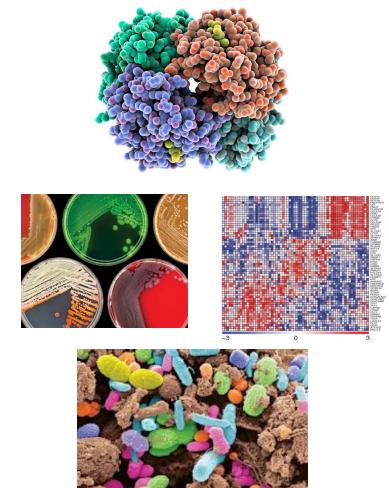


## De las redes intracelulares a las simulaciones de sistemas multicelulares



Profesor: Dr. Miguel Ponce de León ([miguel.ponce@bs.es](mailto:miguel.ponce@bs.es)) - BSC

Coordinador: Dr. Flavio Pazos ([flavio.pazos@gmail.com](mailto:flavio.pazos@gmail.com)) - IIBCE/IP



Apoyan:



# Tema 4

- Redes de información
- Redes de señalización y regulación
- Modelos Booleanos

# Regulatory networks

**Regulatory networks:** Protein-DNA interactions are an important and common class of interactions. Most DNA-binding proteins are transcription factors that regulate the expression of target genes. Combinatorial use of transcription factors further complicates simple interactions of target genes for a given transcription factor. A regulatory network consists of transcription factors and their targets with a specific directionality to the connection between a transcription factor and its target.

**Signaling networks:** These networks represent signal transduction pathways through protein-protein and protein-small molecule interactions. Nodes represent proteins or small molecules, and links represent signal transduction events.

# Review transcriptional regulation

- **Sensory networks**

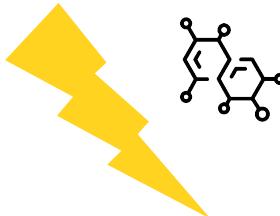
- Present in all forms of cells
- Respond to signals:
  - Stress (heat, pH, etc),
  - nutrients,
  - *quorum sensing*

- **Developmental networks**

- Guide differentiation events
- Cellular differentiation
- Present in multicellular organism

# The cognitive problem of the cell

**Signal (internal/external)**



**Sensor**

**Response**



**Complex environment** (different kind of signals):

- Temperature, pH, Osmotic pressure
- Molecules, other cells...

**Signals also associated with internal state:**

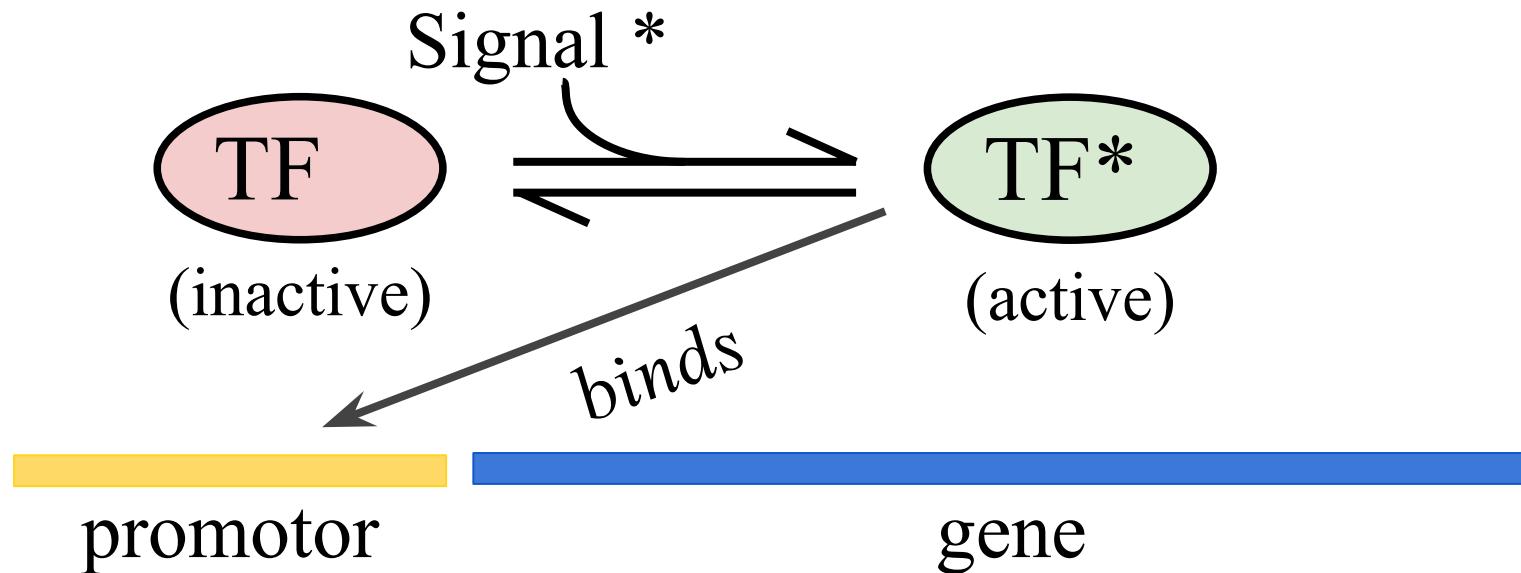
- Metabolite levels
- Internal damage (DNA, protein)

(Producing appropriate proteins that act on the internal or external environment)

How does the cell represent environment (internal or external)?

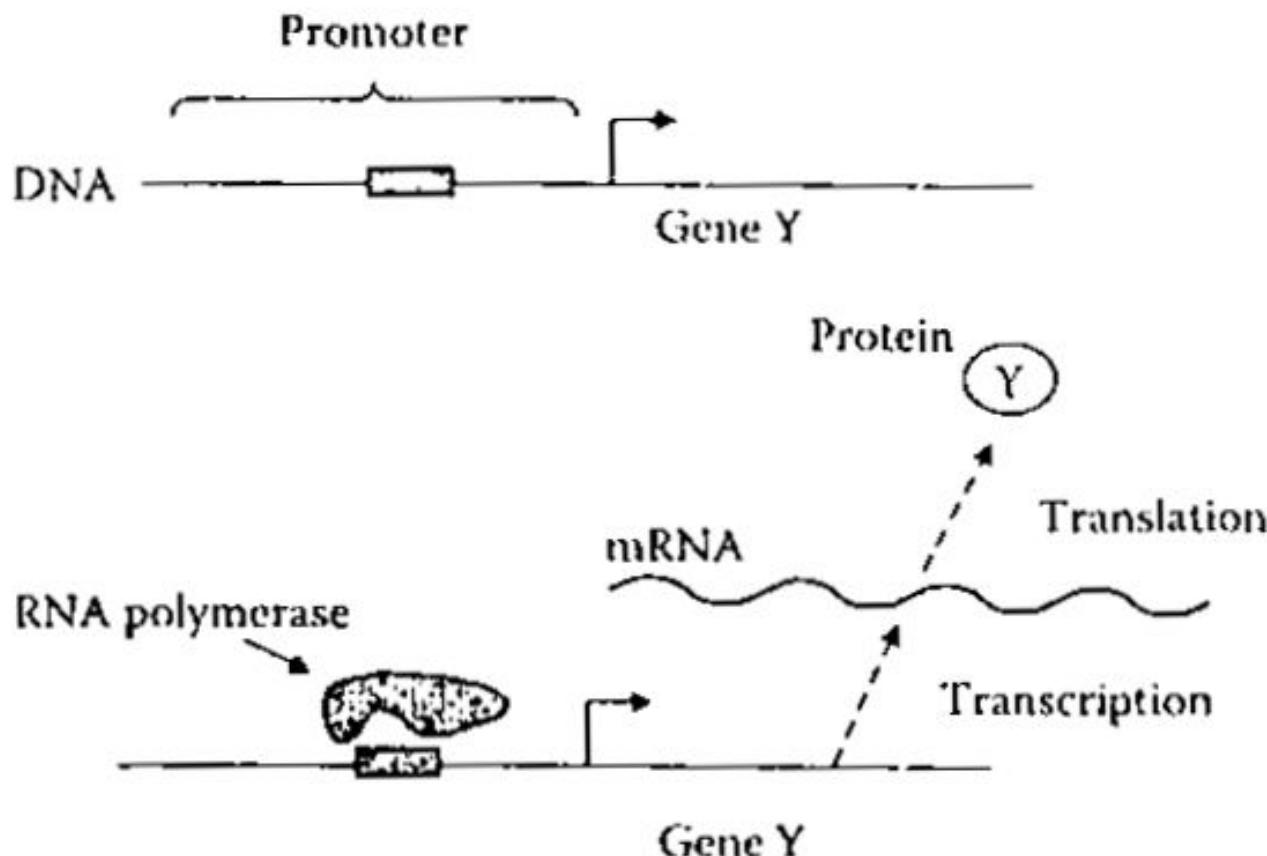
# The cognitive problem of the cell

How does the cell represent environment (int. or ext.)?  
→ Special class of proteins: **Transcription factors**



→ **TF + DNA** modulate the expression of a gene

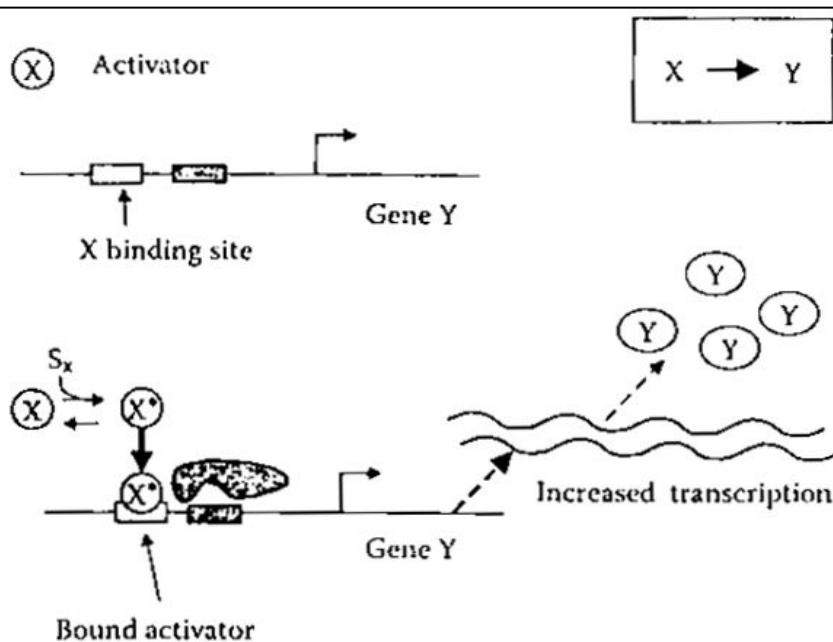
# The regulation of gene expression



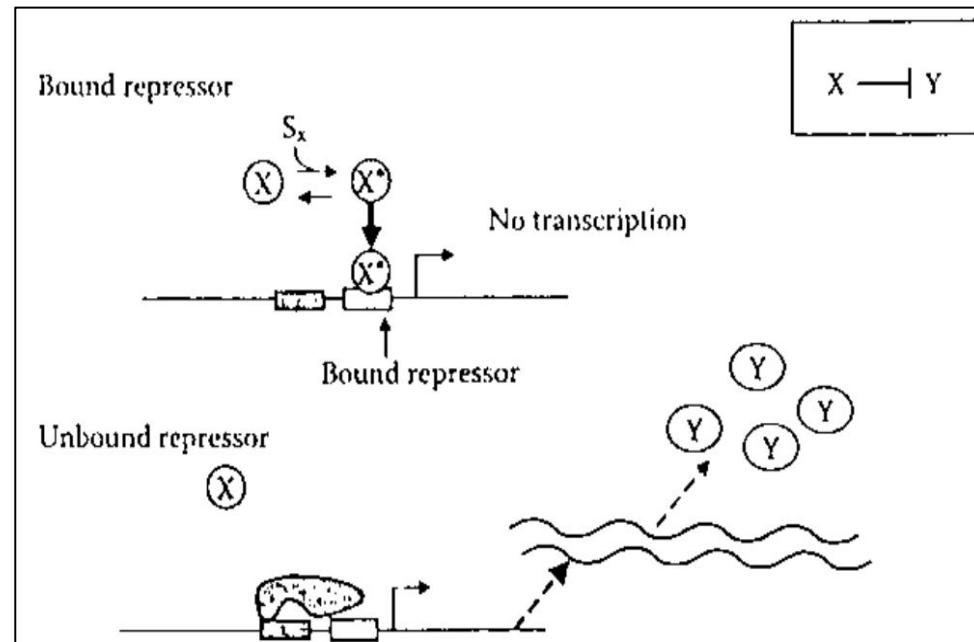
# The regulation of gene expression

There are two type of transcription factors

## Activator



## Inhibitor

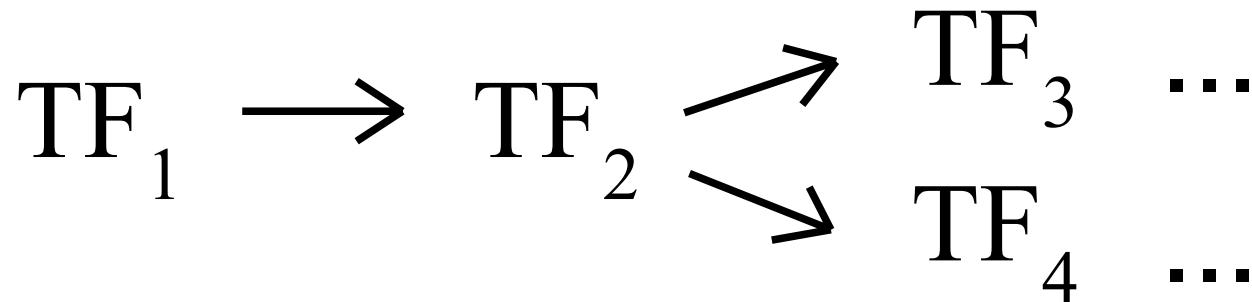


# The regulation of gene expression

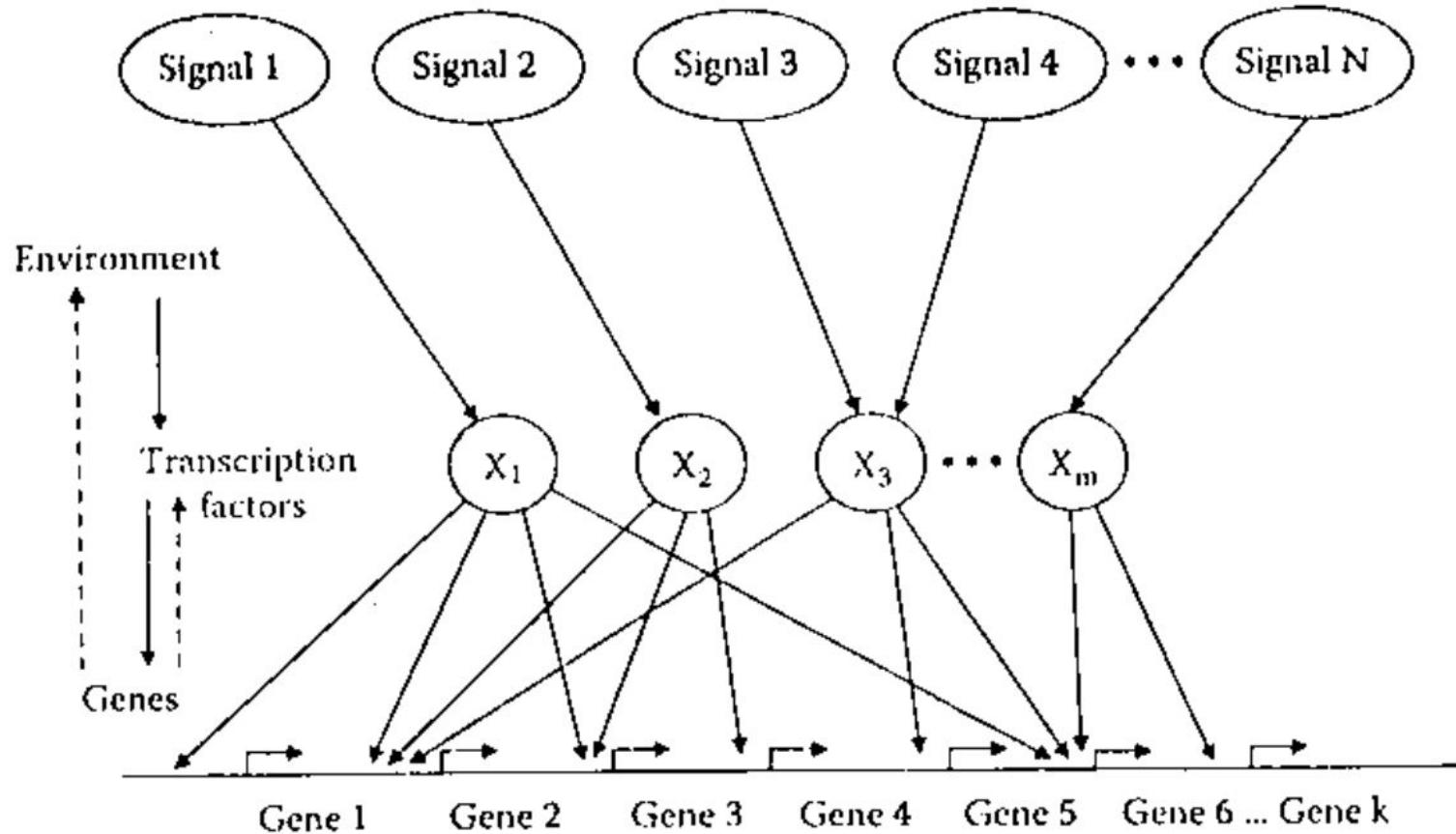
- Cells use **transcription factors** to represent environmental states.
- Designed to **switch** rapidly between **active** & **inactive**.
- **Regulate the rate** of transcription of genes → change the probability per unit time (**affinity**) that *RNApol* binds to the promoter and creates an *mRNA* molecule.
- Two main types of TF: **activators** and **repressors**.

# The regulation of gene expression

- But **transcription factors are proteins**
- Transcription factors are encoded by genes, which are regulated by transcription factors, which are regulated by transcription factors ...
- **Transcription networks** describe all the regulatory transcription interactions in a cell

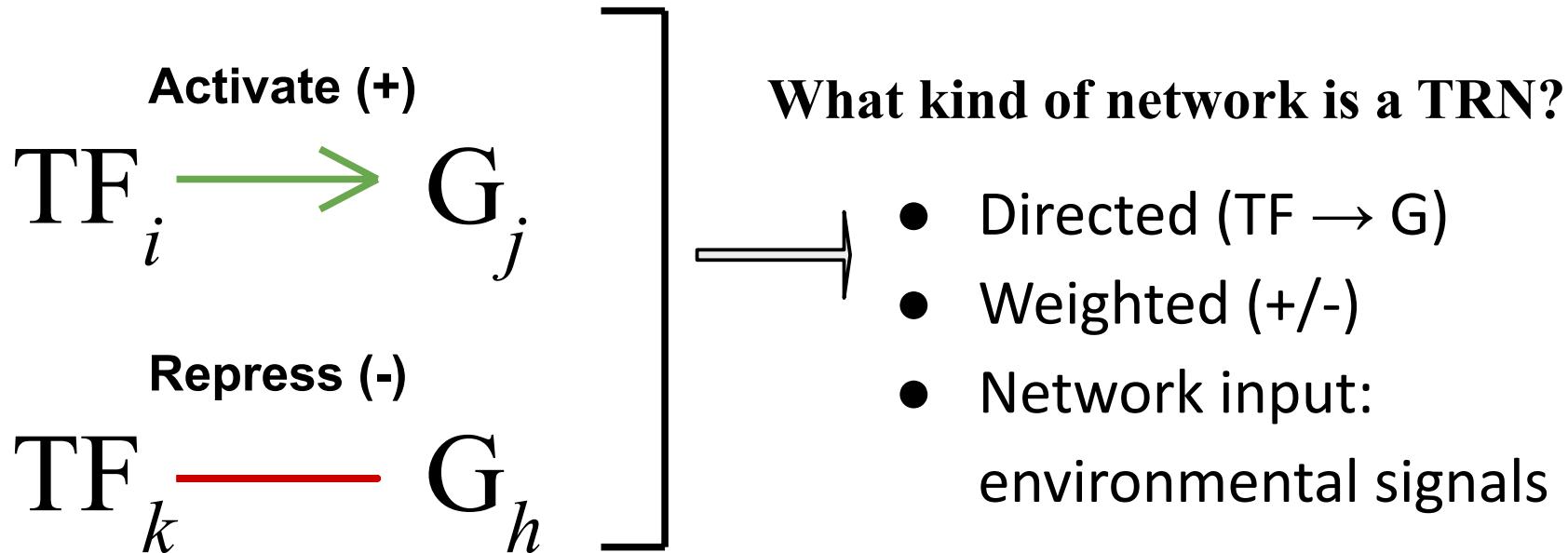


# The regulation of gene expression

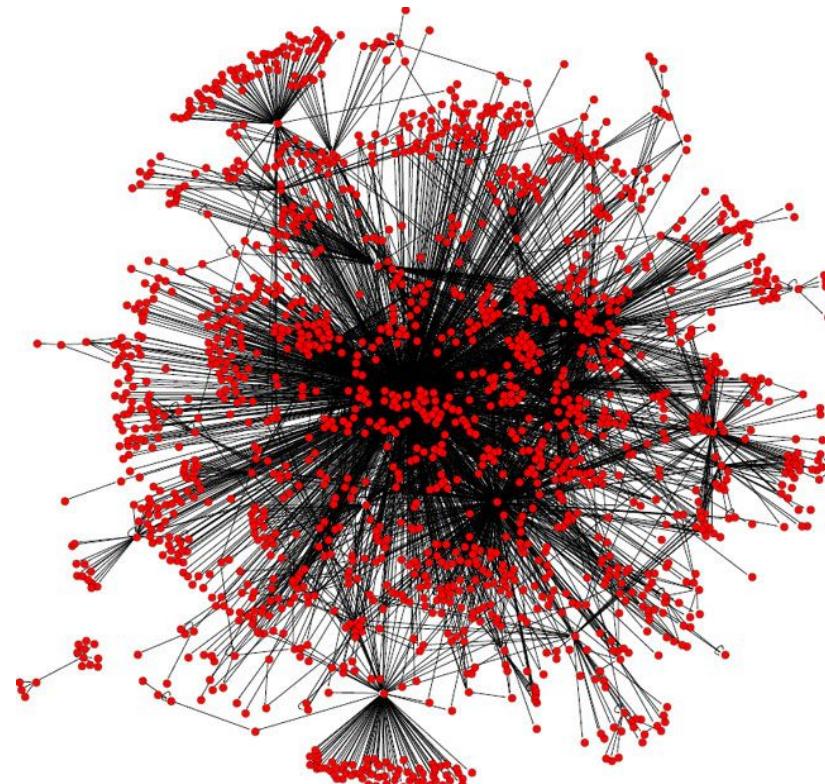


# Transcription regulation as a network

- **Nodes:** represent the different genes
- **Edges:** regulatory relation between a TF and a gene



# Transcription regulation as a network



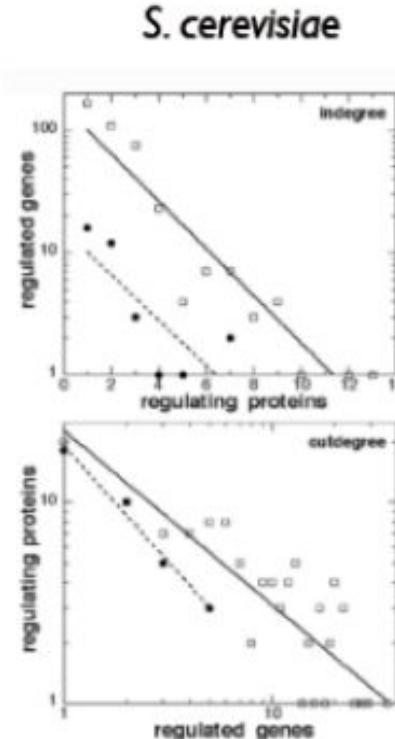
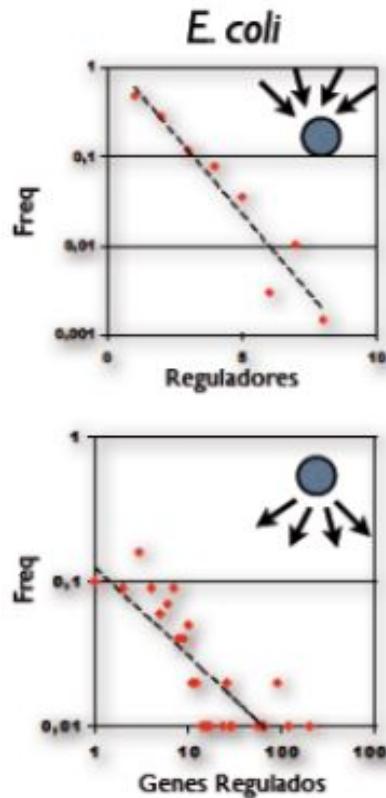
## E. coli contains ~ 300 TF

- Degrees of freedom to represent Internal representation
- Regulates expression of ~4000 proteins
- Example: the activation of one TF means : *I'm starving*

## Some facts

- Out degree sign correlates
- In degree does not
- In general there are more activators than repressors (~60-80% TF (+))

# Network architecture



In-degree: exponential  
 $P(k)=C \cdot e^{-k}$

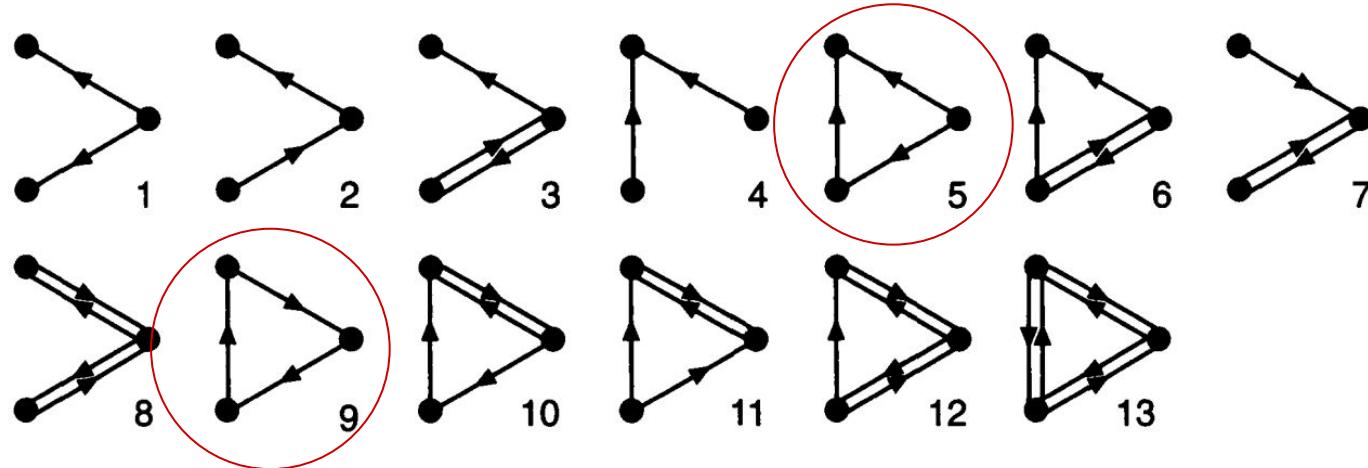
Out-degree: scale-free  
 $P(k)=C \cdot k^{-\gamma}$

Guelzim et al. 2002 Nature Genet. 31:60-63

=> Short paths, resistance to perturbations/failure, ...

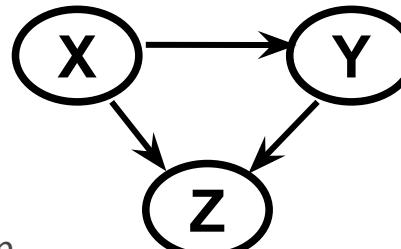
# Motif detection in regulatory networks

All 13 types of three-node connected subgraphs\* (motif)

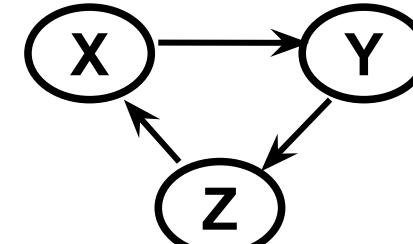


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Feed-forward loop

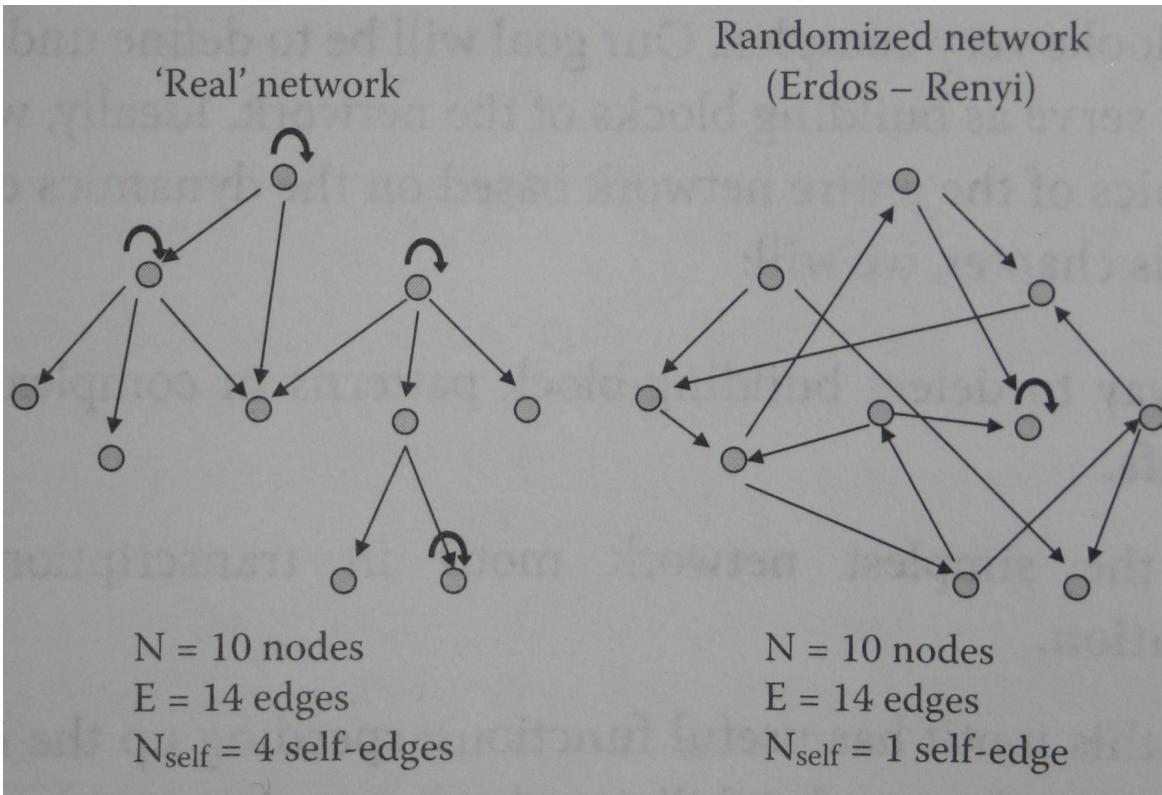


Feed-backward loop



\* not considering sign

# Network randomization → null model



## Randomization

Same number of nodes and edges.

Directed edges assigned at random.

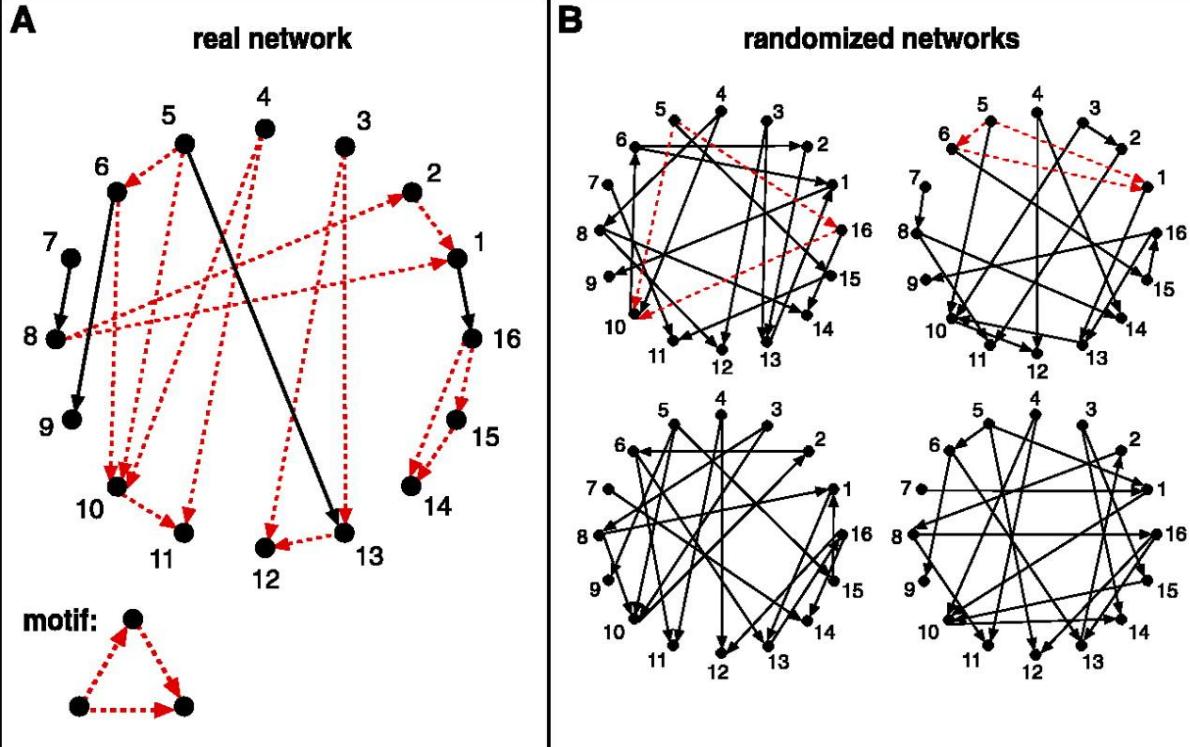
$N$  nodes  $\rightarrow N^2$  possible edges.

Probability edge position is occupied:

$$P = \frac{E}{N^2}$$

The random network is constructed by generating using an ER model

# Motif detection in regulatory networks



Network	Nodes	Edges																																				
<b>Gene regulation (transcription)</b>																																						
<i>E. coli</i>	424	519																																				
<i>S. cerevisiae*</i>	685	1,052																																				
<table border="1"> <thead> <tr> <th><math>N_{\text{real}}</math></th><th><math>N_{\text{rand}} \pm \text{SD}</math></th><th>Z score</th><th><math>N_{\text{real}}</math></th><th><math>N_{\text{rand}} \pm \text{SD}</math></th><th>Z score</th></tr> </thead> <tbody> <tr> <td>X</td><td></td><td></td><td>X</td><td></td><td></td></tr> <tr> <td>V</td><td></td><td></td><td>V</td><td></td><td></td></tr> <tr> <td>Y</td><td></td><td></td><td>Y</td><td></td><td></td></tr> <tr> <td>↓</td><td></td><td></td><td>↓</td><td></td><td></td></tr> <tr> <td>Z</td><td></td><td></td><td>Z</td><td></td><td></td></tr> </tbody> </table>			$N_{\text{real}}$	$N_{\text{rand}} \pm \text{SD}$	Z score	$N_{\text{real}}$	$N_{\text{rand}} \pm \text{SD}$	Z score	X			X			V			V			Y			Y			↓			↓			Z			Z		
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40	7 ± 3	10	203	47 ± 12	13																																	
70	11 ± 4	14	1812	300 ± 40	41																																	
<table border="1"> <thead> <tr> <th><math>N_{\text{real}}</math></th><th><math>N_{\text{rand}} \pm \text{SD}</math></th><th>Z score</th><th><math>N_{\text{real}}</math></th><th><math>N_{\text{rand}} \pm \text{SD}</math></th><th>Z score</th></tr> </thead> <tbody> <tr> <td>X</td><td></td><td></td><td>X</td><td></td><td></td></tr> <tr> <td>Y</td><td></td><td></td><td>Y</td><td></td><td></td></tr> <tr> <td>↓</td><td></td><td></td><td>↓</td><td></td><td></td></tr> <tr> <td>Z</td><td></td><td></td><td>Z</td><td></td><td></td></tr> <tr> <td>W</td><td></td><td></td><td>W</td><td></td><td></td></tr> </tbody> </table>			$N_{\text{real}}$	$N_{\text{rand}} \pm \text{SD}$	Z score	$N_{\text{real}}$	$N_{\text{rand}} \pm \text{SD}$	Z score	X			X			Y			Y			↓			↓			Z			Z			W			W		
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X			X																																			
Y			Y																																			
↓			↓																																			
Z			Z																																			
W			W																																			
Bi-fan																																						

# Motif detection in regulatory networks

## Regulatory considering signed interactions

### Coherent FFL

Coherent type 1



Coherent type 2



Coherent type 3

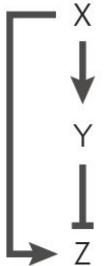


Coherent type 4



### Incoherent FFL

Incoherent type 1



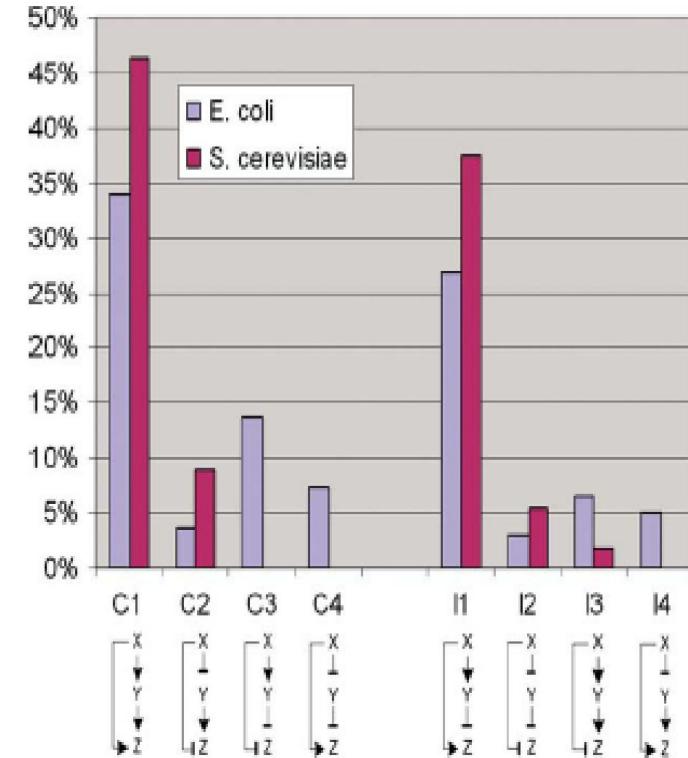
Incoherent type 2



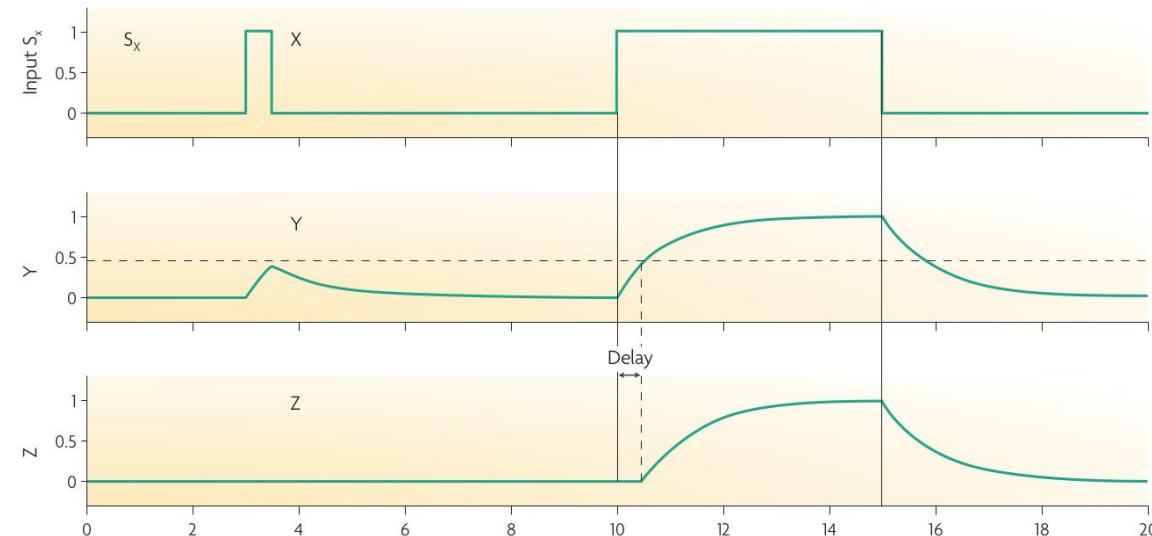
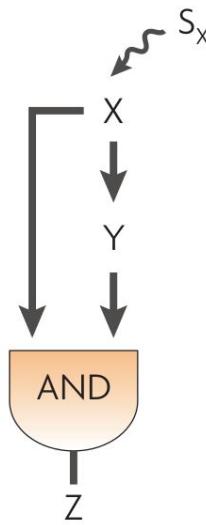
Incoherent type 3



Incoherent type 4



# Coherent Feed-Forward Loop Type 1

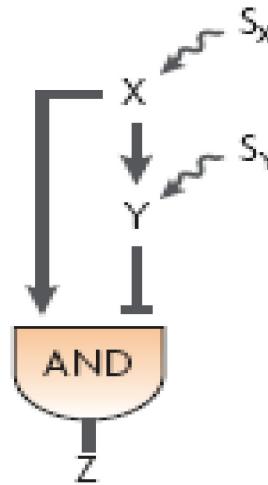


C1-FFL with an AND input function shows:

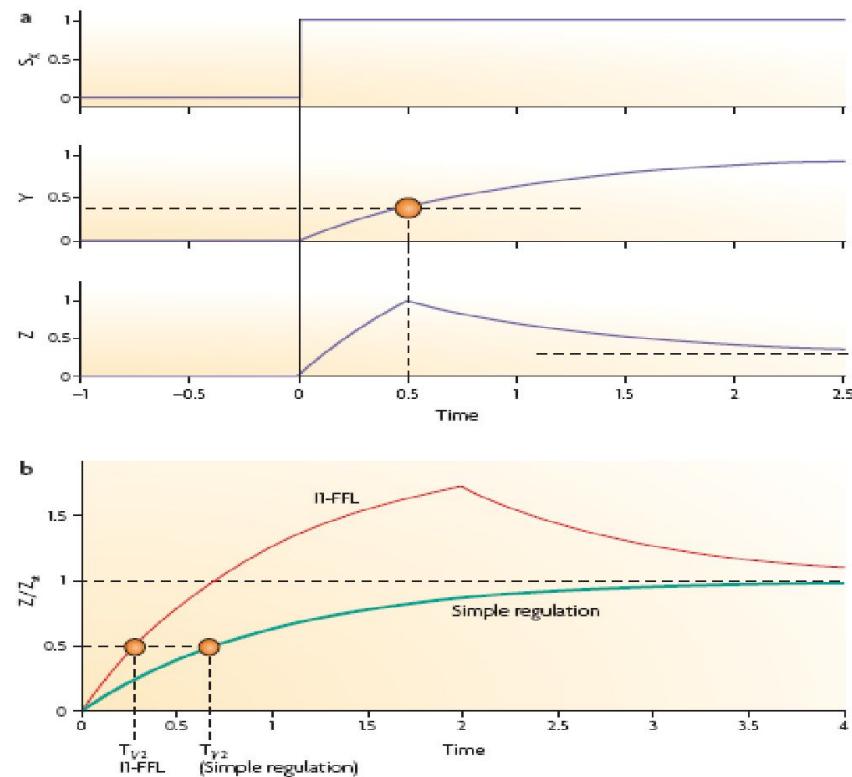
- delay after stimulus ( $S_X$ ) addition
- no delay after stimulus removal

C1-FFL acts as a sign-sensitive filter (responds only to persistent stimuli)

# Incoherent Feed-Forward Loop Type 1



- Two parallel but opposing paths: the direct path activates Z and the other represses Z.
- Z shows high expression when  $X^*$  is bound and low expression when  $Y^*$  is bound.
- Use:** pulse generator & fast response time.



# Summary

- Regulatory networks are directed and weighted networks
- While out-degree follows a typical power-law distribution, the in-degree fits better an exponential distribution (Poisson)
- The regulome is enriched with two motif (FFL and Bi-fan)
- Topological motifs were subject of selection due to their functional properties.
- Motif can work as function modules: filter, amplifier, memory ...

# Reconstructing and representing signaling and regulatory networks

Elucidating gene regulatory networks is crucial for understanding normal cell physiology and complex pathologic phenotypes.

# Cellular signalling networks

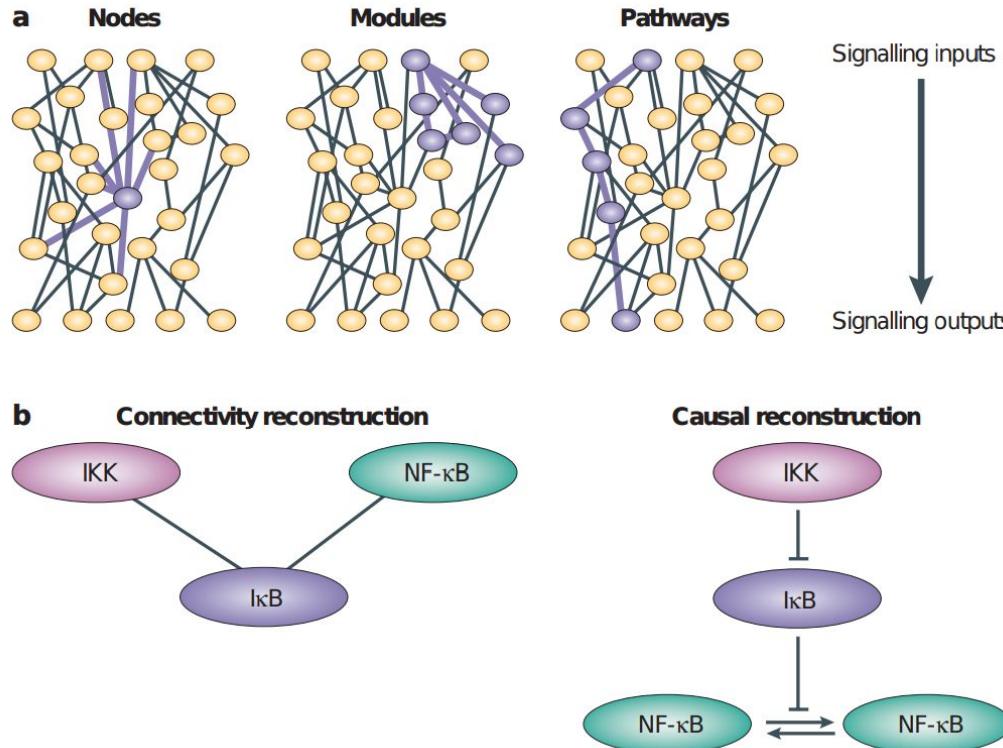
- Cellular signalling networks operate over several orders of magnitude in spatio-temporal scales
- Include extracellular and intracellular signalling mechanisms.
- The endpoints of signalling events include:
  - Quick responses (< 1 seconds)
    - protein modifications
    - changes in Ca<sup>2+</sup> concentrations
  - Slow responses (from minutes to hours)
    - transcriptional regulation
    - cell migration
    - cell-cycle control
    - cell proliferation
    - apoptosis.

## Signaling in numbers

The human cellular signalling network includes genes for **~1,600 signalling receptors**, **~500 protein kinases** and **~150 protein phosphatases**.

The activity of these components can result in the activation (or inhibition) of **~2,000 transcription factors**, which then direct cellular regulatory processes

# Reconstructing a signalling network



## Manual reconstruction:

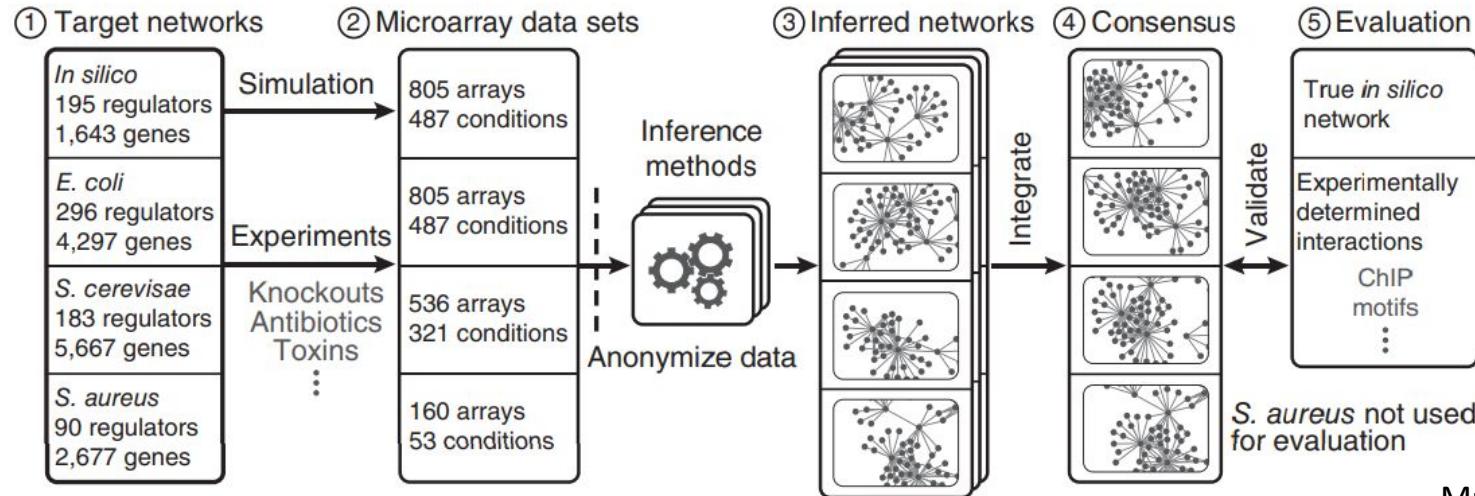
1. Reconstructions of highly connected nodes (**hubs**) in networks.
2. Reconstructing **linear ‘pathways’** that connect signalling inputs to signalling outputs.
3. Identifying **signalling modules** (groups of compounds and proteins that function together under certain conditions)

# Construct the Interaction Network

- Translation of experimental information into edge
- Assembly and refinement of these edges
- Direct information (indicate direct interaction between two components)
  - Physical (Y2H)
  - Biochemical (reaction assay)
  - Perturbation experiment (gene silencing, reaction inhibition)
- Indirect information (Guilty by association principle)
  - Co-expression
  - Co-occurrence (physically, evolutionary)
- Causal relationships are represented as directed (signed) edges between two components:
  - Positive interaction (activation)
  - Negative interaction (inhibition)

# Construct the Interaction Network

- Reconstructing gene regulatory networks from high-throughput data is a long-standing challenge
- Many different methods has been proposed
- Evidence shows that no single inference method performs optimally across all data sets
- Integration of predictions from multiple inference methods shows robust and high-performance across diverse data sets → Wisdom of the crowd



# Automatic inferences

## Reverse Engineering Algorithms:

**ARACNE:** an algorithm for the reconstruction of gene regulatory networks in a mammalian cellular context. Margolin, A. A., et al. (2006).

**MRNET:** Information-theoretic inference of large transcriptional regulatory networks. Meyer, P. E., et al. (2007)

## Correlation-Based Methods:

**WGCNA:** uses weighted correlation network analysis. Langfelder, P., & Horvath, S. (2008).

Spearman Correlation: Feizi, S., et al. (2013). Network deconvolution as a general method to distinguish direct dependencies in networks. *Nature Biotechnology*, 31(8), 726-733.

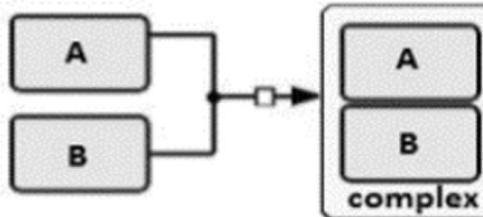
## Machine Learning Approaches:

Support Vector Machines (SVM): Bansal, M., et al. (2006). A community detection algorithm for networks with hierarchical organization. In *Pacific Symposium on Biocomputing* (pp. 6-17).

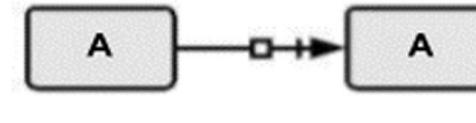
Random Forest: Zhang, X., et al. (2011). Multiscale embedded gene co-expression network analysis. *PLoS Computational Biology*, 7(2), e1001098.

# Representing signaling a regulatory networks

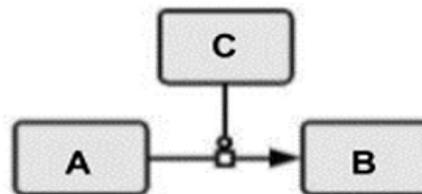
# Systems Biology Graphical Notation (SBGN)



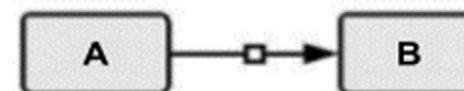
Association



Transport



Catalysis



State transition



Phosphorylated  
protein



Protein

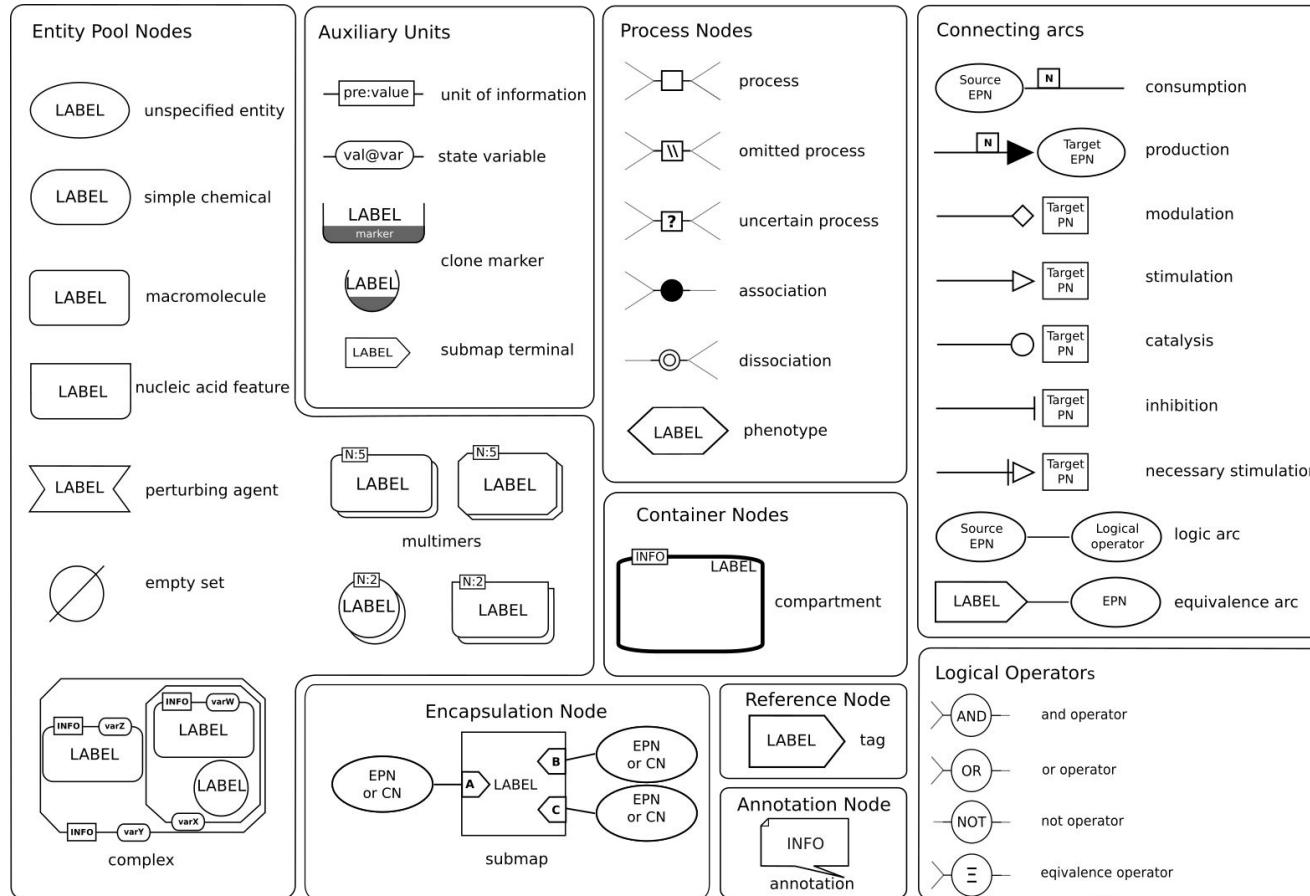


Receptor



Empty set

# SBGN PD Reference Card



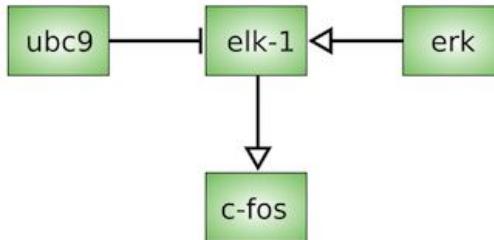
Source: <https://sbgn.github.io/learning>

# Systems Biology Graphical Notation (SBGN)

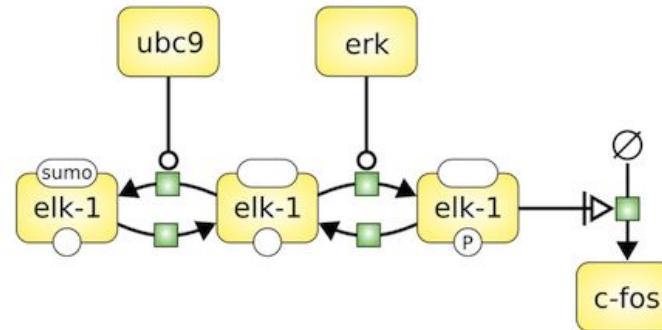
## The three languages of SBGN

The same biological system is shown in different representations depending on the concepts used to describe this system. There are three complementary languages in SBGN:

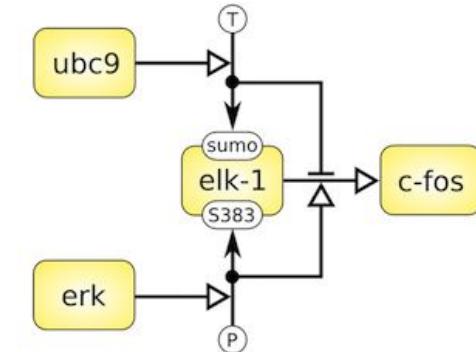
**activity flows**



**process descriptions**



**entity relationships**



directed   
 sequential   
 mechanistic

directed   
 sequential   
 mechanistic

directed   
 sequential   
 mechanistic

# Simulating signaling and regulatory networks

Introduction to Boolean modeling

# Mathematical modeling of regulation

A TF X regulates the expression a gene Y



## Rate equation

$$\frac{dY}{dt} = \beta - \alpha Y$$

$\beta$ : transcription rate

$\alpha$ : degradation rate (deg+dilution)

## Steady state

$$\frac{dY}{dt} = 0 \rightarrow Y_{ss} = \frac{\beta}{\alpha}$$

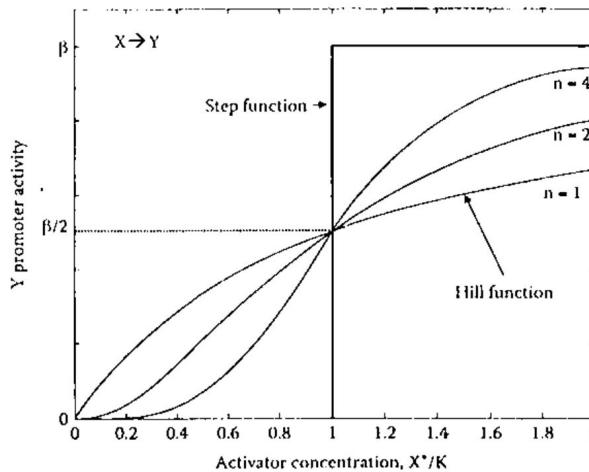
## Time dynamics

$$Y(t) = Y_{ss}(1 - e^{-\alpha t})$$

$$Y(t) = Y_{ss}e^{-\alpha t} \quad \beta = 0 \text{ (not stimulated)}$$

# Mathematical modeling of regulation

**Input function** → strength of the effect of a TF on the transcription rate of target gene.

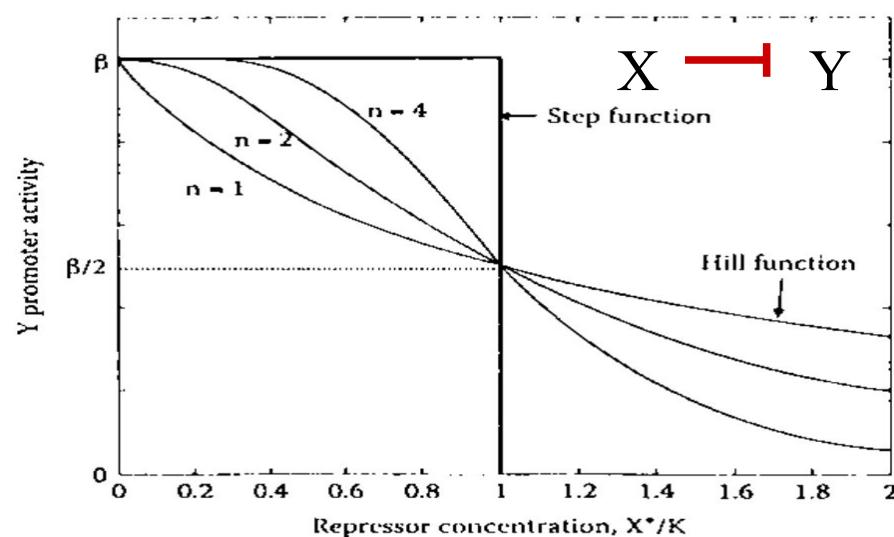


Hill function

$$f(X^*) = \frac{\beta X^{*n}}{K^n + X^{*n}}$$

Logical Function

$$f(X^*) = \beta \theta(X^* > K)$$



Hill function

$$f(X^*) = \frac{\beta}{1 + \left(\frac{X^*}{K}\right)^n}$$

Logical Function

$$f(X^*) = \beta \theta(X^* < K)$$

- All activators present:

$$f(X^*, Y^*) = \beta \theta(X^* > K_X) \theta(Y^* > K_Y)$$

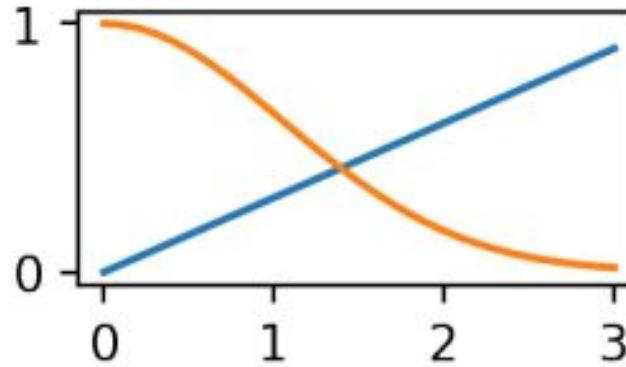
- At least one activator present:

$$f(X^*, Y^*) = \beta \theta(X^* > K_X \text{ OR } Y^* > K_Y)$$

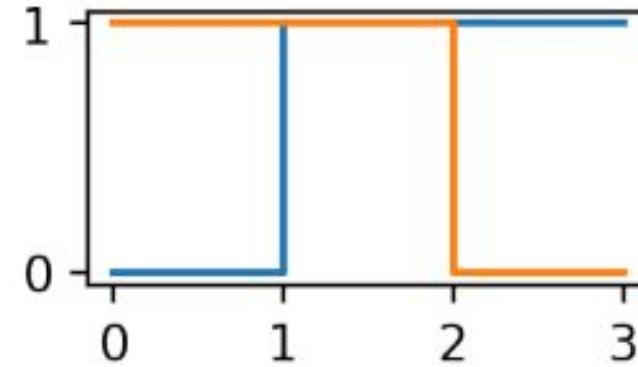
- Non Boolean  $\beta_X X^* + \beta_Y Y^*$

# Quantitative v.s. Qualitative modeling

## Quantitative



## Qualitative



- Values can be any quantity
- Continuous time
- Difficult to write
- Difficult to simulate large models

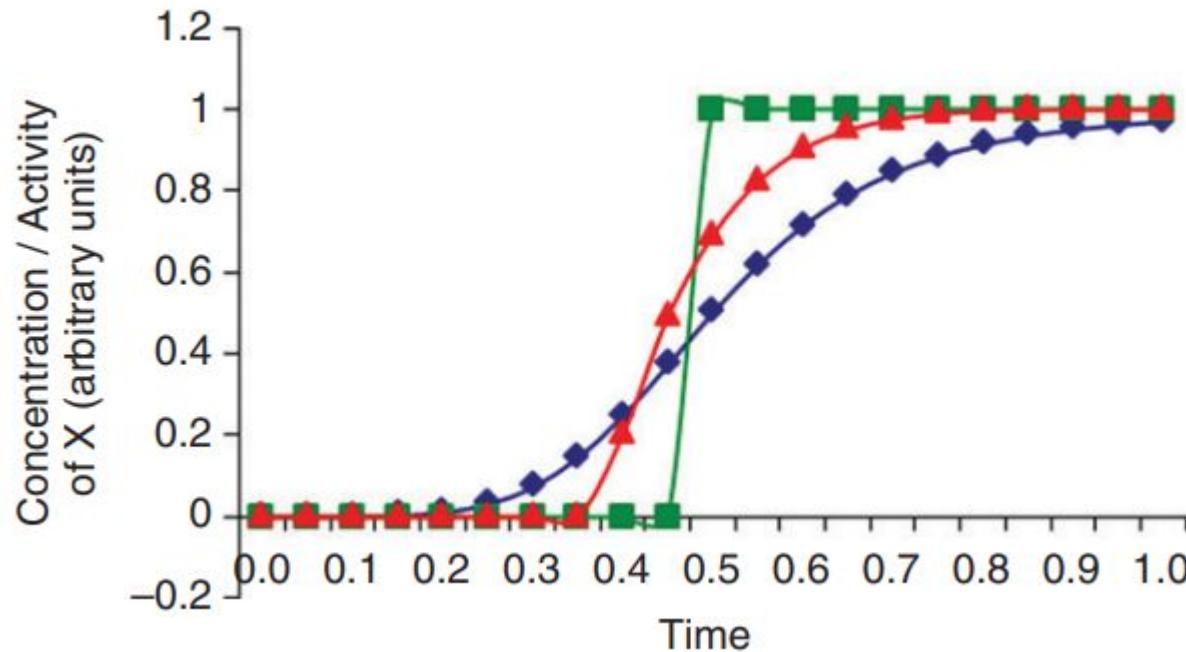
- Values are true/false
- Sequences of events
- Easy to write
- Can simulate large models

# Boolean Modeling

**Source:** *Boolean modeling: a logic-based dynamic approach for understanding signaling and regulatory networks and for making useful predictions*

Réka Albert and Juilee Thakar paper

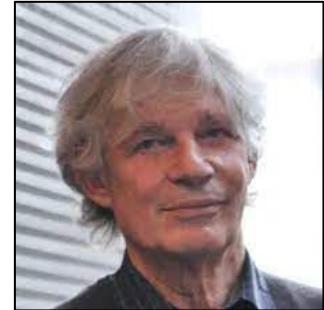
# Transition between a continuous and a Boolean description



$$\frac{d[X]}{dt} = \frac{(t)^n}{(t)^n + (0.5)^n} - [X],$$

# A bit of history....

*J. Theoret. Biol.* (1969) **22**, 437–467



## **Metabolic Stability and Epigenesis in Randomly Constructed Genetic Nets**

S. A. KAUFFMAN

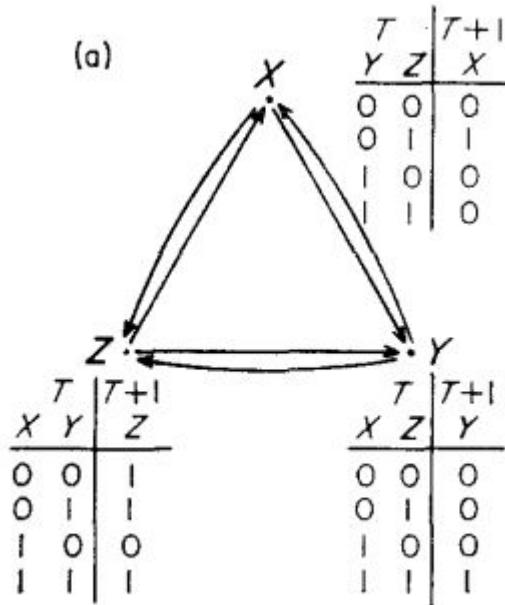
*Department of Anatomy, University of California Medical School,  
San Francisco, California, U.S.A.*

*and*

*Research Laboratory of Electronics, Massachusetts Institute of Technology,  
Cambridge, Massachusetts, U.S.A.†*

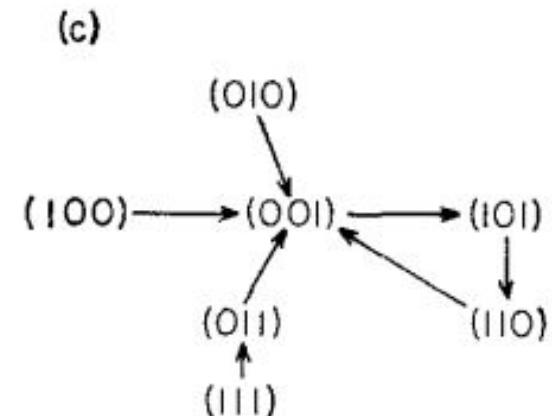
# Metabolic Stability and Epigenesis in Randomly Constructed Genetic Net

S. Kauffman introduced all the elements of Boolean modeling in 1968  
 (Long before the omic revolution and the raise of systems biology)

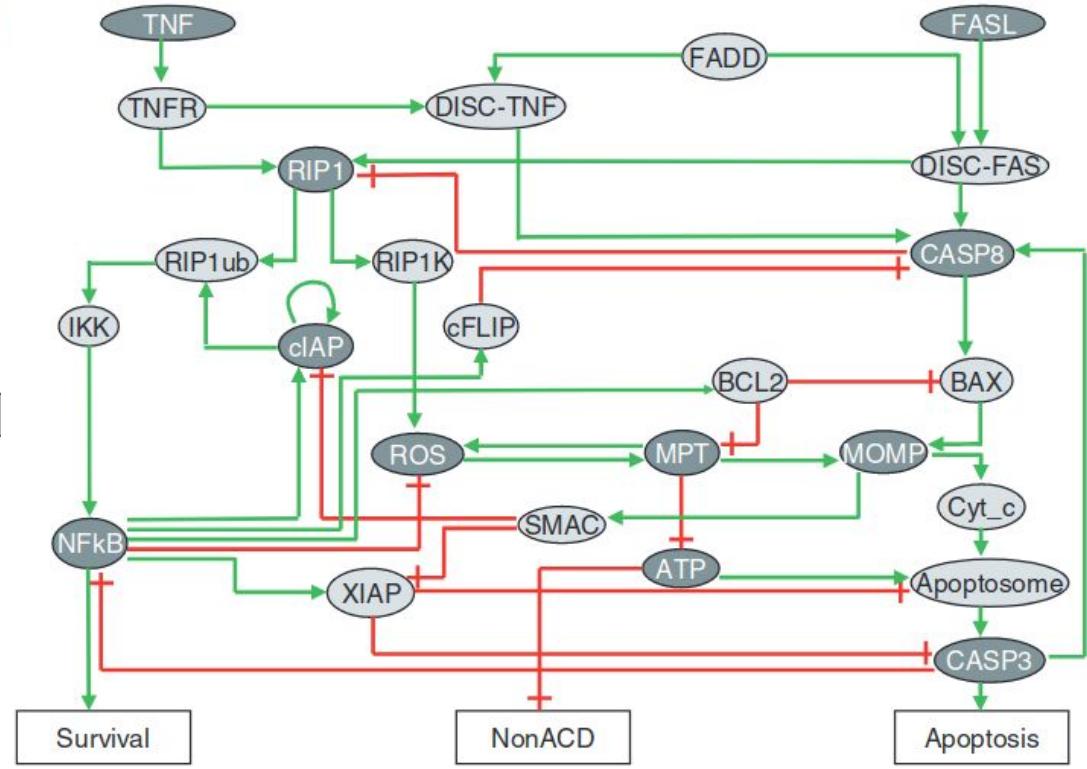
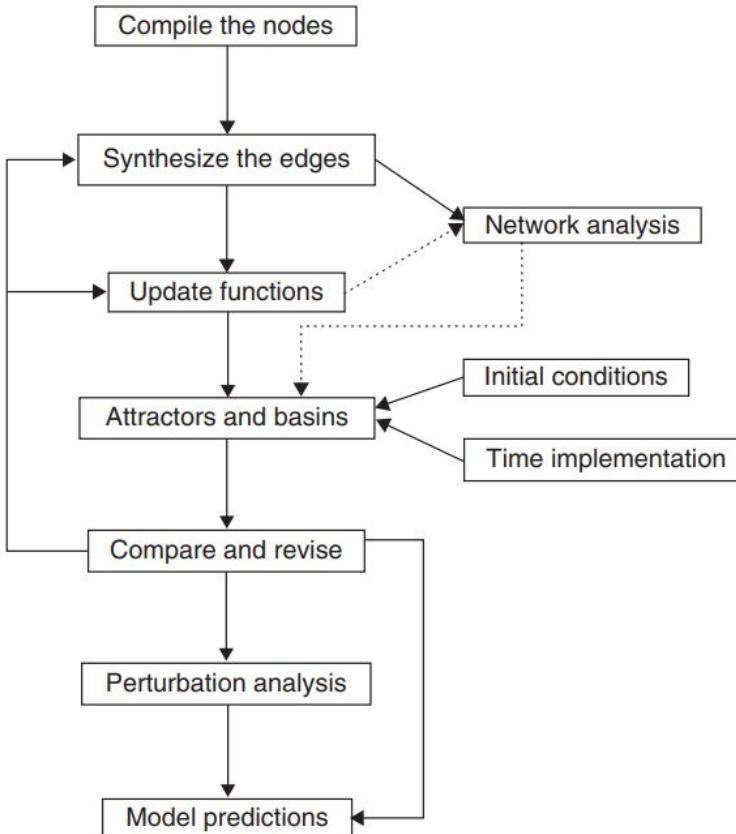


(b)

X	T	Y	Z	X	T+1	Y	Z
0	0	0	0	0	0	1	
0	0	1	1	1	0	1	
0	1	0	0	0	0	0	1
0	1	1	0	0	0	0	1
1	0	0	0	0	0	0	0
1	0	1	1	1	1	1	0
1	1	0	0	0	0	0	0
1	1	1	1	1	0	1	1



# Constructing a boolean dynamic model of a biomolecular interaction network



# Compile Components

It is unfeasible to comprehensively model the dynamics of genome-scale regulatory networks.

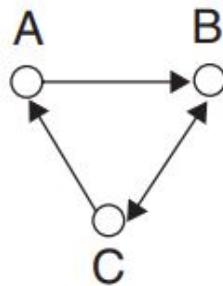
1. Models focus on a single behavior or outcome, e.g., segmentation in *Drosophila melanogaster*, differentiation of T cells.
2. Include all the genes and gene products involved in the relevant outcome or behavior
3. Starts from a core set known from the literature, and expands it by including additional information.

# Determine the Boolean Functions

- Determine the Boolean functions of each node that will depend on the node's regulators indicated in the network. In addition, the function is informed by an interpretation of experimental observations in the literature regarding the node and its regulators
- For nodes with multiple regulators knowledge of the incoming edges (positive and negative regulators) does not uniquely determine the dependency relationships among node states
- When such information is not available, one can construct several variants of the Boolean rules and determine the one that best reproduces the known properties of the real system. If this is not feasible, the OR operator may be used as a default, and the model can be updated once additional information is obtained

# Analyse the Interaction Network

(a) Network



(b) Updating functions

$$B_A = S_C$$

$$B_B = S_A \text{ OR } S_C$$

$$B_C = S_B$$

## Boolnet format

targets, factors

A, C

B, A | C

C, B

(c) Truth tables

$S_C$	$B_A$
0	0
1	1

$S_A$	$S_C$	$B_B$
0	0	0
0	1	1
1	0	1
1	1	1

$S_A$	$B_B$
0	0
1	1

## Boolnet format

targets, factors

G1, G2 & !G3

G2, !G1

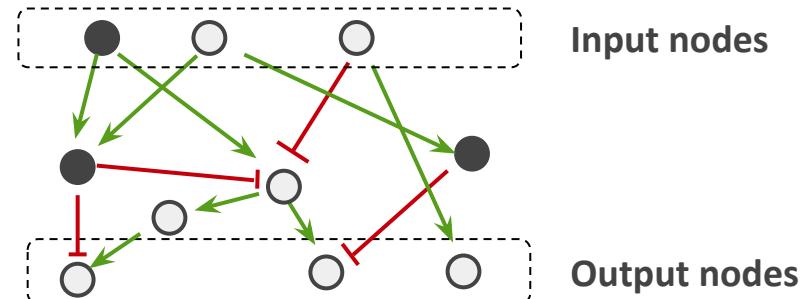
G3, !G3 | G2

# Boolean modelling in a nutshell

## Individual components

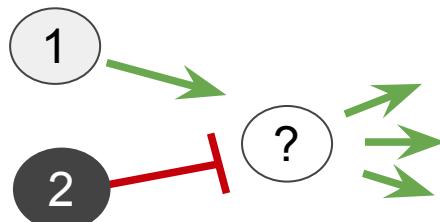
- Protein/complex → Active (1)
- Protein/complex → Inactive (0)
- Activator interaction
- Inhibitory interaction

## Signalling network: structure



## The activation function

A node state at ( $t+1$ ) is updated based on its regulators states at ( $t$ ) and a *logical rule*.



$$x_{t+1}^{(i)} \leftarrow a^{(4)}(x_t^{(1)}, x_t^{(2)})$$

$$x_{t+1}^{(i)} \leftarrow x_t^{(1)} \text{ and not } x_t^{(2)}$$

*Activation function for node i (?)*

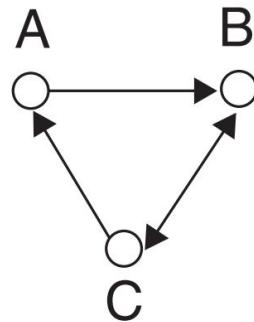
# Choose a Time Implementation

Boolean formalism, synchronous vs. asynchronous strategy

- **Computation of trajectories in the state space:** calculation of sequences of states where each member of the sequence is a logical successor of the previous one
- The **state space  $S$**  of a network with  $N$  nodes is the set  $S$  is and  $N$ -dimensional set  $S = \{0,1\}^N$
- The mathematical definition of the trajectories assumes an **updating rule** for the variables
- Two main strategies are used to simulate Boolean models:
  - **Synchronous:** all variables are updated simultaneously (determinist dynamics).
  - **Asynchronous:** only one component is updated at each time (non-deterministic).
    - i. compute the state  $F(x) = (f_1(x), \dots, f_n(x))$
    - ii. select the indices  $i$  such that  $x_i \neq f_i(x)$
    - iii. for all such indices  $i$ , the state  $(x_1, \dots, f_i(x), \dots, x_N)$  is an asynchronous successor of  $x$

# State Space and State Transition Graph

## Topology



The symbols correspond to the states of the system, indicated in the order A, B, C

Thus, 000 represents:

- SA = 0
- SB = 0
- SC = 0

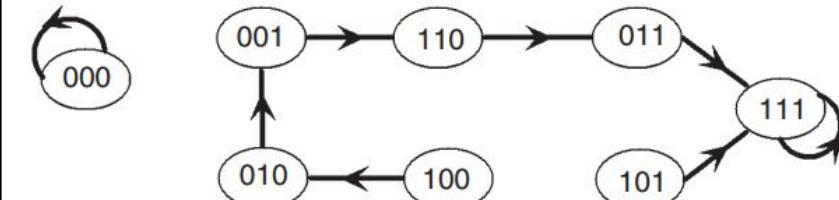
## Boolean functions

$$B_A = S_C$$

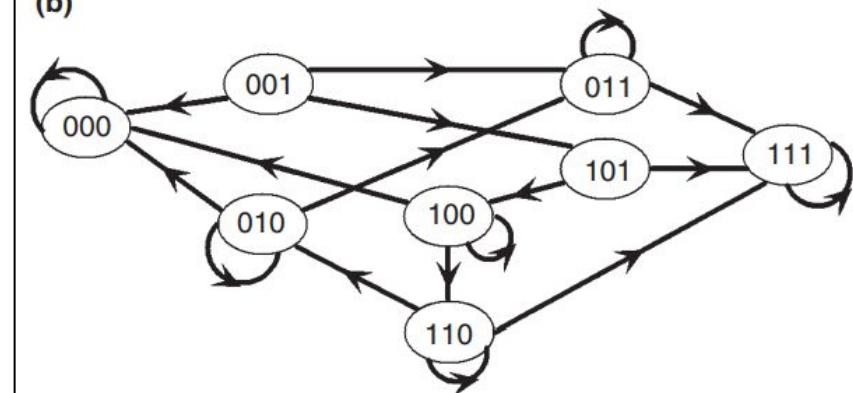
$$B_B = S_A \text{ OR } S_C$$

$$B_C = S_B$$

(a) simultaneously

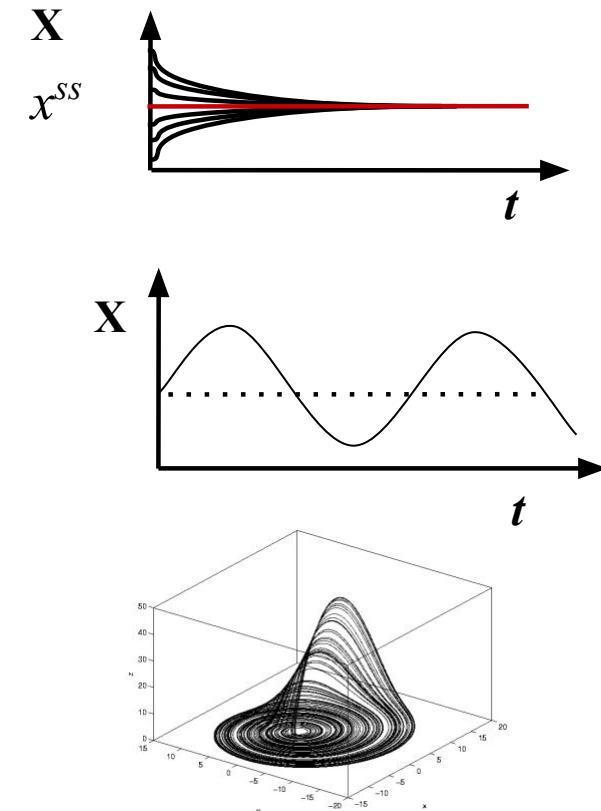


(b)



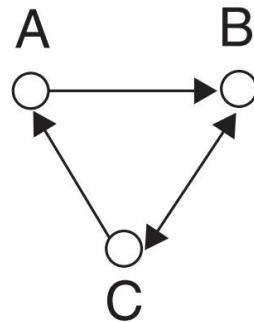
# Dynamical analysis

- **Steady-state** → dynamic concept
  - Fixed point (variables do not change)
  - Structural stability
- **Oscillations** → cyclic behavior
  - Period (variables oscillate)
- **Complex behavior** → Chaos
  - Complex attractors
  - Sensitivity to initial conditions
  - Unpredictability (butterfly effect)



# Determine the Initial Condition

## Topology



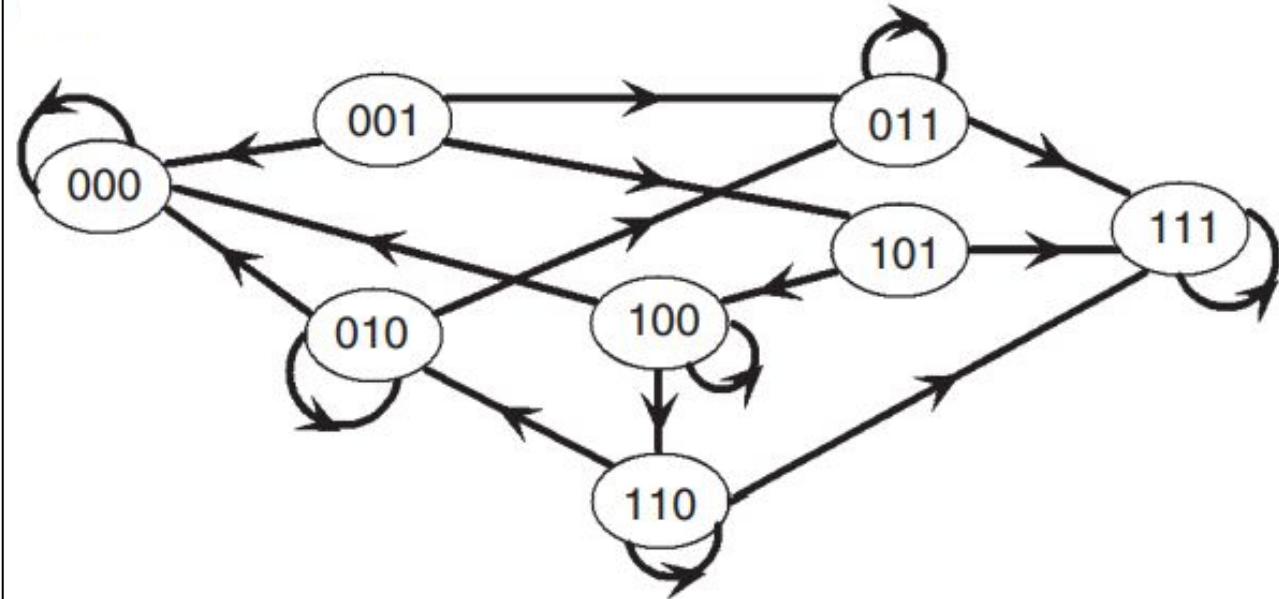
## Boolean functions

$$B_A = S_C$$

$$B_B = S_A \text{ OR } S_C$$

$$B_C = S_B$$

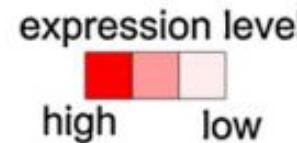
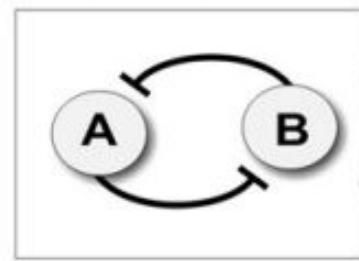
## State Transition Graph



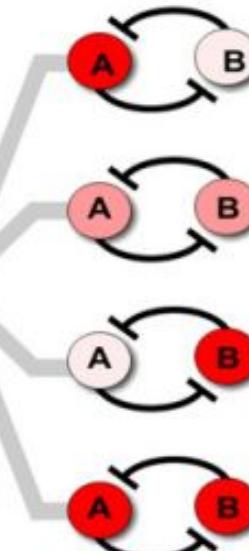
# Attractor Analysis

## SMALL CIRCUIT (2-gene genome)

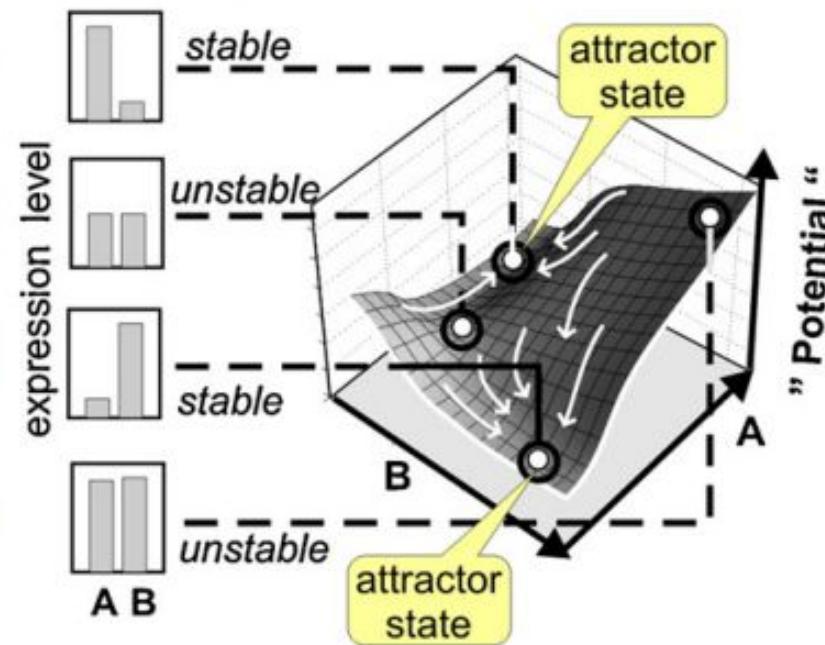
Architecture  
("wiring diagram")



network states  
= gene expression profiles



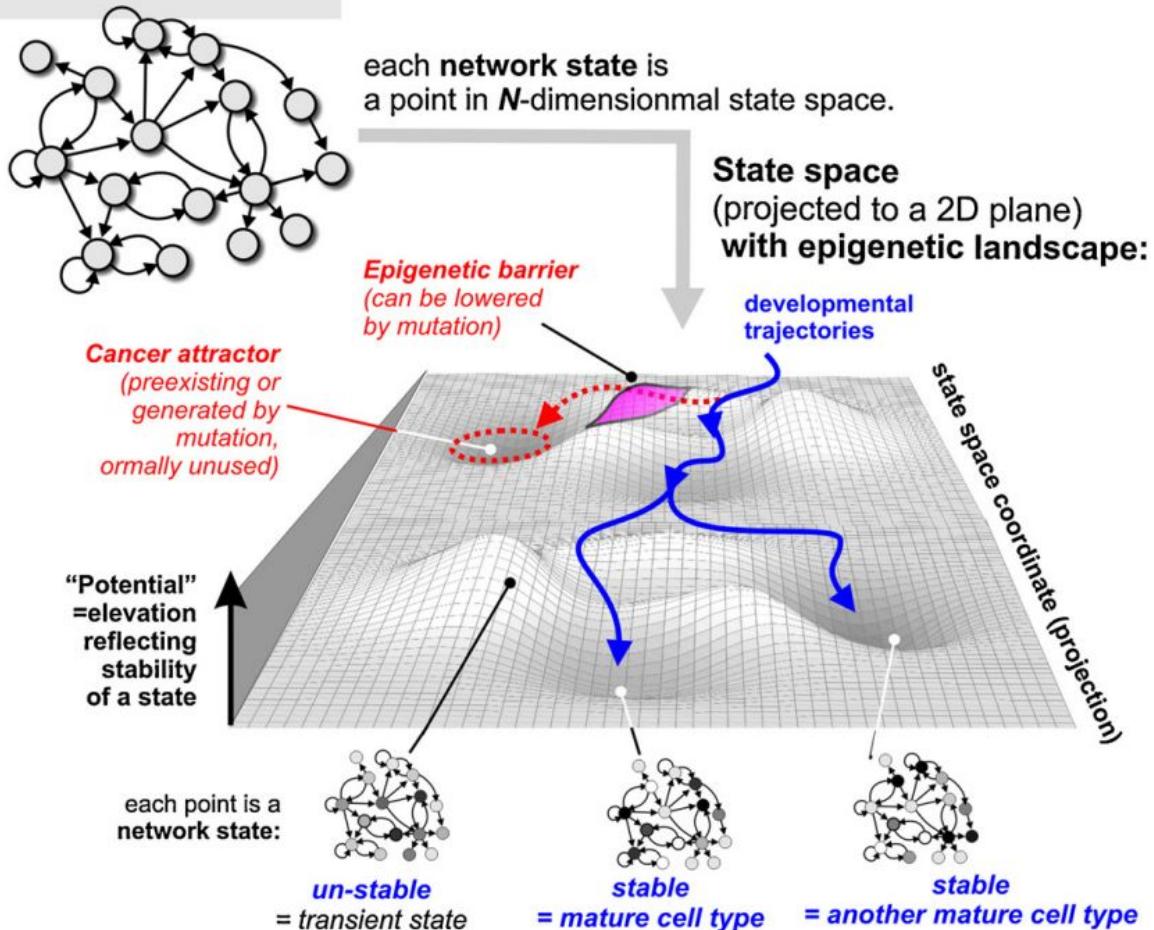
state space  
with  
epigenetic landscape



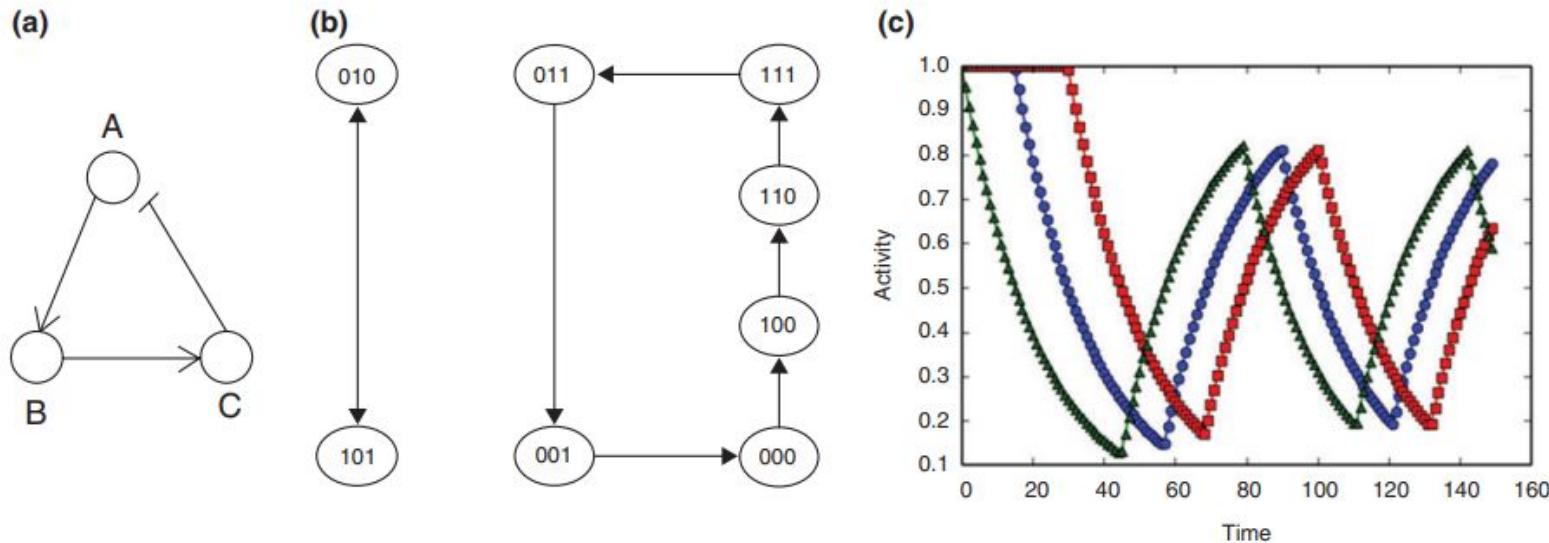
## COMPLEX NETWORK

( $N$  gene genome)

# Attractor Analysis



# From boolean to more quantitative modeling frameworks



**FIGURE 6 |** Illustration of the dynamics of a piecewise linear model. (a) The directed network associated with the model forms a negative feedback loop. The Boolean update functions are completely determined by the network. (b) The state transition graph of a synchronous Boolean model of the network. The symbols correspond to the states of the system indicated in the order ABC. The Boolean model has two limit cycle attractors (sustained oscillations). (c) The time-course of the continuous variables associated with nodes A (green triangles), B (blue circles), and C (red squares) according to a piecewise linear model with  $\gamma = 1$  and  $\theta = 0.5$ . The sustained oscillations agree with the six-state limit cycle of the synchronous Boolean model.

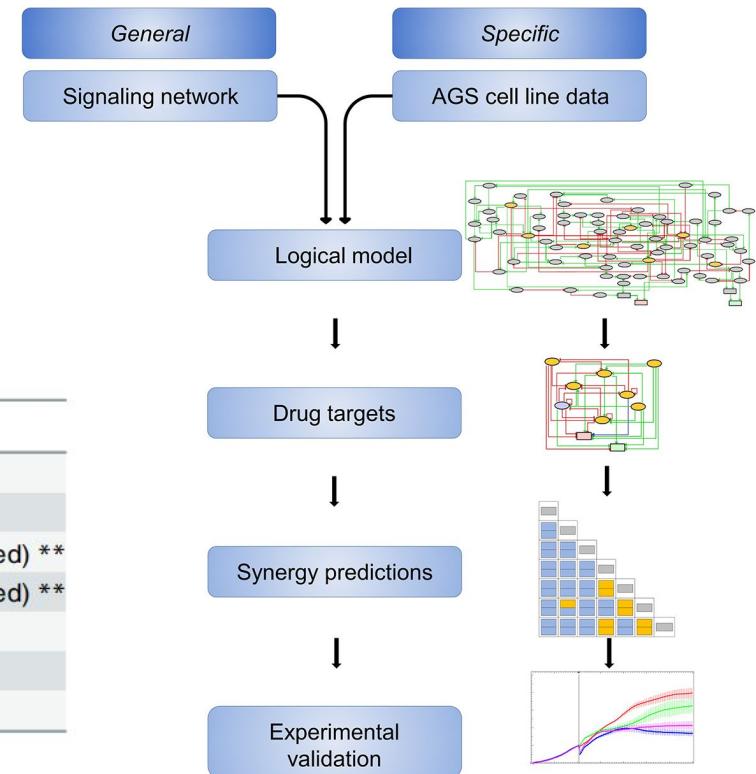
# Applications to cancer treatment

*Discovery of Drug Synergies in Gastric Cancer Cells Predicted by Logical Modeling*

# Discovery of Drug Synergies in Gastric Cancer Cells Predicted by Logical Modeling

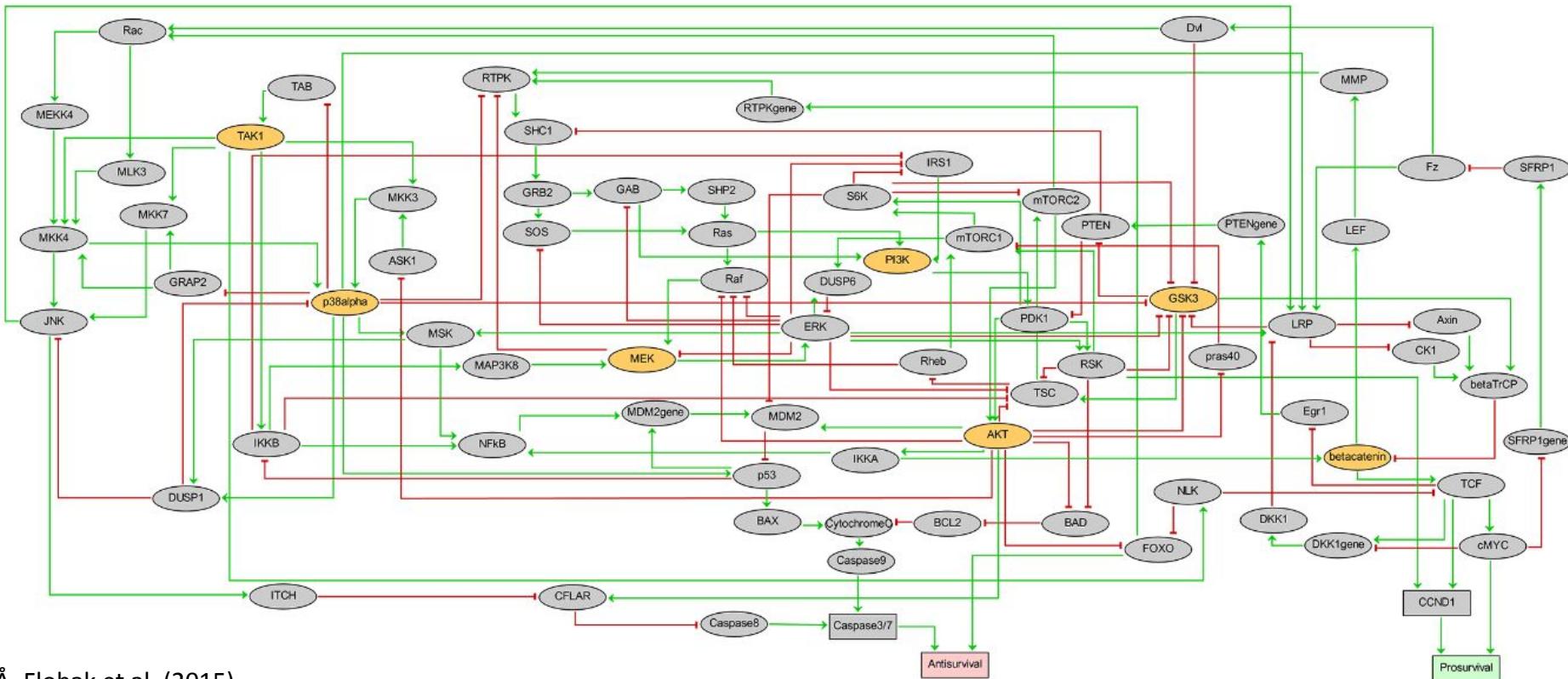
<b>Chemical inhibitor</b>	<b>Target name</b>
(5Z)-7-oxozeanol	TAK1
AKTi-1,2 (AKT inhibitor VIII)	AKT1/2
BIRB0796	p38 MAPK
CT99021	GSK3
PD0325901	MEK
PI103	PI3K
PKF118-310	$\beta$ -catenin

<b>Chemical inhibitor</b>	<b>Target HGNC symbol</b>	<b>GI50*</b>
(5Z)-7-oxozeanol	MAP3K7	0.5 $\mu$ M
AKTi-1,2 (AKT inhibitor VIII)	AKT1, AKT2	10 $\mu$ M
BIRB0796	MAPK14	N/A (5 $\mu$ M used) **
CT99021	GSK3A, GSK3B	N/A (5 $\mu$ M used) **
PD0325901	MAP2K1, MAP2K2	35 nM
PI103	PIK3CA	0.7 $\mu$ M
PKF118-310	CTNNB1	150 nM



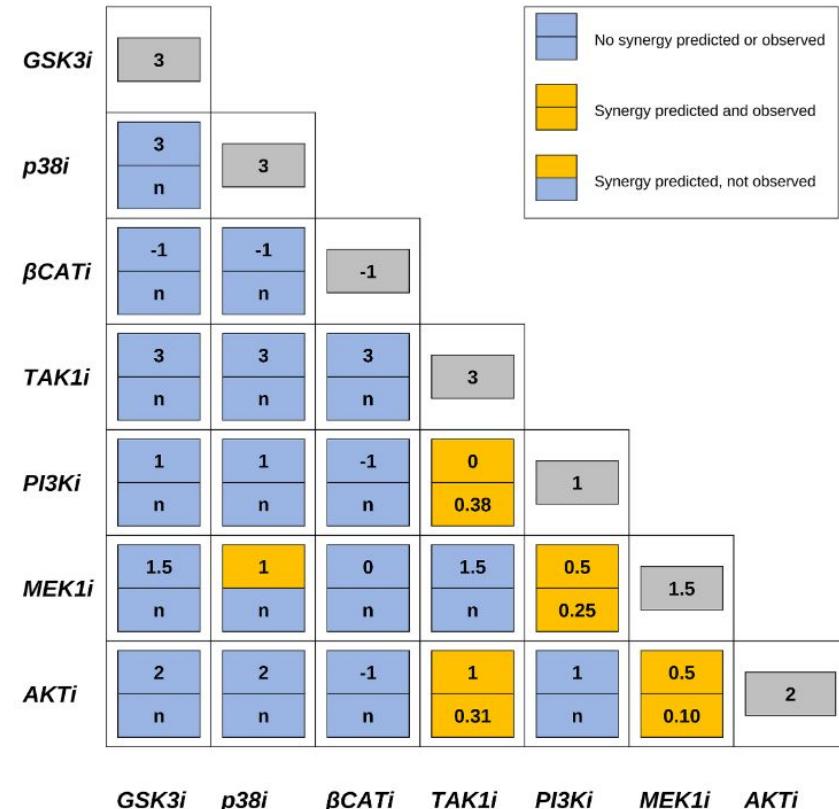
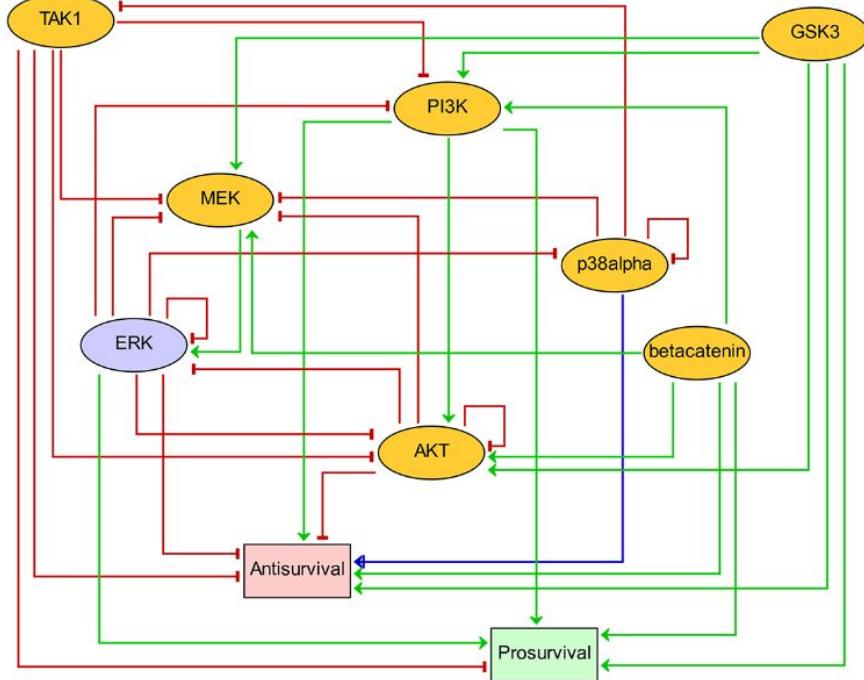
# Applications to cancer treatment

*Discovery of Drug Synergies in Gastric Cancer Cells Predicted by Logical Modeling*

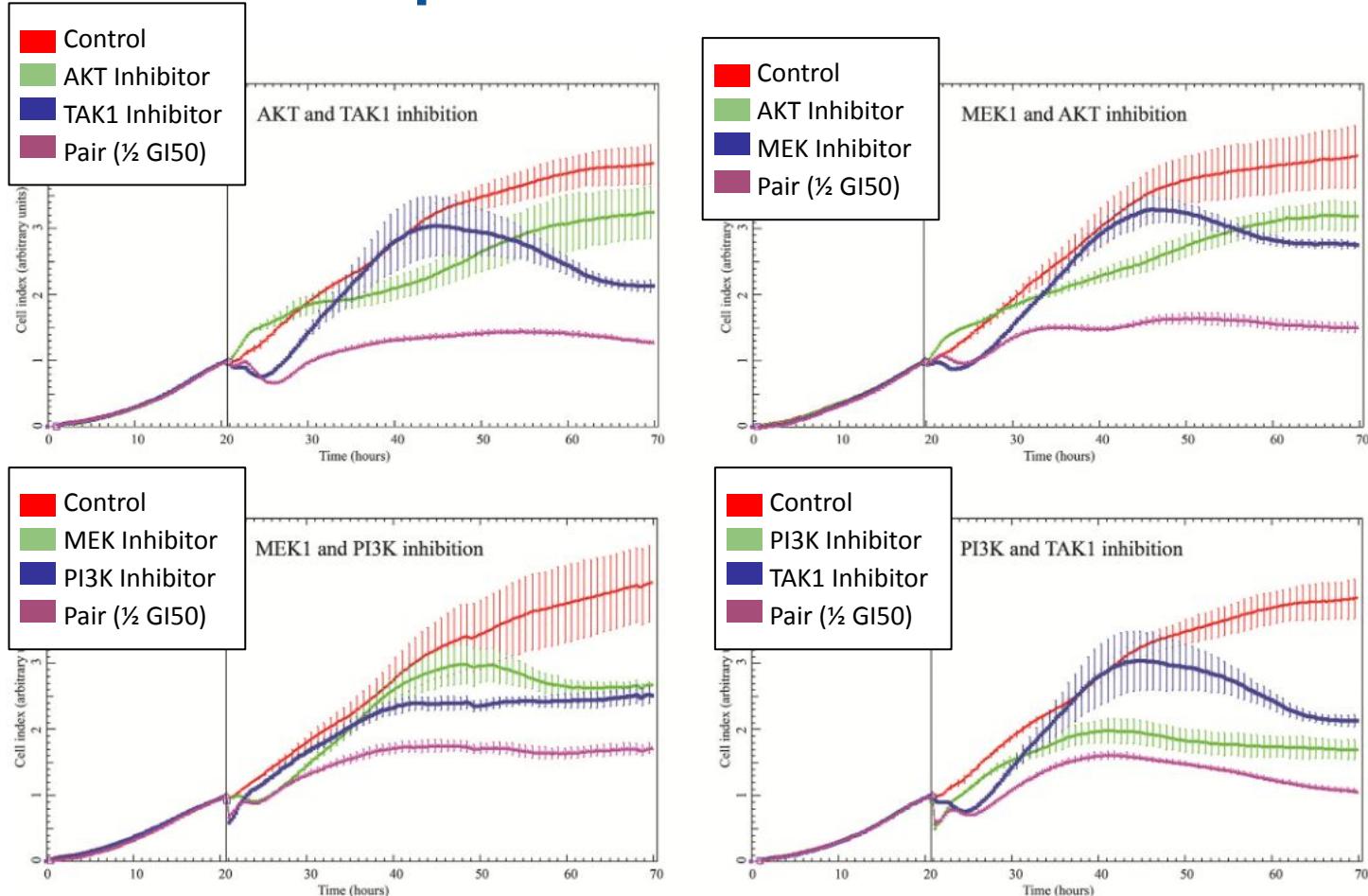


# Applications to cancer treatment

## Discovery of Drug Synergies in Gastric Cancer Cells Predicted by Logical Modeling



# Experimental validation



# Applications to cancer treatment

*Discovery of Drug Synergies in Gastric Cancer Cells Predicted by Logical Modeling*

- Four of the five synergies predicted by the logical model were confirmed experimentally, with CI values well below 0.5, which indicates strong synergy
- Synergies of PI3K-MEK or AKT-MEK inhibitions have already been observed in a variety of tumor cells providing further evidence for the predictions.
- The model-based suggestion that FOXO activation may be important for synergistic growth inhibition does find experimental support in numerous accounts of FOXO
- the dynamical behavior of the logical model recapitulates generic properties that may be relevant for a range of different tumor types.

# Final remarks

- Although Boolean network models have a limited capacity to describe the quantitative characteristics of dynamic systems, they do exhibit considerable dynamic richness
- Boolean models do not require the knowledge of kinetic parameters
- BM were proven effective in describing the qualitative behaviors of biological systems
- BM were proven successful in predicting the key components of signal transduction and gene regulatory networks (drug synergy effects)
- In practice, qualitative and quantitative models are complementary
- Boolean networks can serve as a foundation of modeling regulatory and signaling networks on which more detailed continuous models can be built as kinetic information and quantitative experimental data become available

# References

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