Discrete dynamic modeling elucidates the outcomes of signal transduction networks and helps to control them

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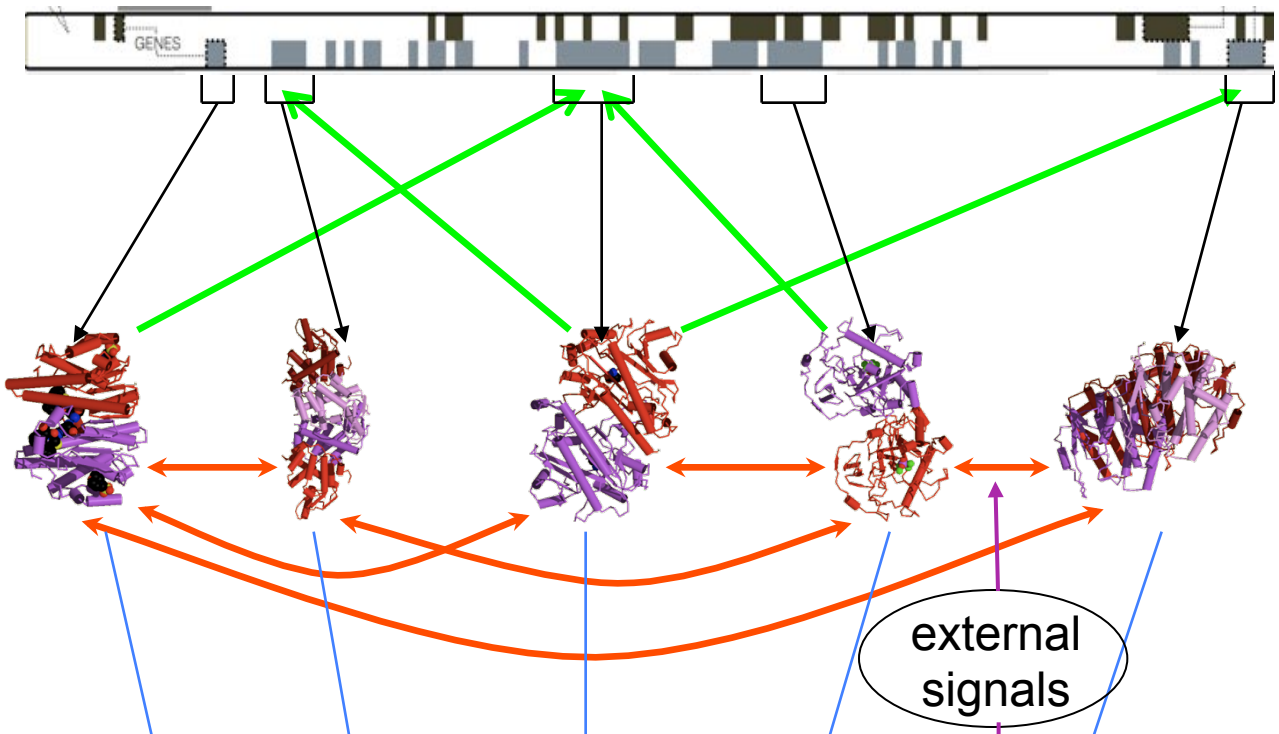
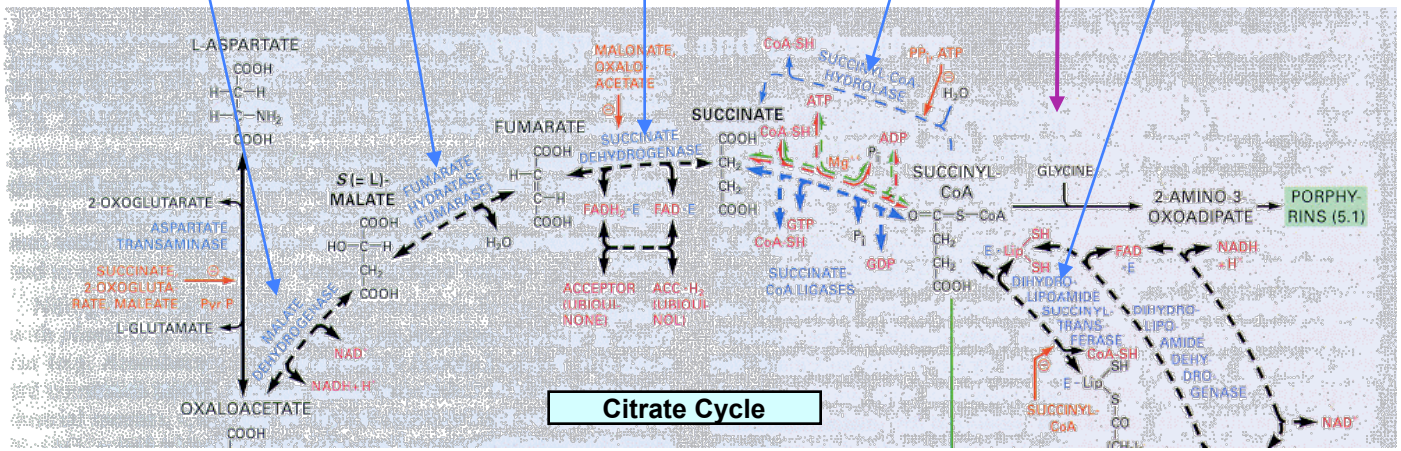
Interaction networks within cells





gene regulatory network

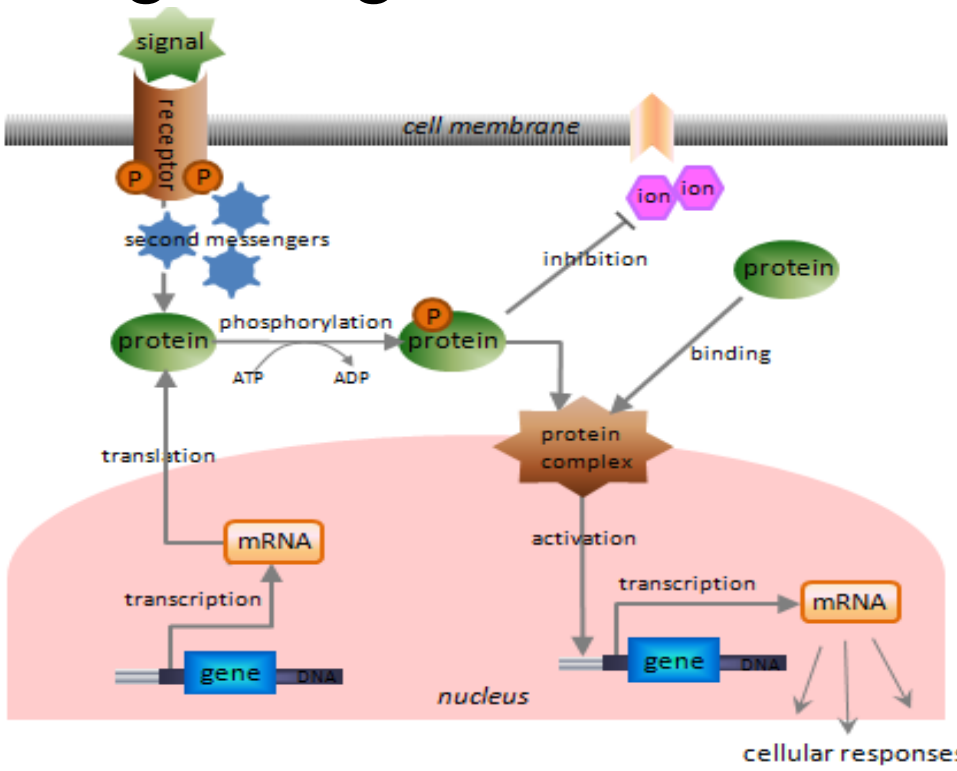
protein-protein interaction network

signal transduction network

biochemical reaction network

Hawoong Jeong

Signaling networks are the most diverse

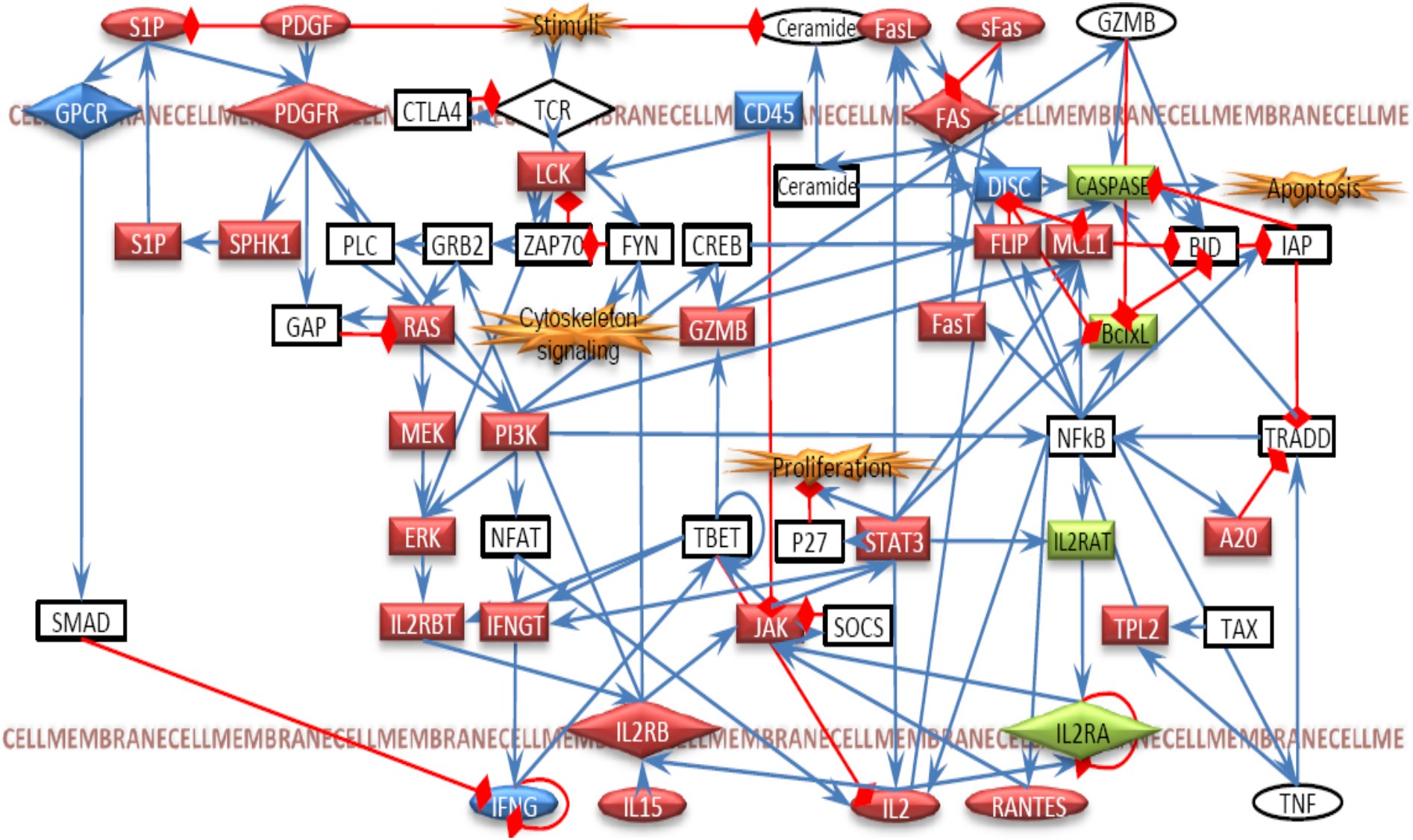


Information starting from outside of the cell propagates through the network and leads to certain outcomes.

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

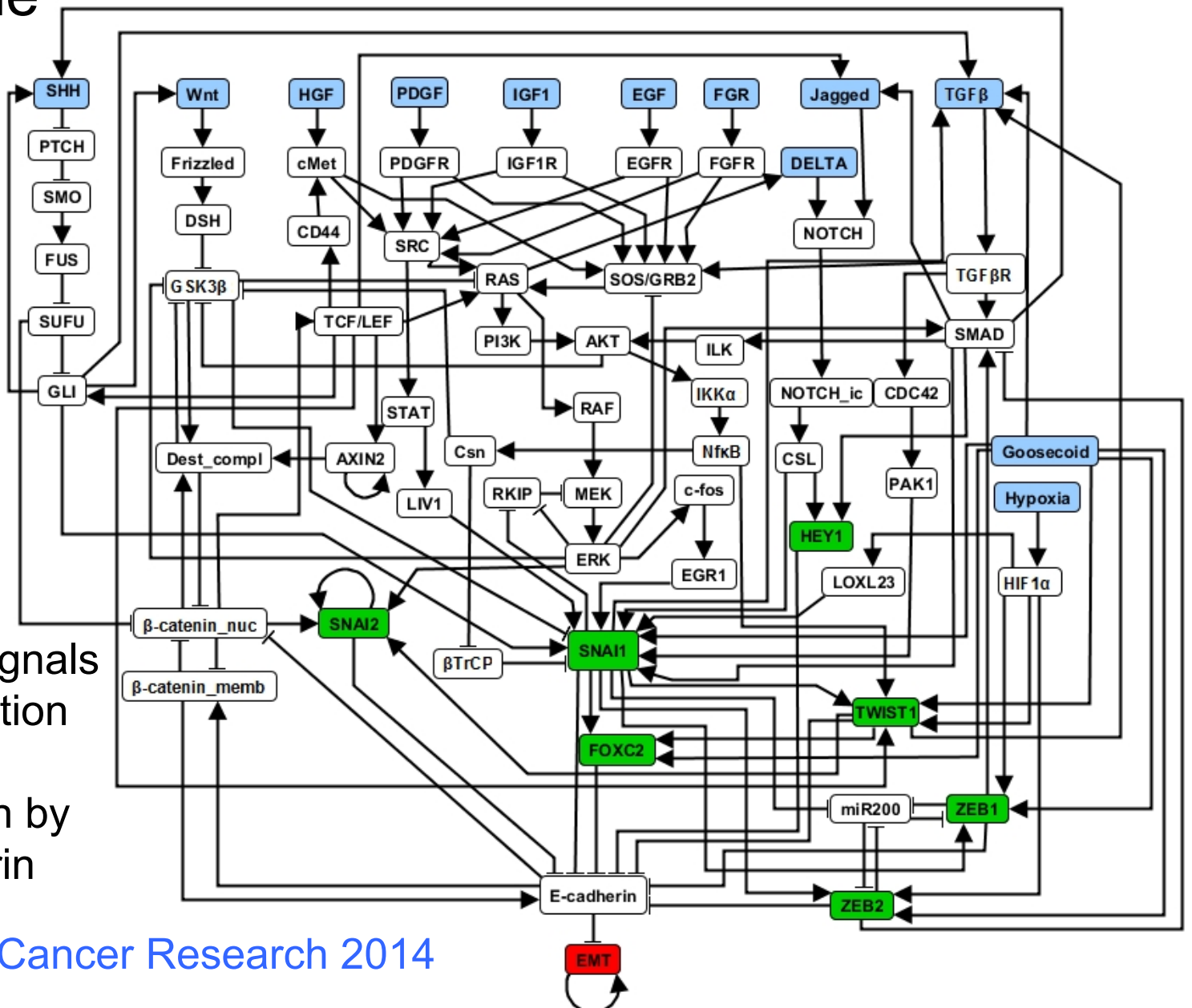
Who can interact with whom is bounded by the laws of physics and chemistry.
But the interactions are usually not known at the single reaction level.
No high-throughput methods exist to map signaling networks; they have to be constructed from the literature

T cell survival signaling network: three signals, three outcomes



Rectangle: intracellular; ellipse: extracellular; diamond: receptor.
Upregulated, downregulated, deregulated node; Activation, inhibition edge

Epithelial to mesenchymal transition: many signals, one outcome



Blue: external signals
Green: transcription factors
Red: EMT driven by loss of E-cadherin

Steinway et al, Cancer Research 2014

Connect within-cell networks to cell behavior through dynamic modeling

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.

Each component is characterized with a state and with a regulatory function that connects the state of its regulators to its own future state.

Mutations and deregulations are incorporated by altered node states or altered regulatory functions.

The long-term states of a subset of the nodes can be related to cell behavior.

How do properties of within-cell networks relate to cell behavior?

A parsimonious dynamic modeling approach: Boolean modeling with stochastic update

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

The future state of a regulated node depends on the current state of its regulators in the network.

This dependence is described by Boolean logic (using NOT, AND, OR)

Node states are updated probabilistically (up to two classes).

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).

In1	*In2*	*Out*
0	0	0
0	1	1
1	0	1
1	1	1

Out= In1 OR In2

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

Validation: reproduces the two known outcomes (apoptosis and abnormal survival state); reproduces known key mediators

Predicts: minimal initial condition necessary for survival state

10 new manipulations that ensure apoptosis of T-LGL cells

12 additional deregulations in survival state

Several predictions were validated experimentally

Implications: identifying therapeutic targets for T-LGL leukemia

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)

Example: Modeling epithelial to mesenchymal transition

Phenomenon: cell fate change that starts cancer metastasis

Constructed: signaling network leading to loss of E-cadherin

Hypotheses: two protein/mRNA states, two timescales

Validation: reproduces known molecular markers and known key mediators of EMT

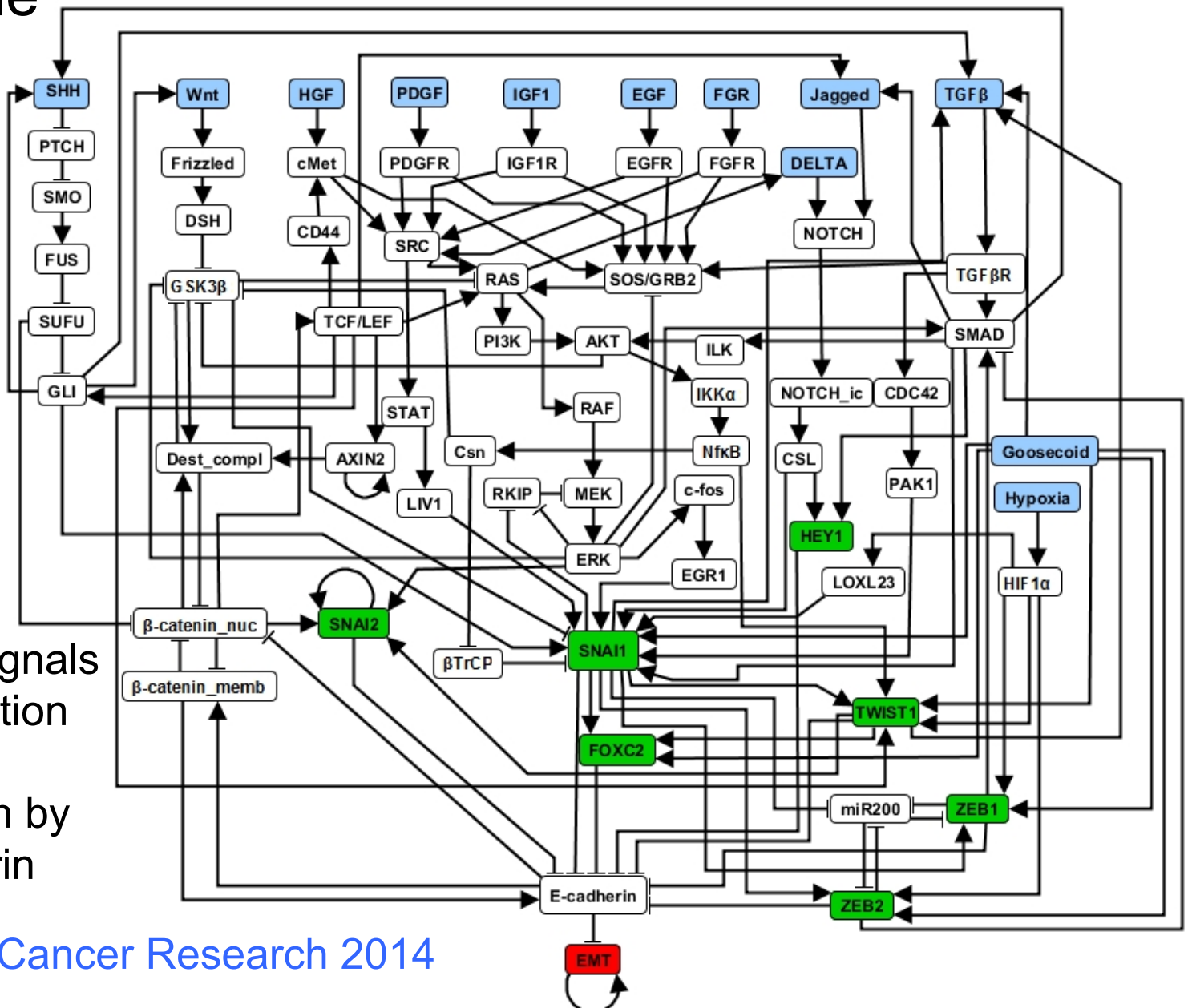
Predicts: activation of several unexpected pathways
successful combinatorial interventions that block EMT

Several predictions were validated experimentally

Implications: identifying therapeutic targets to prevent cancer metastasis

SN Steinway, JGT Zanudo, W Ding, CB Rountree, D Feith, TP Lougran, R. Albert, Cancer Reseach (2014).
SN Steinway, JGT Zanudo, PJ Michel, D Feith, TP Loughran, R. Albert, submitted

Epithelial to mesenchymal transition: many signals, one outcome



Blue: external signals
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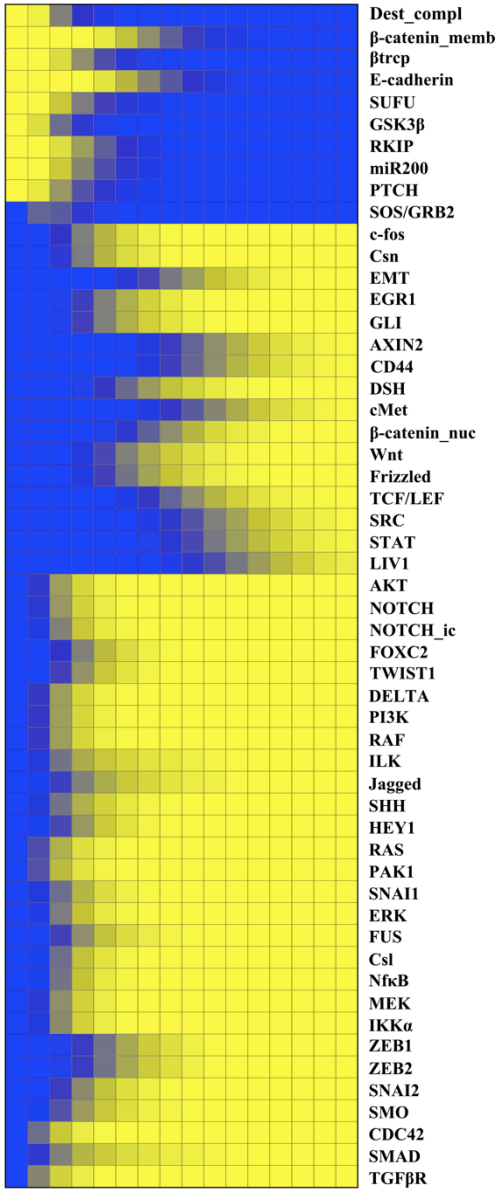
Steinway et al, Cancer Research 2014

Dynamics of the EMT network



Color coded node activity

Epithelial

+TGFβ

Time steps

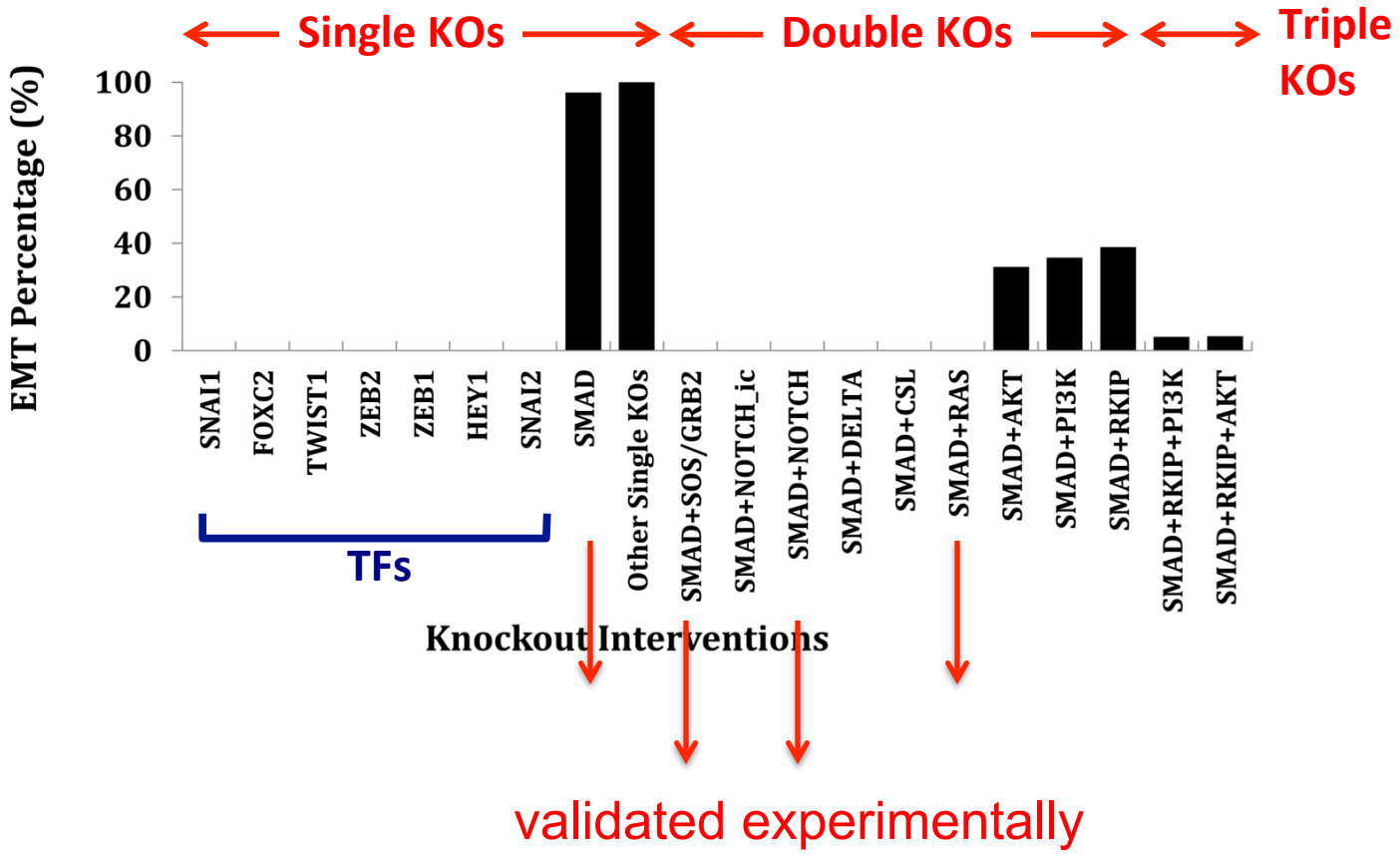
Mesenchymal

- Two steady states without external signals:
- Epithelial state (E)
- Mesenchymal state (M)
- Epithelial + TGFβ → Mesenchymal
- The model reproduces the known aspects of EMT
- The model predicts activation of multiple signaling pathways during TGFβ induced EMT.

SN Steinway, JGT Zañudo, W Ding, CB Rountree, D Feith, TP Loughran, R Albert. Cancer Res 74 (21) (2014).

How do we disrupt the network to suppress EMT?

Try all combinations of knockouts/overexpressions in the model (up to 4 nodes).



How do properties of within-cell networks relate to cell behavior?

Which nodes/groups of nodes determine cell behavior (attractors)?

Can we answer this question without sampling the state space and without considering timing ?

Yes, through an integration of the interaction network and of the Boolean regulatory rules

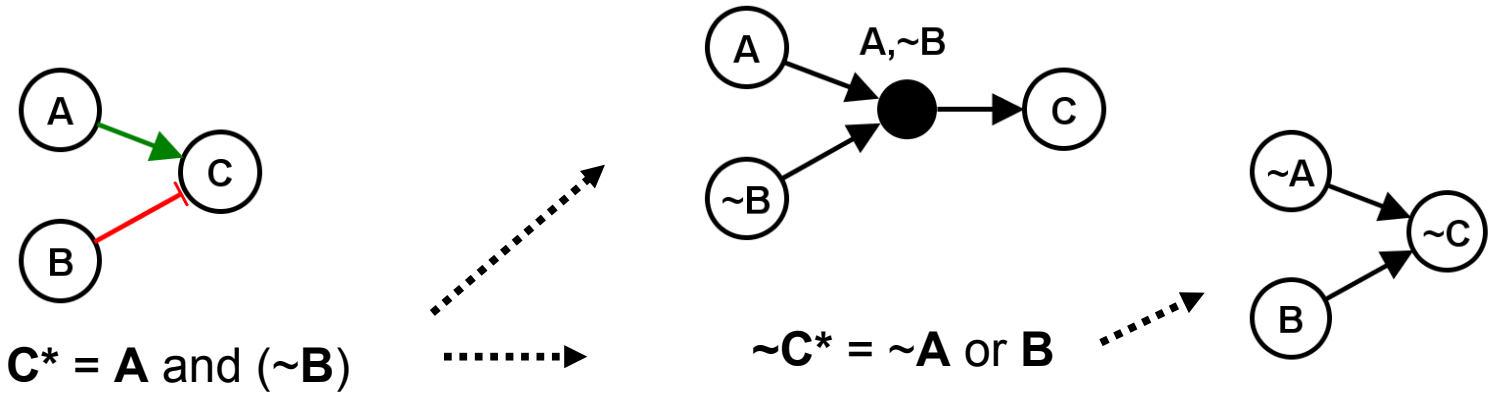
Can be used to

- Determine each node's contribution to outcome
- Find the centers of stability in the network
- Simplify the original network
- Drive the system to or away from attractors

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

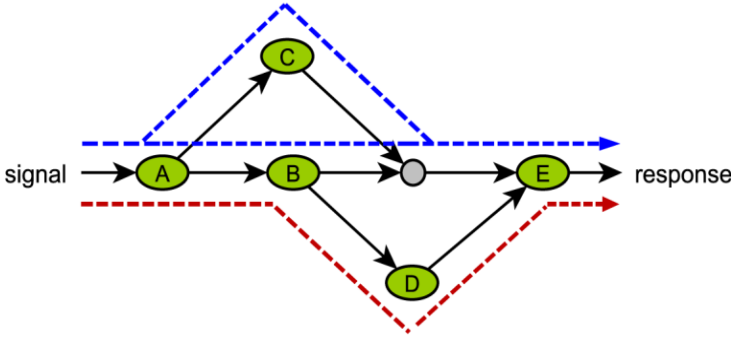


R.S. Wang and R. Albert (2011), BMC Systems Biology 5(44)

Similar method of eliminating ambiguity as an “AND NOT” network (Veliz-Cuba & Laubenbacher)

Elementary signaling modes

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



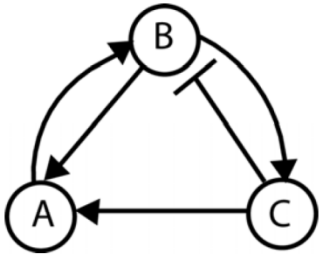
- Node importance measured by the change in the number of ESMs after the node's loss is as successful as importance measures based on Boolean dynamic models.

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.

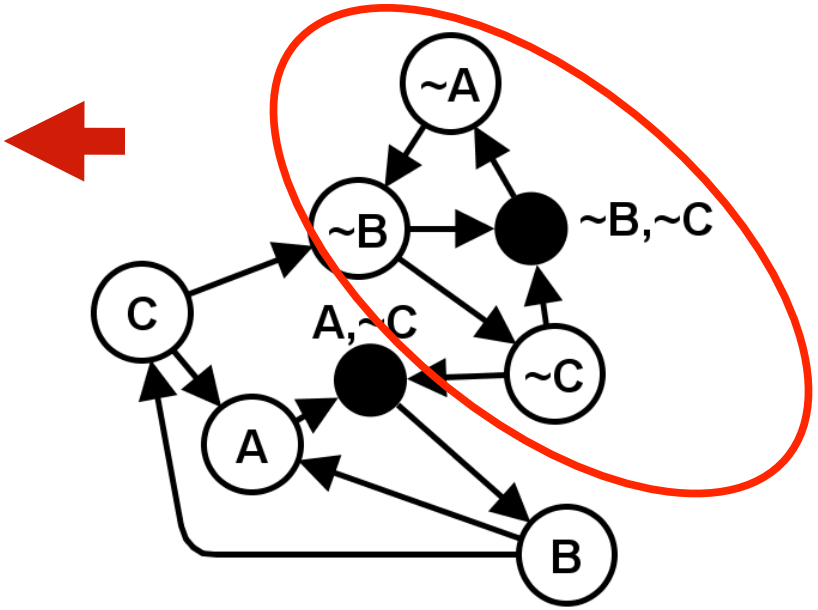
The nodes of a stable motif will have a steady state in any attractor of the network.

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

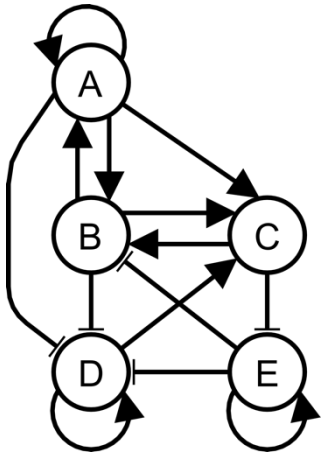


Node	Boolean rule
A	A* = B OR C
B	B* = A AND (NOT C)
C	C* = B

A = 0
B = 0
C = 0

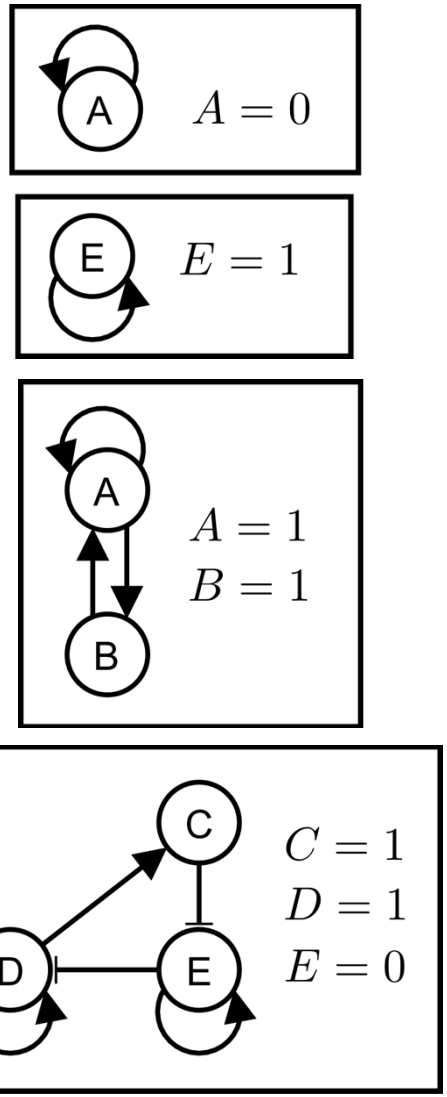


Boolean network

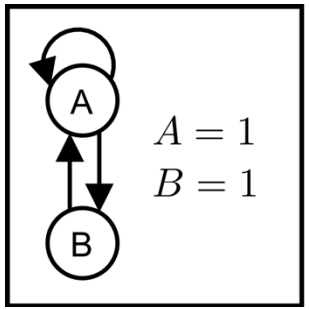


$f_A = A$ AND B
$f_B = A$ OR C OR NOT E
$f_C = (A$ AND $B)$ OR D
$f_D = ($NOT B AND NOT $A)$
OR $(D$ AND NOT $A)$
OR $(D$ AND NOT $B)$
OR NOT E
$f_E = E$ OR NOT C

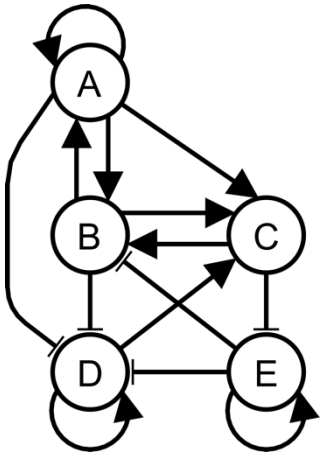
Stable motifs



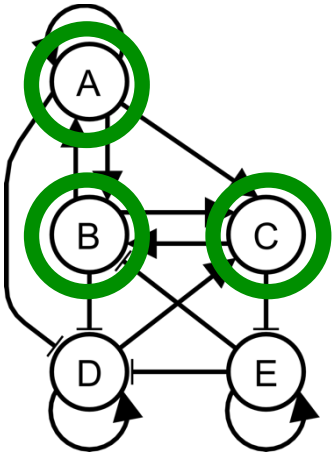
(3) Reduce network using the state of one of the stable motifs.



Plug in



$$
\begin{aligned}
f_A &= A \text{ AND } B \\
f_B &= A \text{ OR } C \text{ OR NOT } E \\
f_C &= (A \text{ AND } B) \text{ OR } D \\
f_D &= (\text{NOT } B \text{ AND NOT } A) \\
&\quad \text{OR } (D \text{ AND NOT } A) \\
&\quad \text{OR } (D \text{ AND NOT } B) \\
&\quad \text{OR NOT } E \\
f_E &= E \text{ OR NOT } C
\end{aligned}
$$



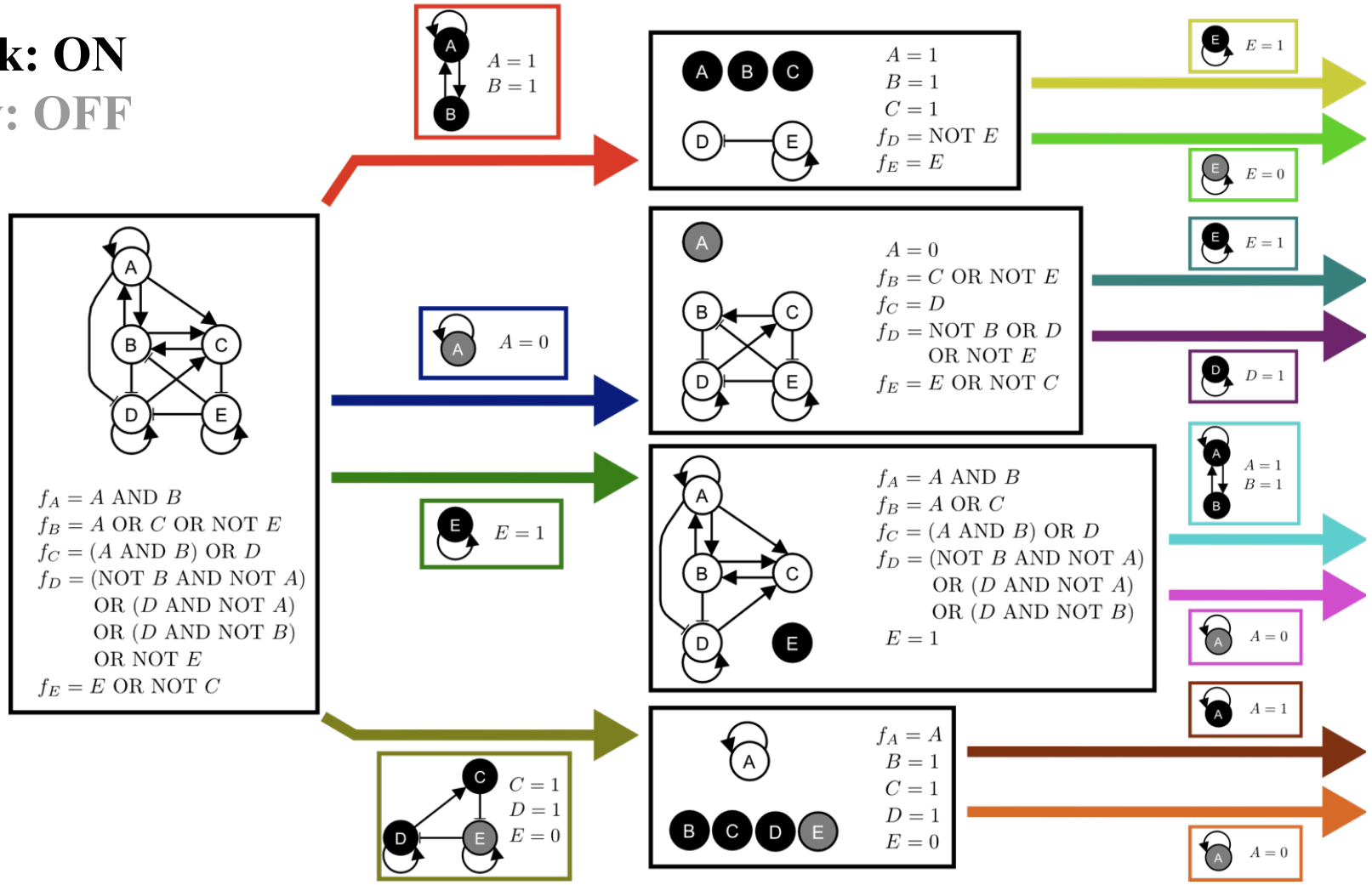
$$
\begin{aligned}
f_D &= \text{not } E \\
f_E &= E
\end{aligned}
$$

Reduced network

Repeat as necessary

Black: ON

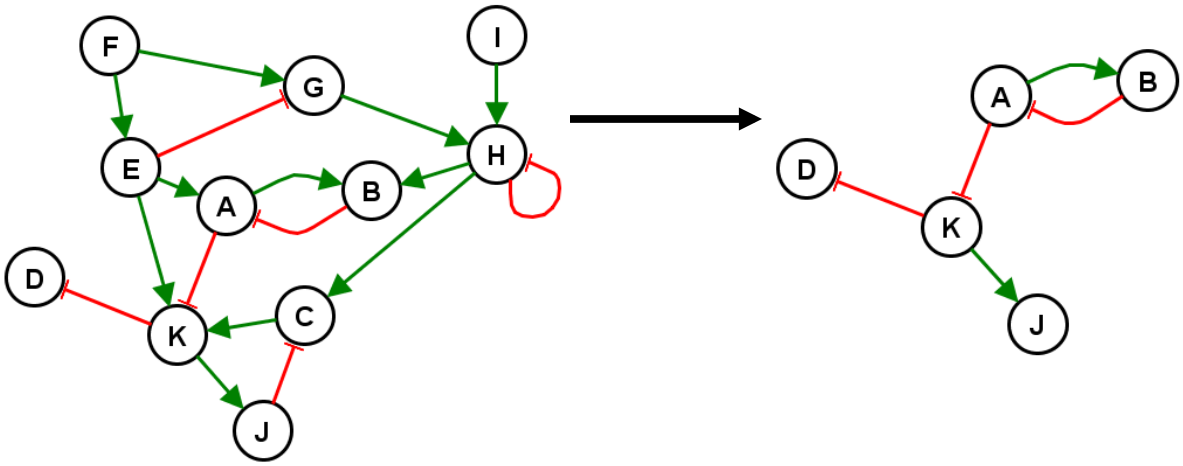
Grey: OFF



The final result 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.

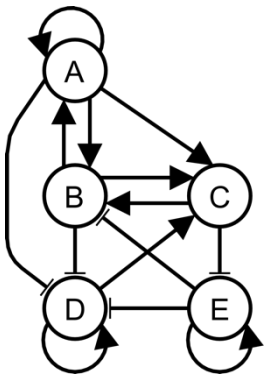


Quasi-attractor

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

J. G. T. Zañudo, R. Albert, Chaos 2013

The stable motif succession diagram reflects the autonomous dynamics of the system



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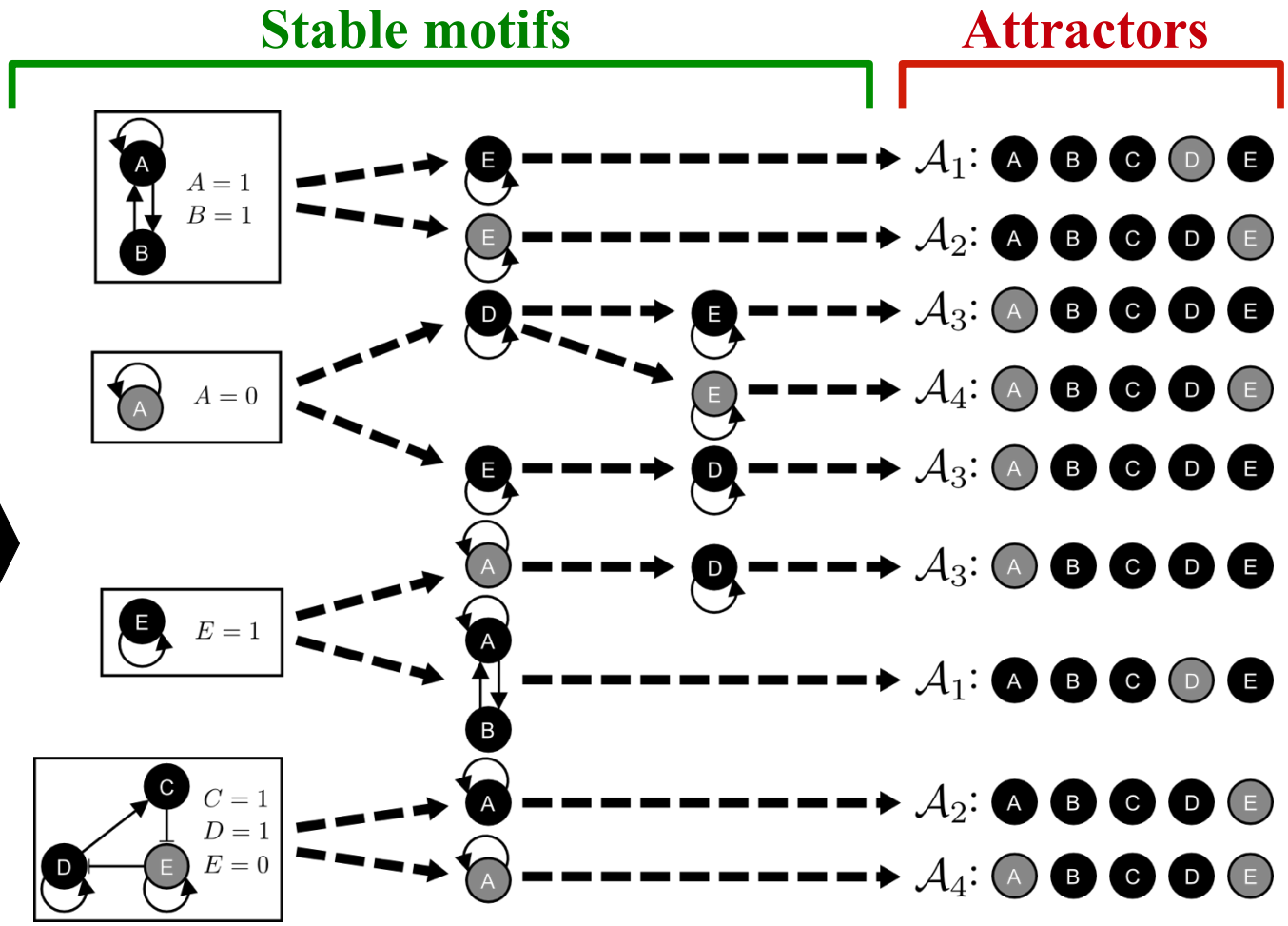
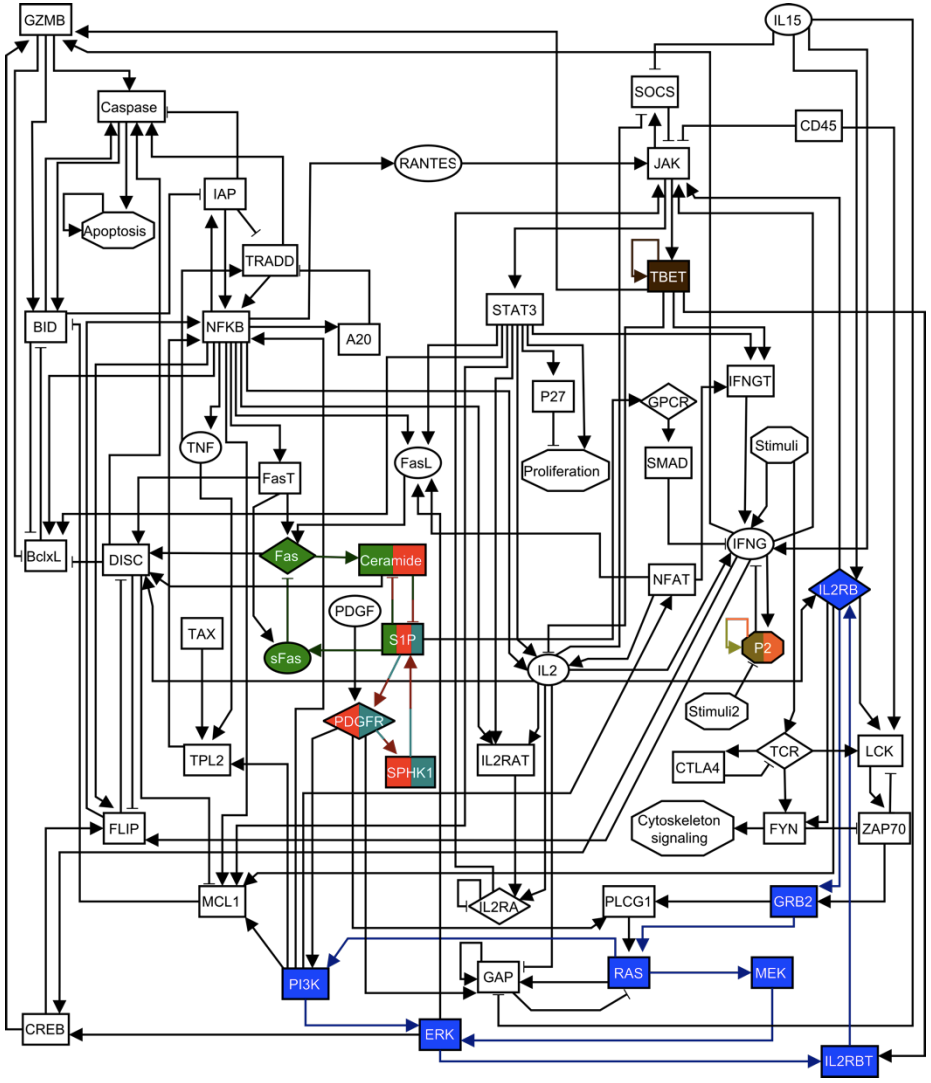
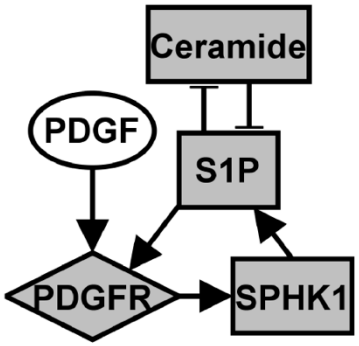


Black: ON

Grey: OFF

Stable motifs in the T-LGL network



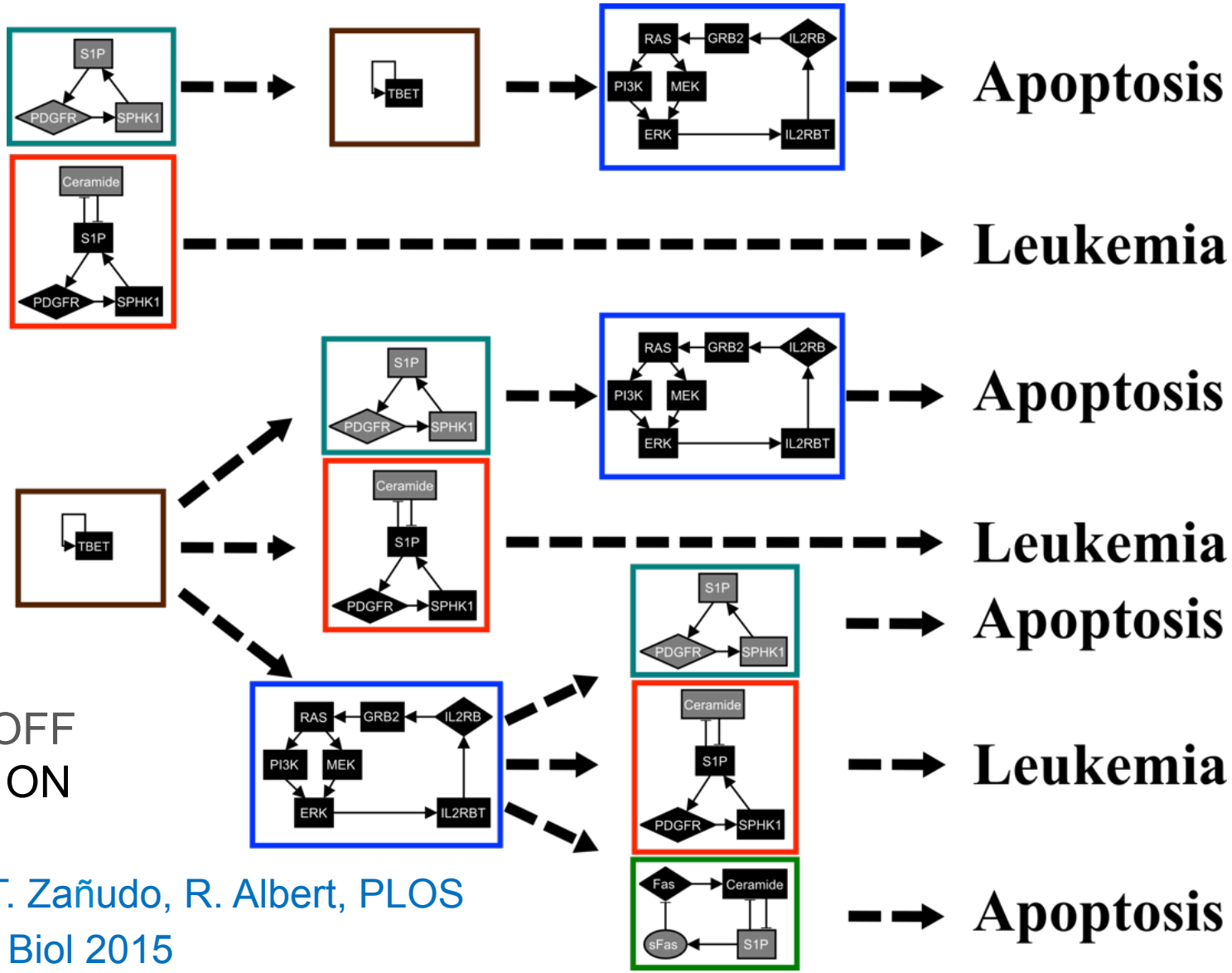


Ceramide = OFF
S1P = ON
PDGFR = ON
SPHK1 = ON

S1P = OFF
PDGFR = OFF
SPHK1 = OFF

J. G. T. Zañudo, R. Albert, Chaos 2013

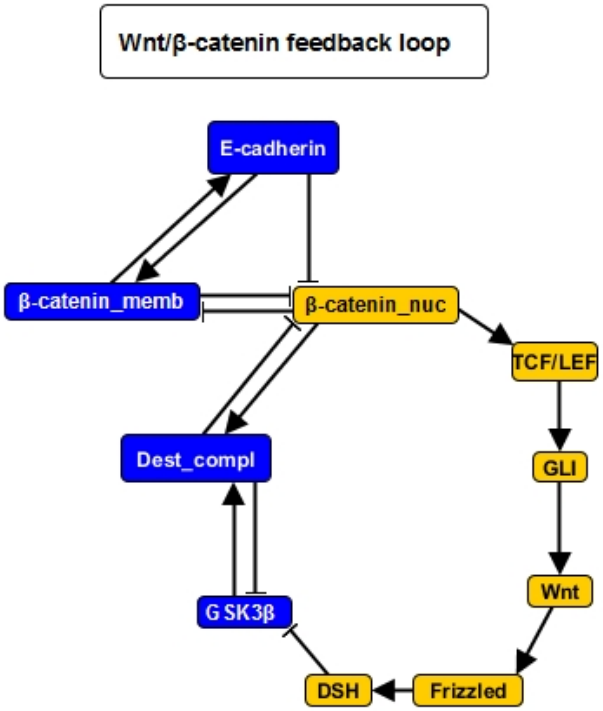
Stable motif succession diagram summarizes all trajectories to the two outcomes

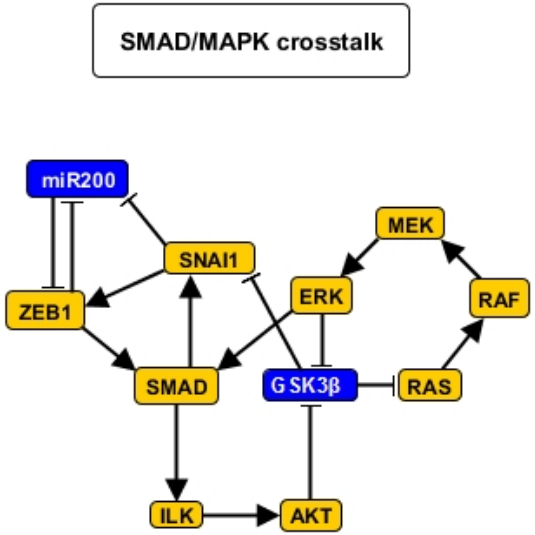


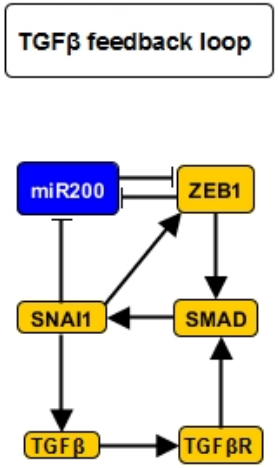
grey: OFF
black: ON

J. G. T. Zañudo, R. Albert, PLOS Comp Biol 2015

Any of the EMT network's motifs can independently drive the mesenchymal state

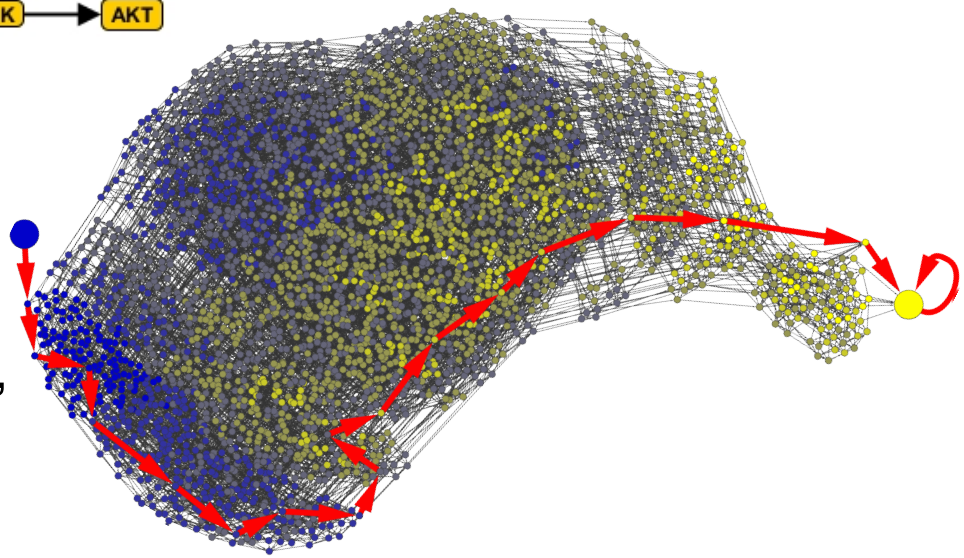




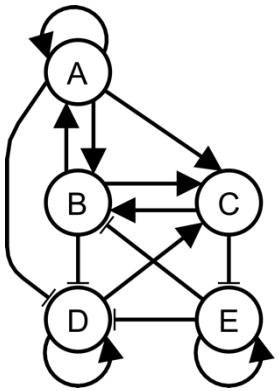


Blue: node OFF
Yellow: node ON

The network has a single attractor, reachable through a multitude of trajectories



A sequence of stable motifs determines an attractor



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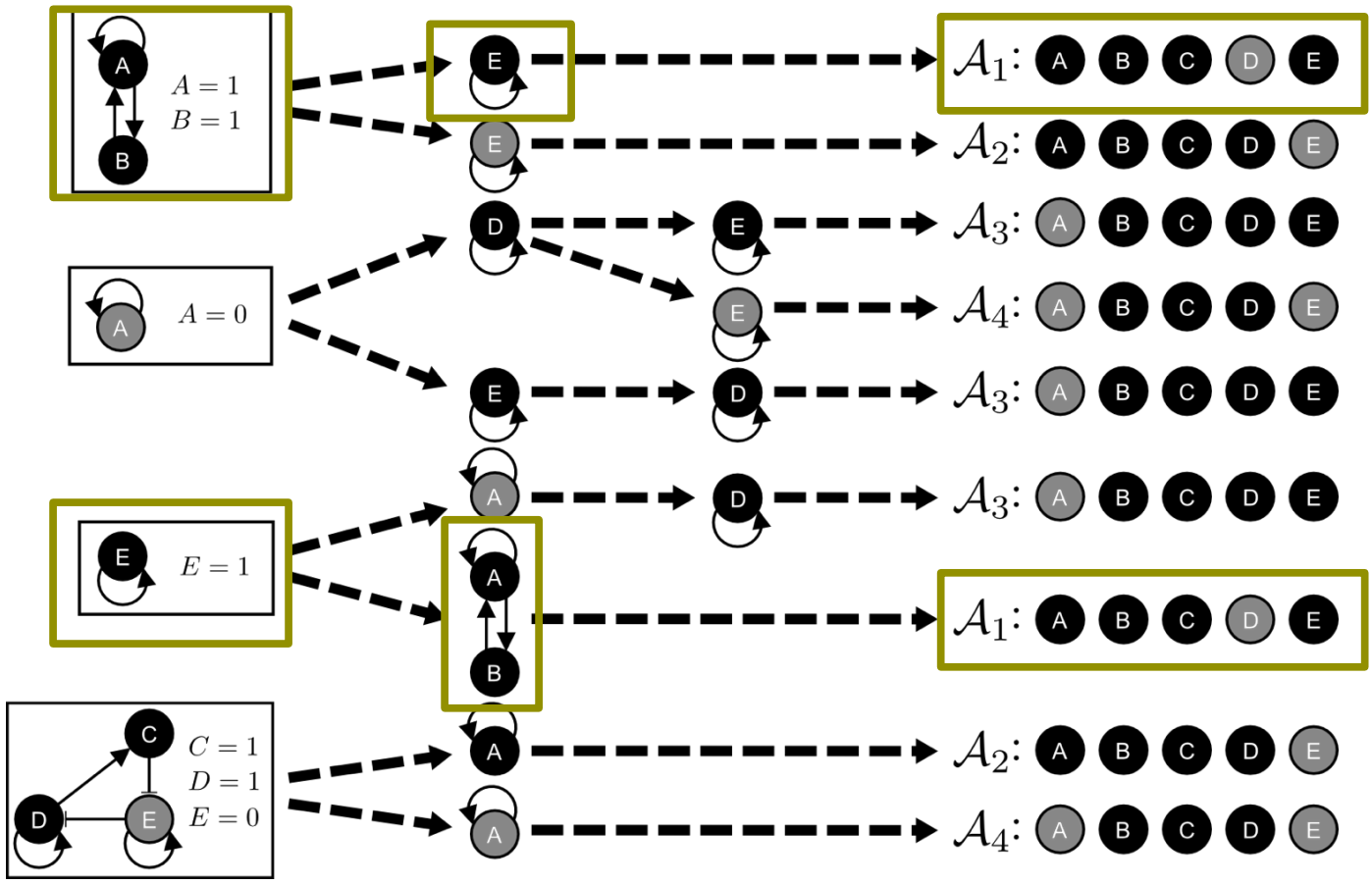
OR NOT E
$f_E = E$ OR NOT C

JGT Zañudo and R Albert. PLoS Comput Biol 11(4): e1004193 (2015).

Stable motifs **Attractors**



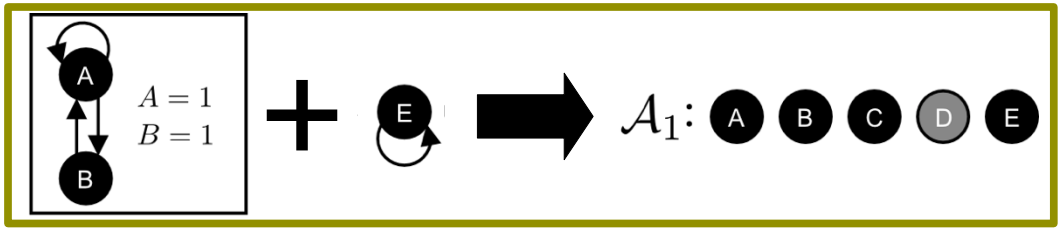
Dynamics

Black: ON
Grey: OFF

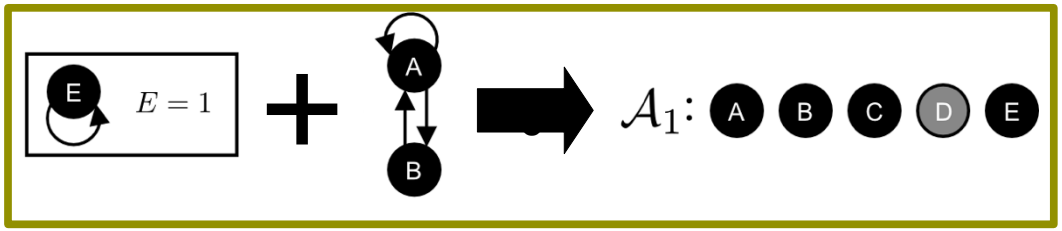
Stable motif control

- Fix the state of all stable motifs in a sequence → all initial conditions go to target attractor.
- Reduce the number of nodes whose state needs to be fixed.

Motif sequence 1



Motif sequence 2



Sequences that lead to A_1

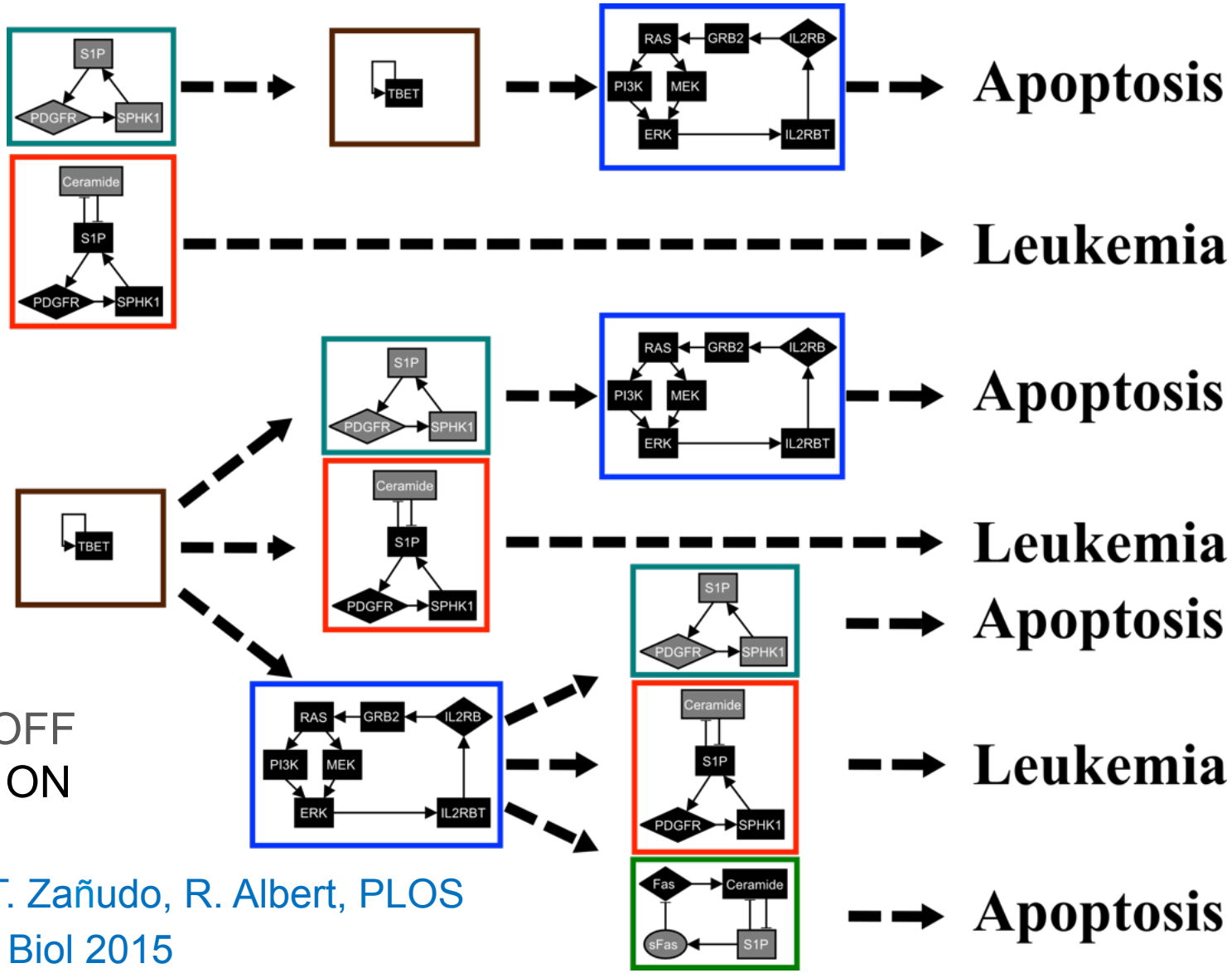
- Simplify sequences (remove unnecessary motifs)
- Find subset of nodes required to stabilize motif

Black: ON
Grey: OFF

Control set for A_1: {A=1, E=1}

JGT Zañudo and R Albert. PLoS Comput Biol 11(4): e1004193 (2015).

Stable motif succession diagram reflects the trajectories to the two outcomes



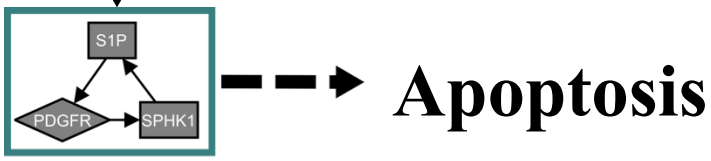
grey: OFF
black: ON

J. G. T. Zañudo, R. Albert, PLOS Comp Biol 2015

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

grey: OFF
black: ON

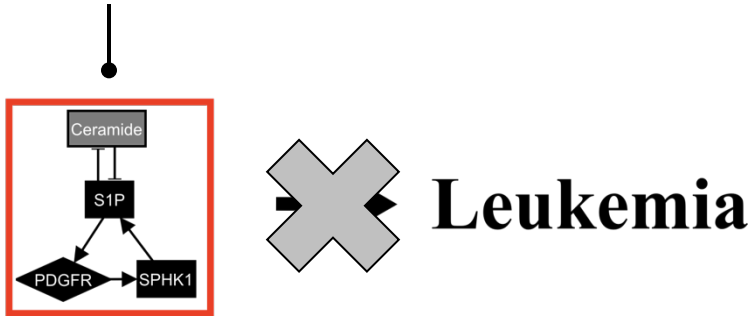
S1P = OFF



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

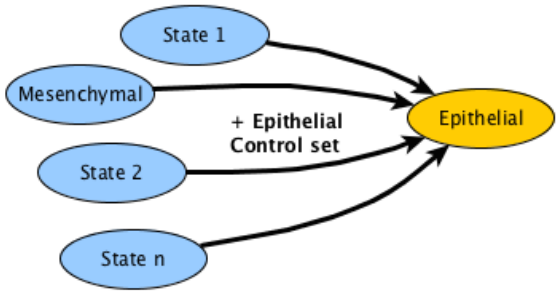
Ceramide = ON



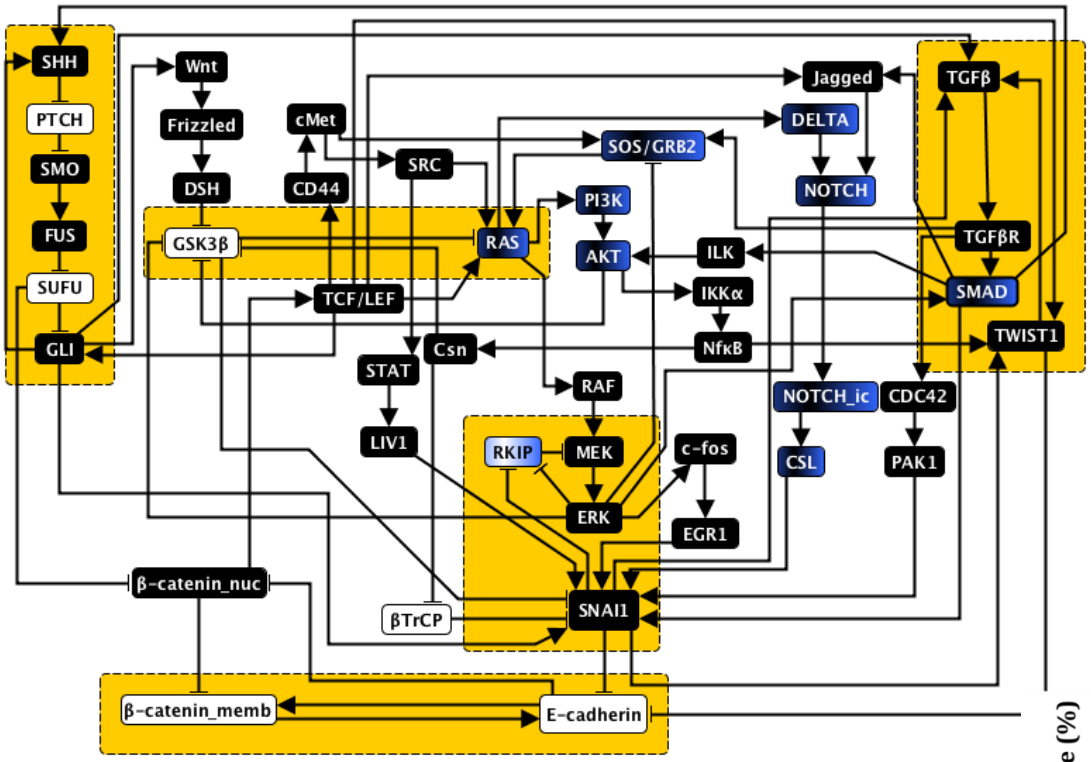
7 interventions that block
the T-LGL attractor with >90%
effectiveness, one effective
when non-permanent.

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

Control sets to reach the epithelial state even in the presence of TGFβ

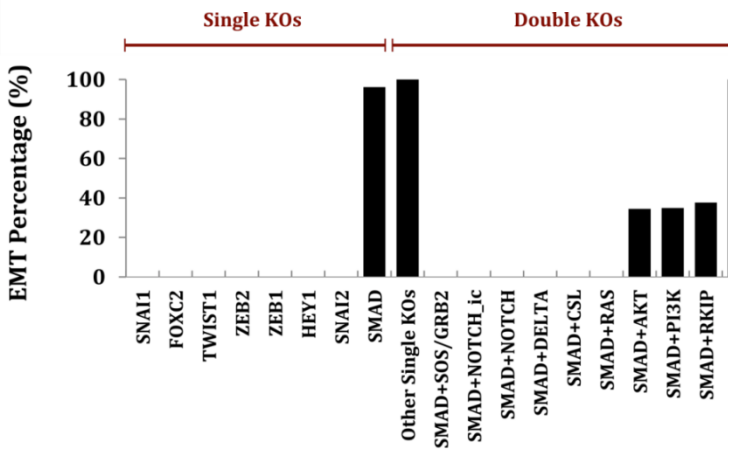


Stable motif associated with the E state



Control of one node in each yellow rectangle is sufficient for convergence to the epithelial state.

Subsets are also effective.



Black: OFF in the E state

White: ON in the E state

Blue: part of a combinatorial intervention

Steinway et al, submitted

The logic-expanded network and motif succession diagram offer new ways of connecting molecular network structure and cellular outcomes.

Java libraries:
github.com/jgtz/BooleanDynamicModeling
github.com/jgtz/StableMotifs

Jorge G T Zañudo	Stable motif analysis, control
Steven Steinway	EMT model and experiments
Ranran Zhang	T-LGL model and experiments
Ruisheng Wang	Key mediator analysis

National Science Foundation