Bioinformatics 3

V10 – Simulating the Dynamics of Gene Regulatory Networks by Boolean Networks

Mon, Nov 28, 2016

Quorum sensing of *Vibrio fischeri*

V. fischeri has a microbial **symbiotic relationship** with the squid *Euprymna scolopes*.
The bacterium exists in **small amounts** in the ocean (10^2 cells/ml) and in **large amount** in the light organs of the **squid** (10^{10} cells/ml).

At low concentrations, *V. fischeri* does not produce luminescence.

At **high cell density** these bacteria emit a **blue-green light**.

The light organ of the squid provides to the bacteria all the **nutrients** that they need to survive.

The squid benefits from the bacteria's quorum sensing and **bioluminescence** abilities.

https://www.bio.cmu.edu/courses/03441/TermPapers/99TermPapers/Quorum/vibrio_fischeri.html

Quorum sensing of *Vibrio fischeri*

The cell density-dependent control of gene expression is activated by a transcriptional activator protein that is coupled to a signal molecule (**autoinducer**).

The autoinducer is released by the bacteria into its surrounding **environment** and taken up from there.

During the day, the squid keeps the bacteria at lower concentrations by expelling some of them into the ocean during regular intervals.

At night however, the bacteria are allowed to accumulate to about 10^{10} cells/ml so that they will emit blue-green light.

https://www.bio.cmu.edu/courses/03441/TermPapers/99TermPapers/Quorum/vibrio_fischeri.html

Vibrio fischeri helps with Camouflage

This is perfect for the squid because it is a night feeder.

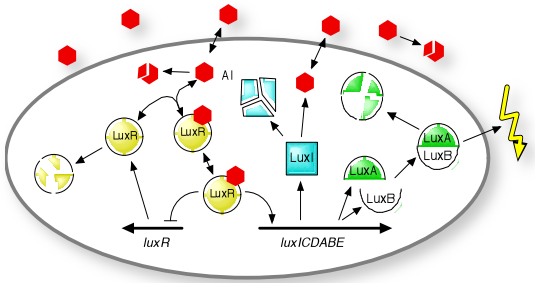
In the **moonlight**, the swimming squid would normally cast a **shadow** beneath itself making it a perfect target for squid-eating organisms.

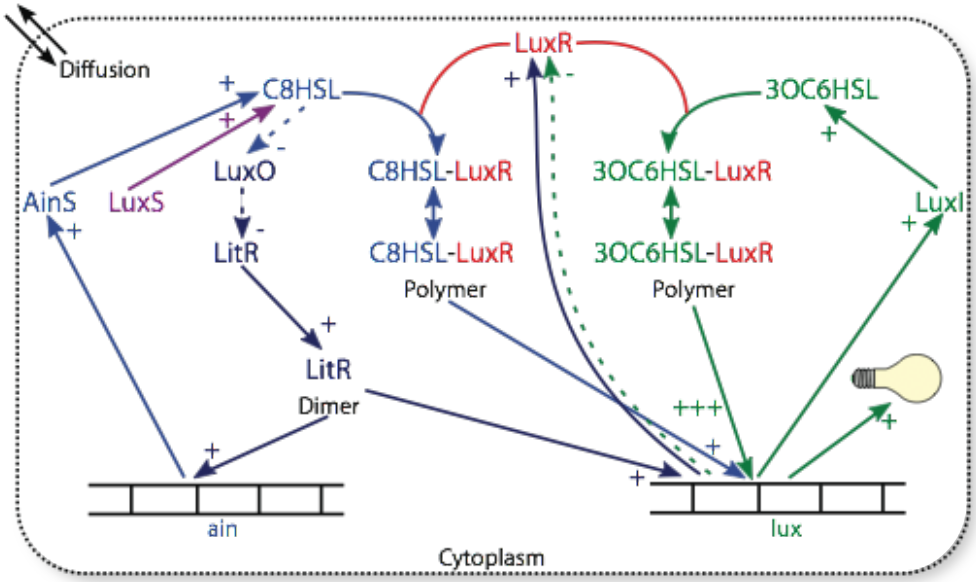
However, the bacterial glow will counter the shadowing effect the moon makes and mask the squid from its predators.

In the **morning**, the squid expels some bacteria into the ocean to a concentration where they will not generate light anymore so as to conserve energy.

https://www.bio.cmu.edu/courses/03441/TermPapers/99TermPapers/Quorum/vibrio_fischeri.html

Quorum sensing of *Vibrio fischeri*





Boolean Networks

Dependencies between variables can be formulated as **conditional transitions**

- "If LuxI is present, then AI will be produced…"
- "If there is AI and there's no LuxR:AI bound to the genome, then LuxR will be expressed and complexes can form…"
- "If LuxR:AI is bound to the genome, then LuxI is expressed…"

Simplified mathematical **description** of the dependencies:

Densities of the species	<=>	discrete states: on/off, 1/0
Network of dependencies	<=>	condition tables
Progress in time	<=>	discrete propagation steps

Boolean Networks II

State of the system: described by **vector** of **discrete** values

$S_i = \{0, 1, 1, 0, 0, 1, \ldots\}$

$S_i = \{x_1(i),\ x_2(i),\ x_3(i), \ldots\}$

fixed number of species with **finite number** of states each

→ finite number of system states

→ periodic trajectories

→ **periodic** sequence of states = **attractor**

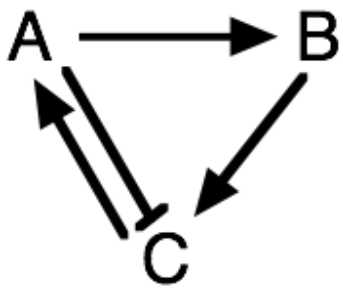
→ all states leading to an attractor = **basin of attraction**

Propagation:

$S_{i+1} = \{x_1(i+1),\ x_2(i+1),\ x_3(i+1),\ \ldots\}$

$x_1(i+1) = f_1(x_1(i), x_2(i), x_3(i), \ldots)$ with f_i given by condition tables

A Small Example



State vector $S = \{A, B, C\} \rightarrow$ 8 possible states

Conditional evolution:

A is on if C is on

A_{i+1}	C_i
0	0
1	1

A activates B

B_{i+1}	A_i
0	0
1	1

C is on if (B is on && A is off)

C_{i+1}	A_i	B_i
0	0	0
1	0	1
0	1	0
0	1	1

Start from {A, B, C} = {1, 0, 0}

#	S_i	A	B	C
0	S_0	1	0	0
1	S_1	0	1	0
2	S_2	0	0	1
3	$S_3 = S_0$	1	0	0

assume here that inhibition through A is stronger than activation via B

periodic orbit of length 3

Test the Other Starting Conditions

Test the other states

#	A	B	C
0	1	1	1
1	1	1	0
2	0	1	0
3	0	0	1
4	1	0	0
5	0	1	0

A_{i+1}	C_i
0	0
1	1

B_{i+1}	A_i
0	0
1	1

C_{i+1}	A_i	B_i
0	0	0
1	0	1
0	1	0
0	1	1

#	A	B	C
0	1	0	1
1	1	1	0

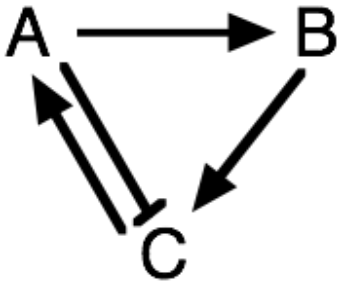
#	A	B	C
0	0	1	1
1	1	0	1

Same attractor as before:

100 → 010 → 001 → 100

is also reached from:

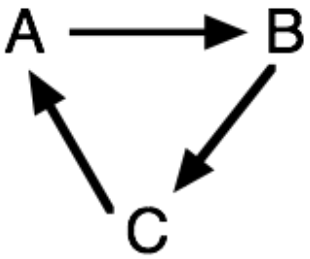
110, 111, 101, 011



#	A	B	C
0	0	0	0
1	0	0	0

→ **Either all off or stable oscillations**

A Knock-out Mutant



A_{i+1}	C_i
0	0
1	1

B_{i+1}	A_i
0	0
1	1

C_{i+1}	B_i
0	0
1	1

Attractors:

#	A	B	C
0	1	0	0
1	0	1	0
2	0	0	1
3	1	0	0

#	A	B	C
0	1	1	0
1	0	1	1
2	1	0	1
3	1	1	0

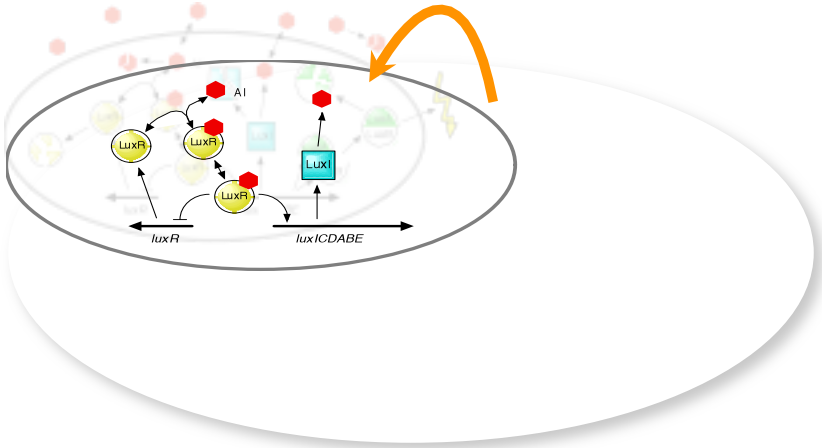
#	A	B	C
0	1	1	1
1	1	1	1

#	A	B	C
0	0	0	0
1	0	0	0

no feedback

→ no stabilization, network just "rotates"

Boolean Network of QS



Minimum set of species:

LuxR, AI, LuxR:AI, LuxR:AI:genome, LuxI

Here: Light signal (LuxAB) α LuxI

Condition tables: describe the state of a species in the next step given the current states of all relevant species.

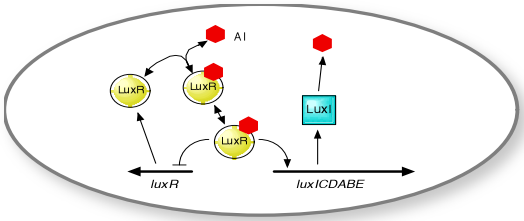
LuxI	LuxR:AI:Genome
0	0
1	1

How does LuxI depend on LuxR:AI:Genome?

LuxR:AI:Genome	LuxR:AI
0	0
1	1

How does LuxR:AI:Genome depend on LuxR:AI?

Condition Tables for QS II



LuxR	LuxR	AI	LuxR:AI:Genome	
1	0	0	0	When LuxR:AI:Genome is empty, LuxR is produced in next step
1	1	0	0	
1	0	1	0	
1	1	1	0	
0	0	0	1	
1	1	0	1	Comment: LuxR present, no AI available
0	0	1	1	
0	1	1	1	LuxR present, binds AI in next step, no LuxR is produced because LuxR:AI:Genome inhibits LuxR production

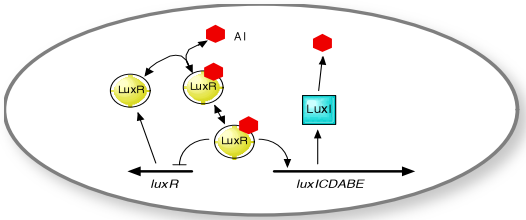
LuxR:AI	LuxR	AI	LuxR:AI:Genome
0	0	0	0
0	1	0	0
0	0	1	0
1	1	1	0
0	0	0	1
0	1	0	1
0	0	1	1
1	1	1	1

→

LuxR:AI	LuxR	AI	LuxR:AI:Genome
0	x	x	x
1	1	1	x

Note: no dissociation
(LuxR:AI:Genome → LuxR:AI + Genome)
only degradation of AI in this model
LuxR:AI:Genome → LuxR + Genome

Condition tables for QS III



AI	LuxR	AI	LuxI
0	0	0	0
0	1	0	0
1	0	1	0
0	1	1	0
1	0	0	1
1	1	0	1
1	0	1	1
1	1	1	1

→

AI	LuxR	AI	LuxI
1	x	x	1
0	x	0	0
1	0	1	0
0	1	1	0

Scanning for Attractors

States of *V. fischeri* QS system are mapped onto integers

{LuxR (LR), LuxR:AI (RA), AI, LuxR:AI:Genome (RAG), LuxI (LI)}

= {1, 2, 4, 8, 16} - current state can be interpreted as binary number!

For each **attractor**:

- periodic orbit and its length (period)
- basin of attraction and its relative size (32 states in total)

→ how likely will the system end up in each of the attractors?

Attractor 1: orbit: 1 → period 1

states: 0, 1 → size 2, 2/32 = 6.25 %

start from state 0:

#	LR	RA	AI	RAG	LI	–	state	
0	.	.	.	.	.	–	0	
1	X	.	.	.	.	–	1	<= attractor
2	X	.	.	.	.	–	1	

States: named by reading occupancies as **binary numbers in reversed order**.

Scanning for Attractors II

Attractor 2: orbit: 3, 9, 17, 5 → period 4

states: 2, 3, 5, 8, 9, 16, 17 → size 7, 21.9 %

start from state 8:

#	LR	RA	AI	RAG	LI	–	state
0	.	.	.	X	.	–	8
1	.	.	.	.	X	–	16
2	X	.	X	.	.	–	5
3	X	X	.	.	.	–	3
4	X	.	.	X	.	–	9
5	X	.	.	.	X	–	17
6	X	.	X	.	.	–	5

Attractor:
17 returns to 5

averaged occupancies in this periodic orbit:

LR	RA	AI	RAG	LI
4/4 = 1	1/4 = 0.25	1/4 = 0.25	1/4 = 0.25	1/4 = 0.25

Attractors III

Attractor 3: period 4, basin of 16 states → 50 %

#	LR	RA	AI	RAG	LI
	.	X	X	.	.
	.	X	X	X	.
	.	.	X	X	X
	.	.	X	.	X

Attractor 4: period 4, basin of 4 states → 12.5 %

#	LR	RA	AI	RAG	LI
	X	X	X	.	.
	X	X	.	X	.
	X	.	.	X	X
	X	.	X	.	X

Attractor 5: period 2, basin of 3 states → 9.4 %

#	LR	RA	AI	RAG	LI
	X	.	X	X	.
	.	X	.		X

Classifying the Attractors

→ Interpret the system's behavior from the properties of the attractors

Attractor	period	basin size	<LuxR>	<LuxR:AI>	<AI>	<LuxR:AI:Gen>	<LuxI>
1	1	6.25 % (2)	1	0	0	0	0
2	4	21.9% (7)	1	0.25	0.25	0.25	0.25
3	4	50 % (16)	0	0.5	1	0.5	0.5
4	4	12.5 % (4)	1	0.5	0.5	0.5	0.5
5	2	9.4% (3)	0.5	0.5	0.5	0.5	0.5

There exist three **regimes**:

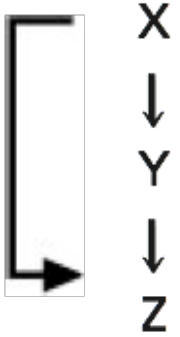
dark: LuxI = 0 — free LuxR, no AI

intermediate: LuxI = 0.25 — free LuxR + little AI

bright: LuxI = 0.5 — little free LuxR (0.24) + much AI (0.85)

The Feed-Forward-Loop

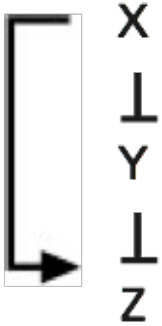
External signal determines state of X
→ response Z for short and long signals X



condition tables:

Y	X
0	0
I	I

Z	X	Y
0	0	0
0	0	I
0	I	0
I	I	I



Y	X
I	0
0	I

Z	X	Y
0	0	0
0	0	I
I	I	0
0	I	I

Signal propagation

Left column: external signal

Response to signal X(t)

X	Y	Z
0	0	0
I	0	0
0	I	0
0	0	0
I	0	0
I	I	0
I	I	**I**
0	I	**I**
0	0	**0**
0	0	0

Short Signal

Long signal

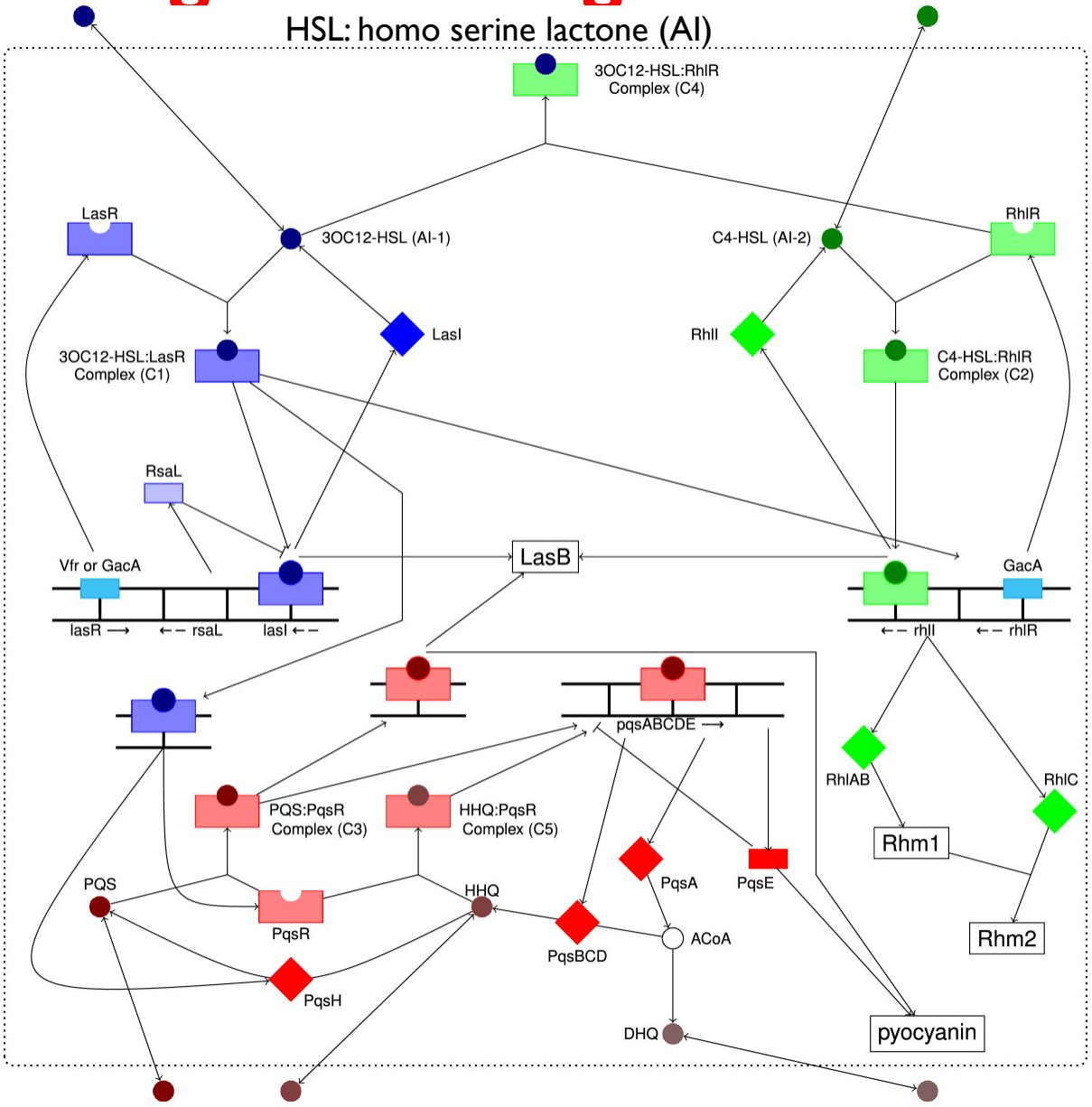
X	Y	Z
0	I	0
I	I	0
0	0	0
0	I	0
I	I	0
I	0	0
I	0	**I**
0	0	**I**
0	I	**I**
0	I	0

Quorum Sensing in *P. aeruginosa*

In the human pathogen *P. aeruginosa*, the QS network consists of 3 systems termed *las*, *rhl*, and *pqs* that are organized hierarchically.

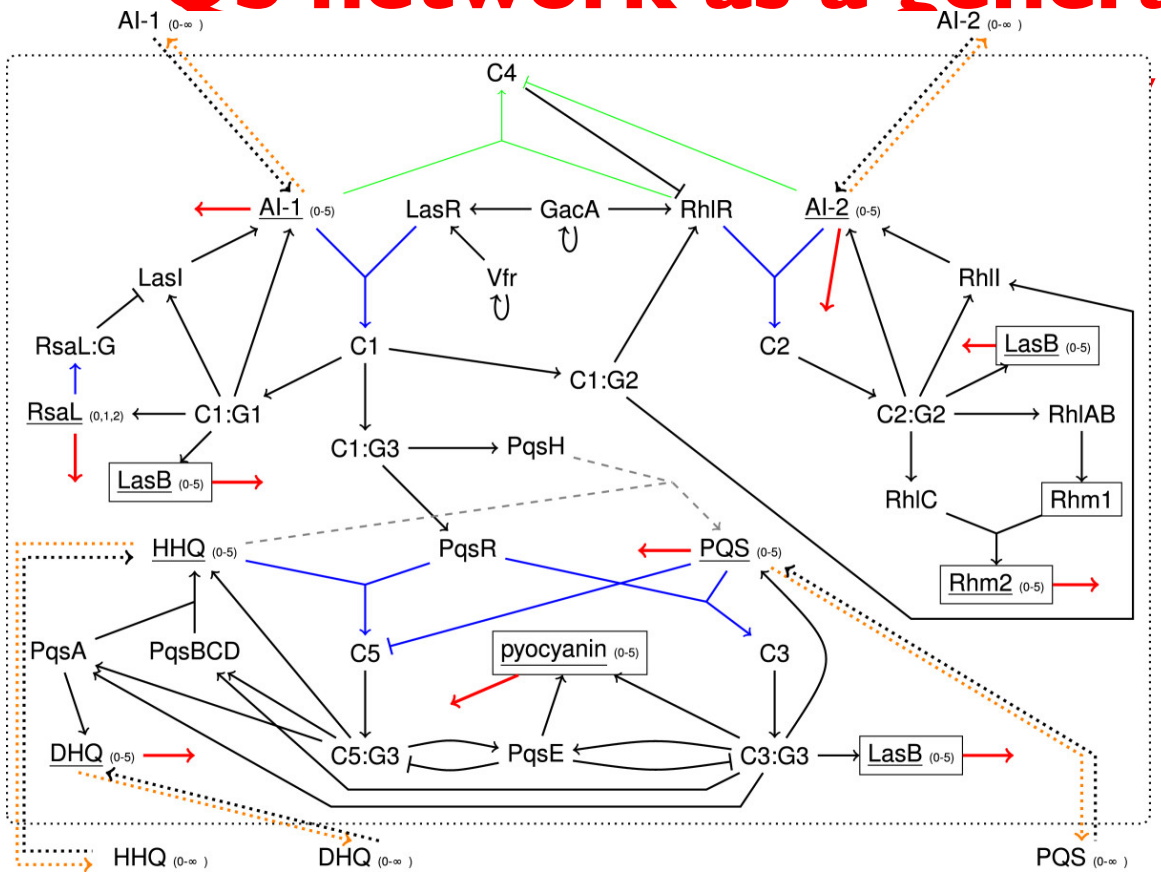
Idea: selectively targeting the QS machinery by signaling molecule inhibitors may avoid development of resistance mutations.

Aim: develop simple computational model that can account for effects of small-molecule inhibitors and resistance mutations.



Schaadt et al. BMC Systems Biol. (2013) 7:81

QS network as a generalized Boolean



We need > 2 levels of selected variables to generate sequential switching of 3 QS systems.

Nodes named C represent a complex between autoinducer and receptor, C:G is the complex bound to an operon.

black edge = threshold is 1

blue edge = state of underlined node must be ≥ 2;

orange edge = state of underlined node must be ≥ 3;

thin **green** edge = state of underlined node must be ≥ 4;
numbers denote possible states for a node;

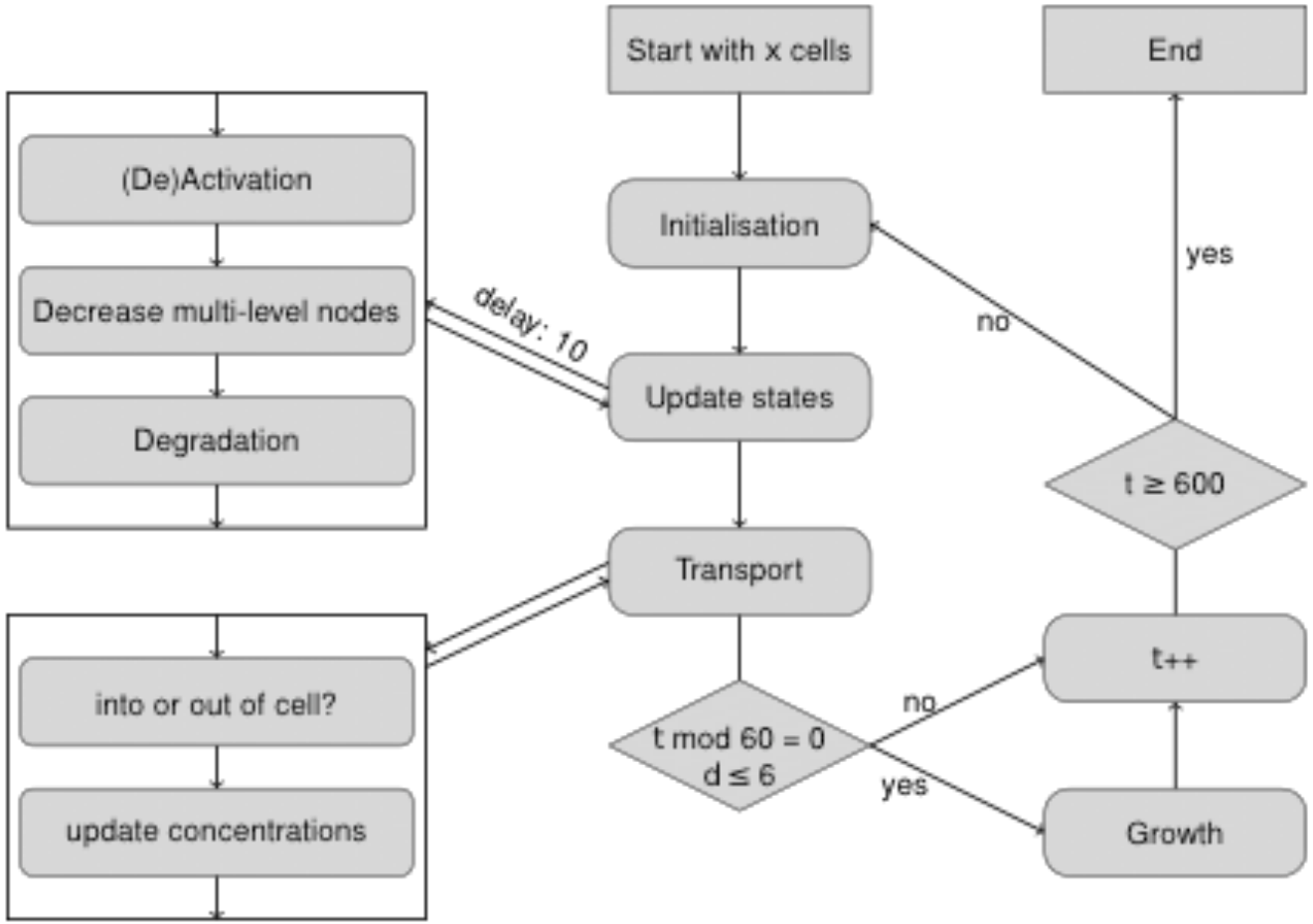
dotted arrows : transport processes

thick **red** edge : happens after a certain number of time steps (degradation).

dashed **grey** arrows : reaction that occurs by chance with a certain probability.

Schaadt et al. BMC Systems Biol. (2013) 7:81

Network propagation



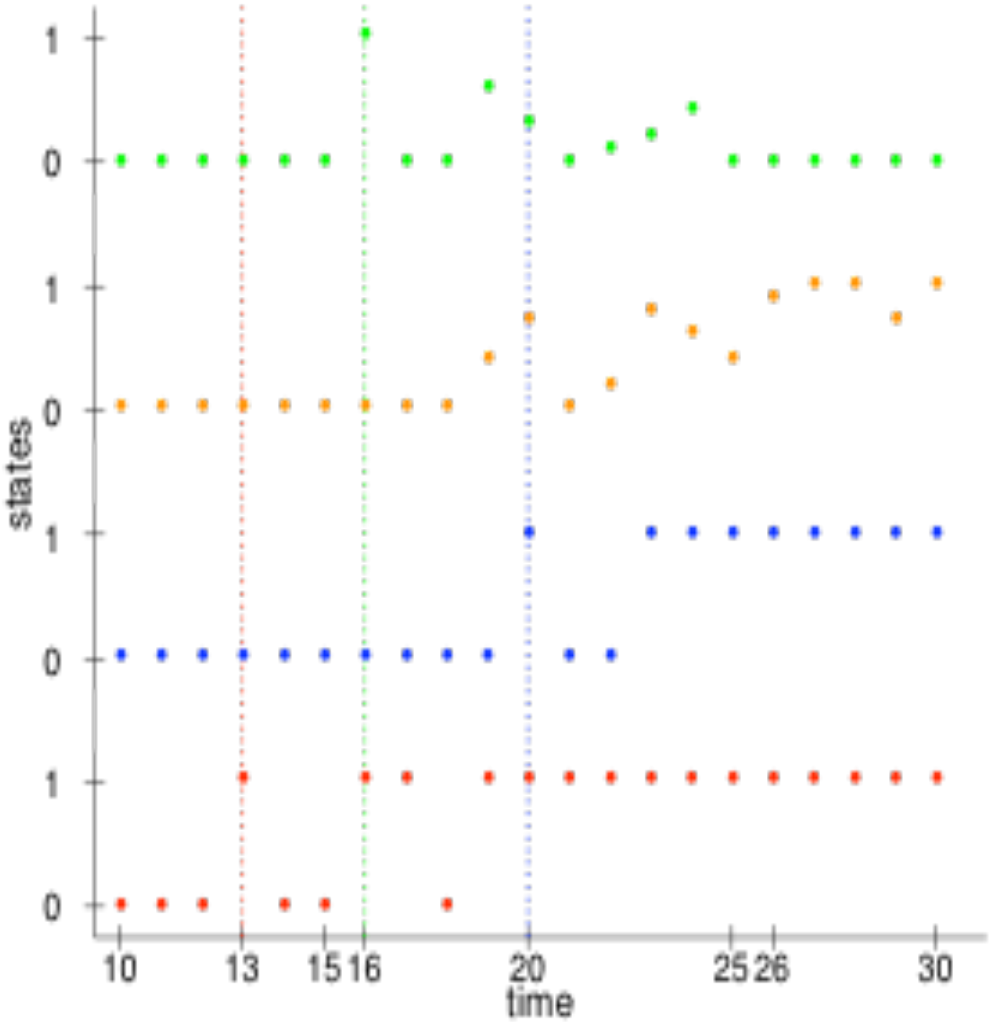
„Growth“ : cell divides into 2 cells

Simulation is stopped after 10 generations (600 iterations)

Sample trajectory

time step	HHQ	PQS	C3	C5	C3:G3	C5:G3	PqsA	PqsBCD	PqsE
10	0	0	0	0	1	1	0	0	0
11	1	1	0	0	0	0	1	1	1
12	2	1	0	0	0	0	0	0	0
13	2	1	0	0	0	0	0	0	0
14	2	1	0	0	0	0	0	0	0
15	2	1	0	0	0	0	0	0	0
16	0	2	0	1	0	0	0	0	0
17	0	2	0	0	0	1	0	0	0
18	1	2	0	0	0	0	1	1	1
19	2	1	1	0	0	0	0	0	0
20	0	1	0	1	1	0	0	0	0
21	0	2	0	0	0	1	1	1	1
22	2	1	1	0	0	0	1	1	1
23	2	1	0	1	0	0	0	0	0
24	0	2	0	1	0	1	0	0	0
25	1	1	1	0	0	1	1	1	1
26	2	2	0	0	0	0	1	1	1
27	1	2	1	0	0	0	0	0	0
28	0	2	1	0	1	0	0	0	0

Simulation start



Start from minimal initial conditions (no complexes formed) that get the QS system started.

Green: complex C5 between HHQ and PqsR.

Orange: complex C3 of the *pqs* system between PQS and **PqsR**

Blue: second complex of AI–2 and **RhlR**.

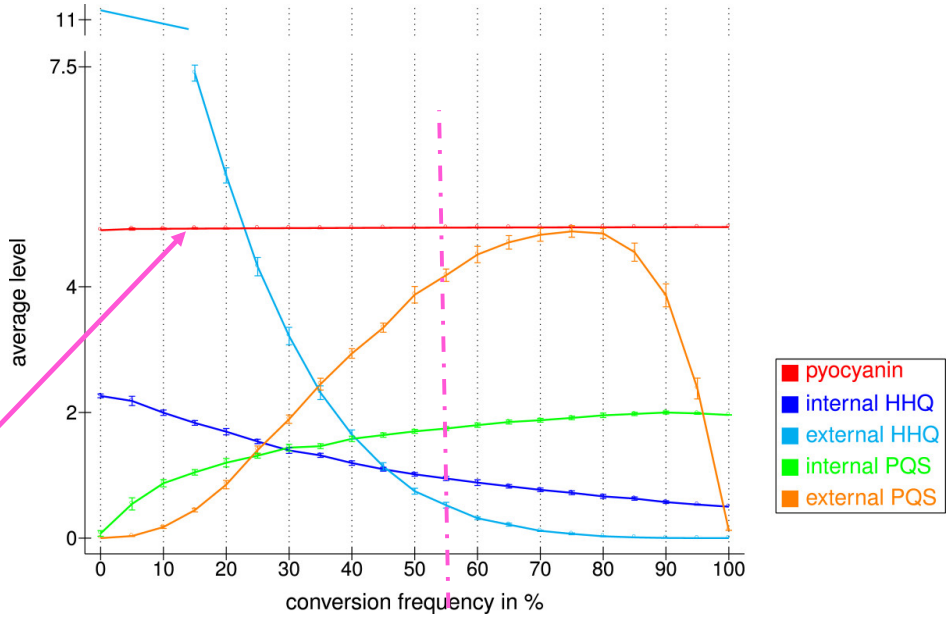
Red: first complex of AI–1 and **LasR**

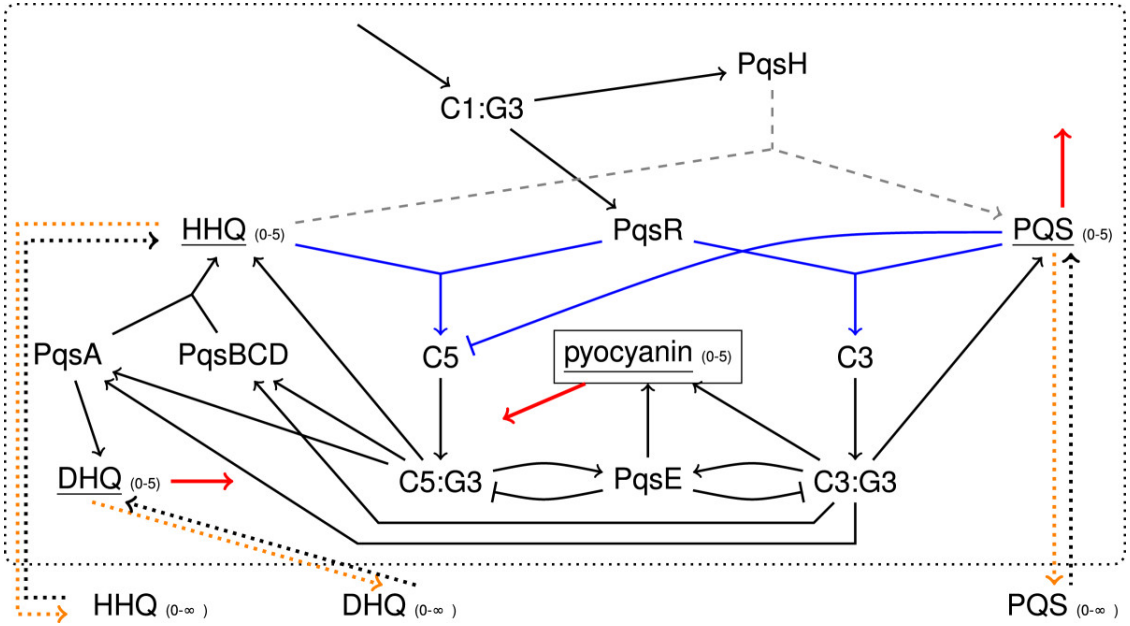
Tune PQS production rate

The conversion of HHQ into PQS (dashed grey line in bottom Fig.) was designed to **occur randomly** with a certain **probability**.

The right figure shows how the autoinducer HHQ and pyocyanin levels depend on the reaction rate of this process.

Due to the activation of pyocyanin biosynthesis by PqsE and the production of PqsE by the complex C5 using HHQ, the pyocyanin level (red) is **independent** of the reaction rate.

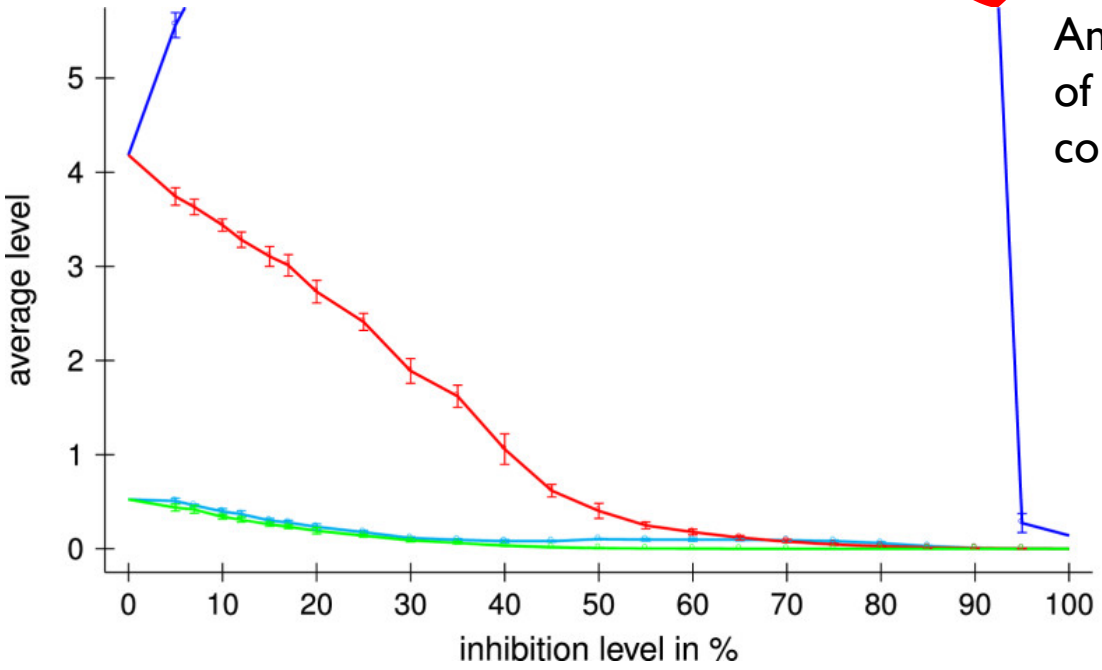




Kesarwani *et al.* reported that the HHQ concentration is about 12% of the PQS concentration in the beginning of the stationary growth phase.

To match this experimental finding, we used a conversion frequency of 55%.

Inhibitors affect PQS and pyocyanin



An inhibition level of 40% means that 40% of all receptors or enzymes of the corresponding type are blocked.

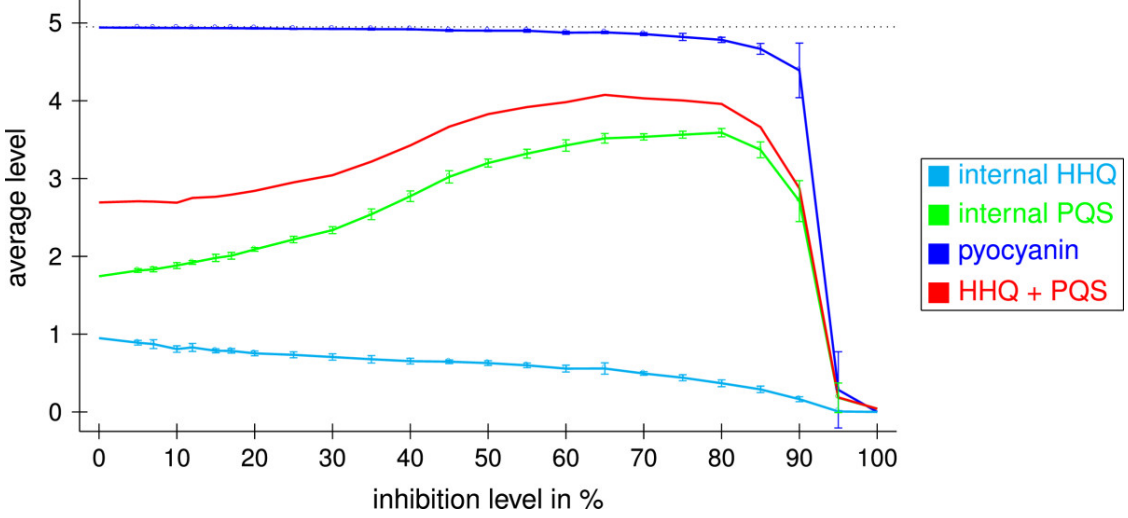
external HHQ in PqsBCD inhibitor
external PQS in PqsBCD inhibitor

In the presence of PqsBCD inhibitors, the external HHQ and PQS levels are noticeably decreased with a high dependence of the inhibition level.
E.g. a PqsBCD inhibitor with inhibition level of 30% reduces the external HHQ level by ca. 80% and the external PQS level by ca. 55% (relative to 0 % inhib).

Indeed, in experiments a PqsD inhibitor reduced the HHQ concentration by 77% and that of PQS by 42%. → Good match!

Enzyme inhibition. Inhibition of PqsBCD with varying inhibition levels.

Fig. shows the predicted effect of weak and strong PqsR receptor antagonists on the internal HHQ and PQS levels and on the pyocyanin level.



As long as < 60% of PqsR is blocked, the pyocyanin level is only very slightly decreased.

In contrast, there **exist** PqsR antagonists with affinity in the low micromolar range that indeed reduce the pyocyanin concentration (IC_{50}: 87 μ M).

Further, it was reported that PqsR antagonists with K_D values in a low nanomolar range **reduce** the pyocyanin formation by about 75% at 3 μ M.

This **discrepancy** suggests that pyocyanin production is co–regulated by further functionally unknown proteins that may or may not be connected to the *pqs* system.

Add new putative reactions to form pyocyanin

Table 1 Possible reactions to form pyocyanin

Notation	Reaction
R1	PqsE ⟶ pyocyanin
R2	C3:G3 ⟶ pyocyanin
R3	C5:G3 ⟶ pyocyanin
R4	PqsR ⟶ pyocyanin
R5	PqsH ⟶ pyocyanin
R6	PqsA ⟶ pyocyanin
R7	PqsBCD ⟶ pyocyanin
R8	PqsR + DHQ ⟶ pyocyanin
R9	PqsH + HHQ ⟶ pyocyanin
R10	PqsH + PQS ⟶ pyocyanin
R11	PqsH + DHQ ⟶ pyocyanin
R12	PqsA + HHQ ⟶ pyocyanin
R13	PqsA + PQS ⟶ pyocyanin
R14	PqsA + DHQ ⟶ pyocyanin
R15	PqsBCD + HHQ ⟶ pyocyanin
R16	PqsBCD + PQS ⟶ pyocyanin
R17	PqsBCD + DHQ ⟶ pyocyanin
R18	PqsE + HHQ ⟶ pyocyanin
R19	PqsE + PQS ⟶ pyocyanin
R20	PqsE + DHQ ⟶ pyocyanin
R21	PqsR + PqsE + HHQ ⟶ pyocyanin
R22	PqsR + PqsE + PQS ⟶ pyocyanin
R23	PqsR + PqsE + DHQ ⟶ pyocyanin
R24	PqsR + PqsE ⟶ pyocyanin

Pyocyanin is positively regulated by PqsE (and therefore also PqsR) in reactions labeled as underlined, by PqsA and PqsBCD (and therefore also PqsR) in reactions labeled in bold, as well as by both in reactions labeled in italic.

Table 1 shows all theoretically possible reactions to form pyocyanin whereby pyocyanin is regulated either via PqsE and therefore by PqsR (labeled as underlined) or via PqsA, PqsBCD, and therefore PqsR (as bold).

Behavior of updated networks

Table 2 Behavior of updated networks

Network	Used reactions	Results			
		$PqsA^-$–$PqsBCD^-$	$PqsE^-$	PqsR antagonists	PqsBCD inhibitors
N1	R1, R2	-----	Pyocyanin	Pyocyanin	-----
N2	R18	-----	-----	Pyocyanin	-----
N3	R19	-----	-----	Pyocyanin	PQS
N4	R20, R2	-----	Pyocyanin	Pyocyanin	-----
N5	R1, R2, R4	Pyocyanin	Pyocyanin	Pyocyanin	-----
N6	R18, R4	Pyocyanin	Pyocyanin	Pyocyanin	-----
N7	R19, R4	Pyocyanin	Pyocyanin	Pyocyanin	PQS
N8	R20, R2, R4	Pyocyanin	Pyocyanin	Pyocyanin	-----
N9	R21	-----	-----	-----	-----
N10	R22	-----	-----	-----	PQS
N11	R23, R2	-----	Pyocyanin	Pyocyanin	-----
N12	R24, R2	-----	Pyocyanin	Pyocyanin	-----

Table entries denote deviations (too high levels) from the expected behavior.

We consider the networks N9 and N10 as being the closest to literature.

Results / Conclusions

Results

•rule–based simulations fulfill the behavior expected from literature considering the external level of autoinducers.

•In the presence of PqsBCD inhibitors, the external HHQ and PQS levels are indeed clearly reduced. The magnitude of this effect strongly depends on the inhibition level.

•It seems that the pyocyanin pathway is incomplete.

Conclusions

•To match experimental observations we suggest a modified network topology in which PqsE and PqsR act as receptors and an autoinducer as ligand that up–regulate pyocyanin in a concerted manner.

•While the PQS biosynthesis is more appropriate as target to inhibit the HHQ and PQS formation, blocking the receptor PqsR that regulates the biosynthesis reduces the pyocyanin level stronger.

Can Boolean Networks be predictive?

Generally: → quality of the **results** depends on the quality of the **model**

→ quality of the model depends on the quality of the **assumptions**

Assumptions for the Boolean network description:

(• subset of the species considered → reduced system state space)

• only discrete density levels → dynamic balances lost, reduced to oscillations

• conditional yes–no causality → no continuous processes

• discretized propagation steps → timing of concurrent paths?

"You get what you pay for"

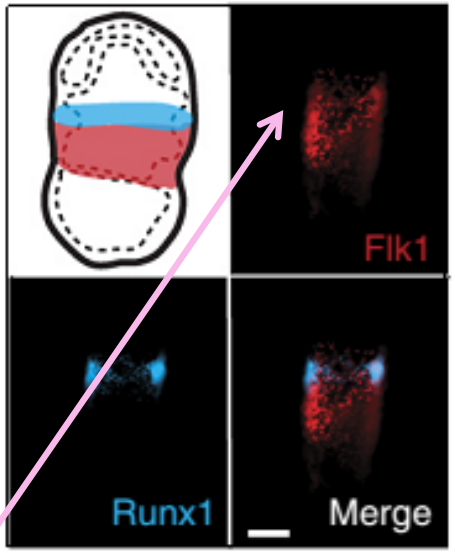
Understand Blood development (hemato-poeisis) with the help of Boolean Networks

Blood development represents one of the **earliest stages** of **organogenesis**. The production of primitive erythrocytes is required to support the growing embryo.

Blood has long served as a model to study organ development owing to the **accessibility** of blood cells and the availability of markers for specific cell populations.

Blood development is initiated at **gastrulation** from multipotent Flk1$^+$ mesodermal cells
(Flk1$^+$ is a marker gene for this developmental stage.)

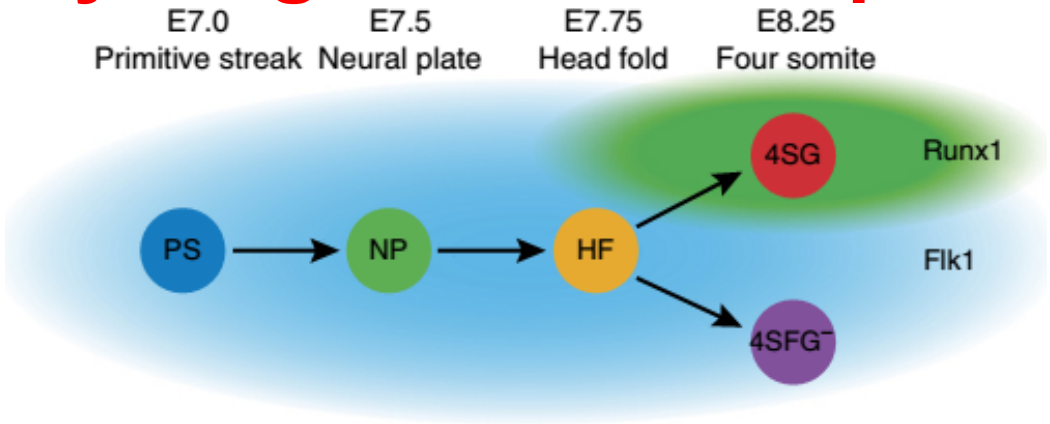
These cells initially have the potential to form either blood, endothelium and smooth muscle cells.



Flk1 and Runx1 staining in E7.5 mesoderm and blood band, respectively

Moignard et al., Nature Biotech. 33, 269 (2015)

Early stages of hematopoesis



The first wave of primitive hematopoiesis originates from Flk1$^+$ mesoderm, with all hematopoietic potential in the mouse contained within the Flk1$^+$ population from E7.0 onwards.

In this study, cells were flow sorted into single Flk1$^+$ cells at E7.0 (primitive streak, PS), E7.5 (neural plate, NP) and E7.75 (head fold, HF) stages.

E8.25 cells were subdivided into putative blood and endothelial populations by isolating GFP$^+$ cells (four somite, 4SG) and Flk1$^+$GFP$^-$ cells (4SFG−), respectively

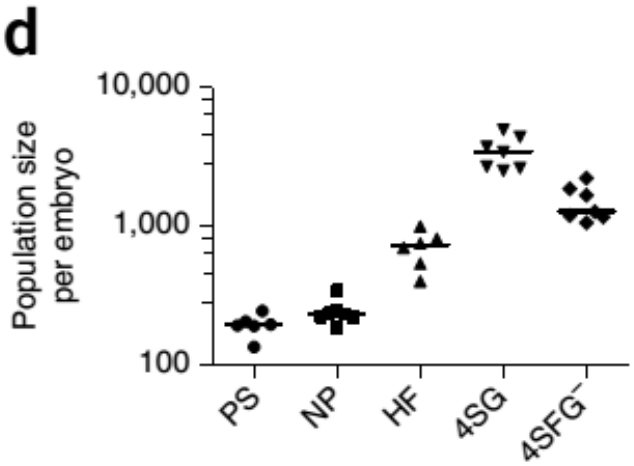
Moignard et al., Nature Biotech. 33, 269 (2015)

Studied cells

Cells were sorted from multiple embryos at each time point, with 3,934 cells going on to subsequent analysis.

Cell type	Number of embryos	Cells sorted	Cells retained	Percentage retained
PS	12	725	624	86.1
NP	9	637	552	86.7
HF	8	1,184	1,005	84.9
4SG	3	1,085	983	90.6
4SFG$^-$	4	858	770	89.7
Total	36	4,489	3,934	87.6

Total cell numbers and numbers of cells of appropriate phenotypes present in each embryo were estimated from fluorescence-activated cell sorting (FACS) data.



Number of cells grows as embryo development progresses

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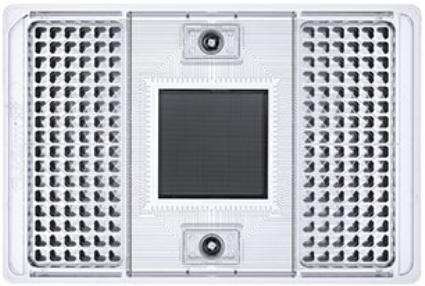
Assay gene expression in single cells

Cell type	Number of embryos	Cells sorted	Cells retained	Percentage retained
PS	12	725	624	86.1
NP	9	637	552	86.7
HF	8	1,184	1,005	84.9
4SG	3	1,085	983	90.6
4SFG$^-$	4	858	770	89.7
Total	36	4,489	3,934	87.6

Discard cells that did not express all 4 house-keeping genes, or for which their expression was more than 3 standard deviations from the mean.

Gene expression in single cells assayed with PCR for:

- 33 **transcription factors** known to be involved in endothelial and hematopoietic development
-9 **marker genes** (needed for FACS-sorting)
-4 **house-keeping genes** (needed for quality checks and normalization)



www.fluidigm.com

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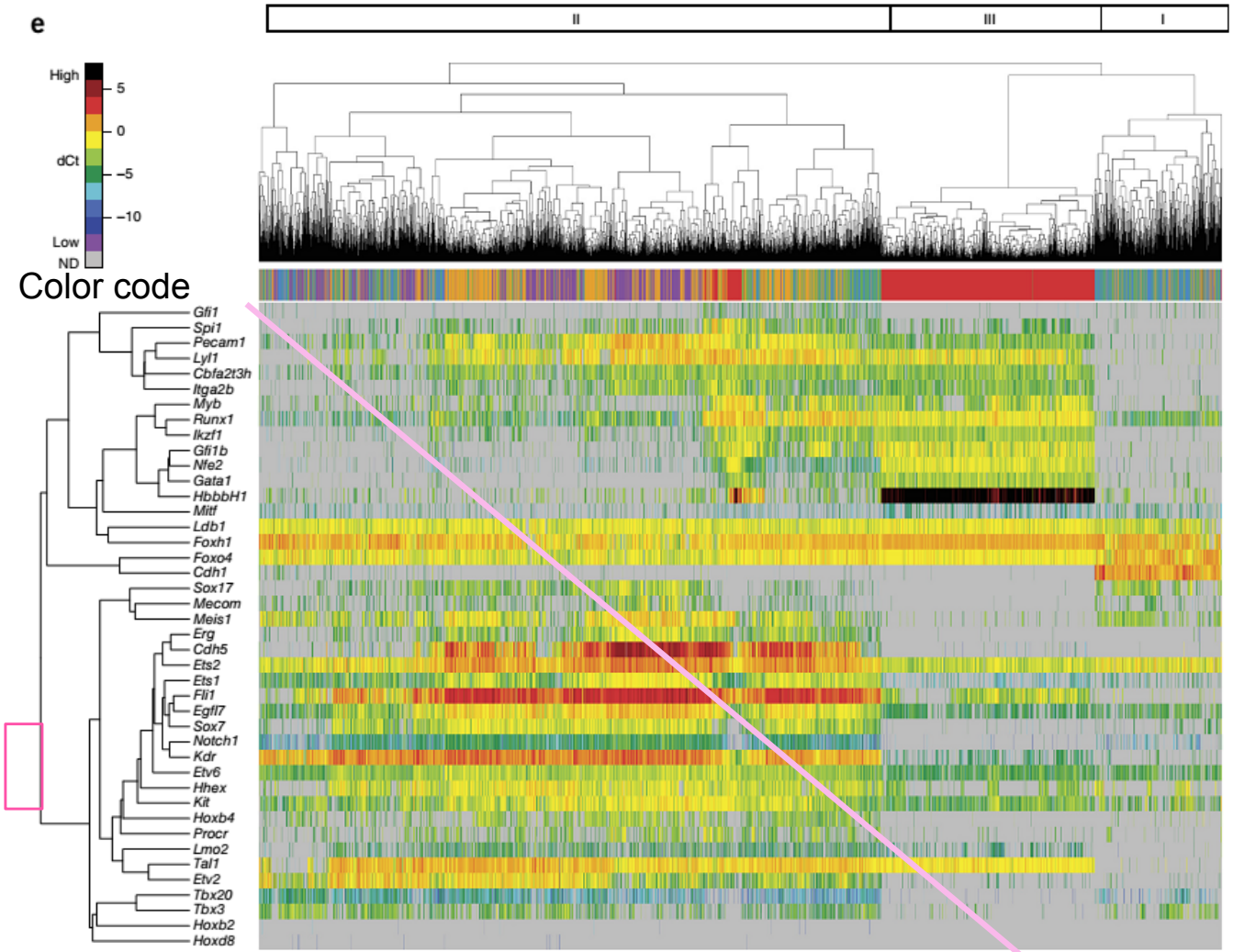
Hierarchical clustering of gene expression data



3 main clusters:

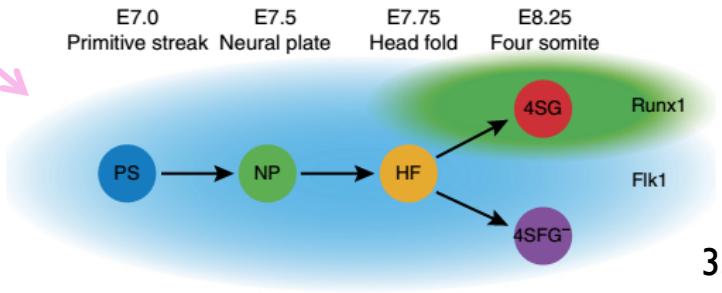
Cluster I (right side) contains mostly PS and NP cells (**green**/**blue**)

Cluster III contains exclusively 4SG cells (**red**)

Cluster II (left side) is mixed (NF, 4SFG$^-$, …)

→Cell differentiation progresses asynchronously

← Single cells →



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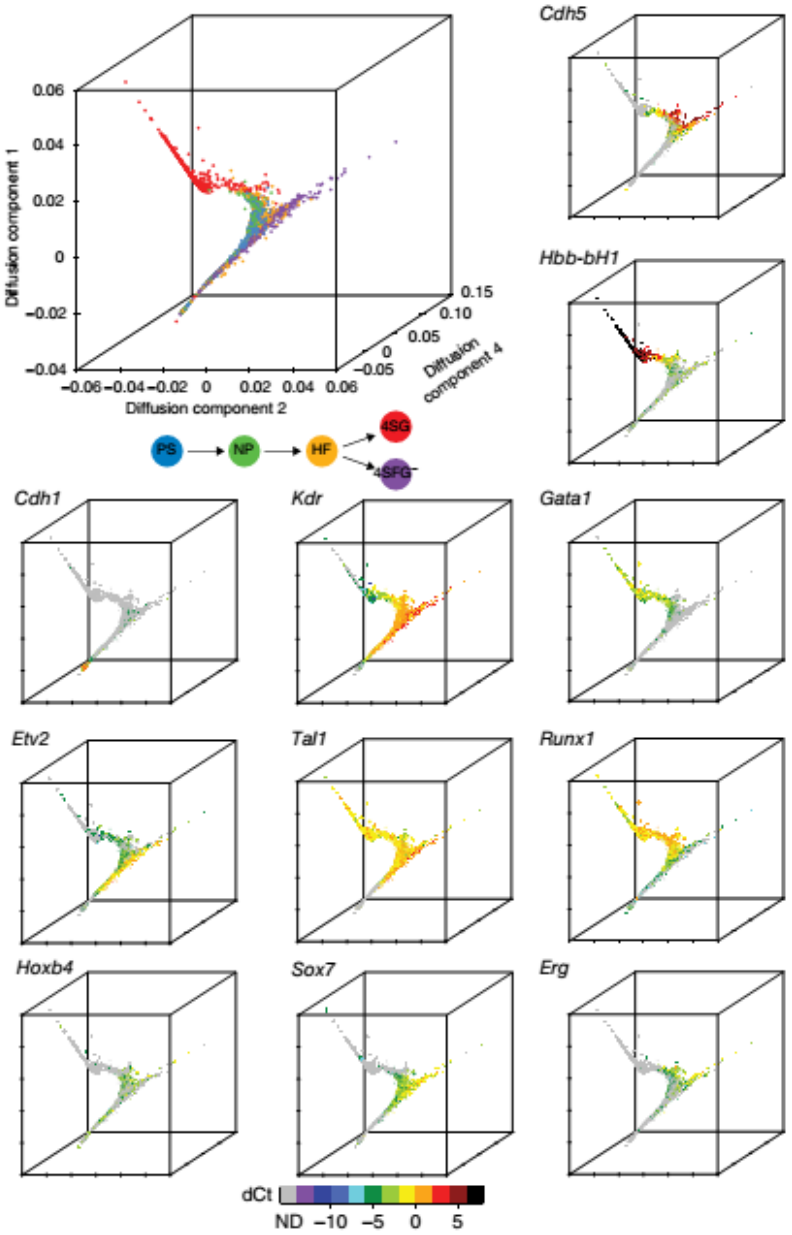
Dimensionality reduction: diffusion maps



Similarity of expression in cells *i* and *j* :

$$P(i,j) = \frac{1}{Z_i} \exp\left(\frac{-\left(x_i - x_j\right)^2}{\varepsilon} \right)$$

P(i,j) is normalized so that $\sum_{i=1} P(i,j) = 1$

The cells are organized in 2D or 3D such that the Euclidean distance between the cells corresponds to the diffusion metric *P*(*i*,*j*) .

The quantity *P*(*i*,*j*) can then be interpreted as the **transition probability** of a diffusion process between cells.

Axes: eigenvectors of matrix P with largest eigenvalues.

Moignard et al.,
Nature Biotech.
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Who regulates hematopoiesis?
Design Boolean Network

33 transcription factors
3,934 cells
= 129,822 RTqPCRs

Binary discretization →

Possible binary states =	$2^{33} \approx 8{,}589 \times 10^6$
Measured binary states =	3,934
Observed unique binary states =	3,070
Largest connected component =	1,448

Determine suitable **expression thresholds** for each gene to categorize its expression levels into **binary** on / off states.

Note that less than 0.1% of the possible states has been observed.

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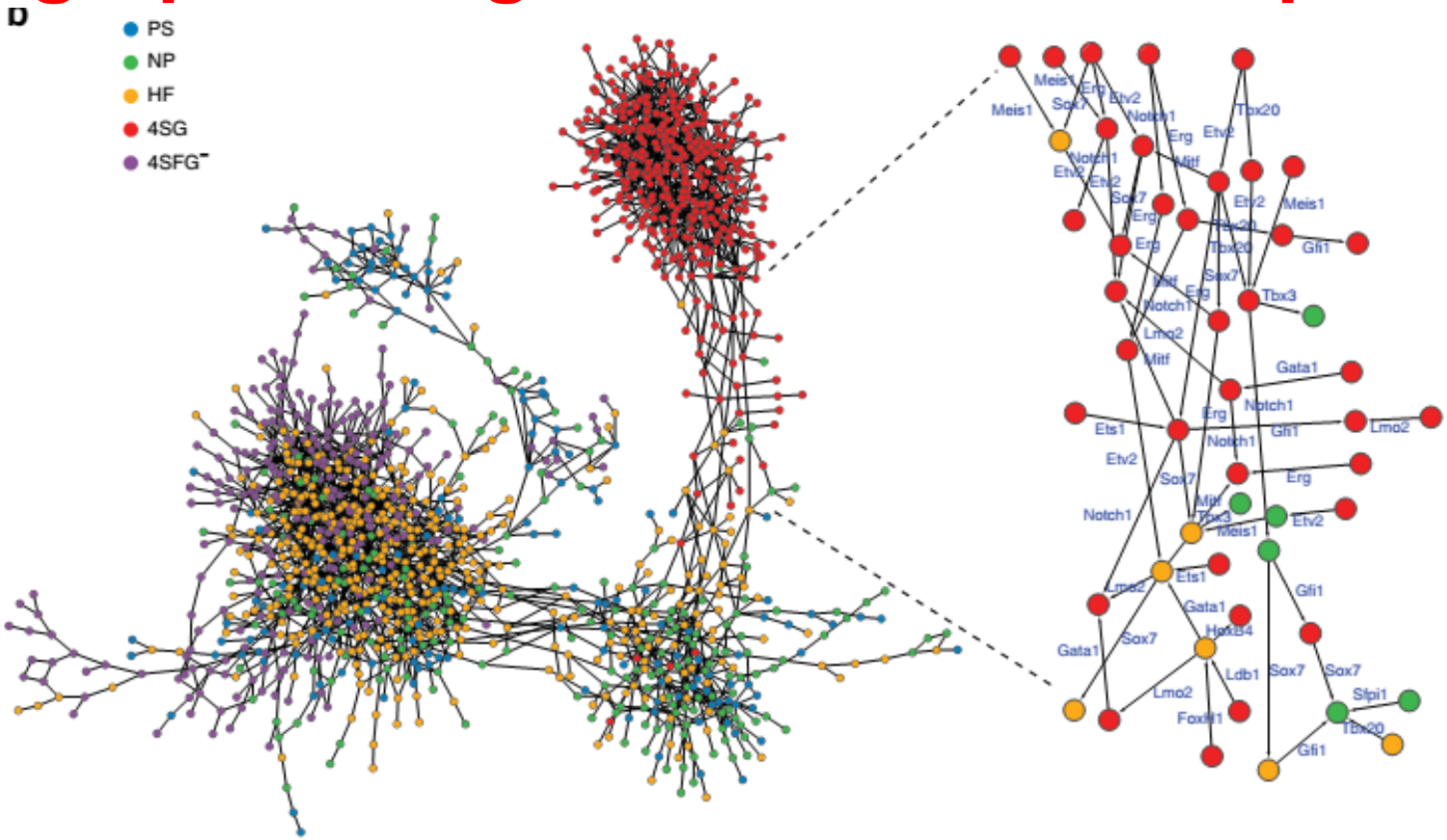
State graph of largest connected comp.



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State graph (largest connected component) of 1448 states reaching all 5 stages.

Add **edges** to connect all those pairs of states that differ in the on/off levels of a single gene (and are identical otherwise), see right side with labeled edges.

Idea behind this: these transitions can be best interpreted.

Automatic derivation of rules for Boolean Network

We are given:

-a set of variables V, corresponding to **genes**,

- an undirected graph $G = (N,E)$
  where each node $n \in N$ is labeled with a **state** $s: V \rightarrow \{0,1\}$, and
  each edge $\{s_1,s_2\} \in E$ is labeled with the **single variable**
  that **changes** between state s_1 and s_2.

We are also given a designated set $I \subseteq N$ of **initial vertices**
and a designated set $F \subseteq N$ of **final vertices**,
along with a **threshold** t_i for each variable $v_i \in V$.

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Optimality criteria for rules

Our rule synthesis method searches for an orientation of G, along with an update function u_i:$\{0,1\}^n \rightarrow \{0,1\}$ for each variable $v_i \in V$, such that the following conditions hold:

1. For each edge (s_1,s_2) labeled with variable v_i in the orientated graph, the update function for v_i takes state s_1 to state s_2: $u_i(s_1) = s_2(i)$.

2. The number of states in which no transitions induced by the update functions are **missing** is **maximized**.

3. Every **final vertex** $f \in F$ is **reachable** from some initial vertex $i \in I$ by a directed path in the orientated graph.

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Allowed complexity of the rules

We restrict the update function u_i to have the form:

$$f_1 \wedge \neg f_2$$

where f_j is a Boolean formula that has

and-nodes of in-degree two,

or-nodes of arbitrary in-degree, and

where f_1 has a maximum depth of N_i and f_2 has a maximum depth of M_i.

N_i and M_i are given as parameters to the method.

The search for edge orientations and associated Boolean update rules is encoded as a Boolean satisfiability (SAT) problem.

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Generated rules for Boolean Network

Gene	Synthesised update functions	% Non-observed transitions disallowed (N_i)	Motifs present
Scl	*Fli1*	98	Yes
Etv2	*Notch1*	96	Yes
Fli1	*Etv2*	96	Yes
	Sox7	97	Yes
Lyl1	*Sox7*	92	Yes
Sox7	*Sox17* ∨ *HoxB4*	82	No (Sox missing)
Erg	(*HoxB4* ∧ *Lyl1*) ∨ *Sox17*	84	Yes
	(*HoxB4* ∧ *Tal1*) ∨ *Sox17*	83	Yes
Notch1	*Sox7*	94	Yes
Gata1	*Gfi1b* ∧ *Lmo2*	86	Yes
	Gfi1b ∧ *Hhex*	84	No (Hhex missing)
	Gfi1b ∧ *Ets1*	84	Yes
HoxB4	(*Lyl1* ∧ *Ets1*) ∧ ¬*Gata1*	65	Yes
	(*Lyl1* ∨ *Nfe2*) ∧ ¬*Gata1*	65	Yes
	(*Lyl1* ∨ *Ikaros*) ∧ ¬*Gata1*	65	No (Ikaros missing)
Sox17	*Lyl1* ∧ ¬*Gfi1b*	77	No (Gfi missing)
	(*Eto2* ∧ *Sox7*) ∧ ¬*Gfi1b*	76	No (Gfi missing)
	(*Eto2* ∧ *Tal1*) ∧ ¬*Gfi1b*	75	No (Gfi missing)
Ets1	*Notch1*	96	Yes
Gfi1	*Gata1* ∧ ¬*Sox17*	88	Yes
	Nfe2 ∧ ¬*Sox17*	88	Yes
Gfi1b	*Nfe2* ∧ *Myb*	87	Yes
	Pu.1 ∧ *Ikaros*	86	No (Ikaros missing)
	Pu.1 ∧ *Nfe2*	86	Yes
	Pu.1 ∧ *Myb*	86	Yes
Eto2	*Sox7*	93	No (Sox missing)
	Hhex	92	No (Hhex missing)
	Ets1 ∧ *Fli1*	94	No (Ets missing)
Hhex	*Sox7*	97	No (Sox missing)
	Notch1	93	No (Rbpj missing)
Ikaros	*Nfe2* ∨ *Gfi1b*	84	Yes
	Nfe2 ∨ *Gata1*	83	Yes
	Nfe2 ∨ *Gfi1*	82	Yes
Lmo2	*Sox7* ∨ *Gfi1*	79	Yes
	Sox7 ∨ *Erg*	79	Yes
	Sox7 ∨ *HoxB4*	77	Yes
Nfe2	*Ikaros*	78	Yes
Pu.1	*Gfi1* ∨ *Erg*	67	Yes
Myb	*HoxB4*	64	Yes

Additional validity check of the postulated rules:

check whether regulated genes contain **TF-binding motifs** in their promoters (right column).

This is the case for 70% of the rules.

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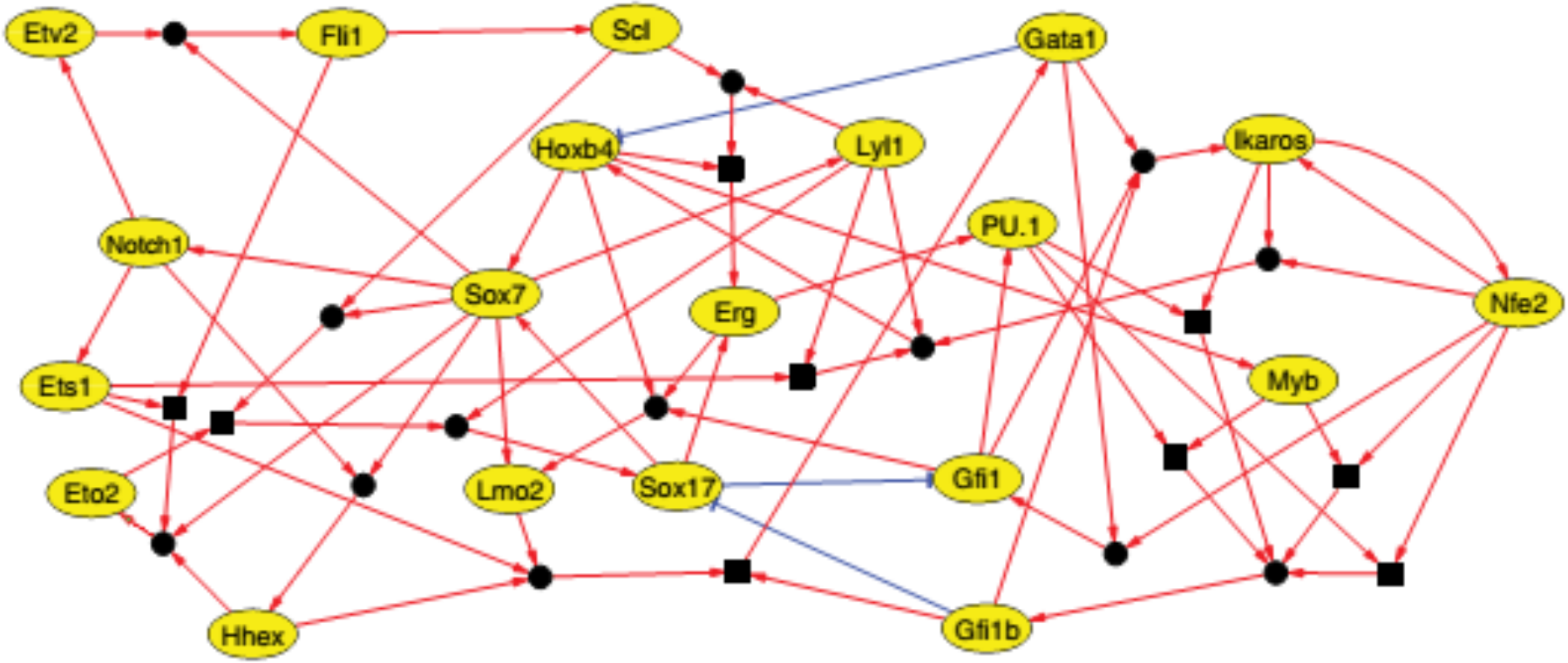
Core network controlling hematopoiesis



Derived core network of 20 TFs.

Red edges: activation

Blue edges: repression

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Predict effects of perturbations as validation

Simulate overexpression and knockout experiments for each TF.

Assess ability of the network to reach wildtype or new stable states.

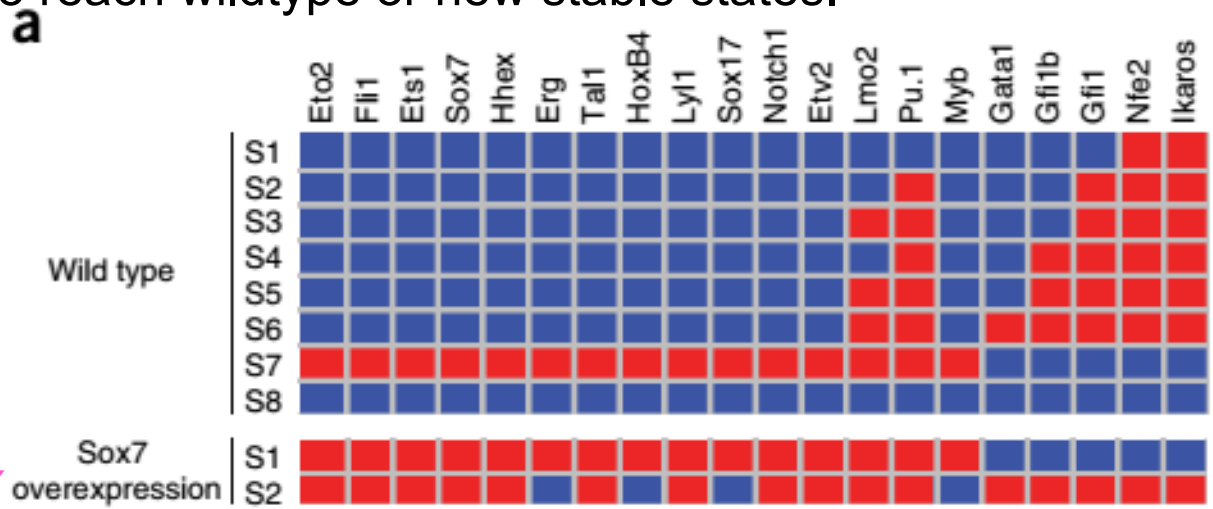
Red : gene expressed;

blue : gene not expressed.

S2-S6: blood-like

S7: endothelial-like

S8 : no activity



Network stable states for wt and Sox7 overexpression.

Enforced expression of Sox7 (that is normally downregulated) stabilized the endothelial module and an inability to reach any of the blood-like states.

Sox7 is predicted to regulate more targets than any other TF, suggesting that perturbing its expression could have important downstream consequences

Moignard et al., Nature Biotech. 33, 269 (2015)

Control experiments

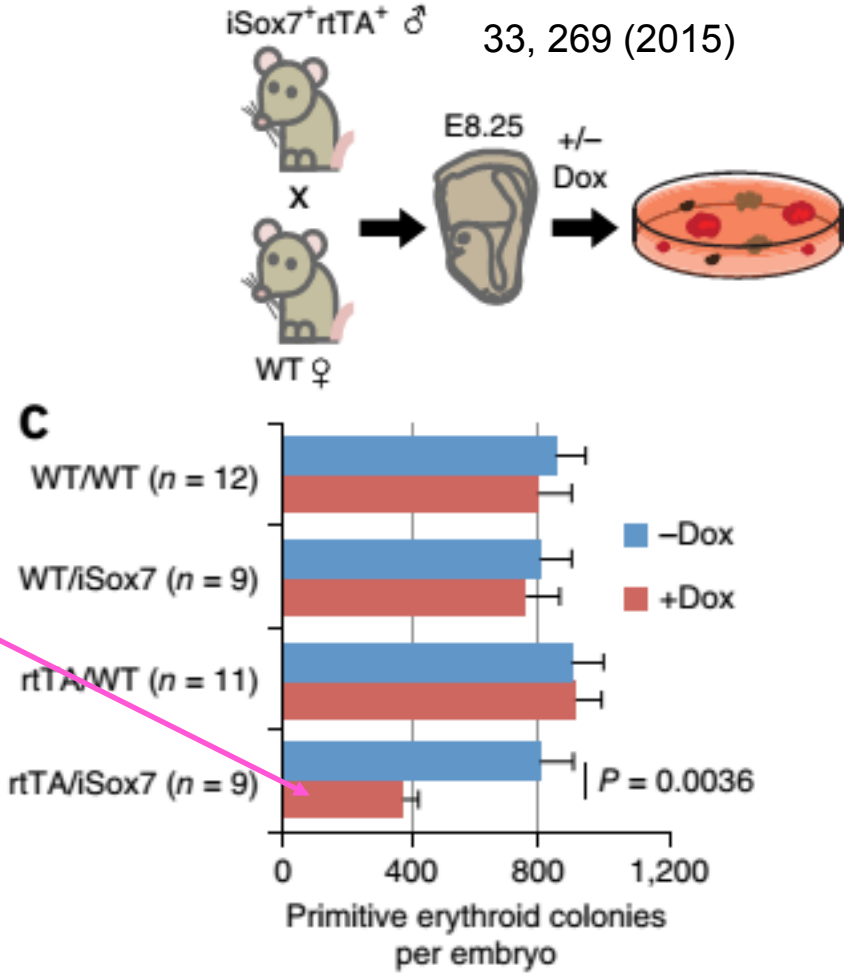
Moignard et al., Nature Biotech. 33, 269 (2015)

(**b**) Colony assays with or without doxycycline from genotyped E8.25 embryos from iSox7^{+}rtTA^{+} mice crossed with wild types.

(**c**) Quantification of primitive erythroid colonies after 4 days.

Embryos carrying both transgenes (rtTA/iSox7) showed a **50% reduction of primitive erythroid colony formation** following doxycycline-induced *Sox7* expression compared to controls.

This suggests, in agreement with modeling data and gene expression patterns, that downregulation of *Sox7* is important for the specification of primitive erythroid cells.



In iSox7-mouse, overexpression of Sox7 is stimulated by inducing the Sox7-promoter by addition of the chemical doxycycline (+Dox).

Conclusions

Cells destined to become blood and endothelium arise at all stages of the analyzed time course rather than in a synchronized fashion at one precise time point, consistent with the gradual nature of gastrulation.

Using an automated Boolean Network synthesis toolkit we identified a core network of 20 highly connected TFs, which could reach 8 stable states representing blood and endothelium.

We validated model predictions to demonstrate e.g. that Sox7 blocks primitive erythroid development.

→ Boolean Networks can be predictive and may guide experiments.

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