Connecting network structure and dynamics through stable motifs

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My group's research focus: modeling the dynamics of systems of interacting elements

- Interactions among species underlie ecological communities. We modeled the assembly of plant-pollinator communities.

- Cellular behaviors and phenotypes arise from the interactions of numerous (macro)molecular components.

- The higher-level behavior/phenotype is an emergent property; it arises from the dynamics of the lower-level elements and interactions.

- Not all the elements and interactions are equally important – use a network framework to identify the key connectivity patterns.

I teach a course on network analysis of biological systems; connecting structure and dynamics is a key topic

Wk	Monday	Wednesday	Friday
1	Introduction, motivation	Network examples	Biological network examples
2	From network structure to dynamics	Network measures: node degree	Network measures: connectivity, distances
3	Labor Day – no class	Network measures: betweenness centrality	Negative edges, edge weights
4	Software for network analysis	Definition and interpretation of cell biological networks	Analysis of cell biological networks
5	Properties shared by various networks: degree distribution	Properties shared by various networks: distances	Network motifs
6	Network modularity	Network benchmarks: random graphs	Random graphs
7	Network benchmarks: scale-free networks	Network resilience	Network resilience
8	Spreading processes on networks	Spreading processes on networks	Dynamic modeling concepts
9	Modeling dynamic processes in molecular/cellular networks	Discrete dynamic modeling: Boolean functions, update	Discrete dynamic modeling: state transition graph
10	Discrete dynamic modeling: attractors and basin of attraction	Solving Boolean equations	Examples of discrete dynamic models of cell biological systems
11	Continuous and deterministic modeling: ligand binding	Continuous and deterministic modeling: Michaelis-Menten kinetics	Continuous and deterministic modeling: steady state analysis
12	Examples of continuous and deterministic models of cell biological systems	Parameter estimation and sensitivity analysis	Compare continuous and discrete models
13	Connections between network structure and dynamics	Connections between network structure and dynamics	Network control: structural methods
14	Network control via stable motifs	Network inference from causal information	Network inference from state information
15	Network inference from state information: continuous methods	Bayesian network inference	Boolean network inference

A good amount is known about the necessary conditions of multi-stability and sustained oscillations

René Thomas et al.: The existence of a positive feedback loop in the interaction network is a necessary condition for the existence of multiple stable steady states. The existence of a negative feedback loop is a necessary condition for sustained oscillations.

Corollary: a network that has a linear or tree-like structure has a unique attractor, which is a stable steady state.

Atsushi Mochizuki et al.: The nodes of the feedback vertex set (whose removal makes the network acyclic) are determinant nodes: the controlled system in which their values are fixed has a unique attractor.

Eduardo Sontag et al.: If the interaction network does not have any negative feedback loops or incoherent feed-forward loops, the dynamical system will not have any chaotic or oscillatory behavior.

Connect within-cell networks to cell behavior through discrete dynamic modeling

The model is built from experimental data and validated by experimental data.

Synthesize the relevant, focused network - from the literature or databases. The nodes are molecular species. The edges need to be directed and causal/mechanistic.

Each node is characterized with a small set of states. It is also characterized with a regulatory function that connects the state of its direct regulators to its own future state.

Software to infer regulatory functions from a prior network and state information: CellNOpT, *caspo-ts,* BooleaBayes.

Deregulations and interventions are represented as constitutive node states.

The long-term states of the nodes, or of a **subset**, are representative of the cellular phenotype.

The dynamic model is built from experimental data and is tested on experimental data
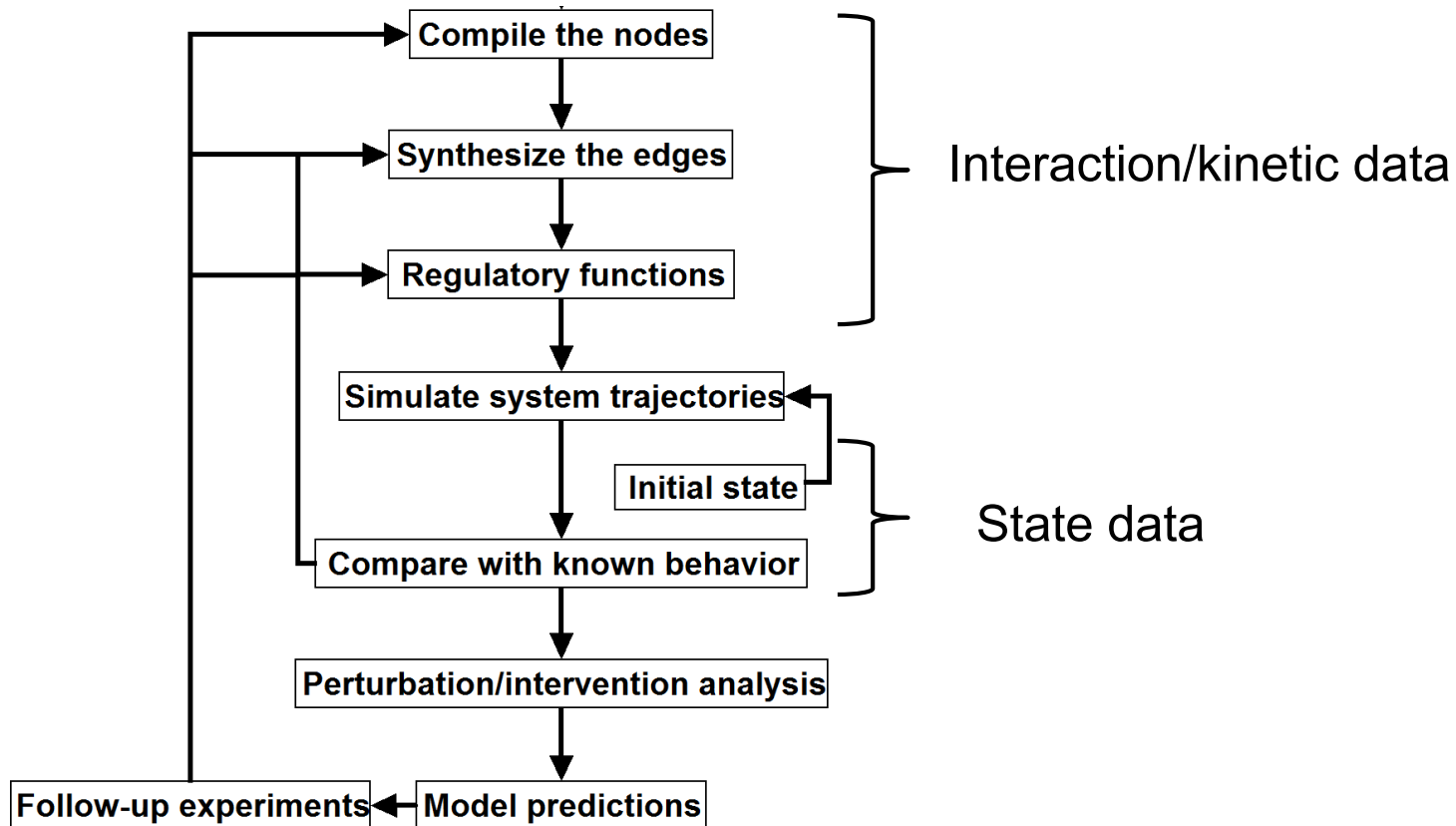
A parsimonious and informative modeling approach: discrete dynamics (logical modeling)

Assumes a small number of states: (active, inactive), (low, medium, high)
The available experimental data inform the states and the regulatory functions.

The regulatory functions of each node can be specified
as tables or using Boolean logic.
NOT- inhibitors;
OR- independent regulators;
AND- conditional regulators

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

Out= In1 OR In2

Time is an implicit variable; there usually is an element of randomness in its implementation.
Replicate simulations (potentially from different initial conditions) yield continuous results.

Logical modeling has had great successes over the last ~20 years.
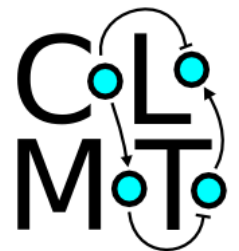
Model – experiment cycles involving our group

Signaling in plant guard cells (with Sally Assmann)
Predicted and validated new interactions, synergies, feedback loops.

Host-pathogen interactions (with Juilee Thakar, Eric Harvill)
Predicted and validated faster clearance of a second infection, synergy between the immune response to pathogens in different organs.

T cell survival signaling (with Ranran Zhang, Thomas Loughran)
Predicted and validated multiple new cell death-inducing therapeutic interventions.

Epithelial to mesenchymal transition in liver cancer (with Steven Steinway, J. Zañudo)
Predicted and validated combinatorial interventions to block TGFβ-induced EMT.

Signaling in breast cancer (with Jorge Zañudo)
Predicted and validated drug resistance mechanisms, combinatorial drug therapies.

Yeast to hyphal transition in *C. albicans* (with David Wooten, Jorge Zañudo, Clarissa Nobile)
Predicted and validated interventions that block the transition.

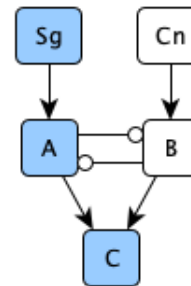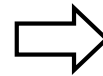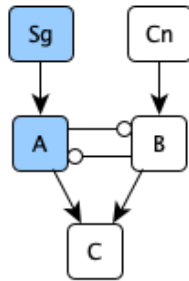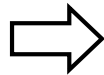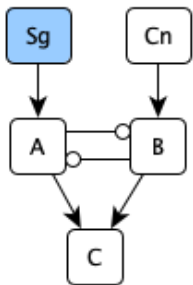Fasting and oxidative stress signaling in *C. elegans* (with Eyleen O'Rourke)
Explained and validated the seemingly contradictory role of FOXO/DAF-16.

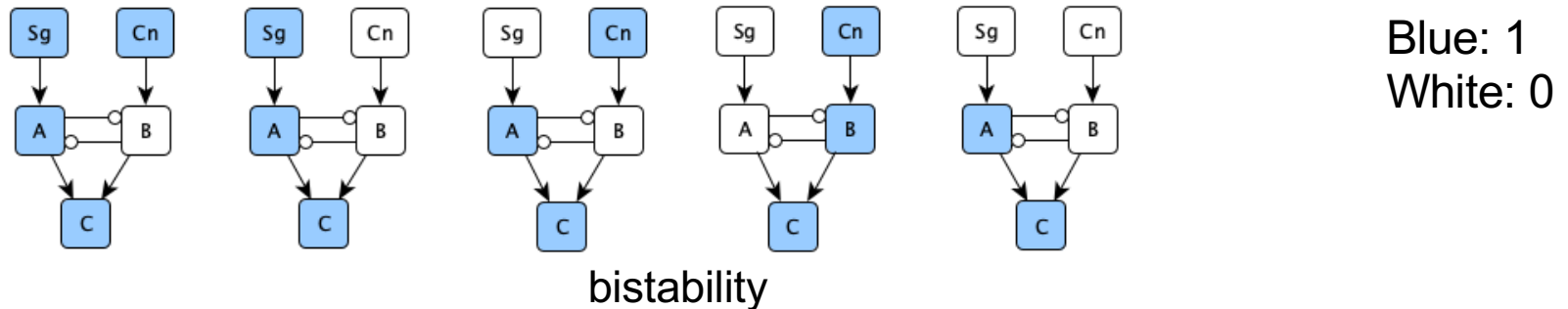# Information propagation in a simple system

mutual inhibition

$f_A = Sg\ or\ not\ B$ A — B $f_B = Cn\ and\ not\ A$

$f_C = A\ or\ B$ C
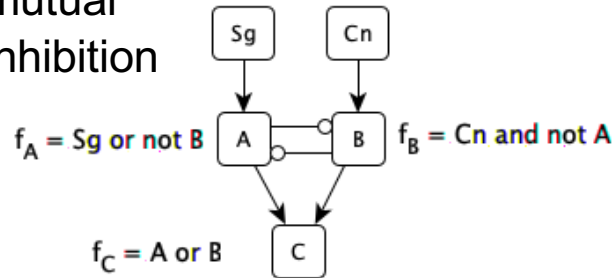
Sg represents an external signal,
Cn represents a cellular context.

point attractor
(steady state)

Blue: 1
White: 0

The model identifies all the attractors (phenotypes)

bistability

Blue: 1
White: 0

Each attractor of the system, or group of attractors that are identical in terms of the internal nodes, can be associated to a phenotype.

P1: A=1, B=0, C=1
P2: A=0, B=1, C=1

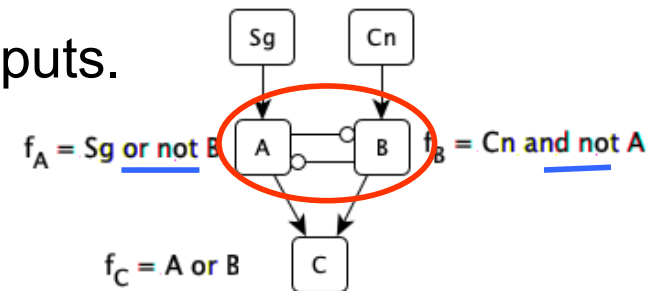The model can describe how the trajectories change in case of deregulations or interventions, e.g. A=0.

What determines the attractor repertoire of a network?

The signals, contexts, deregulations that act as inputs.

Feedback loops in the network.

The logic of the combined effects of interactions.

f_A = Sg or not B f_B = Cn and not A f_C = A or B

My group's work over the years has shown that the attractor repertoire can be identified by analysis of a network derived from the regulatory functions.

The network can be constructed for discrete or continuous systems.

Albert & Othmer J Theor Bio 2003, Zanudo & Albert Chaos 2013 (special issue edited by Leon Glass), Gan & Albert Phys Rev E 2018, Rozum & Albert PLOS Comp Bio 2018, Deritei et al Sci Rep 2019, Rozum et al Sci. Adv. 2021

Analysis of the network also indicates the inevitable consequences of sustained signals or deregulations – logic domain of influence of a node state

Wang & Albert BMC Sys Bio 2011, Yang, Zanudo, Albert Frontiers Phys. 2018

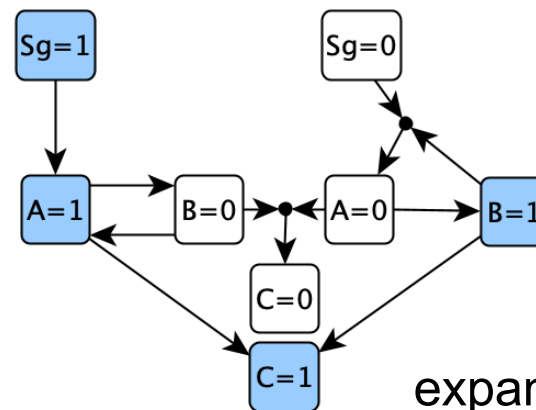# Integration of the interaction network and of the regulatory functions allows causal insight

Represent each node state by virtual nodes, e.g. A=2, A=1, A=0.
AND gates represented by hyperedges, shown here with composite nodes, •
Each (hyper)edge represents logic sufficiency.

Cn=1

f_A = Sg or not B

f_B = not A

f_C = A or B
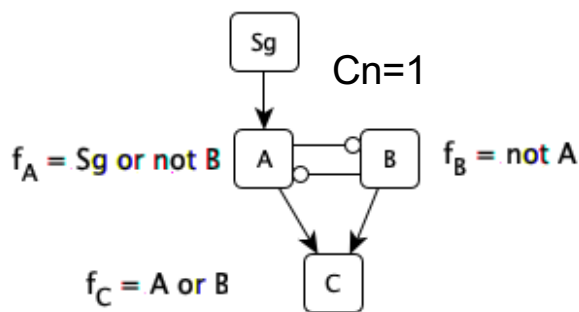
interaction network
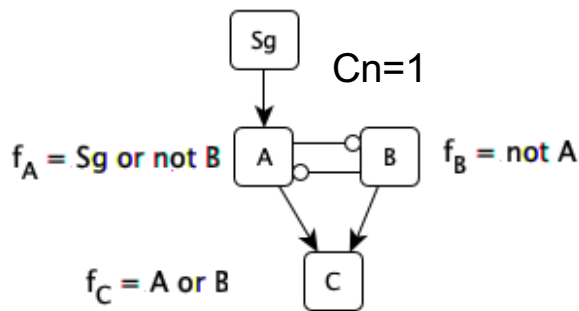
A=1 can be
maintained
by Sg=1
or by B=0.

A=0 can be
maintained if
Sg=0 and B=1

expanded network

Albert & Othmer J Theor. Bio (2003), Wang & Albert BMC Syst Bio (2011),
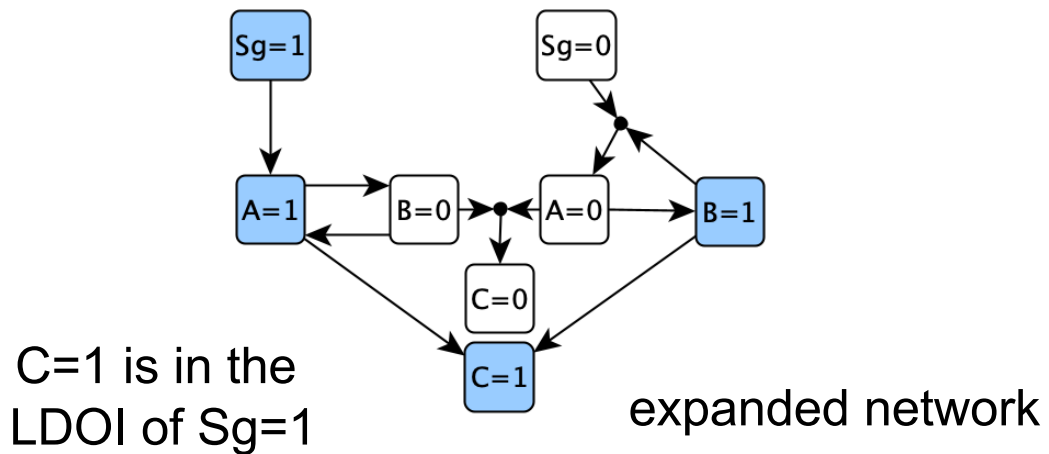Zanudo & Albert Chaos (2013), Rozum Science Advances (2021)

Integration of the interaction network and of the regulatory functions allows causal insight

Represent each node state by virtual nodes, e.g. A=2, A=1, A=0.
AND gates represented by hyperedges, shown here with composite nodes, •
Each (hyper)edge represents logic sufficiency.

interaction network

C=1 is in the
LDOI of Sg=1

expanded network

Albert & Othmer J Theor. Bio (2003), Wang & Albert BMC Syst Bio (2011),
Zanudo & Albert Chaos (2013), Rozum Science Advances (2021)

Stable motifs: self-sustaining positive feedback loops

<u>Stable motif</u>: a cyclic sufficiency relationship.
Stable motifs arise from positive feedback circuits in the interaction network.

The nodes of a stable motif can maintain an associated steady state regardless of the rest of the network. This traps the system into a subspace.

stable motif

conditionally
stable motif

P1:
A = 1
B = 0
C = 1

P2:
A = 0
B = 1
C = 1
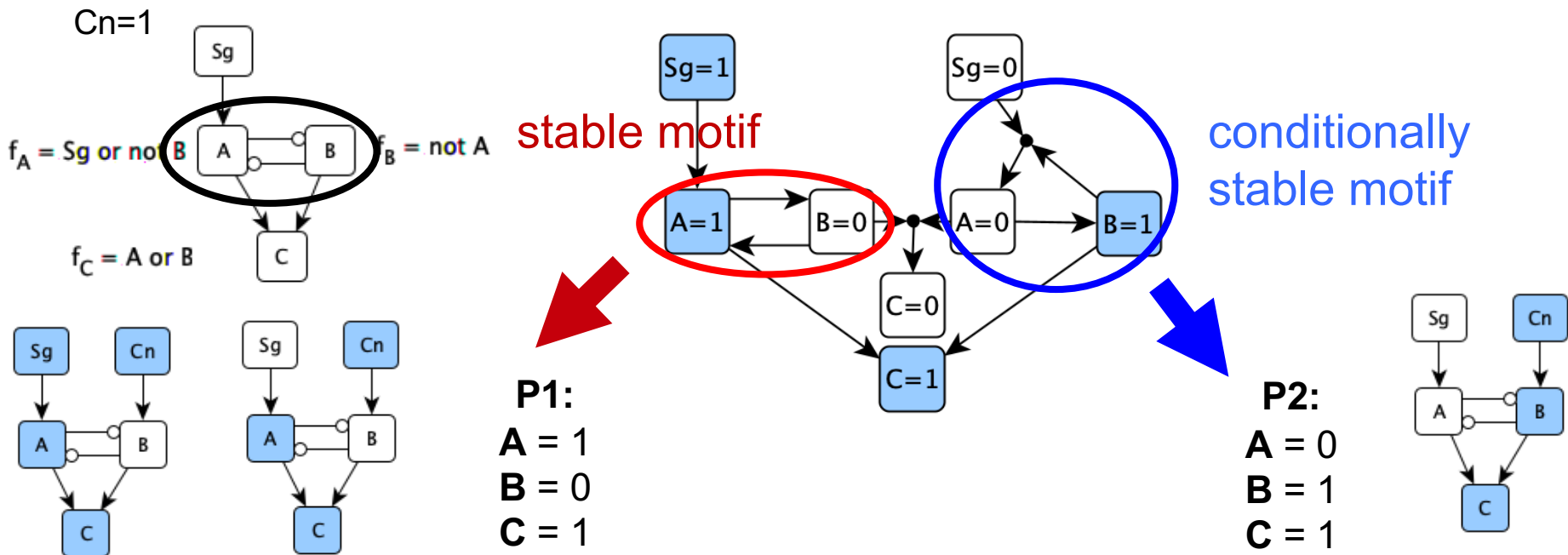
The concept of stable motif is preserved in continuous dynamical systems

ODEs of the form $\dot{x}_i = f_i(\boldsymbol{x}) - g_i(x_i)$, where g_i and f_i are monotonic in their arguments and f_i is bounded.

Each node of the (non-unique) expanded network is a threshold statement about a continuous variable x_i. Edges mean maintenance of truth.

Stable motif: a consistent strongly connected component of the expanded network.

If a system satisfies the statements that form a stable motif, it will keep satisfying them regardless of the rest of the network. This traps the system into a subspace.
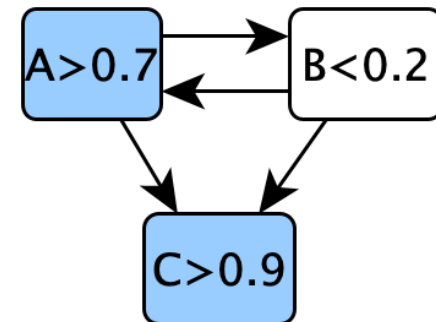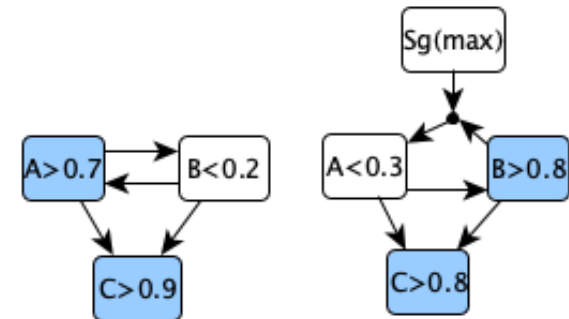

J. Rozum & R. Albert, PLOS Comp Bio 2018, J. Theor. Biol 2018, Adv. Complex Syst. 2019

Determining stable motifs in continuous dynamical systems

Each positive circuit of the interaction network provides two candidate stable motifs.

For any incident edges we consider the worst case for these edges.

We determine the thresholds using methods for monotone input-output systems by Angeli & Sontag. Two solutions indicate bistability.

D. Angeli, E. D. Sontag, Syst. & Control Let. (2004), J. Rozum & R. Albert, PLOS Comp Bio 2018, J. Theor. Biol 2018

The stable motifs of Boolean, Hill and mass action kinetics models of the restriction switch are functional in >20% of the parameter space.
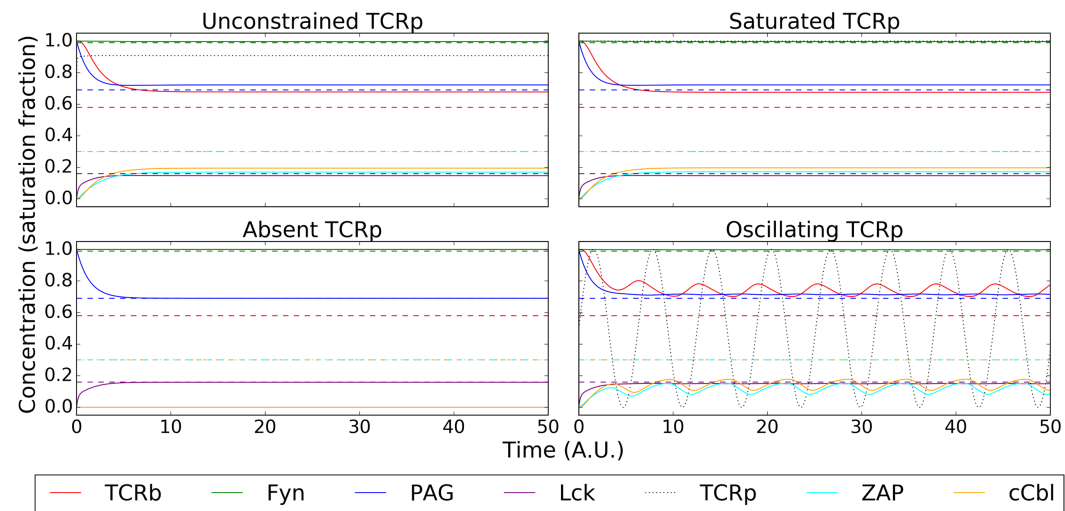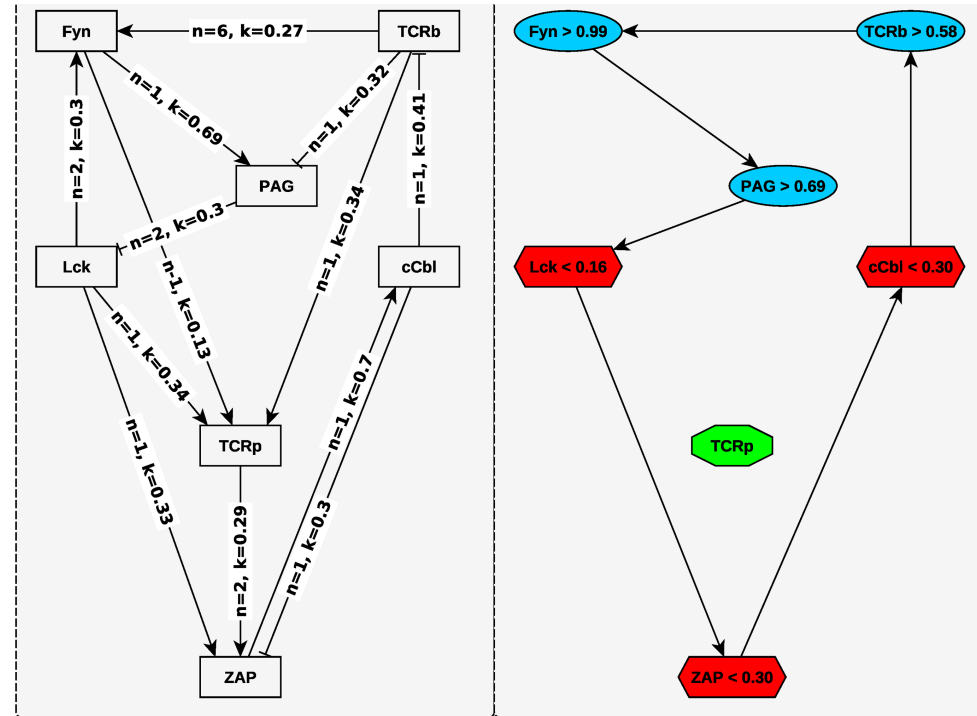
J. Rozum & R. Albert, Adv. Complex Syst. 2019

Verification of stable motifs in ODE systems

Control of a node (TCRp) that does not participate in the stable motif is not able to destabilize the stable motif.

Model: Wittman et al. BMC Systems Biology 2009

J. Rozum & R. Albert, PLOS Comp Bio 2018

Stable motifs and conditionally stable motifs

Conditionally stable motif: a cyclic sufficiency relationship that is maintained as long as a node external to the motif maintains a fixed state.

The condition may be a signal, a cellular context, or the prior lock-in of another stable motif.

Stable motifs are like irreversible switches: after they lock in, a change in signal or context does not destabilize them. Conditionally stable motifs are like reversible toggle switches.
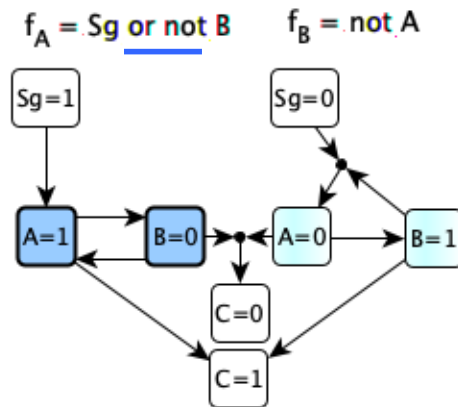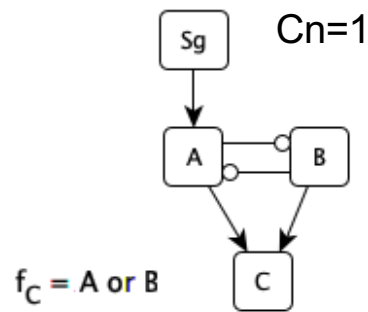
Recognize the existence of stable motifs from input-output curves.
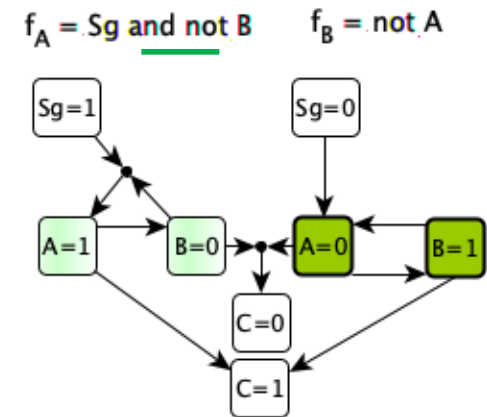
J. Tyson et al., Curr. Opin. Cell Biology (2003)

Deritei et al Sci. Rep 2019, Maheshwari et al Front. Phys. 2020
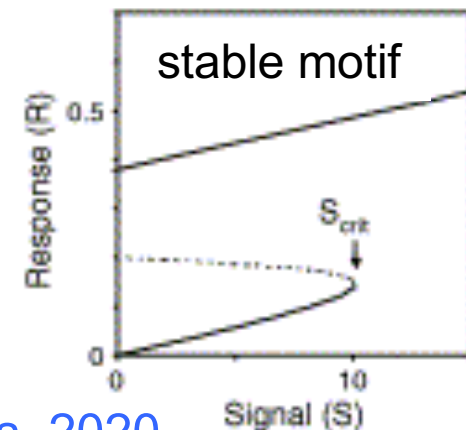
For different regulatory functions the same positive circuit yields a different pair of (conditionally) stable motifs

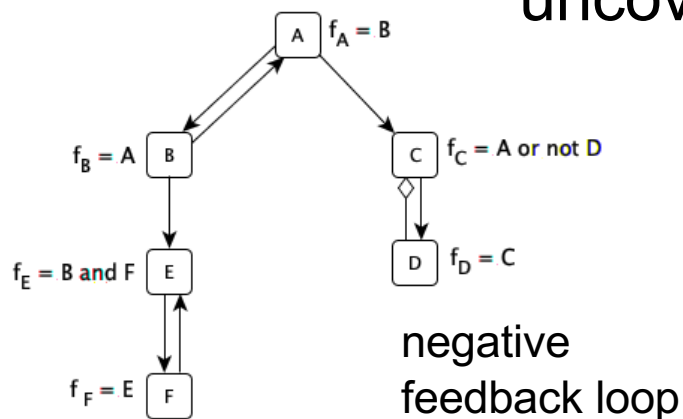Cn=1

$f_C = A\ or\ B$

$f_A = Sg\ or\ not\ B$ $f_B = not\ A$

$f_A = Sg\ and\ not\ B$ $f_B = not\ A$

P1 for both values of Sg,
P2 for Sg=0;
0(P2)→1(P1) →0(P1)
hysteresis

P1 for Sg=1
P2 for both values of Sg;
1(P1)→0(P2) → 0(P2)
hysteresis

Mapping of stable motif-determined trap spaces uncovers the phenotype repertoire of the system

negative feedback loop

oscillation
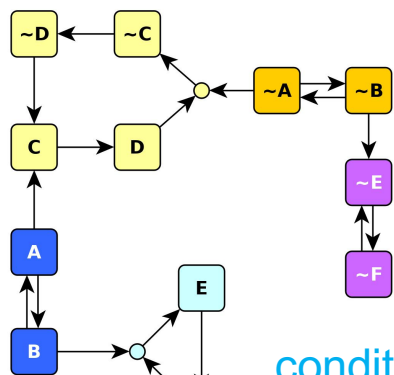
~X means X=0

conditionally stable motif

Stable motif succession diagram

Attractor 1
A=B=C=D=E=F=1

Attractor 2
A=B=C=D=1; E=F=0

Attractor 3
A=B=E=F=0; C,D Osc.

Motif avoiding attractor?
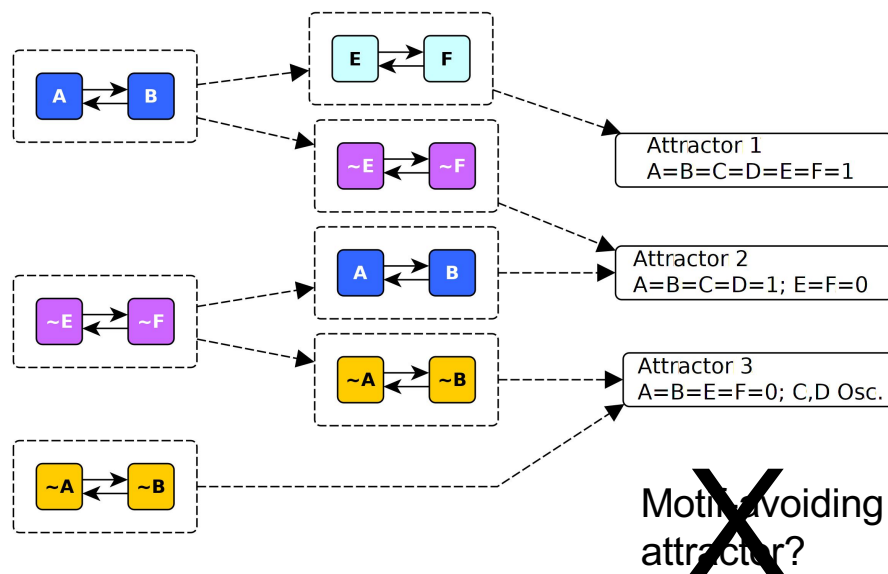
Zanudo & Albert Chaos (2013), Deritei et al Sci. Rep. (2019), Rozum et al Science Advances (2021)

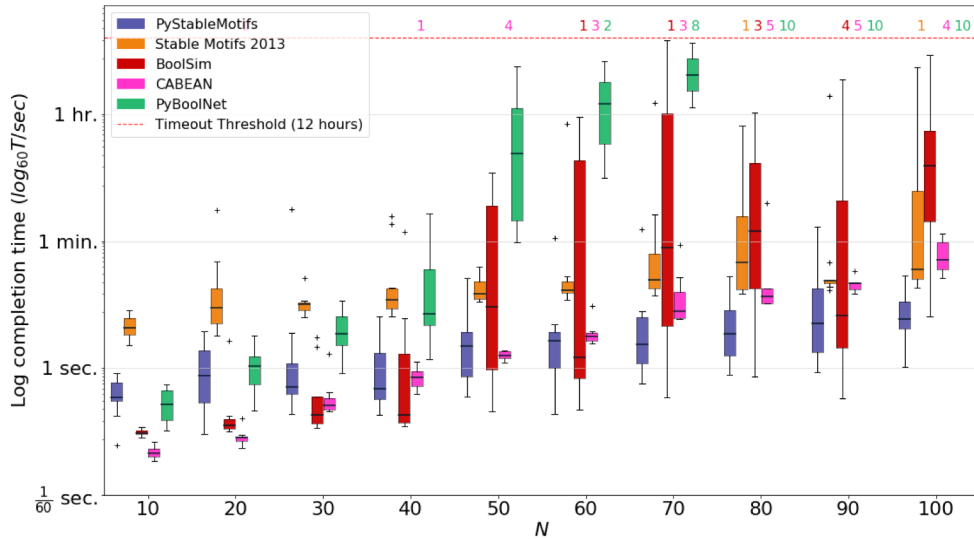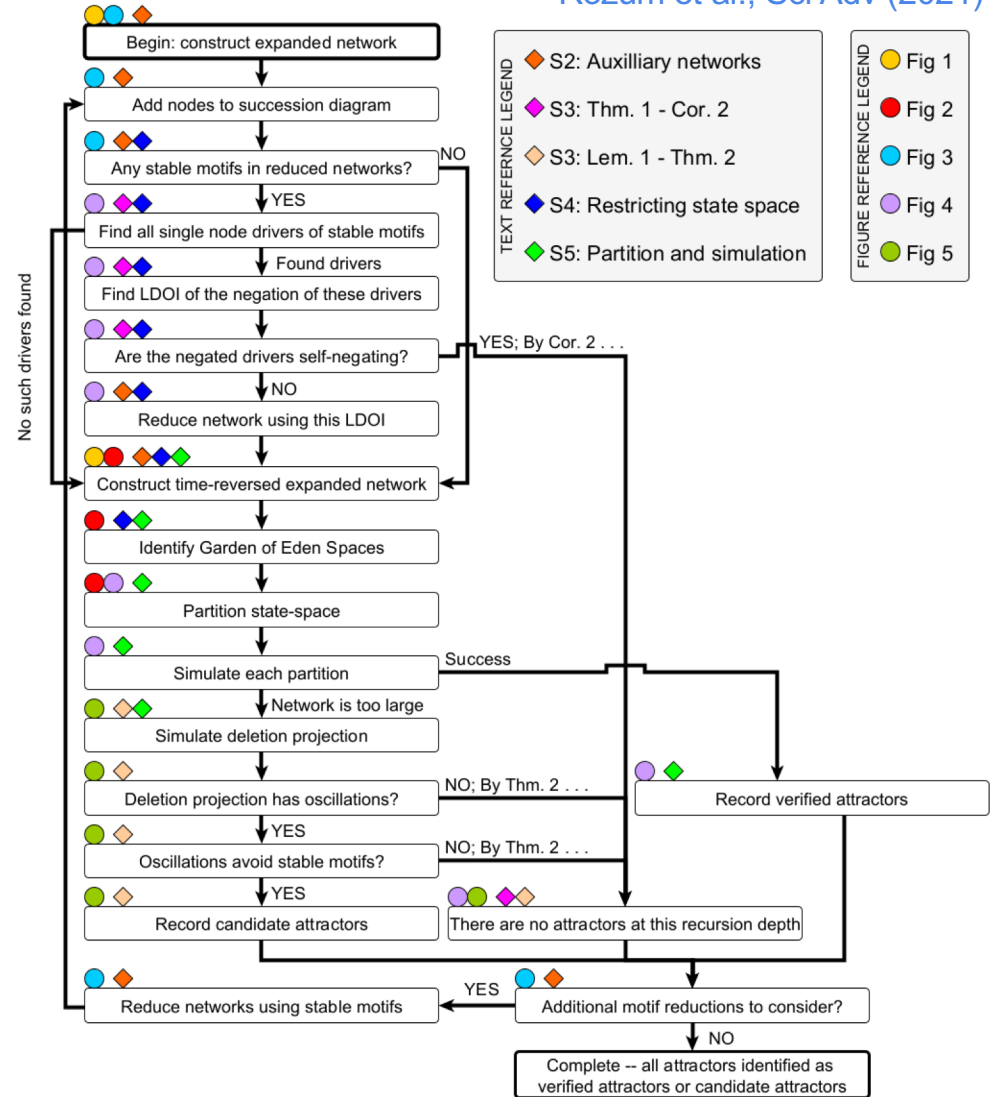Jordan Rozum gathered various Boolean expanded network techniques into a Python library called PyStableMotifs:

https://github.com/jcrozum/PyStableMotifs

Parity Expanded Network
Succession Diagram
Driver Set Constraints
Time Reversal
+
Network Reduction

———————————————

NEW algorithm

TEXT REFERNCE LEGEND
- S2: Auxilliary networks
- S3: Thm. 1 - Cor. 2
- S3: Lem. 1 - Thm. 2
- S4: Restricting state space
- S5: Partition and simulation

FIGURE REFERENCE LEGEND
- Fig 1
- Fig 2
- Fig 3
- Fig 4
- Fig 5

Begin: construct expanded network

Add nodes to succession diagram

Any stable motifs in reduced networks?

No such drivers found

YES

Find all single node drivers of stable motifs

Found drivers

Find LDOI of the negation of these drivers

Are the negated drivers self-negating?

NO

Reduce network using this LDOI

Construct time-reversed expanded network

YES; By Cor. 2 . . .

Identify Garden of Eden Spaces

Partition state-space

Simulate each partition

Success

Network is too large

Simulate deletion projection

Deletion projection has oscillations?

NO; By Thm. 2 . . .

Record verified attractors

YES

Oscillations avoid stable motifs?

NO; By Thm. 2 . . .

YES

Record candidate attractors

There are no attractors at this recursion depth

Reduce networks using stable motifs

YES

Additional motif reductions to consider?

NO

Complete -- all attractors identified as verified attractors or candidate attractors

PyStableMotifs
Stable Motifs 2013
BoolSim
CABEAN
PyBoolNet
Timeout Threshold (12 hours)

Log completion time ($log_{60}T/sec$)

1 hr.
1 min.
1 sec.
$\frac{1}{60}$ sec.

10 20 30 40 50 60 70 80 90 100

N

In the T-LGL network a single locked-in stable motif can yield cell survival
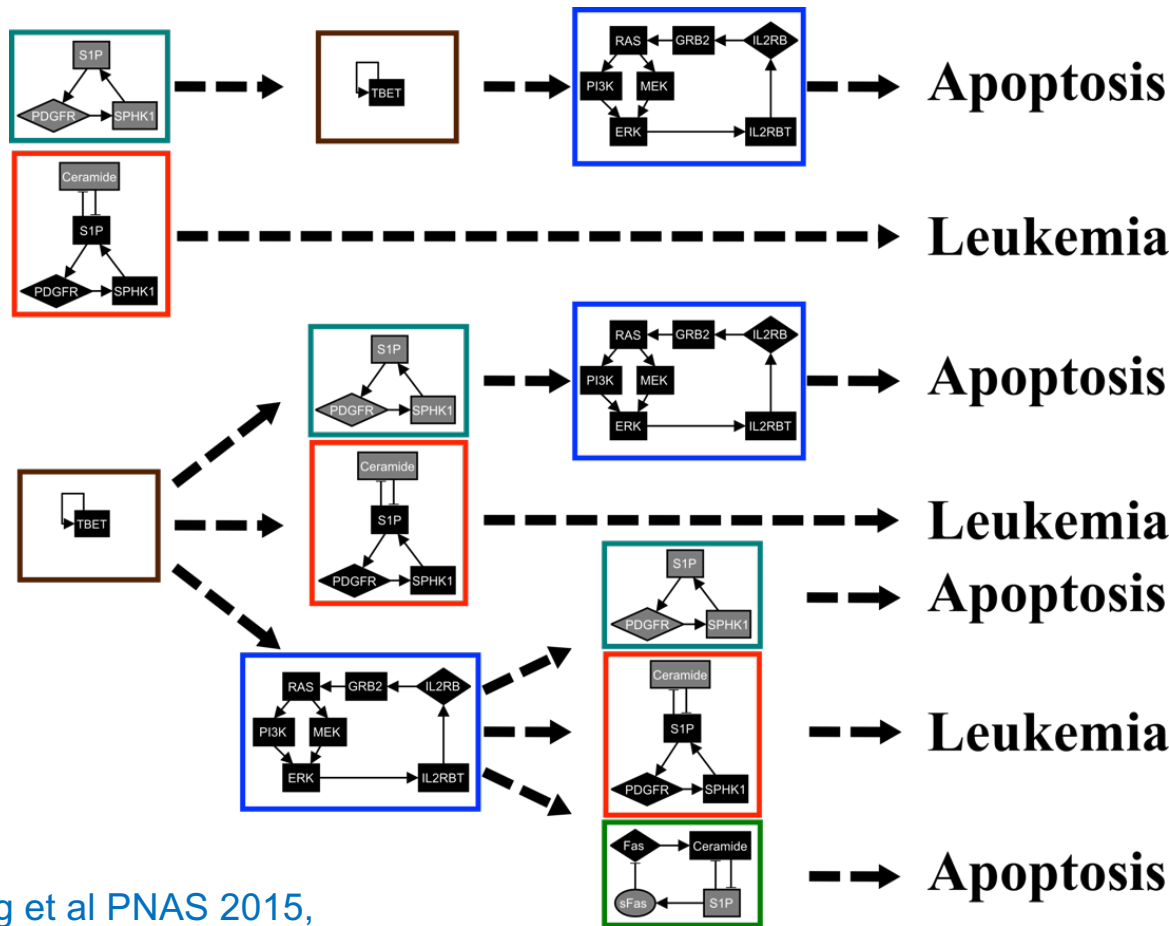
grey: OFF
black: ON

The red stable motif leads to T-LGL Leukemia attractor.

The path to Apoptosis involves the locking- in of three motifs.

No motif-avoiding attractors.

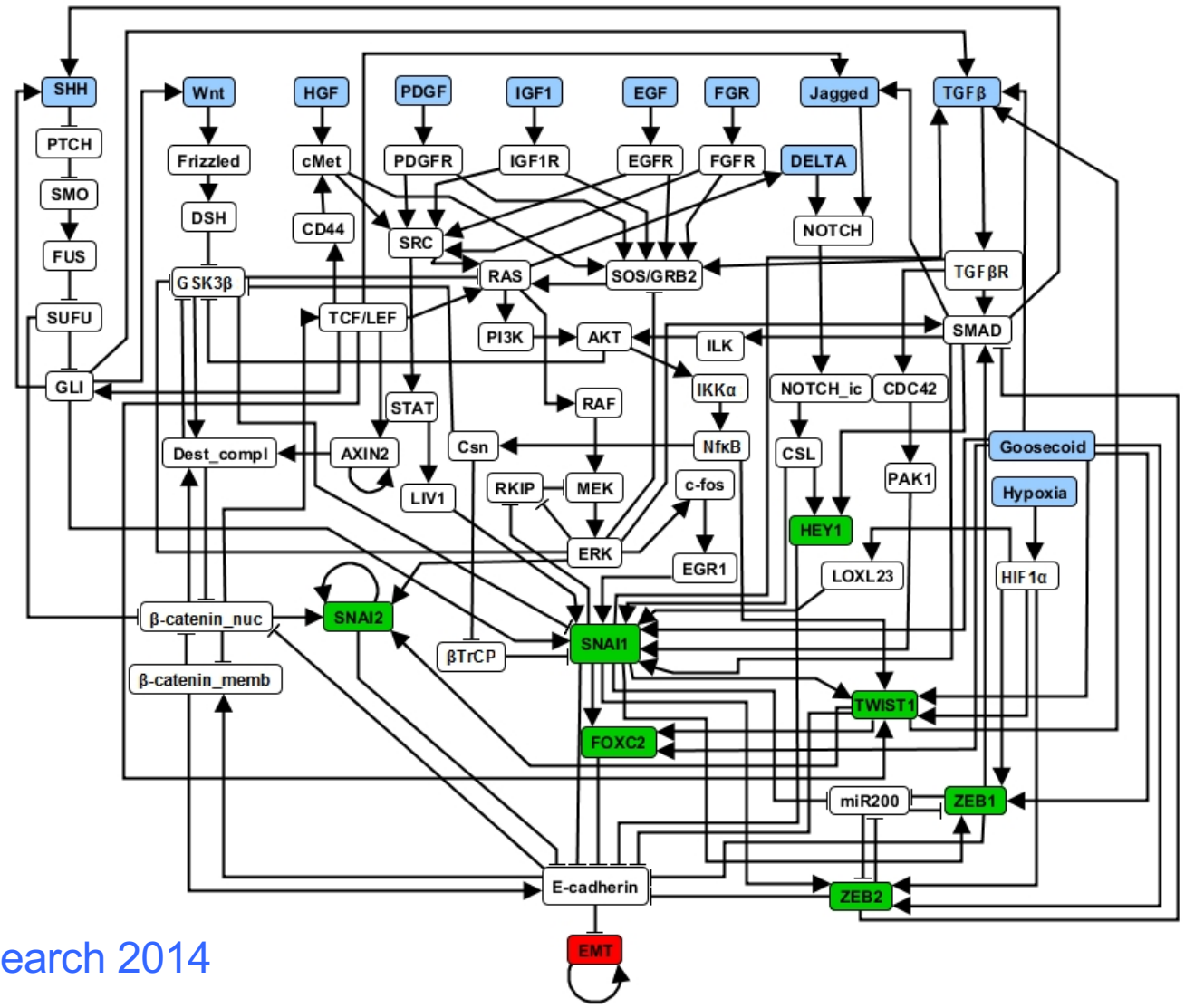Zhang et al PNAS 2015,
Zañudo & Albert PLOS Comp Bio 2015

Boolean model of epithelial to mesenchymal transition (EMT)

Blue: signals from the microenvironment
Green: transcription factors
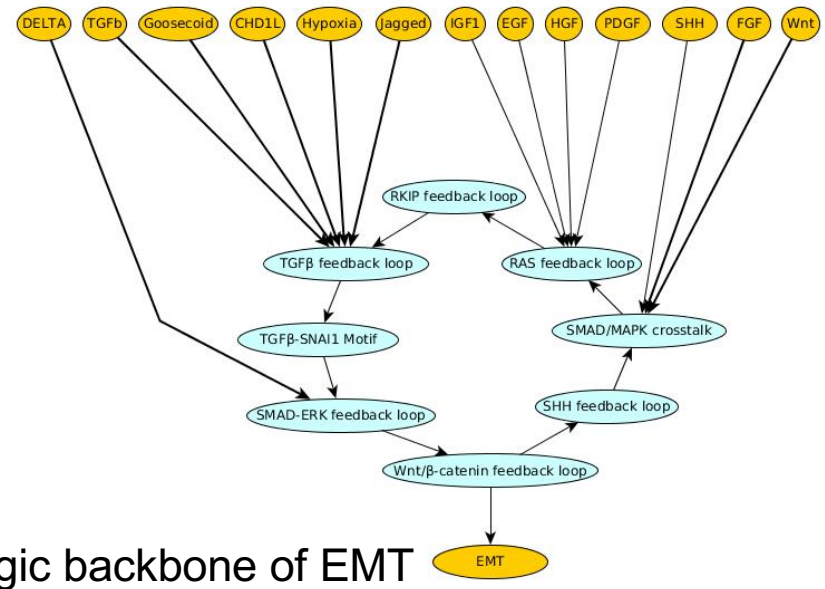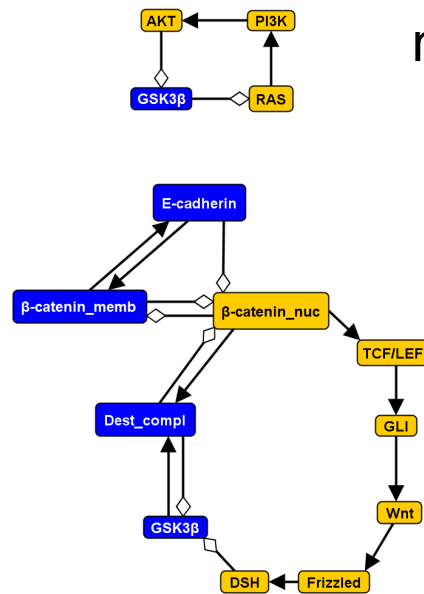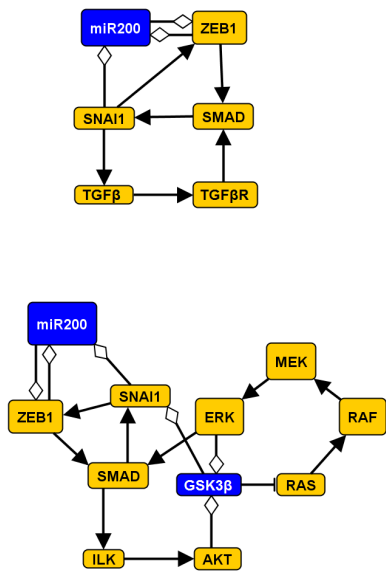Red: EMT driven by loss of E-cadherin

Large feedback-rich component, includes 5 signals (paracrine signaling).

Steinway et al, Cancer Research 2014
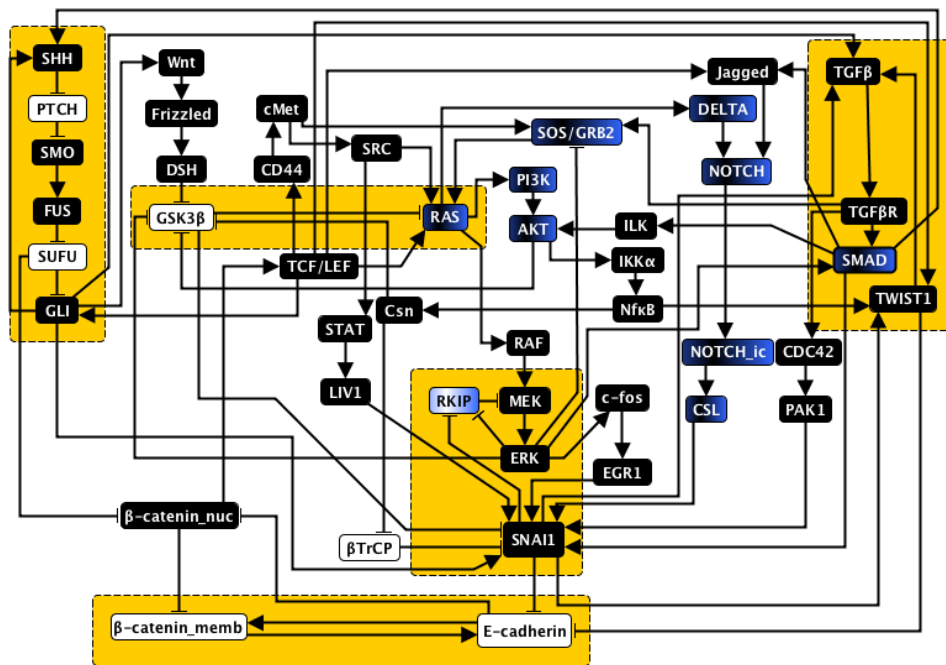
Any one of eight stable motifs yields the M state

Blue: node OFF
Yellow: node ON

Sustained M state of any one of 29 nodes activates one of the stable motifs, which yields the global M state.

Logic backbone of EMT

Steinway et al, Cancer Research 2014, Maheshwari & Albert BMC Syst. Bio 2017

A single stable motif corresponds to the epithelial (E) state

A sustained state of one node in each yellow rectangle ensures convergence to the epithelial state.
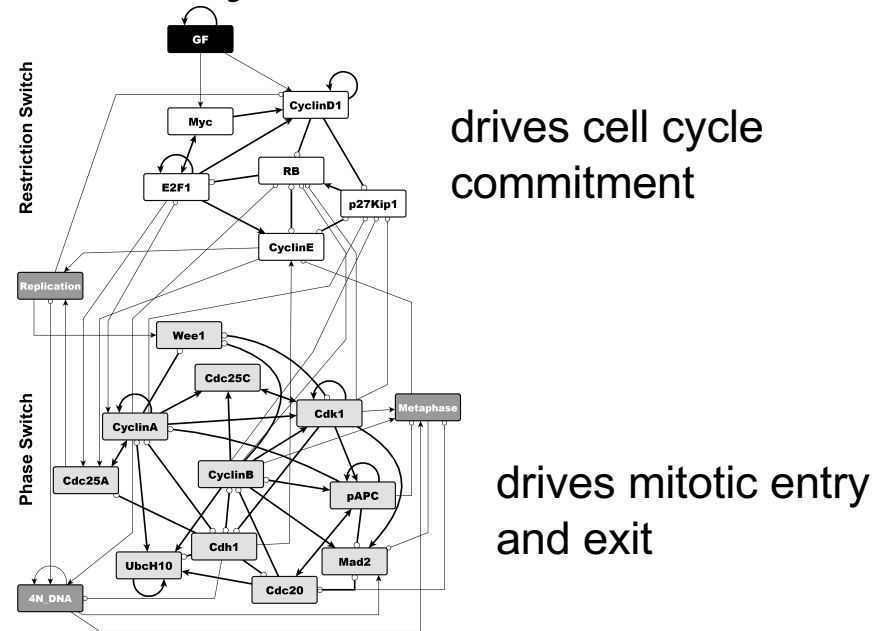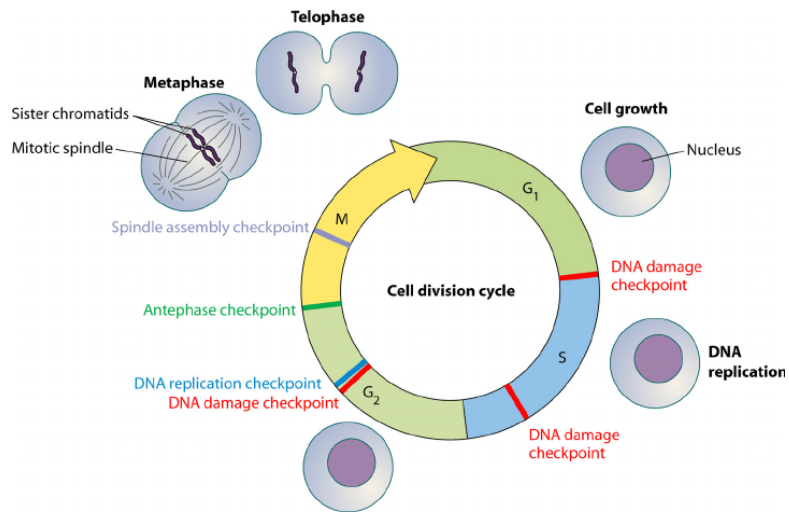e.g. SMAD, SNAI1, RAS, SHH knockout, sustained membrane localization of β-catenin

Black: OFF in the E state
White: ON in the E state
Blue: drivers of the yellow sub-motifs

Analysis of a previous Boolean model of the mammalian cell cycle

drives cell cycle commitment

drives mitotic entry and exit

Depending on the state of the Restriction Switch, the Phase Switch is capable of tristability or oscillations.

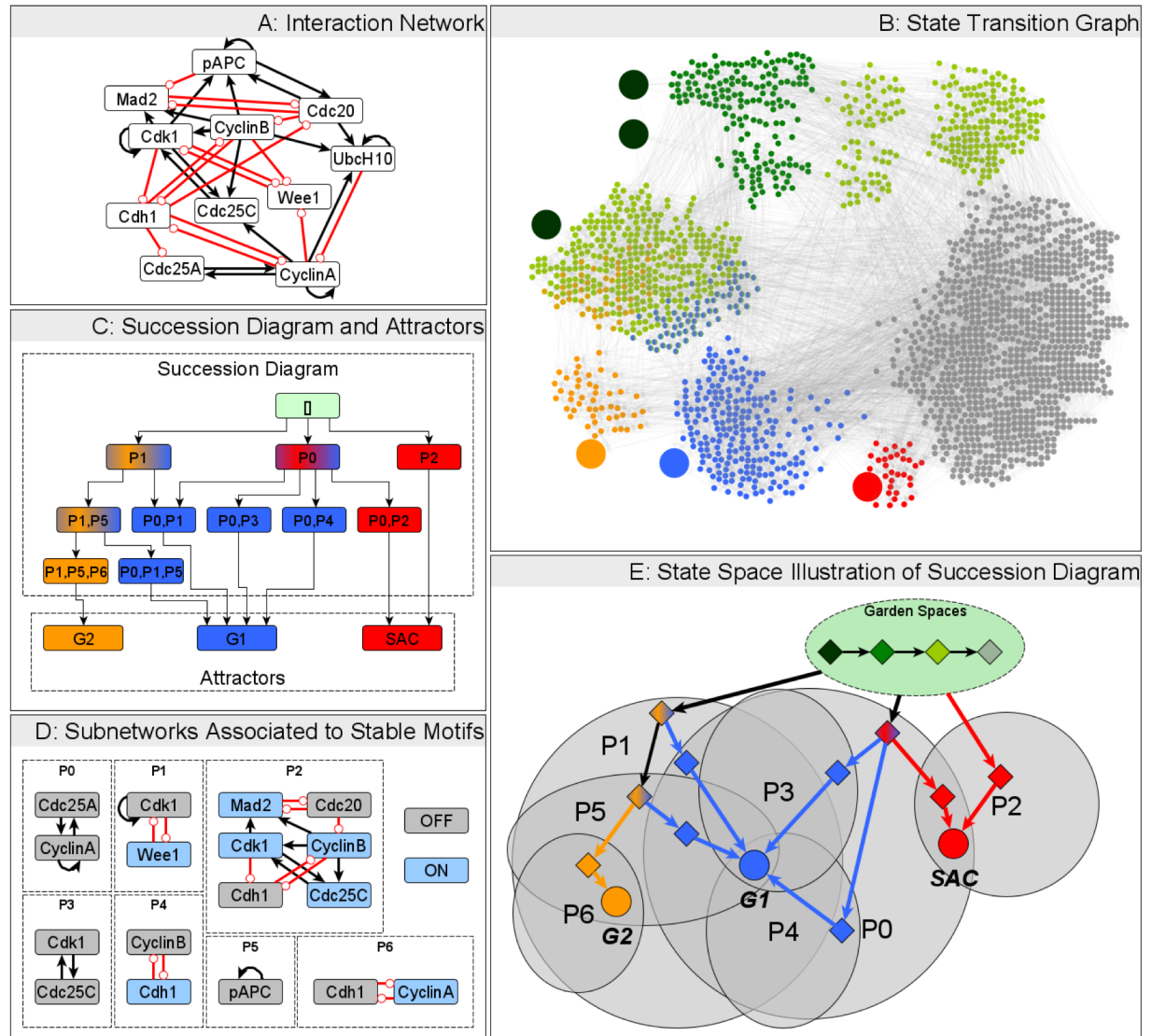D. Deritei, W. Aird, M. Ercsey-Ravasz, E. Ravasz Regan. Sci. Reports 2016

The Phase Switch as a tri-stable system

The isolated Phase Switch has 3 stable motifs and 3 conditionally stable motifs.

The successive lock-in of these motifs traps the system more and more. The three attractors correspond to cell cycle checkpoints.

The stable motifs are conditionally stable motifs of the full system.

Deritei et al., Sci Rep (2019)
Rozum et al., Sci Adv (2021)

A: Interaction Network

B: State Transition Graph

C: Succession Diagram and Attractors

Succession Diagram

Attractors

D: Subnetworks Associated to Stable Motifs

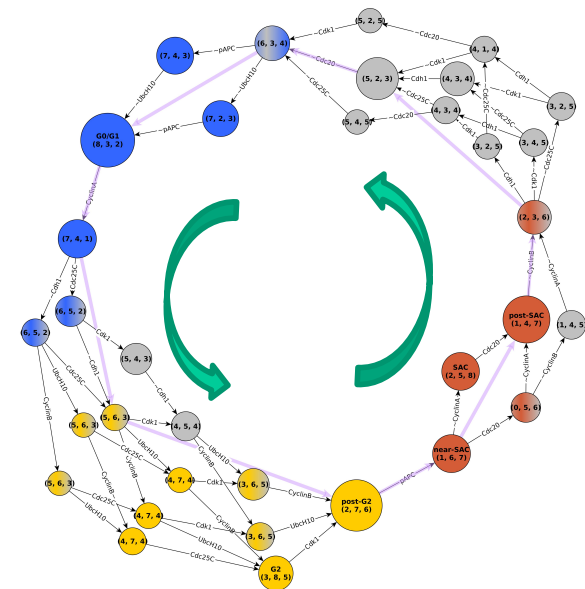E: State Space Illustration of Succession Diagram

The Phase Switch becomes an oscillator if the checkpoint-expressing nodes are disabled

Conditionally stable motif Cyc

Negative feedback
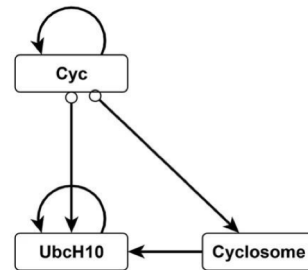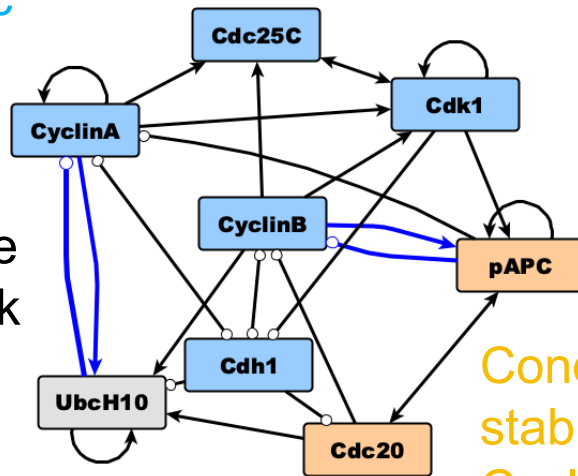
Conditionally stable motif Cyclosome

D. Deritei, J. Rozum, E. Ravasz Regan, R. Albert. Sci Reports 2019

Control of stable motifs ensures a desired fate

A sequence of stable motif lock-ins determines each attractor.

We can ensure that all initial conditions go to a desired attractor by locking in all stable motifs included in the sequences that lead to the attractor.

Reduce the number of nodes whose state needs to be fixed – find the drivers.

S1P=0 **Apoptosis**

Zañudo & Albert PLoS Comp Bio 2015, Yang et al. Front. Phys 2018

The intervention needs to be sustained just long enough to lock in the respective stable motif.

https://github.com/jcrozum/stablemotifs

Conclusions

We are analyzing which connectivity patterns of the network are critical to each attractor.

Our methodologies apply to discrete or continuous dynamics.

Positive feedback loops are important candidate phenotype- drivers.

Efficient Python implementation of attractor identification in Boolean systems:

https://github.com/jcrozum/StableMotifs

If a network has a stable motif, driving the system out of the associated attractor must involve control of nodes or edges of the stable motif.

We also implemented multiple attractor control methods in the StableMotifs library.

Acknowledgements

Jorge G T Zañudo   Stable motif analysis, control

Jordan Rozum   Attractor identification, Stable motifs of continuous systems

Steven Steinway   EMT model and experiments

Xiao Gan   Stable motifs in multi-level dynamics

Dávid Deritei   Conditionally stable motifs

Erzsébet Ravasz- Regan   Cell cycle model

Gang Yang   Logic domain of influence

Parul Maheshwari   Logic backbone

Fatemeh Nasrollahi   Stable motif influence network

David Wooten   Data-driven model construction