biological
computation
group

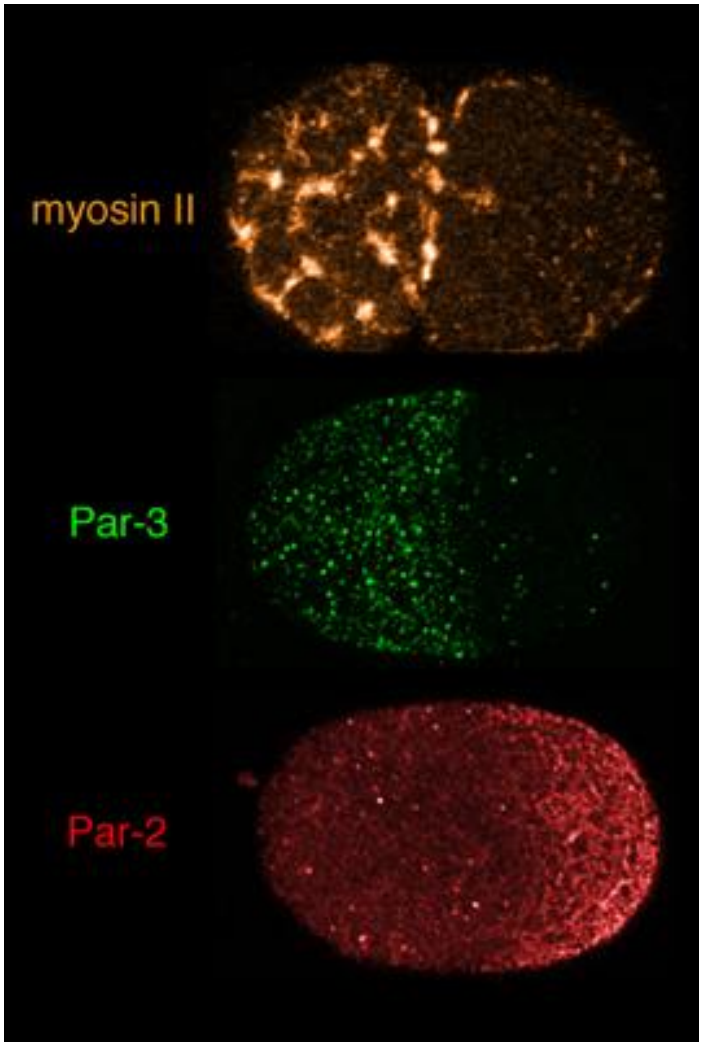
Microsoft Research

Programming Biology

Neil Dalchau

Pattern formation in multicellular systems

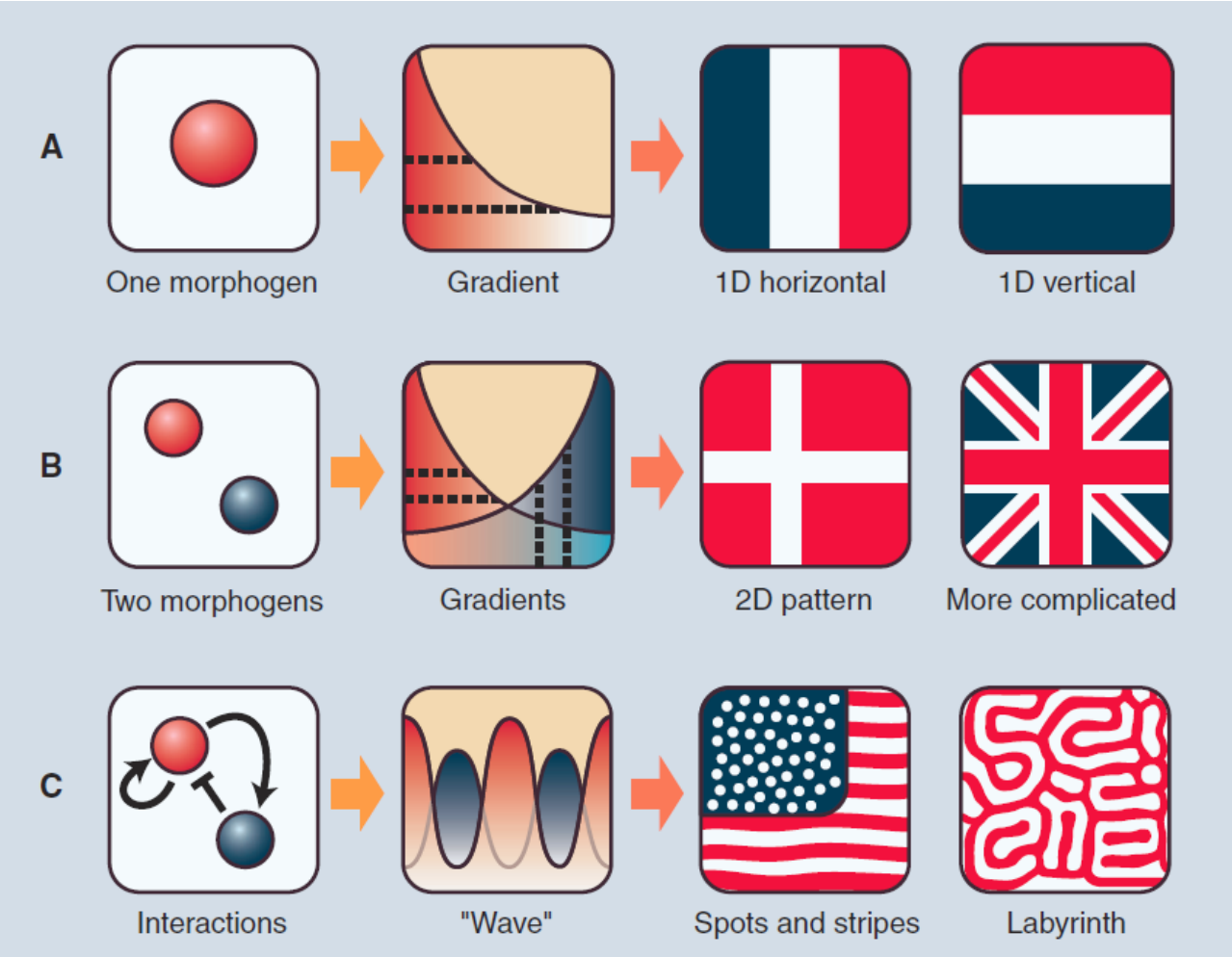
Pattern formation in single cells



munrolab.bsd.uchicago.edu

Even in a single cell, polarization and asymmetrical division can initiate distinct developmental programs

Morphogen gradient vs. Turing model

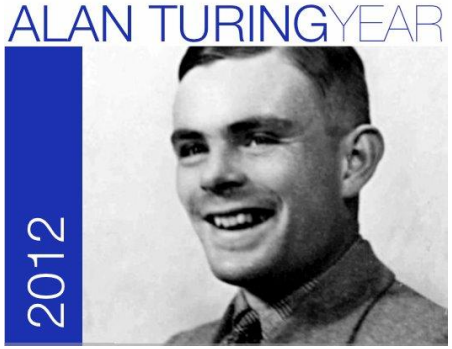


Kondo & Miura, Science 2000

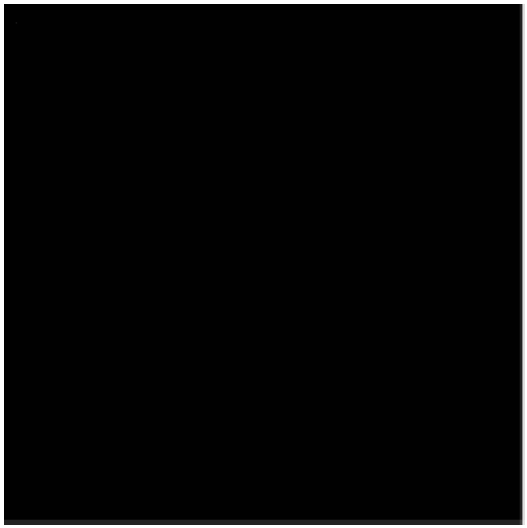
THE CHEMICAL BASIS OF MORPHOGENESIS

By A. M. TURING, F.R.S. *University of Manchester*

Vol. 237. B. 641. (Price 8s.) 5 [Published 14 August 1952



- Alan Turing's seminal work on morphogenesis introduced the idea of diffusible molecules being capable of generating stable spatial patterns
  - Two morphogens
  - Activation-inhibition



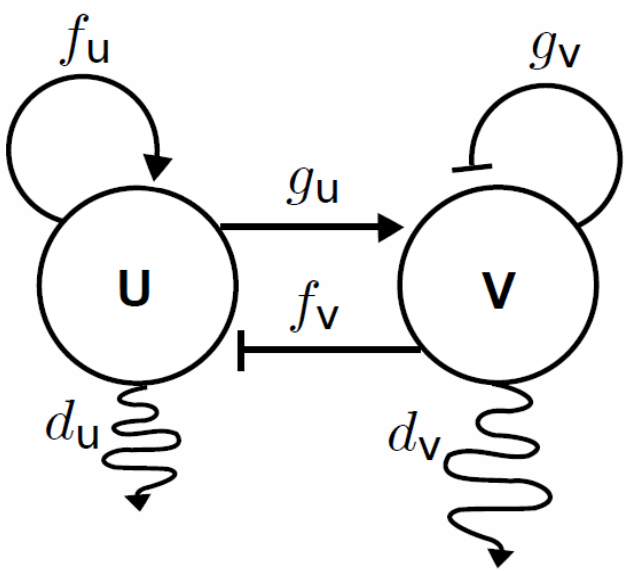
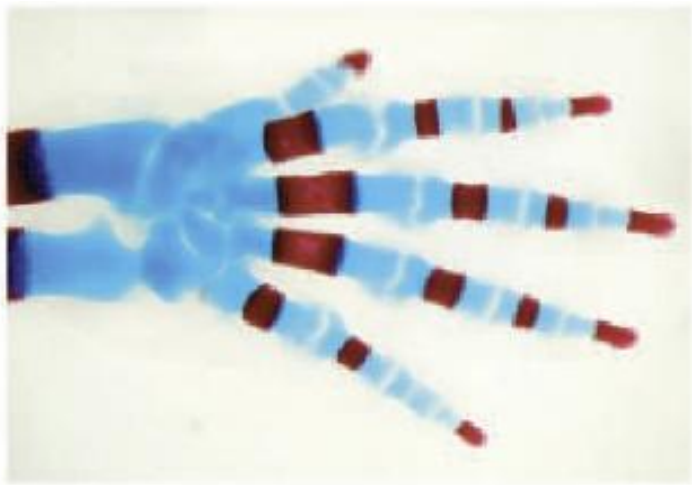
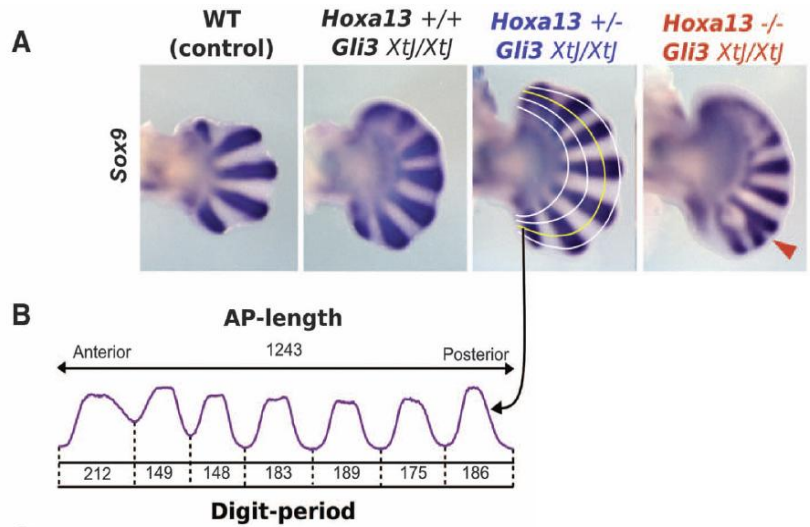


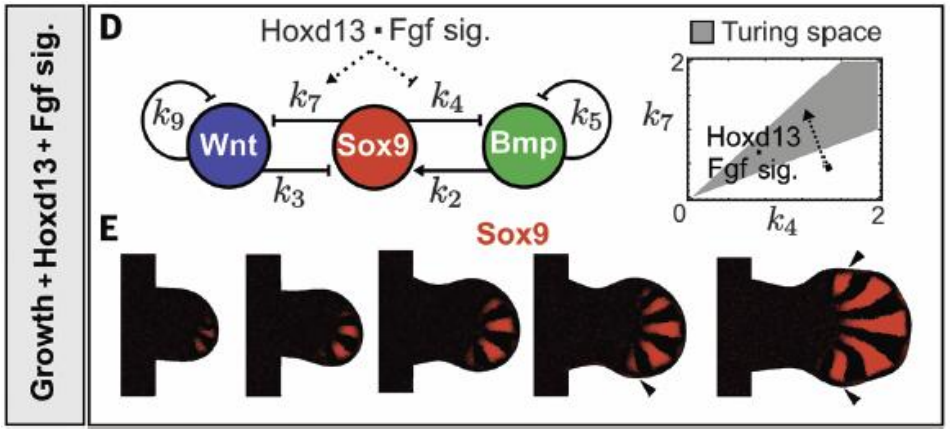
Figure S7: The network of the Activator-Inhibitor model

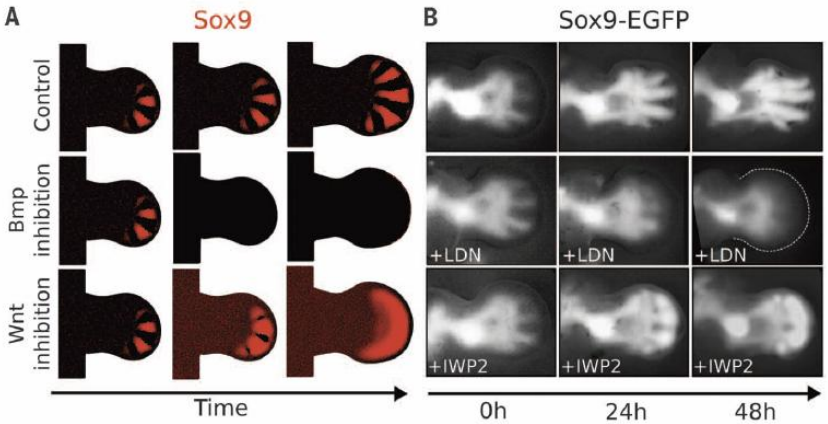
Turing pattern behind digit formation





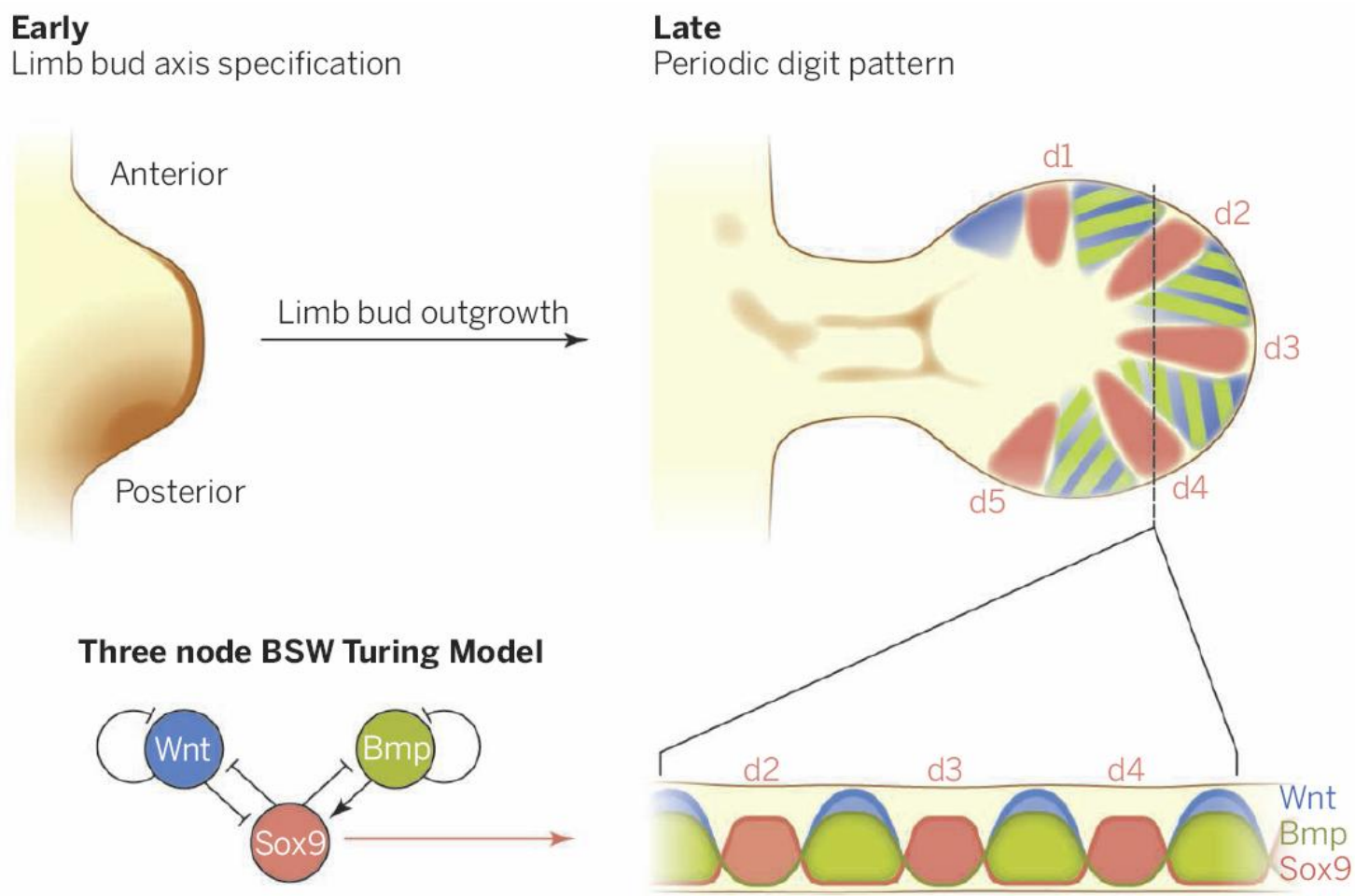
Sheth *et al.*, *Science* 2012





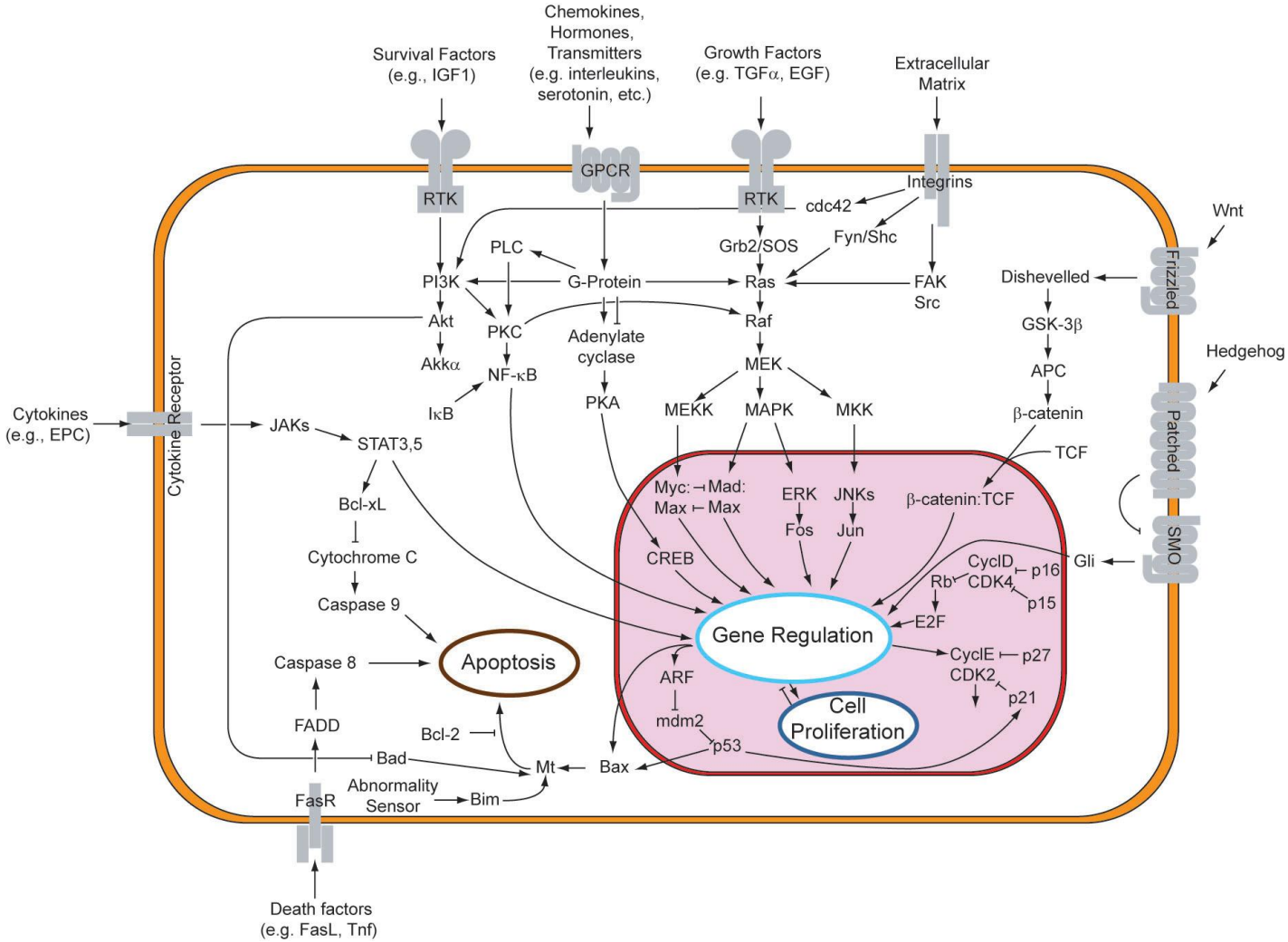
Raspopovic *et al.*, *Science* 2014

Turing pattern behind digit formation



Zuniga & Zeller, *Science* 2014

Complexity of evolved systems

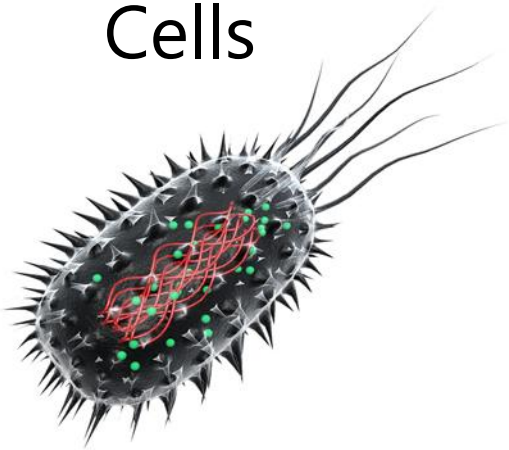


How can we understand biological patterning independent of the complications of evolution and development *in vivo*?

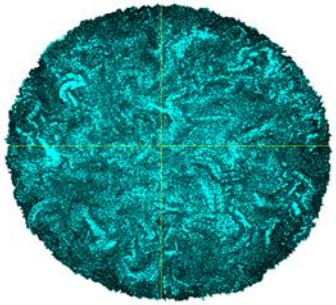
Programming biological systems

Molecules

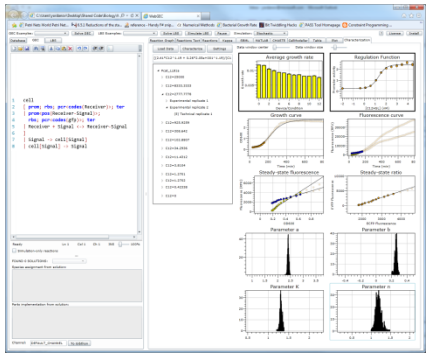
Cells

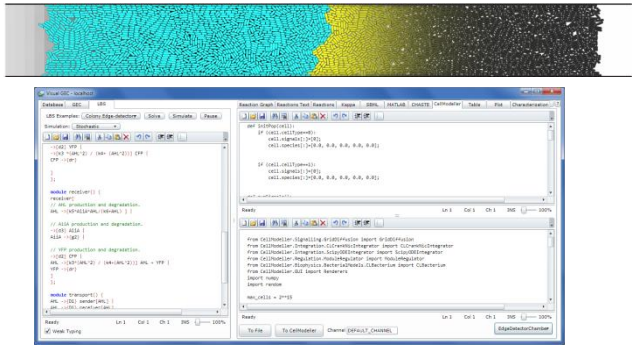


Colonies



Computer Aided Design software





Programming pattern formation in cells

- To what extent can we program static/dynamic/spatial behaviours in cell colonies?

- Can we *implement* **Turing patterns**?
  - Independent cell-cell signalling channels
  - Activation-inhibition network
  - Differential diffusion

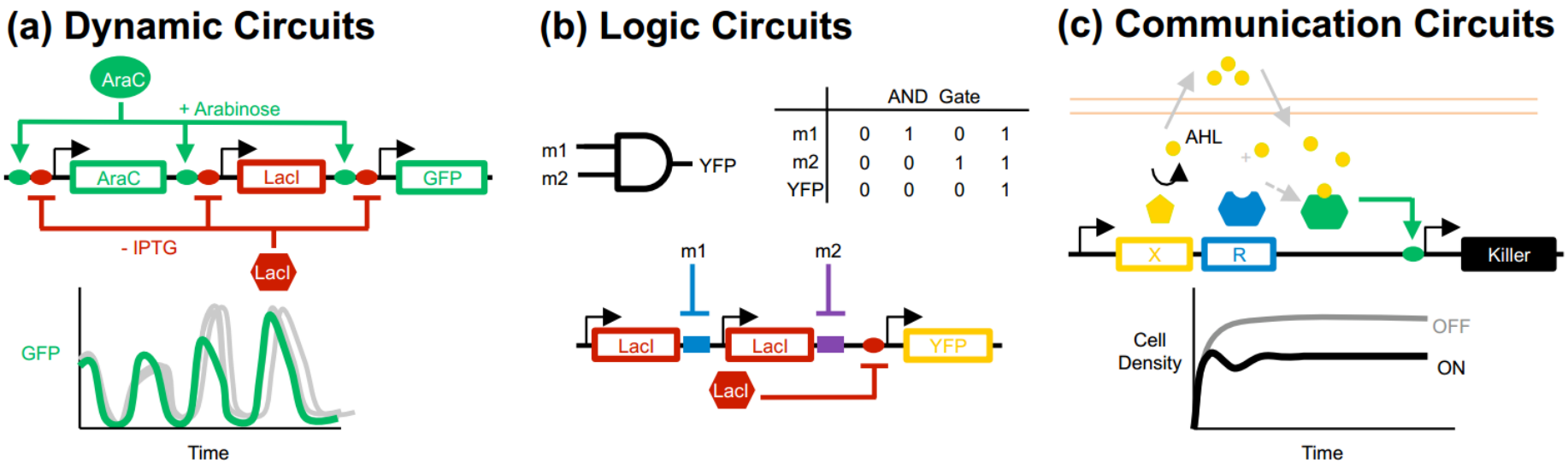
HASELOFF LAB
University of Cambridge

Paul Grant

James Brown

Jim Haseloff

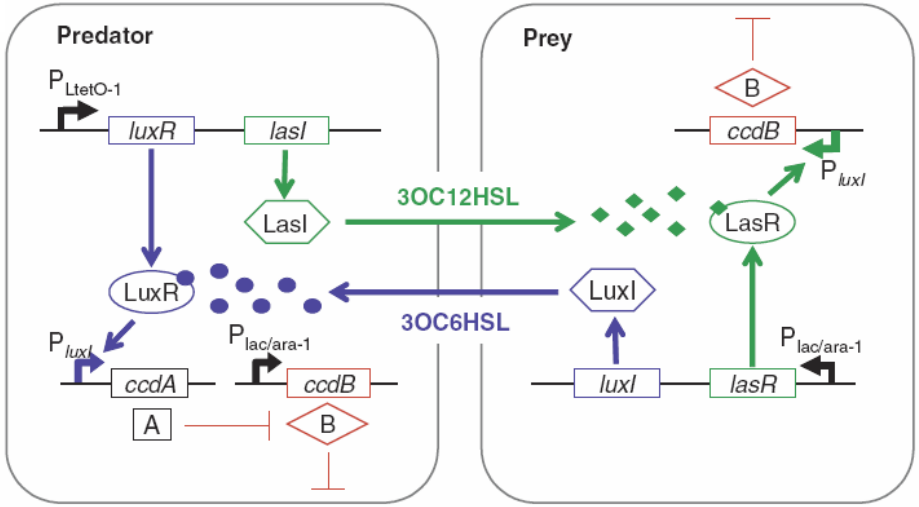
Using established biological components



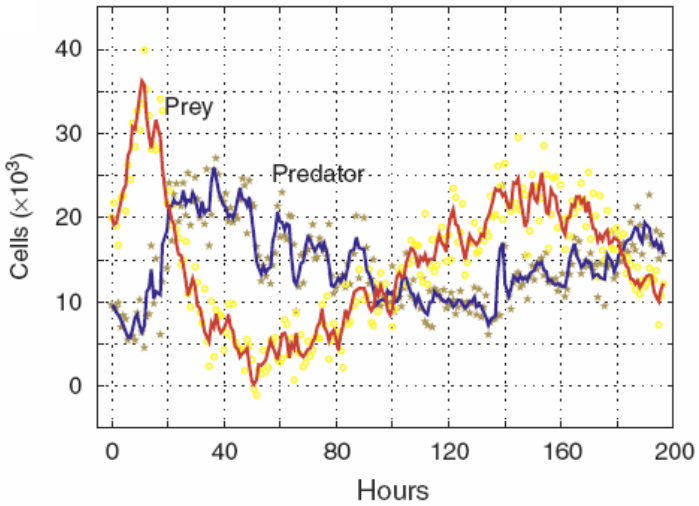
Chen *et al.*, Genome Biology 2012

Multiple signalling channels

Predator-prey interaction based on two AHL communication channels



Cycles of high prey abundance then high predator abundance

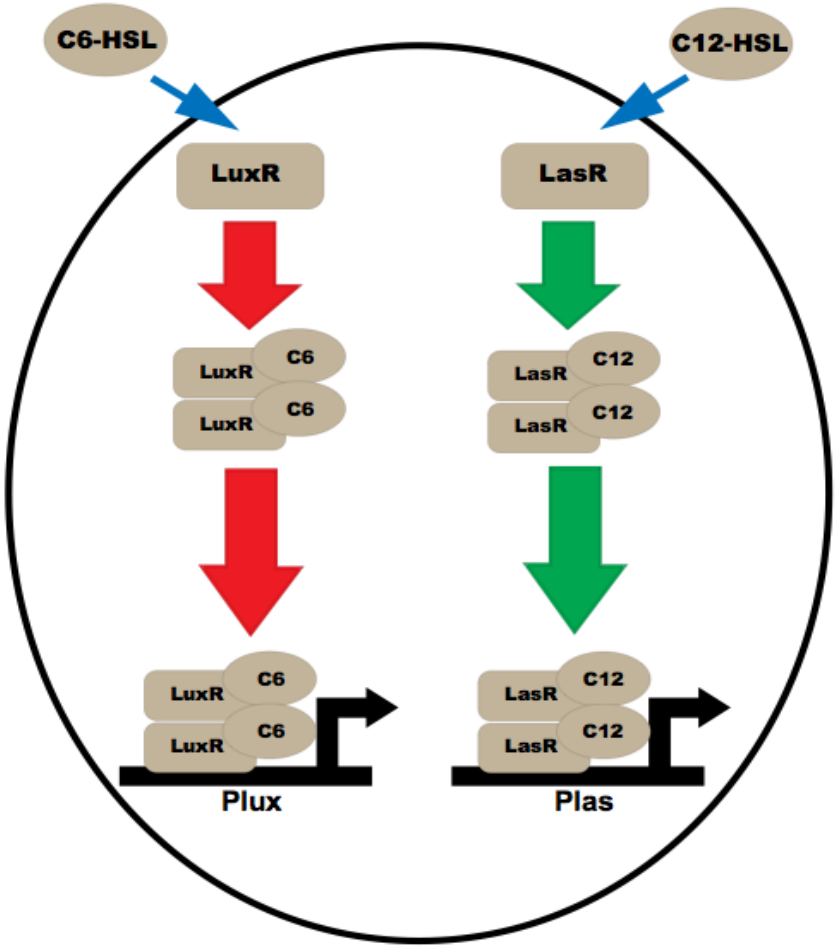


Balagaddé *et al.*, *Mol. Syst Biol.* 2008

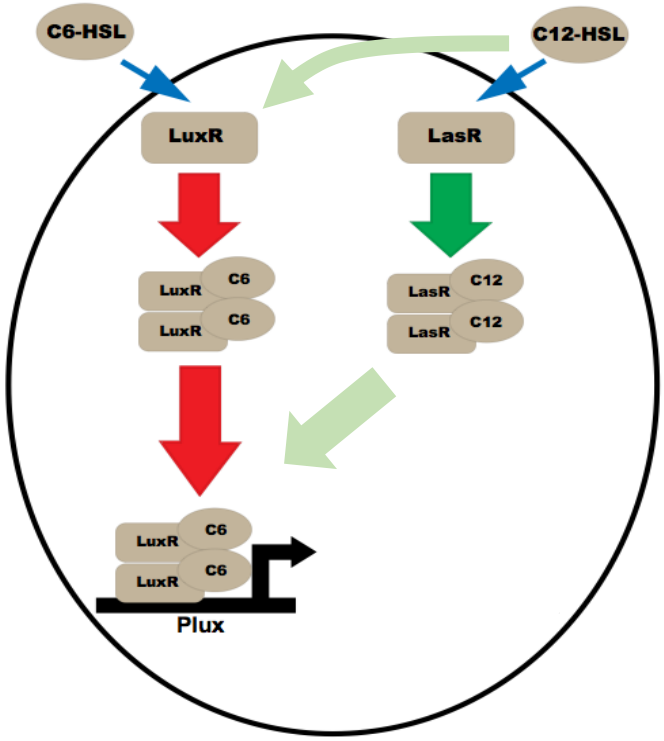
Two signalling channels in the same cell?

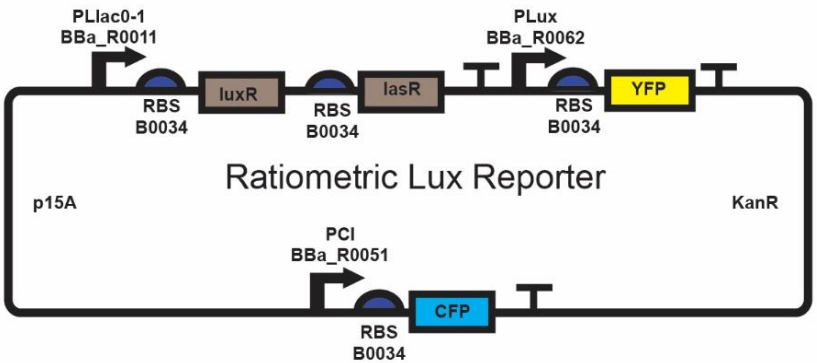
"A central goal of synthetic biology is to achieve multi-signal integration and signal processing in living cells for diagnostic, therapeutic and biotechnology applications."

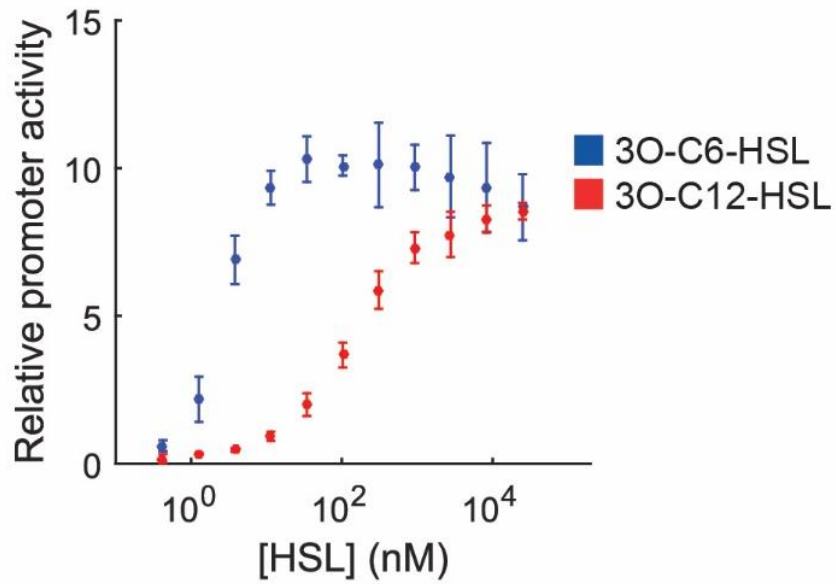
Daniel et al., *Nature* 2012



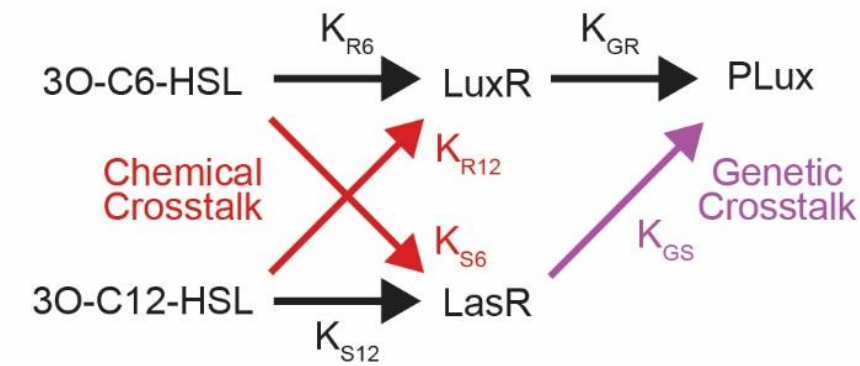
Measuring response of P_{Lux} to HSL signals







Identifying cross-talk



Chemical reaction model

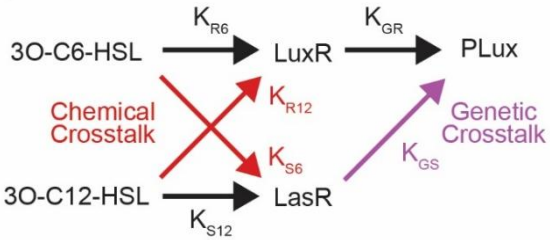
$$
\begin{array}{rcl|rcl}
\emptyset & \xrightarrow{n_R} & R & \emptyset & \xrightarrow{n_S} & S \\
R + C_k & \underset{u_{Rk}}{\overset{b_{Rk}}{\rightleftharpoons}} & R_k & S + C_k & \underset{u_{Sk}}{\overset{b_{Sk}}{\rightleftharpoons}} & S_k \\
R_k + R_k & \underset{u_{Dk}}{\overset{b_{Dk}}{\rightleftharpoons}} & D_k & S_k + S_k & \underset{u_{Ek}}{\overset{b_{Ek}}{\rightleftharpoons}} & E_k \\
G + D_k & \underset{u_{GDk}}{\overset{b_{GDk}}{\rightleftharpoons}} & G.D_k & G + E_k & \underset{u_{GEk}}{\overset{b_{GEk}}{\rightleftharpoons}} & G.E_k \\
G.D_k & \xrightarrow{a_{Rk}} & G.D_k + \text{mRNA} & G.E_k & \xrightarrow{a_{Sk}} & G.E_k + \text{mRNA} \\
G & \xrightarrow{a_0} & G + \text{mRNA} & \emptyset & \xrightarrow{n_G} & G
\end{array}
$$

$$R, S, R_k, S_k, D_k, E_k, G, G.D_k, G.E_k \xrightarrow{\gamma} \emptyset$$

Approximate transfer function

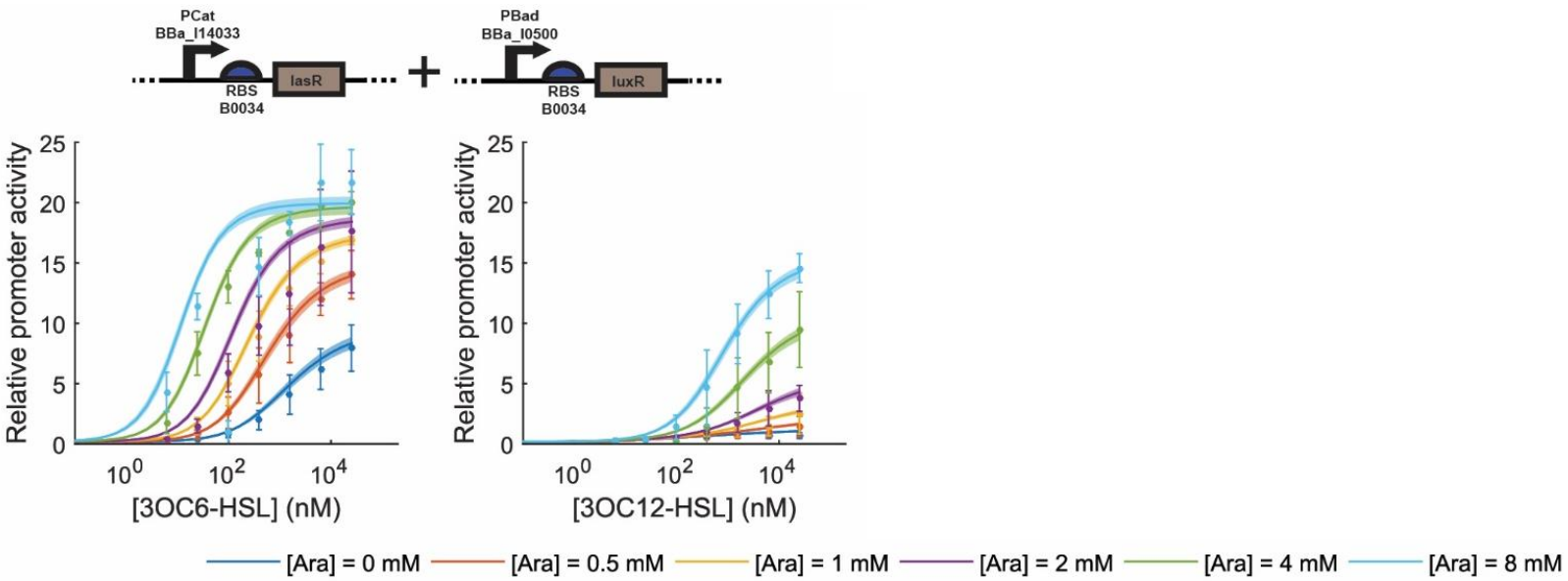
$$
\frac{a_0 + a_{Rk} K_{GRk} r^2 \left(\frac{K_{Rk} C_k}{1 + K_{Rk} C_k} \right)^n + a_{Sk} K_{GSk} s^2 \left(\frac{K_{Sk} C_k}{1 + K_{Sk} C_6} \right)^n}{1 + K_{GRk} r^2 \left(\frac{K_{Rk} C_k}{1 + K_{Rk} C_k} \right)^n + K_{GSk} s^2 \left(\frac{K_{Sk} C_k}{1 + K_{Sk} C_k} \right)^n}
$$

Controlling LuxR and LasR

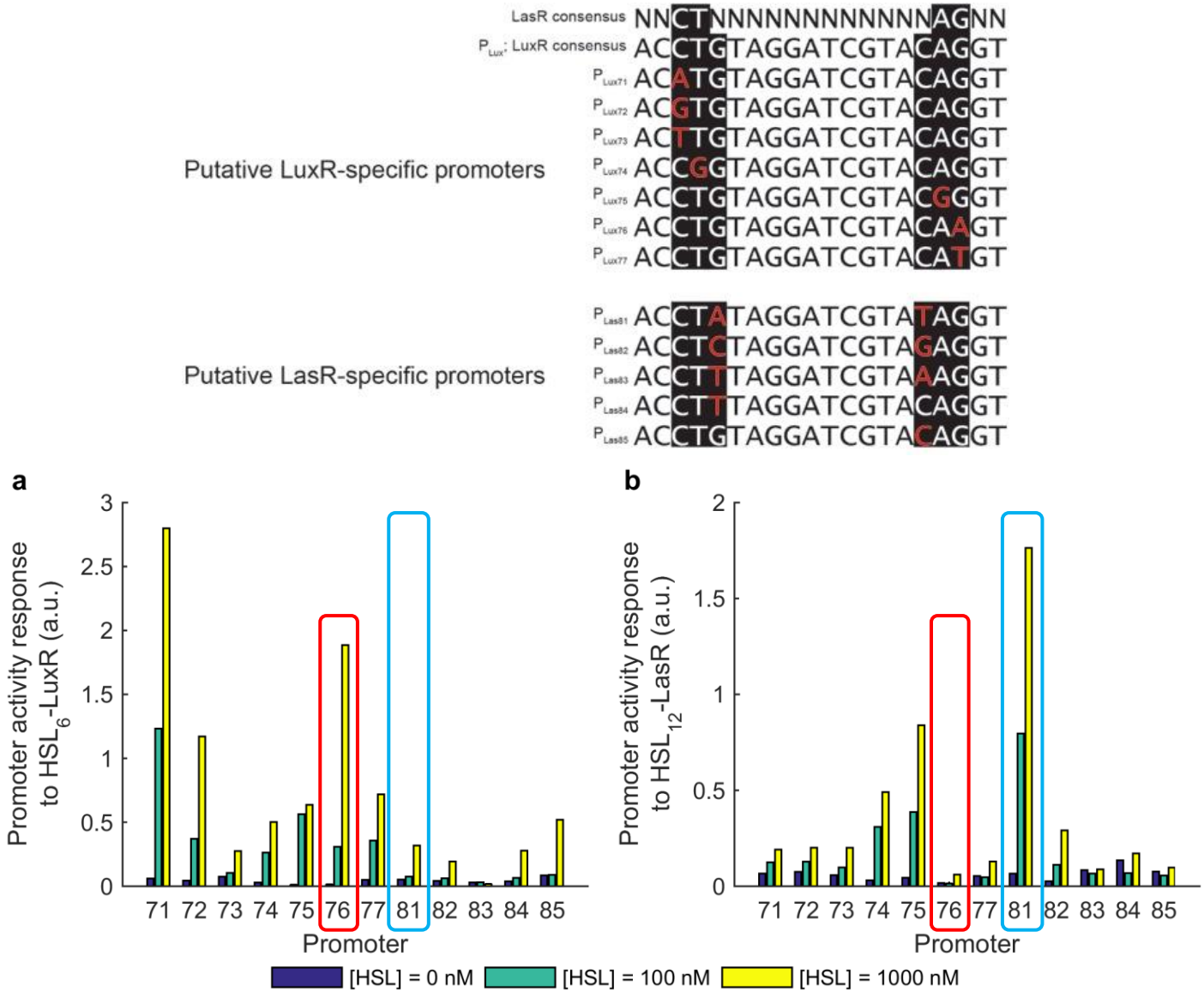


Arabinose-inducible **LuxR**

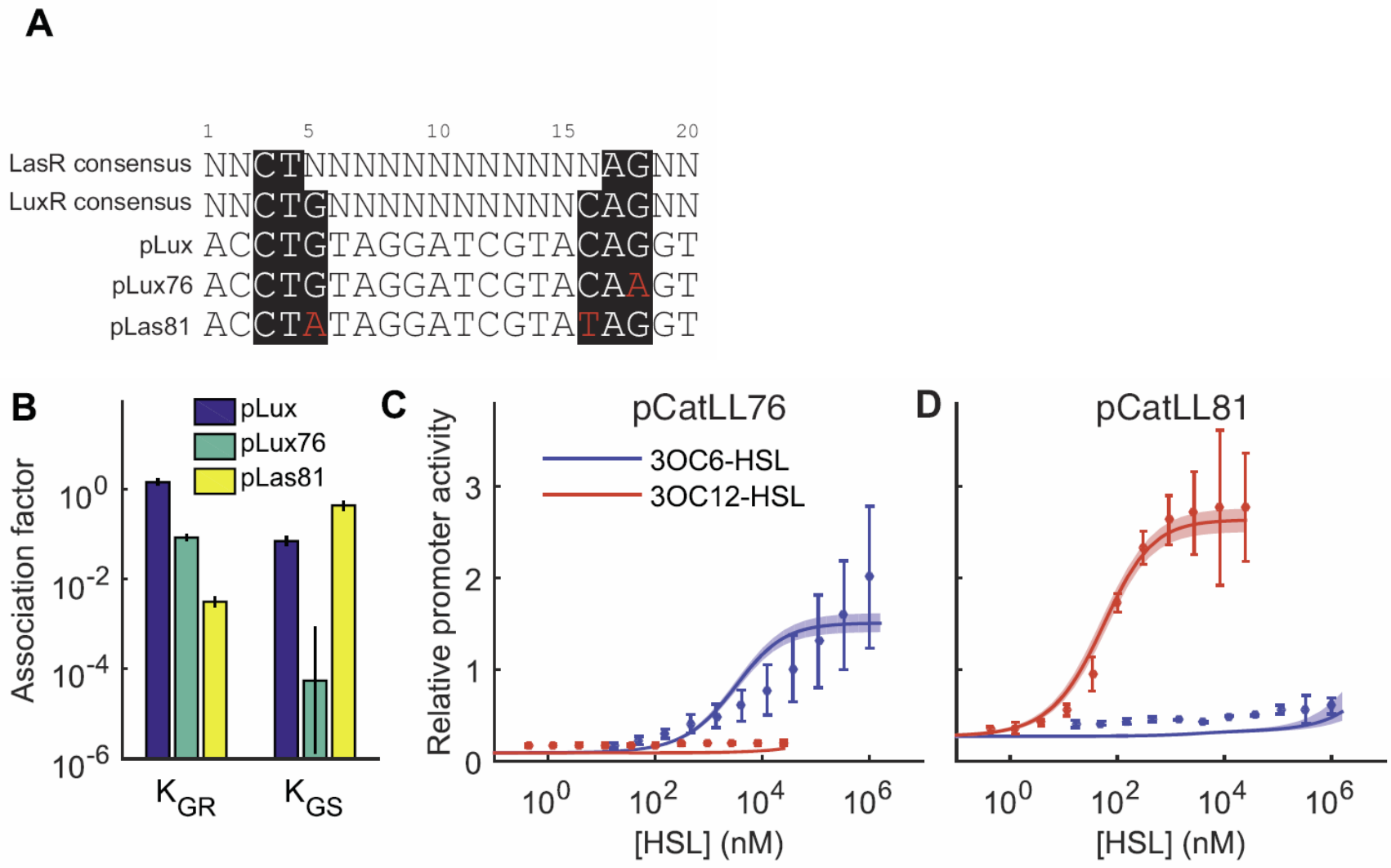
Arabinose-inducible **LasR**



Mutants for exclusive luxR/lasR responses

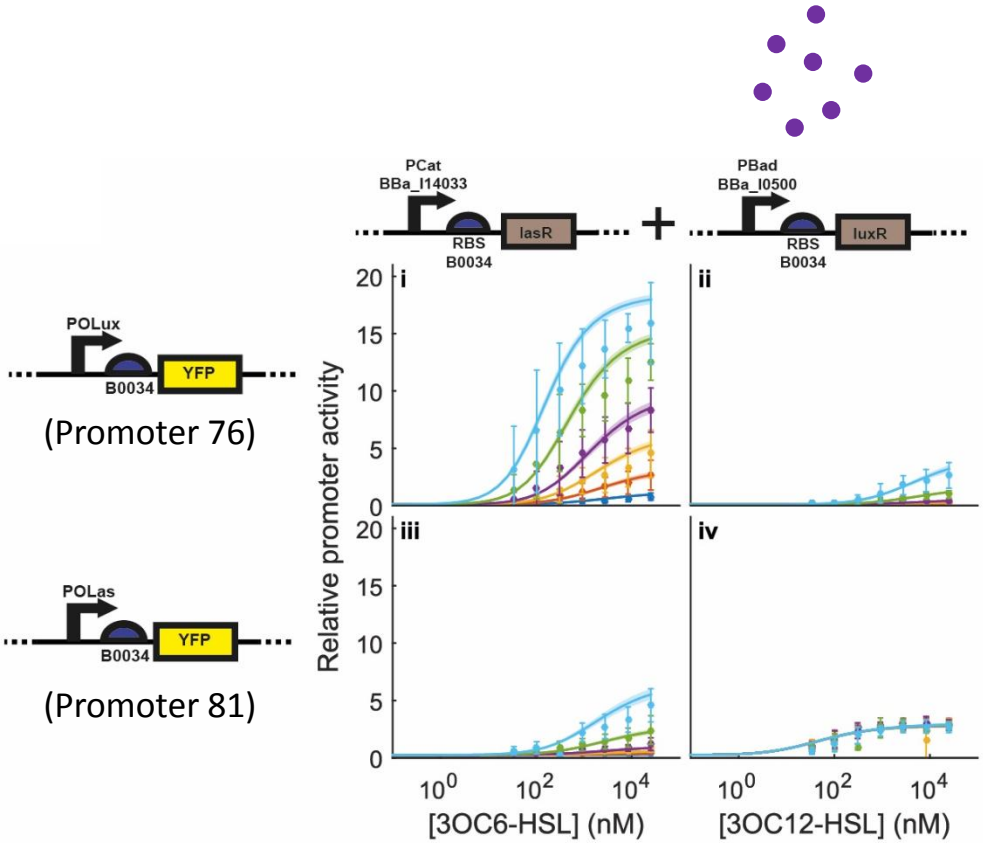


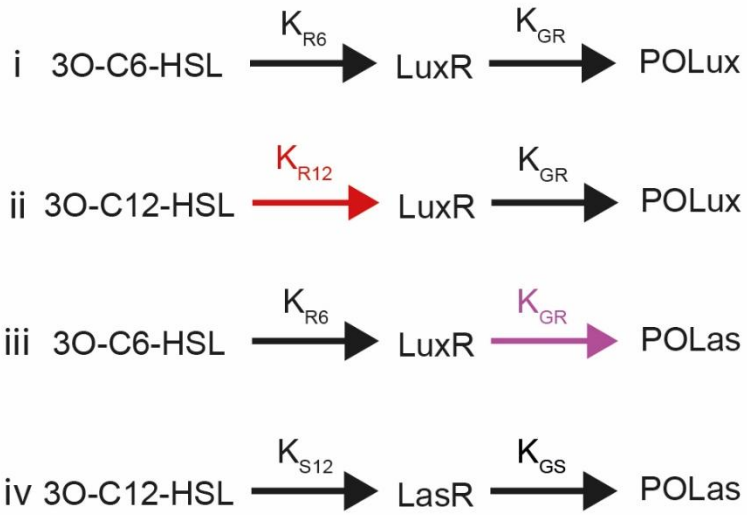
Candidate mutants



Response to increased **LuxR** levels

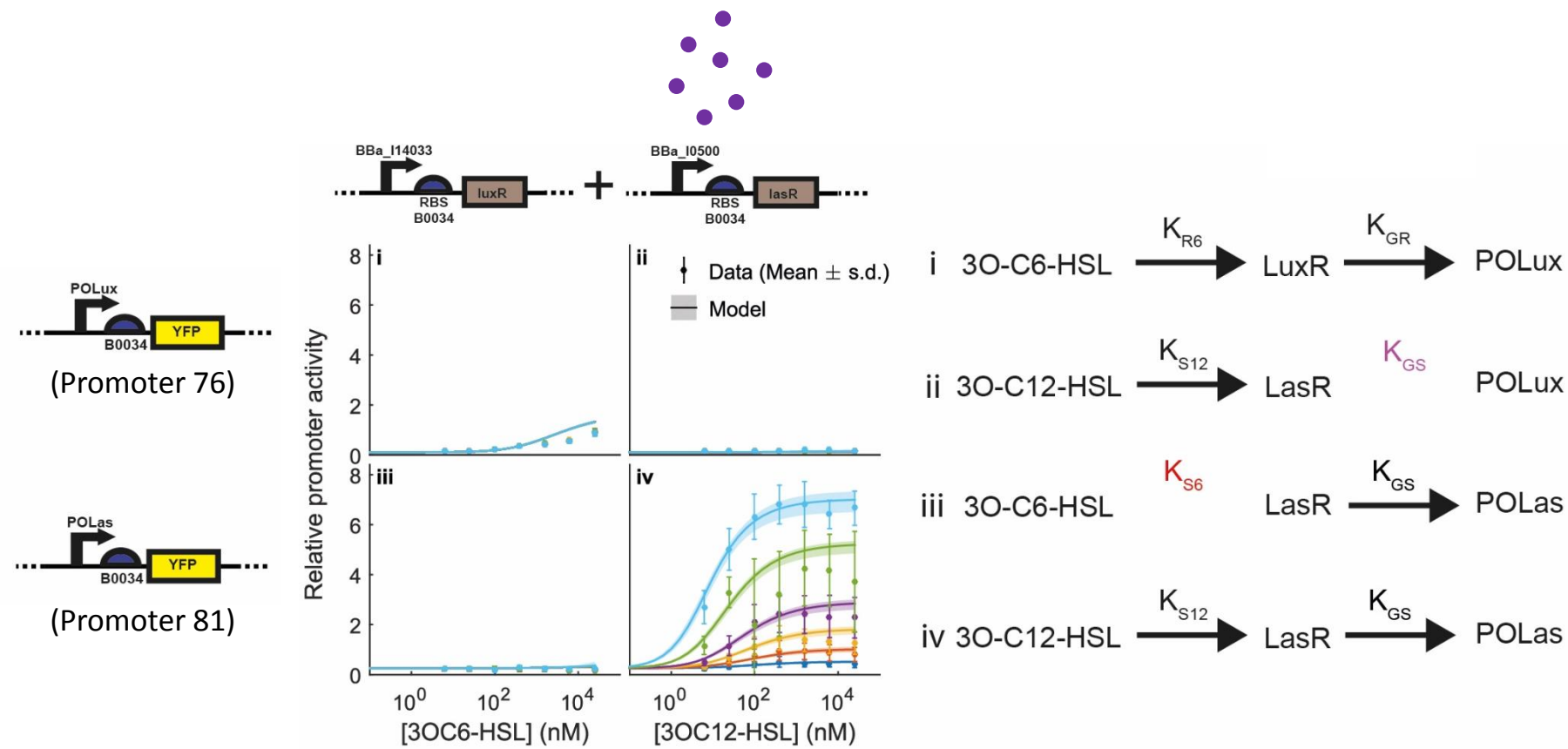
Arabinose-inducible **LuxR**



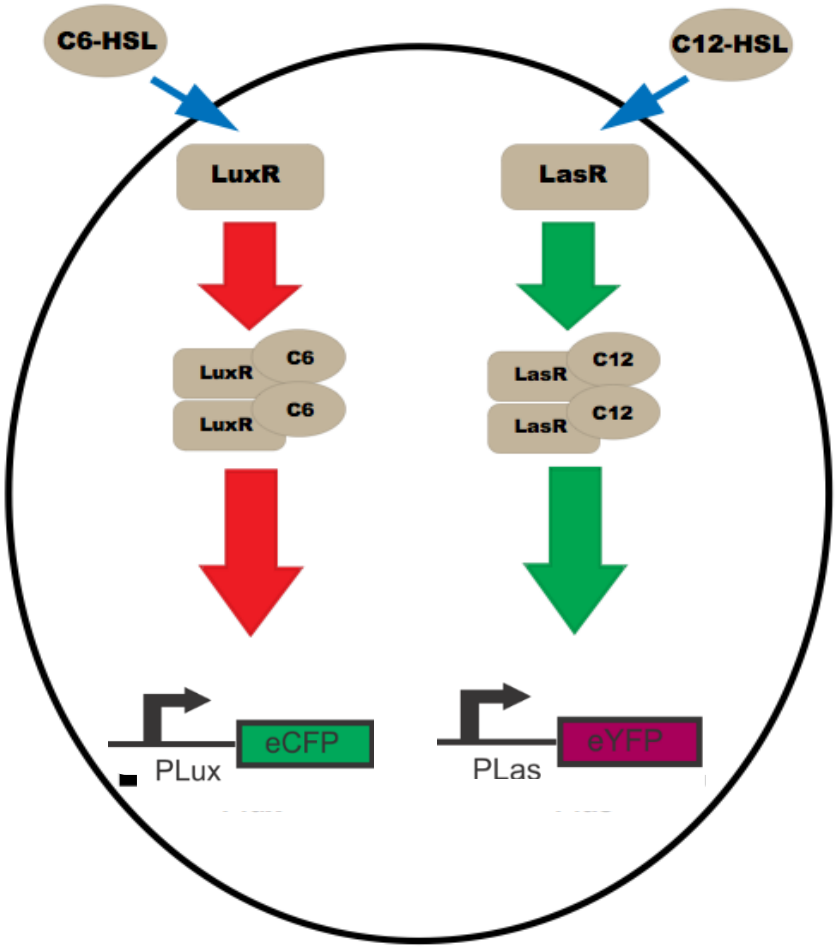


Response to increased **LasR** levels

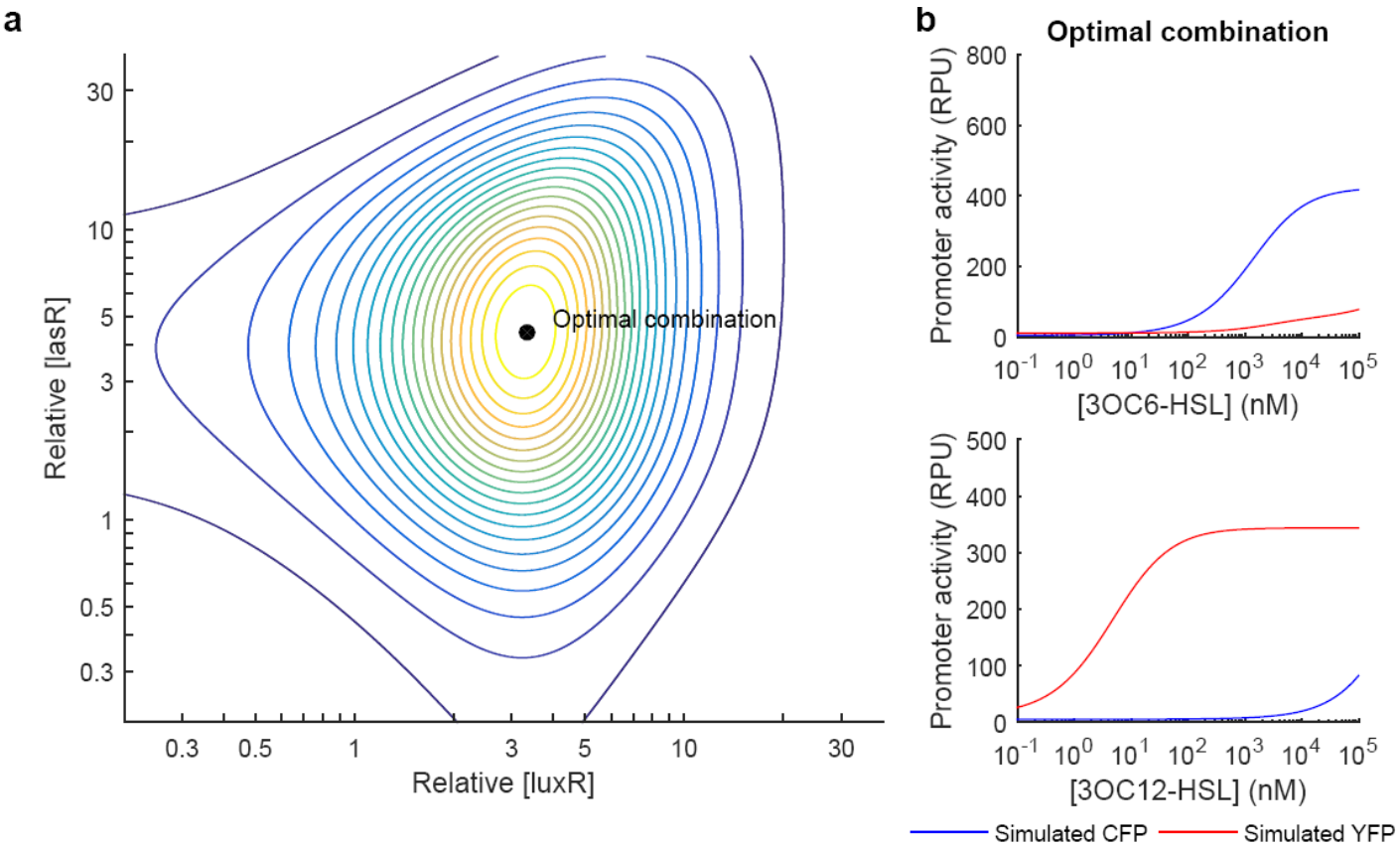
Arabinose-inducible **LasR**



Double receiver cells

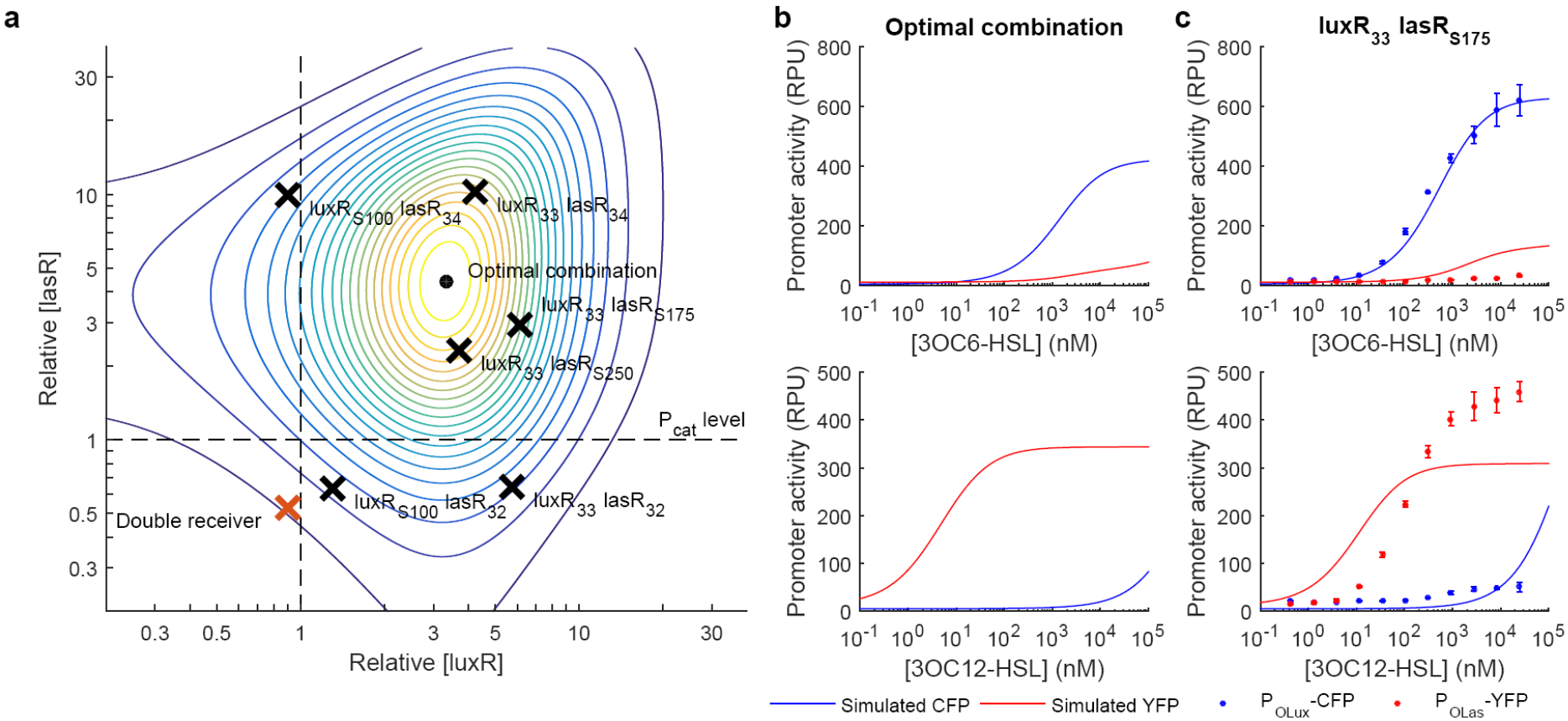


Minimize impact of chemical crosstalk: Selection of optimal luxR/lasR levels



$$\max_{R,S}\left\{\frac{f_6^{P76}(R,S)}{f_{12}^{P76}(R,S)}\cdot\frac{f_{12}^{P81}(R,S)}{f_6^{P81}(R,S)}\right\}$$

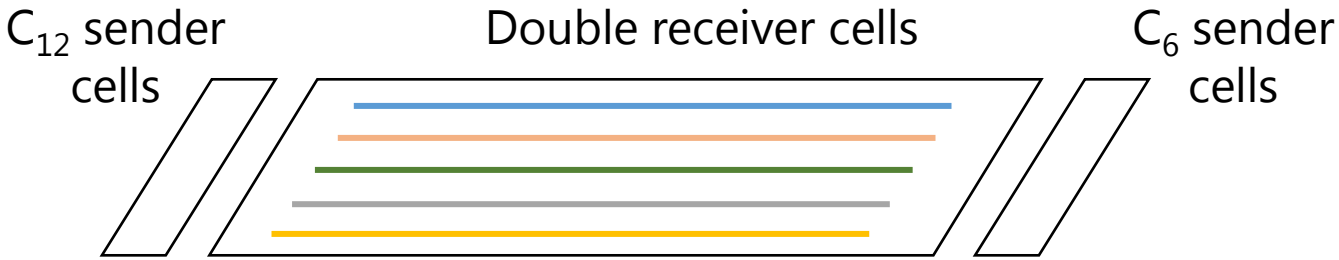
Minimize impact of chemical crosstalk: Selection of optimal luxR/lasR levels



$$\max_{R,S}\left\{\frac{f_6^{P76}(R,S)}{f_{12}^{P76}(R,S)}\cdot\frac{f_{12}^{P81}(R,S)}{f_6^{P81}(R,S)}\right\}$$

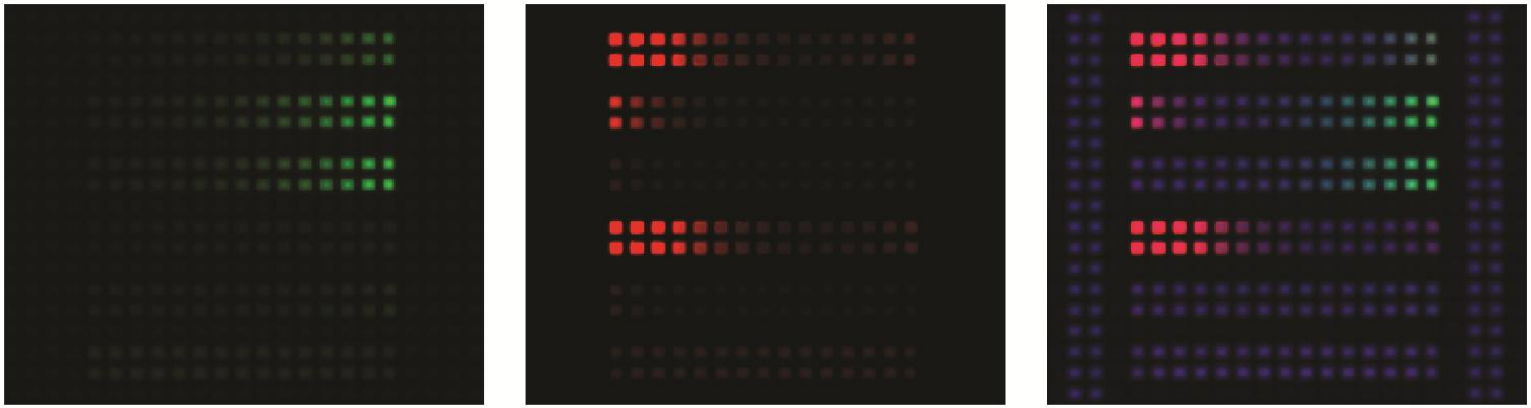
Independent detection of signal propagation

Experiment design



Measurements

CFP (reports C_6) YFP (reports C_{12}) Overlay

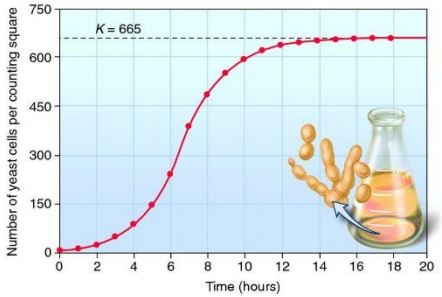


$luxR_{33}$ $lasR_{34}$
$luxR_{33}$ $lasR_{S175}$
$luxR_{33}$ $lasR_{32}$
$luxR_{S100}$ $lasR_{34}$
$luxR_{S100}$ $lasR_{32}$
Constitutive

(Snapshot after 36 hours)

A spatial model

Cell growth
(Logistic)



(http://biology-forums.com)

$$\frac{\partial[N]}{\partial t} = r[N](1-[N])$$

$$\frac{\partial[c_6]}{\partial t} = -\gamma_{\mathrm{HSL}}[c_6] + D_6\frac{\partial^2[c_6]}{\partial x^2}$$

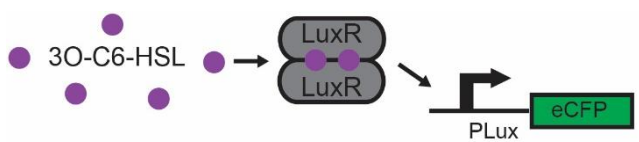
$$\frac{\partial[c_{12}]}{\partial t} = -\gamma_{\mathrm{HSL}}[c_{12}] + D_{12}\frac{\partial^2[c_{12}]}{\partial x^2}$$

Diffusion

$$\frac{\partial[\mathrm{CFP}]}{\partial t} = [N].f_{c6}(R,S,[c_6]) - \gamma_{\mathrm{FP}}[\mathrm{CFP}]$$

$$\frac{\partial[\mathrm{YFP}]}{\partial t} = [N].f_{c12}(R,S,[c_{12}]) - \gamma_{\mathrm{FP}}[\mathrm{YFP}]$$

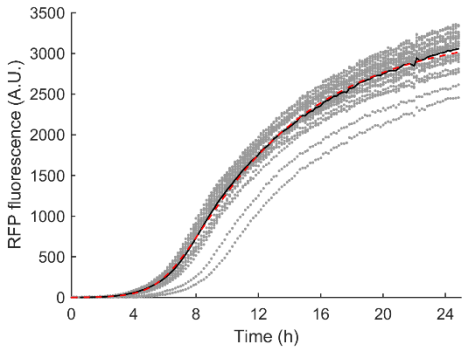
Gene expression

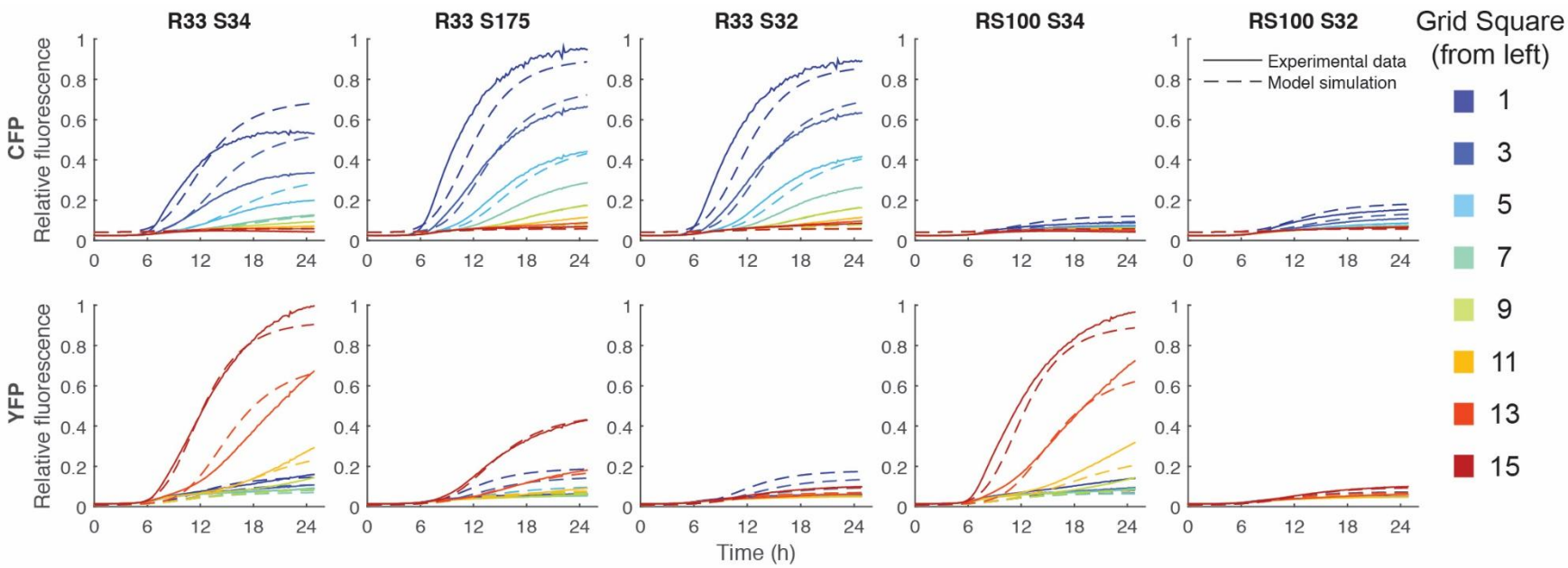


$$\frac{a_0 + a_{Rk}K_{GRk}r^2\left(\frac{K_{Rk}C_k}{1+K_{Rk}C_k}\right)^n + a_{Sk}K_{GSk}s^2\left(\frac{K_{Sk}C_k}{1+K_{Sk}C_6}\right)^n}{1 + K_{GRk}r^2\left(\frac{K_{Rk}C_k}{1+K_{Rk}C_k}\right)^n + K_{GSk}s^2\left(\frac{K_{Sk}C_k}{1+K_{Sk}C_k}\right)^n}$$

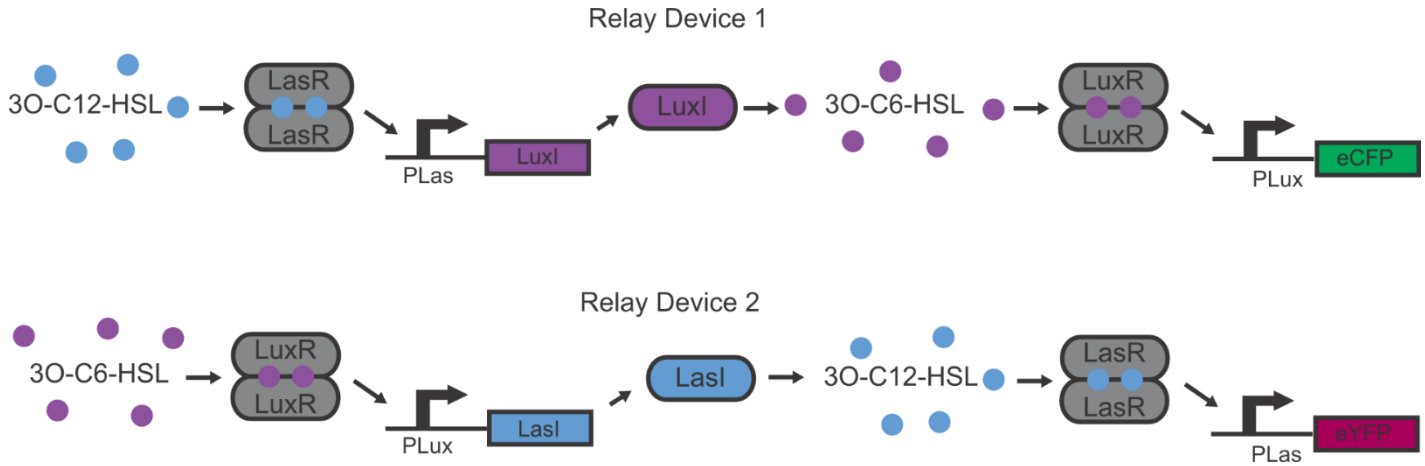
Parameterising the spatial model

- Used RFP measurement to parameterise cell growth
- Fit HSL diffusion and HSL sender cells to the spatial data

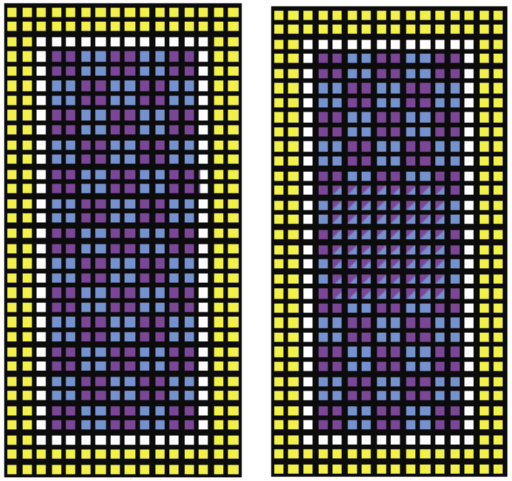




Patterning with intercellular signalling

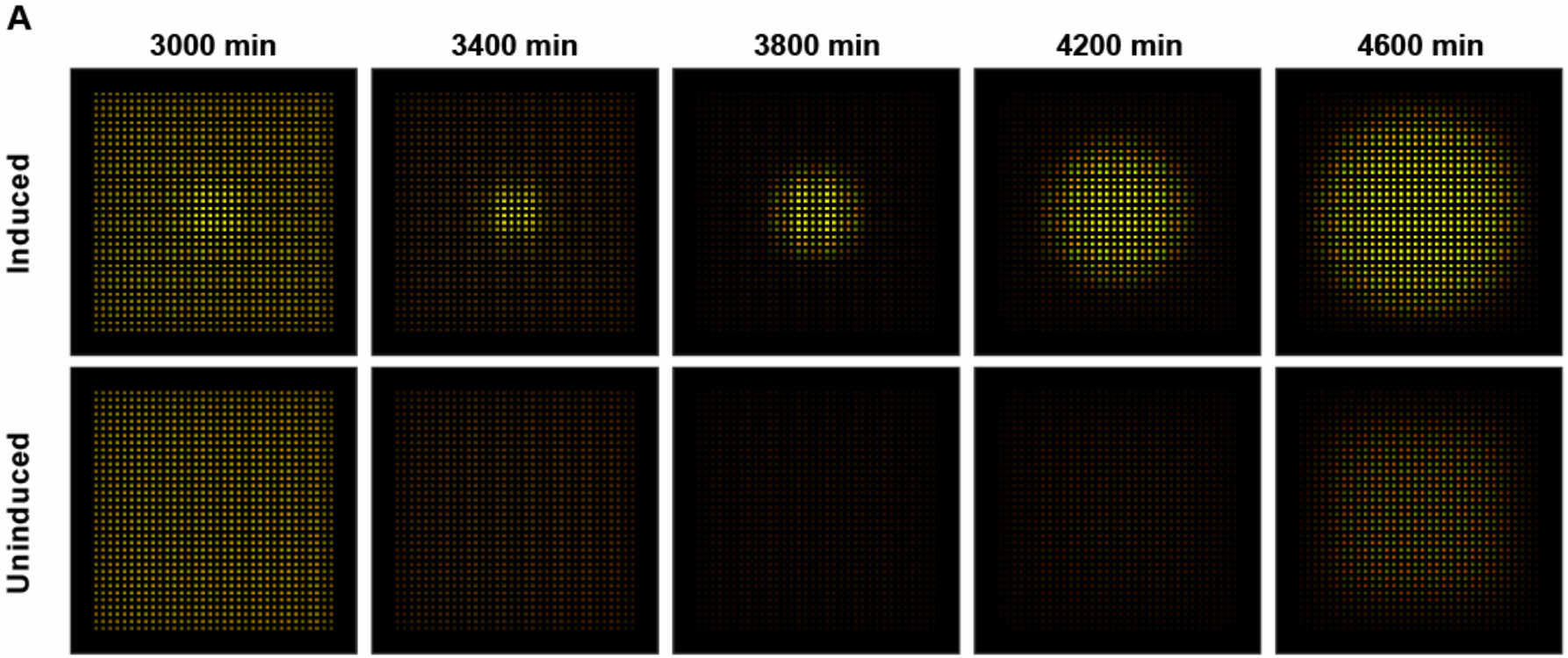


Uninduced *Induced*



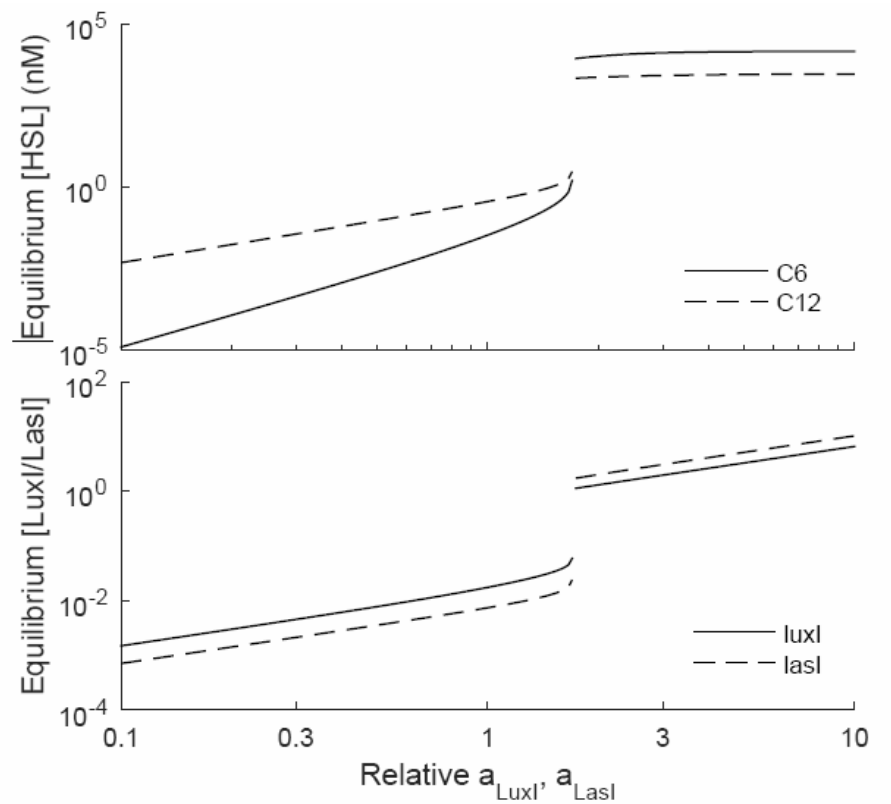
Both cell-types

Model simulation



Model analysis

- Inferred parameters did not show the correct qualitative behaviour
  - We had to increase the HSL synthesis rates
- System is near a bifurcation
  - i.e. inducible signalling proceeds upon reaching a critical threshold



Summary

- By altering promoter sequence, specificity for regulators can be altered
  - We created two mutant promoters: one LuxR-specific and one LasR-specific
  - Mathematical modelling helped to describe and quantify the sources of crosstalk
- By controlling the expression of receiver protein, we can optimise for independent signalling
  - Predicted levels that maximise *signal-to-noise* using the model
- Demonstrated independent signalling by constructing **double reporter** cells, which simultaneously recognise and report two different signals
  - Spatiotemporal dynamics well predicted by the model
- The philosophy behind synthetic biology offers a new way to understand cell biology and developmental patterning
- Next up… Turing patterns?

Acknowledgements

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2. TJ Rudge, JR Brown, F Federici, N Dalchau, A Phillips, JW Ajioka, J Haseloff (2015). Characterization of intrinsic properties of promoters. *ACS Synthetic Biology, in press*.
3. Salis HM, Mirsky EA, Voigt CA (2009) Automated design of synthetic ribosome binding sites to control protein expression. *Nat Biotechnol* **27:**946-950.
4. **PK Grant*, N Dalchau*, JR Brown, F Federici, TJ Rudge, B Yordanov, O Patange, A Phillips, J Haseloff (2015). Orthogonal intercellular signaling for programmed spatial behaviour. *Molecular Systems Biology*, *in press*.**

Tools BETA

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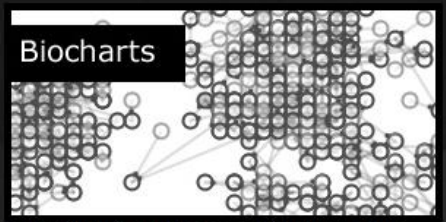
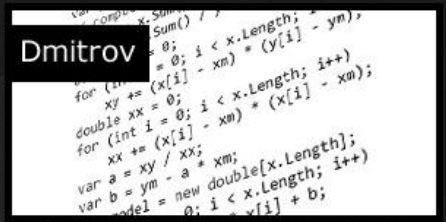


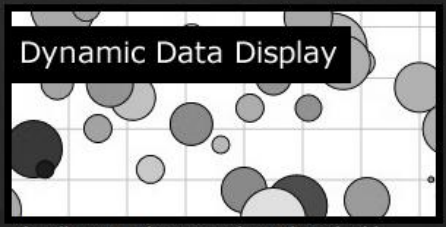
Unify biological hypotheses with models and experiments

Easy visualization of scientific data: drag, drop, filter, slide, view, zoom, share



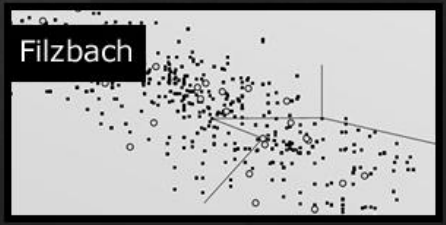
Easily work with multidimensional datasets: NetCDF, text, memory or remote, all from within your code

A language for designing and simulating computational devices made of DNA



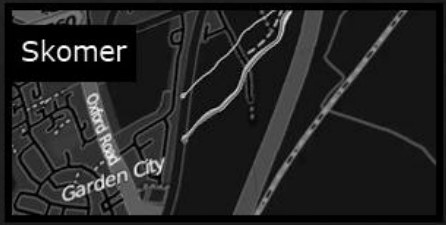
Visualize your data over the web and add complex dynamic graphs and maps to your web applications

Retrieve climatic and environmental information with the click of a button or a few lines of code

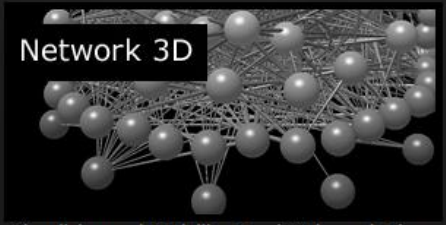


Fit complex models to hetereogenous data: Bayesian and likelihood analysis made easy

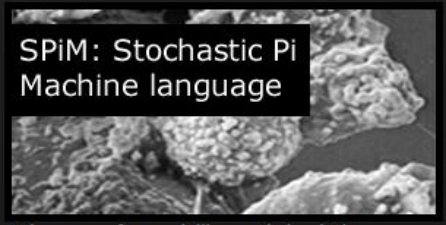
A language for designing and simulating genetic devices to reprogram cell behaviour



Changing our understanding of animal behaviour: novel hardware, analysis and software tools



Visualizing and Modelling Food Webs and other Complex Network



A language for modelling and simulating complex biological processes in a modular way

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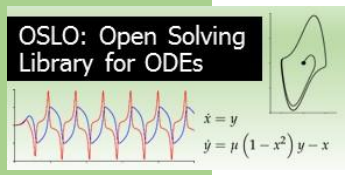
Domain-specific programming languages

Modelling Engine

Simulation

- ODEs
- PDEs
- Stochastic
- Chemical Master Equation



Bayesian inference

Table 1: Datasets used for wild-type promoter. Throughout, the tag "123" indicates the wildtype promoter.

Codename	Description	Files	Platereader
P_{cat}–LL123	luxR and lasR transcribed constitutively via P_{cat}	char130508_Pcat_LL123_CI_Edited.csv	Ajioka
		char130530_Pcat_LL123.csv	Ajioka
		char140624_Pcat_LL123.csv	Haseloff
P_{cat}–R123	luxR transcribed constitutively via P_{cat}	char130607_Pcat_R123.csv	Haseloff
		char130718_Pcat_R123.csv	Ajioka
		char130822_Pcat_R123.csv	Ajioka
		char140625_Pcat_R123.csv	Haseloff
		char140702_Pcat_R123.csv	Haseloff
P_{cat}–S123	lasR transcribed constitutively via P_{cat}	char130614_Pcat_S123.csv	Ajioka
		char130624_Pcat_S123.csv	Haseloff
		char130718_Pcat_S123.csv	Ajioka
		char140702_Pcat_S123.csv	Haseloff
P_{bad}–LuxR–S123	luxR transcribed inducibly via P_{bad} and lasR transcribed constitutively via P_{cat}	char140419_S123.csv	Haseloff
		char140430_S123.csv	Haseloff
		char140507_S123.csv	Haseloff
P_{bad}-LasR–R123	lasR transcribed inducibly via P_{bad} and luxR transcribed constitutively via P_{cat}	char140527_R123.csv	Haseloff
		char140601_R123.csv	Haseloff
		char140612_R123.csv	Ajioka

Table 2: Datasets used for mutant promoter 76.

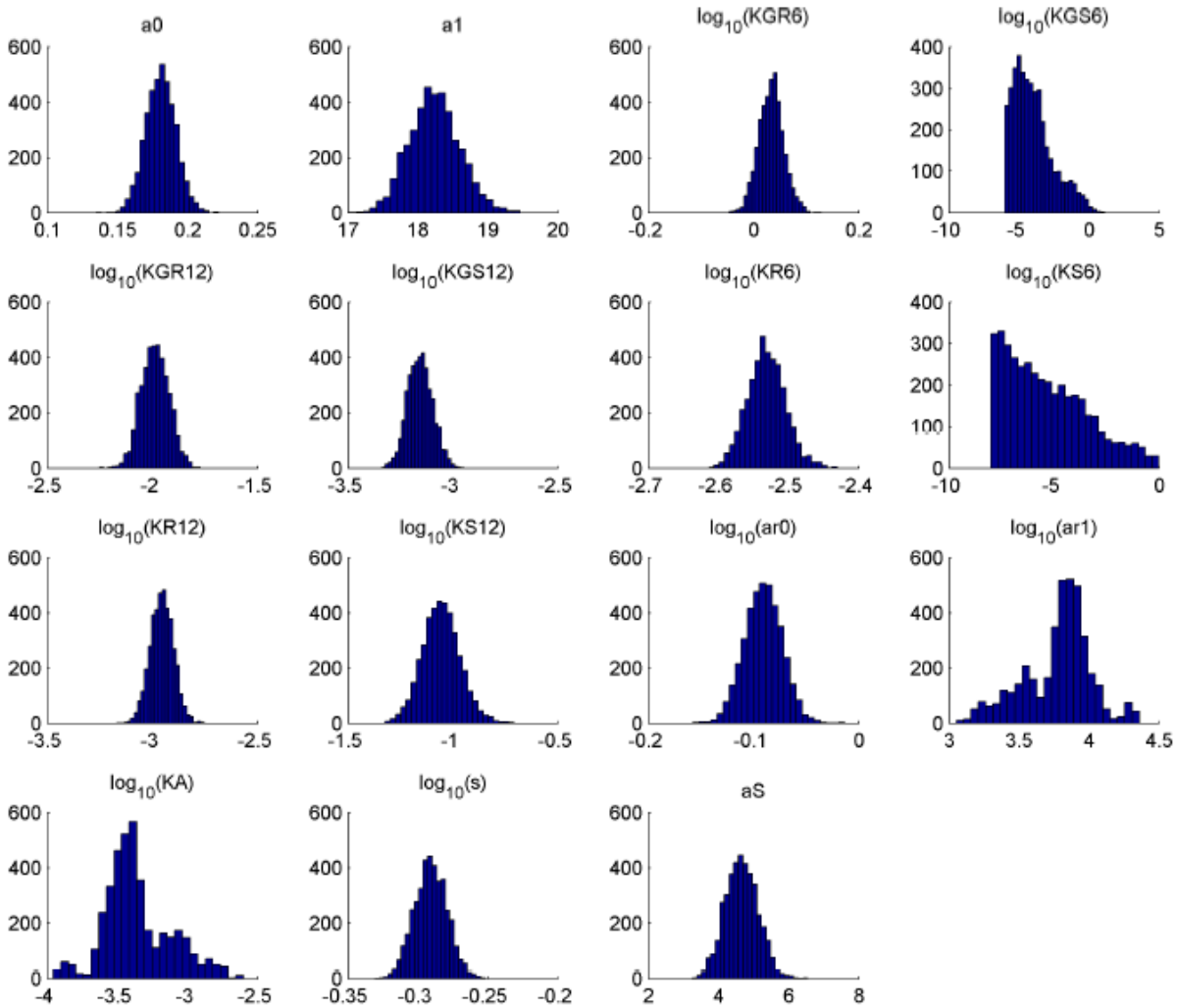
Codename	Files	Platereader
P_{cat}–LL76	char130530_Pcat_LL76_Edited.csv	Ajioka
	char130719_Pcat_LL76.csv	Ajioka
	char130822_Pcat_LL76.csv	Ajioka
	char130923_Pcat_LL76_CI_Edited.csv	Haseloff
	char140702_Pcat_LL76.csv	Haseloff
P_{cat}–R76	char130607_Pcat_R76.csv	Haseloff
	char130719_Pcat_R76.csv	Haseloff
	char140624_Pcat_R76.csv	Haseloff
P_{cat}–S76	char130614_Pcat_S76_CI.csv	Ajioka
	char130624_Pcat_S76.csv	Haseloff
	char140624_Pcat_S76.csv	Haseloff
P_{bad}–LuxR–S76	char140422_Pbad_LuxR_S76.csv	Haseloff
	char140428_Pbad_LuxR_S76.csv	Ajioka
	char140506_Pbad_LuxR_S76.csv	Ajioka
P_{bad}-LasR–R76	char140607_Pbad_LasR_R76.csv	Haseloff
	char140610_Pbad_LasR_R76.csv	Ajioka
	char140611_Pbad_LasR_R76.csv	Haseloff

Table 3: Datasets used for mutant promoter 81.

Codename	Files	Platereader
P_{cat}–LL81	char130530_Pcat_LL81_Edited.csv	Ajioka
	char130822_Pcat_LL81.csv	Ajioka
	char130923_Pcat_LL81_CI_Edited.csv	Haseloff
	char140702_Pcat_LL81.csv	Haseloff
P_{cat}–R81	char130607_Pcat_R81.csv	Haseloff
	char130822_Pcat_R81.csv	Ajioka
	char140625_Pcat_R81.csv	Haseloff
	char140703_Pcat_R81.csv	Haseloff
P_{cat}–S81	char130626_Pcat_S81.csv	Haseloff
	char140625_Pcat_S81.csv	Haseloff
P_{bad}–LuxR–S81	char140424_Pbad_LuxR_S81.csv	Haseloff
	char140429_Pbad_LuxR_S81.csv	Haseloff
	char140502_Pbad_LuxR_S81.csv	Haseloff
P_{bad}-LasR–R81	char140611_Pbad_LasR_R81.csv	Haseloff
	char140612_Pbad_LasR_R81.csv	Haseloff
	char140616_Pbad_LasR_R81.csv	Haseloff

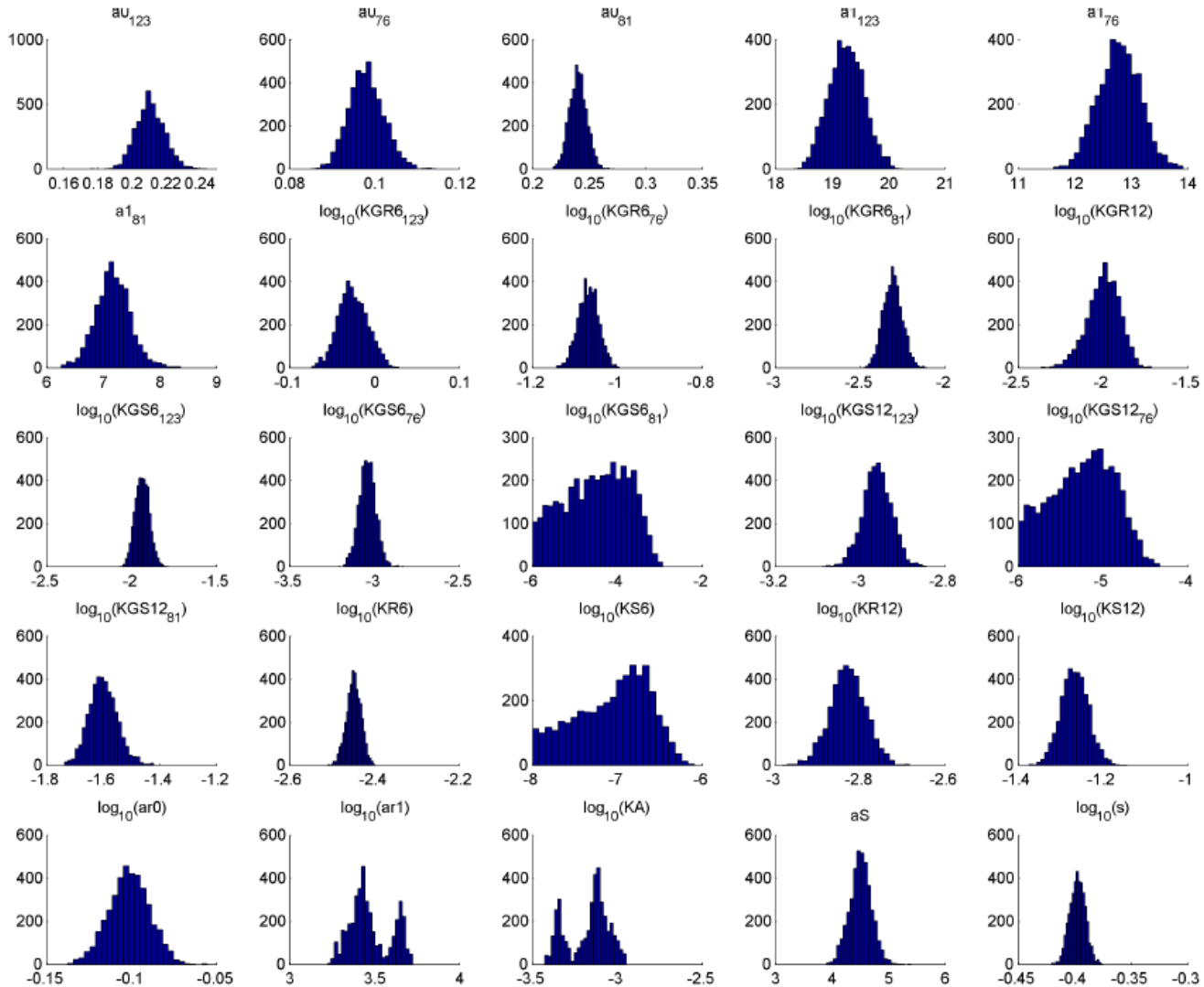
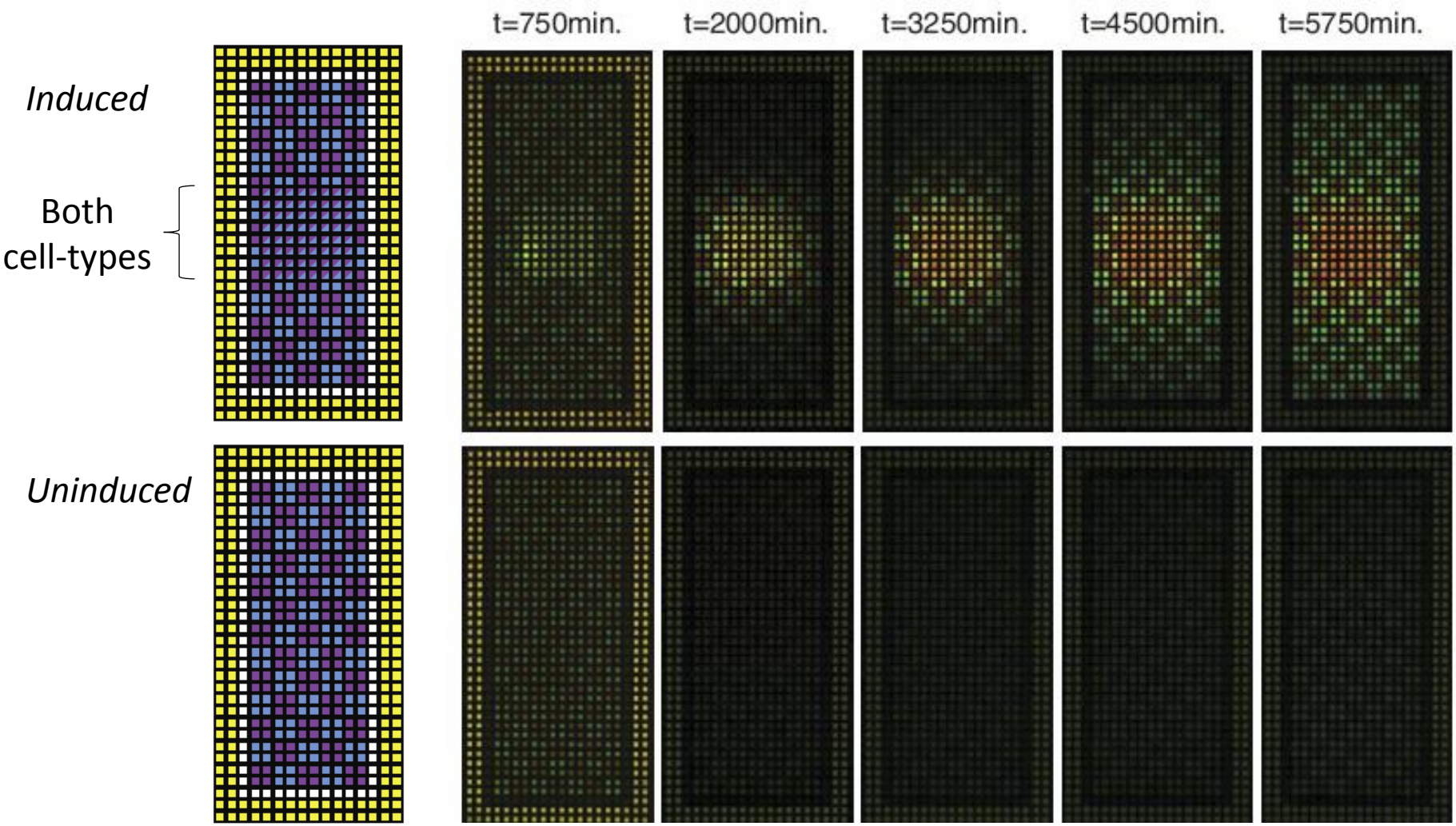
Posterior parameter distributions

Fitting wild-type promoter data alone



Posterior parameter distributions

Fitting wild-type and mutant promoters data together



Relay signal spread