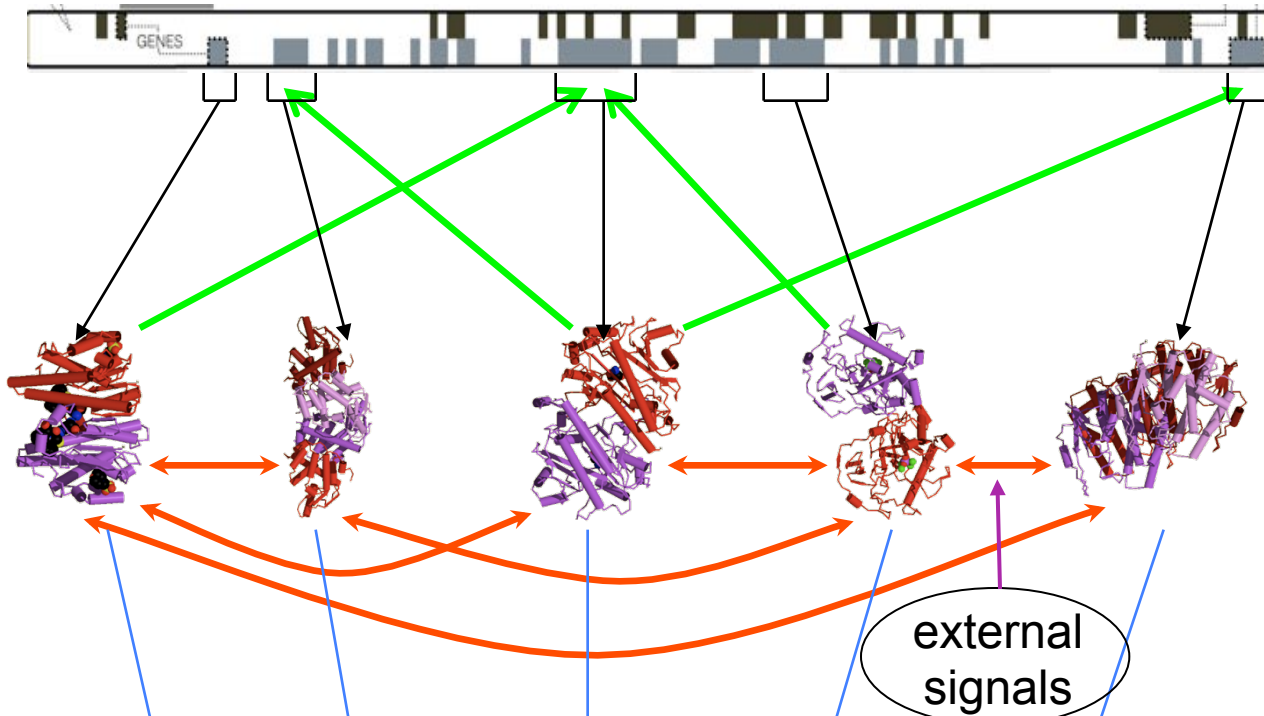


# Network analysis and discrete dynamic modeling elucidates the outcomes of within-cell networks

Réka Albert

Departments of Physics and Biology  
Huck Institutes for the Life Sciences  
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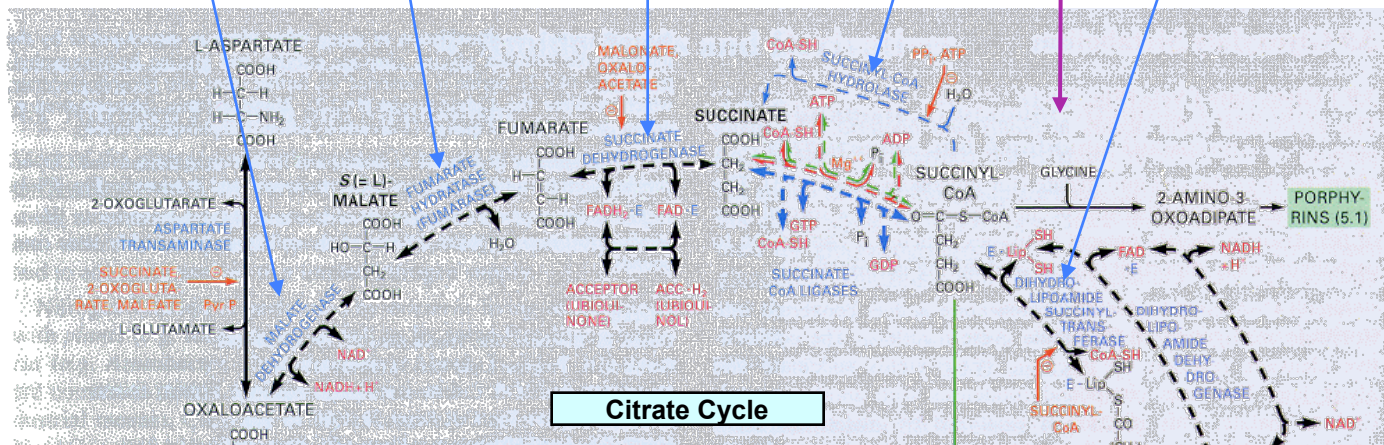
# Interaction networks within cells



gene regulatory network

protein-protein interaction network

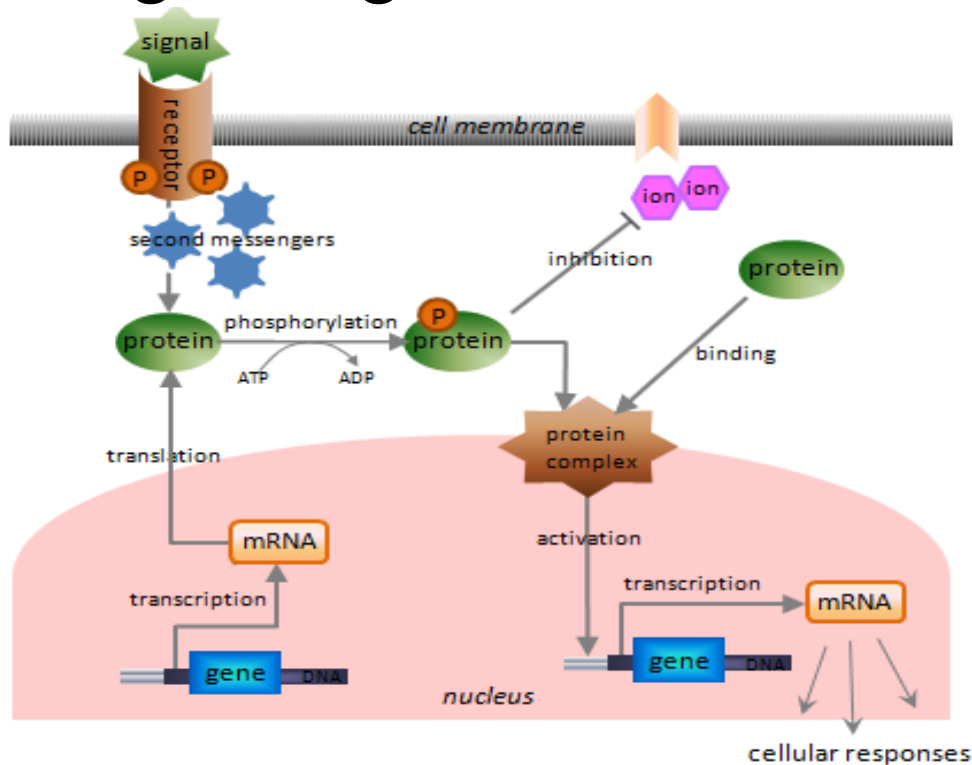
signal transduction network



biochemical reaction network

Hawoong Jeong

# Signaling networks are the most diverse



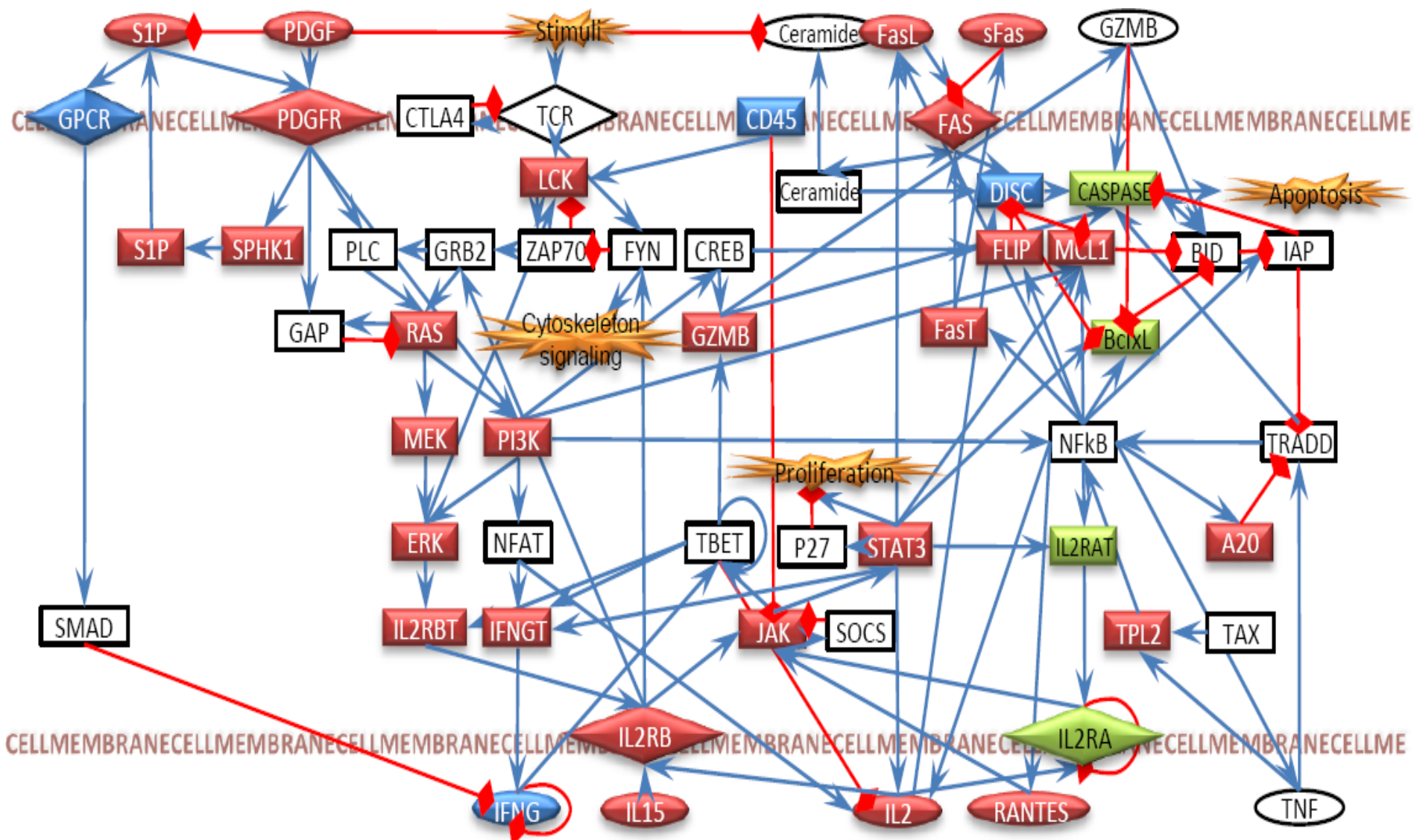
- nodes: molecular species
- edges: interactions, biochemical reactions
- directed and **signed** (positive or negative) edges

Information starting from outside of the cell propagates through the network and leads to certain outcomes.

Who can interact with whom is bounded by the laws of physics and chemistry.

But the interactions are usually not known at the single reaction level.

# T cell survival signaling network: three signals, three outcomes



Rectangle: intracellular; ellipse: extracellular; diamond: receptor.

Upregulated, downregulated, deregulated node; Activation, inhibition edge

# From within-cell networks to cellular outcomes

Cell motion, proliferation, programmed cell death are regulated through signal transduction, gene regulatory or metabolic networks.

Wrong cellular behavior often leads to disease. Conversely, stem cells and cell reprogramming offer the hope of curative therapies.

We connect within-cell networks to cell behavior through dynamic modeling. Each component is characterized with a state and with a regulatory function that connects the state of its regulators to its own future state.

The model can be used to describe the system's behavior, e.g. its steady states and complex attractors.

The attracting states of a subset of the nodes can be related to cell behavior.

# A parsimonious dynamic modeling approach: asynchronous Boolean modeling

Main assumption: components have two main states: ON (1) or OFF (0)

The future state of a regulated node (the output) depends on the current state of its regulators in the network (inputs), and may depend on its own current state.

The input-output relationships (transition functions) are described by Boolean logic.

Time is discretized into time steps. A randomly selected node is update at each step.

Starting from an initial condition, the system's state vector changes in time and eventually settles down into an attractor (a steady state or a complex attractor).

<i>In1</i>	<i>In2</i>	<i>Out</i>
0	0	0
0	1	1
1	0	1
1	1	1

$$Out = In1 \text{ OR } In2$$

# A few examples of Boolean and logical models in biology

Pioneers: S. Kauffman, R. Thomas, L. Glass

Cell & organ development

Sanchez & Thieffry, J Theor Biol 2003

Espinosa-Soto et al. Plant Cell 2004

Mendoza, Biosystems 2006

Naldi et al. PLoS Comput. Biol. 2010

The cell cycle

Li et al. PNAS 2004

Fauré et al. Bioinformatics 2006

Signaling networks

Sahin et al. BMC Syst Biol 2009

Samaga et al. PLoS Comput Biol 2009

Shlatter et al. PLoS Comput. Biol. 2009

Calzone et al. PLoS Comput. Biol 2010

Espinal et al. PLoS ONE 2011

Mbodj et al. Mol. Biosystems 2013

Grieco et al. PLOS Comput. Biol. 2013

Host-pathogen interactions

Franke et al. BMC Syst Biol 2008

# Examples of logical models by our group

## Drosophila embryonic development:

Albert & Othmer, J Theor Biol 2003

Chaves et al. J Theor Biol 2005, IEE Proc Sys Biol 2006,

Chaves & Albert J R S Interface 2008

## Signaling in plants (with Sally Assmann):

Li, Assmann, Albert, PLoS Biology 2006

Saadatpour et al., J Theor Biol 2010

Sun, Jin, Albert, Assmann, PLOS Comput Biol 2014

## Host-pathogen interactions (with Eric Harvill, Isabella Cattadori):

Thakar et al. PLoS Comput Biol 2007, J R S Interface 2009, PLoS Comput. Biol 2012

## T cell survival signaling (with Thomas Loughran):

Zhang et al PNAS 2008, Saadatpour et al PLoS Comp Biol 2011

## Epithelial to mesenchymal transition in liver cancer (with Thomas Loughran):

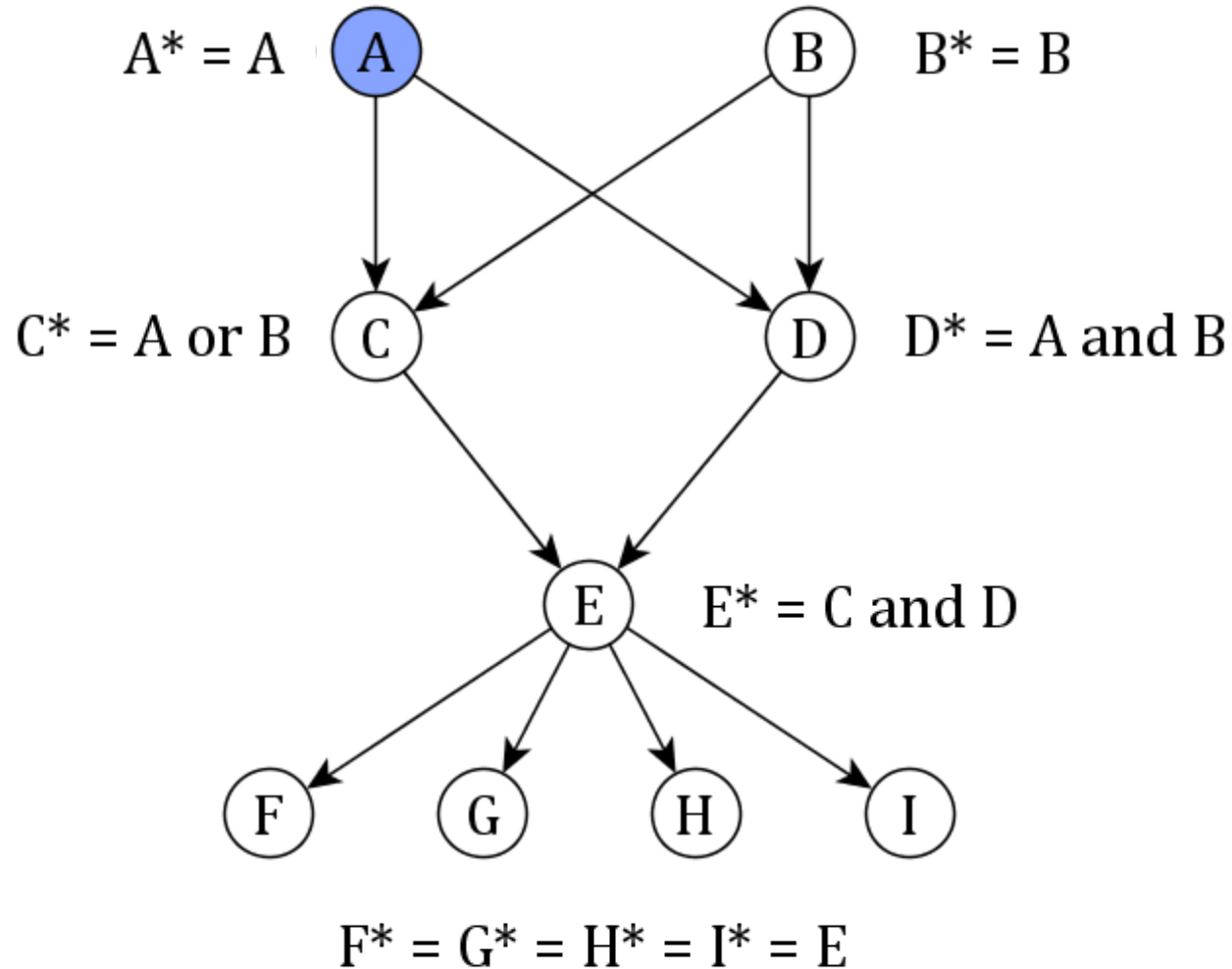
Steinway et al, Cancer Research 2014

## Plant-pollinator communities (with Katriona Shea):

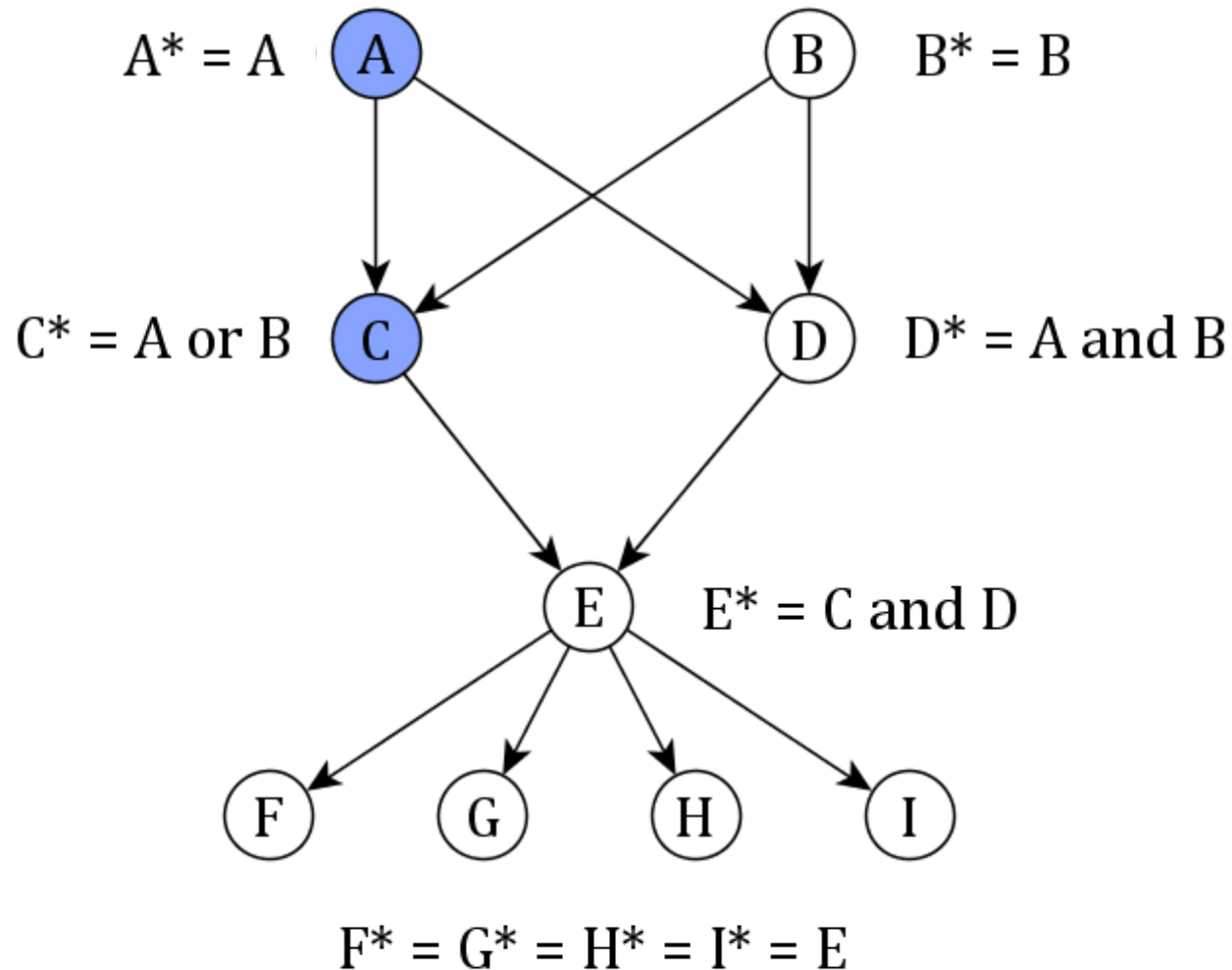
Campbell et al PNAS 2011, PRE 2012, Theor Ecol 2013, Theor Ecol 2014



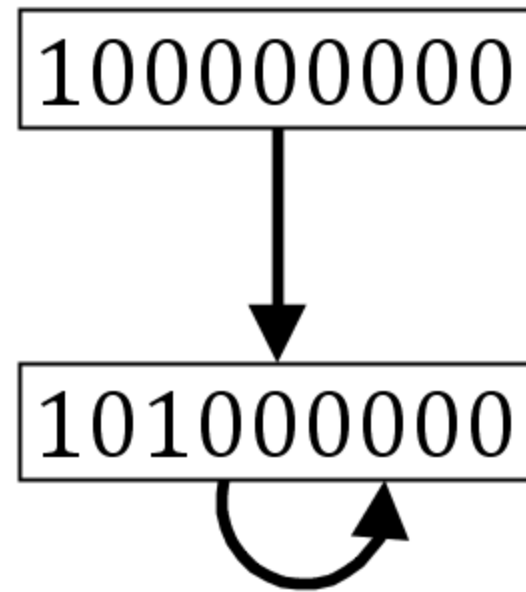
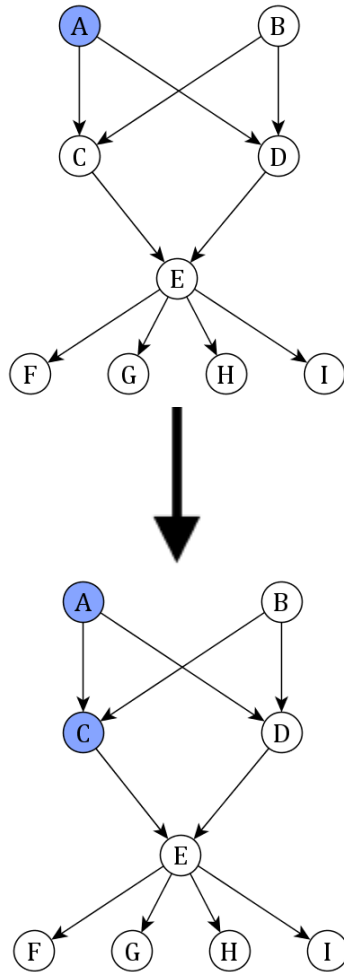
# Example of signal propagation



# Example of signal propagation



The network of transitions among all network states summarizes the dynamics

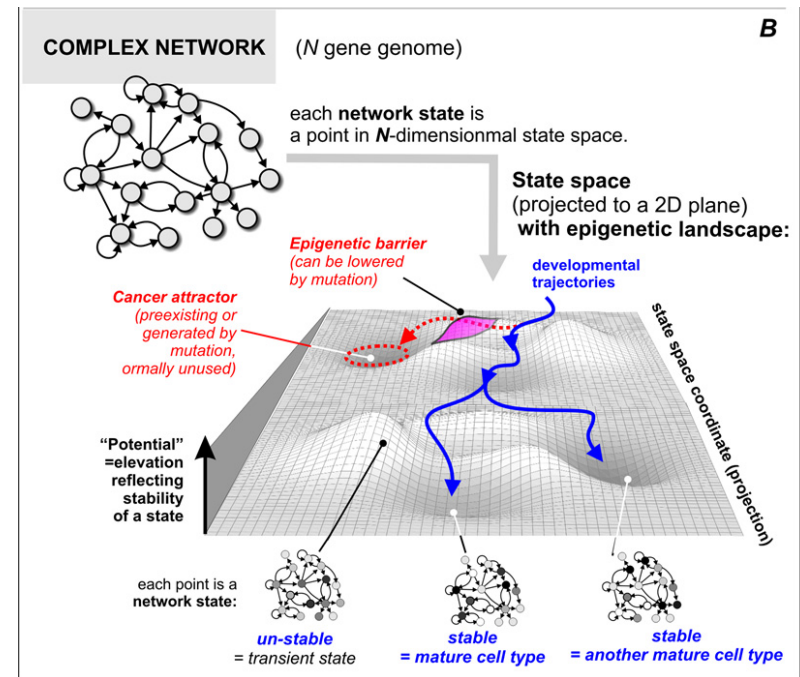
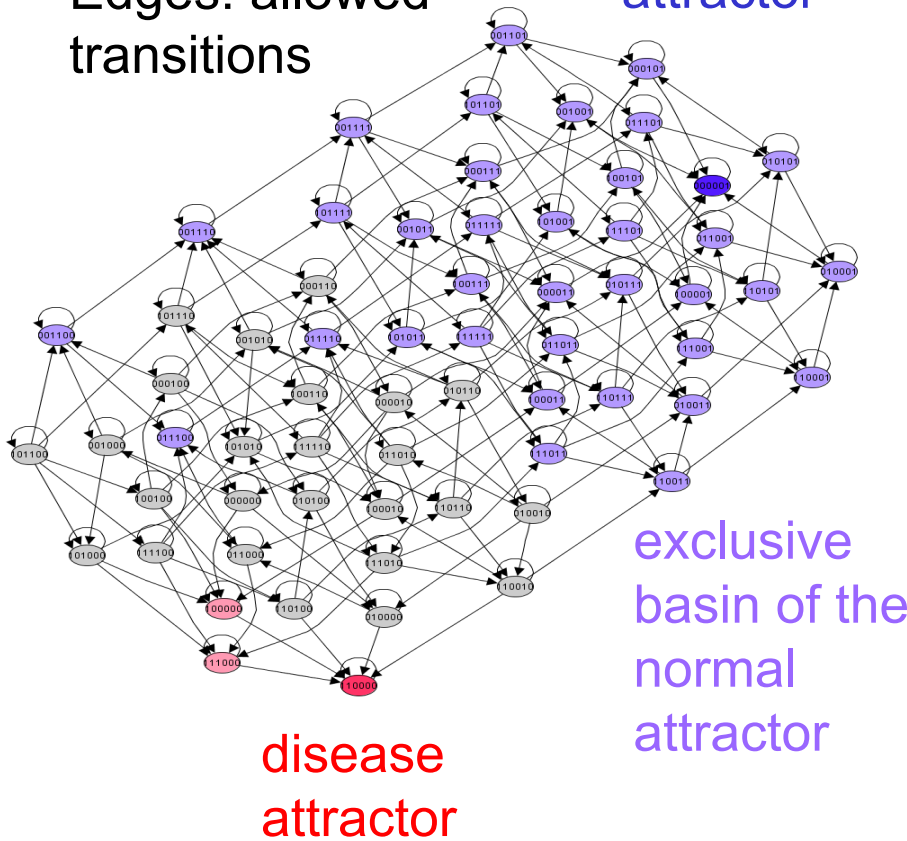


Nodes: states of the system ( $2^N$ )  
Edges: allowed transitions

# A Boolean model's state transition network is a potential embodiment of the epigenetic landscape

Nodes: states  
Edges: allowed transitions

normal  
attractor



Huang, Ernberg, Kauffmann, Sem. Cell & Dev. Biol. 2009

# Steps in constructing a Boolean model of a regulatory or signaling network

1. Input to the model: components; interactions; binary states of components in certain known conditions
2. Construct the interaction network
3. Determine the Boolean update functions for each node. When there are multiple regulators, select the function that best represents the existing knowledge about their combinatorial action.
4. Determine the relevant initial condition(s)
5. Choose a time implementation (update schedule)
6. Analyze the model:
  - determine the attractors,
  - determine the effects of node perturbations on the attractors
7. Compare with known behavior. If there are discrepancies, revise the network and/or the Boolean update functions
8. Use the model to make novel predictions.

# From modeling disease-related signal transduction networks to their control

- Many diseases involve deregulation of the signal transduction networks governing cell proliferation, programmed cell death etc.
- The relevant attractor is the state of a few designated output nodes
- Boolean dynamic modeling provides a practical and useful representation
- Mutations can be represented as stuck-at-zero faults (loss of function mutation), stuck-at-one faults (constitutive expression or activity) or bridging faults (changes in interactions)
- Faults that change the output attractors can be identified
- Interventions that reverse or ameliorate the effect of faults can be proposed.

# Example: Modeling T cell survival

**Phenomenon:** survival of cytotoxic T cells in T-LGL leukemia

**Constructed:** survival signaling network inside T- LGL cells

**Hypotheses:** two protein/mRNA states, timing sampled stochastically

**Validation:** reproduces known deregulations and known key mediators

**Predicts:** minimal initial condition necessary for T-LGL state

10 new manipulations that ensure apoptosis of T-LGL cells

12 additional deregulations

Several predictions were validated experimentally

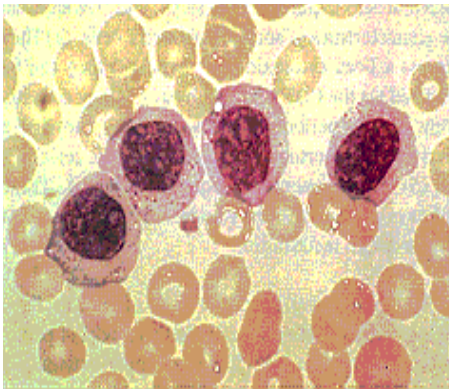
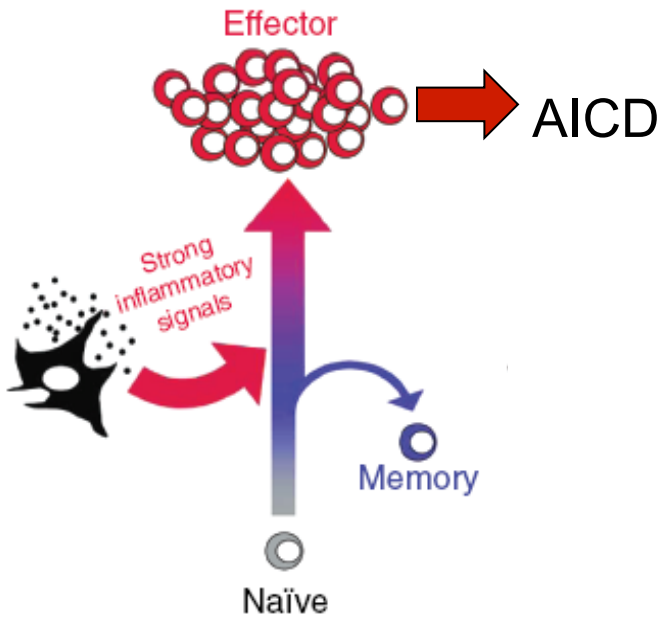
**Implications:** identifying therapeutic targets for T-LGL leukemia

tumor and cancer vaccine development

R Zhang, MV Shah, J Yang, SB Nyland, X Liu, JK Yun, R Albert, TP Loughran, PNAS (2008).

A Saadatpour, RS Wang, A. Liao, X Liu, TP Loughran, I. Albert, R. Albert, PLoS Comp Biol (2011)

# LGL leukemia and activation induced cell death



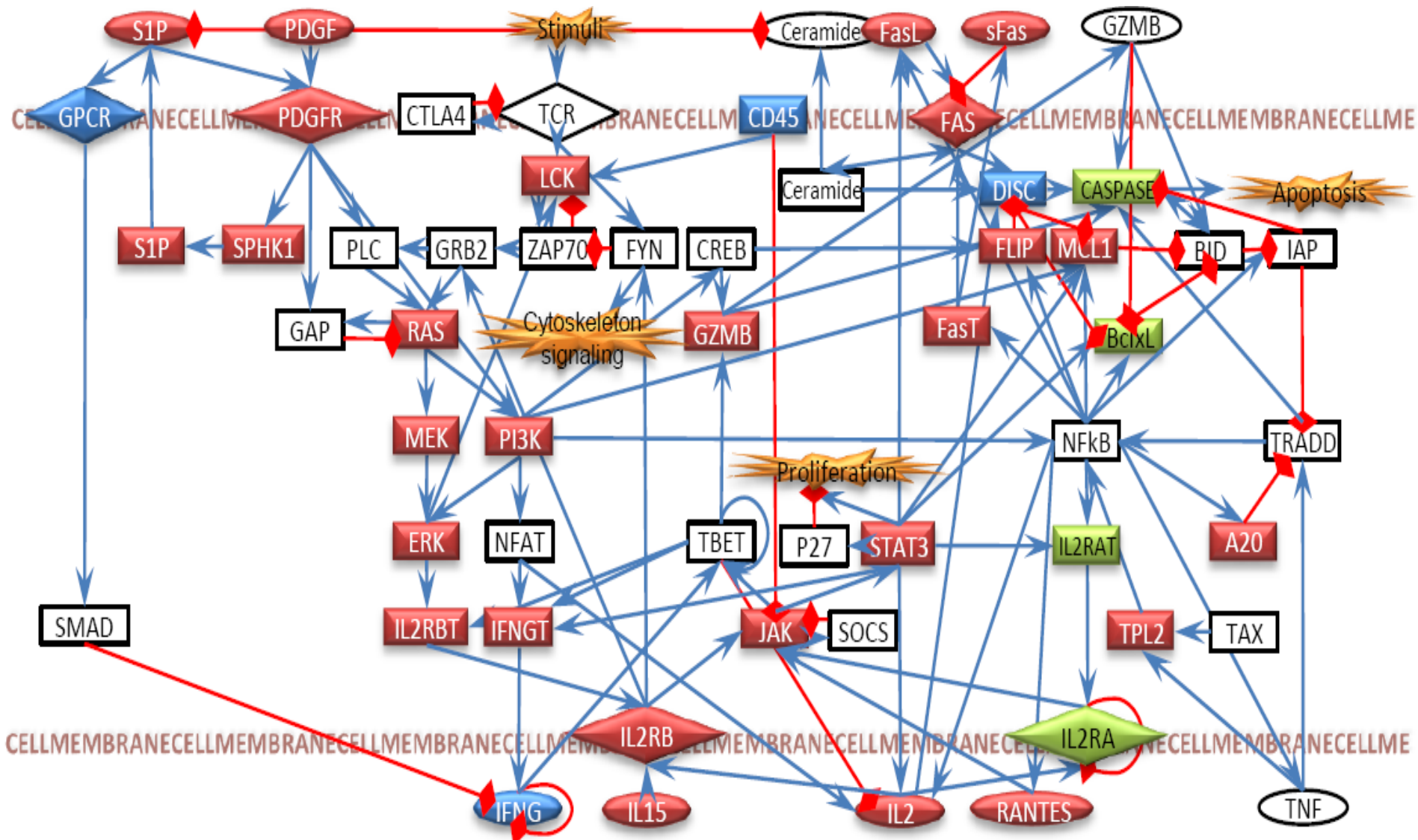
- **cytotoxic T cells** eliminate infected cells and tumor cells
- The majority of activated T cells undergo activation induced cell death (AICD)
- T-LGL: abnormal expansion and survival of active T cells
- Activation of multiple survival pathways (MAPK; JAK-STAT)
- Several other proteins have abnormal levels/activity (are deregulated)
- No known curative therapy

T. Lamy & Thomas P. Loughran, 1998, Cancer Control

Hypothesis: T-LGL is caused by the deregulation of the signaling network responsible for AICD.



# T-LGL CTL survival signaling network



Rectangle: intracellular; ellipse: extracellular; diamond: receptor.

Upregulated, downregulated, deregulated node; Activation, inhibition edge

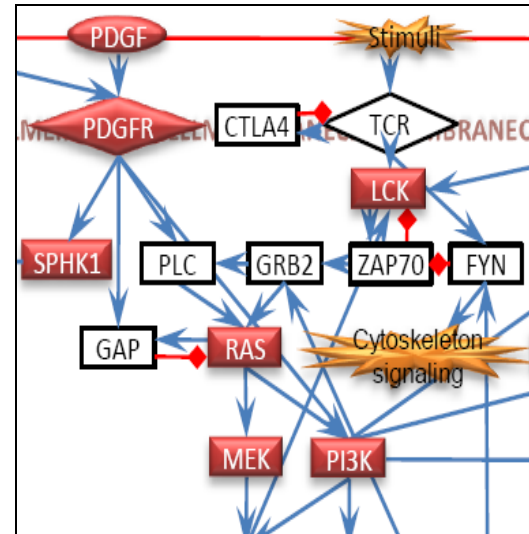
# Stochastic Boolean dynamic model

Two states: ON, OFF

- Inhibitors NOT
- Conditional activation AND
- Independent activation OR

$PI3K^* = PDGFR \text{ OR } RAS$

$TCR^* = \text{Stimuli AND NOT CTLA4}$



Initial state characteristic to resting T cells + Stimuli = ON + ?  
(unknown number of “driver” deregulations)

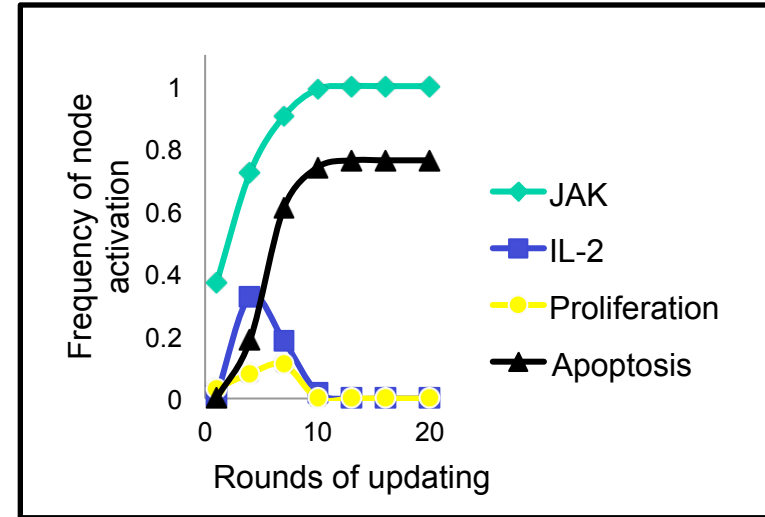
- Stochastic asynchronous update
- Multiple (>300) replicate simulations for each initial condition
- Simulation ends when Apoptosis (cell death) is activated.
- Output of the model: the frequency of node activation among simulations that are still “alive”

# Minimum condition to reproduce a T-LGL-like state

Two attractors:

Normal: Apoptosis=ON (cell death)

T-LGL: Apoptosis=OFF, known deregulated states for other nodes



Minimum condition:

IL-15 constantly ON, PDGF intermittently ON, Stimuli initially ON.

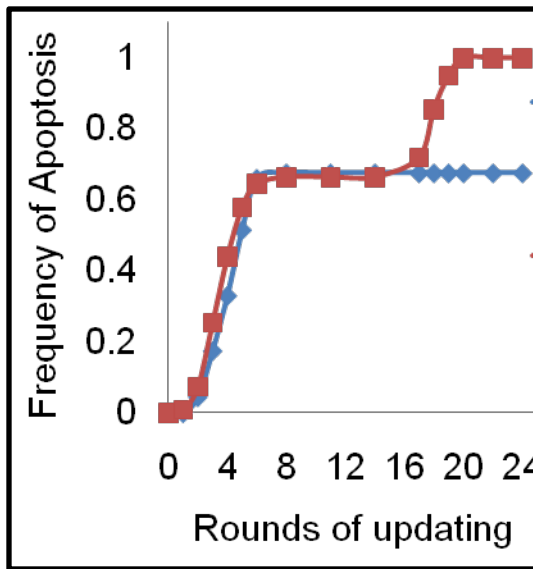
No mutations necessary, it may all be in the environment.

Provision of IL-15 and PDGF may generate long-lived T cells.

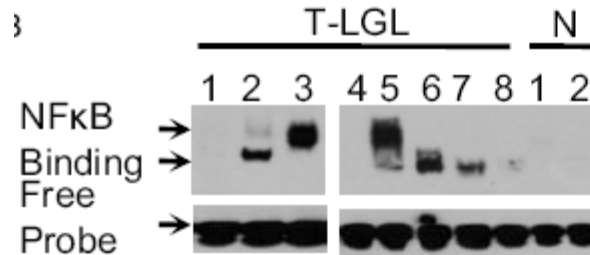
R Zhang, MV Shah, J Yang, SB Nyland, X Liu, JK Yun, R Albert, TP Loughran, PNAS (2008).

# Manipulations that reverse T-LGL survival

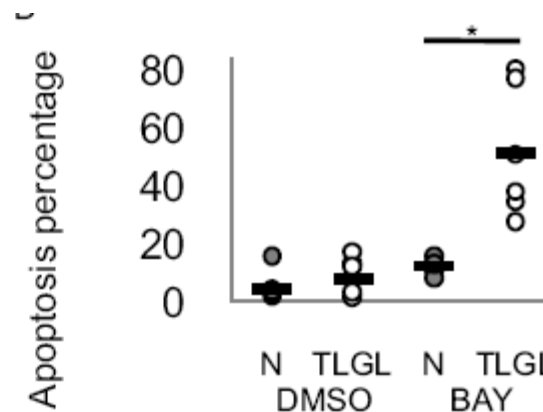
- Assume IL-15, PDGF present
- Permanently reverse the T-LGL state of a single node
- Candidate therapeutic target: altering its state causes apoptosis in all simulations
- Newly identified: **SPHK1, NFκB, S1P, SOCS, GAP, BID and IL2RB**



Model: NFκB stabilizes at ON, setting it OFF causes **total apoptosis**



Validation: NFκB constitutively active in T-LGL



Validation: NFκB inhibition induces apoptosis in T-LGL

# Two ways forward

The analysis so far was simulation-based and looked at specific initial conditions (deregulations).

To explore all trajectories of the system, we need to determine its state transition network, but it is too big ( $2^{60}$  states).

1. Simplify the network without modifying its attractor repertoire

[A. Sadatpour, R. Albert, T. Reluga, SIAM J Appl Dyn Syst \(2013\)](#)

2. Integrate the regulation into the network and analyze this expanded network. This analysis predicts the key mediators and the model's dynamic repertoire

[R.S. Wang and R. Albert, BMC Systems Biology 5, 44 \(2011\)](#)

[J.G.T Zañudo and R. Albert, Chaos 23, 025111 \(2013\).](#)

# Network simplification enables state space analysis

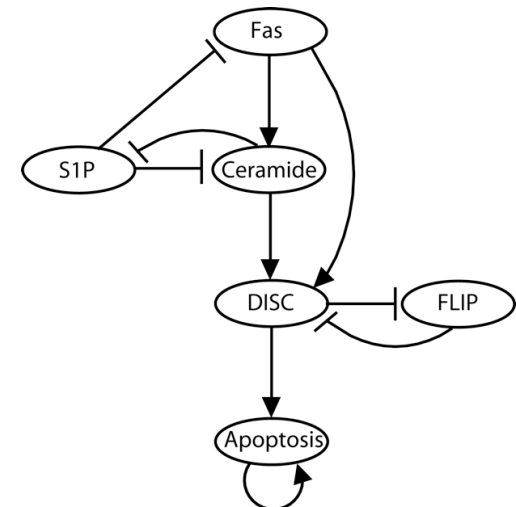
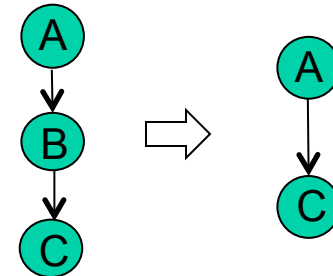
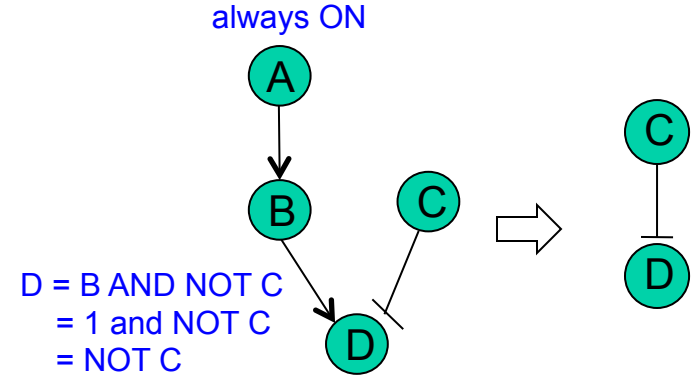
(i) Determine and eliminate the nodes whose states stabilize due to their regulation by sustained signals.

(ii) Iteratively collapse nodes with one incoming and/or one outgoing edge.

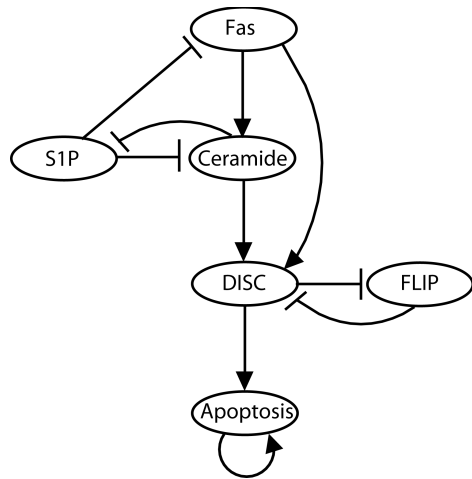
This network reduction is proven to preserve attractors (fixed points and complex attractors) for general asynchronous update.

A. Sadatpour, R. Albert, T. Reluga, SIAM J Appl Dyn Syst 2013

In the T-LGL network removing stabilized nodes due to sustained Stimuli, IL-15 and PDGF, then collapsing simple mediator nodes leads to a reduction of 90%.

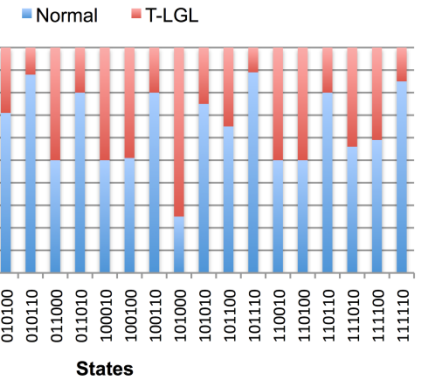
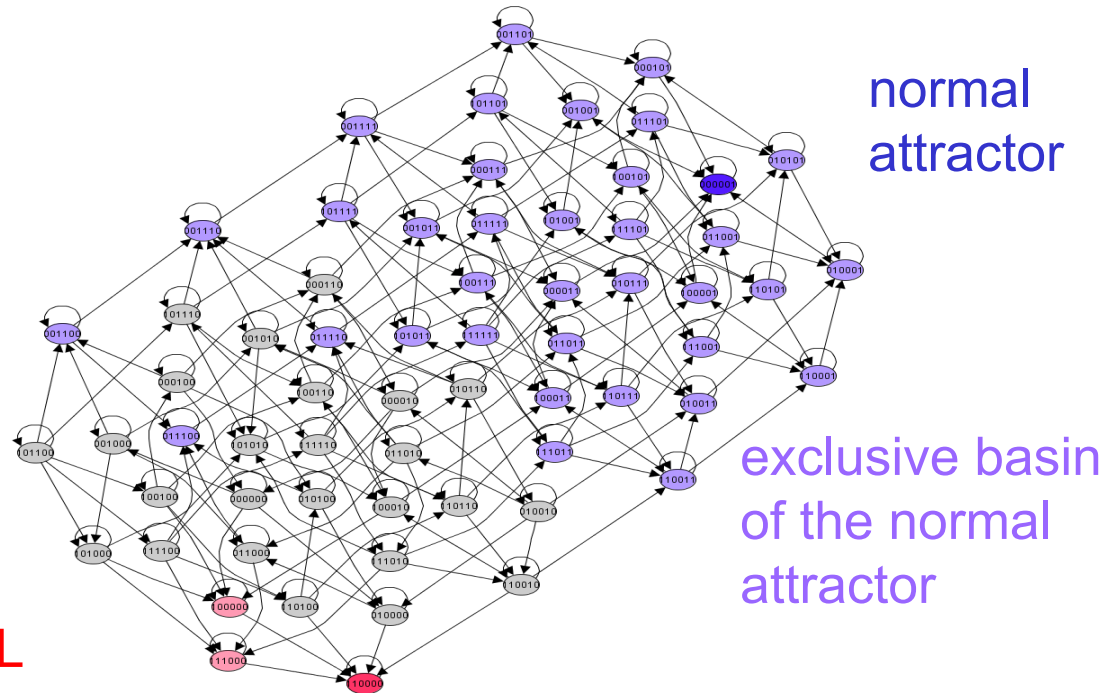


# State space analysis of the simplified T-LGL network



**T-LGL  
attractor**

Probability of  
reaching each  
attractor from  
the grey states



# Perturbation analysis identifies new interventions

Knock out a node that stabilized at ON in the T-LGL fixed point or over-express one that stabilized at OFF

Redo network simplification and state space analysis

Successful manipulation: leads to a large increase in the exclusive basin of the normal steady state

19 targets identified, 68% of which are corroborated by prior experimental evidence (including our own), the rest are new.

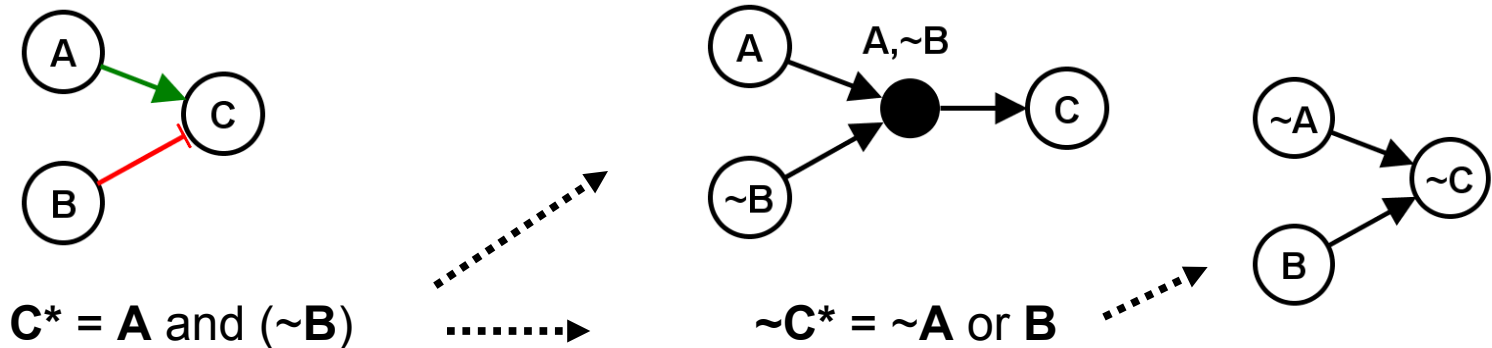
Node	T-LGL state	Fixed point the disruption leads to	Size of exclusive basin of normal fixed point
DISC	OFF	Normal ✓	100%
Ceramide	OFF	Normal ✓	100%
Caspase	OFF	Normal	100%
SPHK1	ON	Normal ✓	100%
S1P	ON	Normal ✓	100%
PDGFR	ON	Normal ✓	100%
GAP	OFF*	Normal	100%
RAS	ON*	Normal	100%
MEK	ON	Normal	100%
ERK	ON	Normal	100%
IL2RBT	ON	Normal	100%
IL2RB	ON	Normal	100%
STAT3	ON	Normal ✓	100%
BID	OFF	Normal	100%
MCL1	ON	Normal ✓	100%
SOCS	OFF*	Both	81%
JAK	ON	Both ✓	81%
PI3K	ON	Both ✓	75%
NFκB	ON	Both ✓	75%
Fas	OFF	Both	72%
sFas	ON	Both	72%
TBET	ON	Both	63%
RANTES	ON	Both	63%
PLCG1	ON*	Both	63%
FLIP	ON	Both	56%
IL2	OFF	Both	56%
IAP	ON*	Both	56%



# Network expansion allows the identification of nodes essential to a cellular behavior

Signal transduction network, its output node corresponds to a cellular outcome.

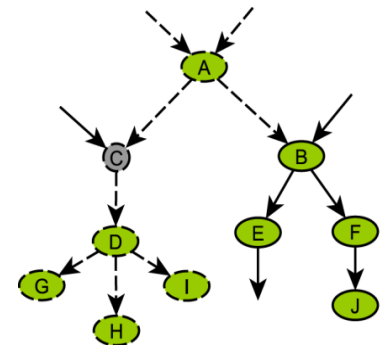
Network expansion: a complementary (negated) node is added for each node; a composite node is added for each AND rule



Evaluate the importance of a node by comparing the expanded network before and after the loss of this node from the network.

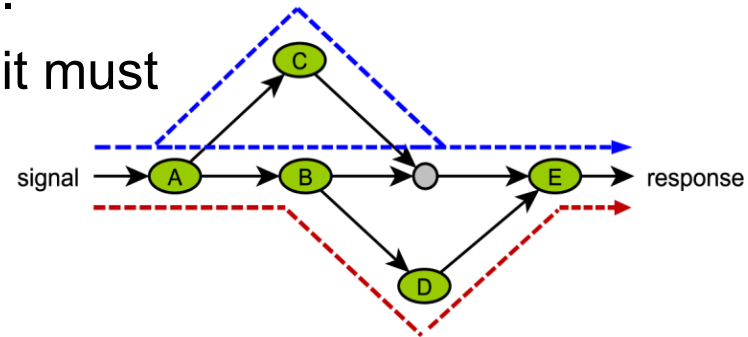
Track the cascading effects of node loss.

R.S. Wang and R. Albert BMC Systems Biology (2011)



# Quantify signal – response connectivity by the number of elementary signaling modes

- ESM: a minimal set of nodes that can mediate signal transduction from input to the output. (analogous to an elementary flux mode).
- If an ESM contains a composite node, it must contain all of its regulators as well



The importance measure based on the reduction of the number of elementary signaling modes after the loss of a node does as well as dynamic perturbation analysis.

For the T-LGL network it predicts the same 19 key mediators as the state space analysis, plus an additional one.

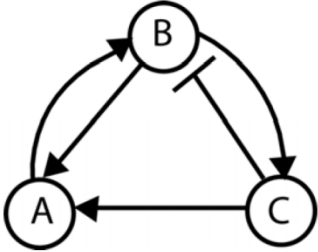
# Expanded network can be used to simplify the network

Stable motif: the smallest strongly connected component that

- Does not contain both a node and its negation.
- If it contains composite nodes, it also needs to contain these nodes' inputs.

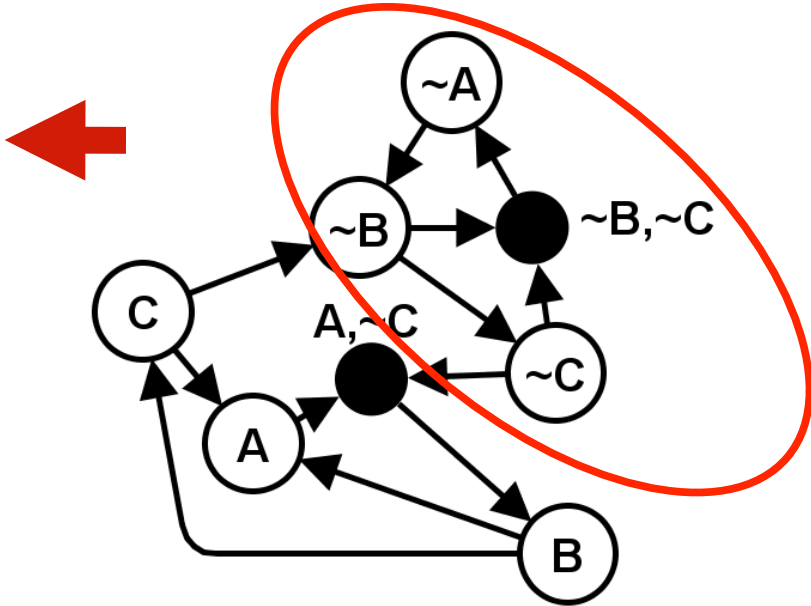
The nodes of a stable motif will have a **steady state** in any attractor of the network.

1. Create expanded network (complementary, composite nodes).
2. Identify stable motifs.
3. Reduce network using the state of one of these stable motifs.
4. Repeat as necessary



**A = 0**  
**B = 0**  
**C = 0**

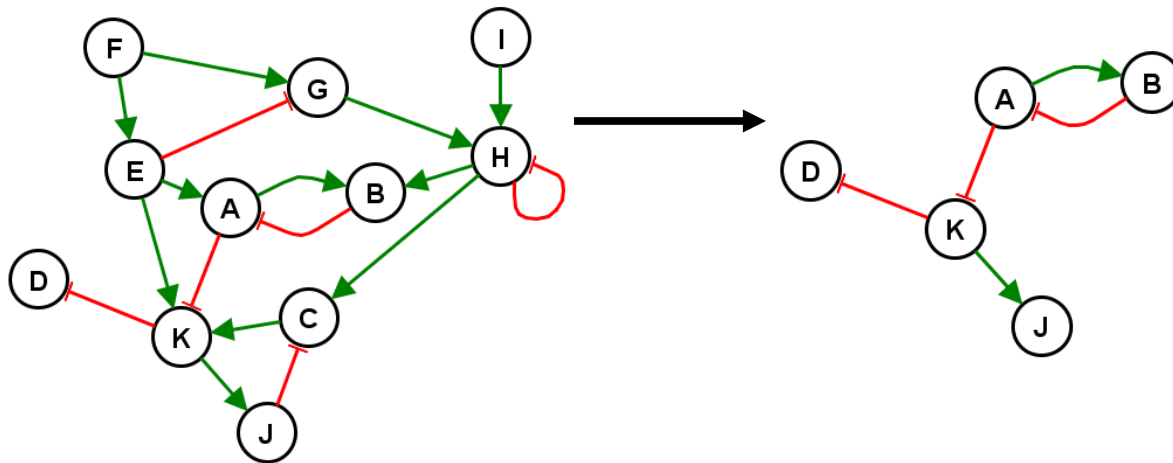
Node	Boolean rule
A	$A^* = B \text{ OR } C$
B	$B^* = A \text{ AND } (\text{NOT } C)$
C	$C^* = B$



# The result of network simplification is a (quasi)attractor

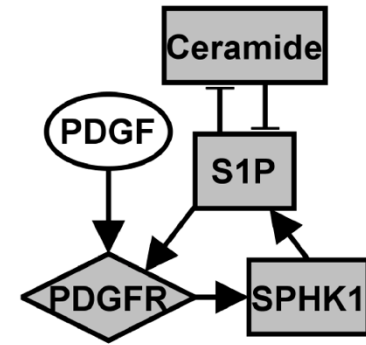
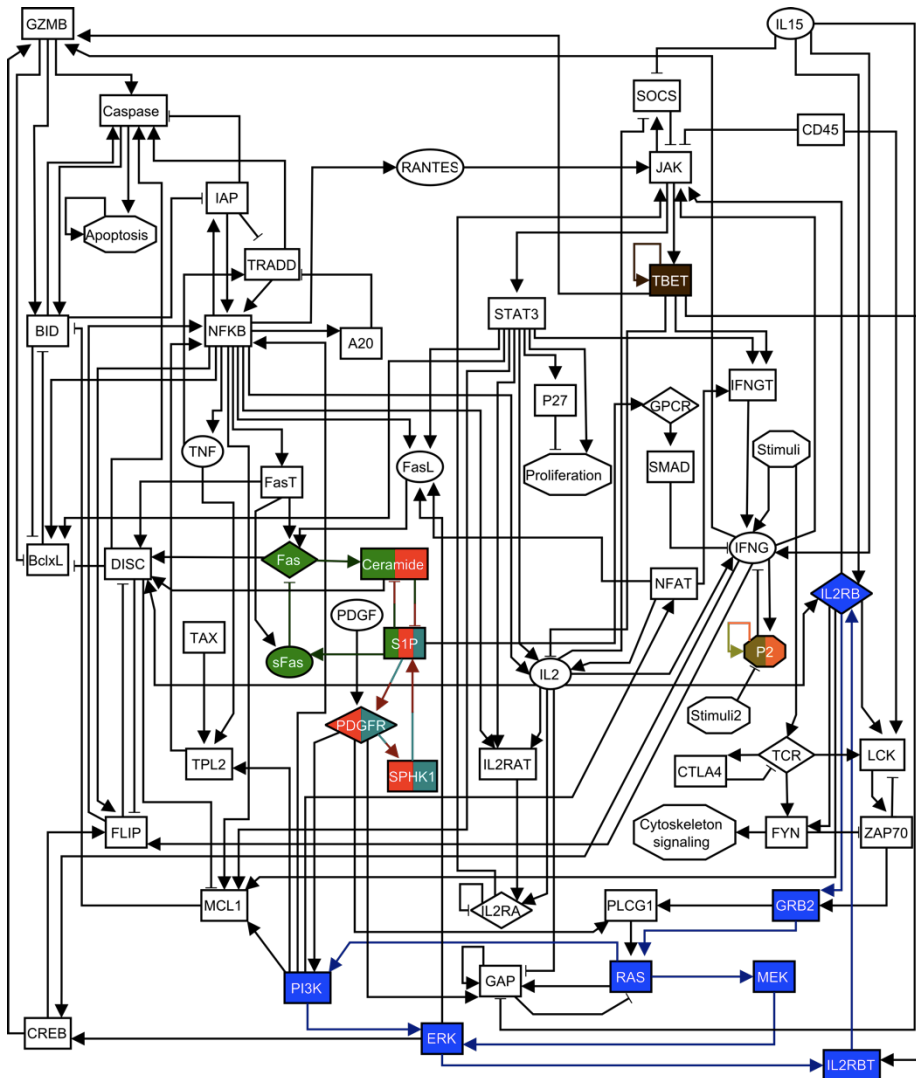
The quasi attractor is either a steady state or a partial steady state, in which some nodes have fixed states and others are oscillating.

The algorithm indicates the nodes with a **predicted fixed state**.  
The nodes whose **state was not predicted** are expected to oscillate.



**Quasi-attractor**  
**A** = ? (Oscillates?)  
**B** = ? (Oscillates?)  
**C** = 1  
**D** = ? (Oscillates?)  
**E** = 0, **F** = 0  
**G** = 0, **H** = 1  
**I** = 1  
**J** = ? (Oscillates?)  
**K** = ? (Oscillates?)

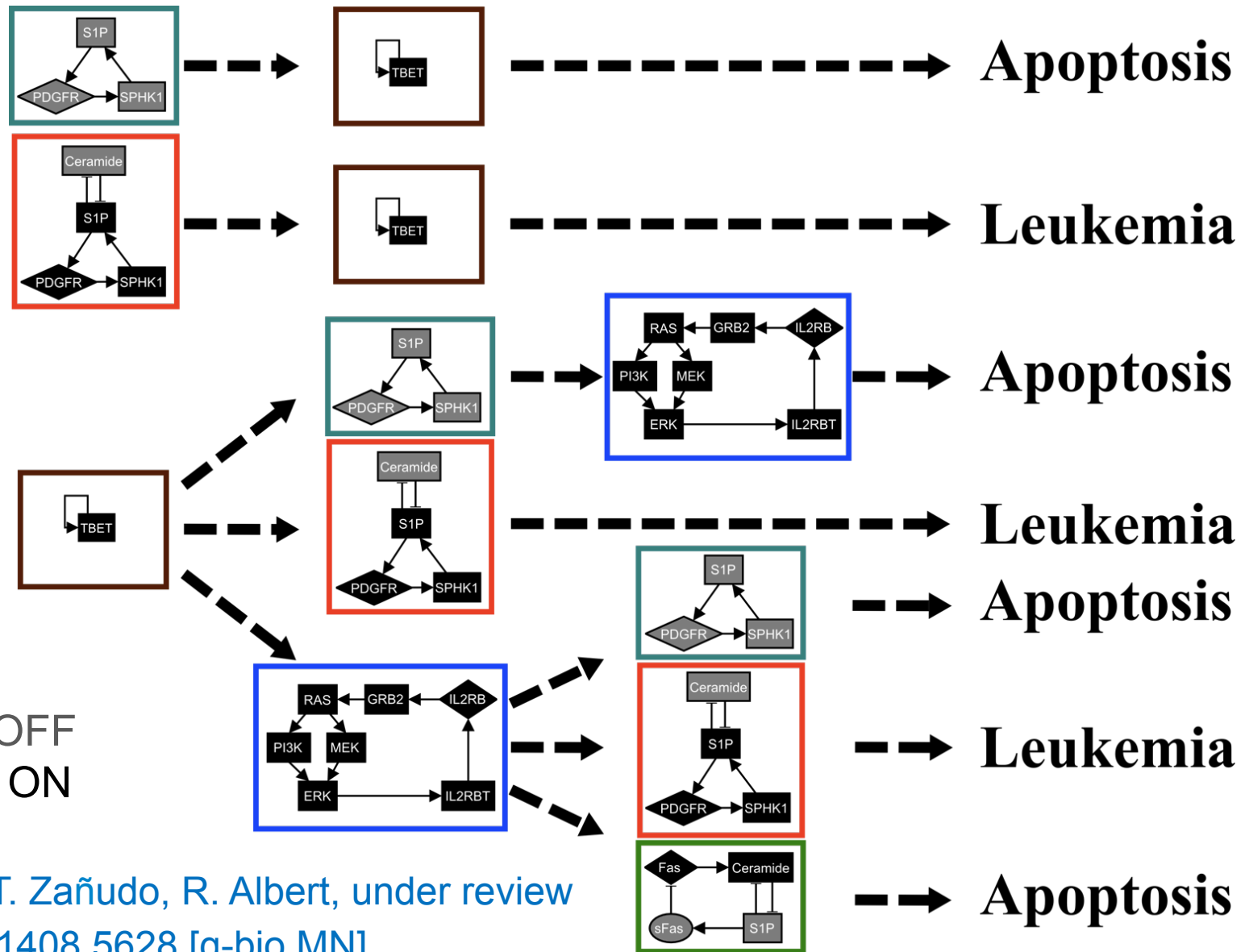
# Stable motifs in the T-LGL network



**Ceramide = OFF**  
**S1P = ON**  
**PDGFR = ON**  
**SPHK1 = ON**

**S1P = OFF**  
**PDGFR = OFF**  
**SPHK1 = OFF**

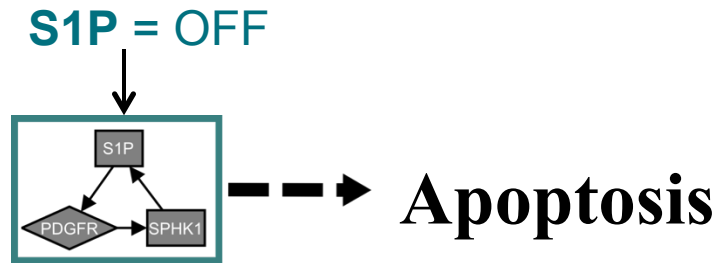
# Motif succession diagram reflects the autonomous dynamics of the system



J. G. T. Zañudo, R. Albert, under review  
 arXiv:1408.5628 [q-bio.MN]

# Setting the state of a motif guides the system to a desired attractor

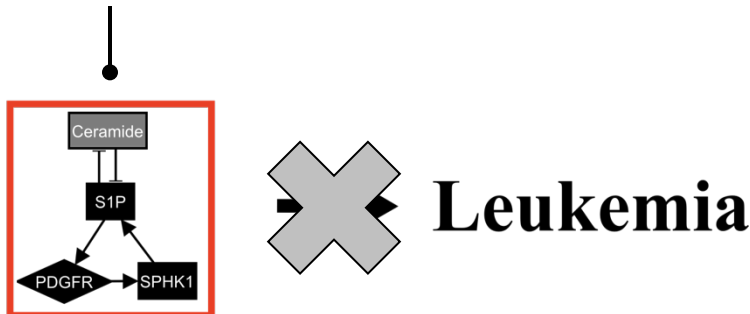
grey: OFF  
black: ON



9 interventions that lead to apoptosis, 6 combinatorial, all 100% effective even when non-permanent.

Opposing the state of a motif may block the system from reaching an undesired attractor

**Ceramide = ON**



7 interventions that block the T-LGL attractor with >90% effectiveness, one effective when non-permanent.

The initial condition of the rest of the nodes does not matter.  
Interventions effective for a continuous version of the model as well.

# Conclusions & acknowledgements

- Stochastic asynchronous Boolean modeling offers a practical representation of qualitative information.
- Integrating the regulatory rules into the network increases the predictive power of network analysis.
- Stable motifs identify the points of no return in the dynamics and serve as control targets.

Ranran Zhang      T-LGL model and experiments

Assieh Saadatpour      Network reduction, attractor analysis

Rui-Sheng Wang      ESMs, key mediator analysis

**Jorge G T Zañudo**      Stable motif analysis, control – **see poster**

