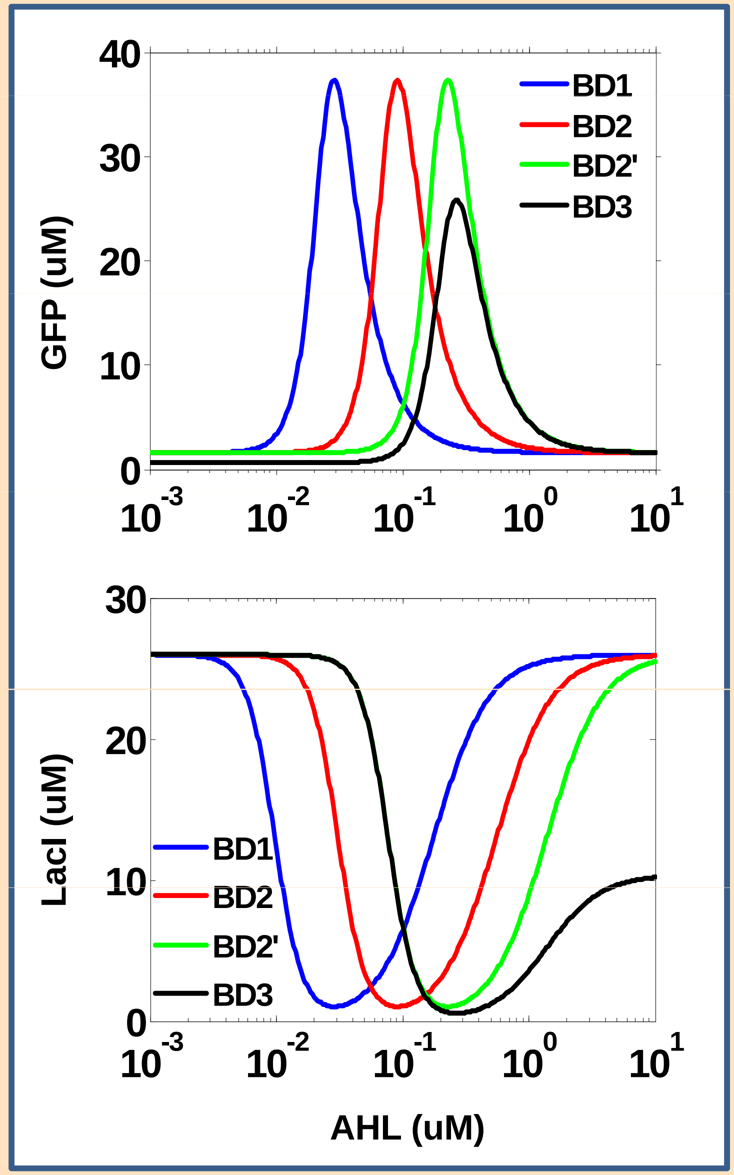
$$[R_{\mathrm{ss}}] = \frac{1}{\gamma_{\mathrm{R}}} \cdot \rho_R \cdot [LuxR_{\mathrm{ss}}]^2 \cdot [A_{\mathrm{ss}}]^2$$

$$[L^*{}_{\mathrm{ss}}] = \frac{1}{\gamma_{\mathrm{L}}} \cdot \frac{\alpha_L{}^* \cdot [R_{\mathrm{ss}}]^{\eta_R}}{[R_{\mathrm{ss}}]^{\eta_R} + \beta_R^{\eta_R}}$$

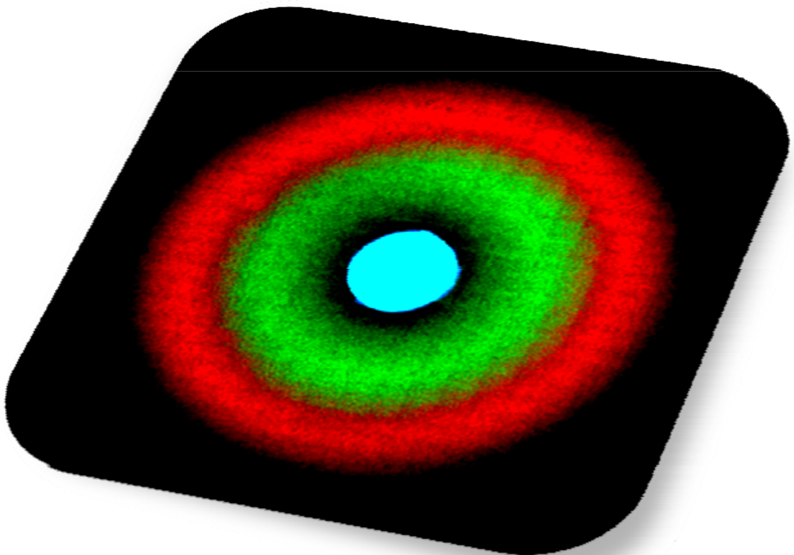
$$[C_{\mathrm{ss}}] = \frac{1}{\gamma_{\mathrm{C}}} \cdot \frac{\alpha_C \cdot [R_{\mathrm{ss}}]^{\eta_R}}{[R_{\mathrm{ss}}]^{\eta_R} + \beta_R^{\eta_R}}$$

$$[L_{\mathrm{ss}}] = \frac{1}{\gamma_{\mathrm{L}}} \cdot \frac{\alpha_L \cdot \beta_C^{\eta_C}}{\beta_C^{\eta_C} + [C_{\mathrm{ss}}]^{\eta_C}}$$

$$[G_{\mathrm{ss}}] = \frac{1}{\gamma_{\mathrm{G}}} \cdot \frac{\alpha_G \cdot \beta_L^{\eta_L}}{\beta_L^{\eta_L} + ([L_{\mathrm{ss}}] + [L^*{}_{\mathrm{ss}}])^{\eta_L}}$$

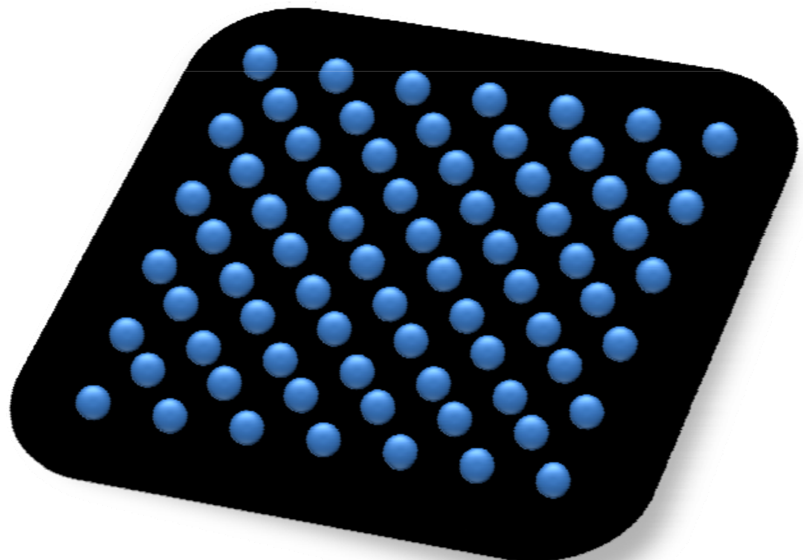


Global behavior ⇌ local rules



compile

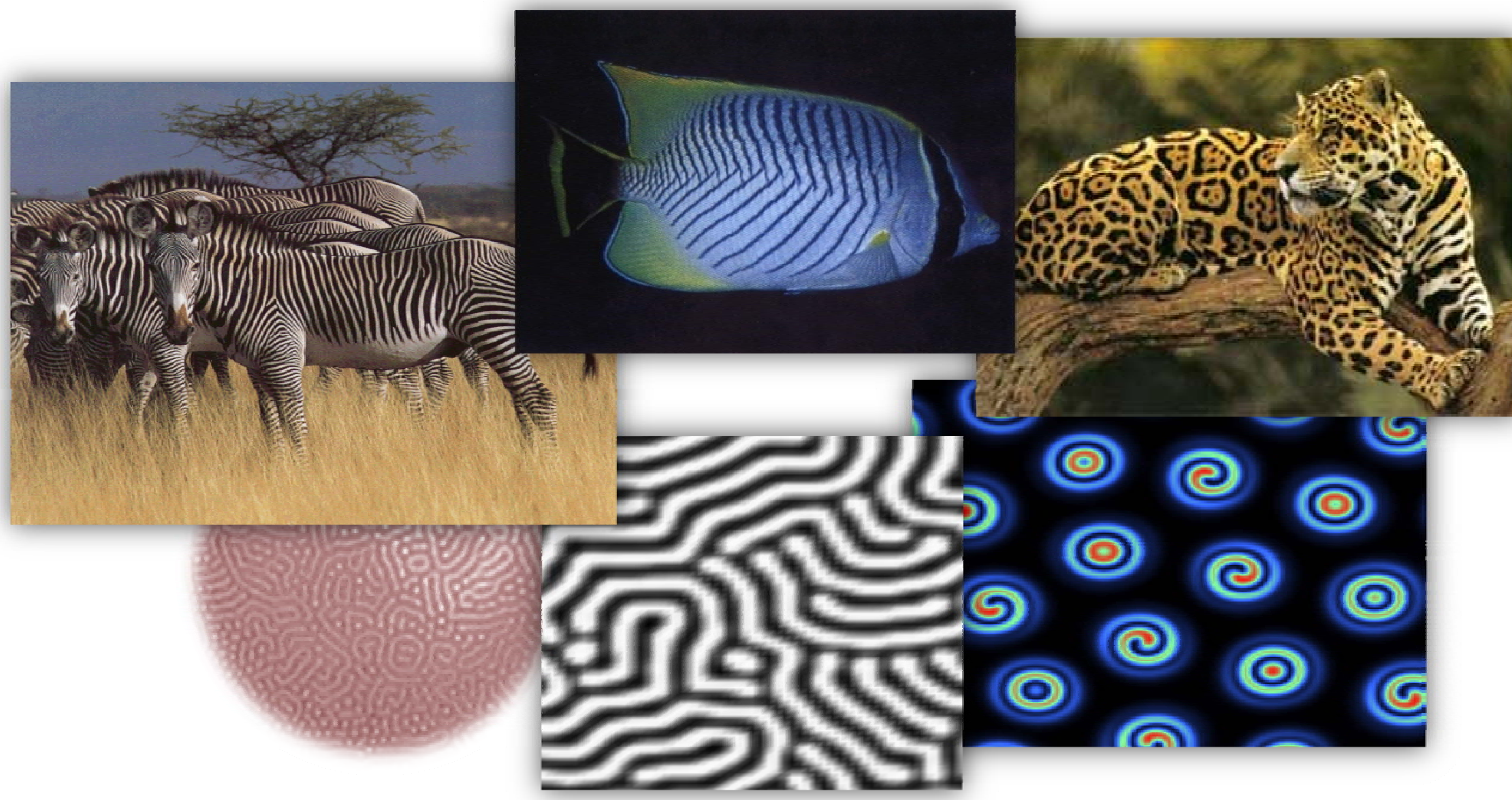
predict



High level
behavioral specification

Local rules of interaction

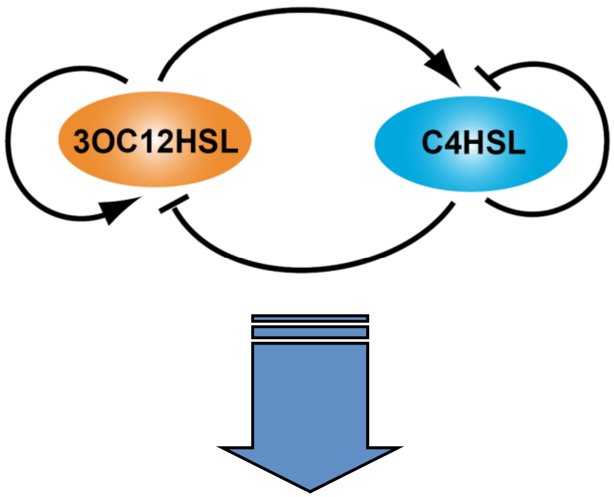
Turing patterns (Ting Lu)



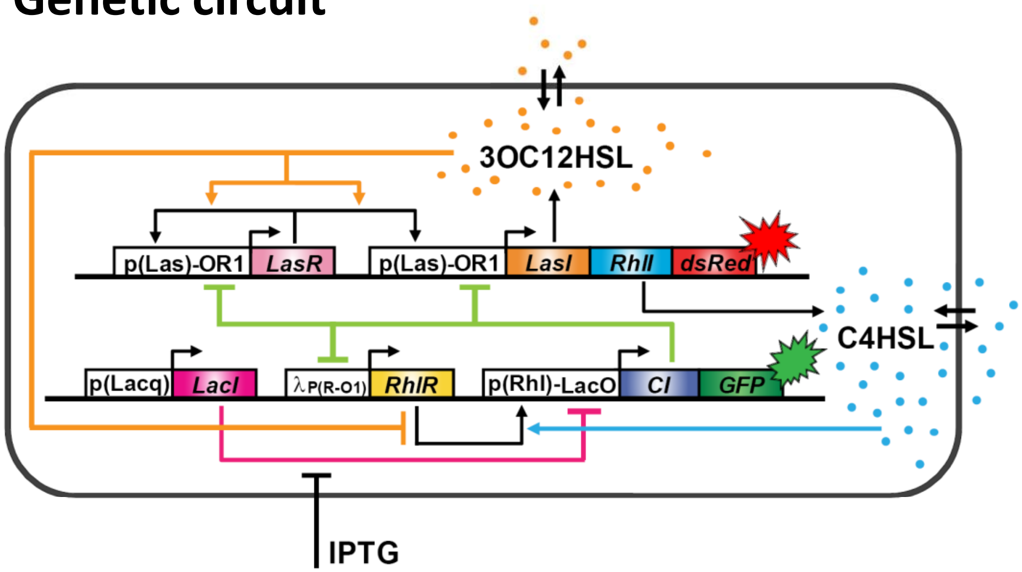
- Simple local rules → complex global behavior
- Non modular system
- Experimentally achievable using a reaction/diffusion system?
- How predictive are the mathematical models?
- Role of intuition and computation in circuit design?

Turing patterns: system design

Abstract design



Genetic circuit



Two morphogens

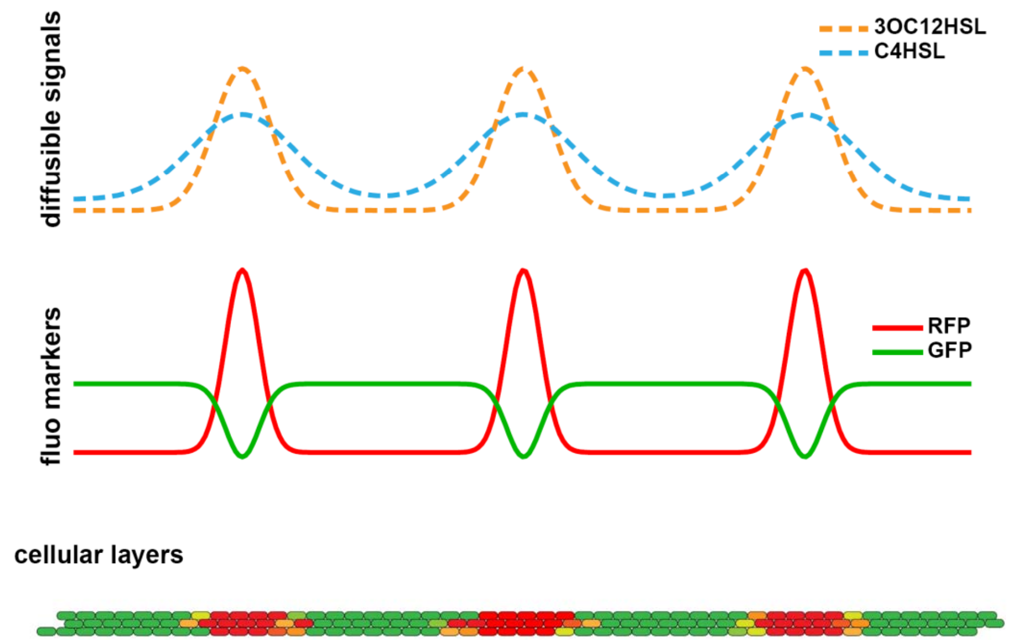
3OC12HSL: self activation, slow diffusion

C4HSL: Inhibition, faster diffusion

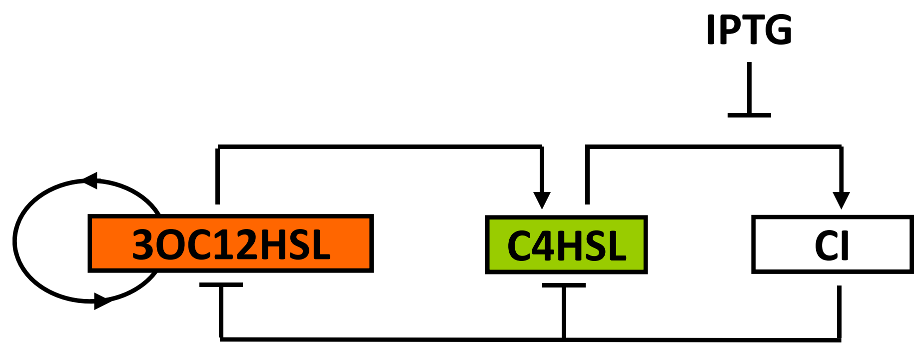
which satisfy ….

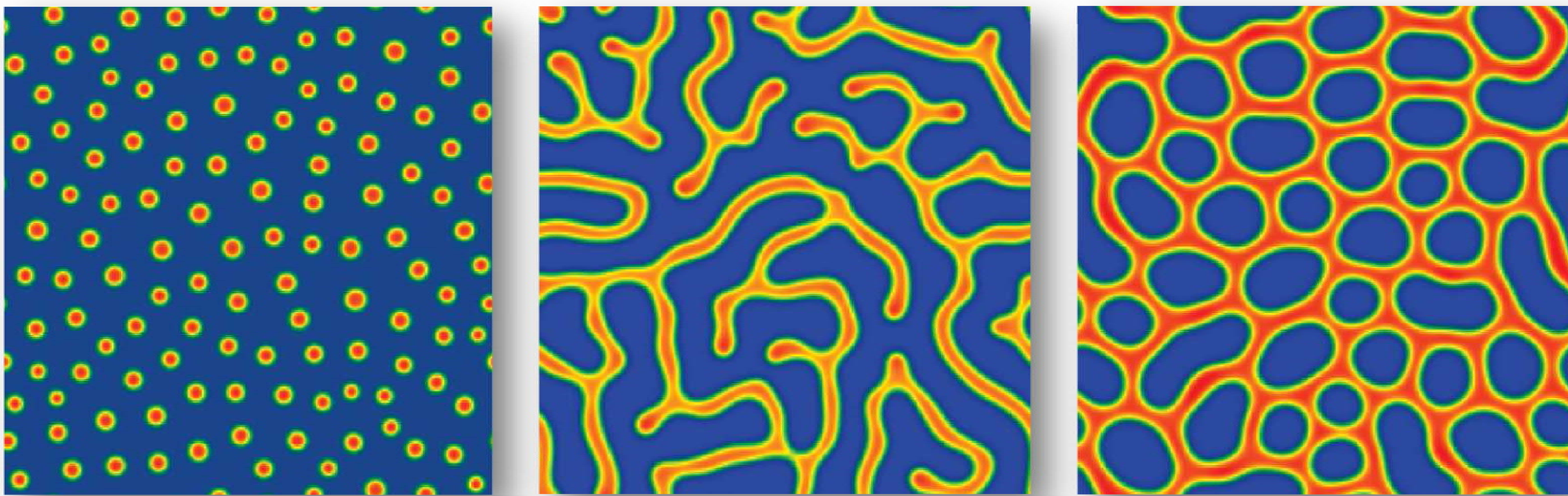
Physical requirements for Turing pattern

- Short-range autocatalytic activation
- Long-range inhibition

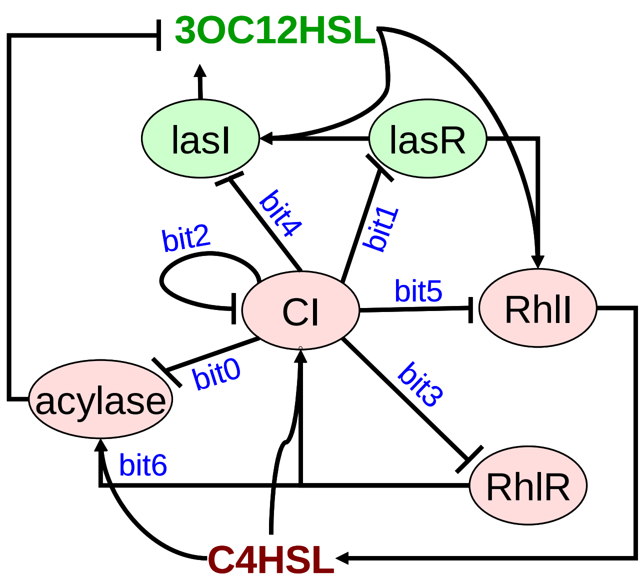


Patterns from different simulation conditions

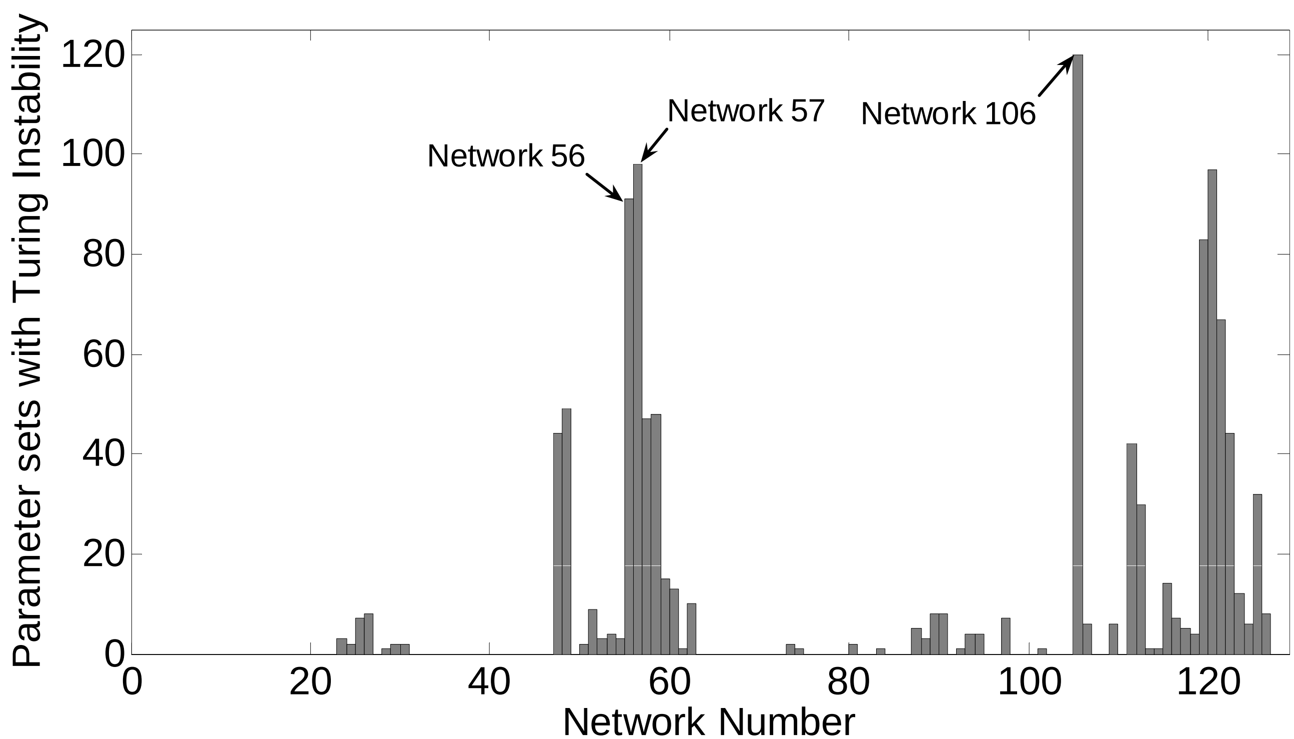


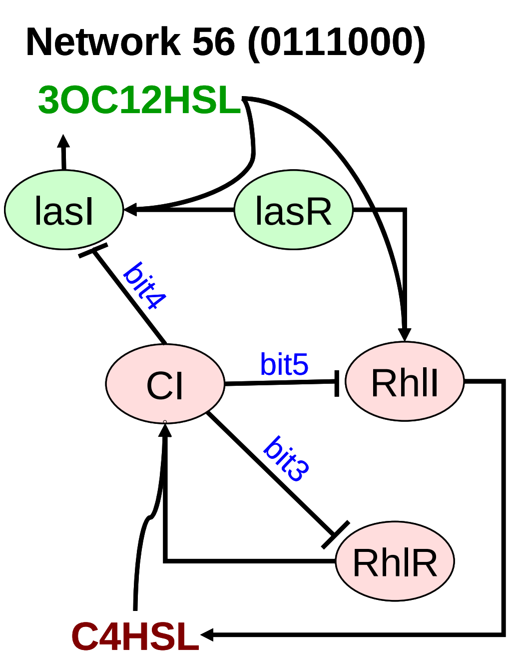


Search for best network structures



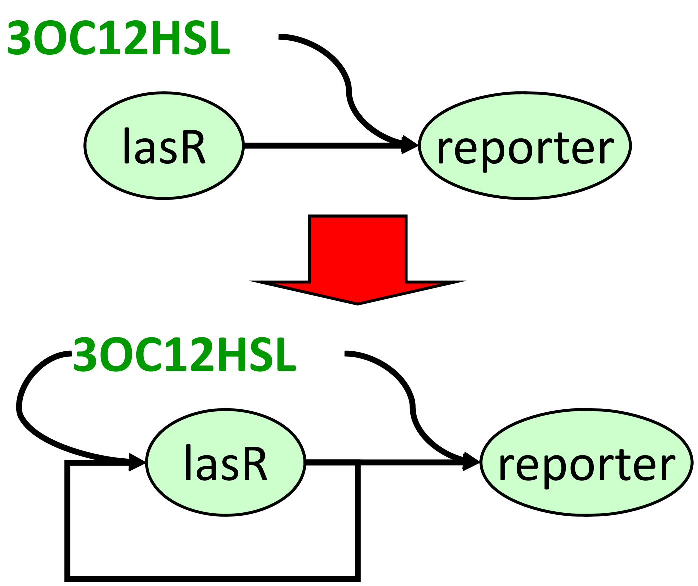
- Computational search for network variations enriched for Turing instabilities
  - Randomly chose parameter sets for exploring different network structures
  - Counted # of sets for each network that formed Turing instabilities

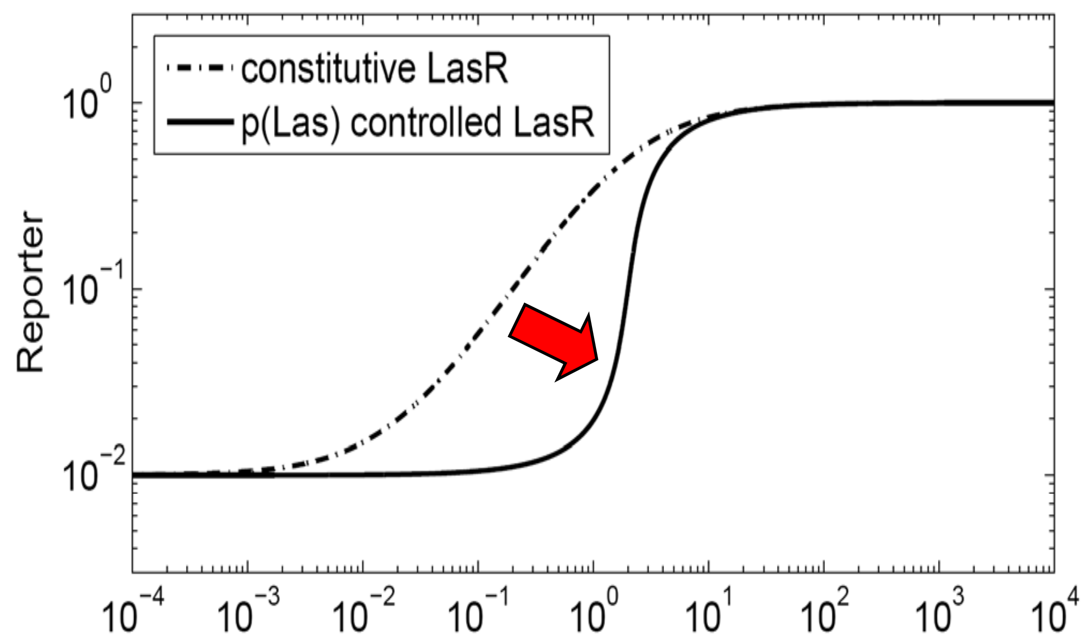




Examine role of parameter values

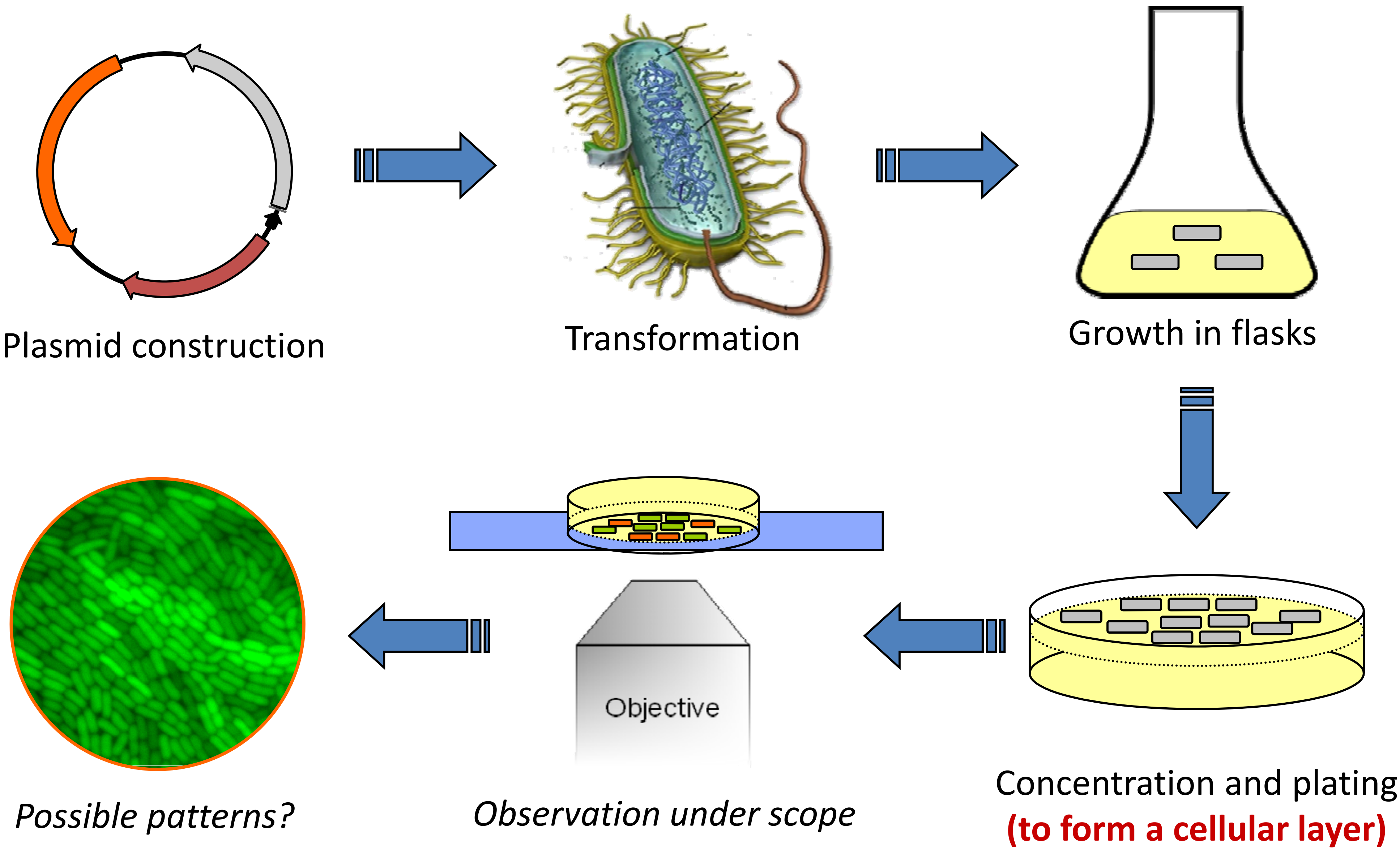
- Similarly, examined the effect of 3 Hill coefficients on pattern formation capability:
  - p(las)/3OC12HSL activation, p(rhl)/C4HSL activation, CI repression
- Results:
  - Hill coefficients of p(las) activation ≤ 1 **never** yield Turing instability
  - For p(rhl) activation, Hill coefficient ≤ 1 is 55% as likely to yield Turing instability as Hill coefficient ≥ 1
  - For CI repression, the relative likelihood is 37%
- Solution:



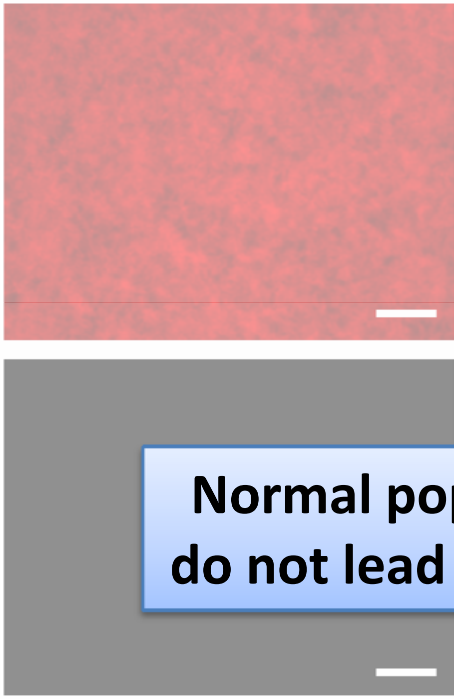


Experimental setup

Schematic illustration of experimental procedures

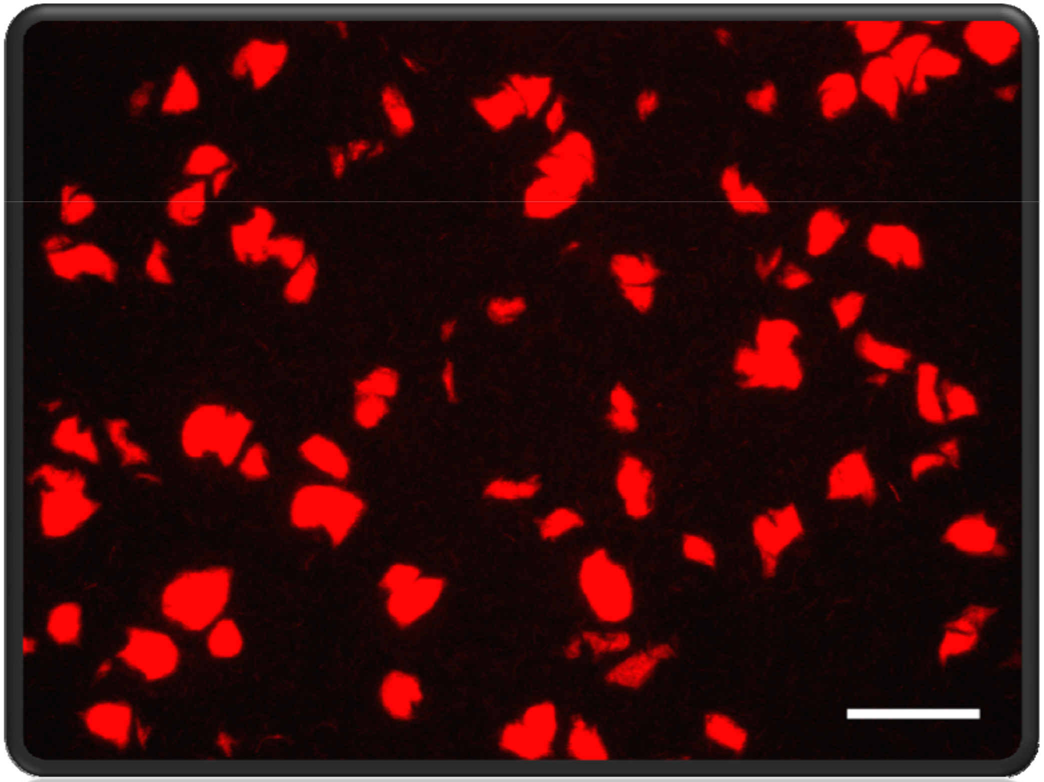


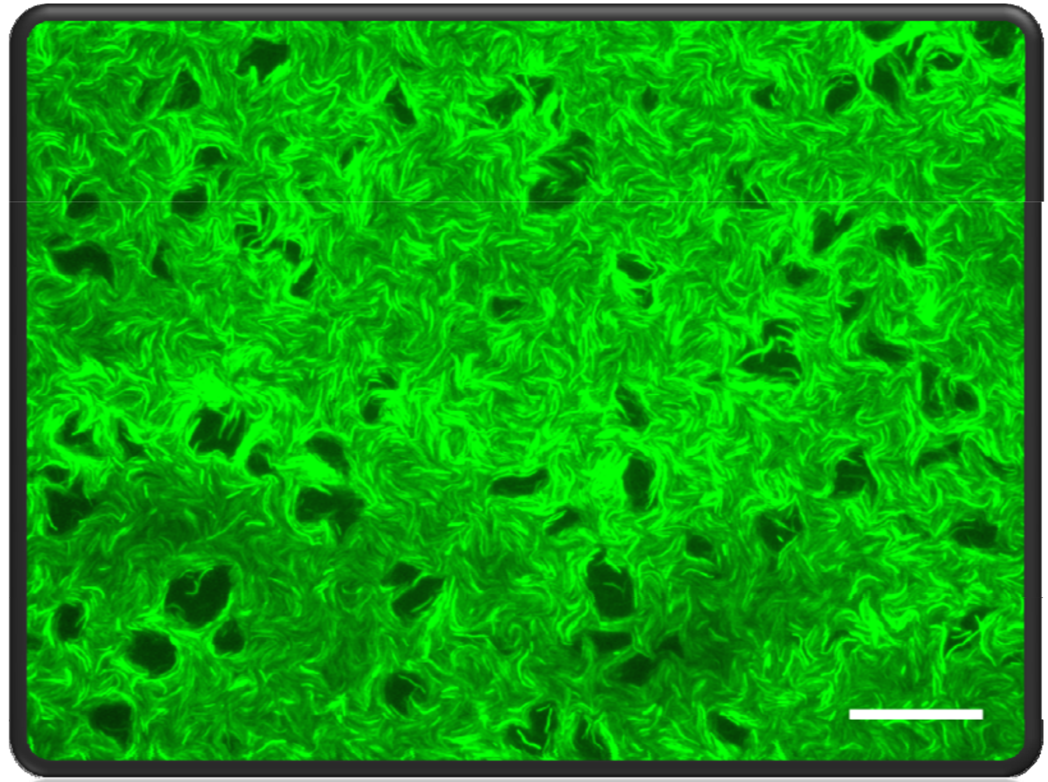
Patterns from various cell types



Normal population growth and initial spatial heterogeneity do not lead to spatial patterns observed in our Turing system

Turing patterns

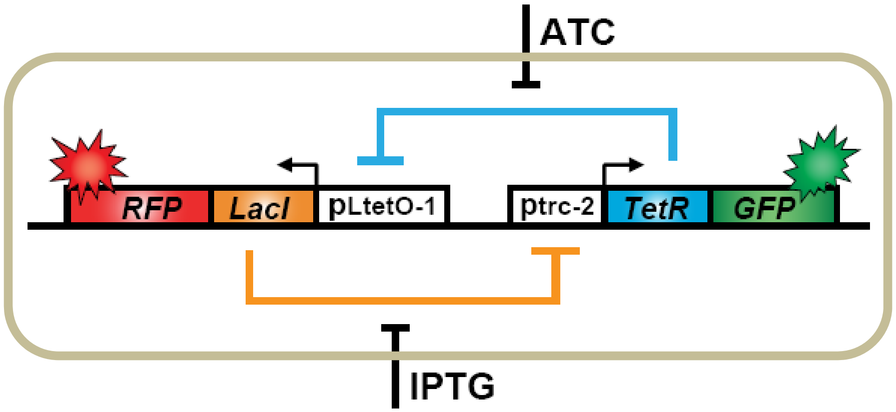




Question: ***Do cells make decisions individually or collectively when forming these spatial patterns?***

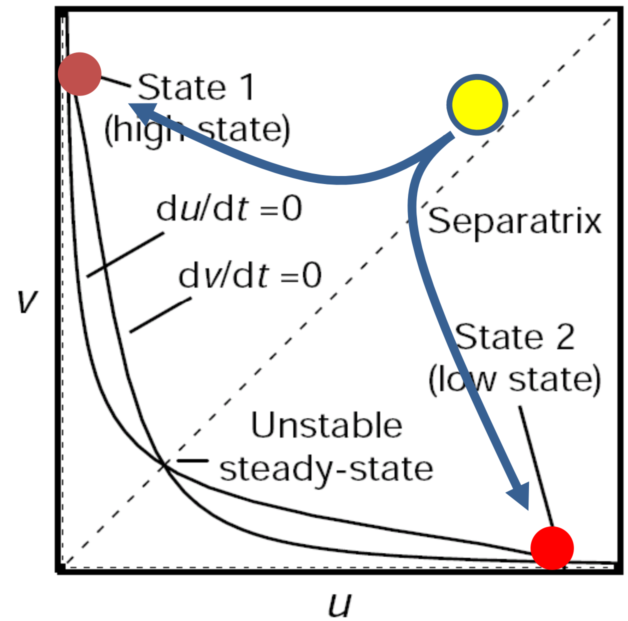
Patterns from toggle switch cells

A two-state switch
provides individual decision making

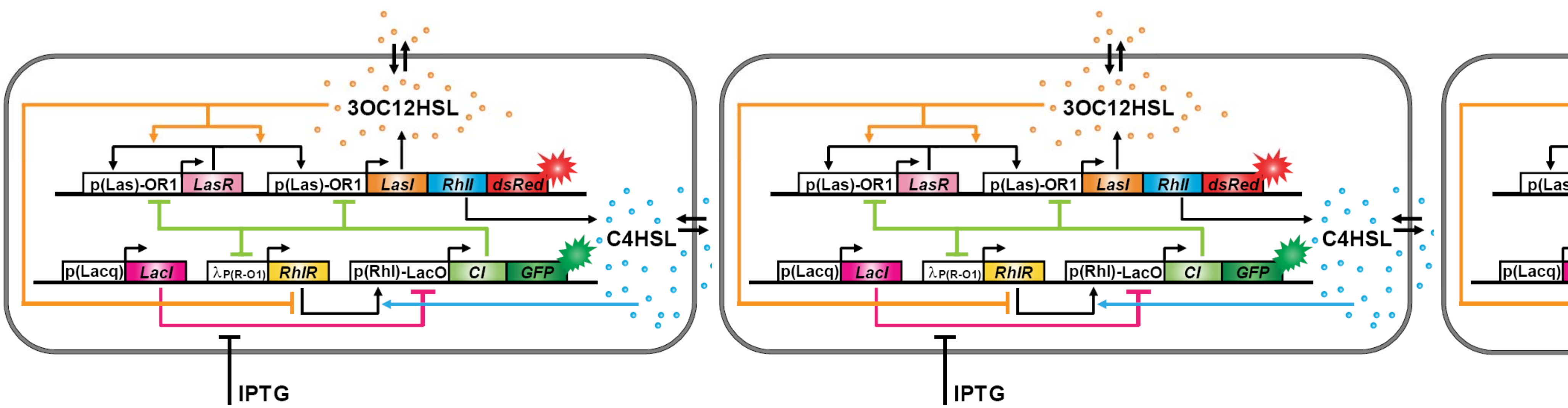


Circuit modified from Gardner et al. *Nature 403* (2000).

Phase diagram for the toggle switch



Our communication-based system



Patterns from toggle switch cells

A two-state switch

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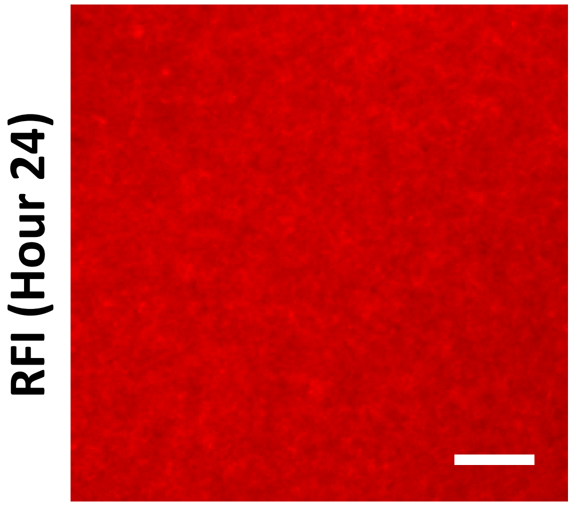
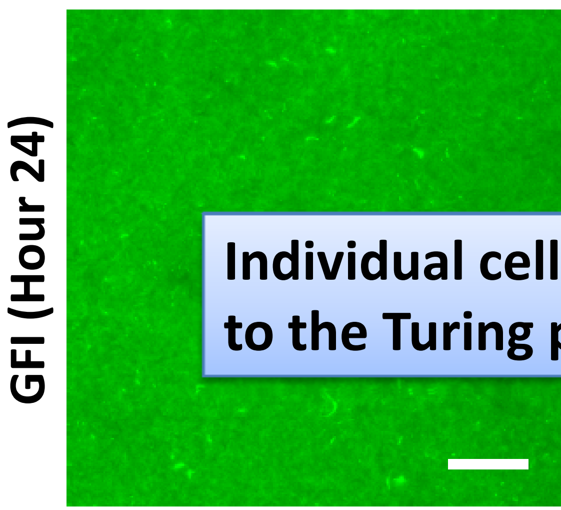
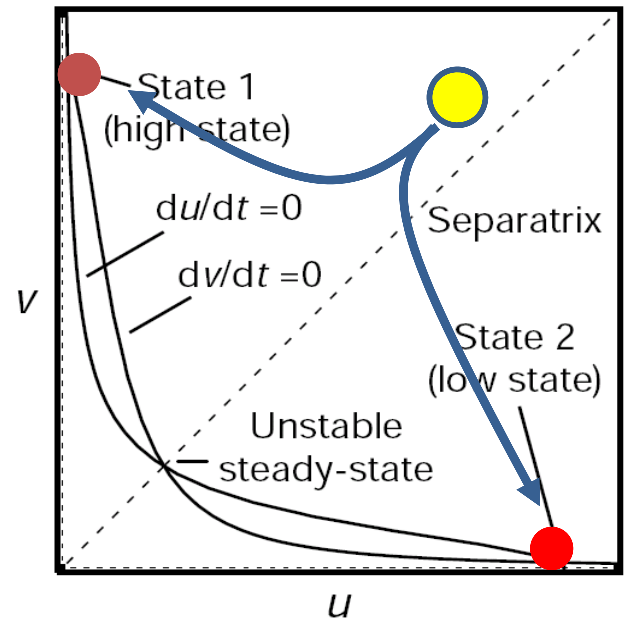
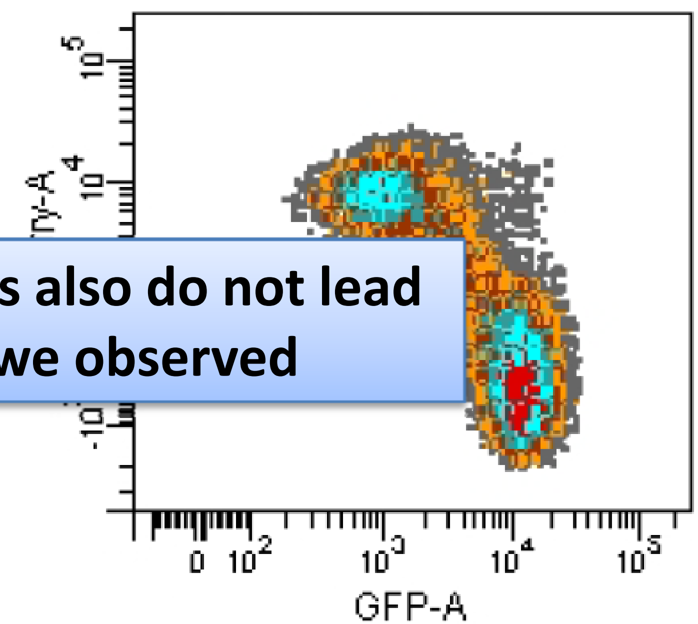




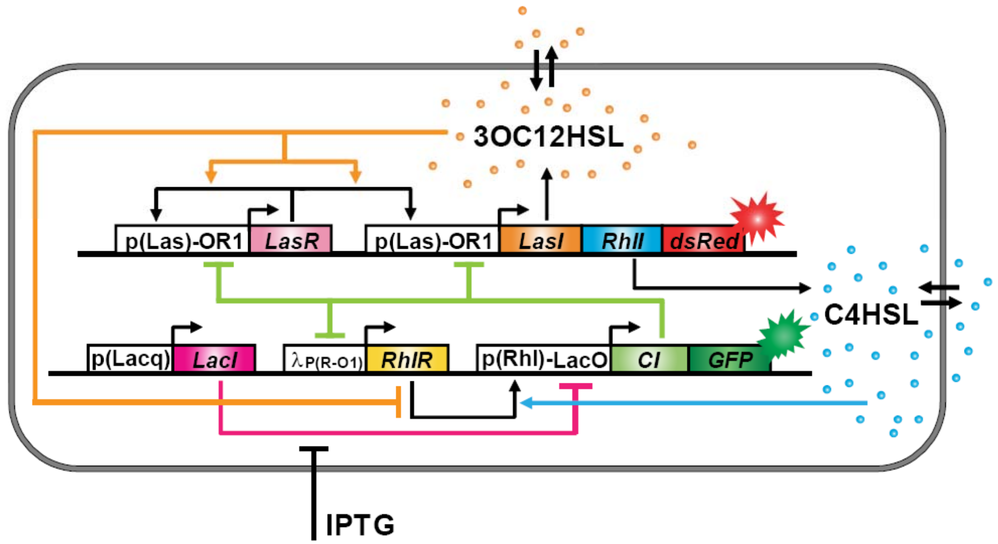


FACS data (at Hour 24)



Individual cell decisions also do not lead to the Turing patterns we observed

Mathematical model for Turing system



A deterministic reaction-diffusion model incorporating the major biochemical reactions and chemical diffusion

3OC12HSL $$\frac{\mathrm{d}U}{\mathrm{d}t} = \alpha_u I_u - \gamma_u U + D_u \nabla^2 U$$

C4HSL $$\frac{\mathrm{d}V}{\mathrm{d}t} = \alpha_v I_v - \gamma_v V + D_v \nabla^2 V$$

LasI $$\frac{\mathrm{d}I_u}{\mathrm{d}t} = \alpha_{iu} F_1(X_1, C) - \gamma_{iu} I_u$$

RhlI $$\frac{\mathrm{d}I_v}{\mathrm{d}t} = \alpha_{iv} F_1(X_1, C) - \gamma_{iv} I_v$$

CI $$\frac{\mathrm{d}C}{\mathrm{d}t} = \alpha_c F_2(X_2, L) - \gamma_c C$$

where,

$$F_1(X_1, C) = \frac{[1 + f_1(\frac{X_1}{K_{d1}})^{\theta_1}][1 + f_2^{-1}(\frac{C}{K_{d2}})^{\theta_2}]}{[1 + (\frac{X_1}{K_{d1}})^{\theta_1}][1 + (\frac{C}{K_{d2}})^{\theta_2}]}$$

$$F_2(X_2, L) = \frac{[1 + f_3(\frac{X_2}{K_{d3}})^{\theta_3}][1 + f_4^{-1}(\frac{L}{K_{d4}})^{\theta_4}]}{[1 + (\frac{X_2}{K_{d3}})^{\theta_3}][1 + (\frac{L}{K_{d4}})^{\theta_4}]}$$

$$X_1 = R_u U$$

$$X_2 = \frac{R_v V}{(1 + U/K_{c3})}$$

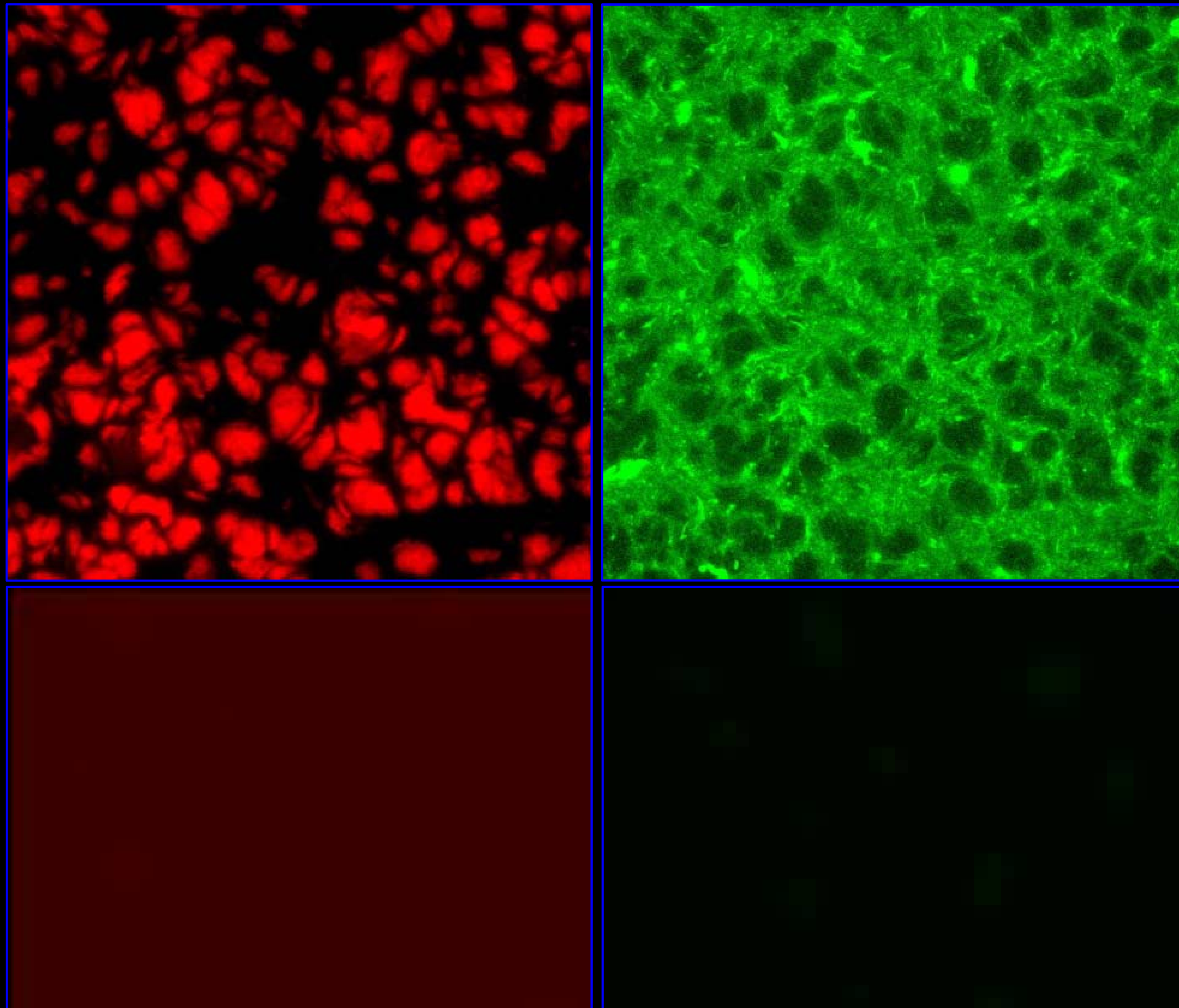
$$R_u = \lambda_u I_u$$

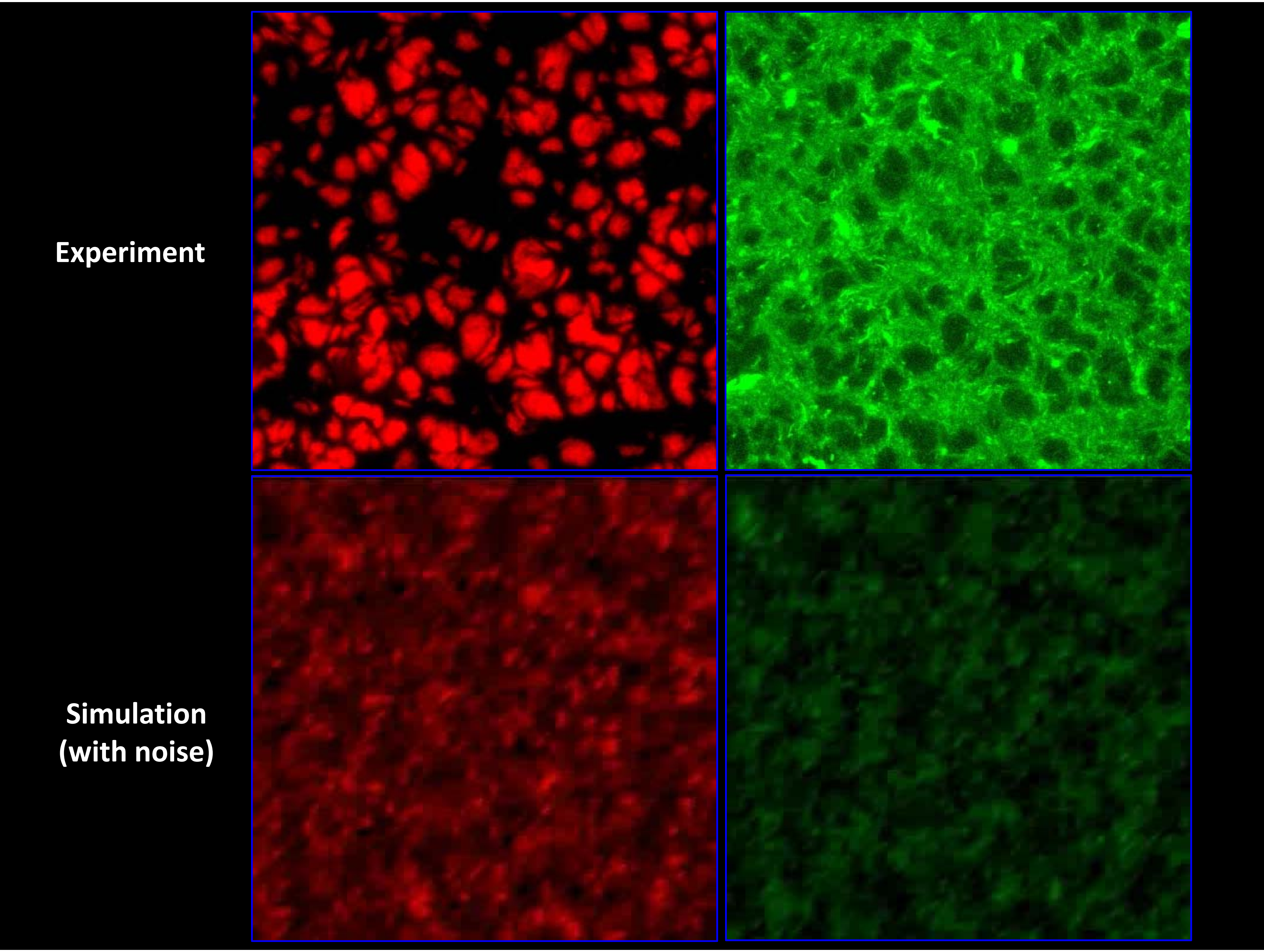
$$R_v = \lambda_v \left(\frac{1 + f_5^{-1} (C/K_{d5})^{\theta_5}}{1 + (C/K_{d5})^{\theta_5}} \right)$$

$$L = \lambda_l \left(\frac{1 + f_6^{-1} \, (I/K_{d6})^{\theta_6}}{1 + (I/K_{d6})^{\theta_6}} \right)$$

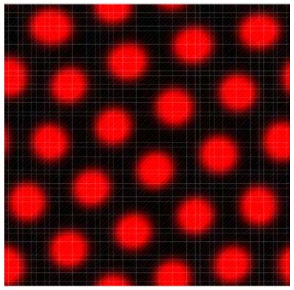
Experiment

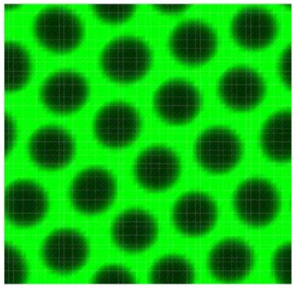
Simulation



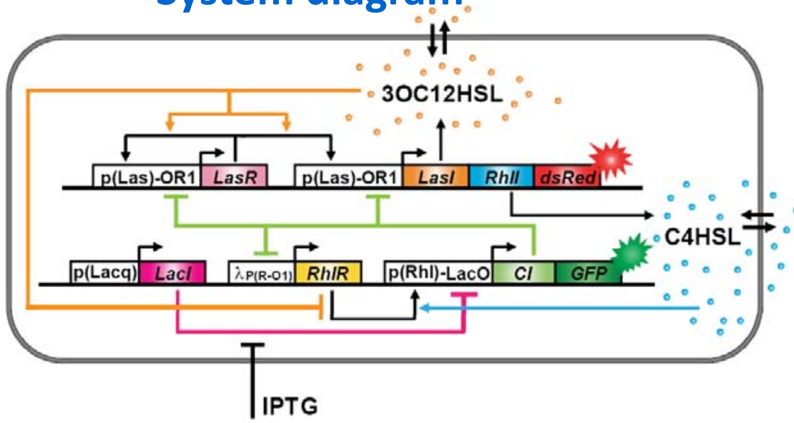

Experiment
Simulation
(with noise)

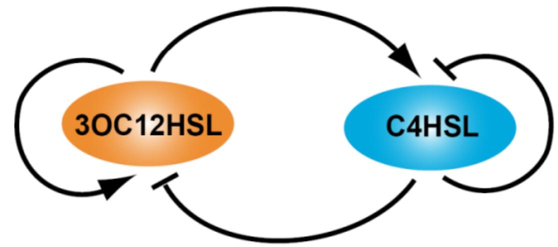
Pattern modulation with IPTG



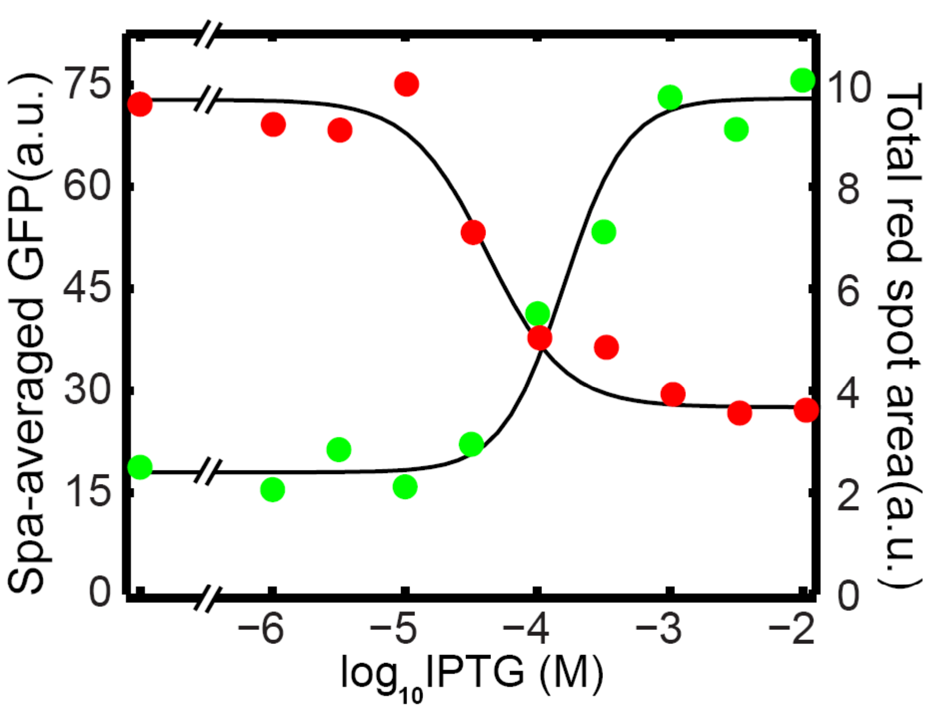


System diagram

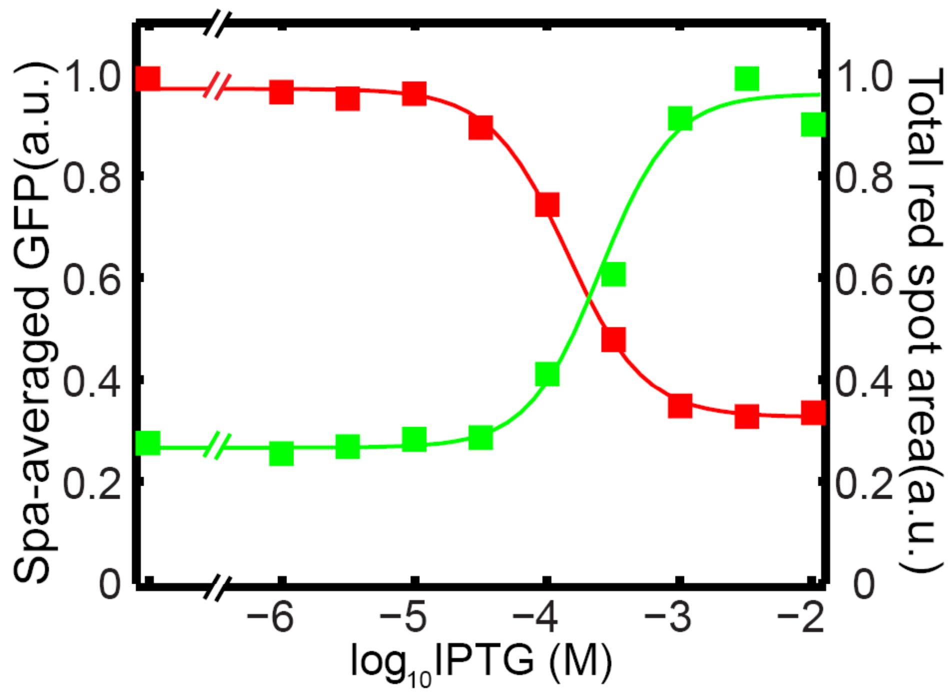




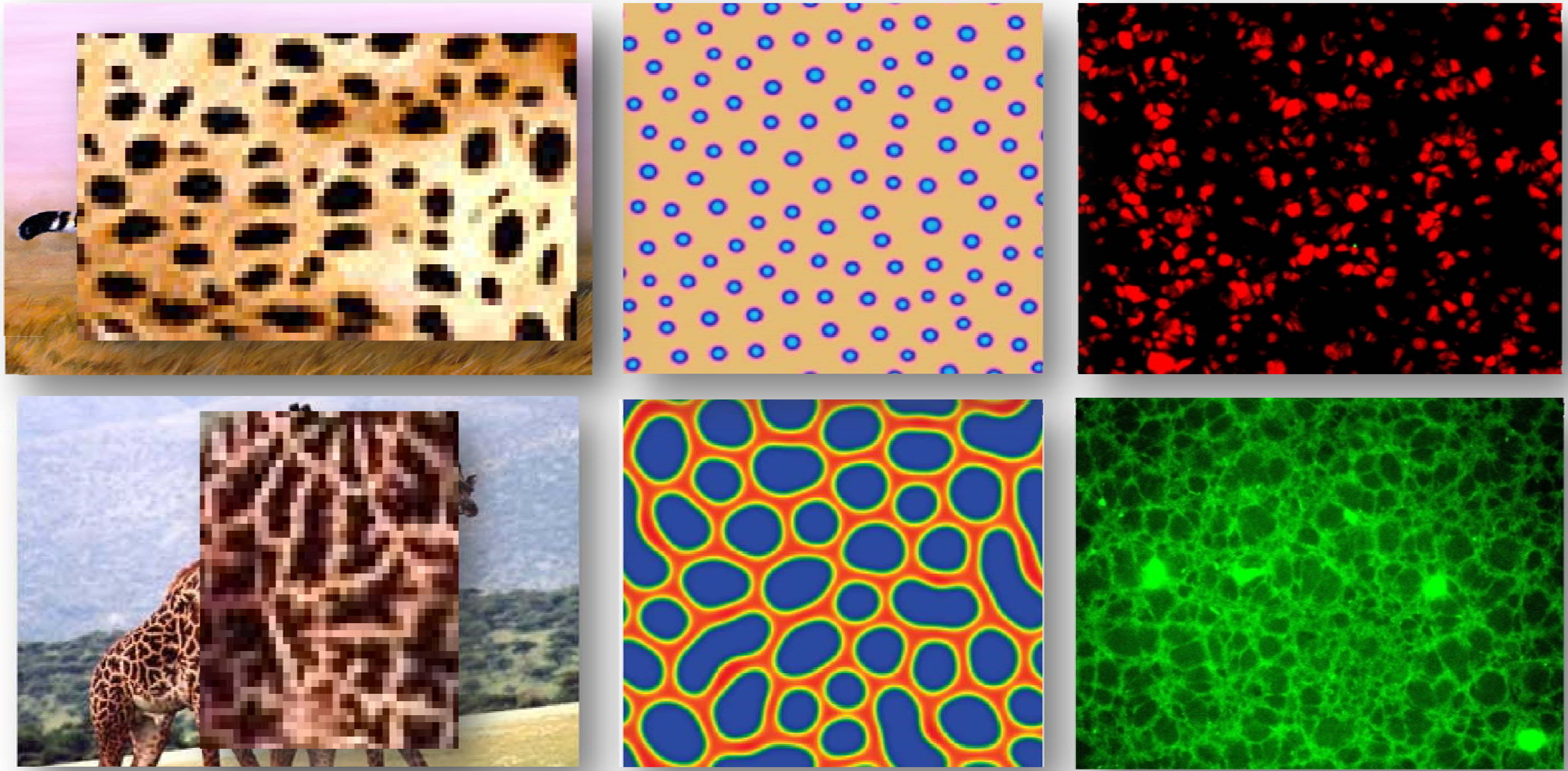
Experimental results



Computational results

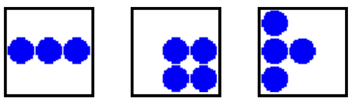
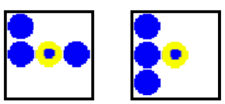
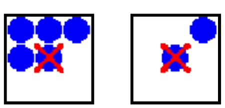


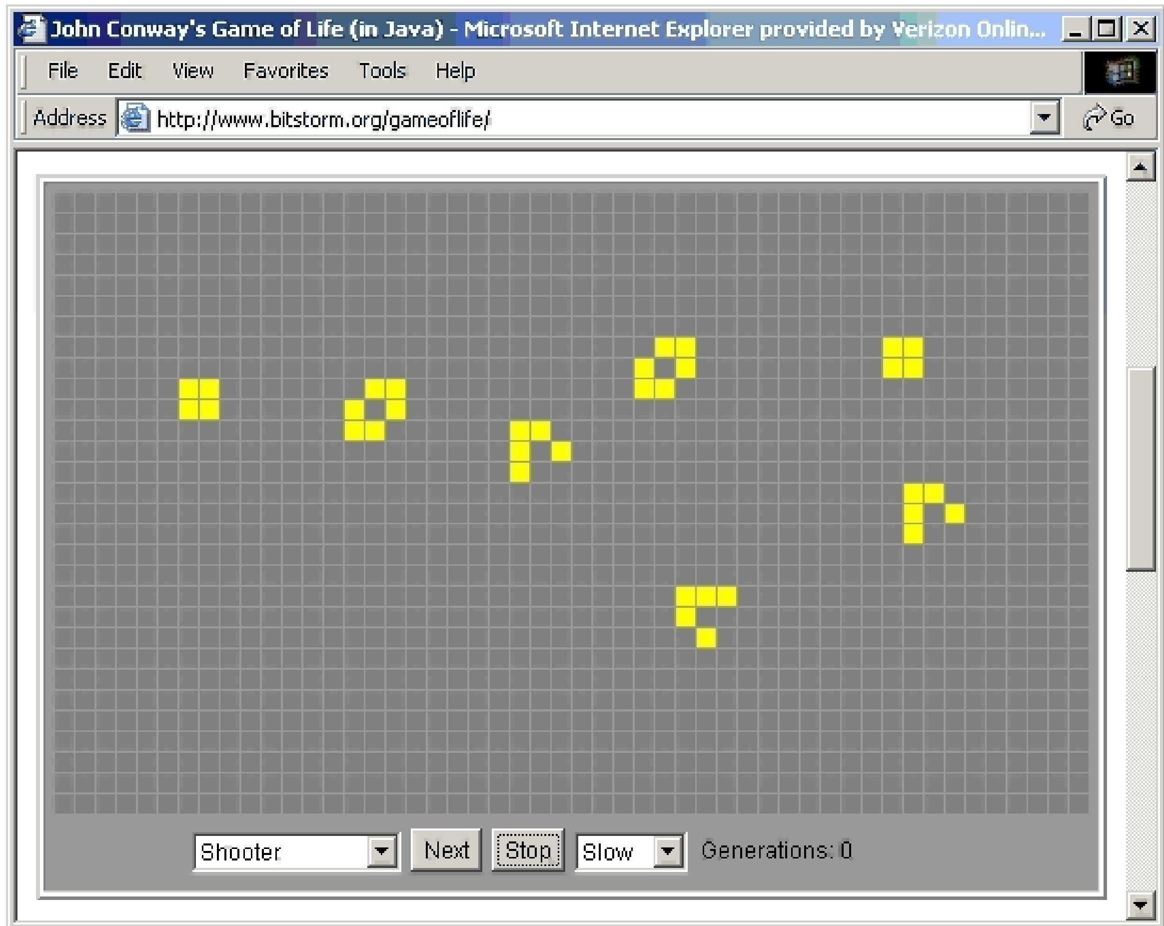
Nature, models, and synthetic biology



- Demonstrated control over emergent patterns
- Models (with noise) correlate well with experiments
  - Performing spectral analysis
- To achieve smoother patterns:
  - Different circuits, perhaps with more signals?
  - What are the limits?

Conway’s Game of Life (Jingjing Sun)

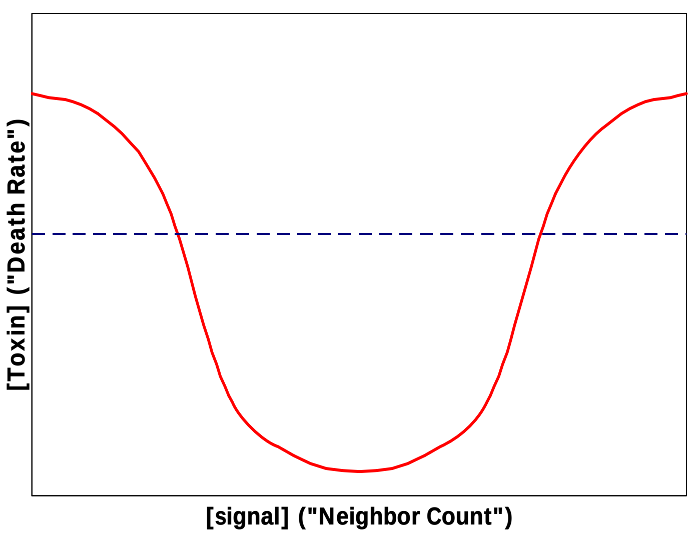
- Interesting dynamics with a single signaling molecule
- Simple local rules → complex global behavior:
  - Survival:
    2/3 neighbors
    
  - Birth:
    3 neighbors
    
  - Death:
    ≥4 or ≤1 neighbors
    

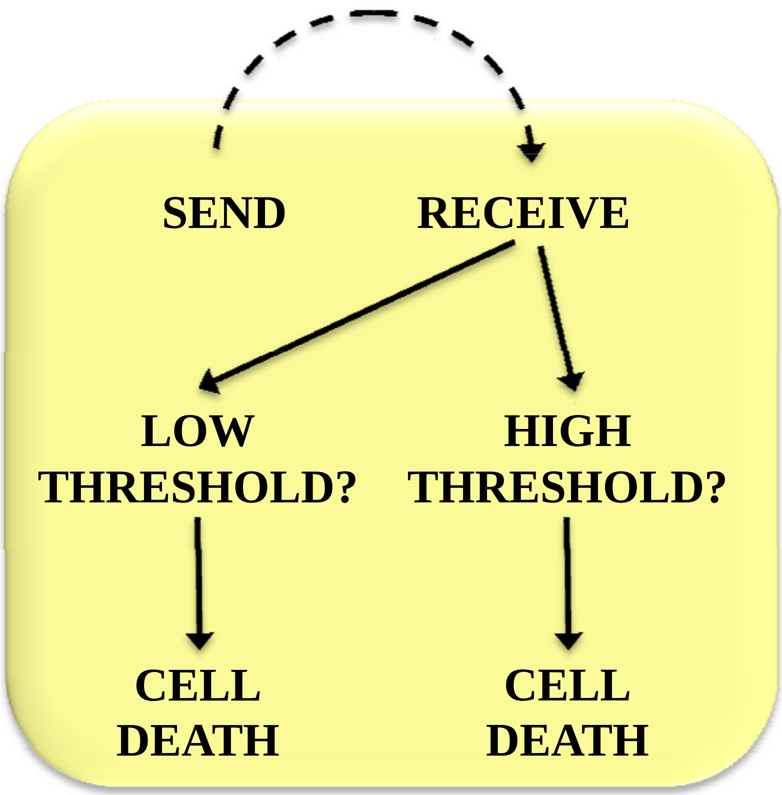


- Global behavior has been proven to be unpredictable

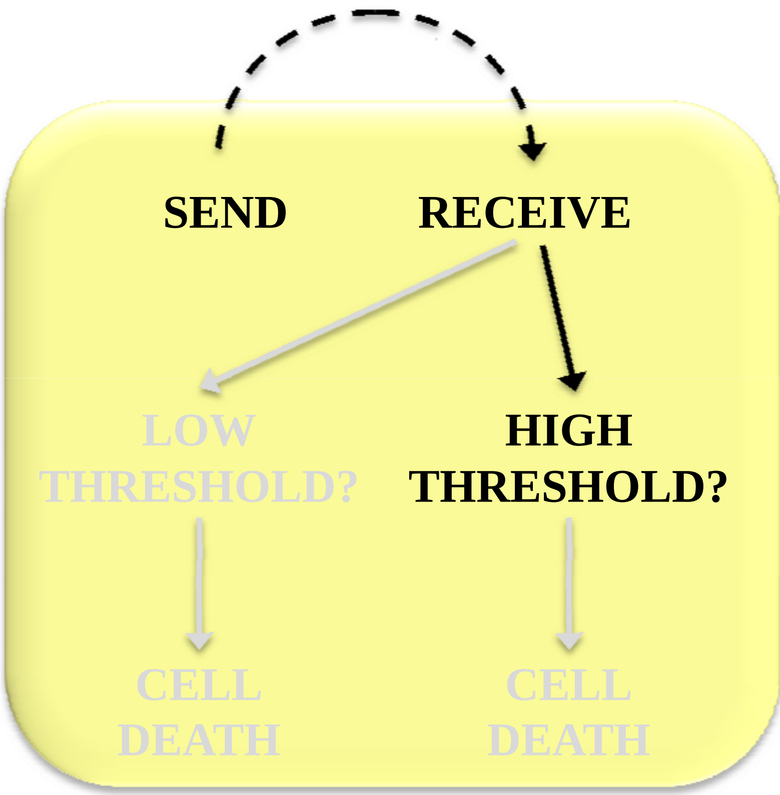
Conway's Game of Life in yeast

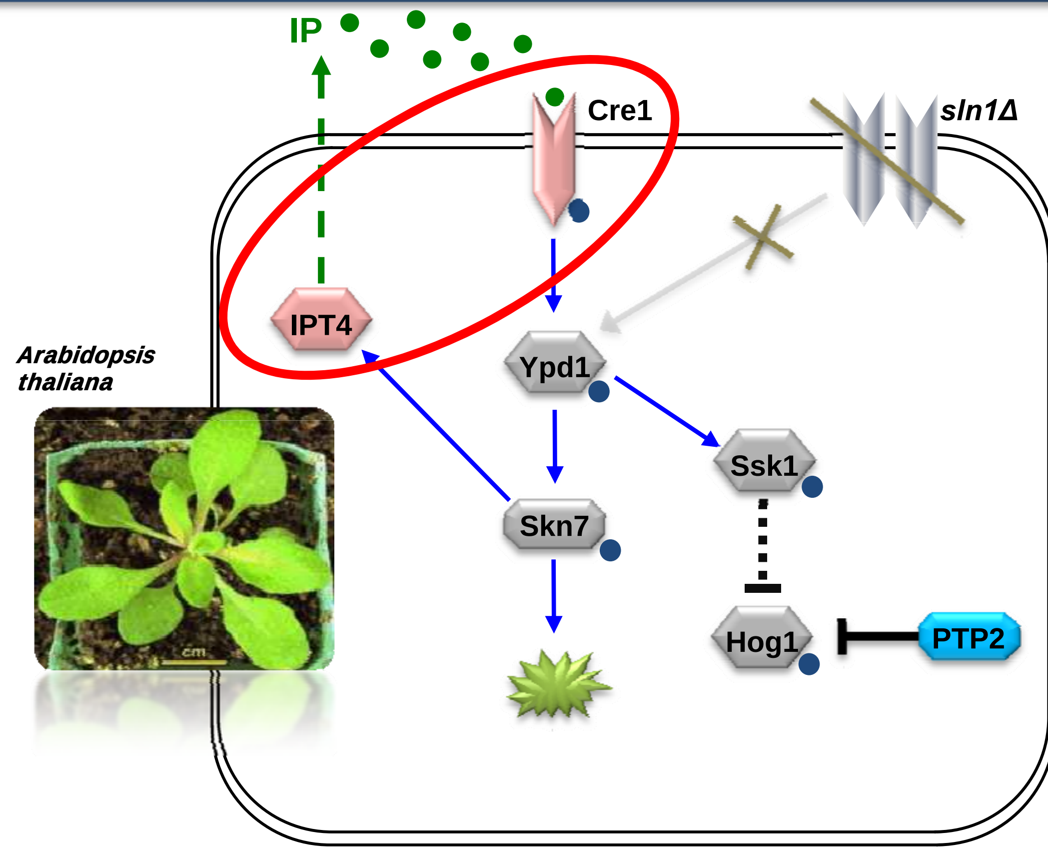
- Artificial cell-cell communication to allow yeast to detect neighboring population density
- Population density circuits
  - High threshold detection
  - Low threshold detection
- Cell killing mechanism

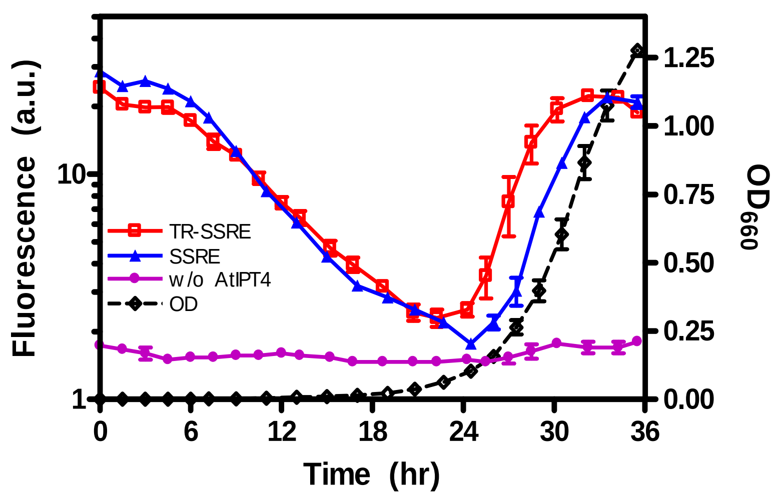


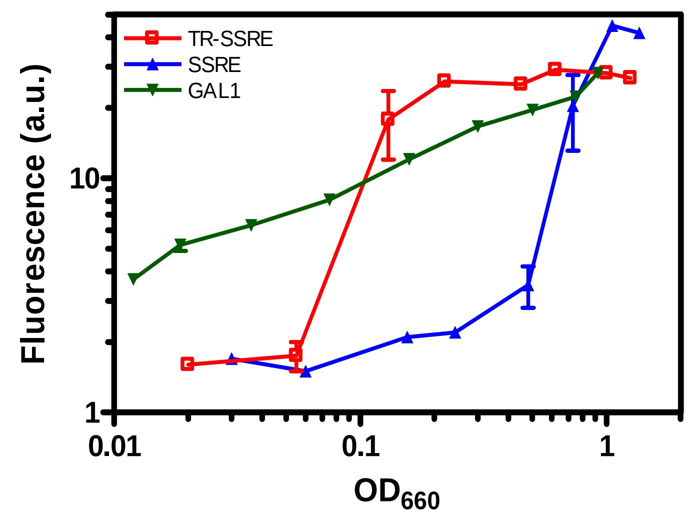


Artificial "quorum sensing" yeast

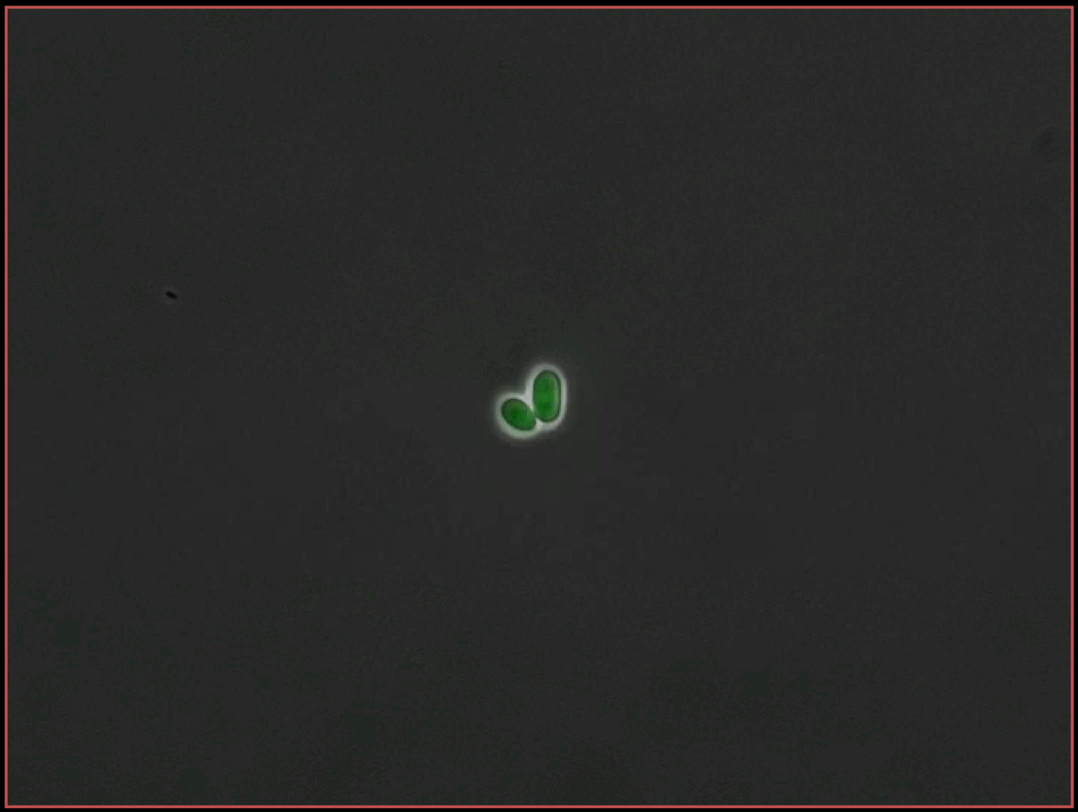


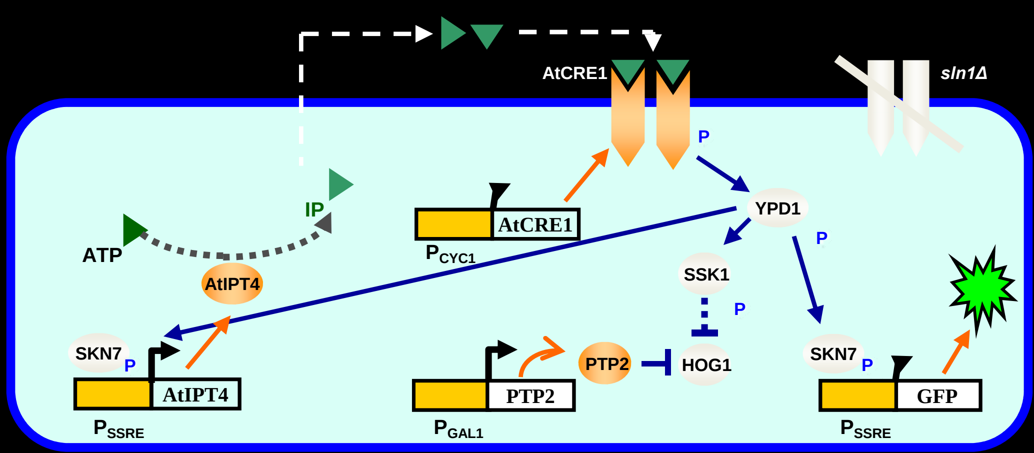


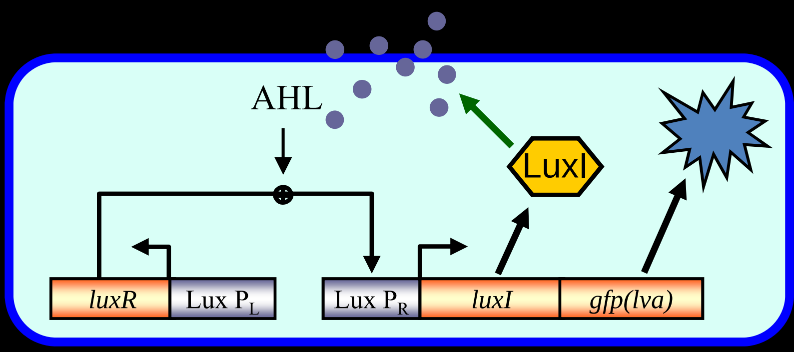




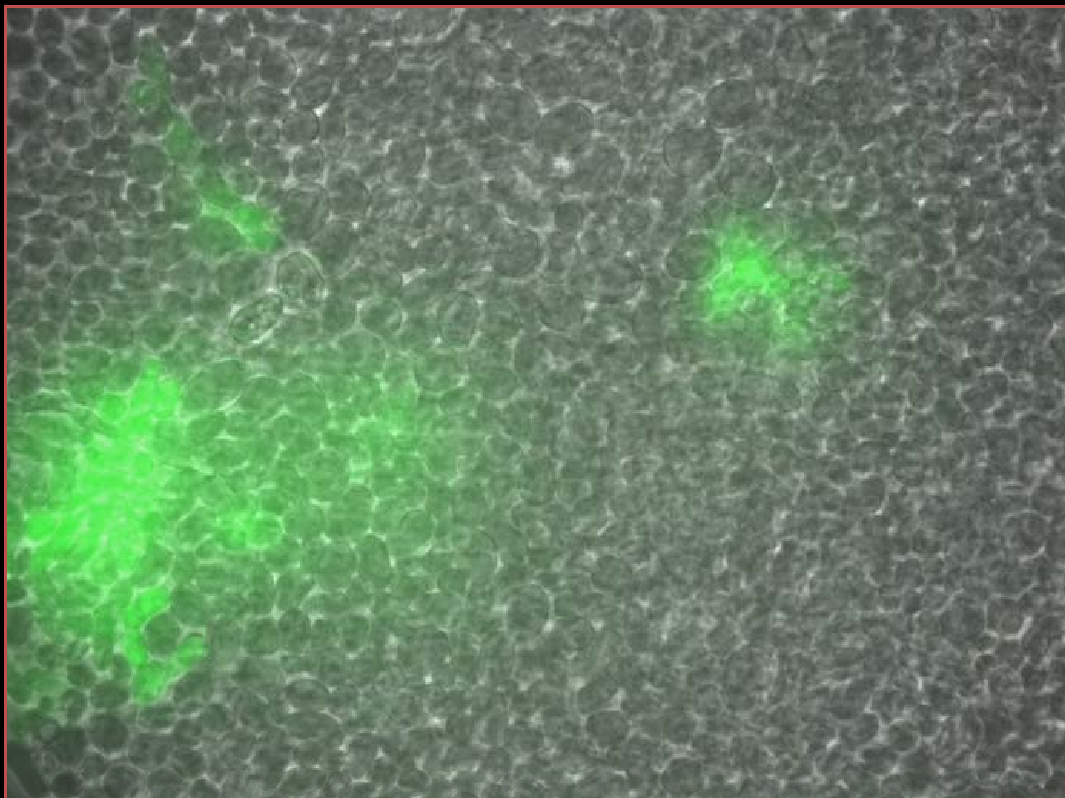
Comparison of quorum sensing

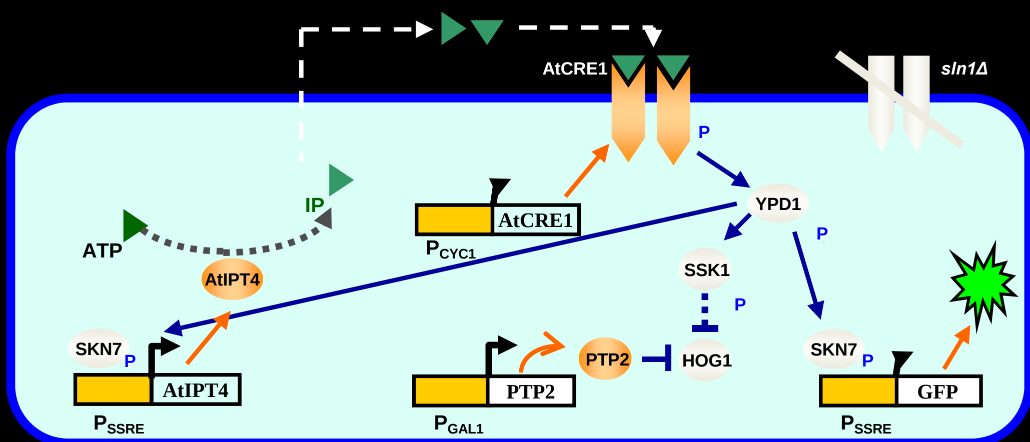


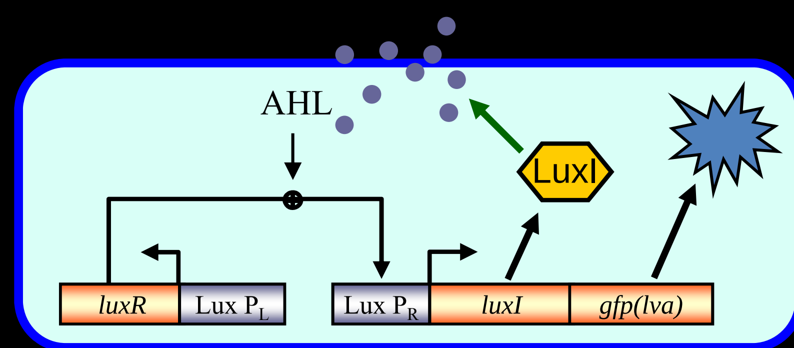




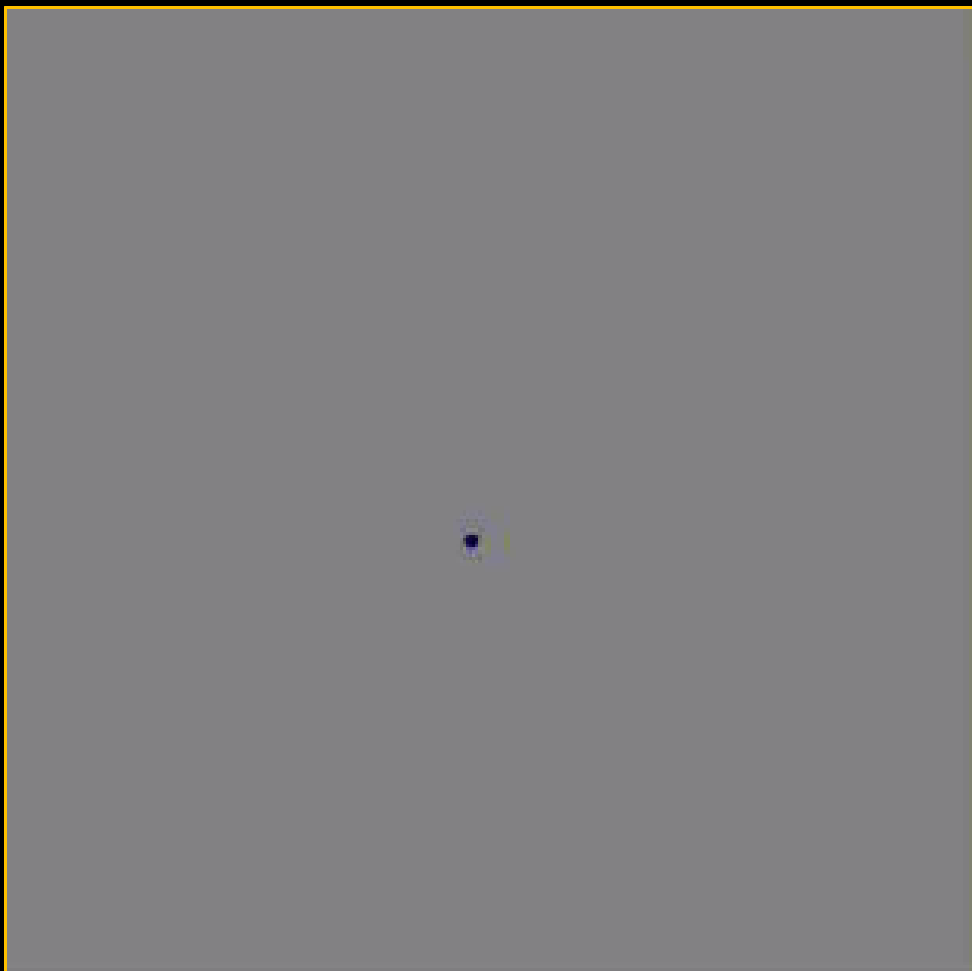
Comparison of quorum sensing



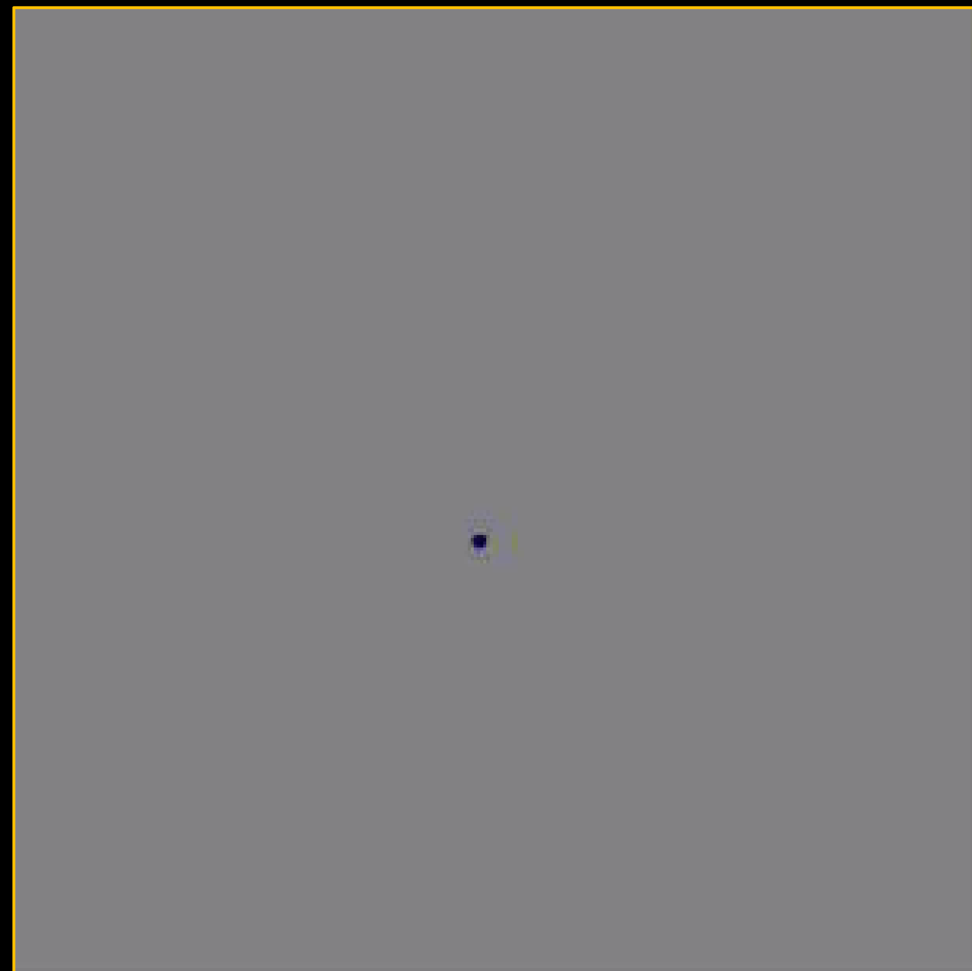




Quorum sensing simulation movies

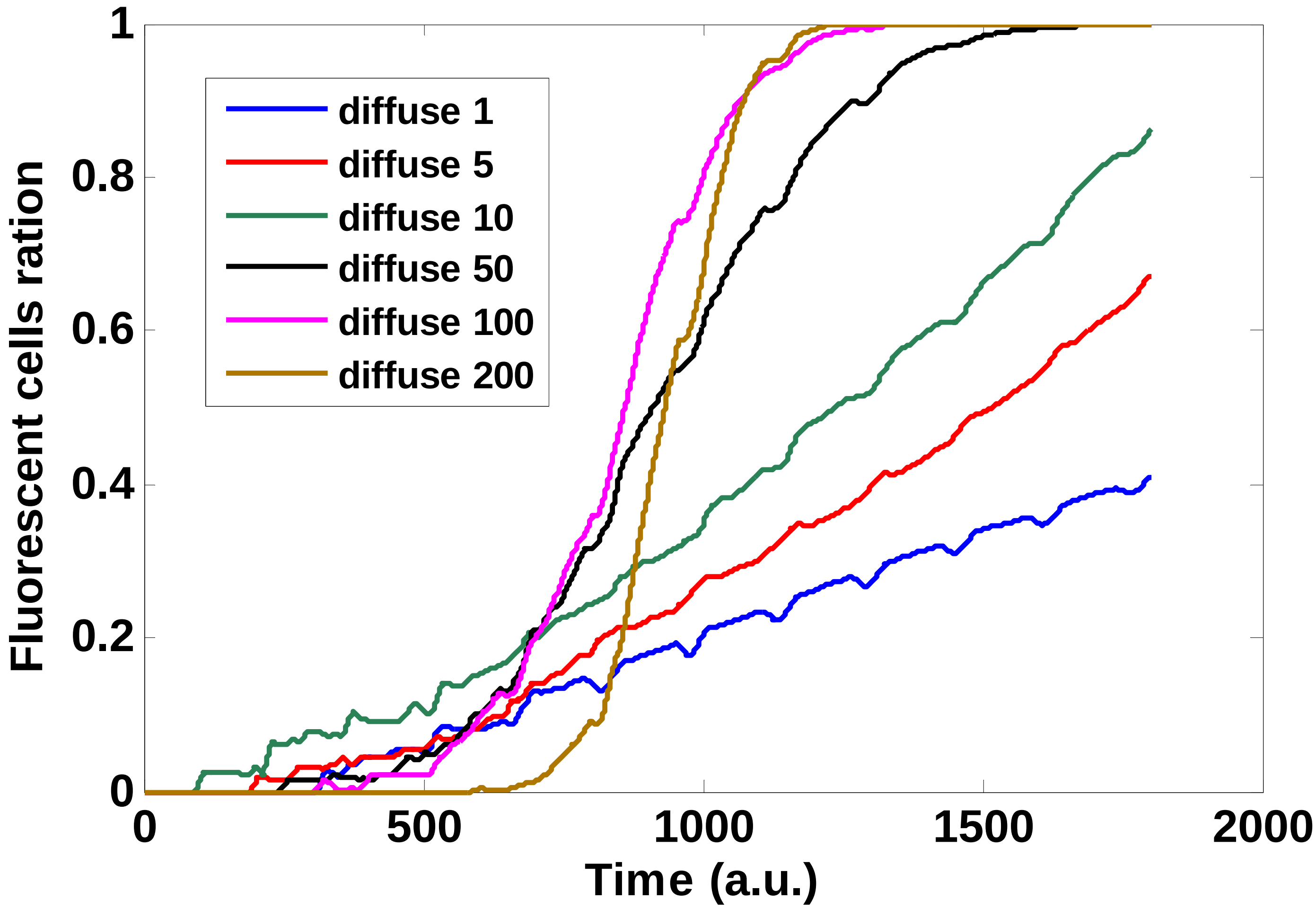


Slow diffusion



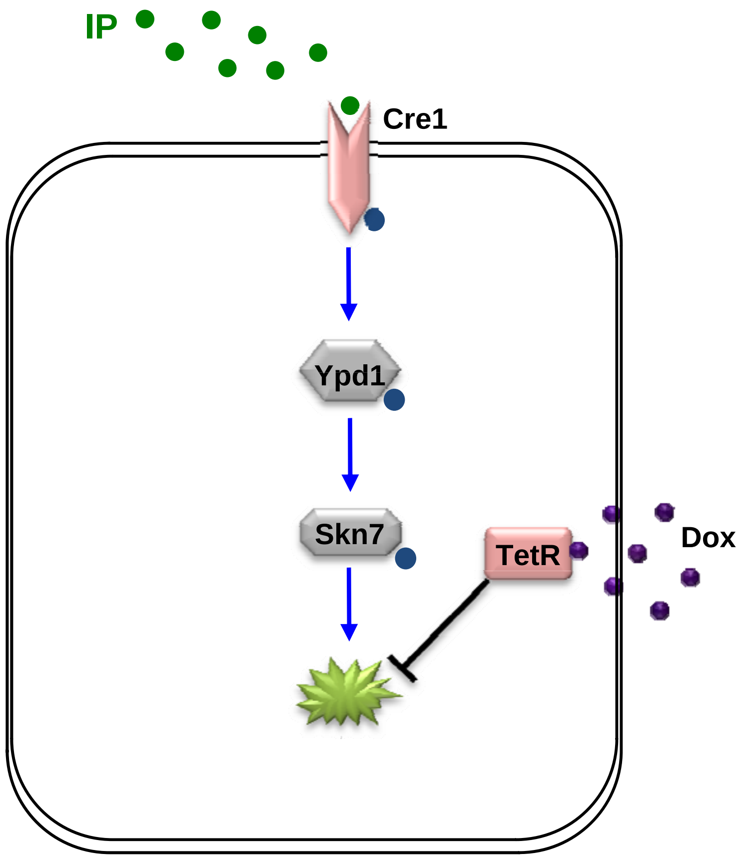
Fast diffusion

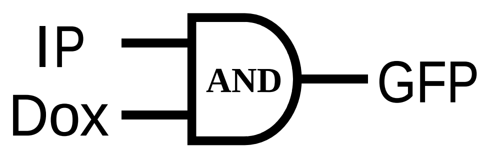
Simulations of domain formation

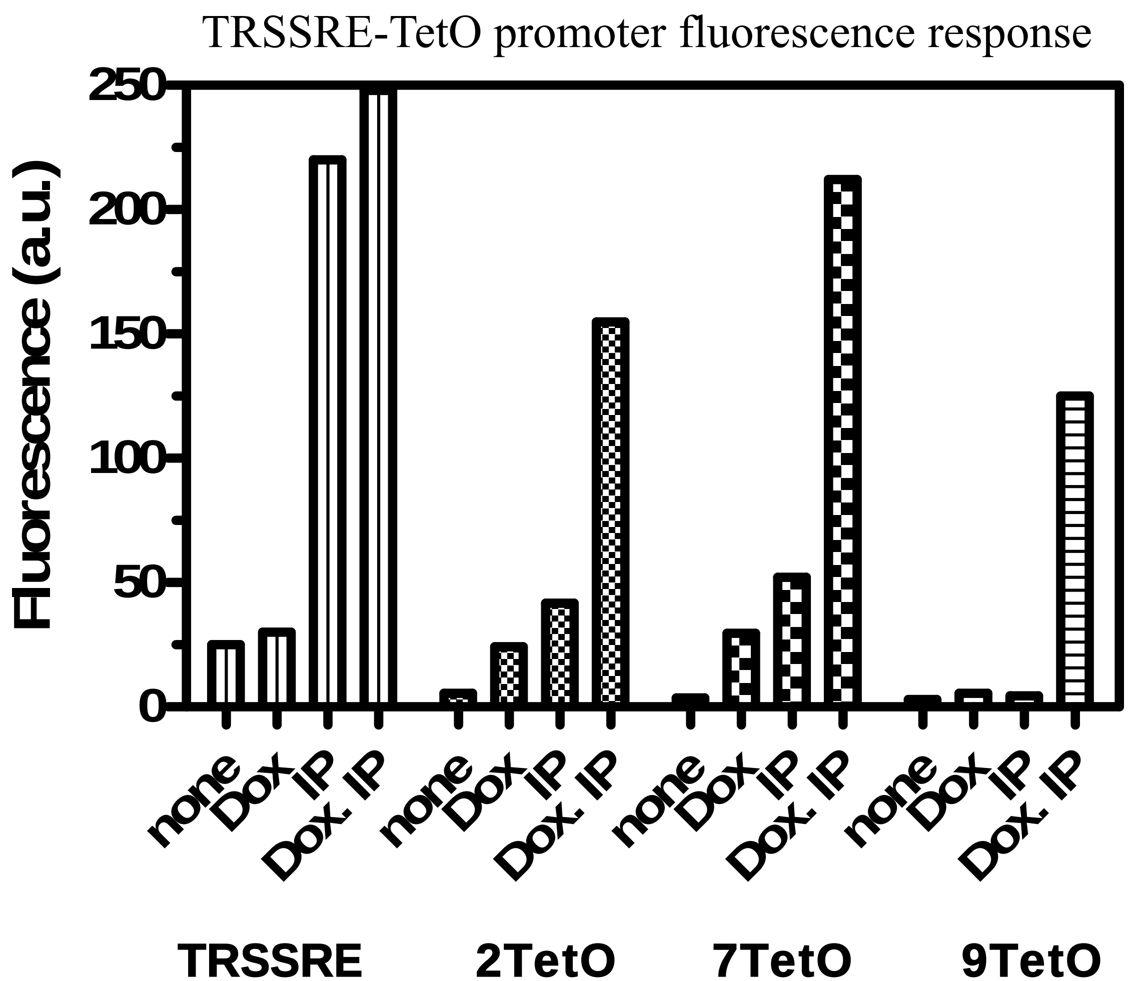


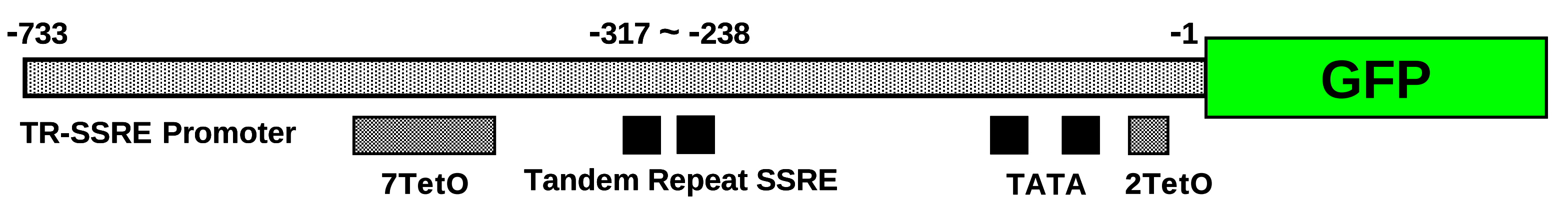
Similar to the experimental observations, the population response becomes more “digital” as the diffusion rate increases.

Doxycycline-regulatable TR-SSRE promoters

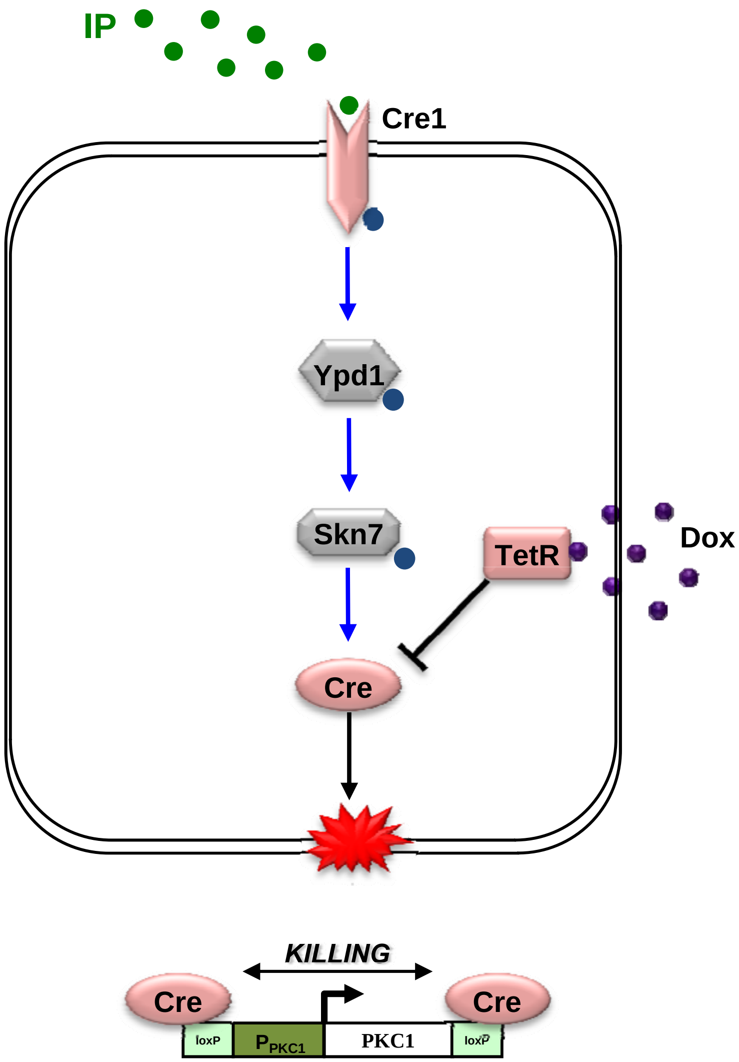


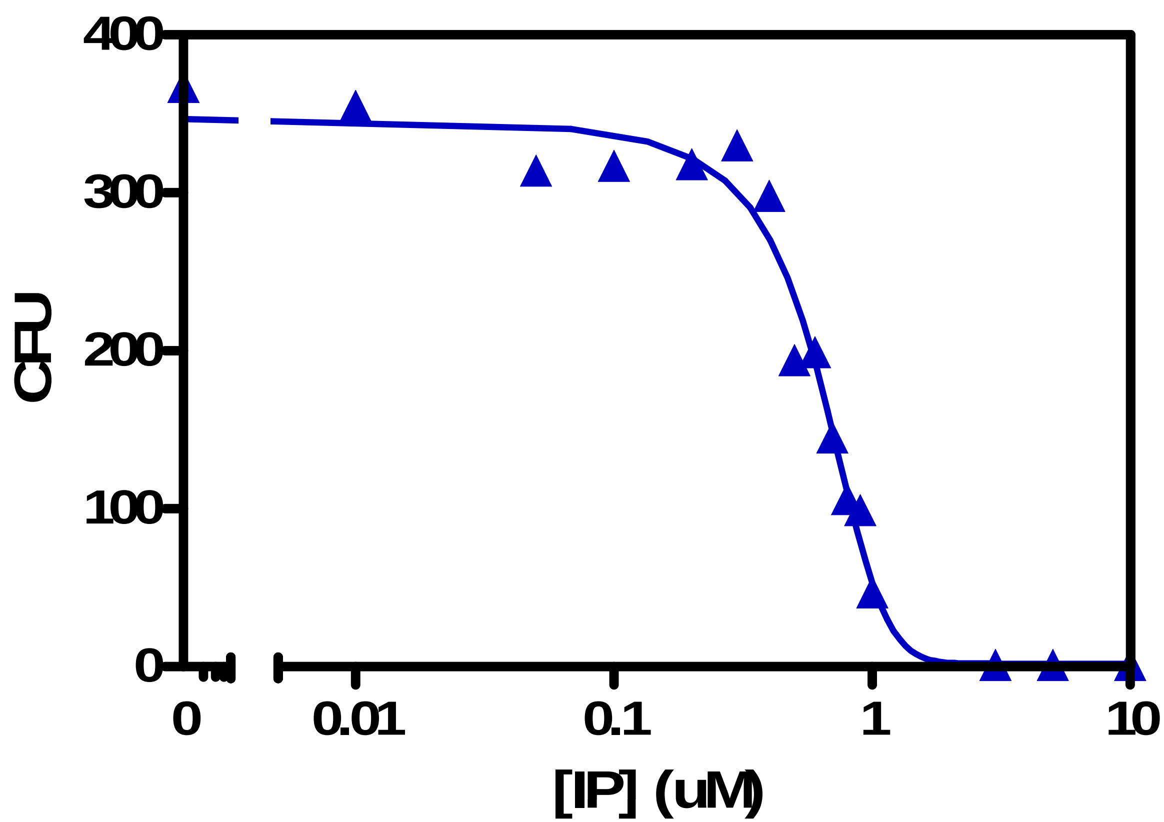






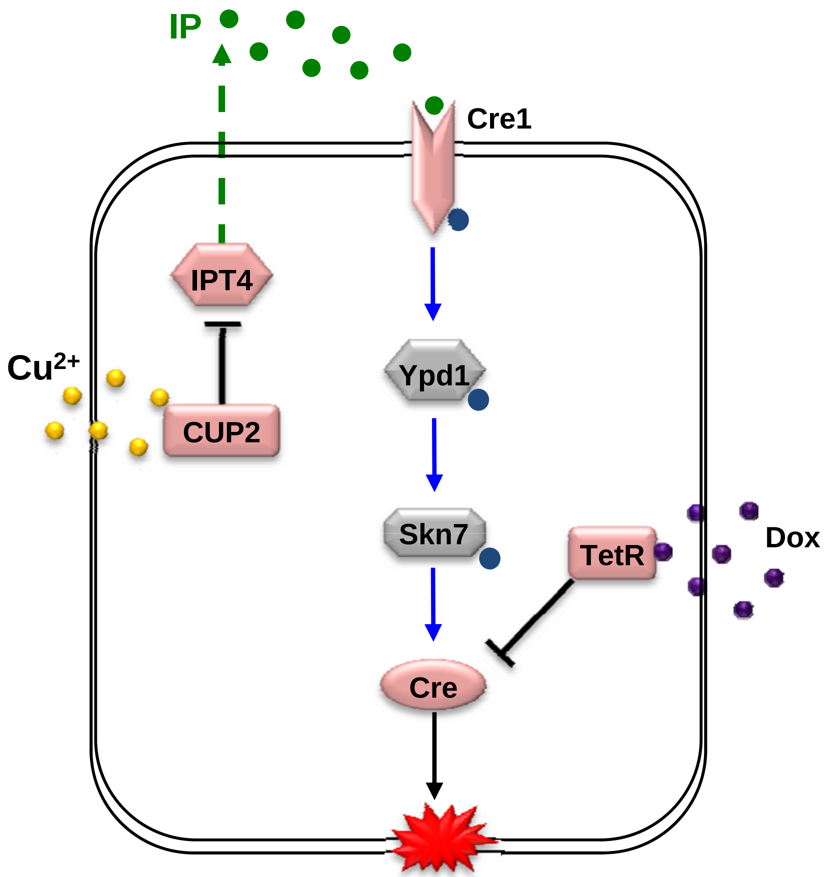
Dense population detection and cell suicide

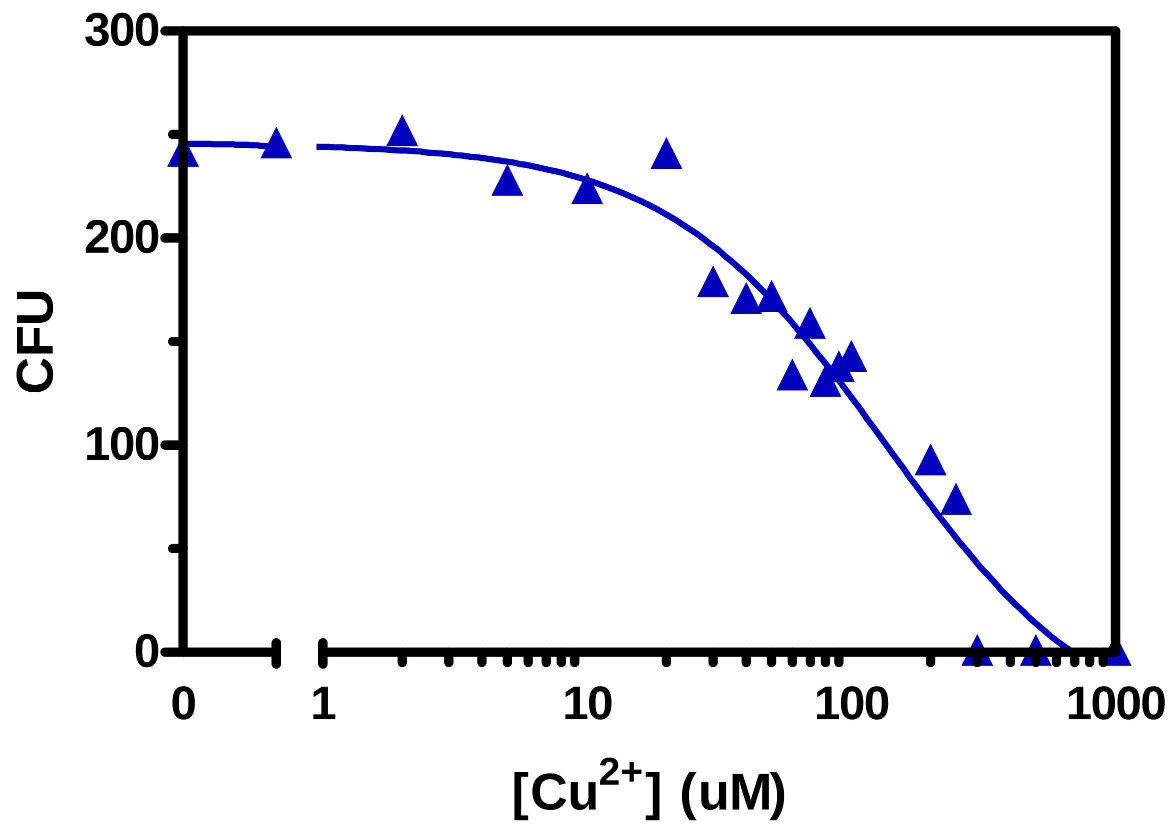




Yeast was transformed with the killer circuit. Cells were taken from initial transformation plate and re-suspended. Around 500 cells were evenly spread on a 100mm petri dish with 10 ug/ul [Dox] and different IP concentrations. The number of surviving colonies is plotted as a function of exogenous [IP]

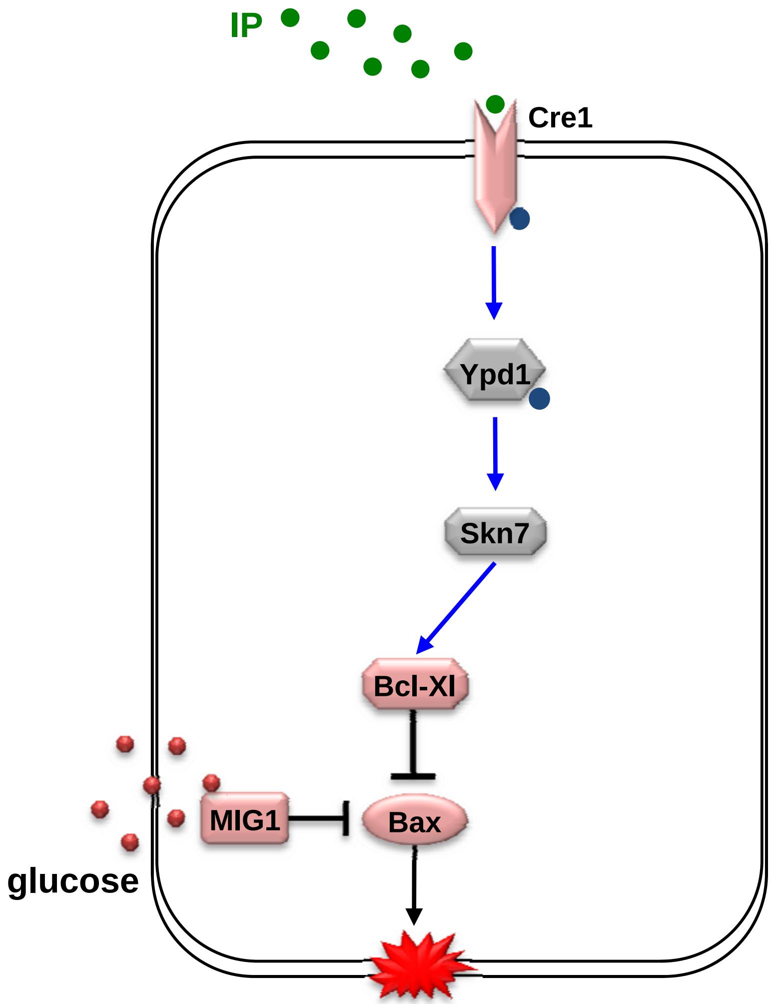
Population control: QS and cell suicide



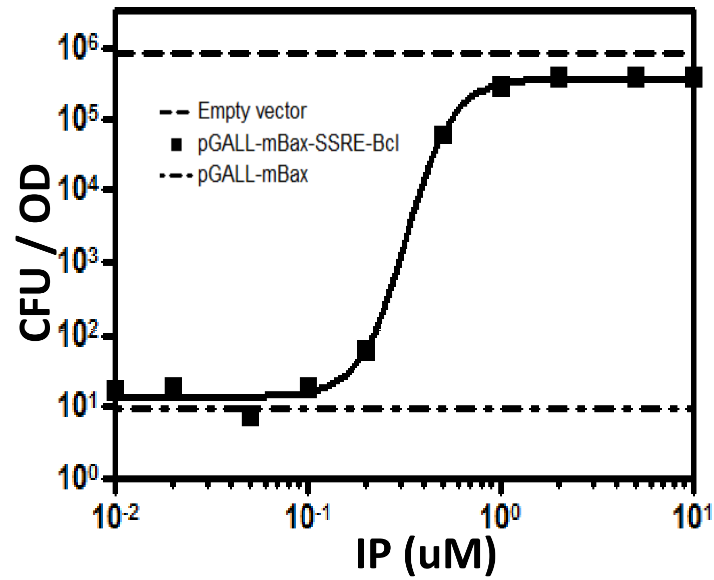


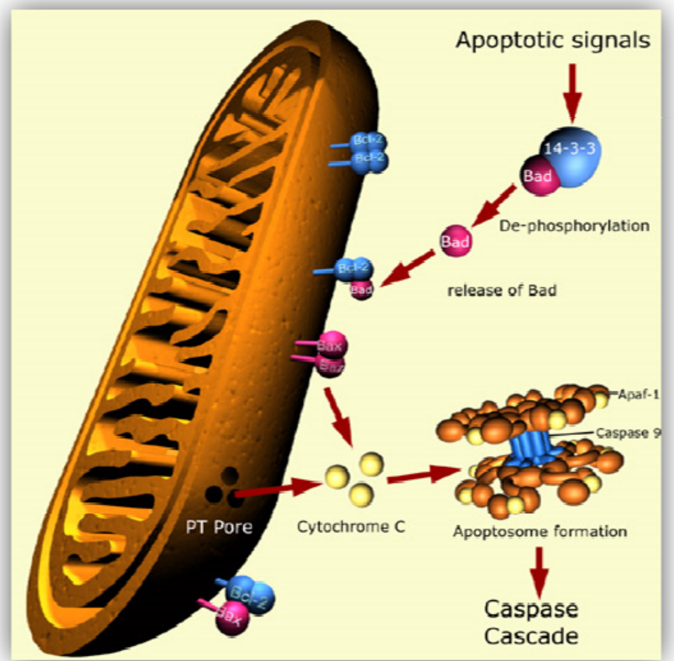
Yeast were transformed with the IP synthesis and killer circuits. Cells were taken from the initial transformation plate and re-suspended in water. Around 500 cells were then evenly spread on a 100mm petri dish with 10 ug/ul [Dox] and different copper concentrations. The number of surviving colonies is plotted as a function of [Cu^{2+}]

LT killing with protein/protein interactions



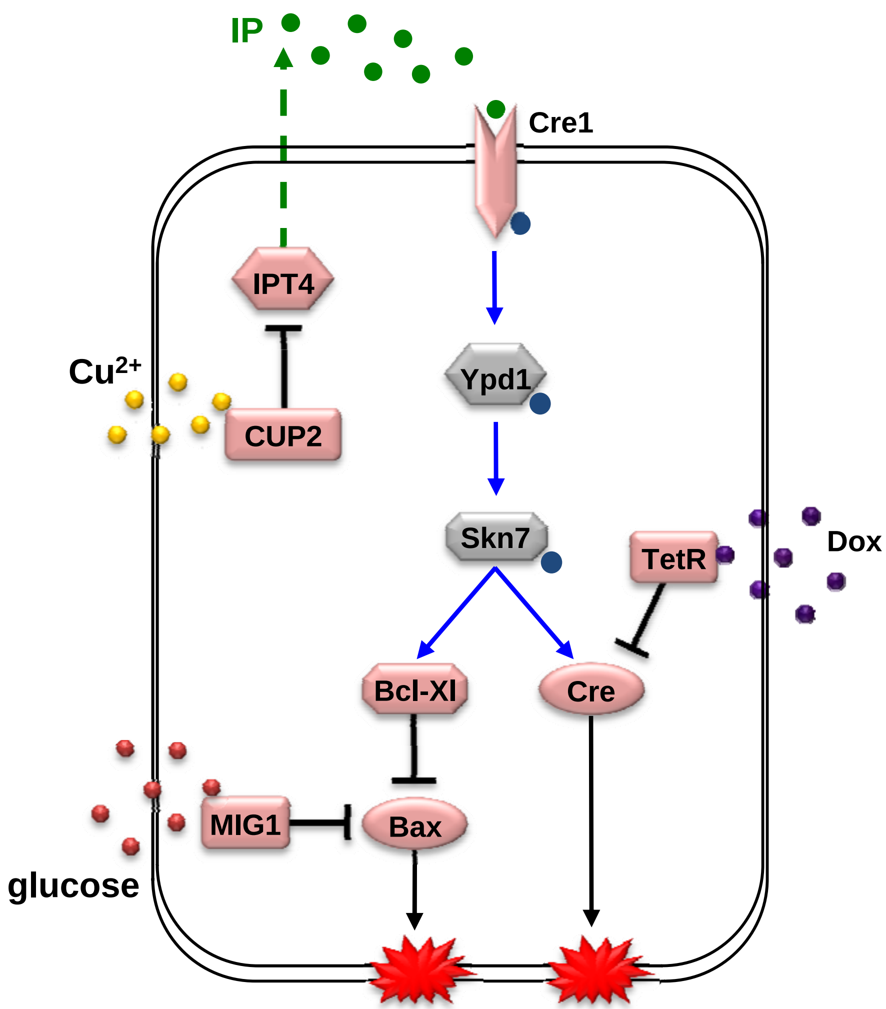
mitochondrial outer membrane permeabilization with Bax

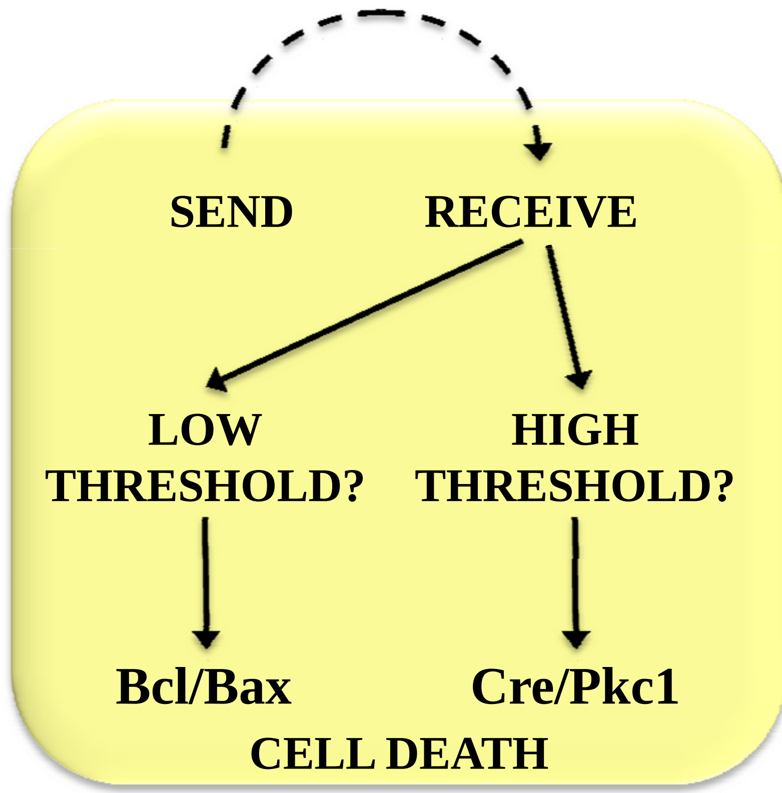




http://www.sgul.ac.uk/depts/immunology/~dash/apoptosis/mito2.jpg

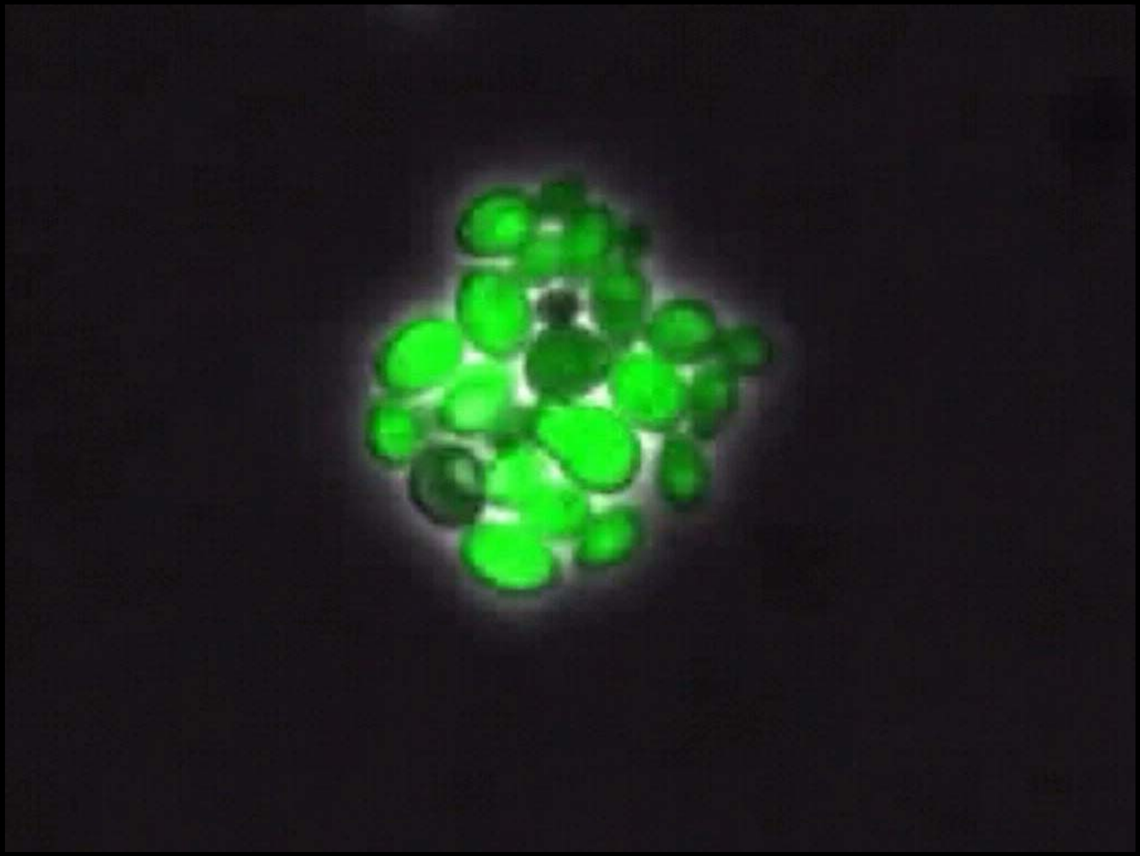
Complete Game-of-Life system





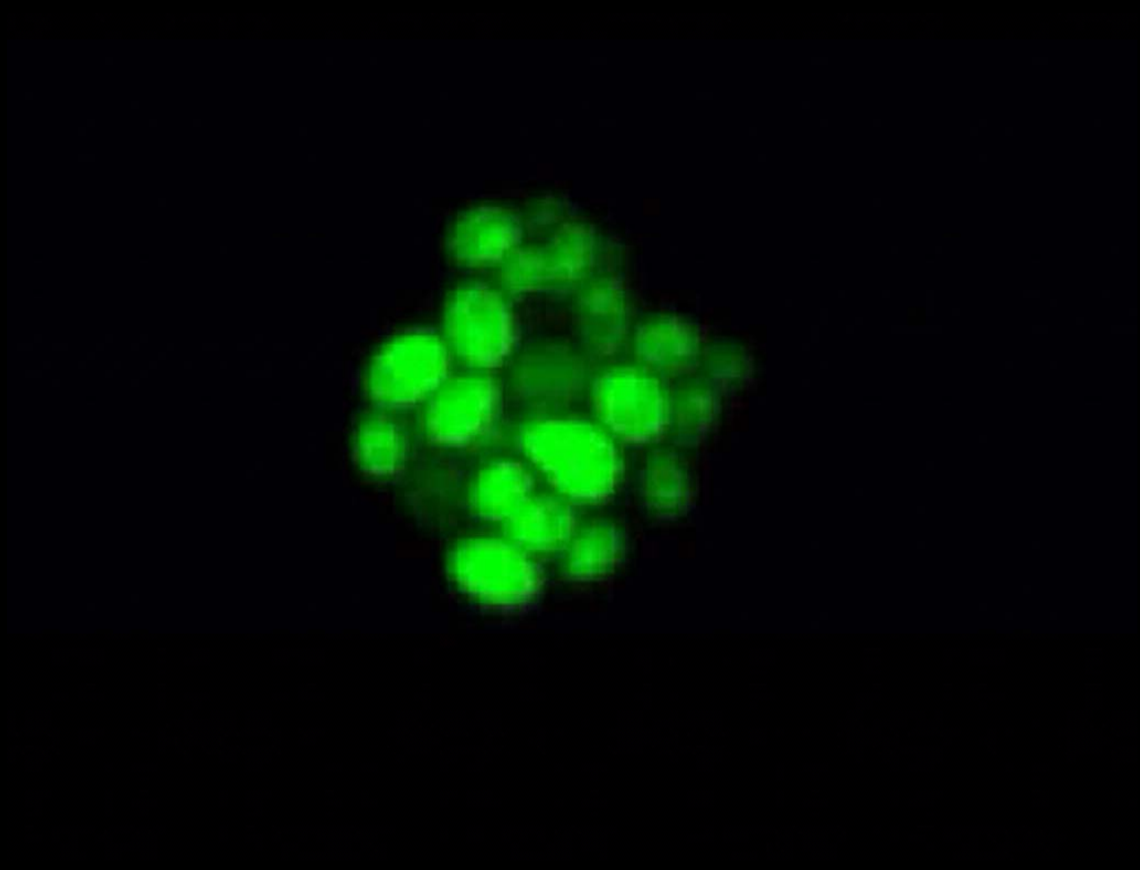
- Survival thresholds can be tuned with Dox, glucose, Cu^{2+sd}
- Integrate many mechanisms: enzymatic synthesis, cell-cell comm., phosphorylation, transcriptional regulation, protein/protein inhibitory binding, cell death

Game of Life (HT) microscope observations



- Time lapse movie of quorum sensing induced cell suicide
- Cell killing results in ‘ghosts’ – these do not appear to alter growth patterns of live cells

Game of Life (HT) microscope observations



- Time lapse movie of quorum sensing induced cell suicide
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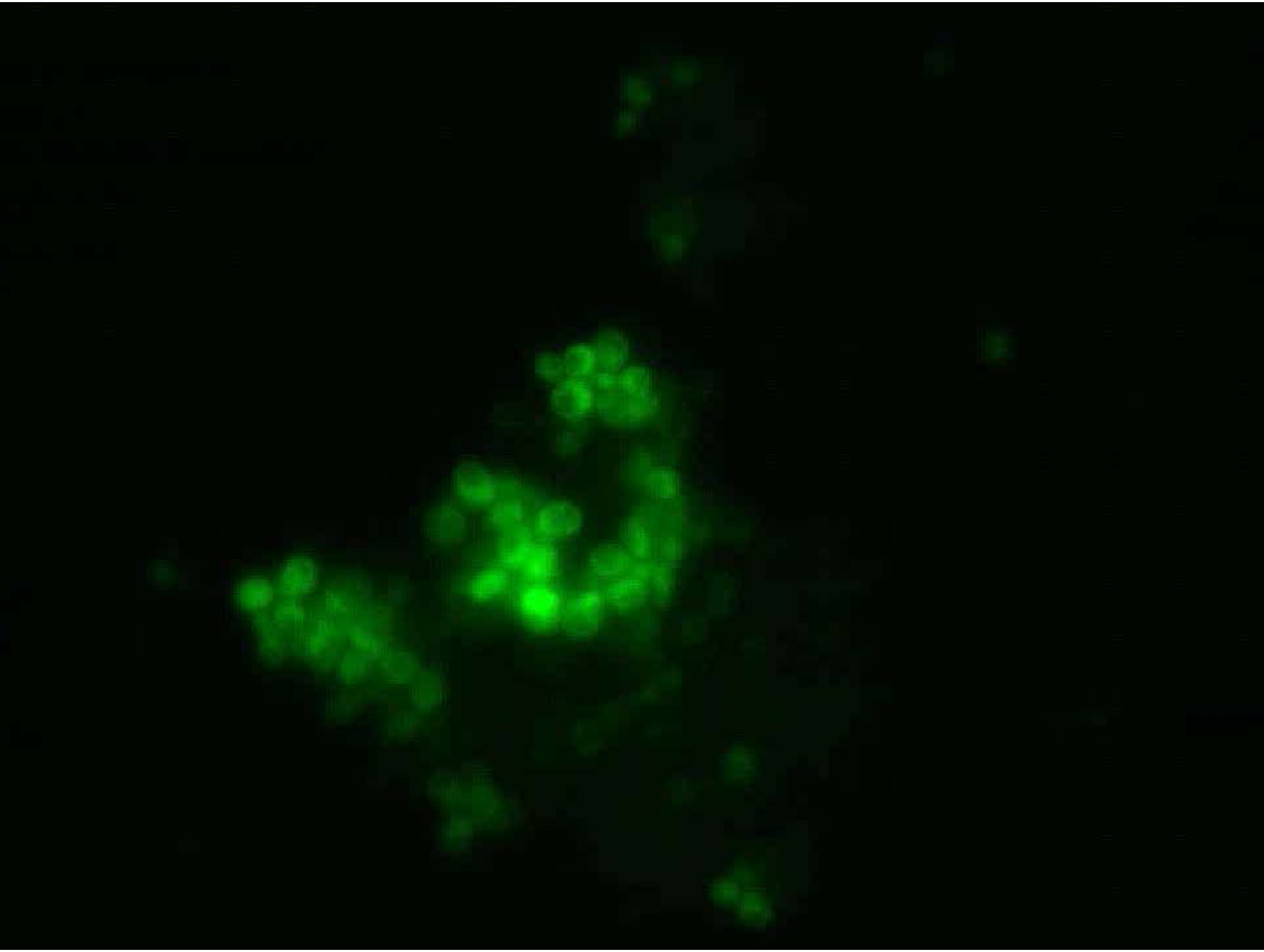
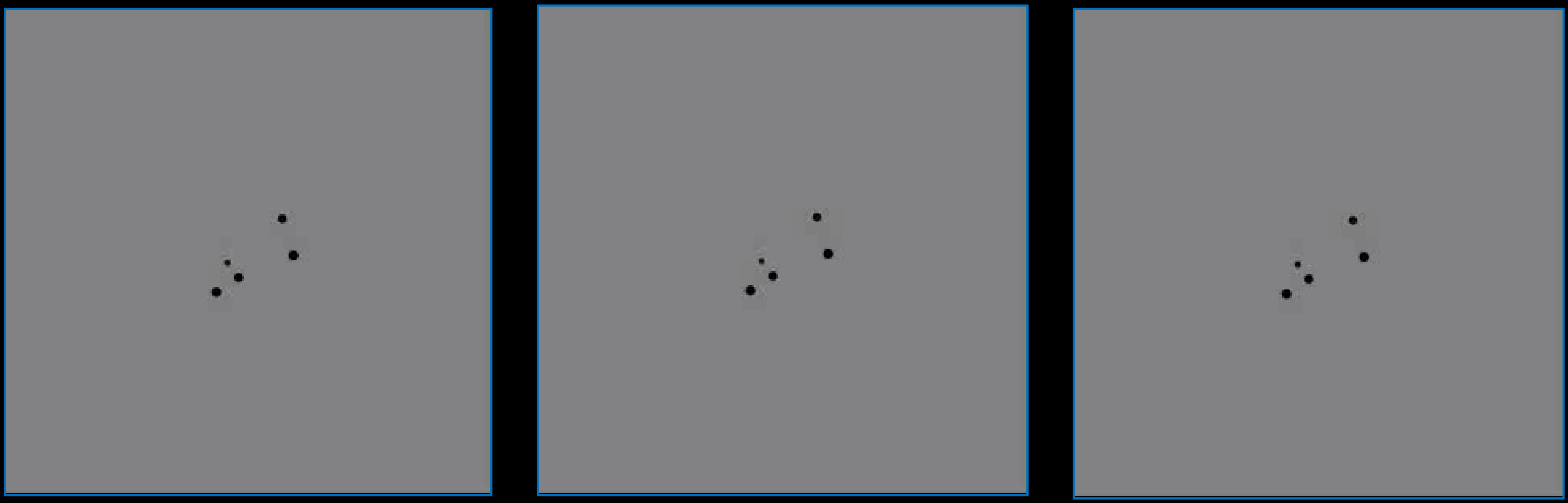


Game of Life agent based simulations

Game of Life agent based simulations



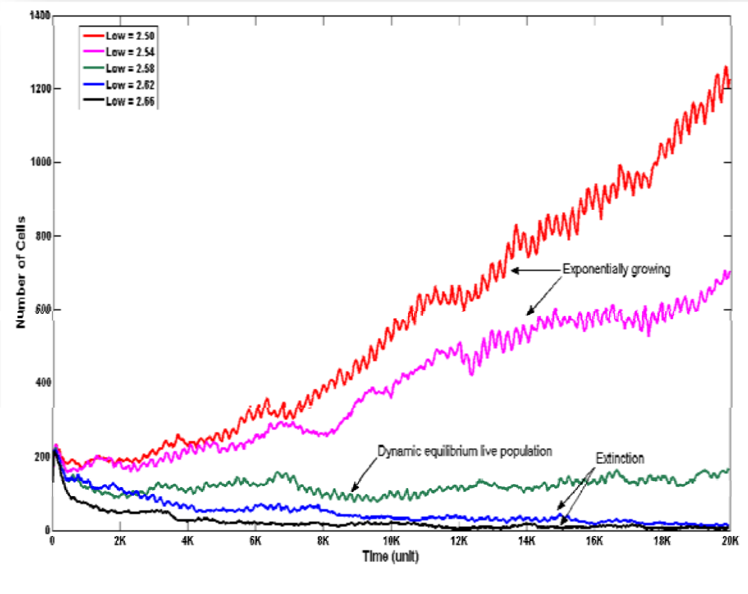
HT = low

HT = medium

HT = high

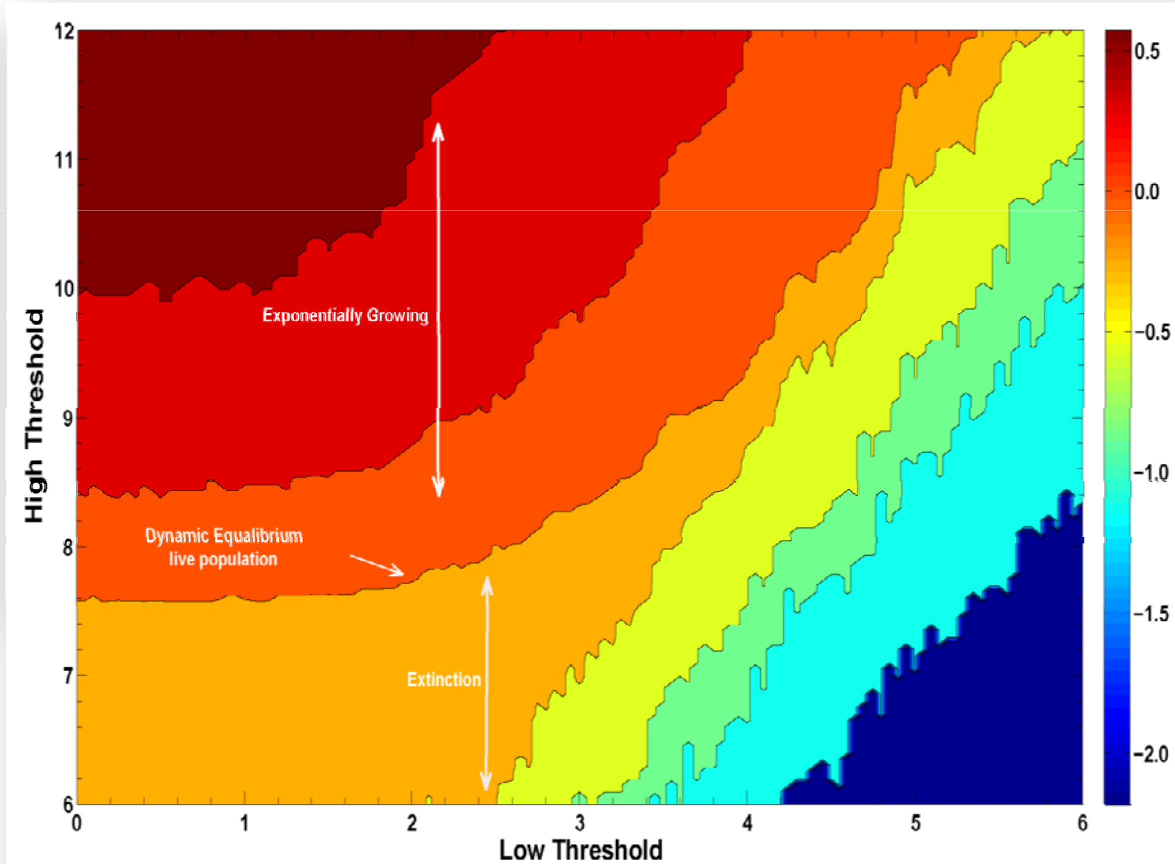
Game of Life simulations

Colony growth vs. LT



High Threshold = 8

Colony growth vs. LT & HT

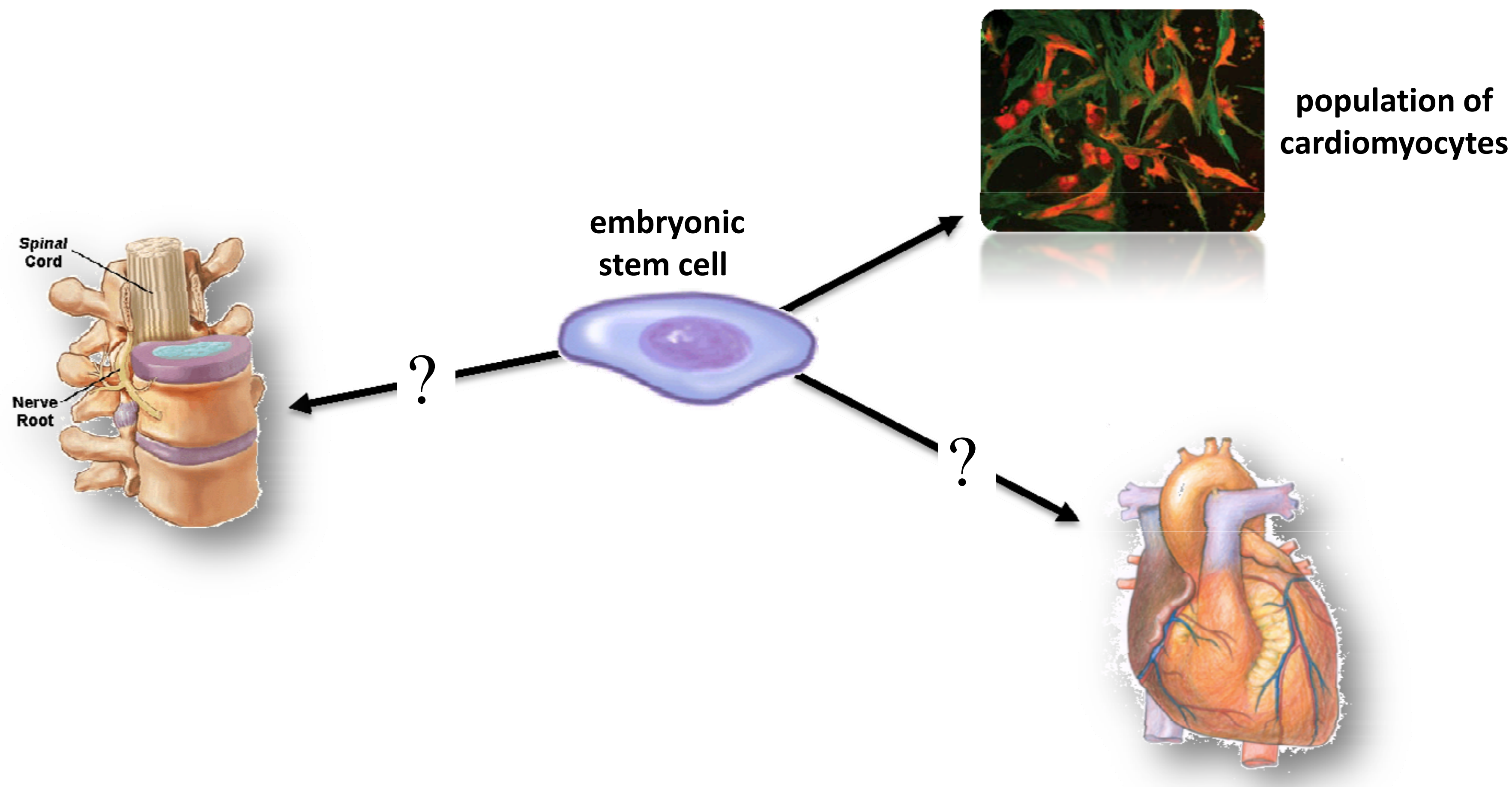


Colony growth rate (log)

While the behavior of any individual cell cannot be predicted, the overall behavior of the population perhaps can be predicted.

Applying synthetic biology to stem cell research

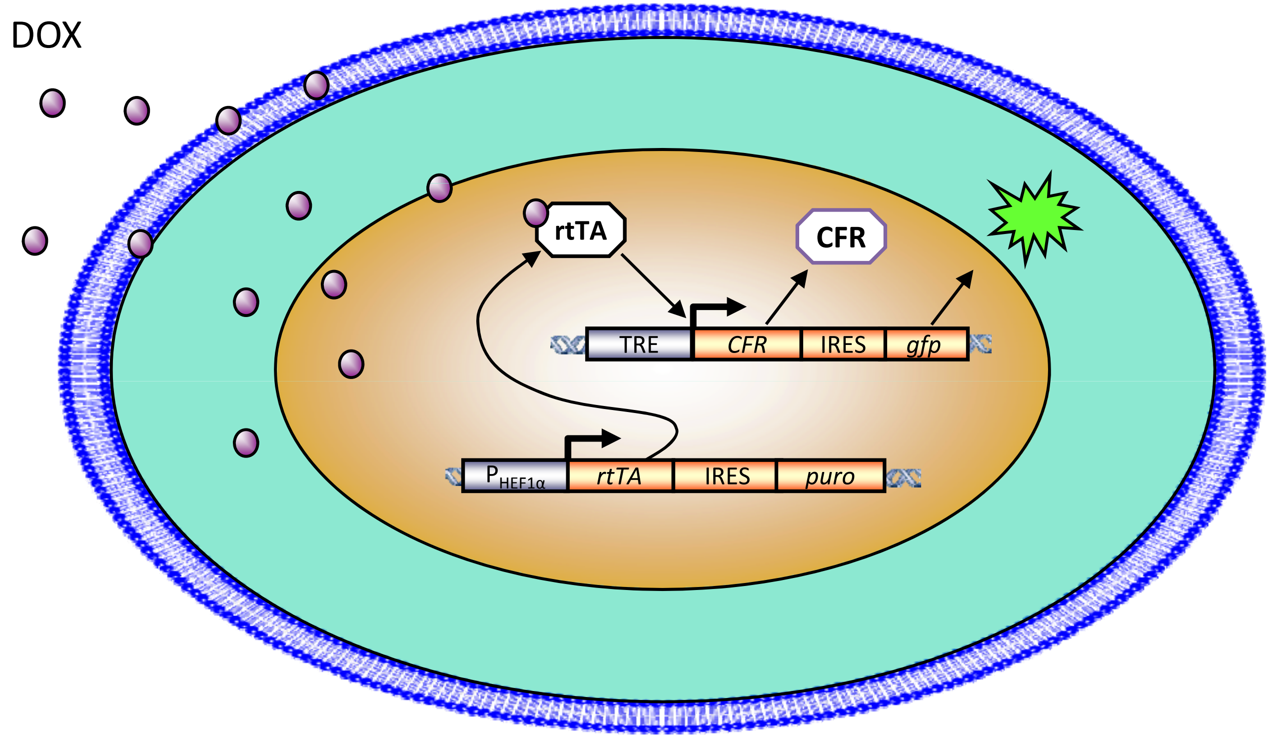
[Patrick Guye, Cil Purnick, Noah Davidsohn, Yingqing Li]



- Fundamental question in tissue engineering:

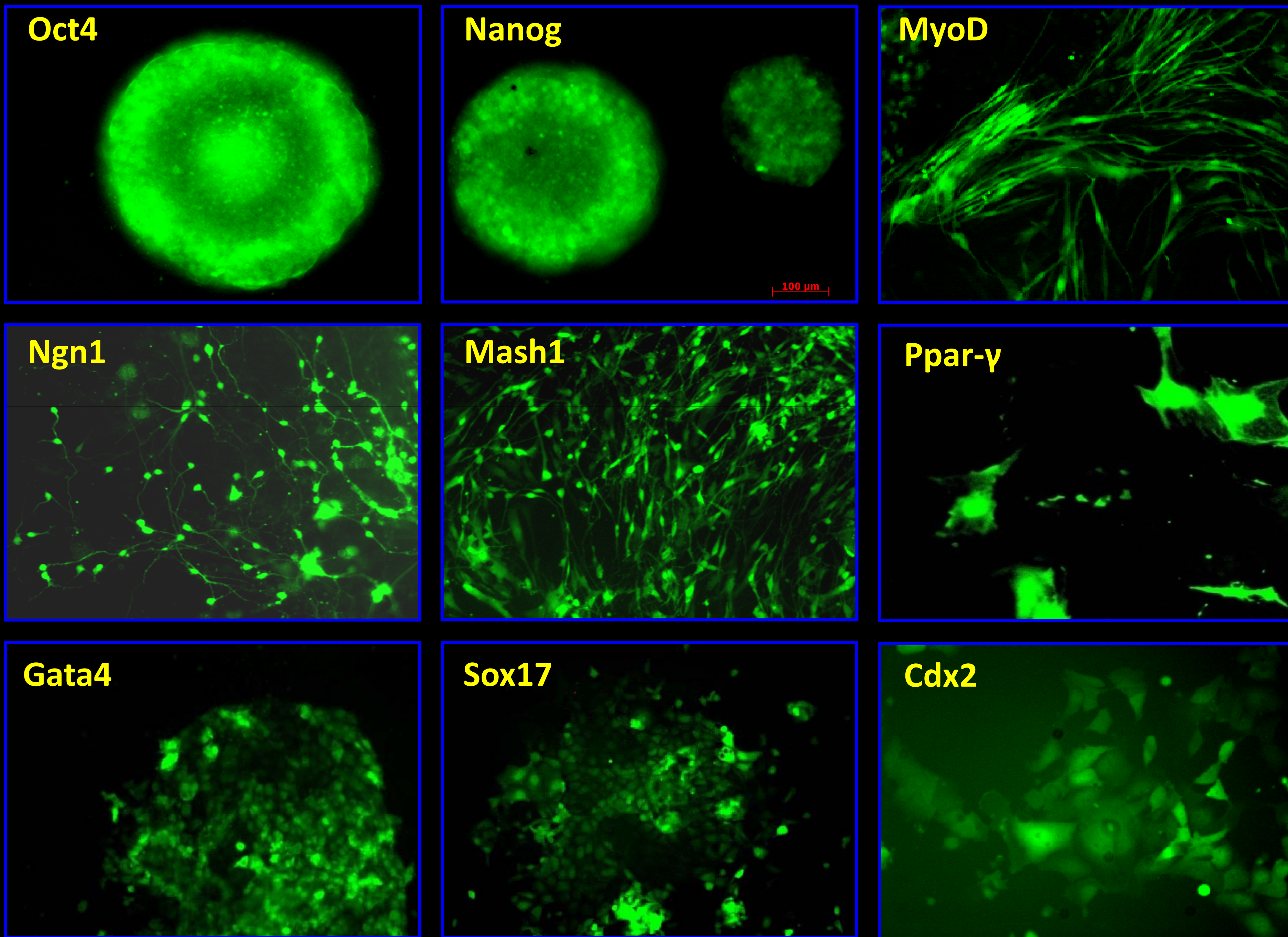
  Can we <u>create</u> and <u>maintain</u> large scale spatially defined functional tissues?

Directed differentiation with cell fate regulators



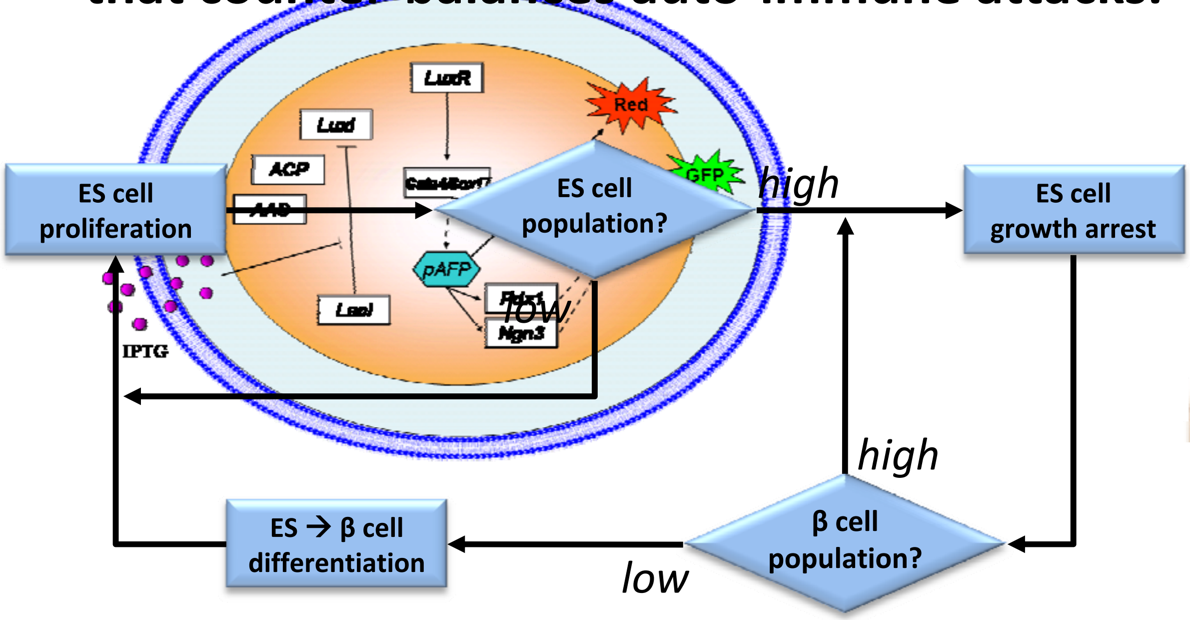
- **Endoderm: Gata4, Gata6, Sox17**
  J. Fujikura, *et al.*, Genes and Development, Vol. 16, pp. 784-789, 2002.
- **Trophectoderm: Cdx2**
  H. Niwa, *et. al*, Cell, Vol. 123(5), pp. 917-929, 2005.
- **Neuronal: Nkx2.2, Nkx6.1, Pax6, Ngn1**
  Ericson, *et al.*, Cell, Vol. 90, pp. 169-180, 1997.
  J. Briscoe, *et al.*, Nature, Vol. 398, p.622-627, 1999.
  T. Jessell, Nature Reviews Genetics, Vol. 1, pp. 21-29, 2000.
  M. McCormick, *et al.*, Mol. and Cell. Bio., Vol. 16(10), pp. 5792-5800, 1996.
- **Muscle: MyoD**
  R. Davis, H. Weintraub, A. B. Lassar, Cell Vol. 51, pp. 987-1000, 1987.
- **Pancreas: Ngn3, Pax4, Nkx2.2, Pdx1**
  G. Gu, *et al.*, Development Vol. 129, pp. 2447-2457, 2002.
  J. Wang, *et al.*, Developmental Biology, Vol. 266, pp. 178-189, 2004.
  N. Lavon, *et al.*, Stem Cells, Vol. 24, pp. 1923-1930, 2006.
  J. Heit, S. Karnik, S. Kim, Ann. Rev. Cell Dev. Biol. 22: 311-338, 2006.
  D. Sinner, *et al.*, Development, Vol. 131, pp. 3069-3080, 2004
  M. Yasunaga, *et al.*, Nature Biotech, Vol. 23(12), 1542-1550, 2005.
- **Adipocytes: PParγ**
  C. Vernochet, *et al.*, FEBS Letters, Vol, 510, pp. 94-98, 2002.

A library of cell fate regulation ‘parts’



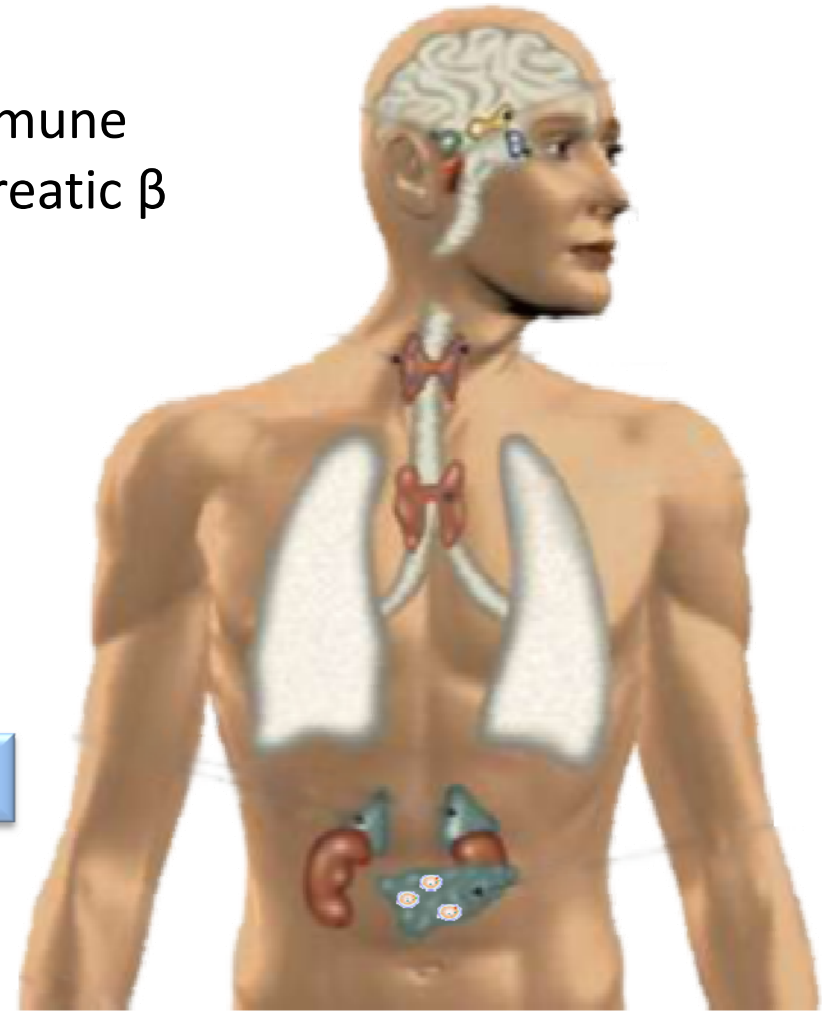
Artificial tissue homeostasis for β cells

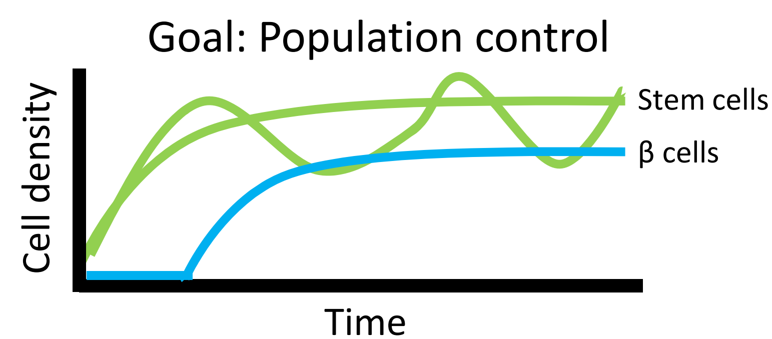
- 7.8% of the US population has diabetes
- In Diabetes Type I (10% of diabetics), auto-immune response (slowly) kills insulin-producing pancreatic β cells.
- **<u>Goal:</u>**
  Maintain population level of β cells using auto-regulated differentiation of ES cells that counter-balances auto-immune attacks.



Complex system with 22 components

<u>Design with 'known' modules!</u>





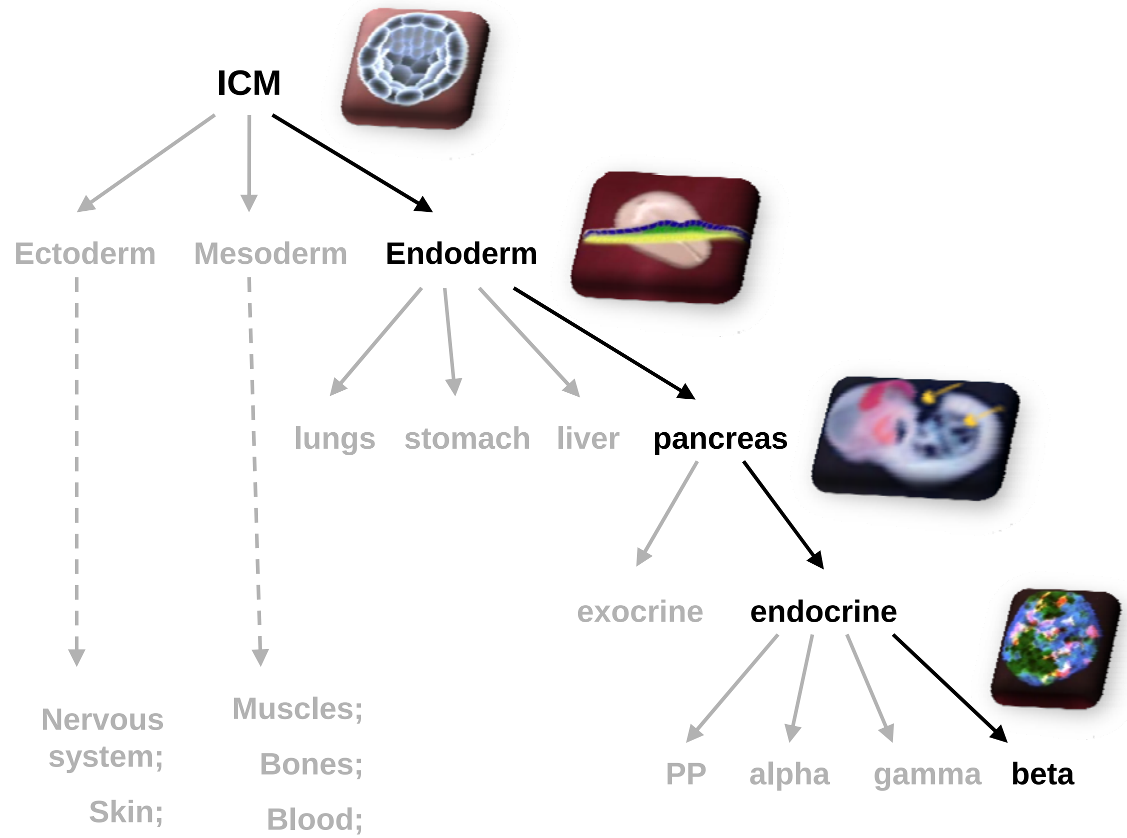
A self-timed genetic program for β cell differentiation

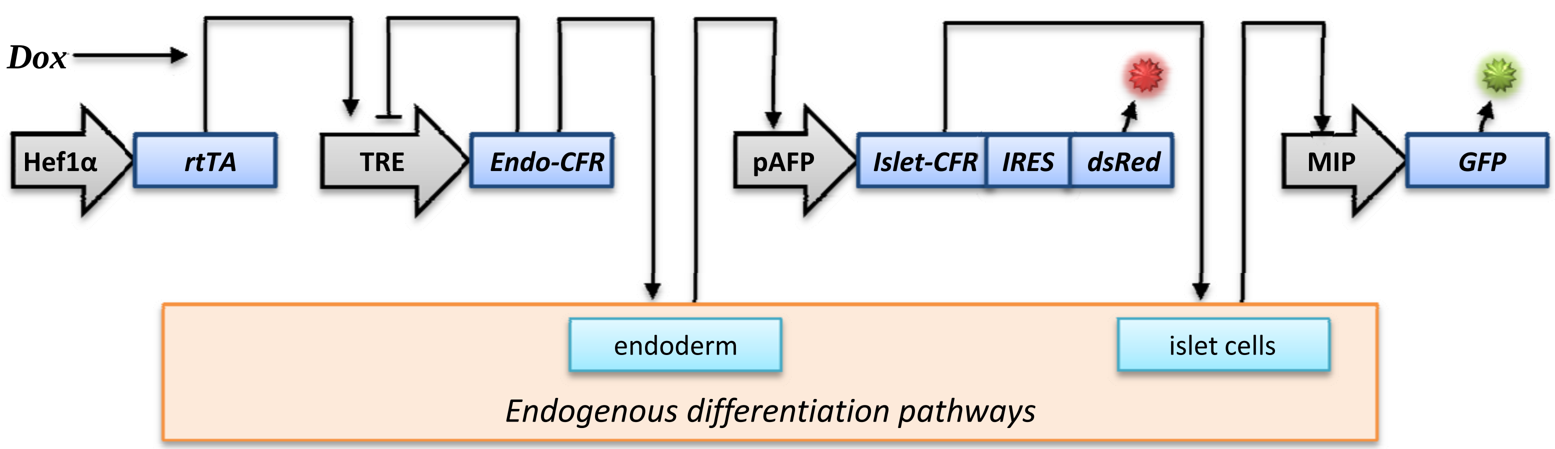
High level behavior:

“Self-timed, multi-step β cell differentiation”

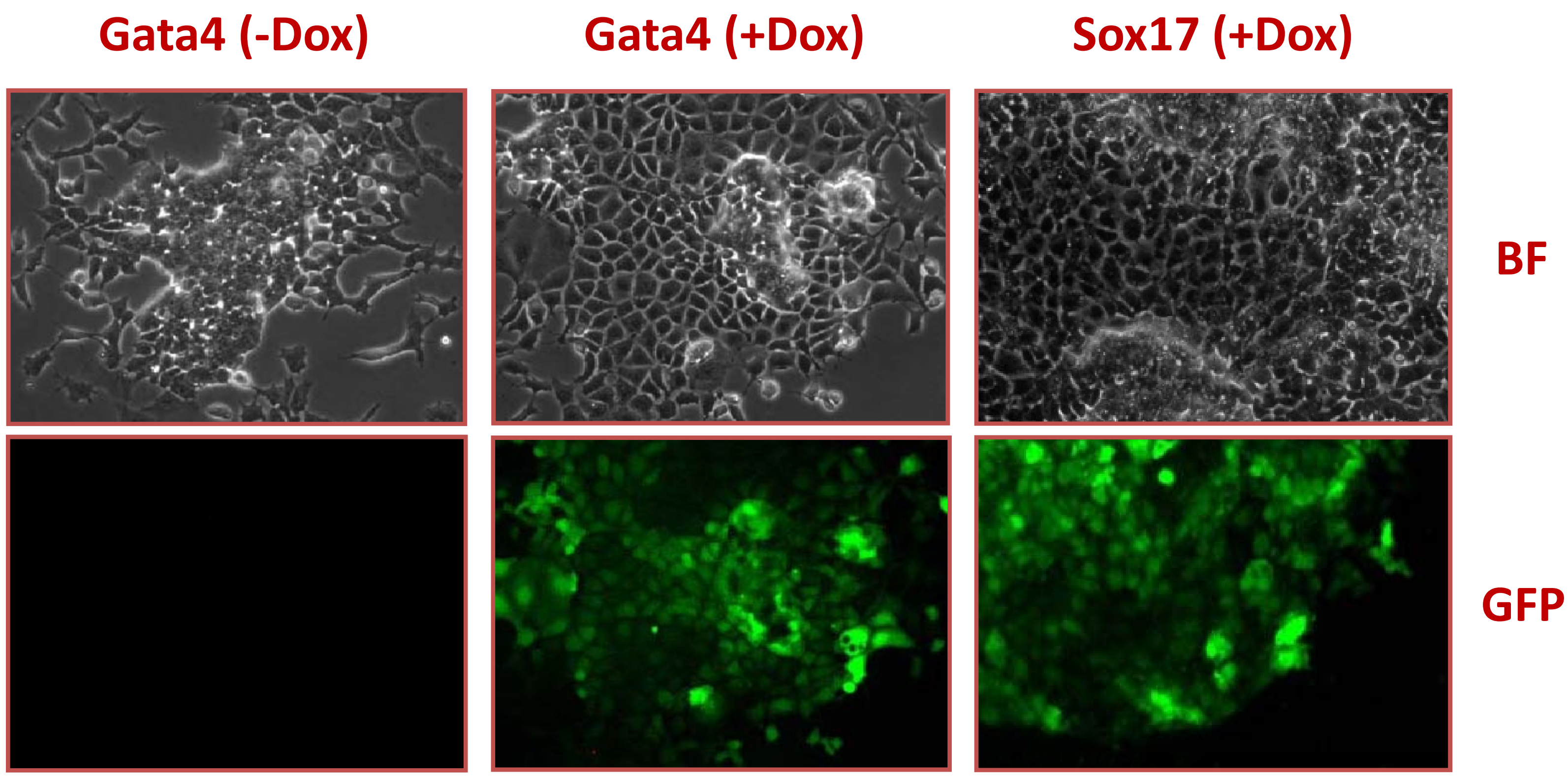
Bio-program:

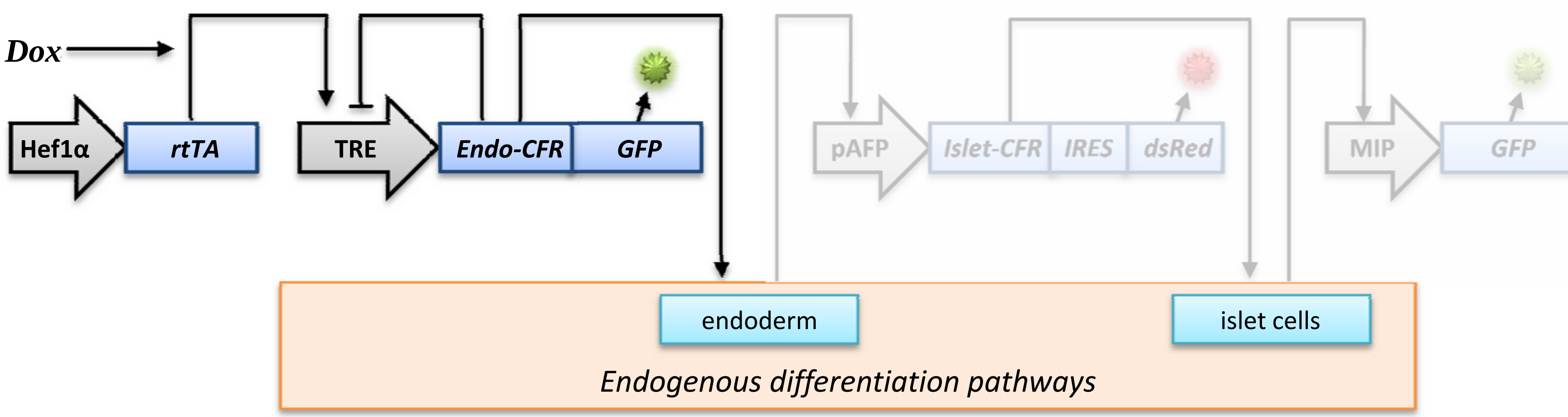
```
input: DOX
if (DOX)
  express(Endo-CFR)
if (Endoderm)
  express(Islet-CFR)
if (Islet)
  express(GFP)
```



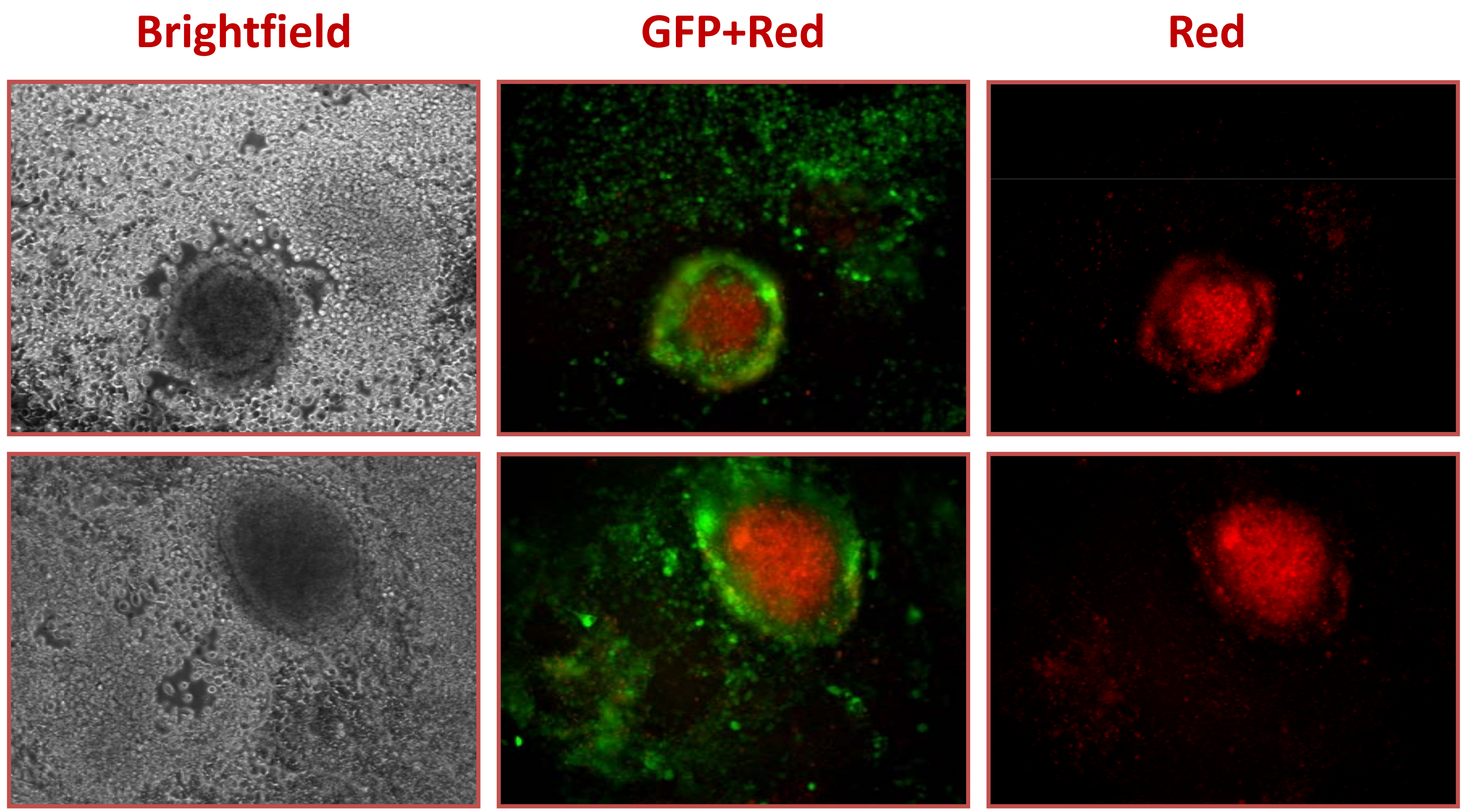


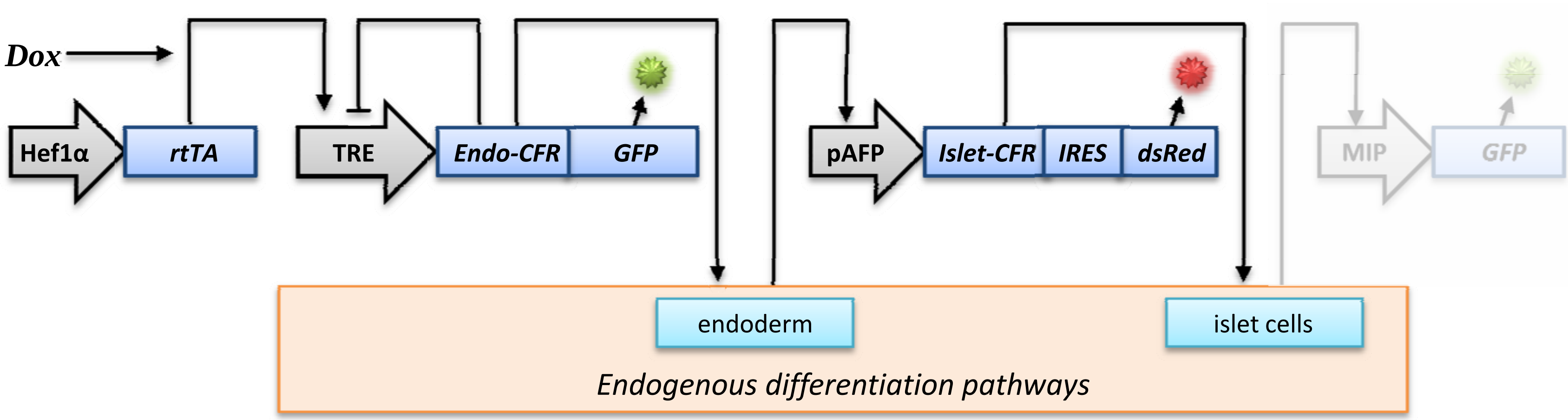
A self-timed genetic program for β cell differentiation





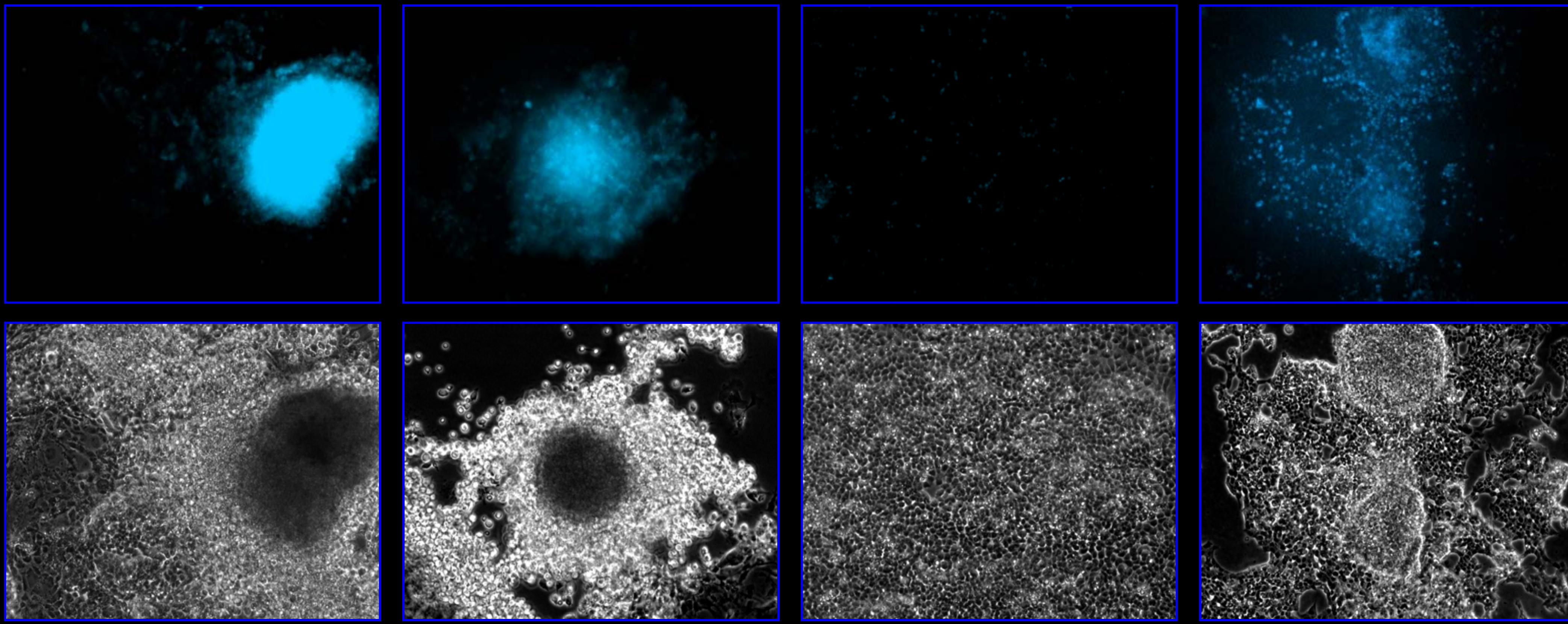
A self-timed genetic program for β cell differentiation





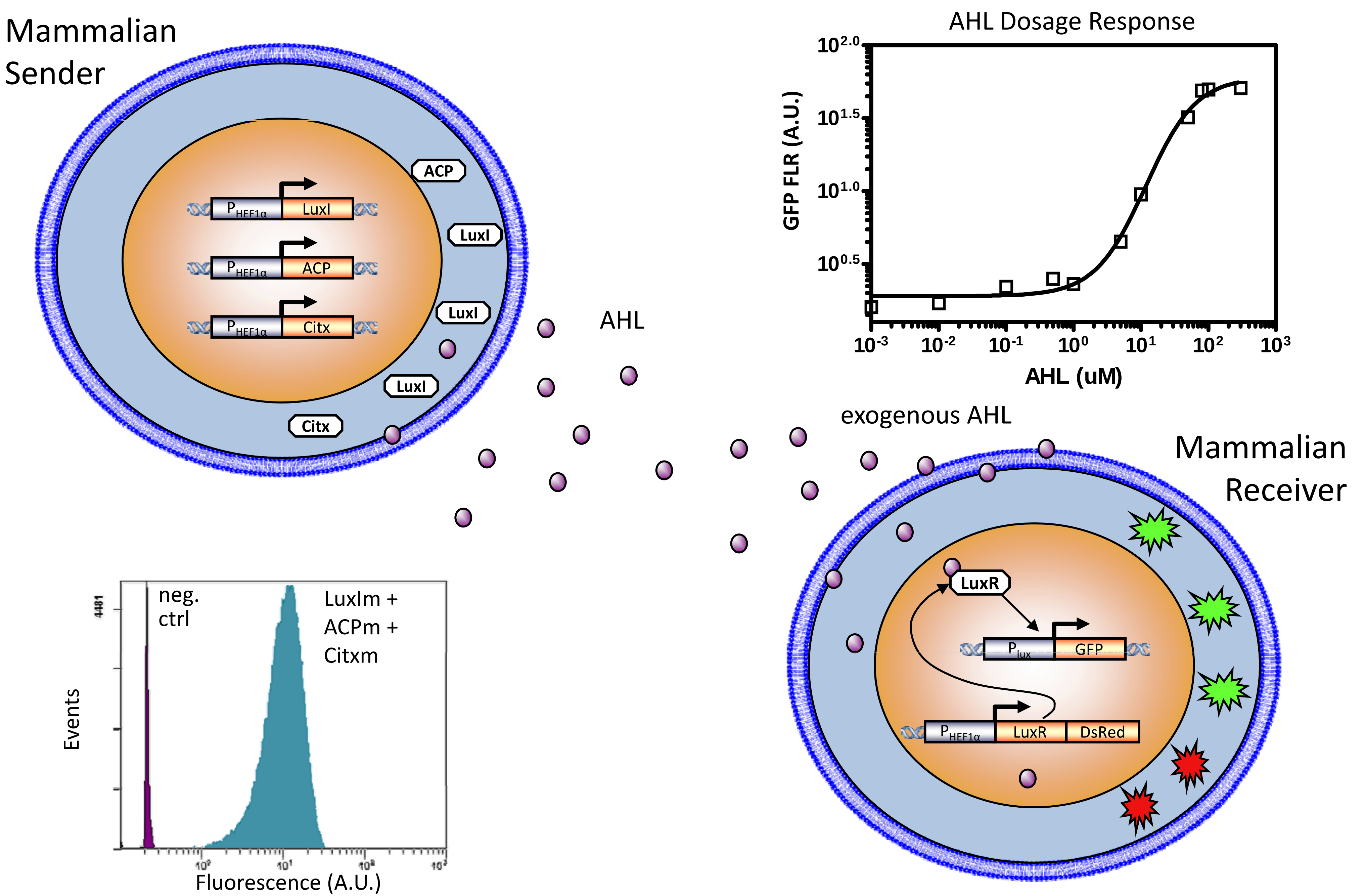
Insulin-production assay with C-peptide antibodies

Program 1	Program 2	Neg. ctrl	Pos. ctrl

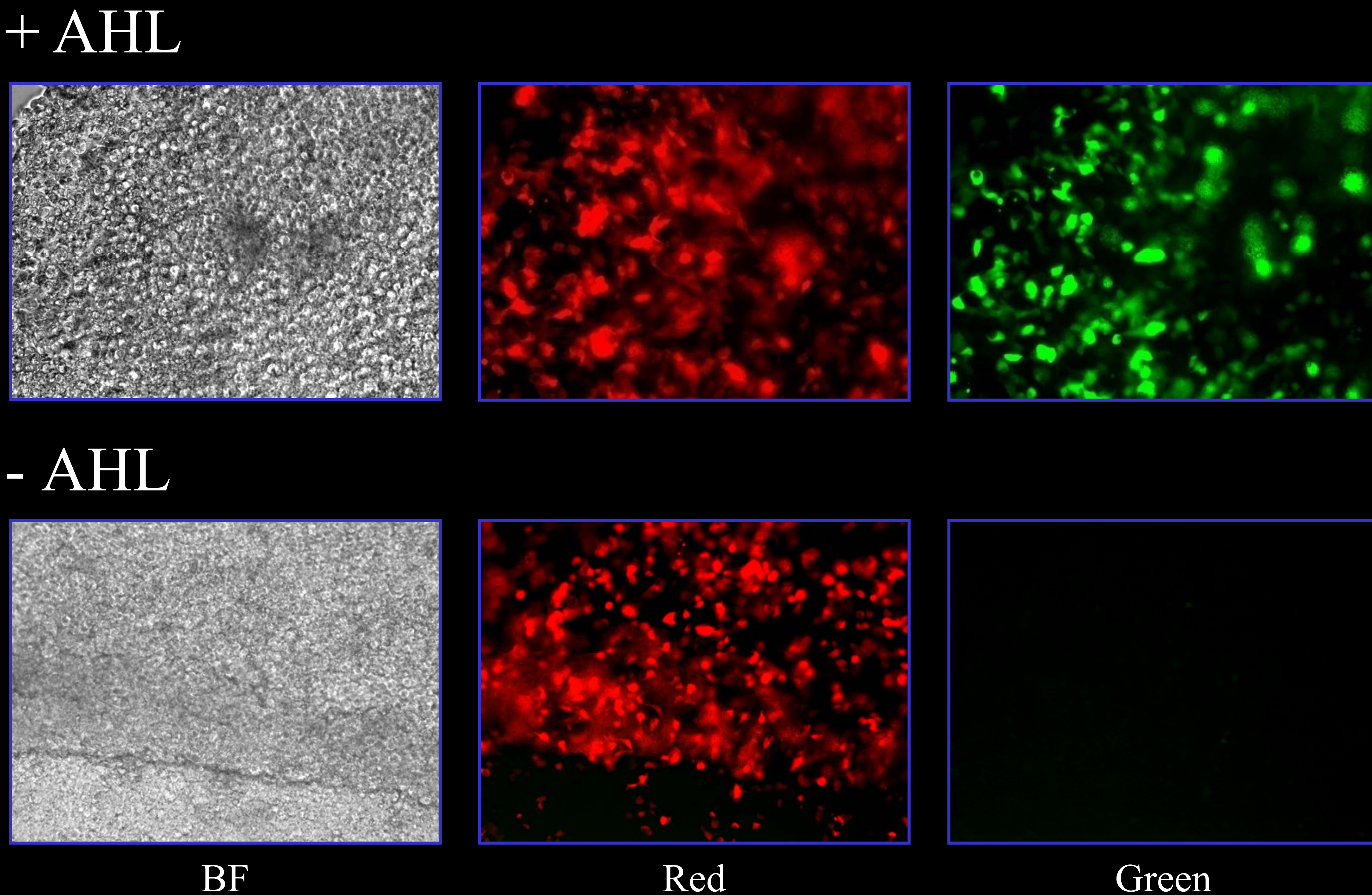


GATA4 Ngn3 Dox	GATA4 Ngn3 Pdx1 Dox/aTc	CCE	Beta TC6

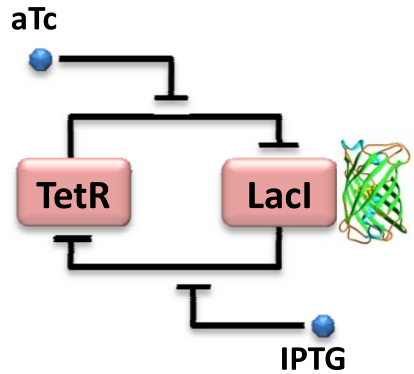
Teaching stem cells a new language

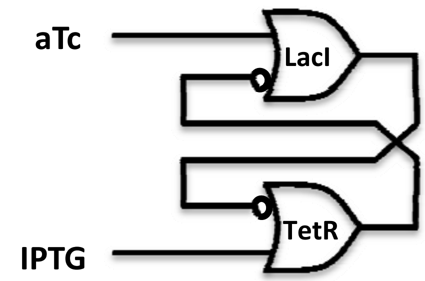


Microscope observations

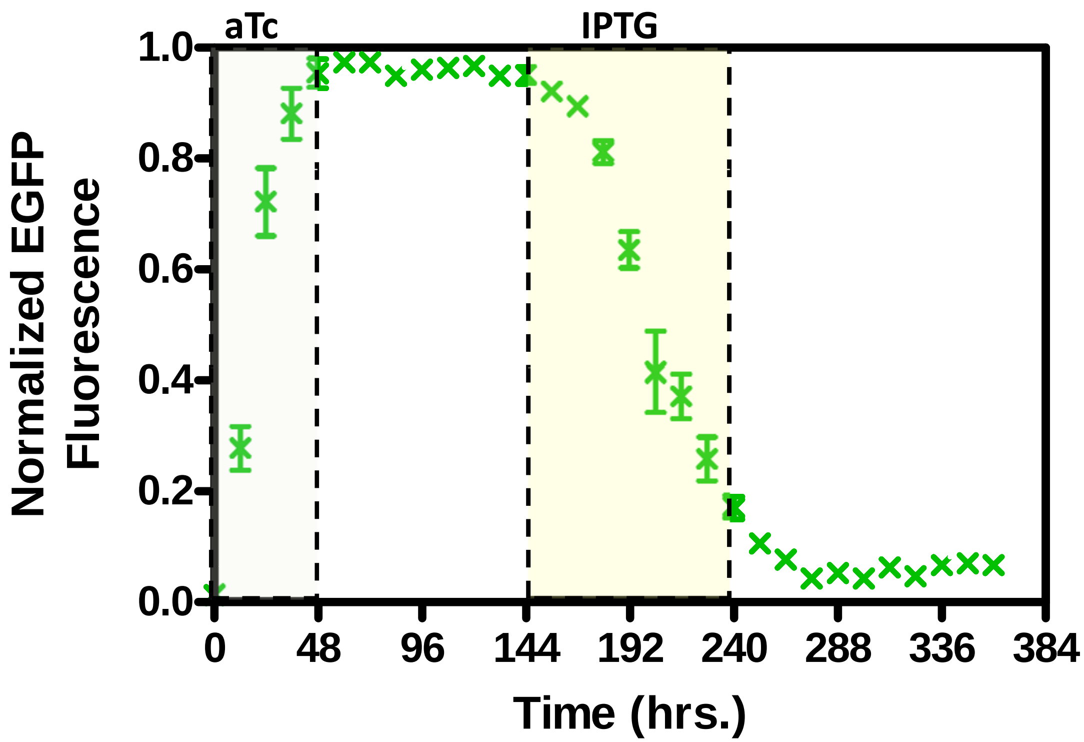


Information processing: toggle switch

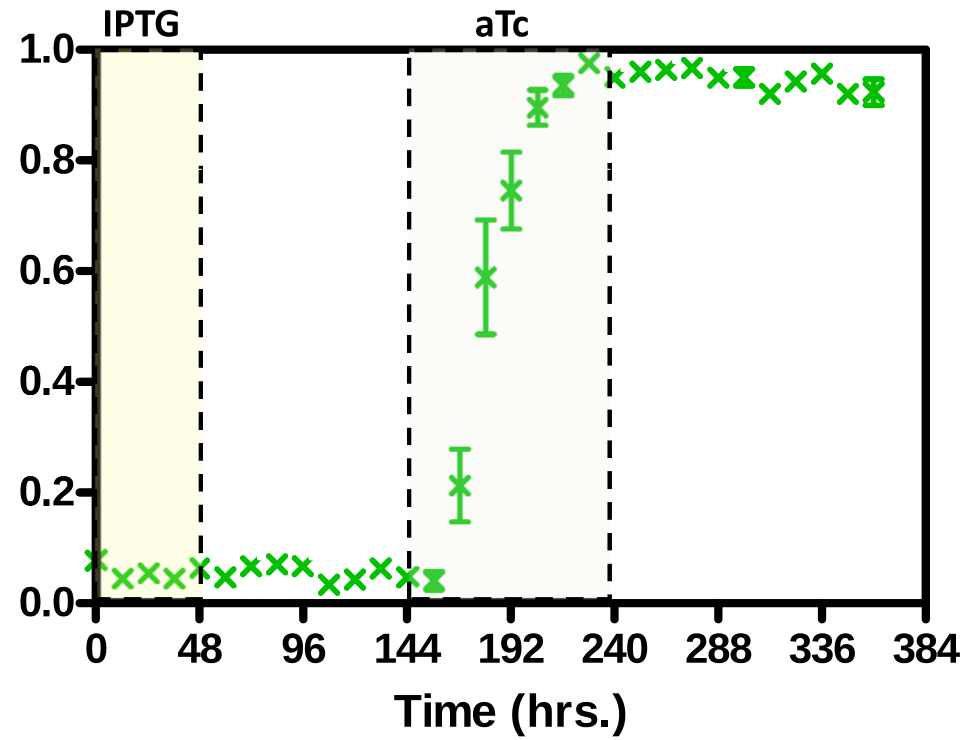




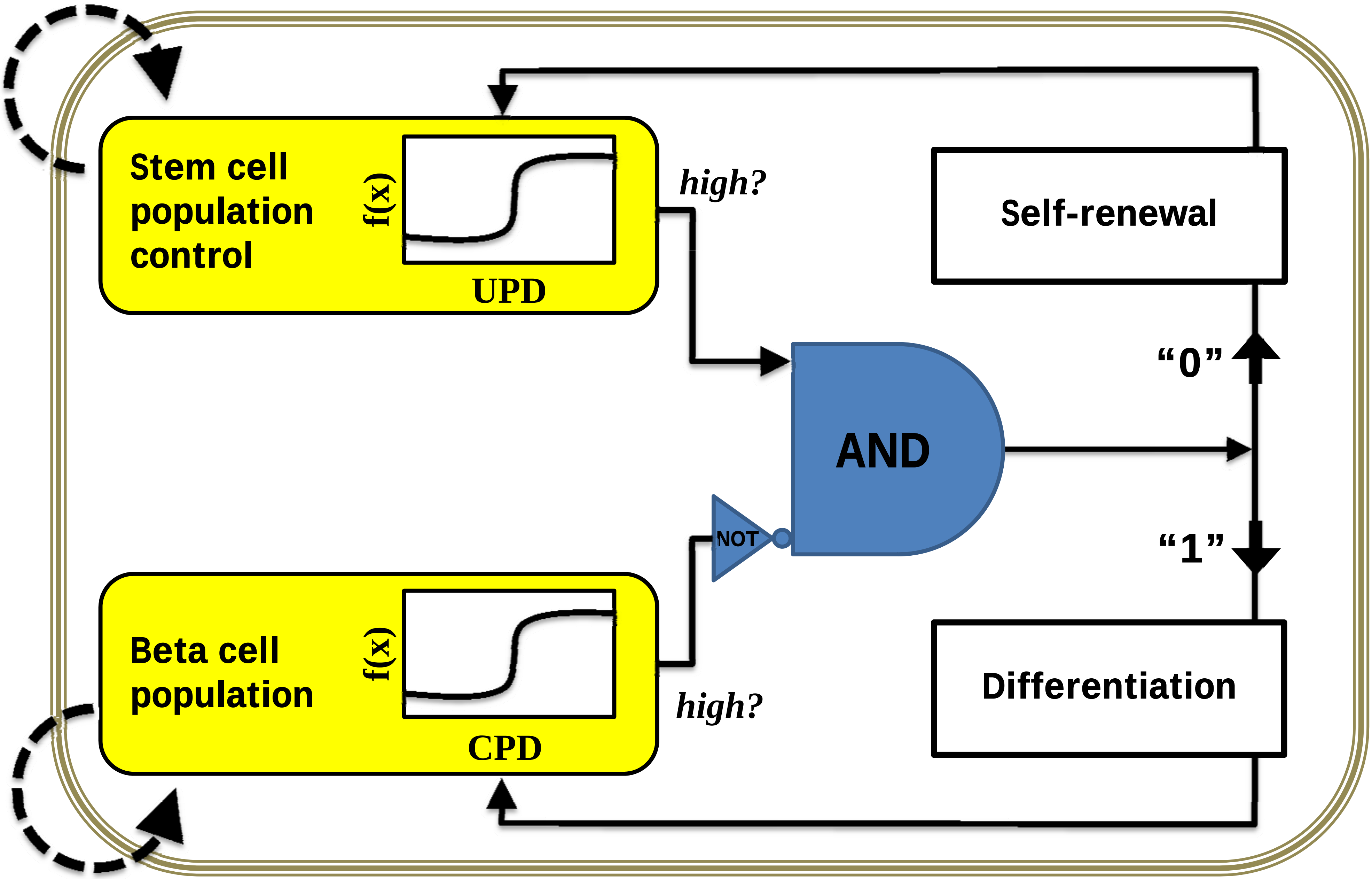
Toggle Switch Bistability
HIGH - LOW



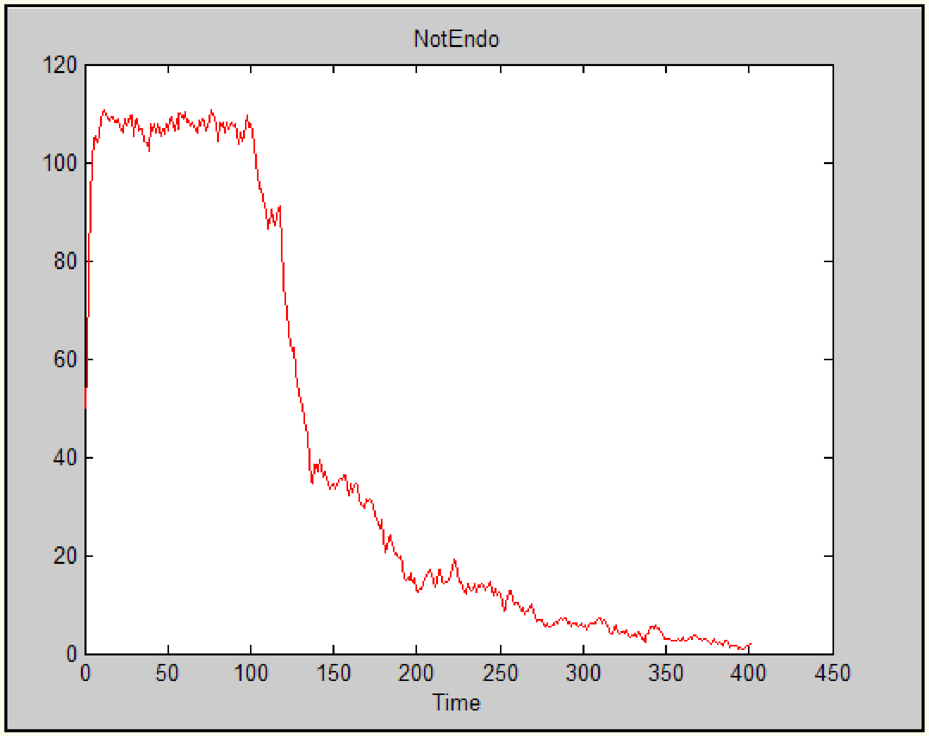
Toggle Switch Bistability
LOW - HIGH



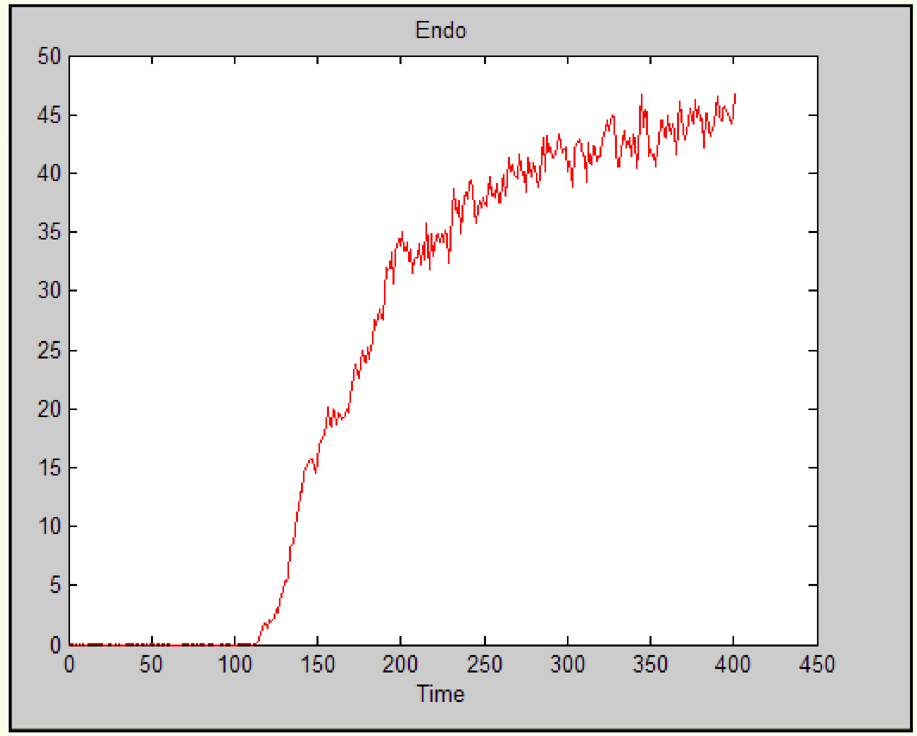
Logic to regulate differentiation



Artificial tissue homeostasis (version 0.1)

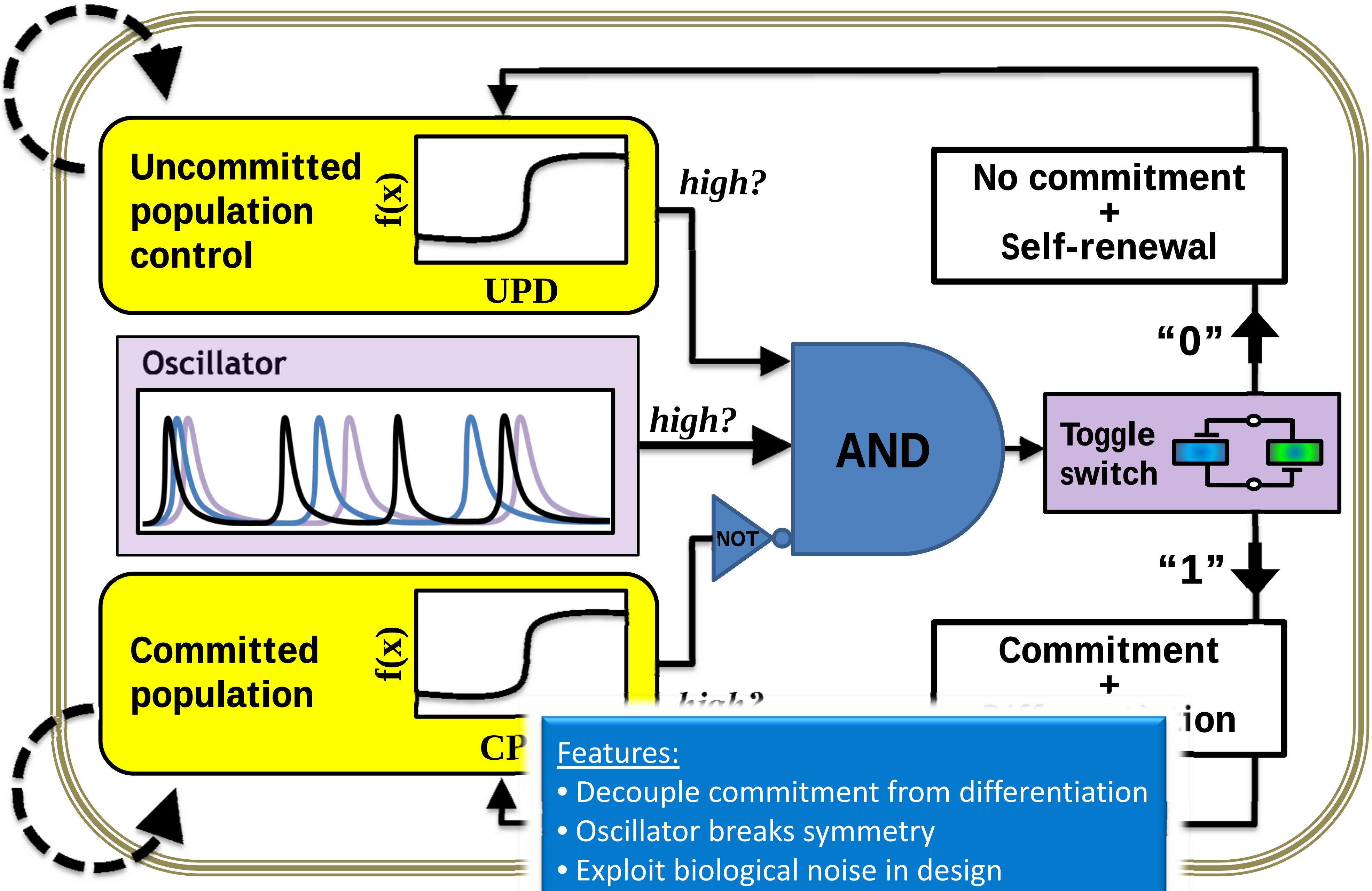


Stem cell indicator



Endoderm indicator

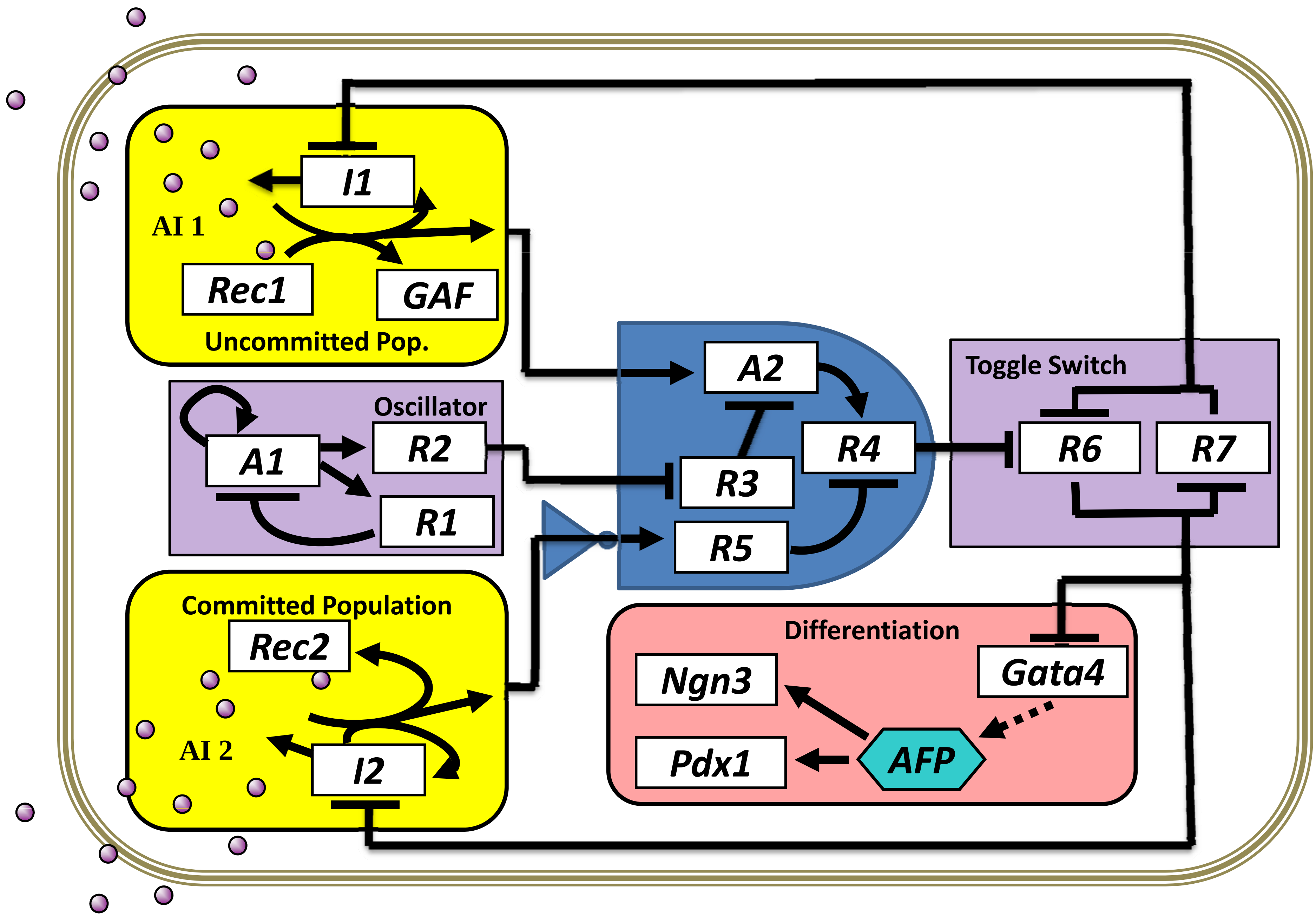
Logic to regulate differentiation



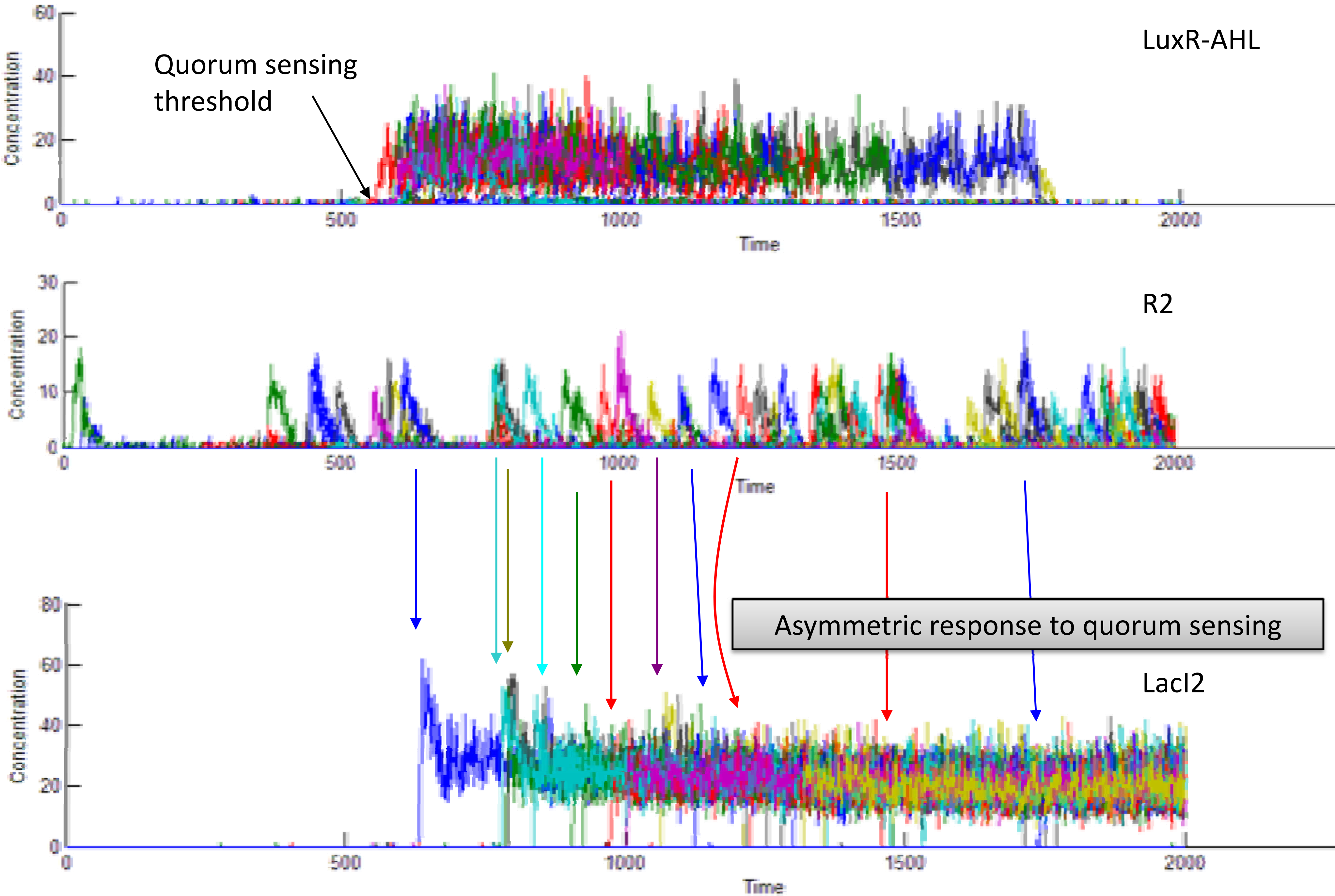
Features:

- Decouple commitment from differentiation
- Oscillator breaks symmetry
- Exploit biological noise in design

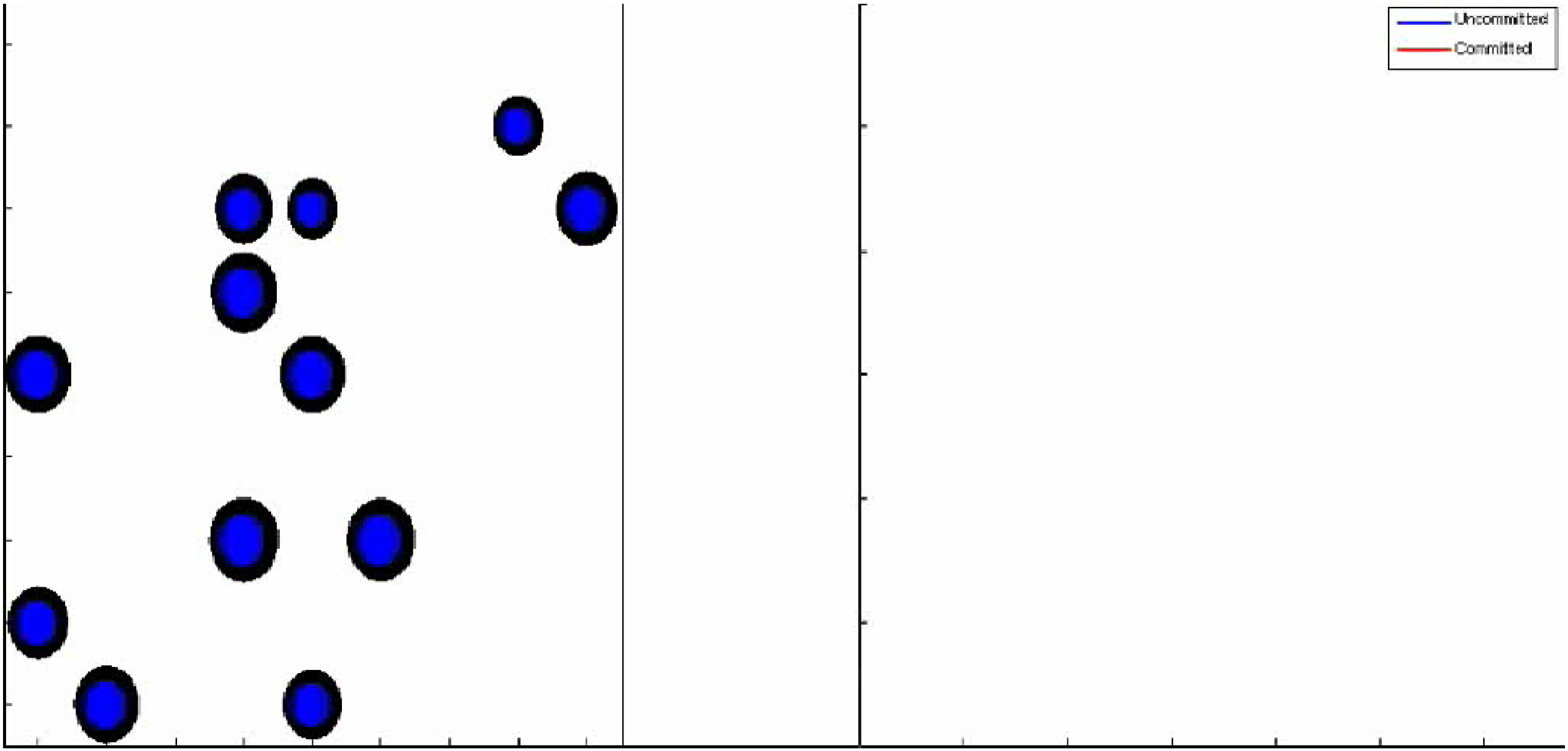
Artificial tissue homeostasis (version 0.2)



Simulation of symmetry breaking + commit



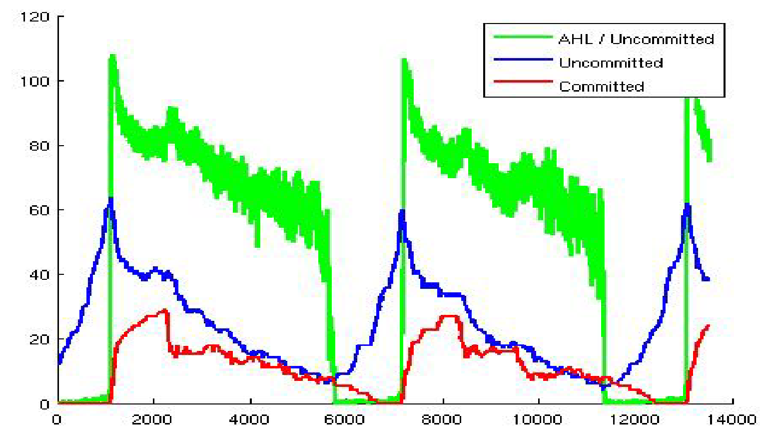
Simulation of artificial tissue homeostasis

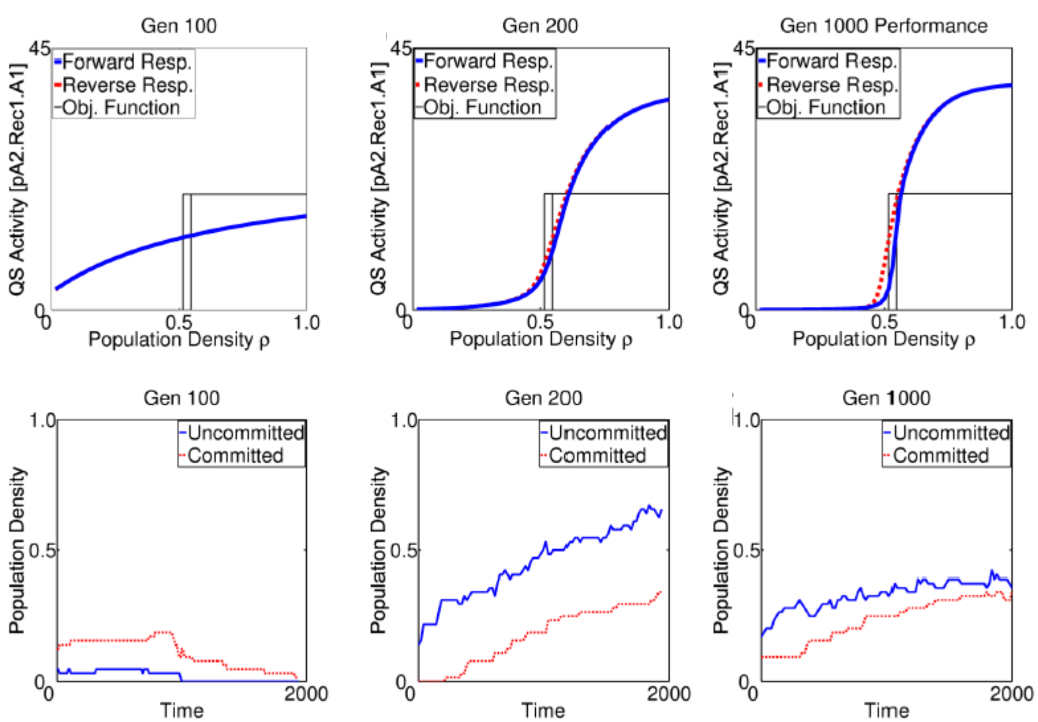


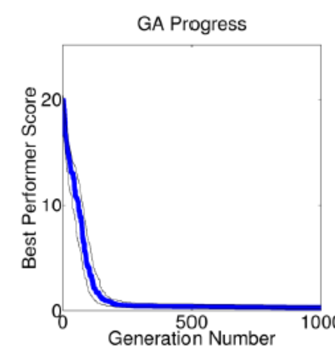
Blue = uncommitted
Red = committed

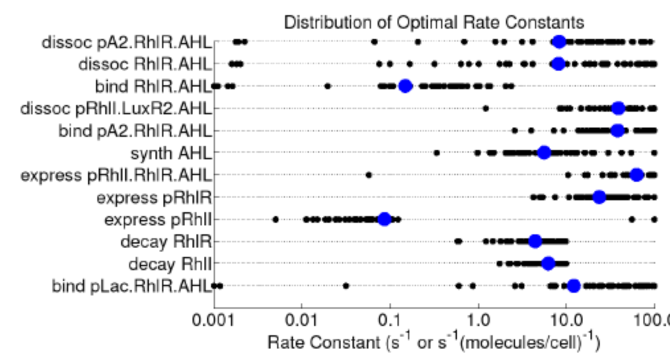
System optimization

- <u>Issue:</u>
  quorum sensing hysteresis results in non-optimal commitment
- <u>Approach:</u>
  Use sensitivity analysis to identify important parameters and genetic algorithms to optimize parameter set and remove hysteresis

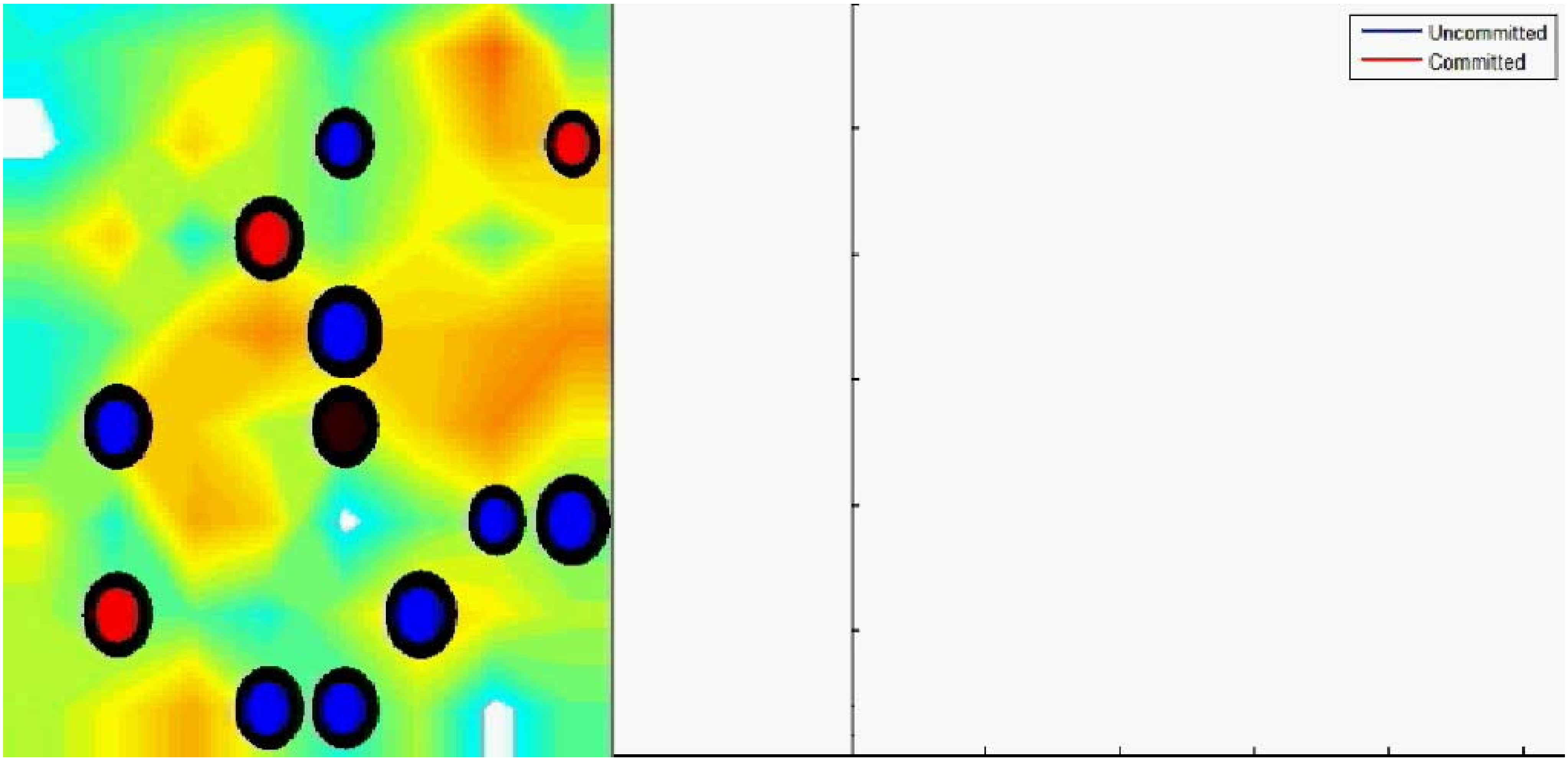








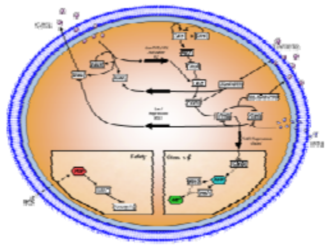
Simulation of artificial tissue homeostasis

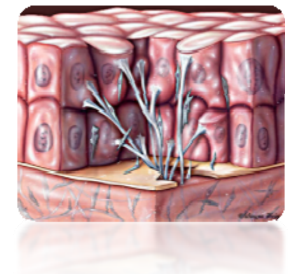


Blue = uncommitted
Red = committed

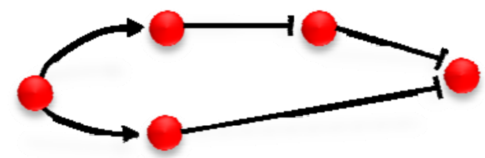
Modules to systems

systems





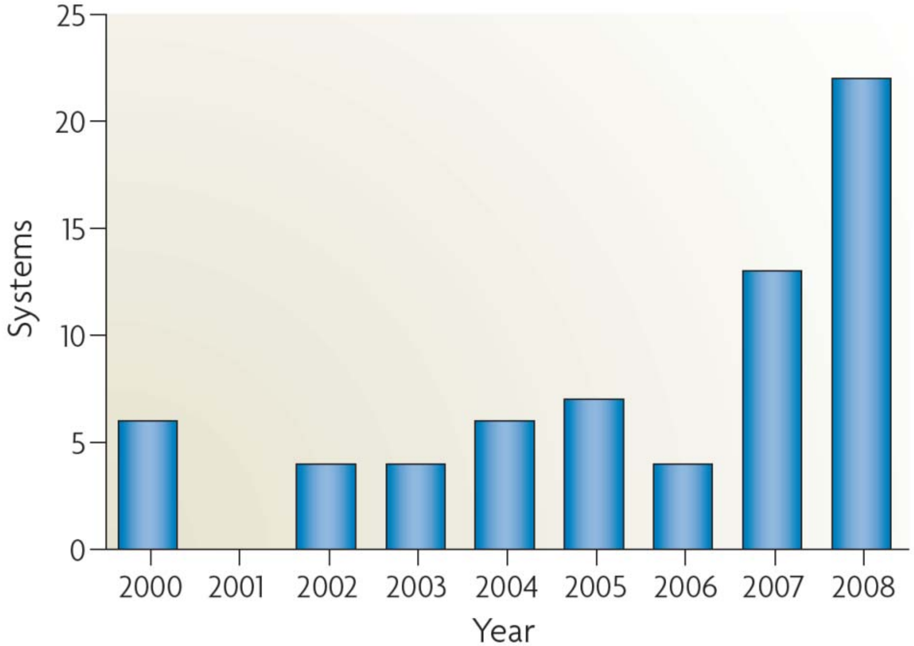
modules

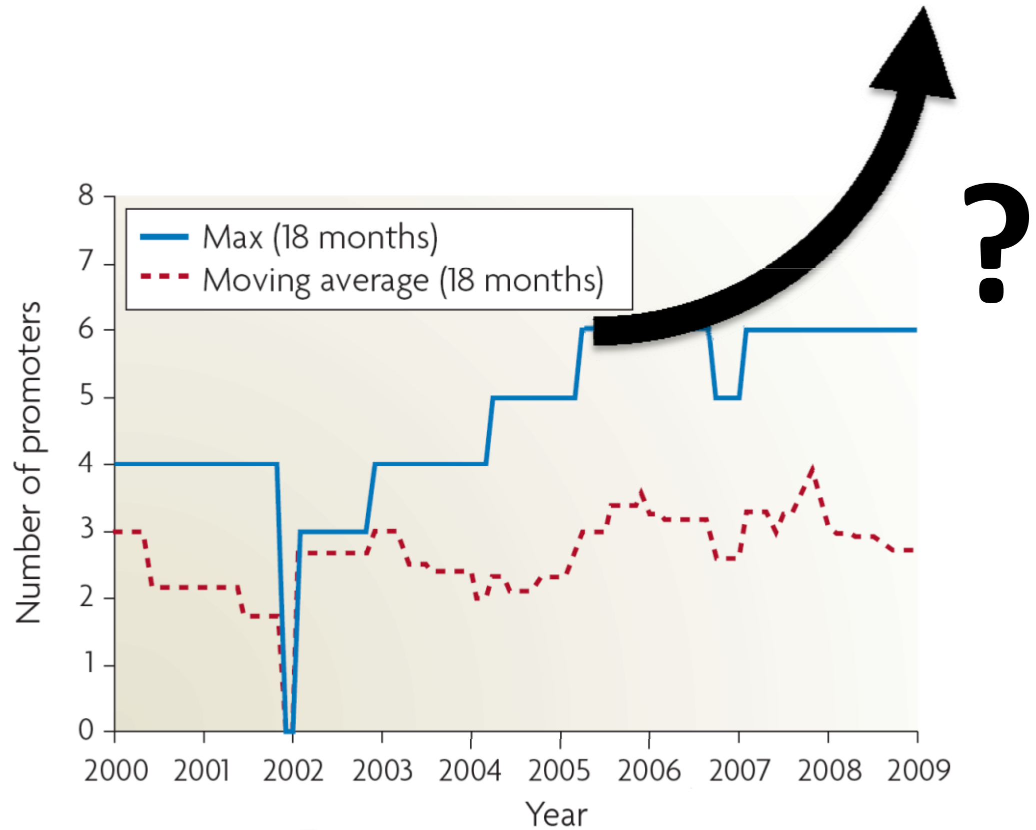


devices

physical

Synthetic biology projects





*Sampling of systems in publications with experimental circuits

Summary

- Implemented a variety of basic modules
  - Digital cascades, pulse generator, band detect, population control, communication, toggle switch
- Building and analyzing local↔global systems
  - Turing patterns, Conway's Game of Life, Predator-prey
- Pursuing applications
  - Programmed tissue regeneration, artificial tissue homeostasis, artificial immune system, RNAi-based cancer therapy
- Exploring system design principles and technologies
  - Hybrid circuits, multicellular, larger scale systems-level integration

(Simplified) Lessons

Be inspired by Biology / CS / EE / MechEng / CivilEng / …

then

Design it your way

but

Don’t underestimate biological complexity!

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Questions?