Analyzing interconnections of asynchronous Boolean networks with biological applications*

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Asynchronous Boolean models of biological regulatory networks

- Each variable is either absent 0 or present 1
  - ✓ State space is finite $\Omega = \{0,1\}^n$
  - ✓ Time is discrete $t = 0,1,2,\dots$
  - ✓ Biological interactions represented by logical rules

- At each time, only one variable is updated
  - ✓ Generate asynchronous transition graph
  - ✓ Contains all possible trajectories
  - ✓ Much more realistic than synchronous updating (takes into account different time scales)

- Apply graph theoretical / probabilistic tools
  - ✓ SCC decomposition + topological sorting
  - ✓ Attractor computation (terminal SCC)
  - ✓ Add probabilities: absorbing Markov chains

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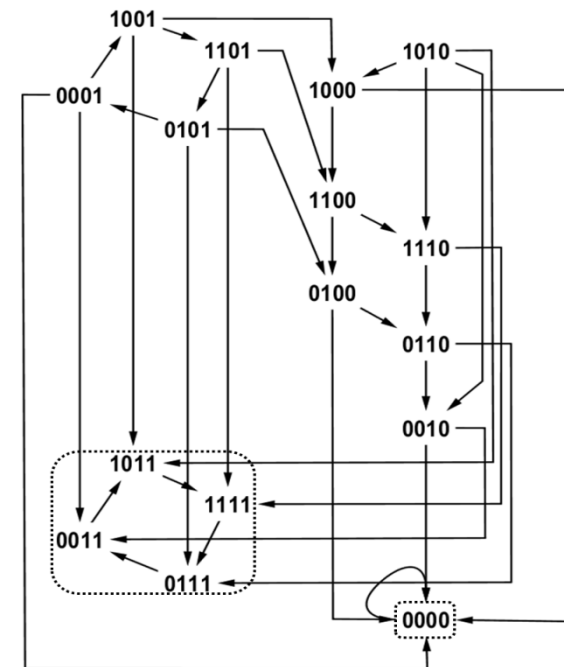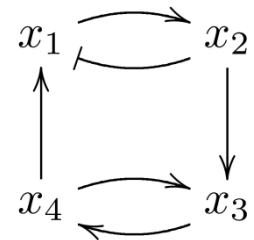
Interconnection of Boolean networks

- ## A computational limitation of Boolean networks

The state space $\Omega = \{0,1\}^n$ is *finite*, but grows **exponentially** with the number of variables: $n \geq 20$ variables, $2^n > 1\,000\,000$ states

- ## An observation: *modularity* of biological networks

Ex: cell cycle & circadian clock are two central regulatory networks in eukaryotic cells: (how) do they dynamically interact? (Icycle Project, 2017-20)

- ## A classical idea (systems theory): interconnections of modules

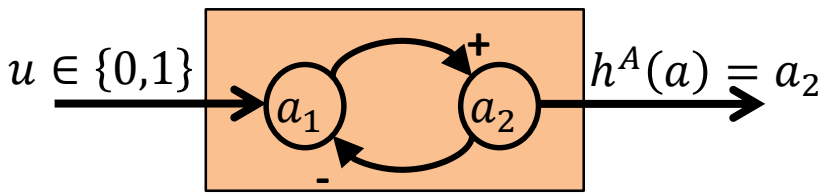Reduce the complexity by "breaking" the network into smaller subnetworks
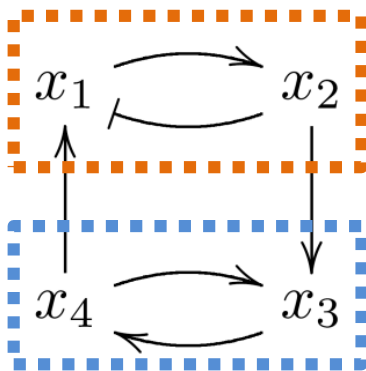
> ➤ Can we obtain some knowledge on the **dynamical behavior** of the interconnected network from the dynamics of each module?

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Predicting the attractors of an interconnection
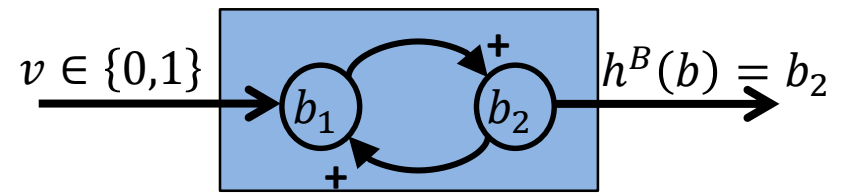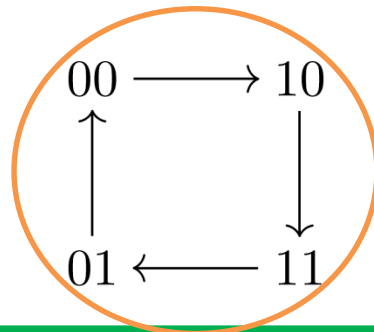

Subnetwork A: a **negative** SISO feedback loop

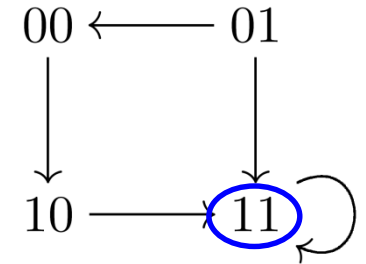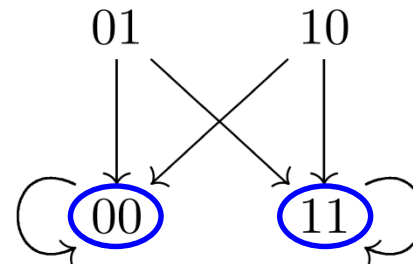$$f_1^A(a,u) = u \wedge \neg a_2$$
$$f_2^A(a,u) = a_1$$

$u = 0$

$u = 1$
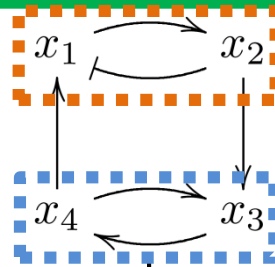
Subnetwork B: a **positive** SISO feedback loop

$$f_1^B(b,v) = v \vee b_2$$
$$f_2^B(b,v) = b_1$$

$v = 0$

$v = 1$

$u \in \{0,1\}$ $h^A(a) = a_2$

$v \in \{0,1\}$ $h^B(b) = b_2$

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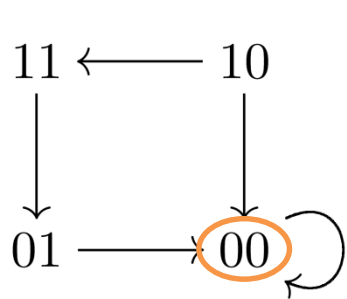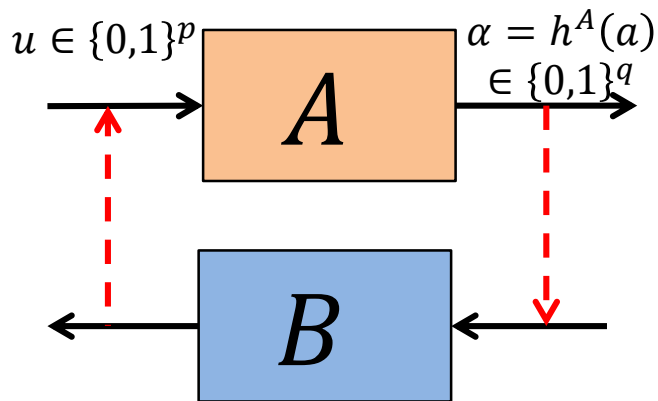
The asymptotic graph method

[Tournier, Chaves. *Automatica*, 2013]
[Chaves, Tournier. *Frontiers in Physiology*, 2018]

$u \in \{0,1\}^p$ → A → $\alpha = h^A(a) \in \{0,1\}^q$

B

Input-output Boolean module:

State space: $a \in \Omega = \{0,1\}^n$

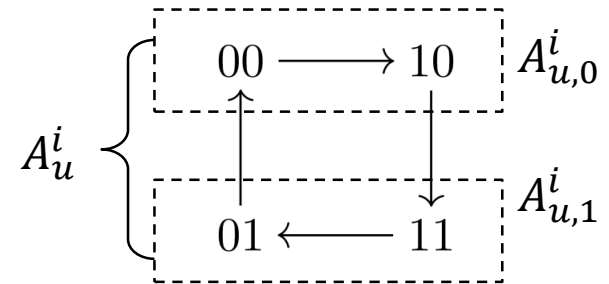Boolean rules: $f^A(a,u) : \{0,1\}^n \times \{0,1\}^p \to \{0,1\}^n$

Boolean output: $h^A(a) : \{0,1\}^n \to \{0,1\}^q$

A_u^i = the i-th attractor of module A under input u

- Separate each attractor into *same-output* sets:

$$A_u^i = \bigcup_\alpha A_{u,\alpha}^i$$

$A_u^i \begin{cases} \\ \\ \end{cases}$

$00 \longrightarrow 10$ $A_{u,0}^i$

$01 \longleftarrow 11$ $A_{u,1}^i$

- Define the set $V^{as} = \left\{ A_{u,\alpha}^i \times B_{v,\beta}^j \right\}$ of all cross-products of semi-attractors

- Next step: build a graph over V^{as} called **asymptotic graph**
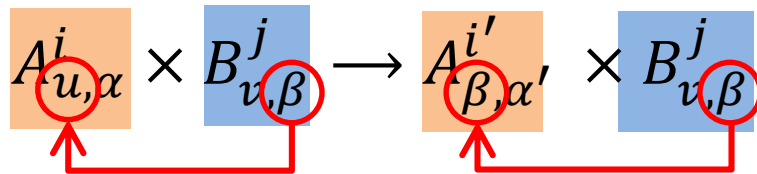
The asymptotic graph method

$$A_{u,\alpha}^i \times B_{v,\beta}^j \longrightarrow A_{u,\alpha}^i \times B_{\alpha,\beta'}^{j'}$$
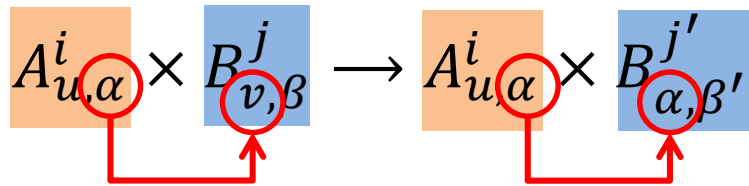
If and only if:

$\exists b \in B_{v,\beta}^j: \exists b' \in B_{\alpha,\beta'}^{j'}$: such that b leads to b' in graph $G^{B,\alpha}$

Generate a graph $\mathbf{G}^{as}$

called **asymptotic graph**

$$A_{u,\alpha}^i \times B_{v,\beta}^j \longrightarrow A_{\beta,\alpha'}^{i'} \times B_{v,\beta}^j$$

If and only if:

$\exists a \in A_{u,\alpha}^i: \exists a' \in A_{\beta,\alpha'}^{i'}$: such that a leads to a' in graph $G^{A,\beta}$
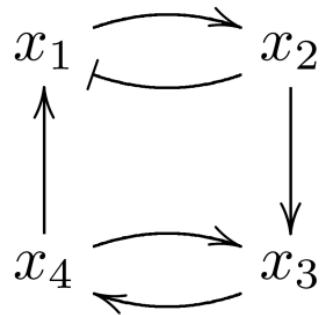
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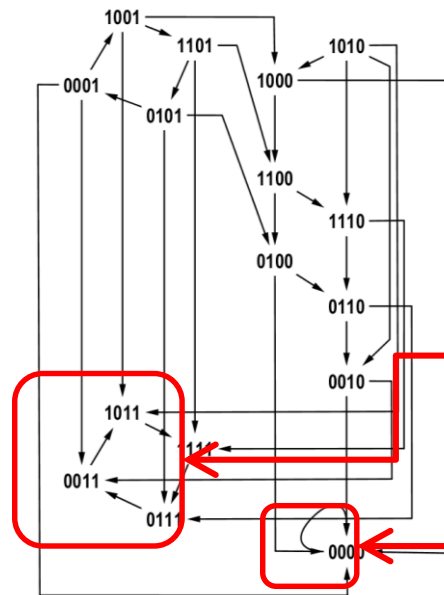
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The asymptotic graph method

Theorem 1. [Tournier & Chaves, *Automatica*, 2013]
If Q is an attractor of the interconnection, then there exists a terminal SCC R of the asymptotic graph such that states$(R) \subseteq Q$.
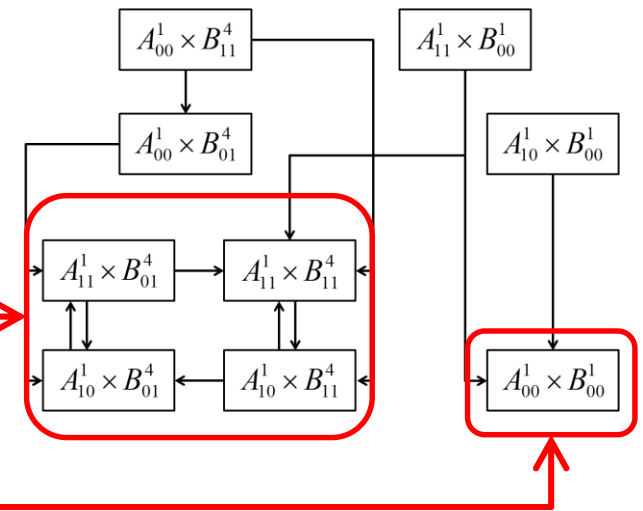
➢ The asymptotic graph contains a representative of **each attractor** of the interconnection. In other words: we do not "miss" any attractor
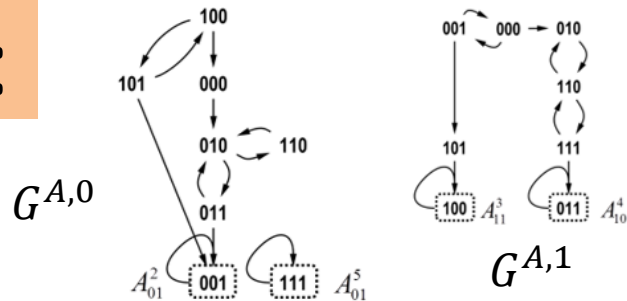

Full dynamics

Asymptotic graph

The asymptotic graph method

Downside: we may get "spurious" attractors

A:

$G^{A,0}$

$G^{A,1}$

B:

$G^{B,0}$

$G^{B,1}$

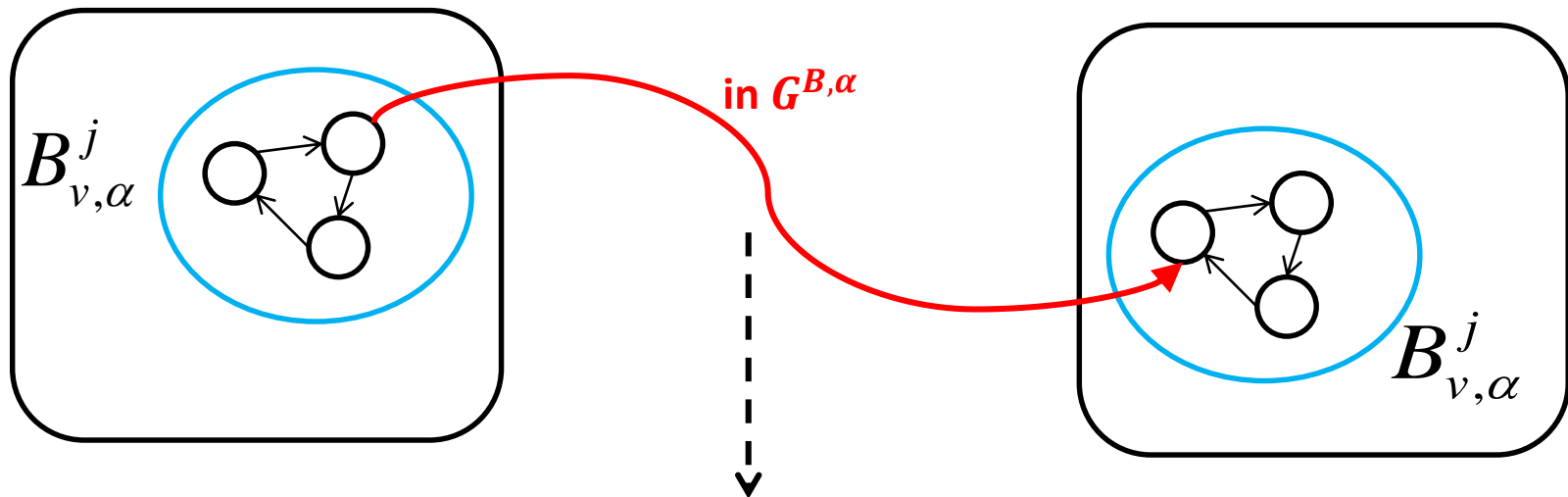Spurious attractor Real attractors
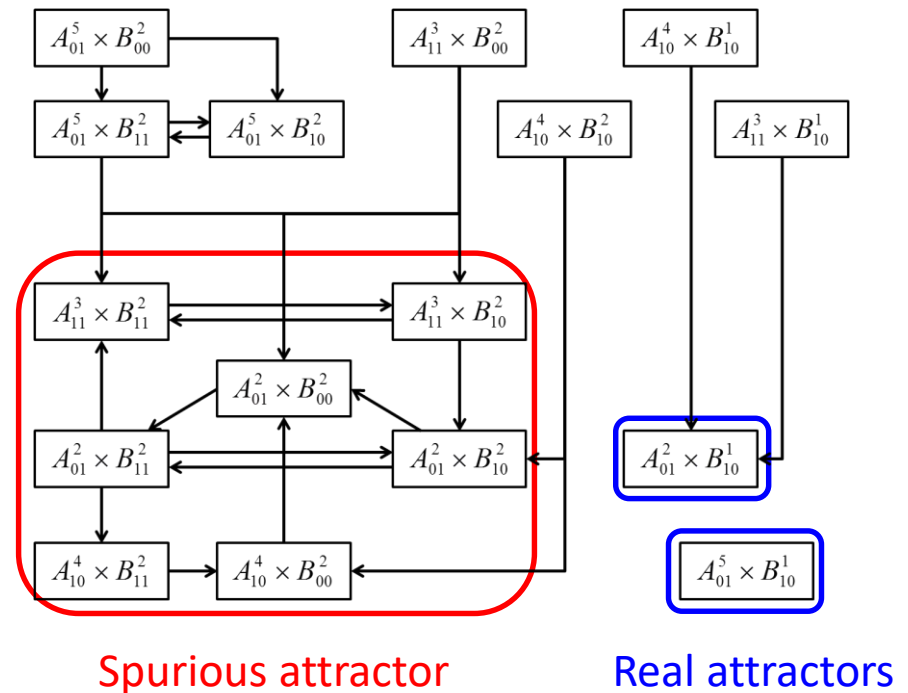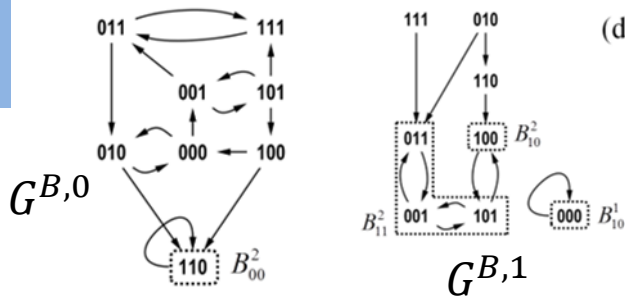
[Tournier & Chaves 2013; Chaves & Carta 2015]: different sufficient conditions to rule out spurious. The most simple one: **singletons** cannot be spurious.

Observation: the asymptotic graph is constructed **only from the asymptotic behaviors** of each module (no transient)

$$A_{u,\alpha}^{i} \times B_{v,\beta}^{j} \longrightarrow A_{u,\alpha}^{i} \times B_{\alpha,\beta'}^{j'} \qquad \Leftrightarrow B_{\alpha,\beta'}^{j'} \text{ is reachable from } B_{v,\beta}^{j} \text{ in } G^{B,\alpha}$$

in $G^{B,\alpha}$

$B_{v,\alpha}^{j}$

$B_{v,\alpha}^{j}$

Assumption: the transient is ignored (*ie*: infinitely rapid)

The cross graph method

- $G^{A,u}$: transition graph of module A under input u.
  SCC decomp. $\rightarrow Z^{A,u} = \{A_u^i, 1 \leq i \leq N^{A,u}\}$ partition of the state space Ω^A

- Define: $Z^A = \bigwedge_{u \in \{0,1\}^p} Z^{A,u} = \{A^1, A^2, \dots\}$
  (the coarsest partition of Ω^A that is finer than every SCC decomposition)

- Further refine Z^A by cutting each set with respect to outputs
  We obtain a final partition $Z_h^A = \{A_\alpha^i : 1 \leq i \leq N^A, \alpha \in \{0,1\}^{q_A}\}$

- Define $V^{cr} = \{A_\alpha^i \times B_\beta^j\}$ and the arcs:

  - $A_\alpha^i \times B_\beta^j \rightarrow A_{\alpha'}^{i'} \times B_\beta^j$ iff $\exists a \in A_\alpha^i, a' \in A_{\alpha'}^{i'}$
    such that $a \rightarrow a'$ in graph $G^{A,\beta}$.

  - $A_\alpha^i \times B_\beta^j \rightarrow A_{\alpha'}^{i'} \times B_{\beta'}^{j'}$ iff $\exists b \in B_\beta^j, b' \in B_{\beta'}^{j'}$
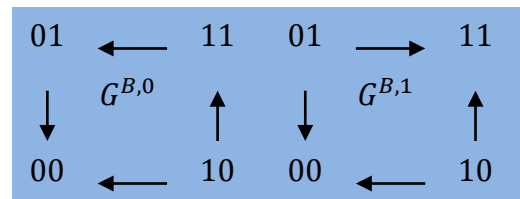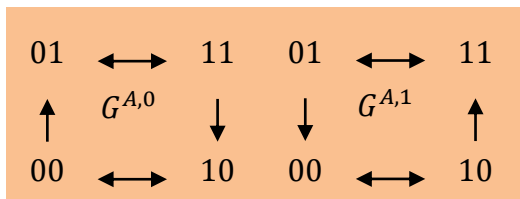   such that $b \rightarrow b'$ in graph $G^{B,\alpha}$.

Cross-graph G^{cr}
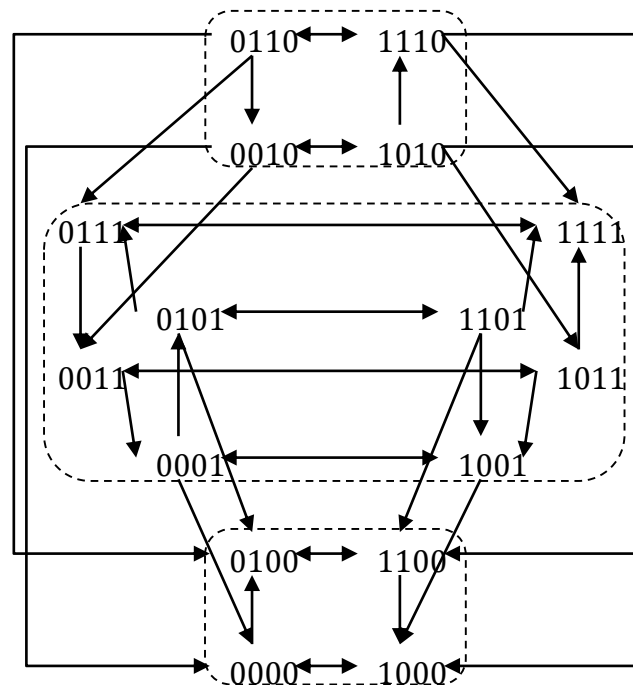
The cross graph method

Theorem 2. [Chaves & Tournier, *Front Physiol* 2018]
The cross-graph G^{cr} and the full transition graph G of the interconnection have the same SCC decomposition. Furthermore, terminal SCCs of G^{cr} fully recover the attractors of the interconnection.
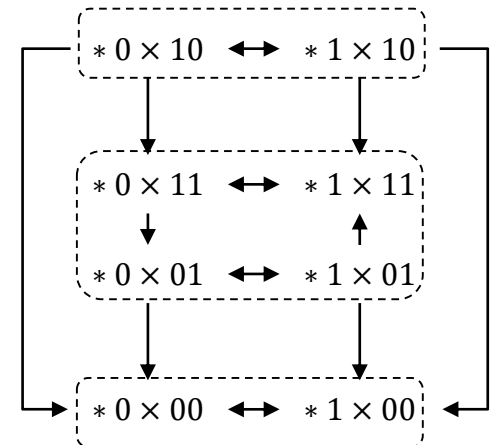
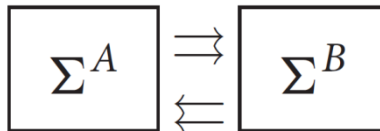

Example

Full dynamics

Cross graph

Performance, complementarity of the methods

Generate random IO networks with average connectivity:

- n variables, p inputs, q outputs
- Inner connectivities k_i picked at random, *e.g.* according to a binomial distribution $\mathcal{B}\left(n + p, \frac{K_{mean}}{n+p}\right)$

Dataset 1: 2000 random interconnections

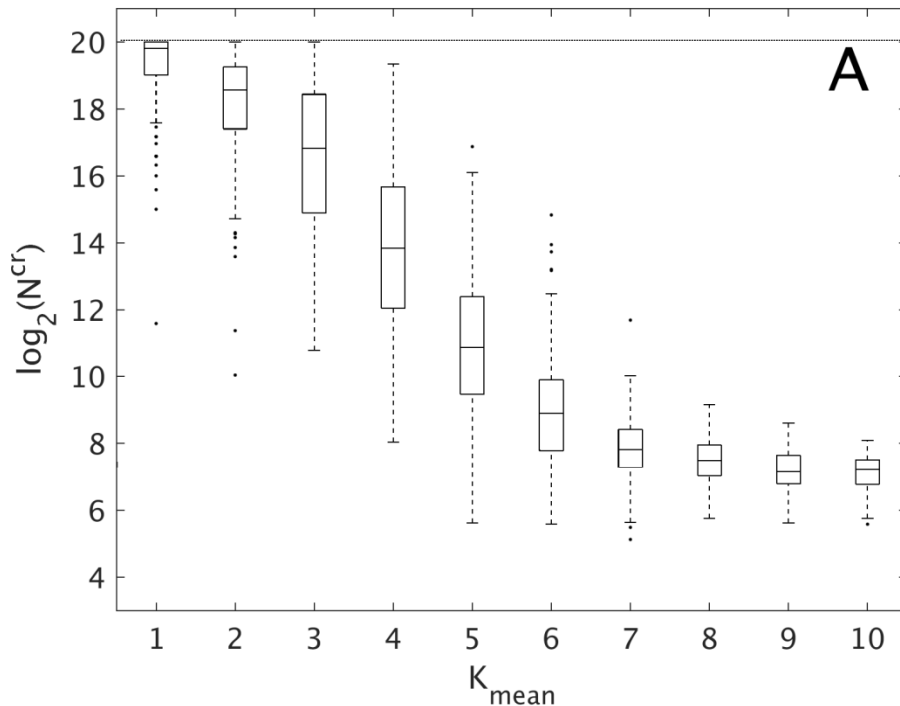Transition Graph G of the full interconnected network: $N(G) = 2^{20}$

With:

- $n_A = n_B = \mathbf{10}$ variables,
- $p_A = p_B = \mathbf{2}$ inputs and $q_A = q_B = \mathbf{2}$ outputs,
- Average connectivity K_{mean} varying in $\{1, \dots, 10\}$

Performance, complementarity of the methods

Size G^{cr}

Size G^{as}
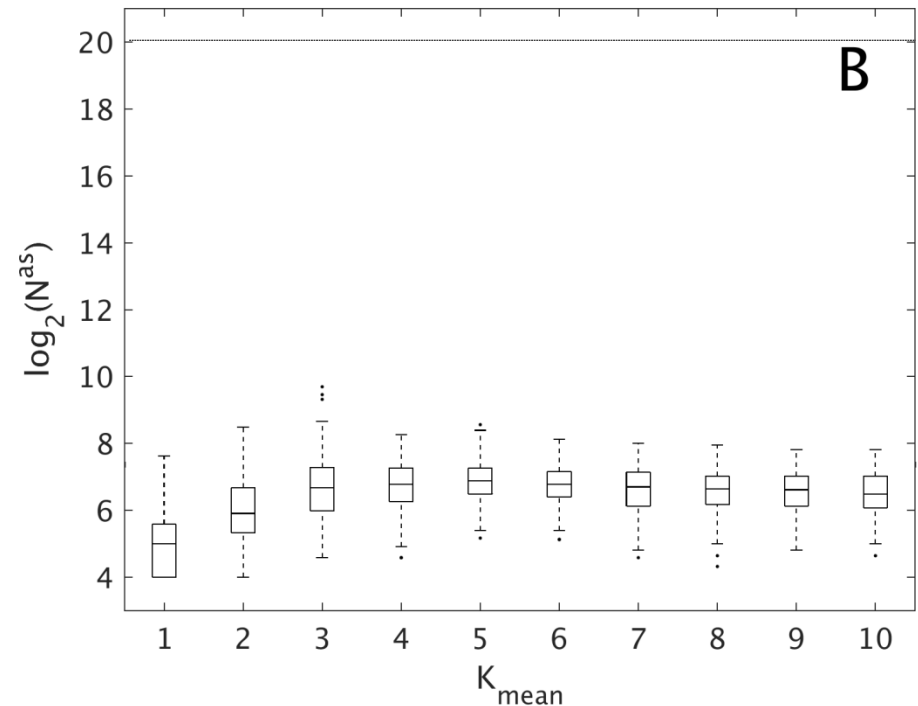
Performance, complementarity of the methods

Execution times

Performance, complementarity of the methods

Quality of the predictions of G^{as}

11 out of 2000 interconnections showed spurious attractors (0.05%)

Mean accuracy = 0.86

Performance, complementarity of the methods

Dataset 2: 200 random interconnections
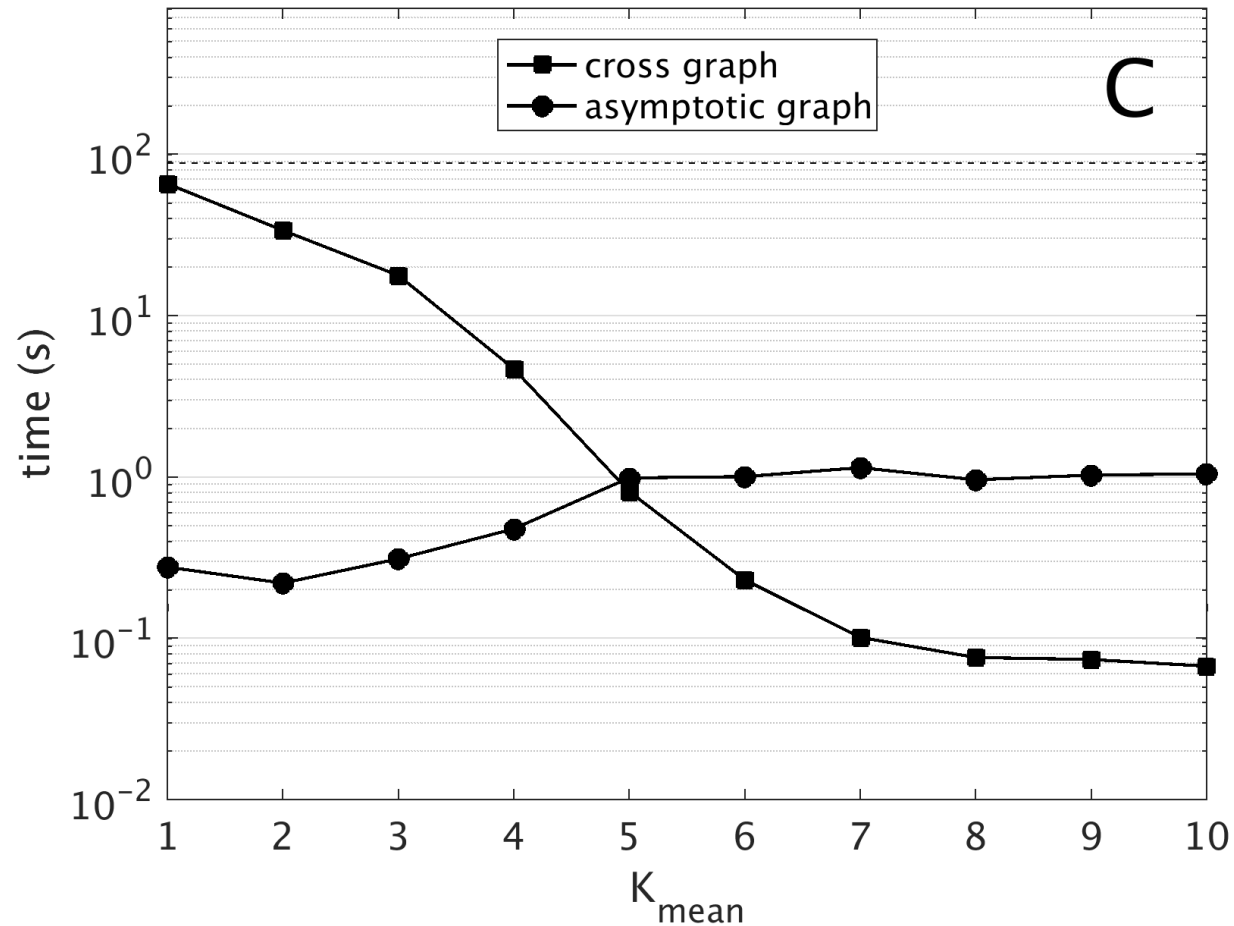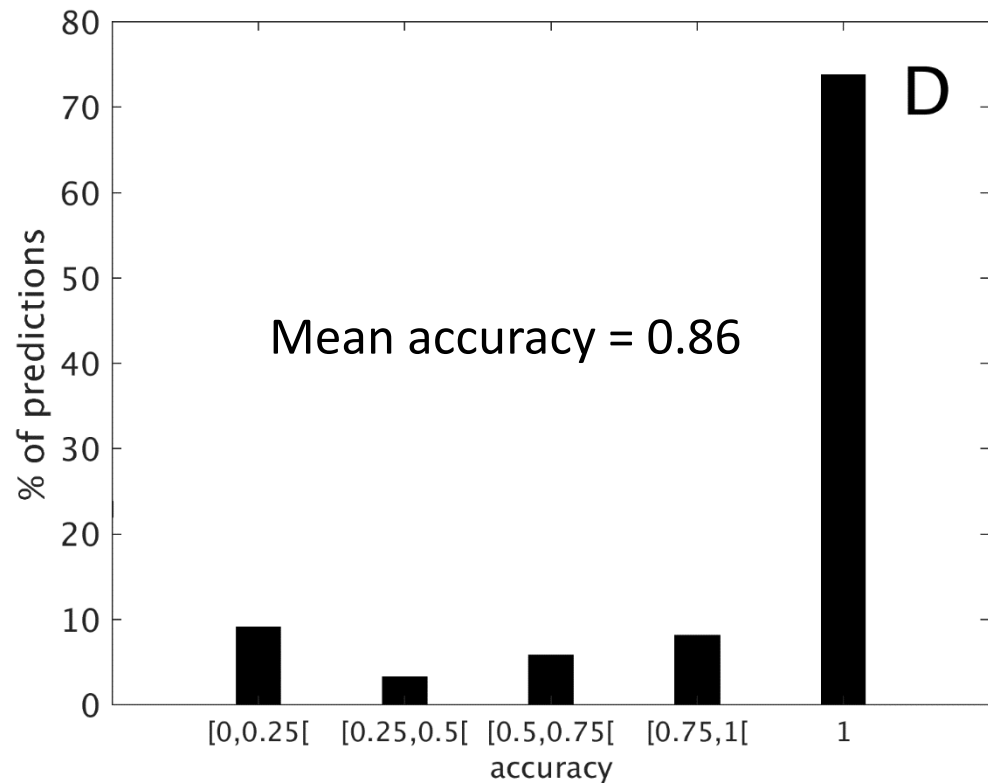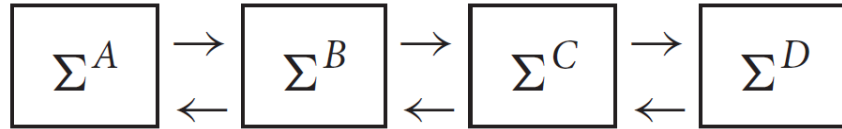
$$\boxed{\Sigma^A} \xrightarrow{\ } \boxed{\Sigma^B} \xrightarrow{\ } \boxed{\Sigma^C} \xrightarrow{\ } \boxed{\Sigma^D}$$

Full state space = $2^{60}(> 10^{18})$!

- $n_A = n_B = n_C = n_D = 15$ variables,
- SISO interconnections
- Average connectivity K_{mean} varying in $\{1, \dots, 5\}$

K_{mean}	$\log_2(N^{cr})$		Time (s)		$\log_2(N^{as})$		Time (s)	
	mean	std	mean	std	mean	std	mean	std
1	57.3	2.3	–	–	8.5	1.4	9	2
2	52.2	4.1	–	–	9.8	1.1	9	7
3	42.4	5.7	–	–	11.0	1.4	63	185
4	29.6	5.9	493	361	11.3	1.1	40	51
5	20.9	4.7	176	223	11.0	1.0	27	38

No spurious were detected (when computable)

Application 1: model reduction
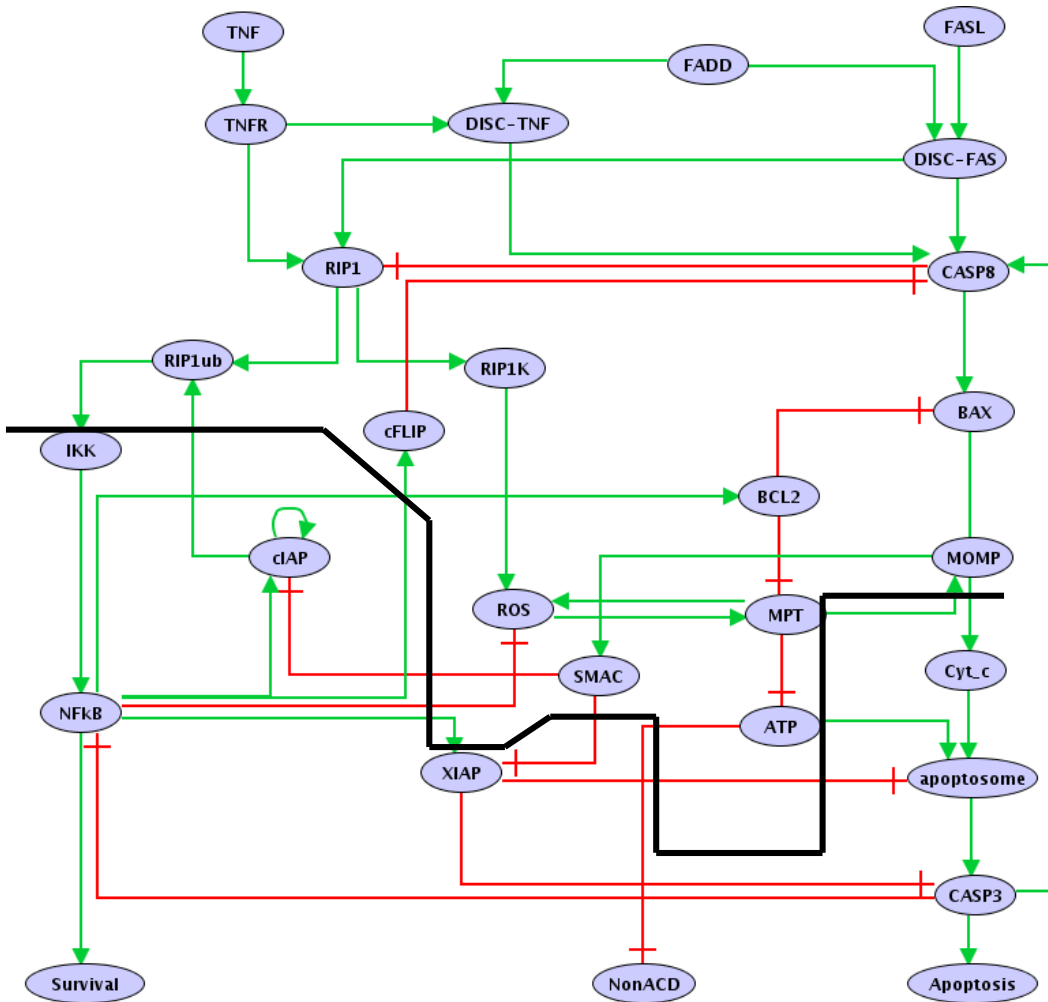
A cell fate decision network (apoptosis/necrosis/survival) [Calzone, Tournier *et al*, *PLoS Comp Biol* 2010]

Full network: $2^{22} \approx 4.10^6$
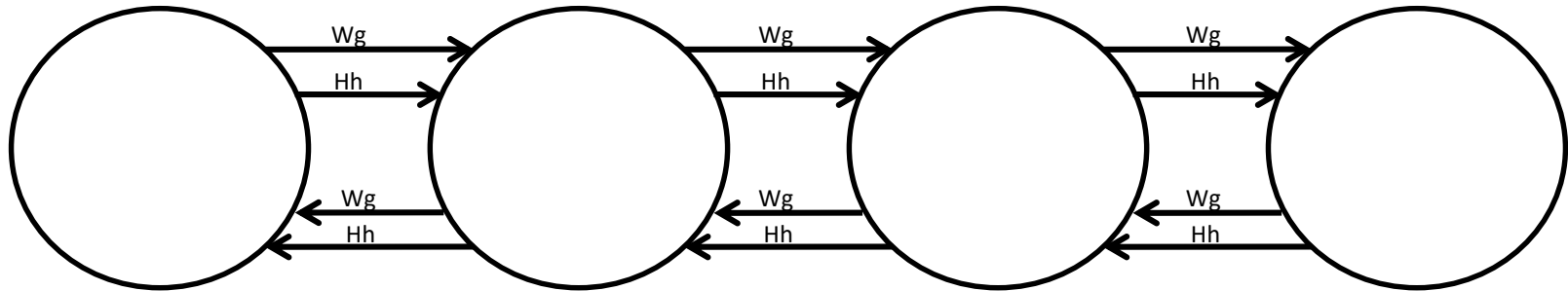
Asymptotic Graph method:

$$2 \times \underbrace{(2^3 \times 2^{11})}_{\text{modules}} + \underbrace{120}_{G^{as}} \approx 33000$$

Remark. How to cut a network efficiently? Graph partitioning / clustering techniques

- Spectral clustering
- Hierarchical clustering
- *etc*

Application 2: multicellular models, morphogenesis

**Segment polarity gene network
in *D. melanogaster***
(adapted from [Albert&Othmer, 2003])
- 7 inner variables
- 2 diffusing proteins: *Wg*, *Hh*

4 cells segment (4x7=28)
- Full network = $2,6.10^8$ states
- Asympt. graph = 7448 nodes

Application 3: connecting known biological modules

ANR Project ICycle: Interconnection and feedback control of two cyclic modules in mammalian cells (cell cycle – circadian clock). Inria-INRA-CNRS
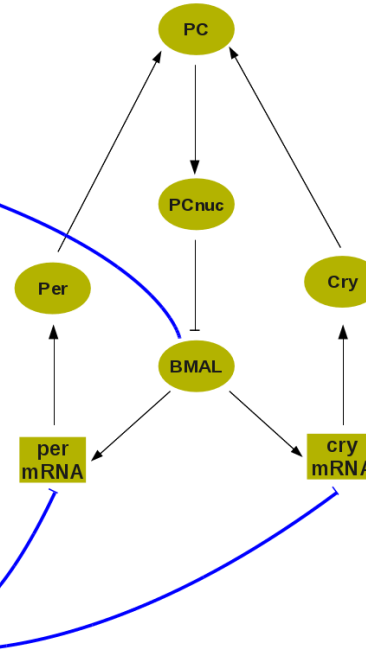
Boolean cell cycle model
[Fauré *et al*, 2006]

- One steady state (G0 quiescent state)
- One 112 states attractor (G1-S-G2-M)
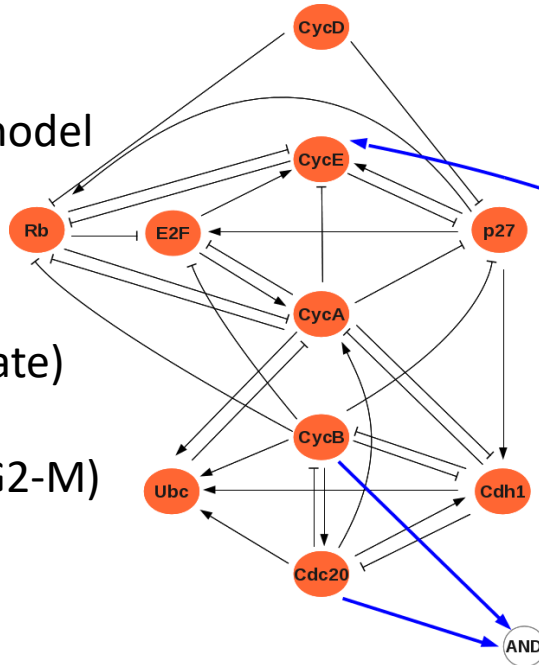
Boolean circadian model
[Comet *et al*, 2012]

- One steady state (mitosis, BMAL=1)
- One 120 states attractor (circ. clock)

Proposed interconnection scheme (modeling choice):

Circ → CC: BMAL acts negatively on G1 phase: $CycE' = \neg u \land (E2F \land \neg Rb)$

CC → Circ: mitosis blocks transcription: $mPER' = mCRY' = \neg v \land BMAL$

Conclusion

- Cross graph, asymptotic graphs: two complementary methods

$$G^{cr} \qquad\qquad\qquad G^{as}$$

+ One-to-one recovery of all dynamics (asymptotic + transient)
+ Exact recovery of attractors
− Computationally costly

+ Computationally efficient
+ Possible to analyze "big" interconnection schemes
− Partial recovery + spurious (rare)

- Interconnection of asynchronous Boolean networks
  - ✓ Efficient model reduction technique (exploiting modularity)
  - ✓ A step towards multi-cellular modeling and cell-to-cell communications
  - ✓ Interconnection of known modules: an efficient framework to test different topologies of regulatory links

References

1. Tournier L, Chaves M. Interconnection of asynchronous Boolean networks, asymptotic and transient dynamics . *Automatica* 2013.
2. Chaves M, Tournier L. Analysis tools for interconnected Boolean networks with biological applications. *Frontiers Physiol* 2018.

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