6e Séminaire DKM

Static Analysis with Abstract Interpretation for the Verification of Dynamical Properties of Biological Regulatory Networks

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Bio-informatics?

[De Jong, *Journal of Computational Biology*, 2002]

Bridge between **Biology** and **Computer Science**

Bio-informatics?

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Many fields:

- Sequencing
- Networks
- Simulation
- Experiments
- Databases
- ...

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  - Differential equations (quantitative)
  - Stochastic/probabilistic (quantitative)
  - Discrete graphs (qualitative)
  - Hybrid
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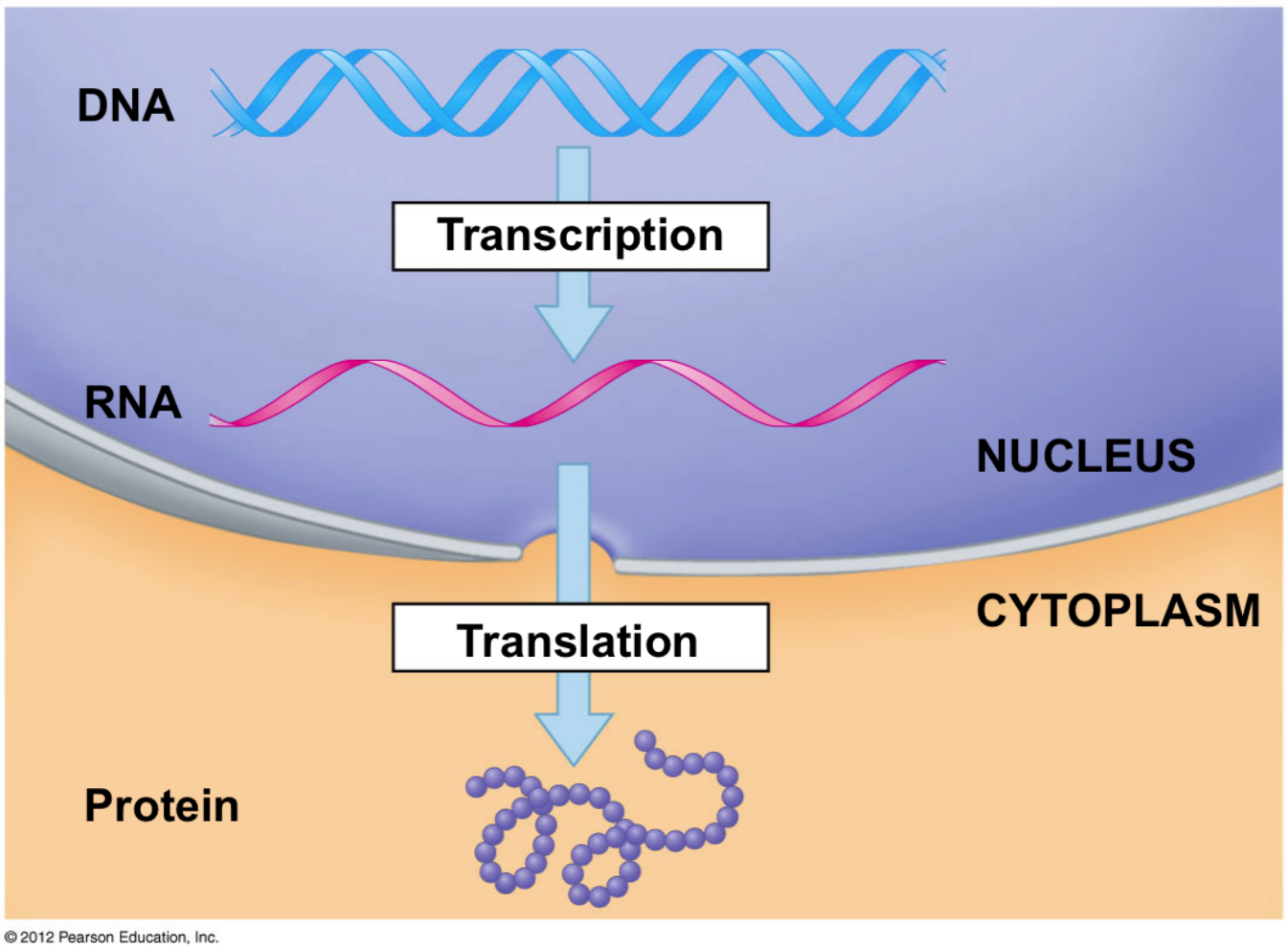
Abstraction



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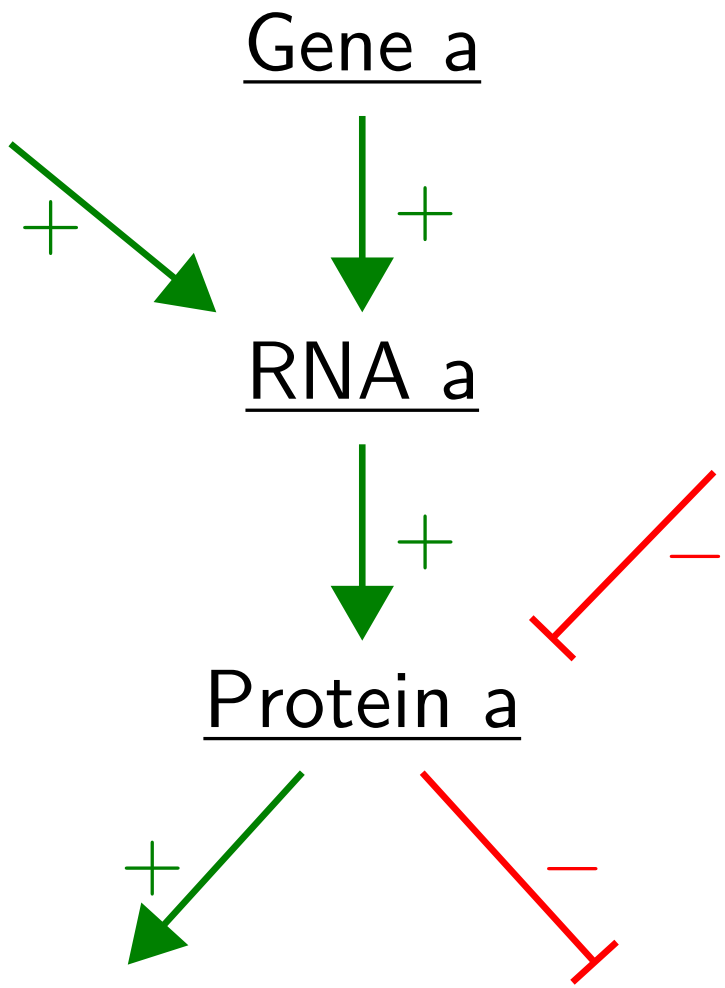

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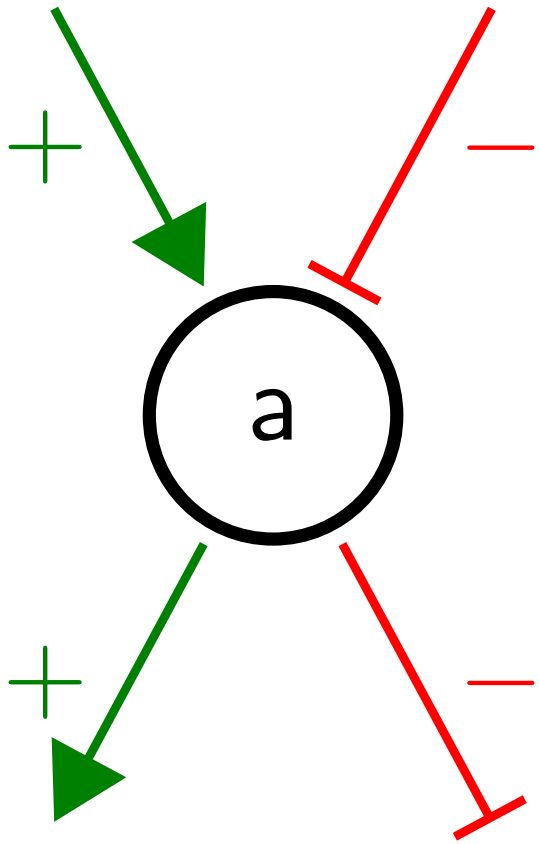

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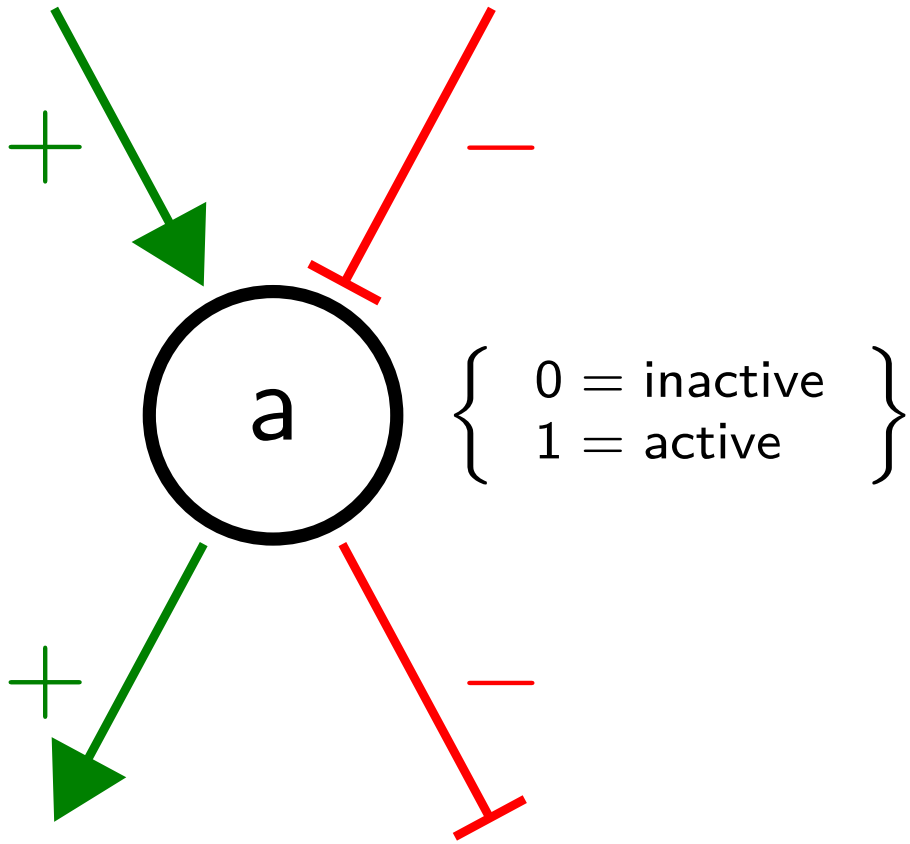

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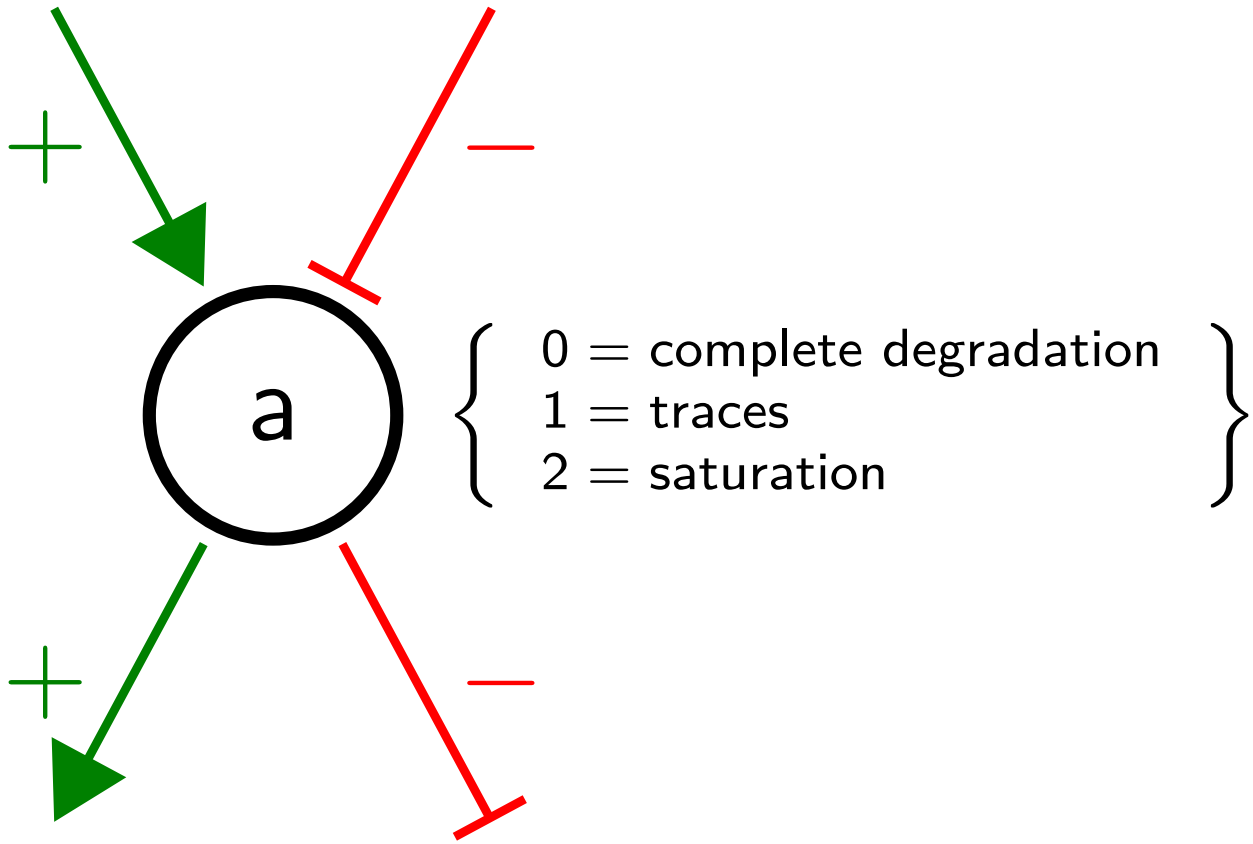
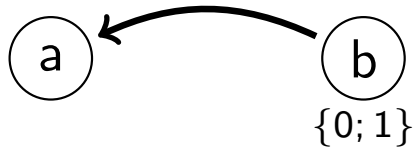
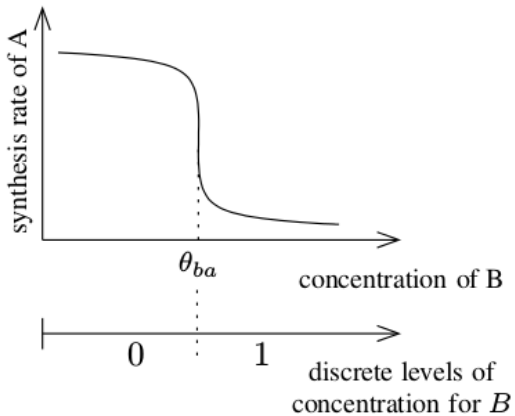


Discretization and Asynchronism

[Richard *et al.*, 2008]





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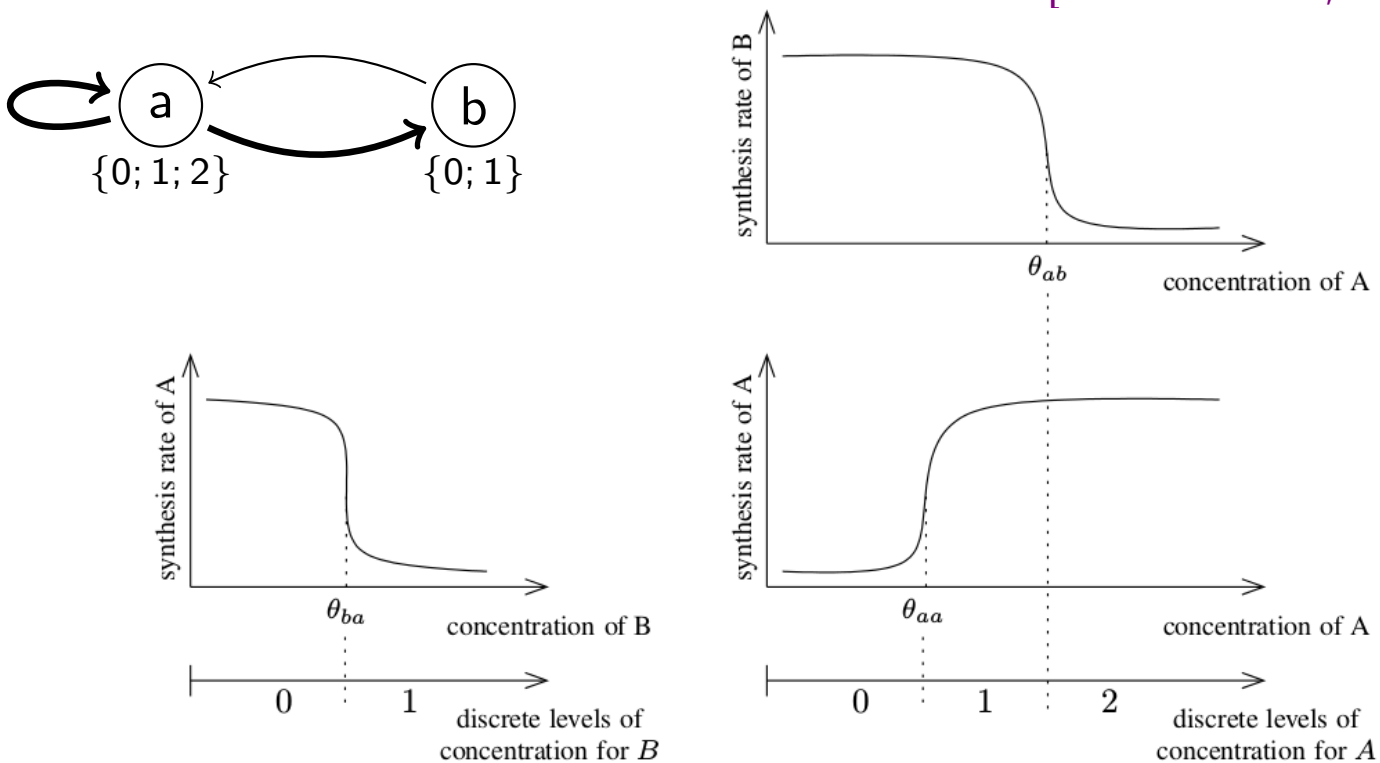

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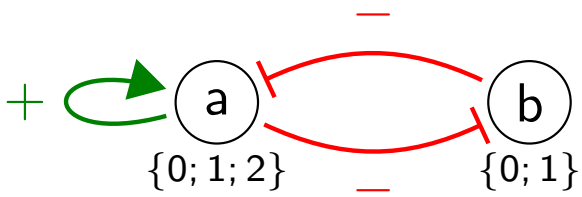

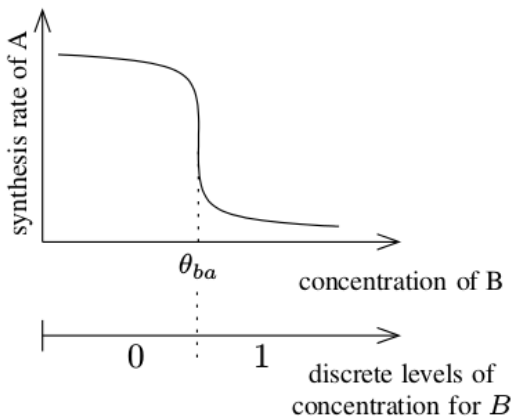

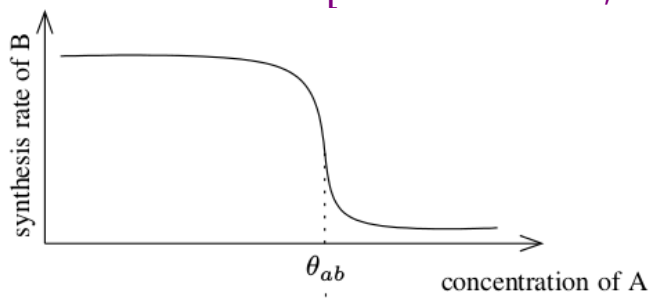

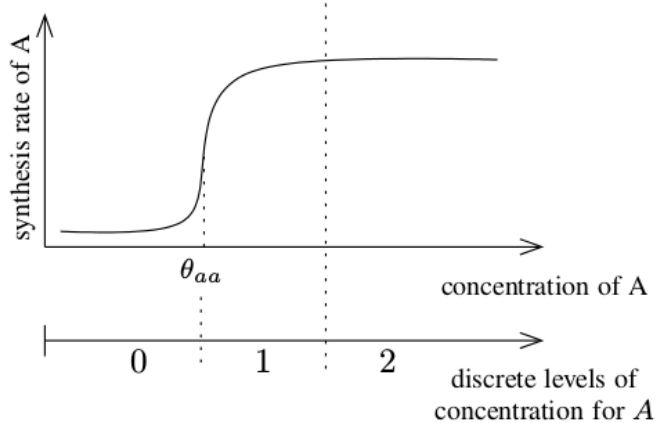

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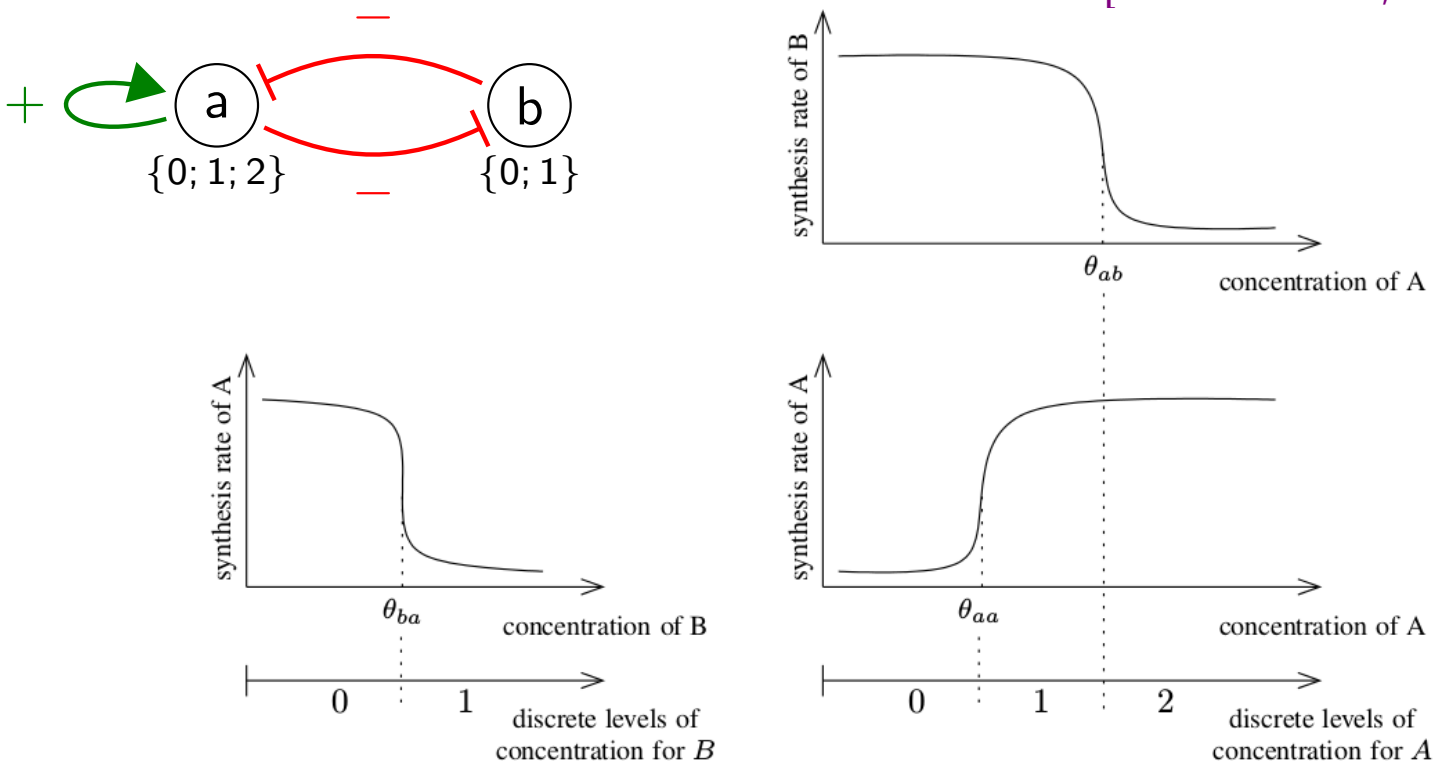

- Unknown real values of concentrations or continuous activity levels
  → Abstracted as **discrete levels** and thresholds

Discretization and Asynchronism

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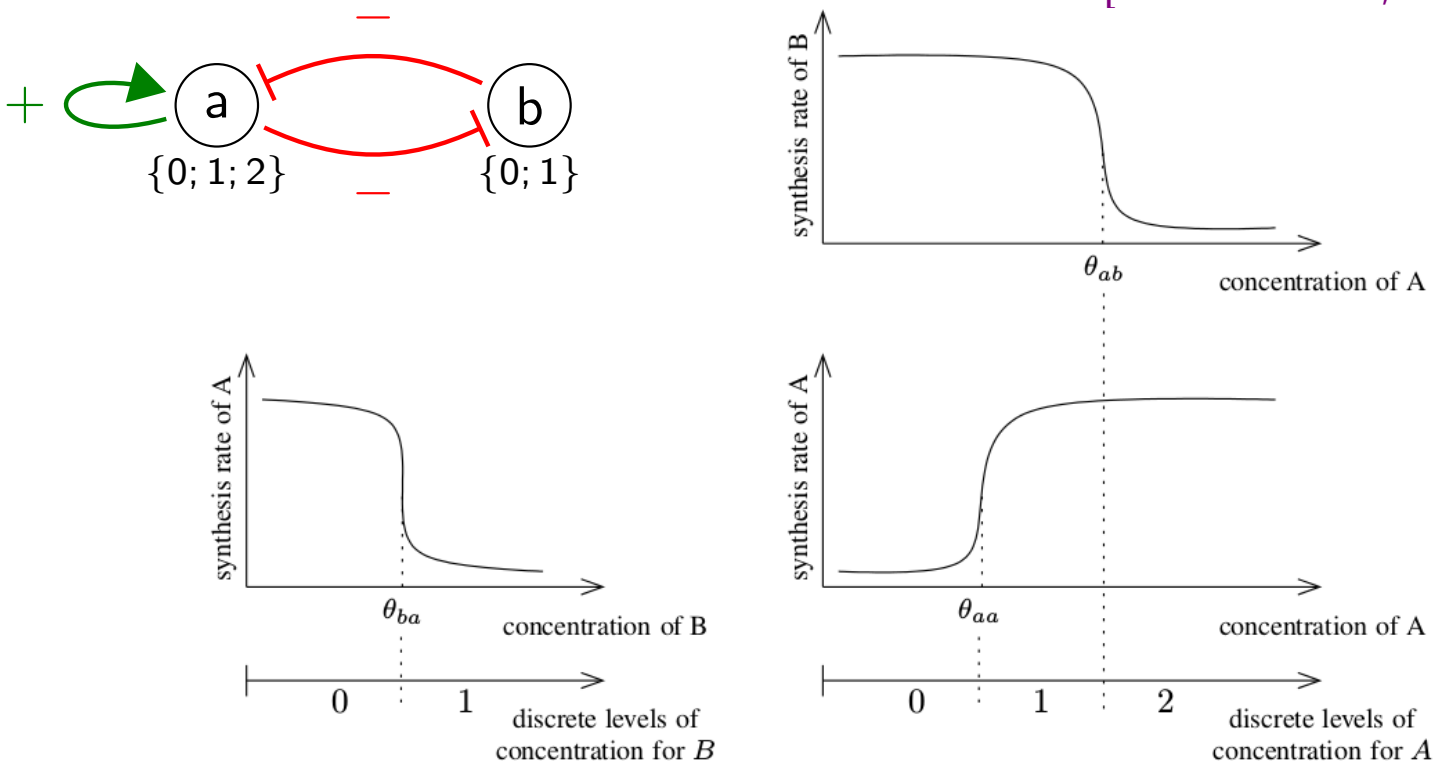

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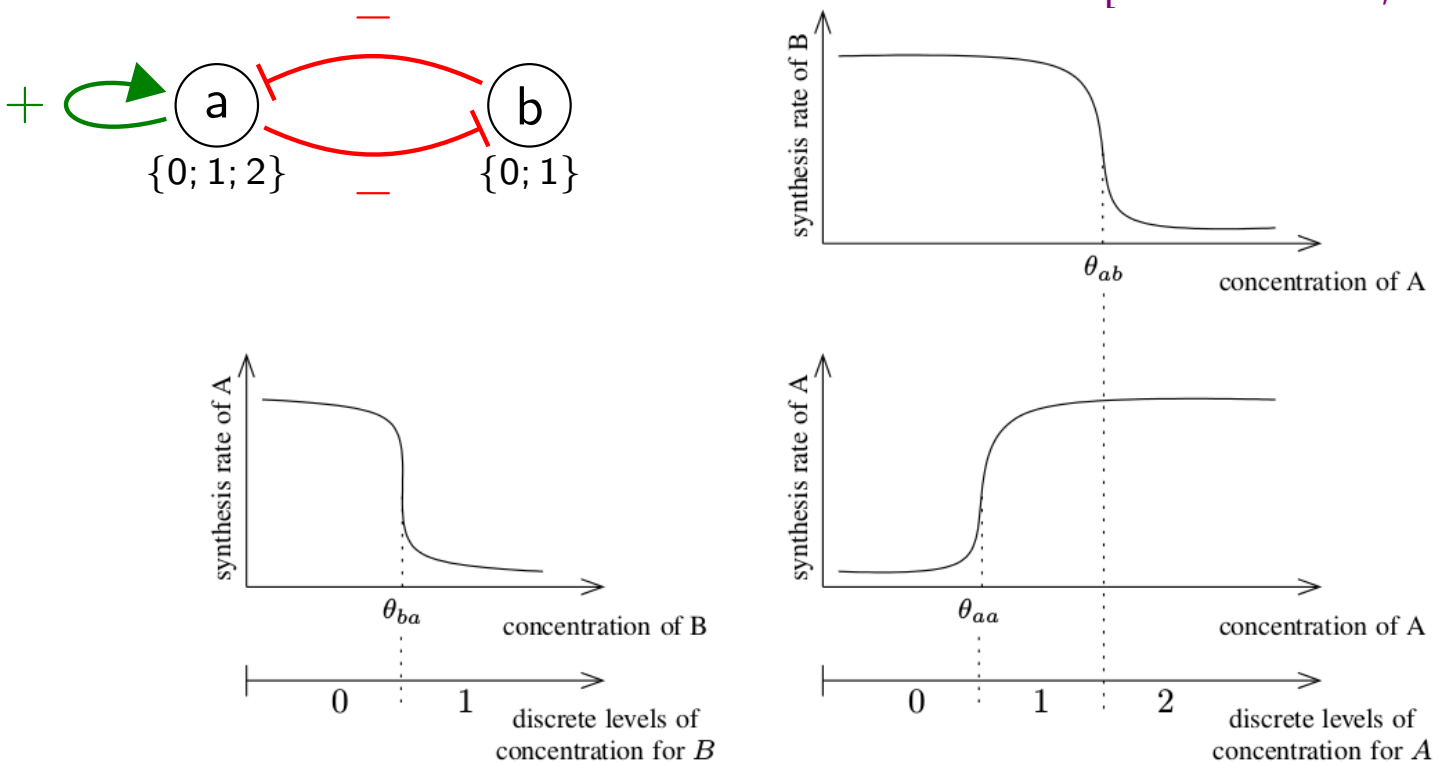
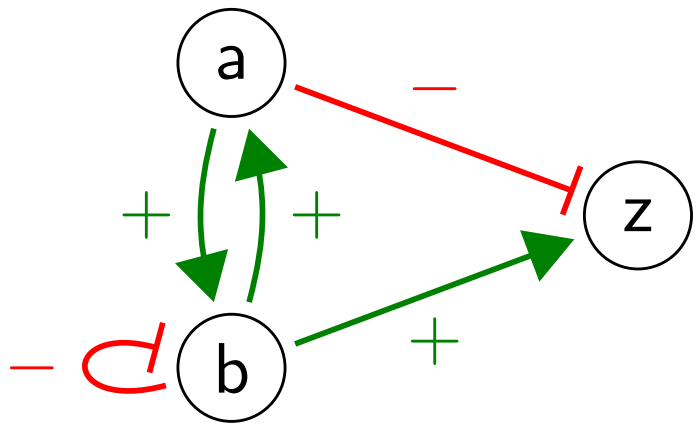


- Unknown real values of concentrations or continuous activity levels
  → Abstracted as **discrete levels** and thresholds
- Continuous variations of the real values
  → **Unitary** dynamics
- Simultaneous crossings of two thresholds never occurs
  → **Asynchronous** dynamics

Modeling of René Thomas

[Thomas, *Journal of Theoretical Biology*, 1973]

Models of interacting components (genes, proteins, ...)



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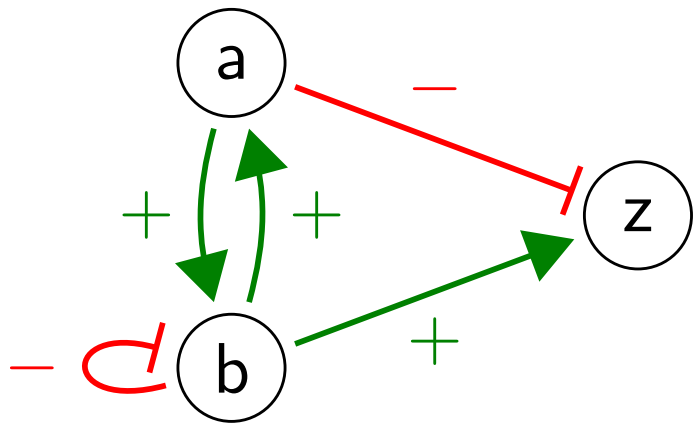


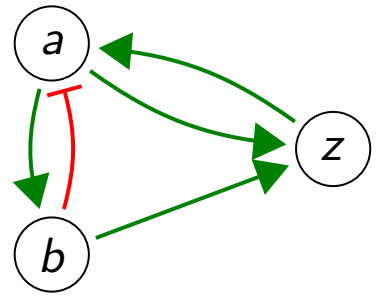
Questions:

- How does (z) **behave**?
- Is it **possible** to make (a) inactive?
- If I **knock-out** (b), what changes?

State-graph

The state-graph depicts explicitly the whole dynamics

abz			
000	010	001	011
100	110	101	111
200	210	201	211



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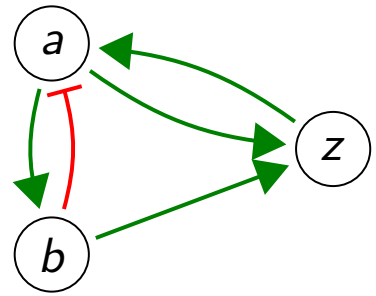

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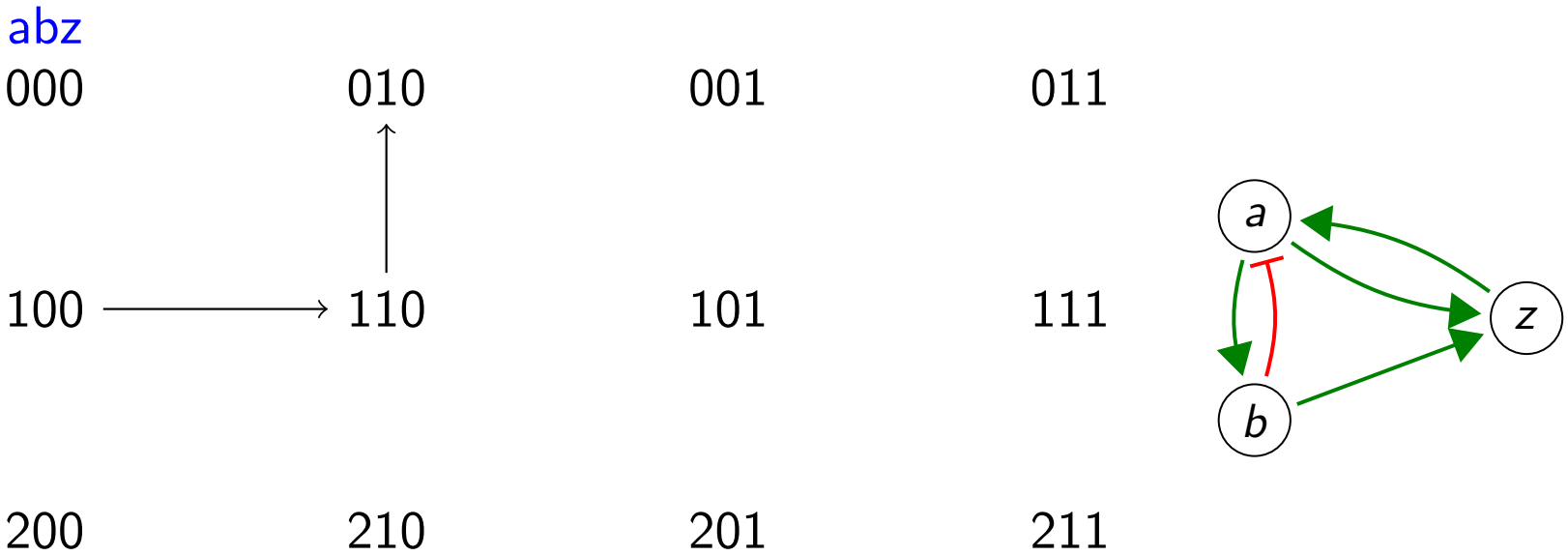

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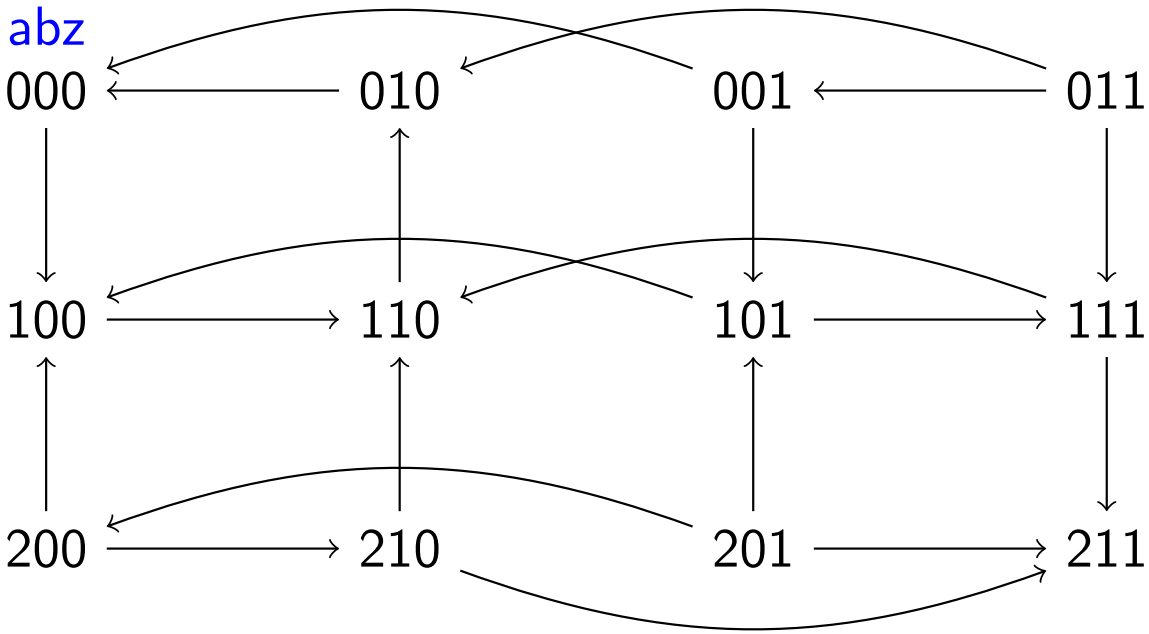

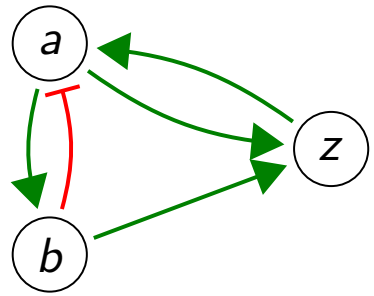

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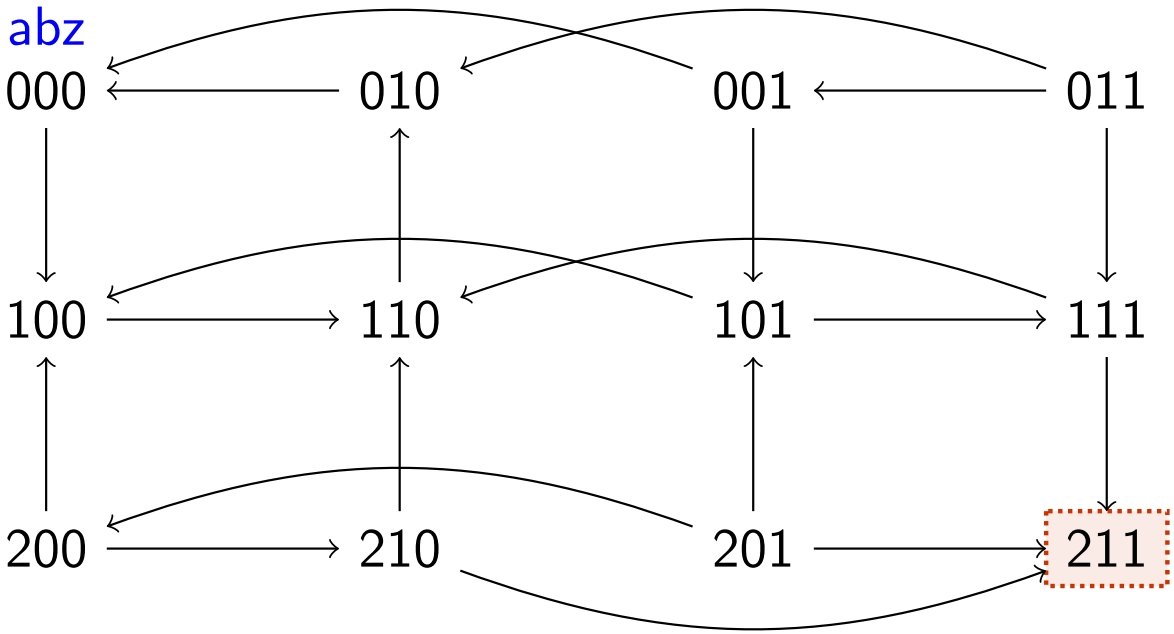

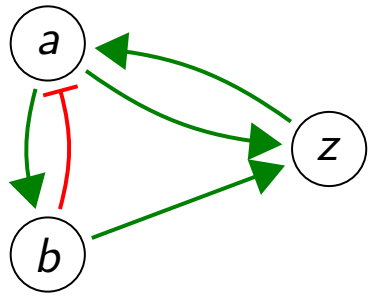

- **Stable state** = state with no successors

State-graph

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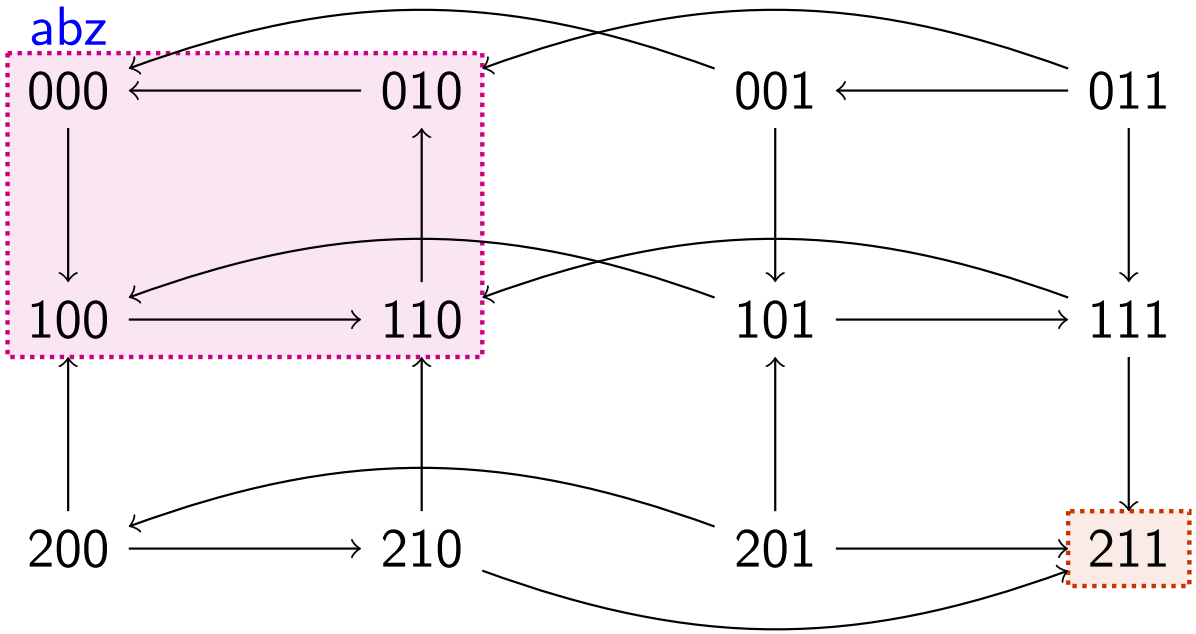

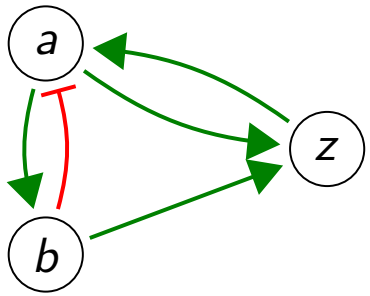

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- **Complex attractor** = minimal loop or composition of loops from which the dynamics cannot escape

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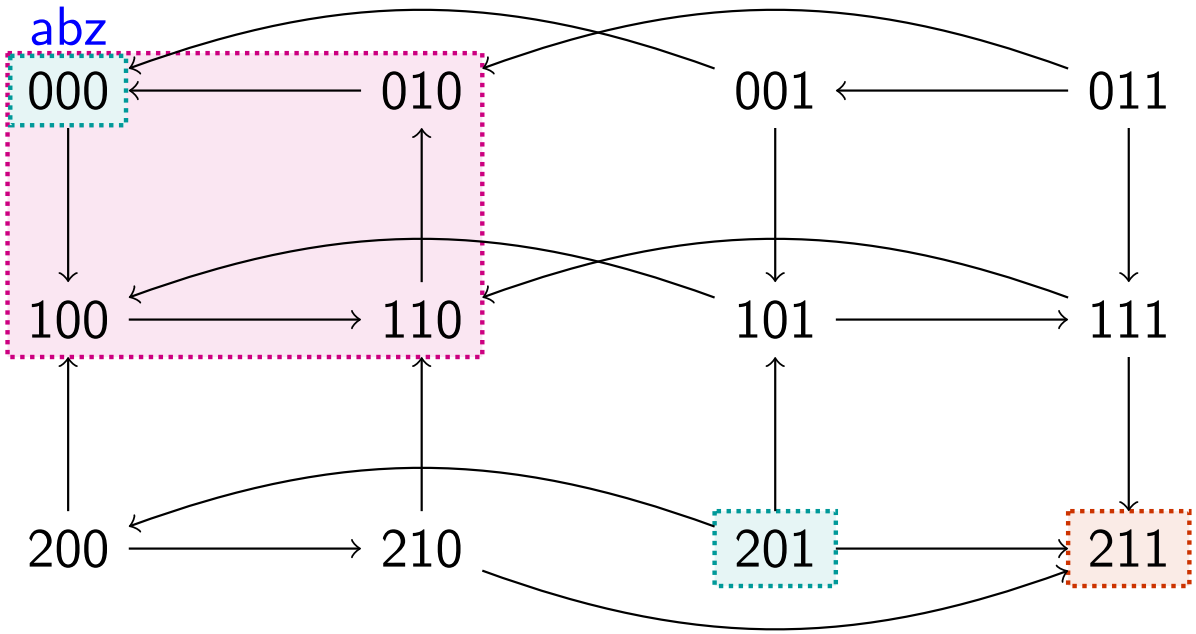

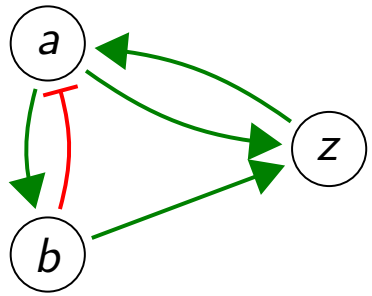

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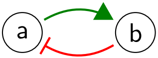
- **Reachability** = from **201**, can I reach **000**?

Combinatorial explosion

Model	Possible states
	4

Combinatorial explosion

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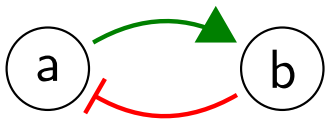
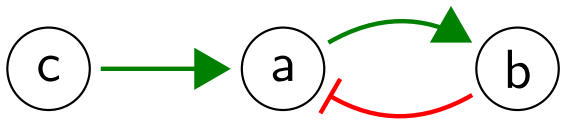
	4
	8

Combinatorial explosion

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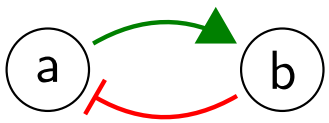
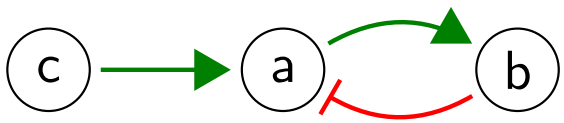
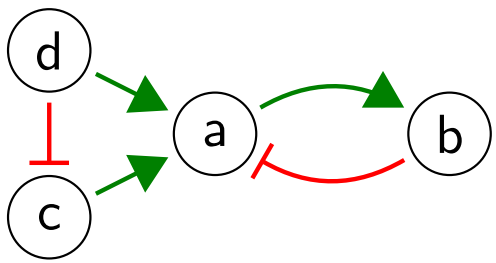
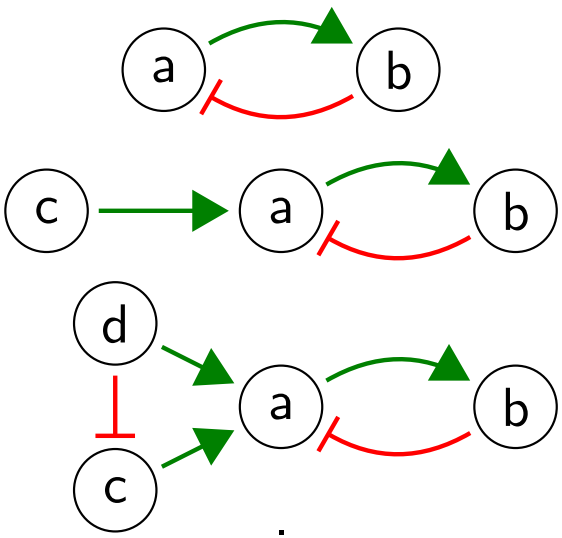
	4
	8
	16

Combinatorial explosion

Model	Possible states
	4
	8
	16
⋮	⋮
(10)	1024

Combinatorial explosion

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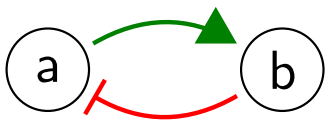
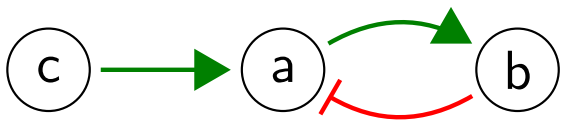
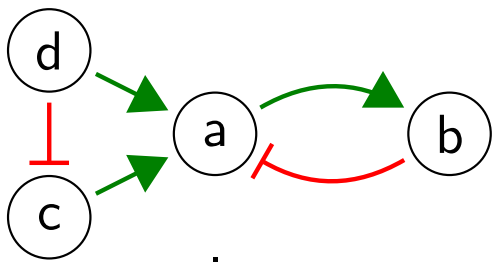
	4
	8
	16
⋮	⋮
(10)	1024
(20)	1048576

Combinatorial explosion

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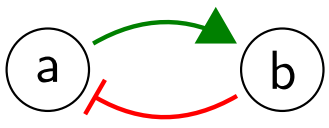
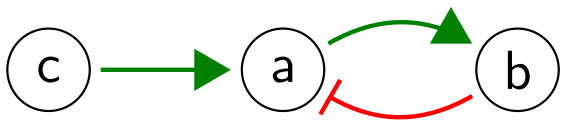
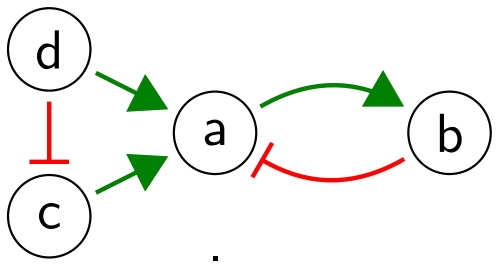
	4
	8
	16
⋮	⋮
(10)	1024
(20)	1048576
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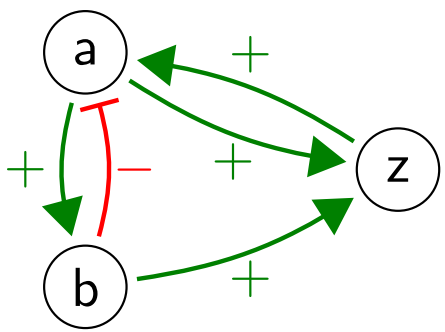
Conjectures of René Thomas

[Thomas, *Num. Methods in the Study of Crit. Phenomena*, 1981]



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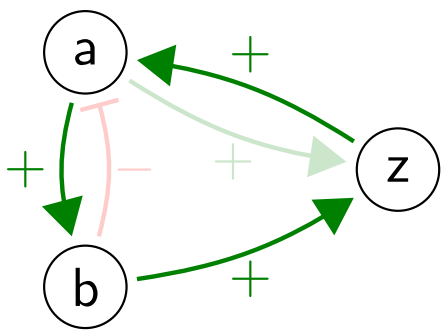

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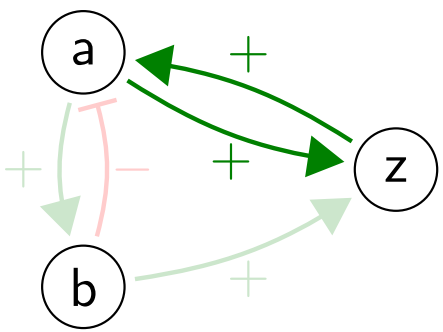

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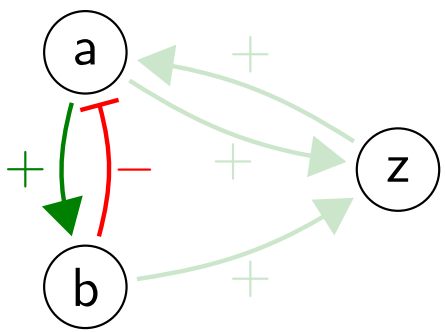
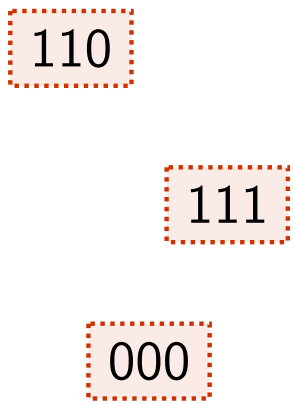
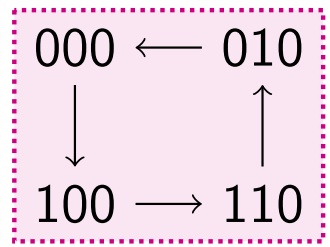
- Multiple **stable states** ⇒ positive cycle
- Sustained oscillations (**complex attractor**) ⇒ negative cycle







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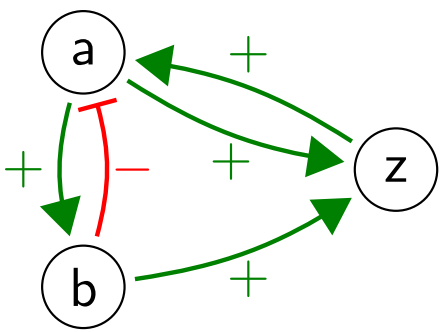
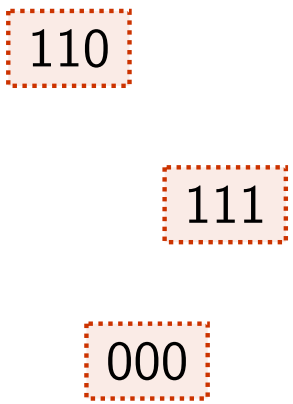
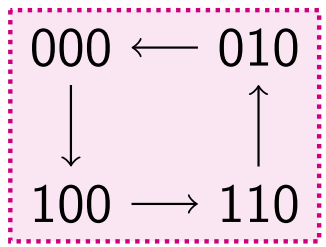




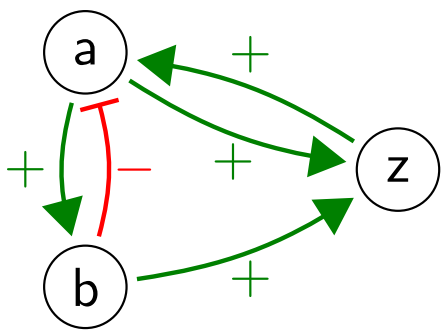


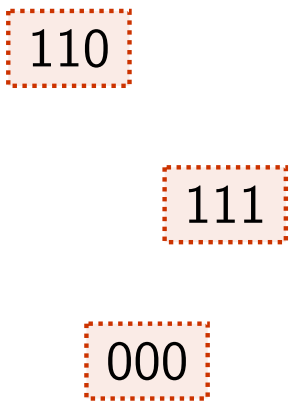
Proofs: [Remy *et al.*, *Advances in Applied Mathematics*, 2008]
[Richard, *Advances in Applied Mathematics*, 2010]
[Richard & Comet, *Discrete Applied Mathematics*, 2007]

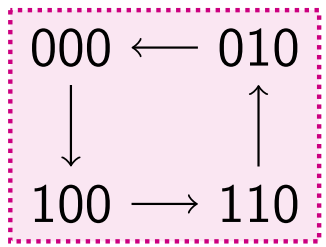
Conjectures of René Thomas

[Thomas, *Num. Methods in the Study of Crit. Phenomena*, 1981]

- Multiple **stable states** ⇒ positive cycle
  No positive cycle ⇒ The stable state (if any) is unique
- Sustained oscillations (**complex attractor**) ⇒ negative cycle





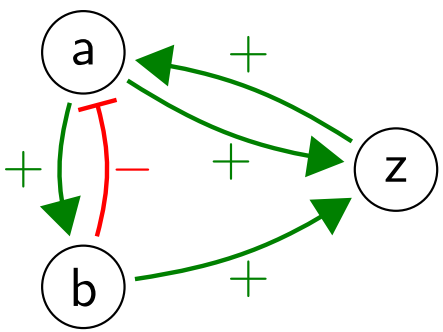


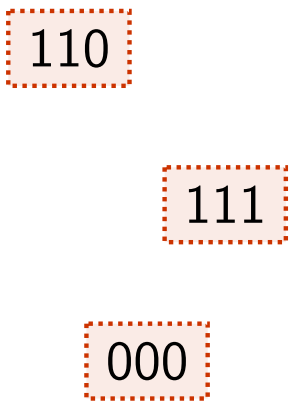
Proofs: [Remy *et al.*, *Advances in Applied Mathematics*, 2008]
[Richard, *Advances in Applied Mathematics*, 2010]
[Richard & Comet, *Discrete Applied Mathematics*, 2007]

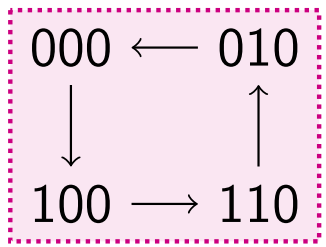
Conjectures of René Thomas

[Thomas, *Num. Methods in the Study of Crit. Phenomena*, 1981]

- Multiple **stable states** ⇒ positive cycle
  No positive cycle ⇒ The stable state (if any) is unique
- Sustained oscillations (**complex attractor**) ⇒ negative cycle
  No negative cycle ⇒ No complex attractor (only stable states)







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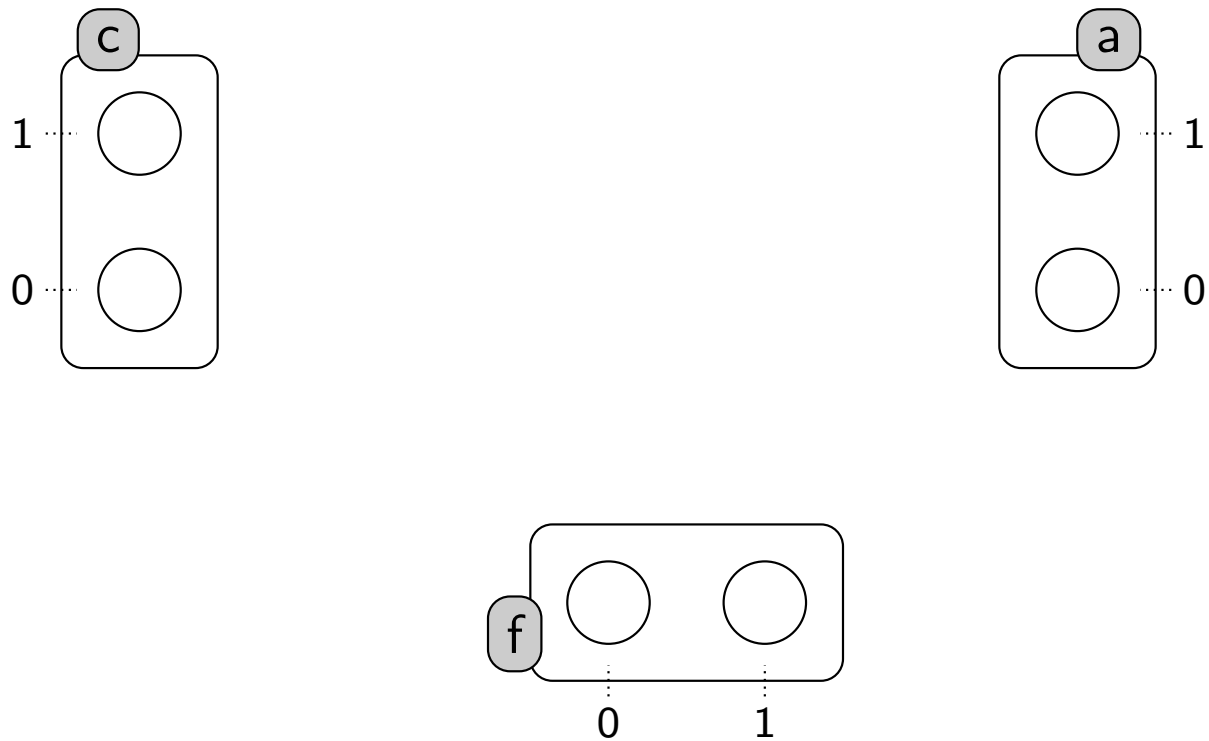
Asynchronous Automata Networks (AAN)

[Paulevé *et al.*, *Transactions on Computational Systems Biology*, 2011]
[Folschette *et al.*, *Theoretical Computer Science*, 2015]



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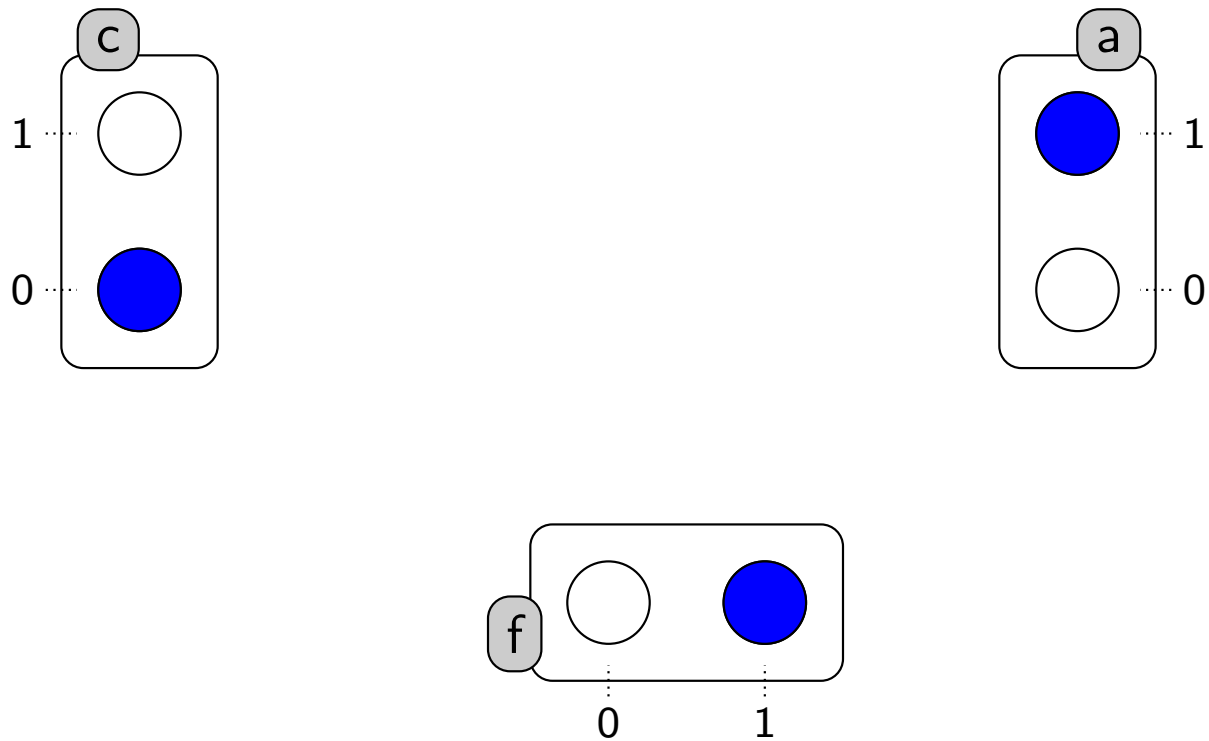

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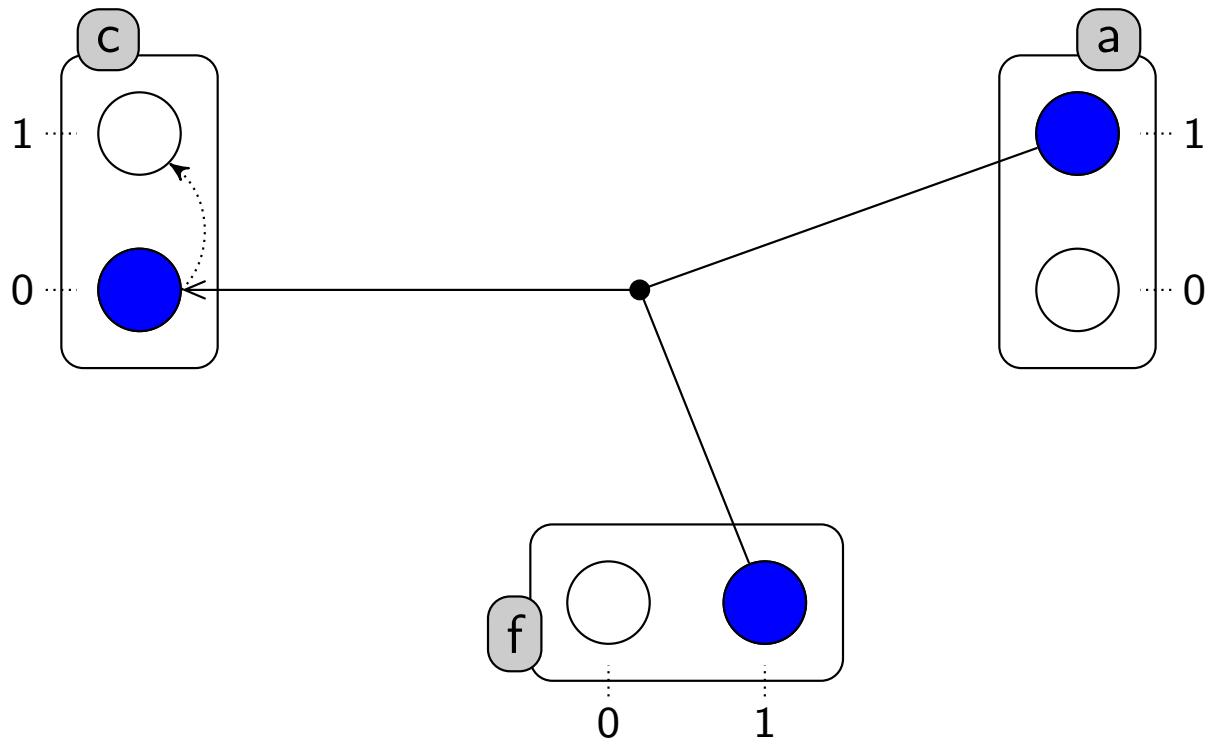

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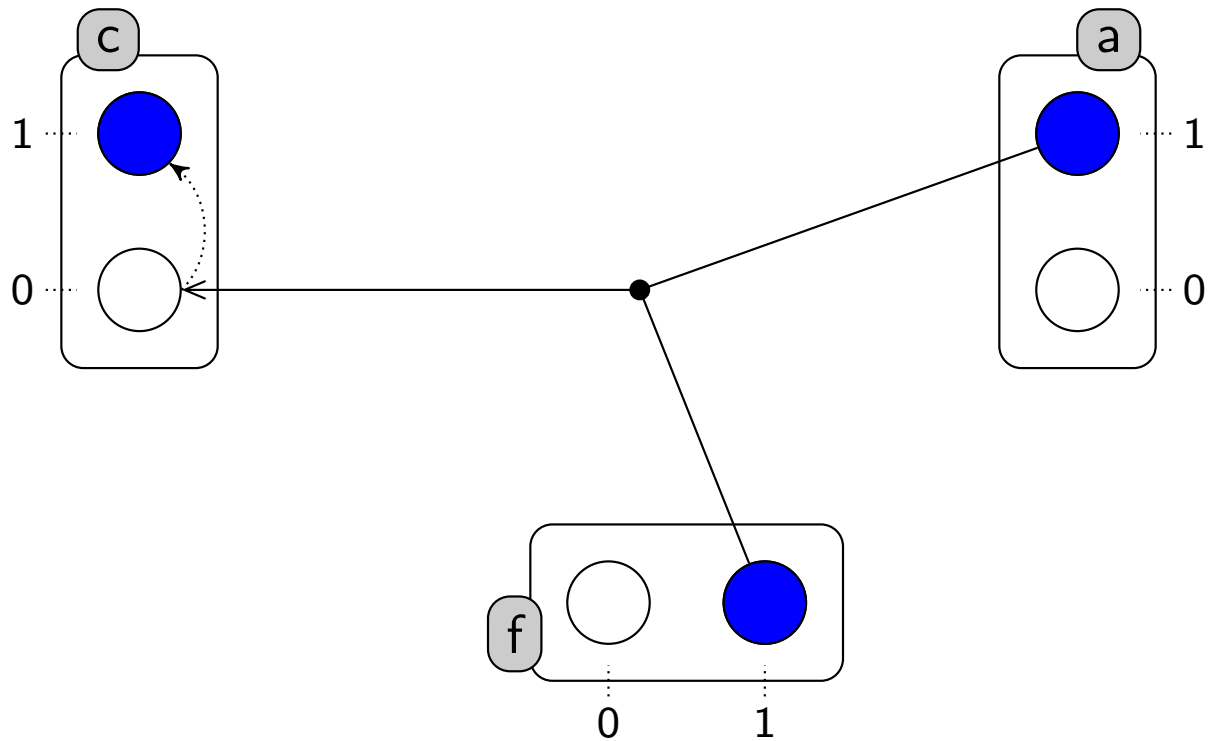

Asynchronous Automata Networks (AAN)

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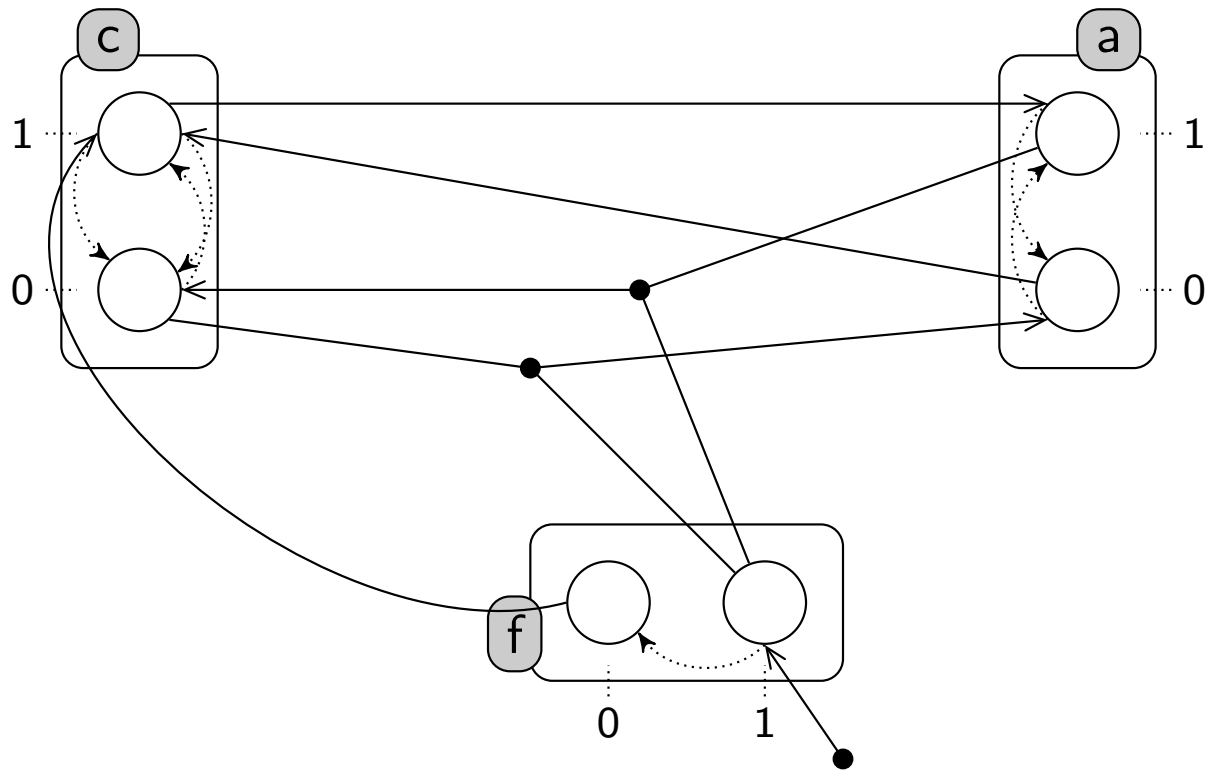
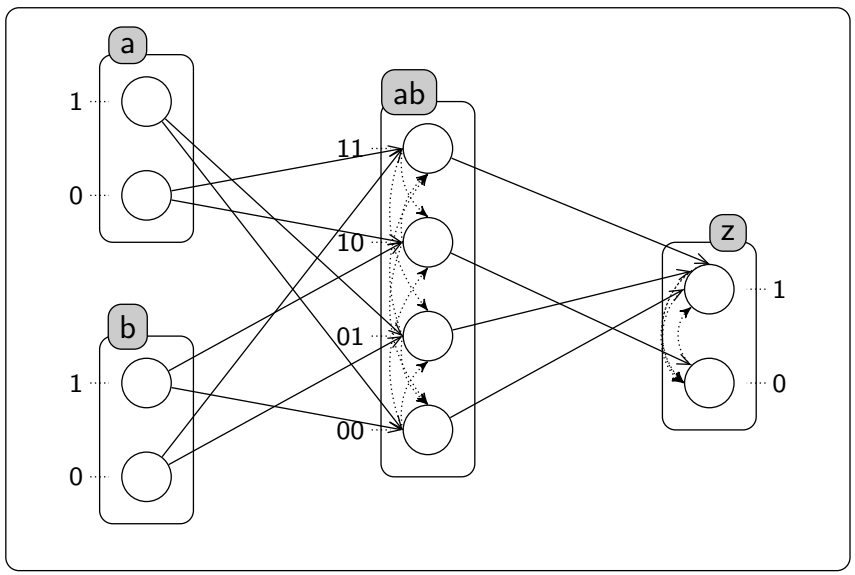
Model from [François *et al.*, *Molecular Systems Biology*, 2007]



Translation of AAN models

[Folschette *et al.*, *Computational Methods in Systems Biology*, 2012]



Process Hitting

Efficient but recent

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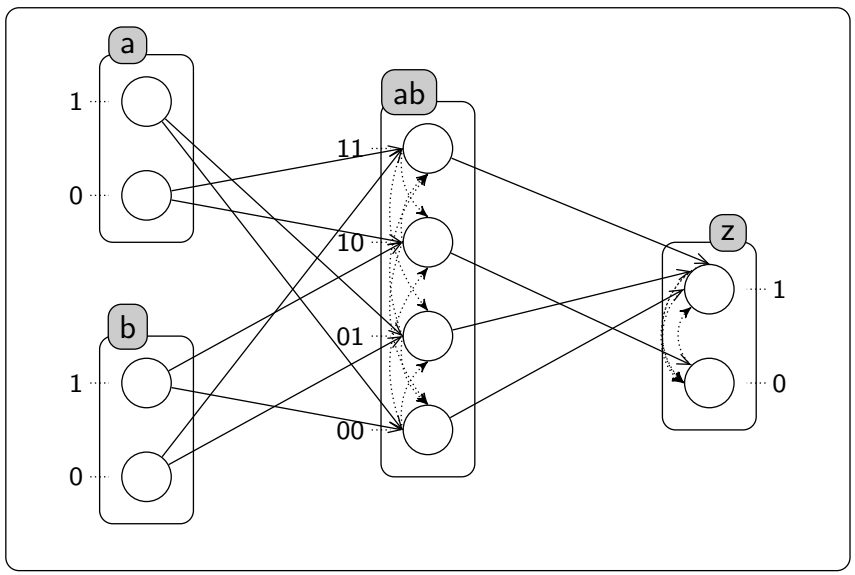

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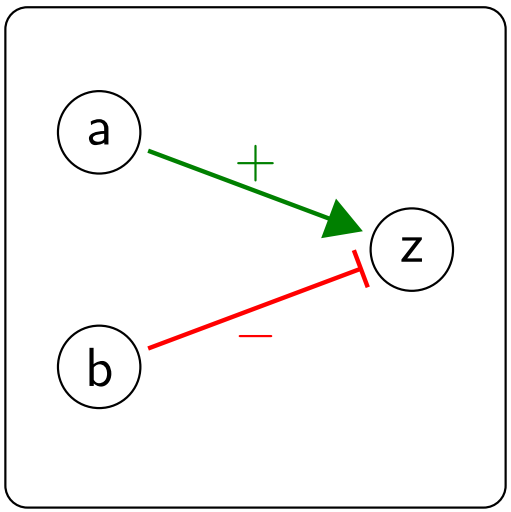

Thomas modeling
Widespread & readable

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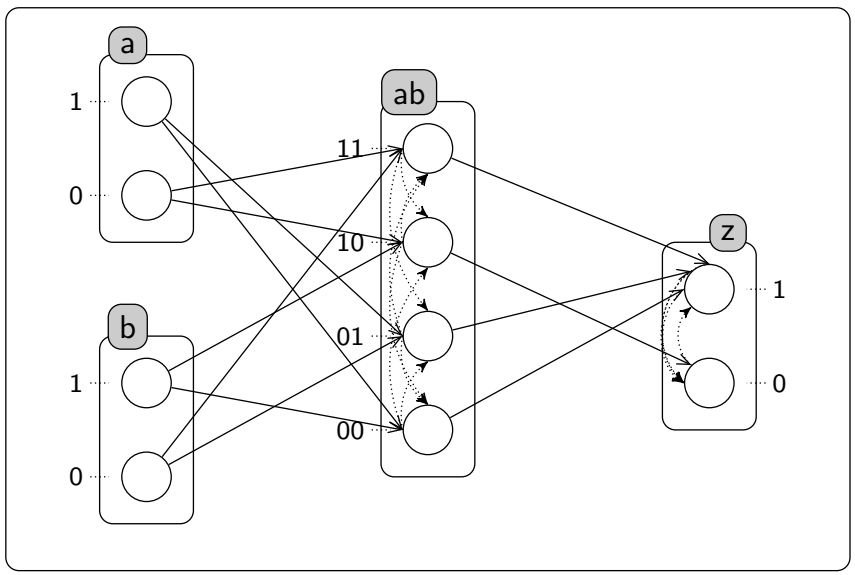

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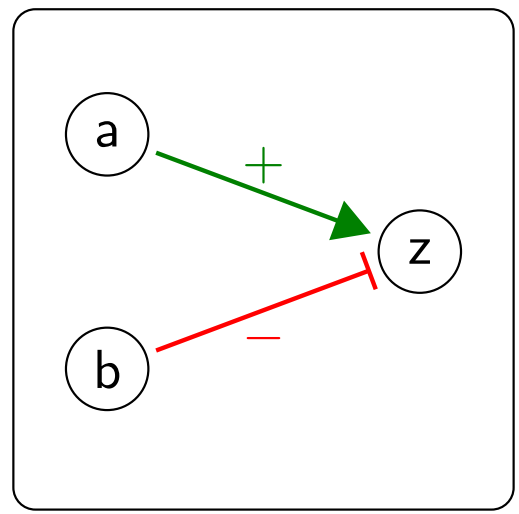

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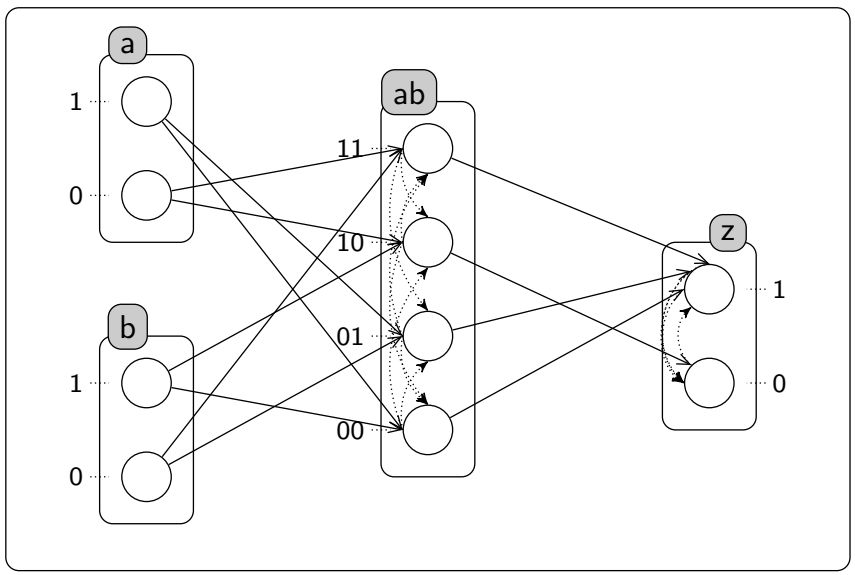

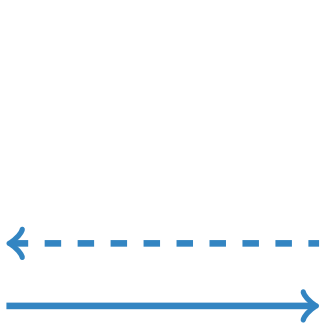

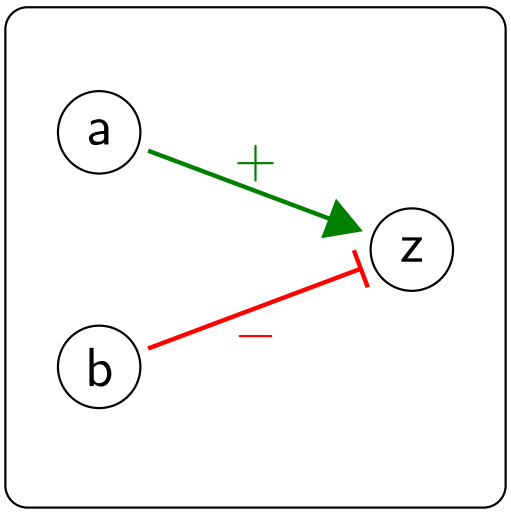

Process Hitting
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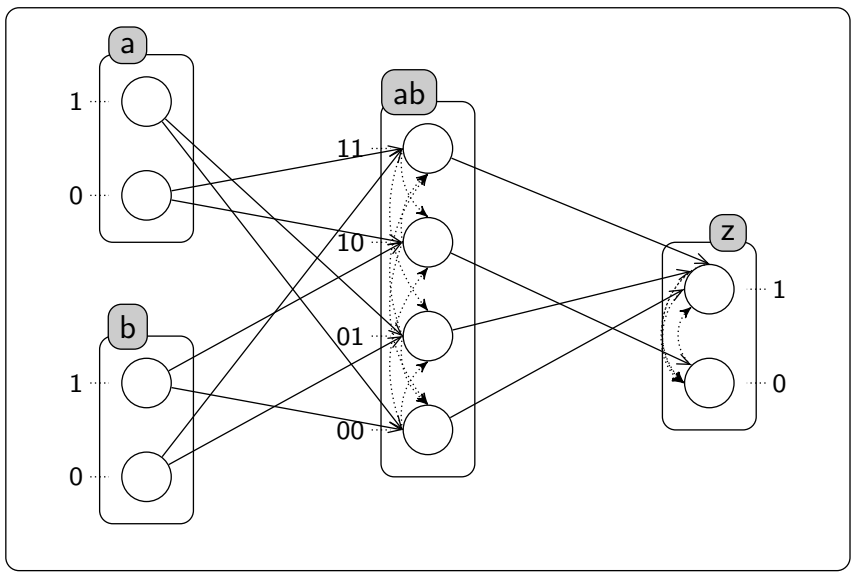
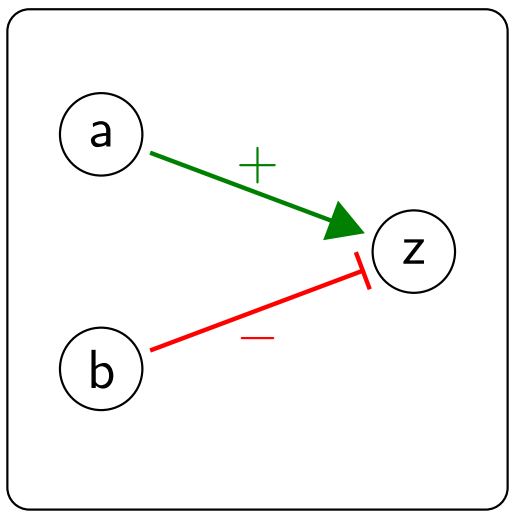
Towards AANs

[Folschette *et al.*, *CS2Bio*, 2013]



Process Hitting
Loose behavior

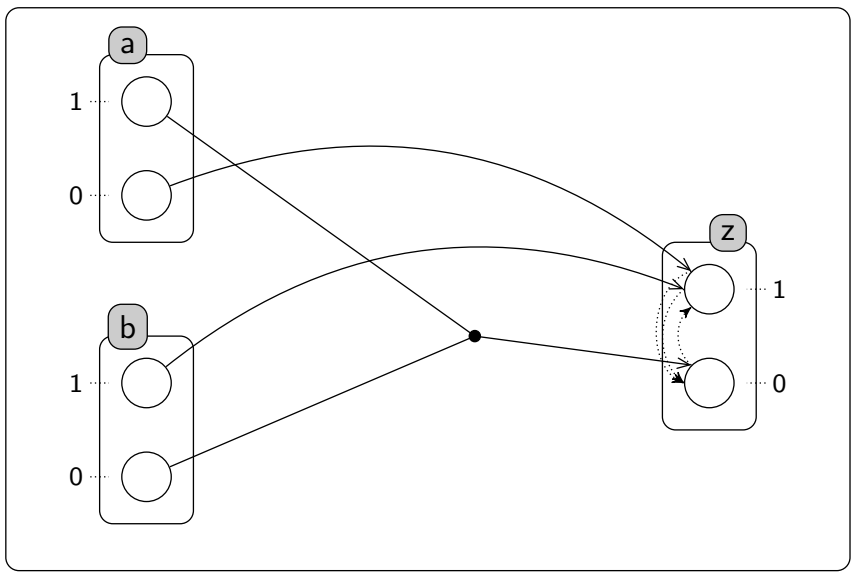
≠



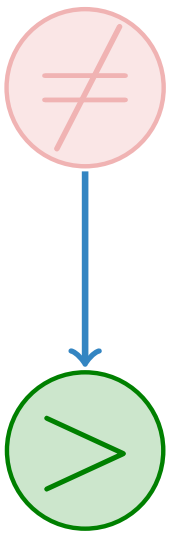
Thomas modeling
Expected behavior

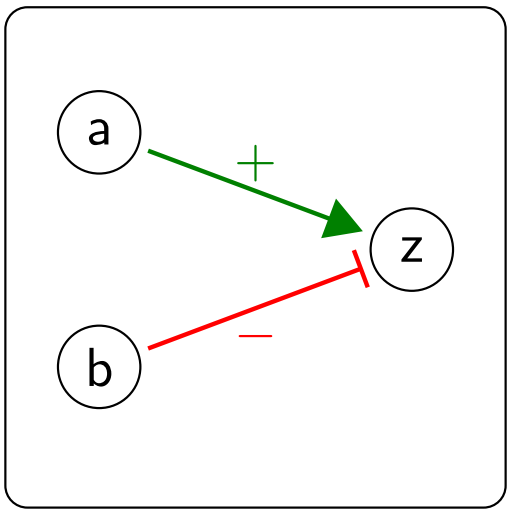
Towards AANs

[Folschette *et al.*, *CS2Bio*, 2013]



AANs
Accurate behavior

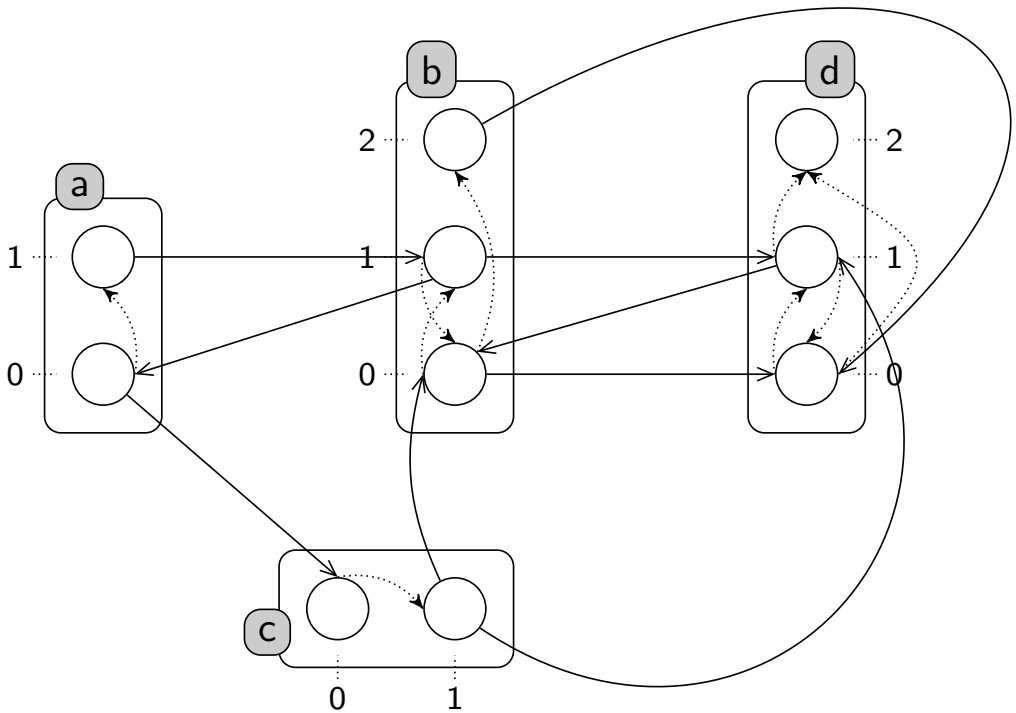




Thomas modeling
Expected behavior

Static analysis: successive reachability

[Paulevé *et al.*, *Mathematical Structures in Computer Science*, 2012]
[Folschette *et al.*, *Theoretical Computer Science*, 2015]



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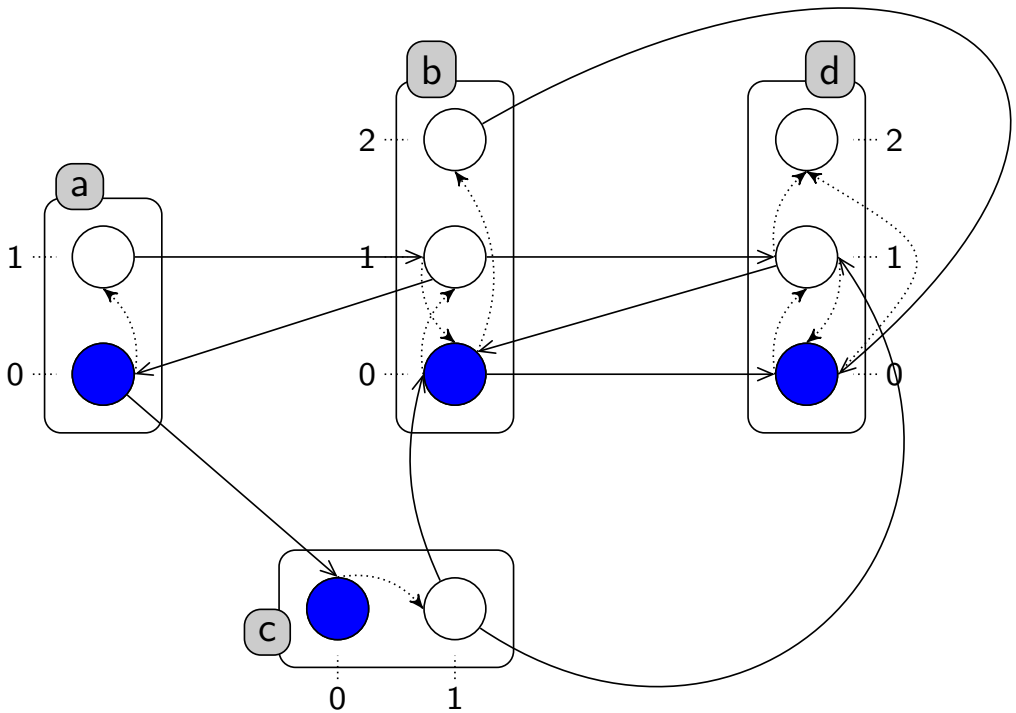

- Initial state

$\langle a_1, b_0, c_0, d_0 \rangle$

Static analysis: successive reachability

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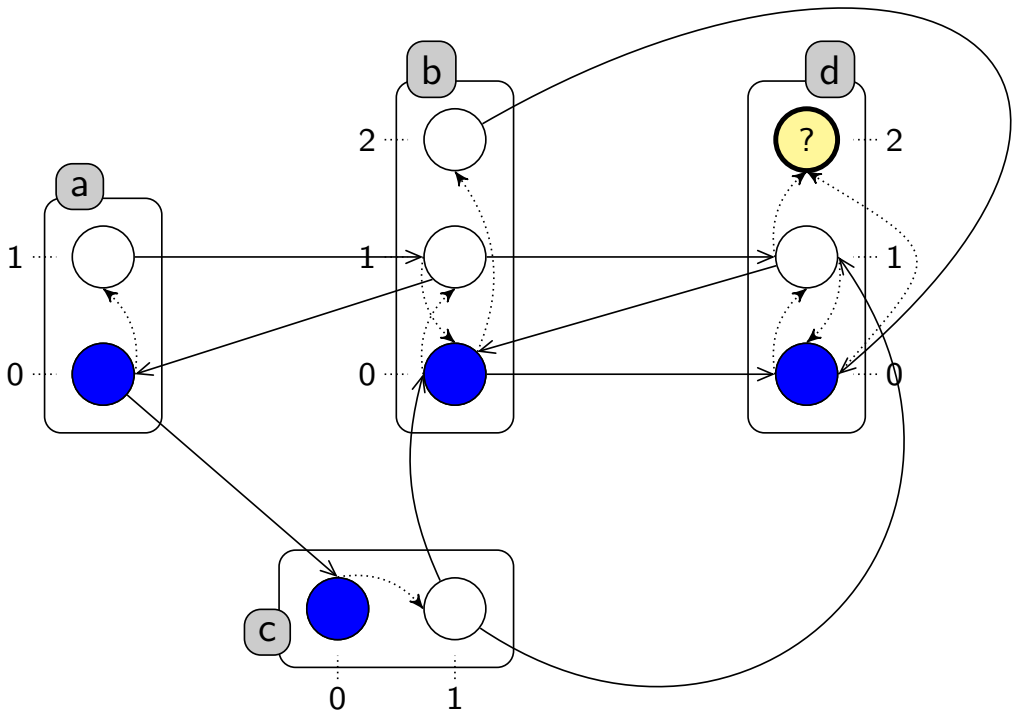

- Initial state

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- Objective(s)

$$[\ \Rsh\ d_2\ ]$$

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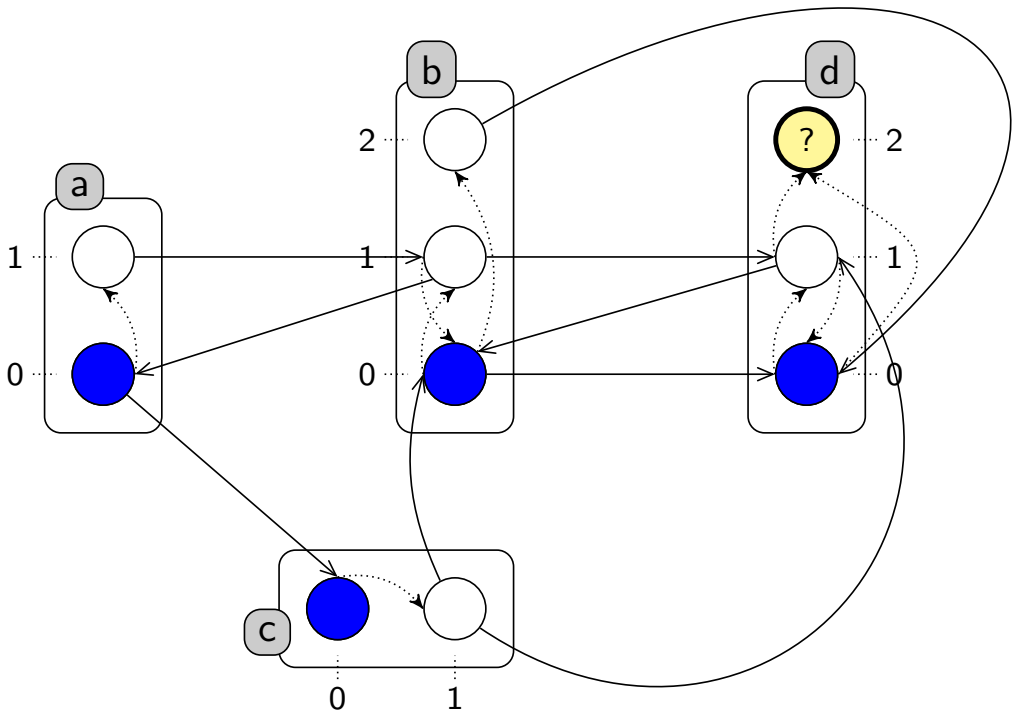

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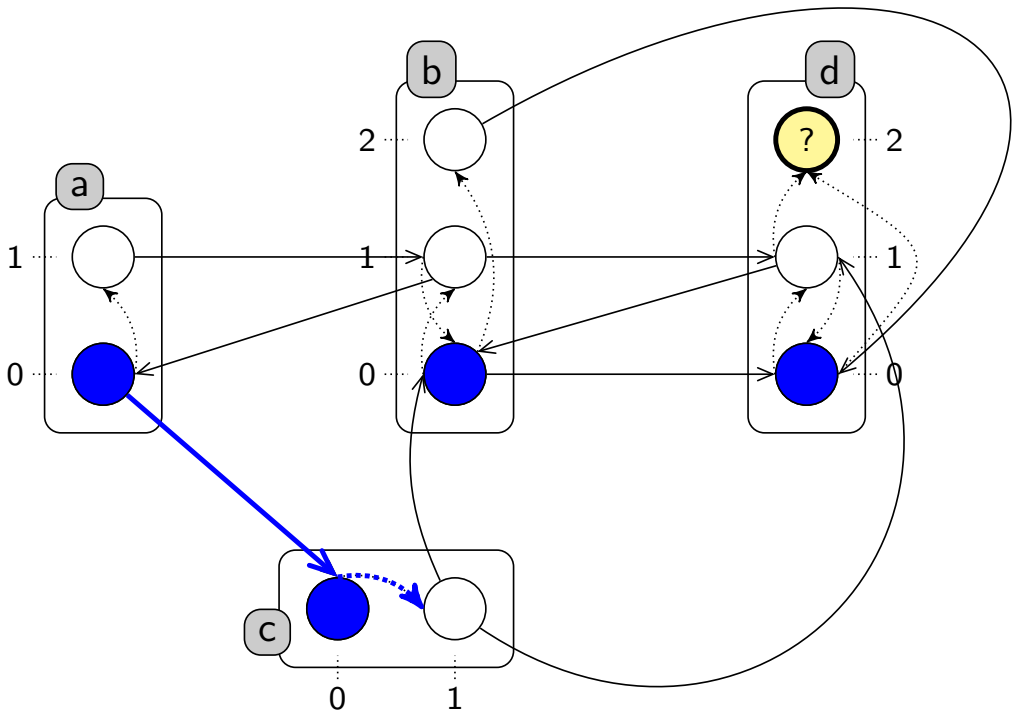

- Initial state

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$\rightarrow$ Sequence of actions?

$a_0 \rightarrow c_0 \Rsh c_1$

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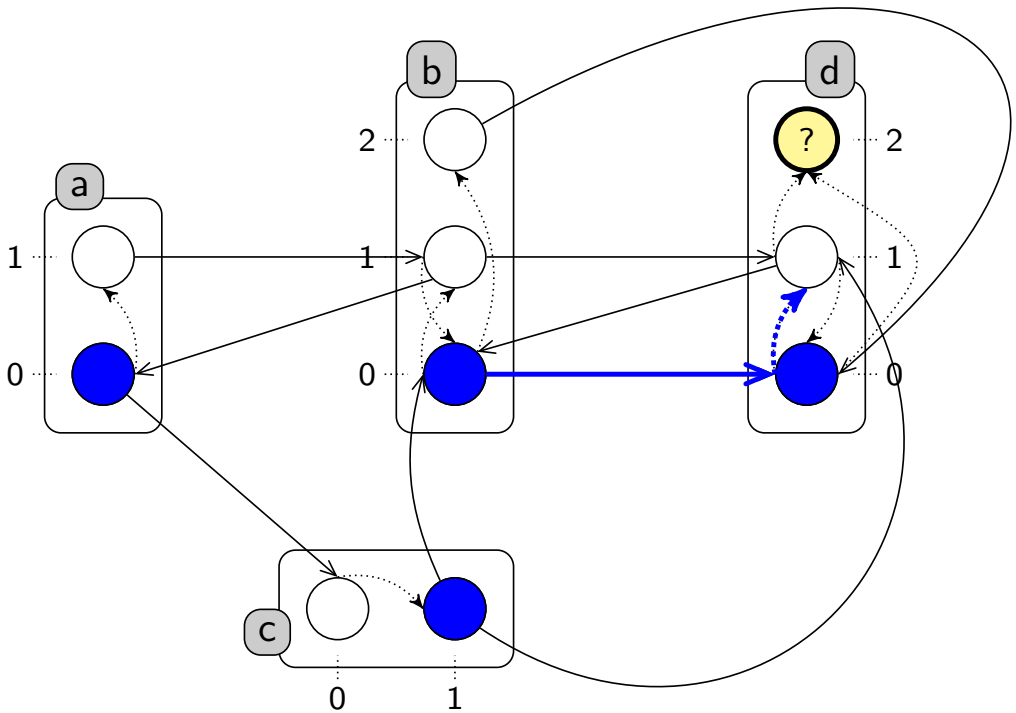

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$a_0 \rightarrow c_0 \Rsh c_1 :: b_0 \rightarrow d_0 \Rsh d_1$

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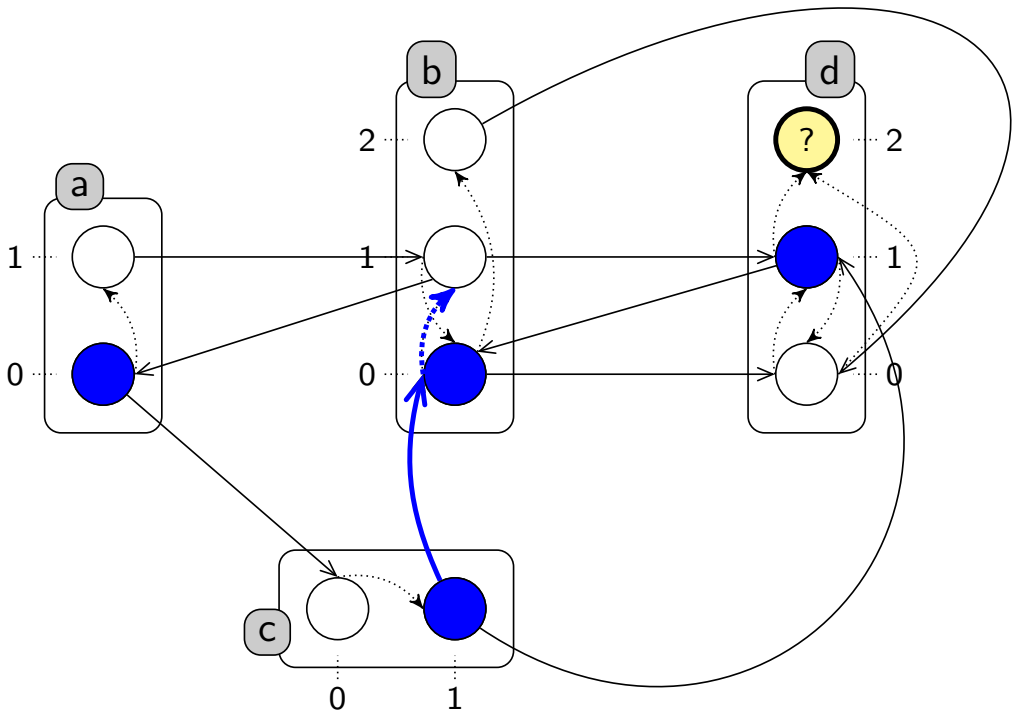

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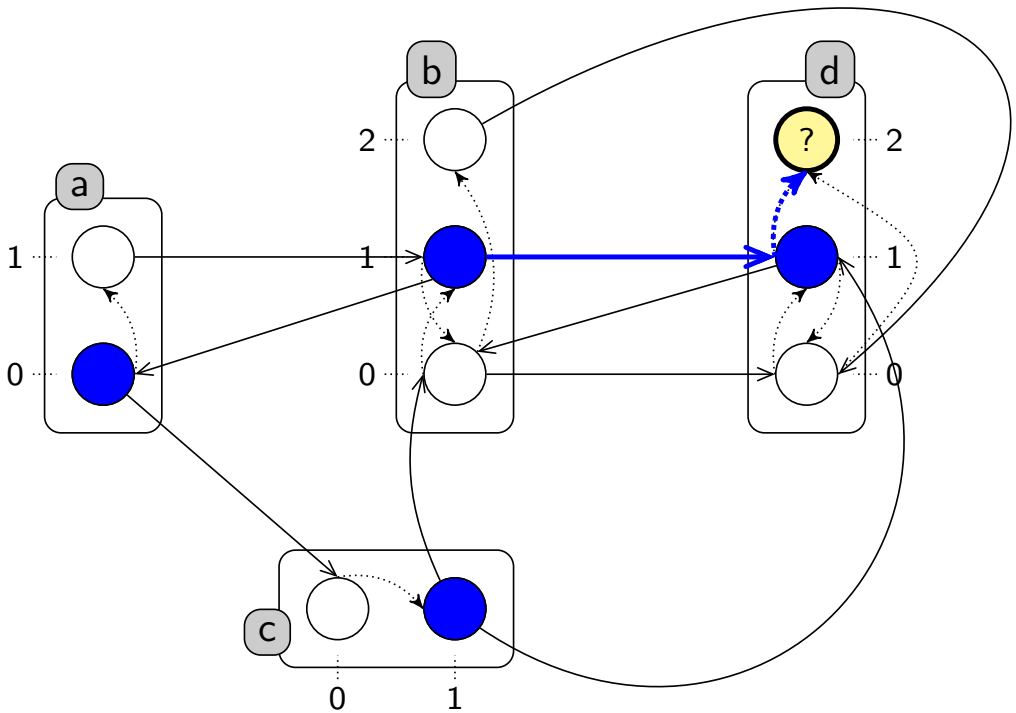

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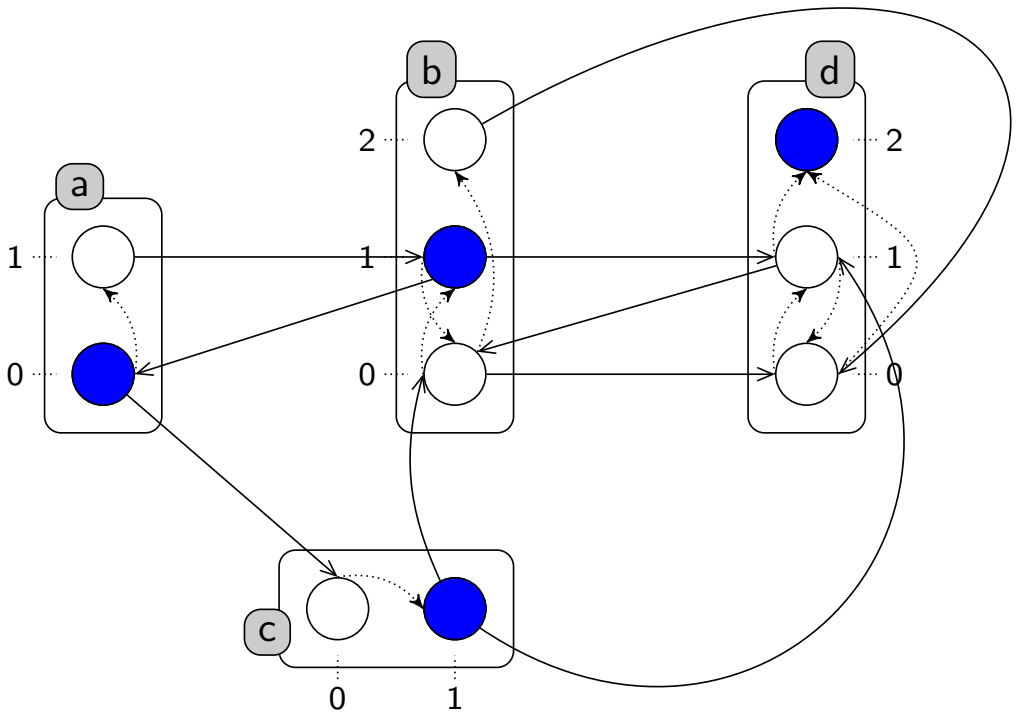

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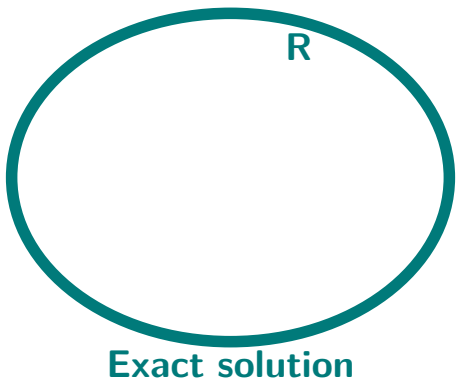
Approximations of the Dynamics

[Paulevé *et al.*, *Mathematical Structures in Computer Science*, 2012]
[Folschette *et al.*, *Theoretical Computer Science*, 2015]

- Directly checking **R** is hard (**exponential**)
- Rather check **approximations** **P** and **Q** so that: $\mathbf{P} \Rightarrow \mathbf{R} \Rightarrow \mathbf{Q}$
  so that computing **P** and **Q** is faster (roughly **polynomial**)



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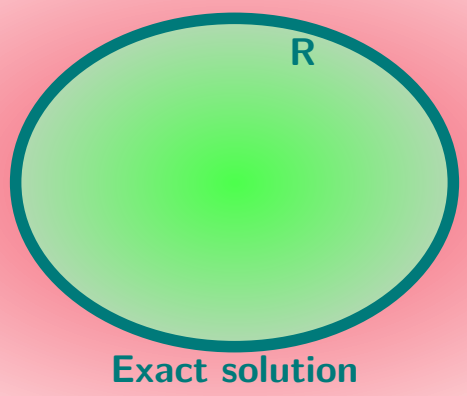

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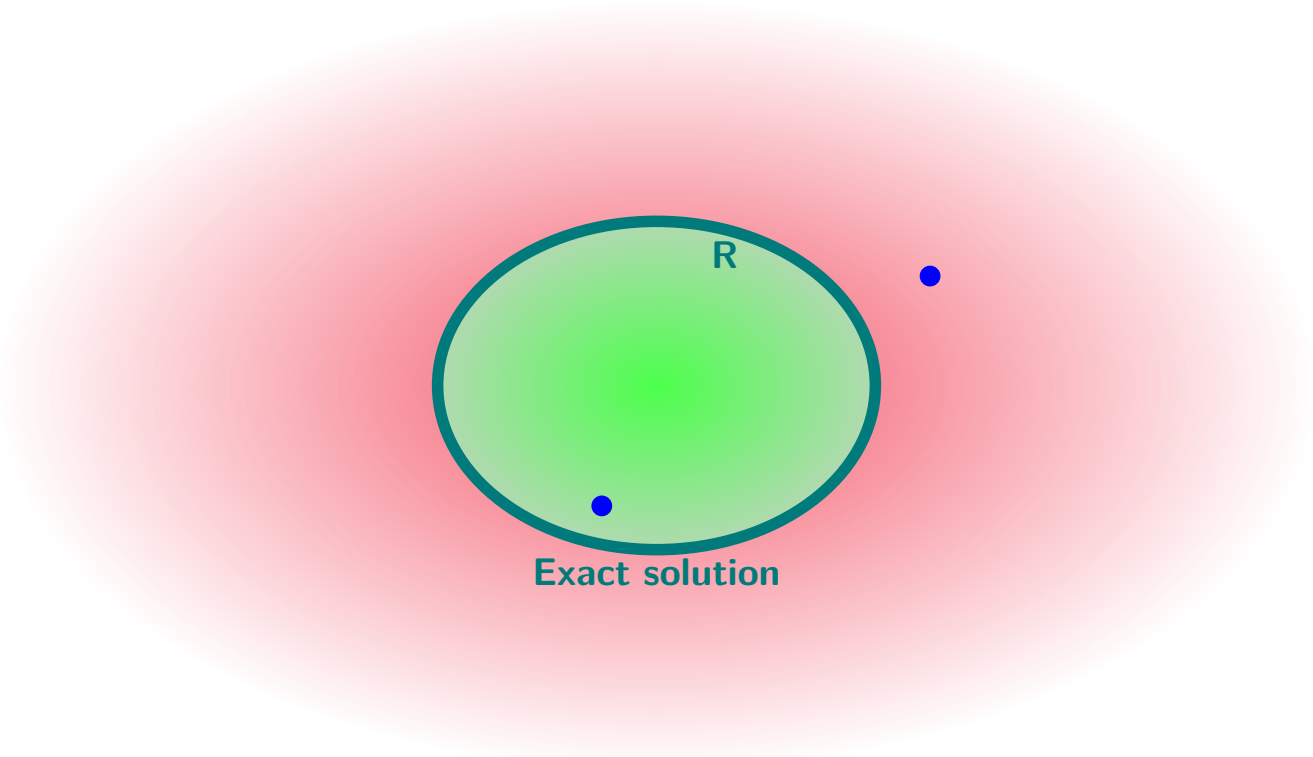

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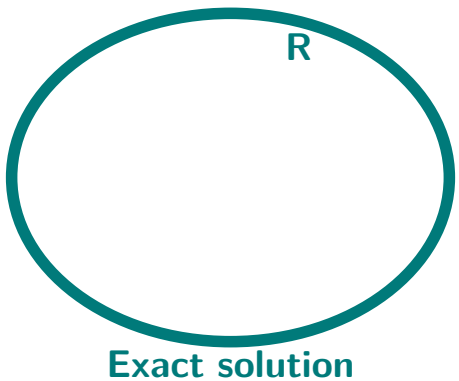

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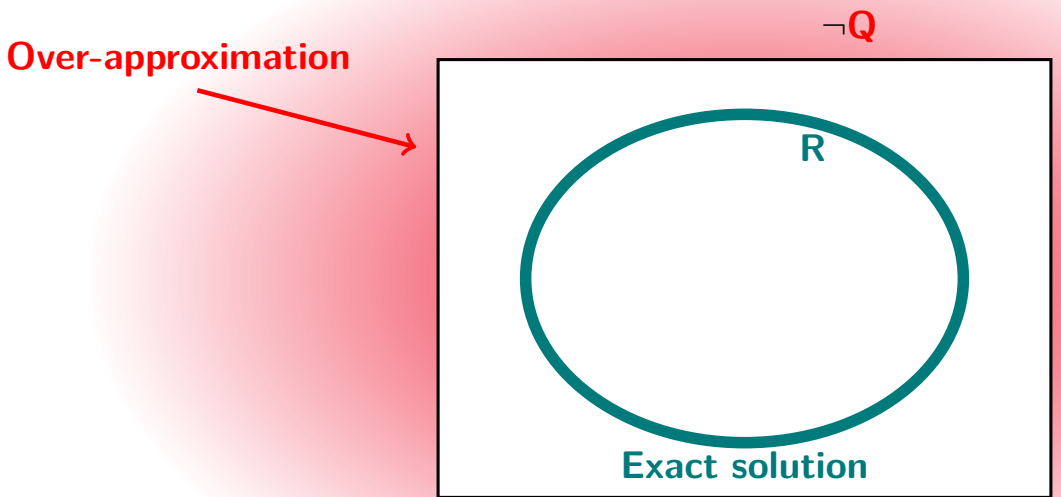

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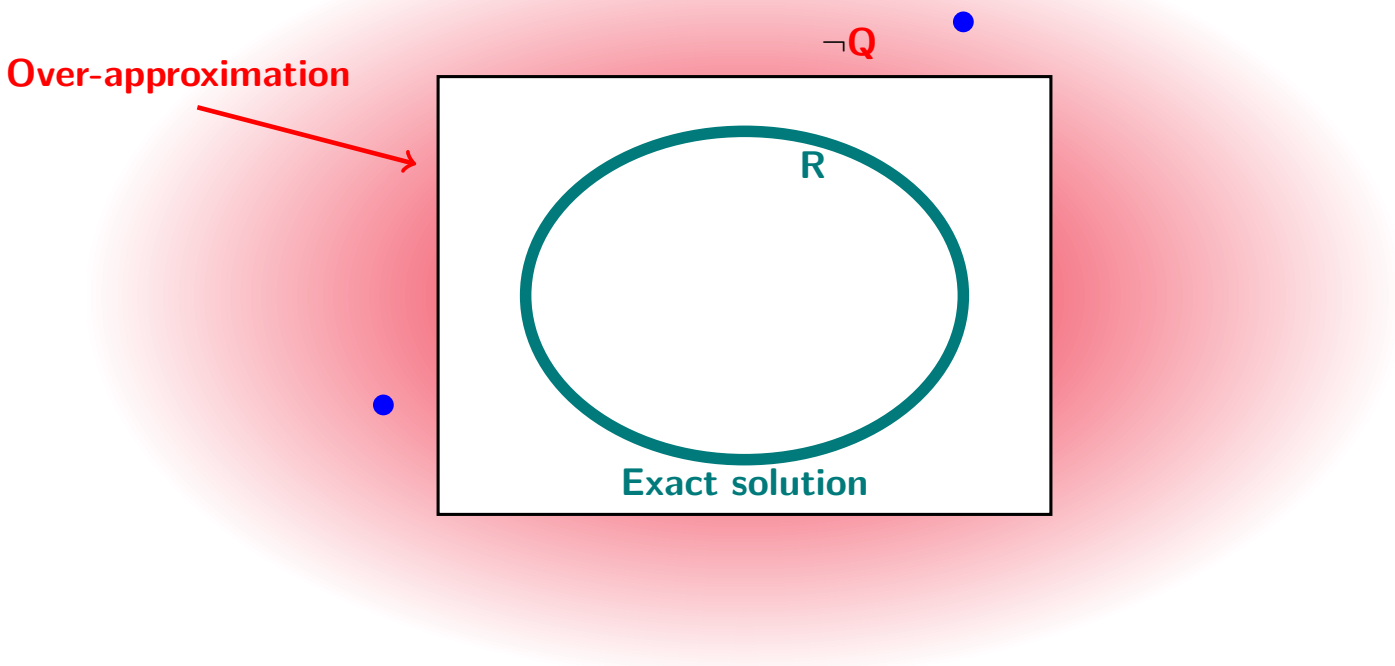

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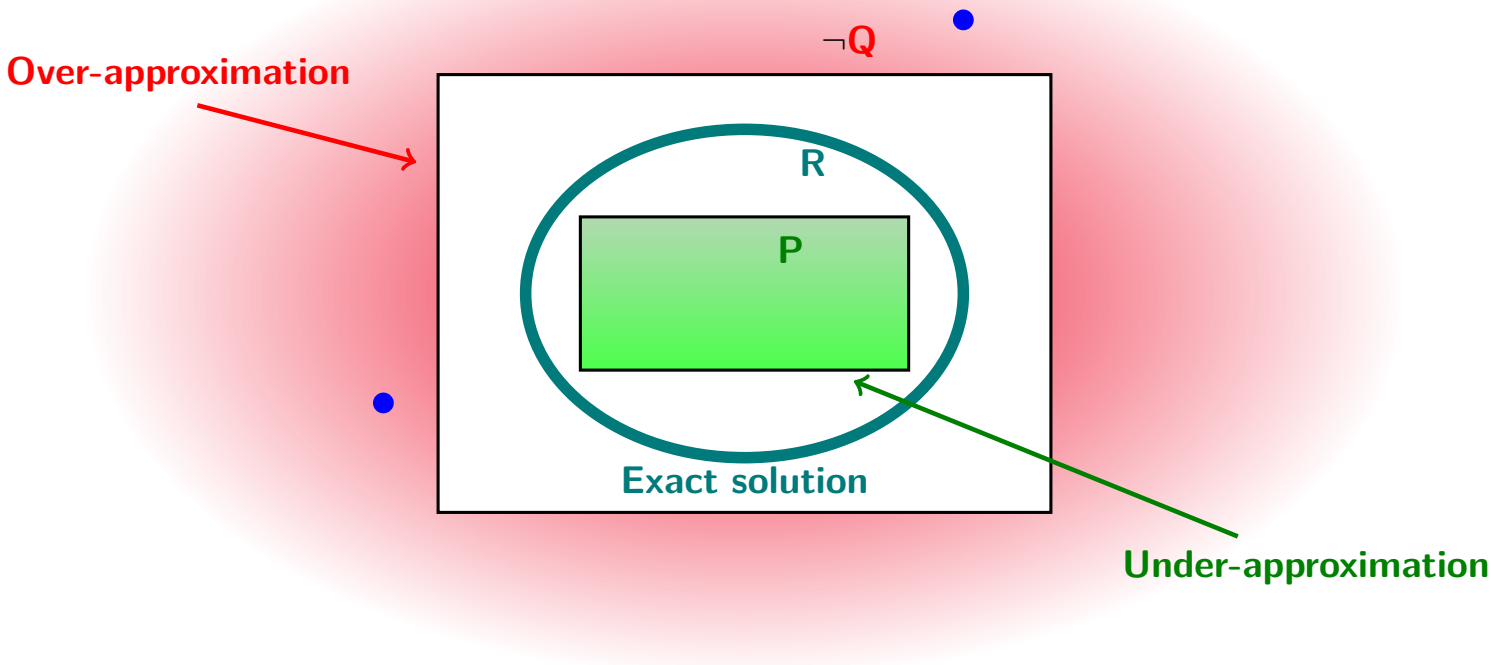

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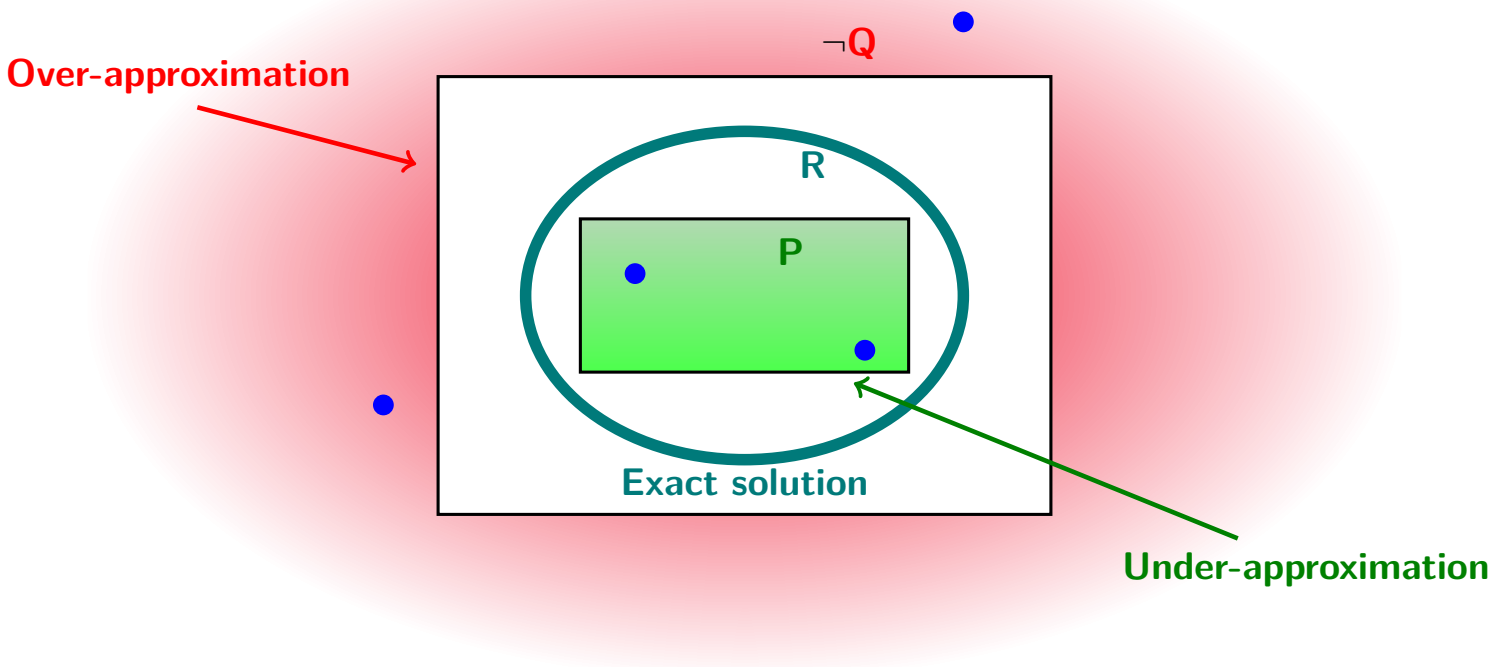

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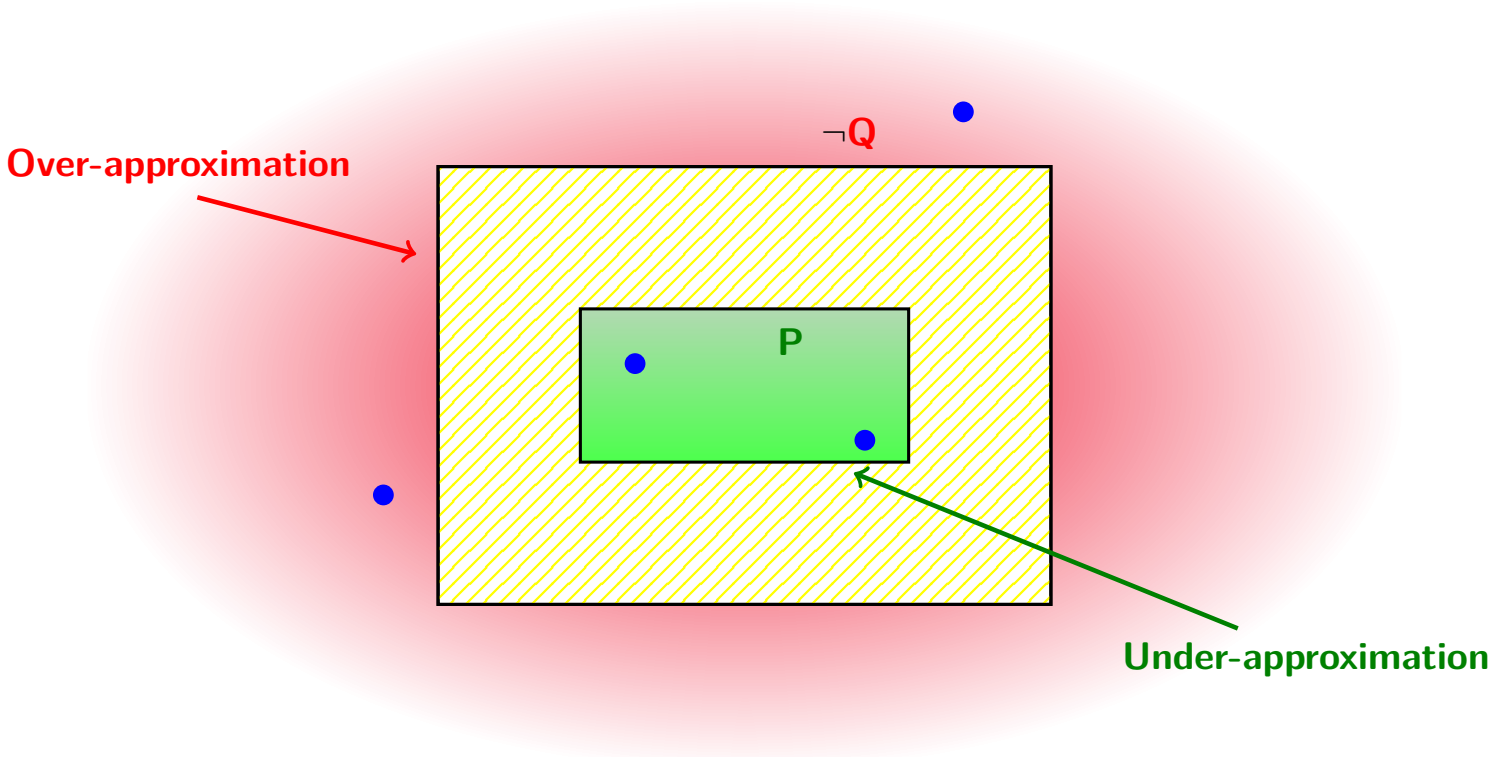
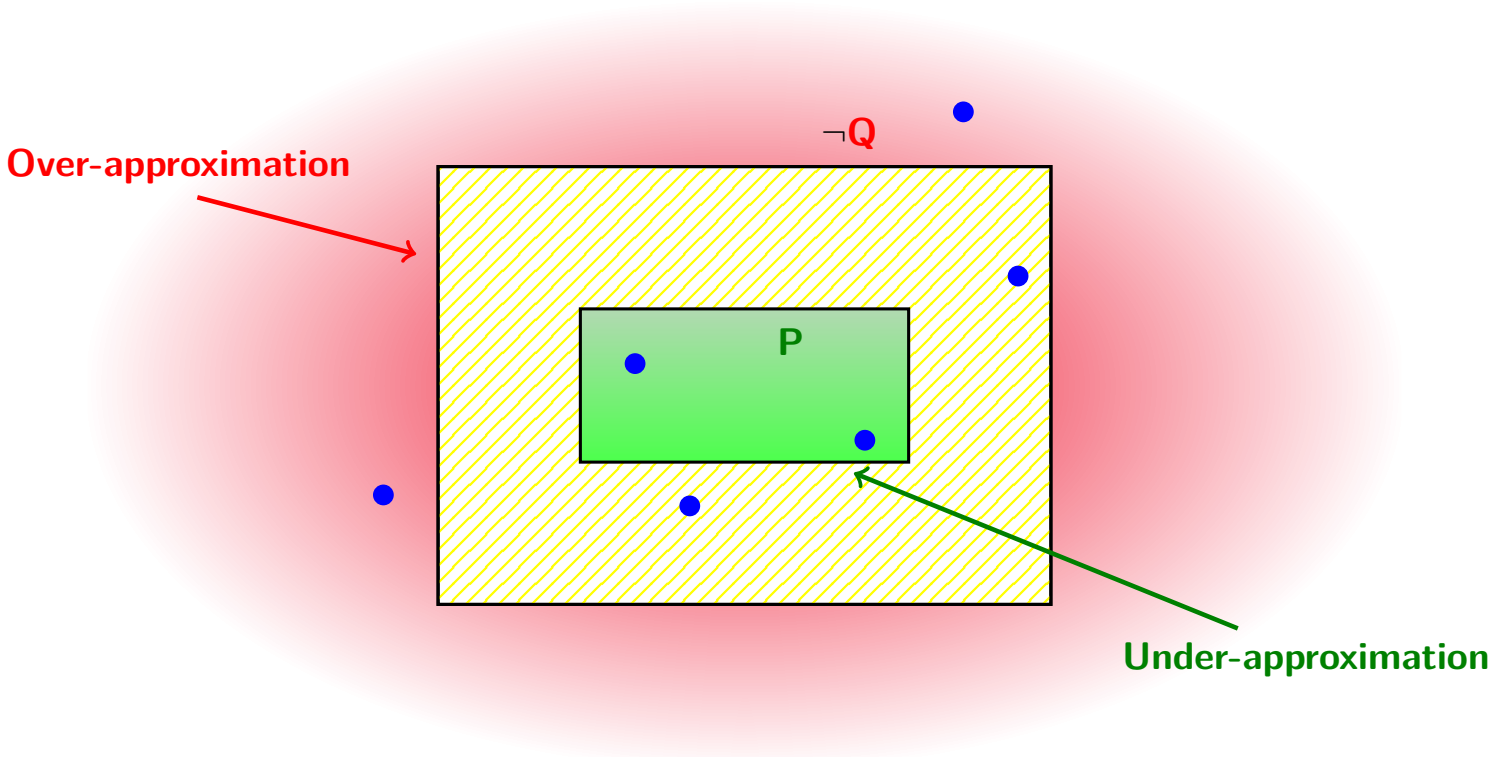


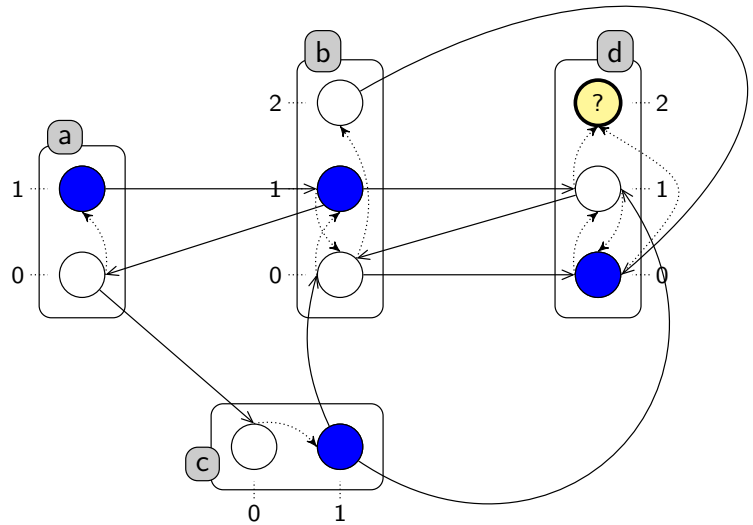
Approximations of the Dynamics

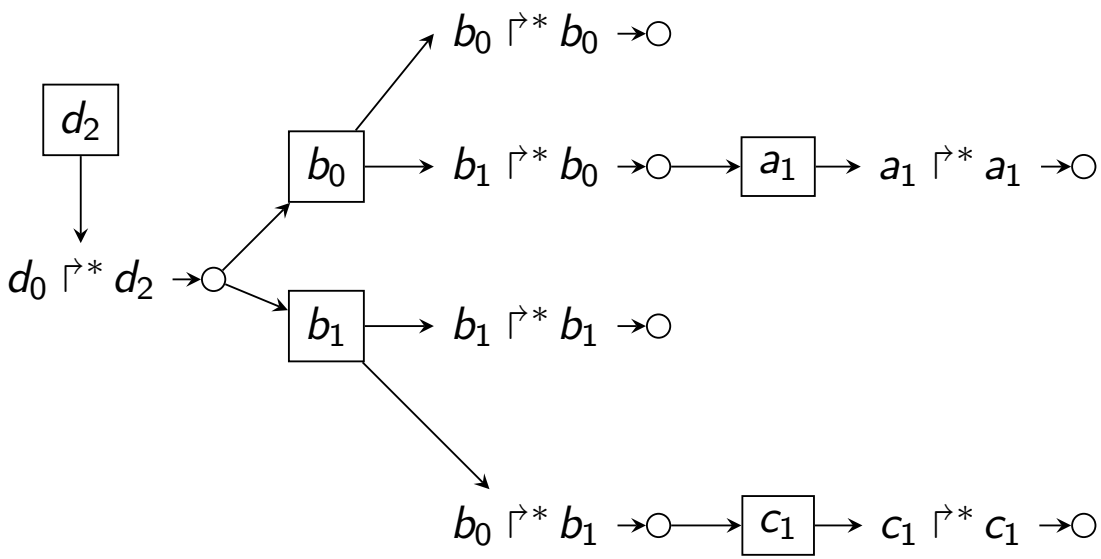
[Paulevé *et al.*, *Mathematical Structures in Computer Science*, 2012]
[Folschette *et al.*, *Theoretical Computer Science*, 2015]

- Directly checking **R** is hard (**exponential**)
- Rather check **approximations** **P** and **Q** so that: **P** ⇒ **R** ⇒ **Q**
  so that computing **P** and **Q** is faster (roughly **polynomial**)



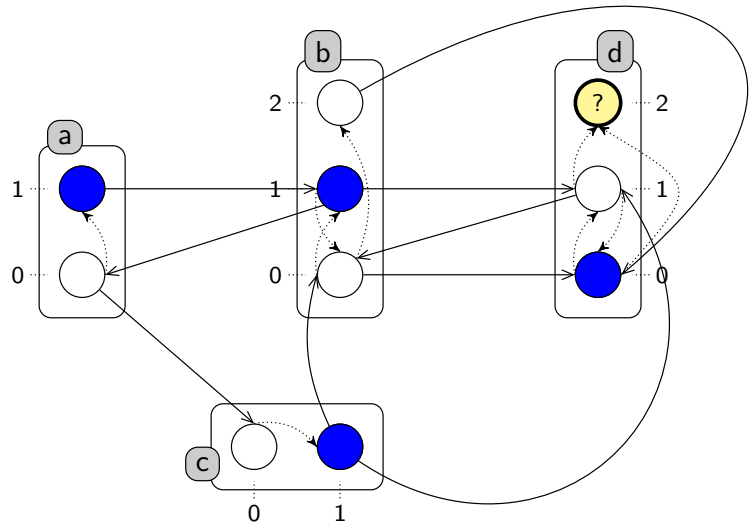
Under-approximation





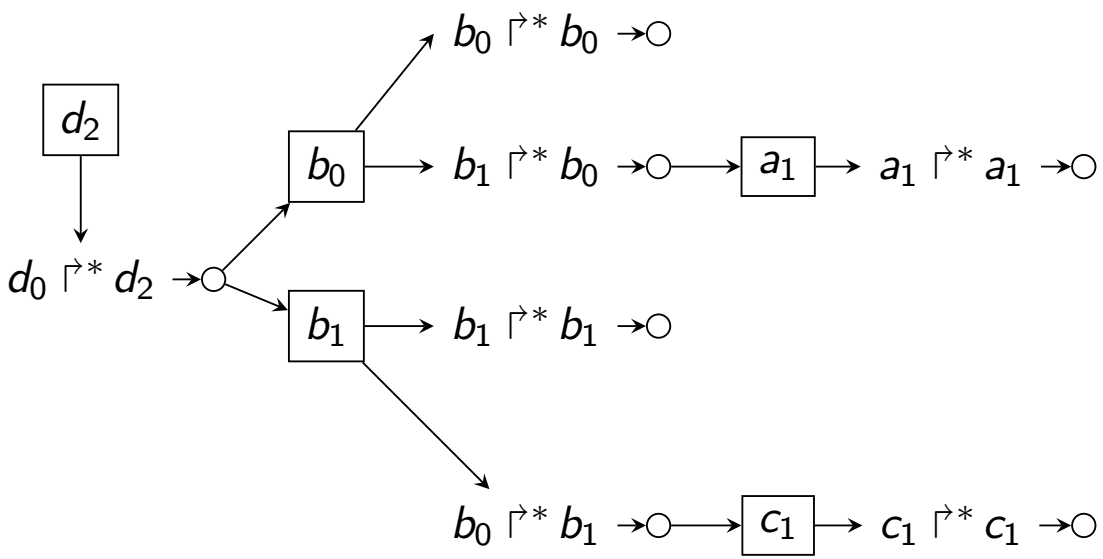
d_2	Required local state
$d_0 \lceil^* d_2$	Objective
○	Solution to an objective

Under-approximation



Sufficient condition P:

- no cycle
- each objective has a solution



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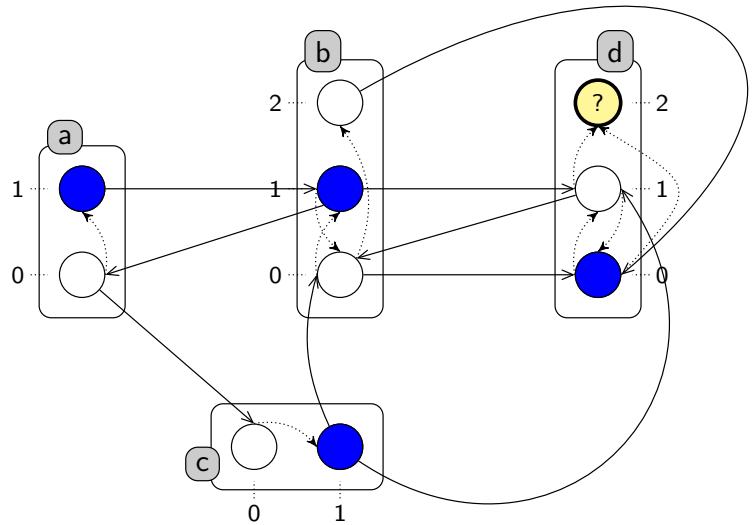

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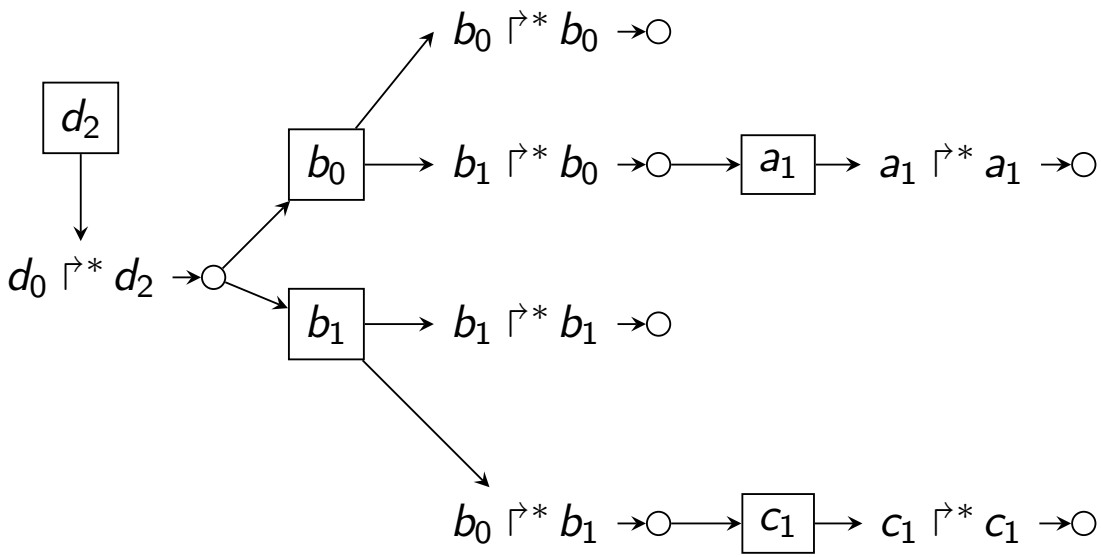
P is true ⇒ R is true



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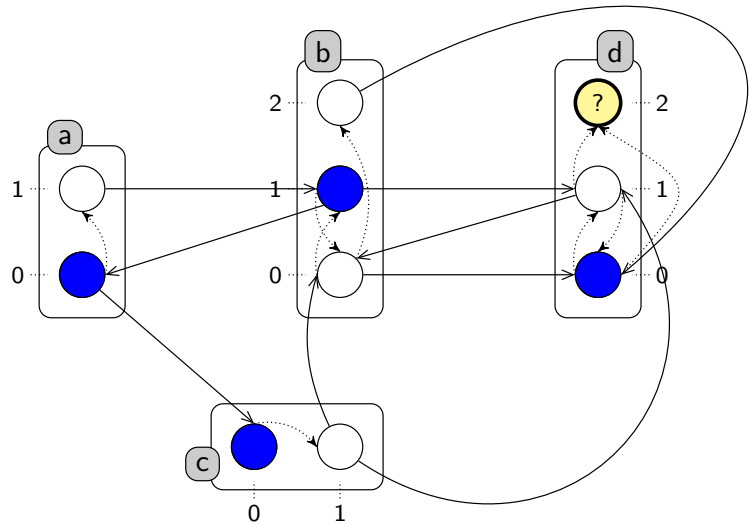

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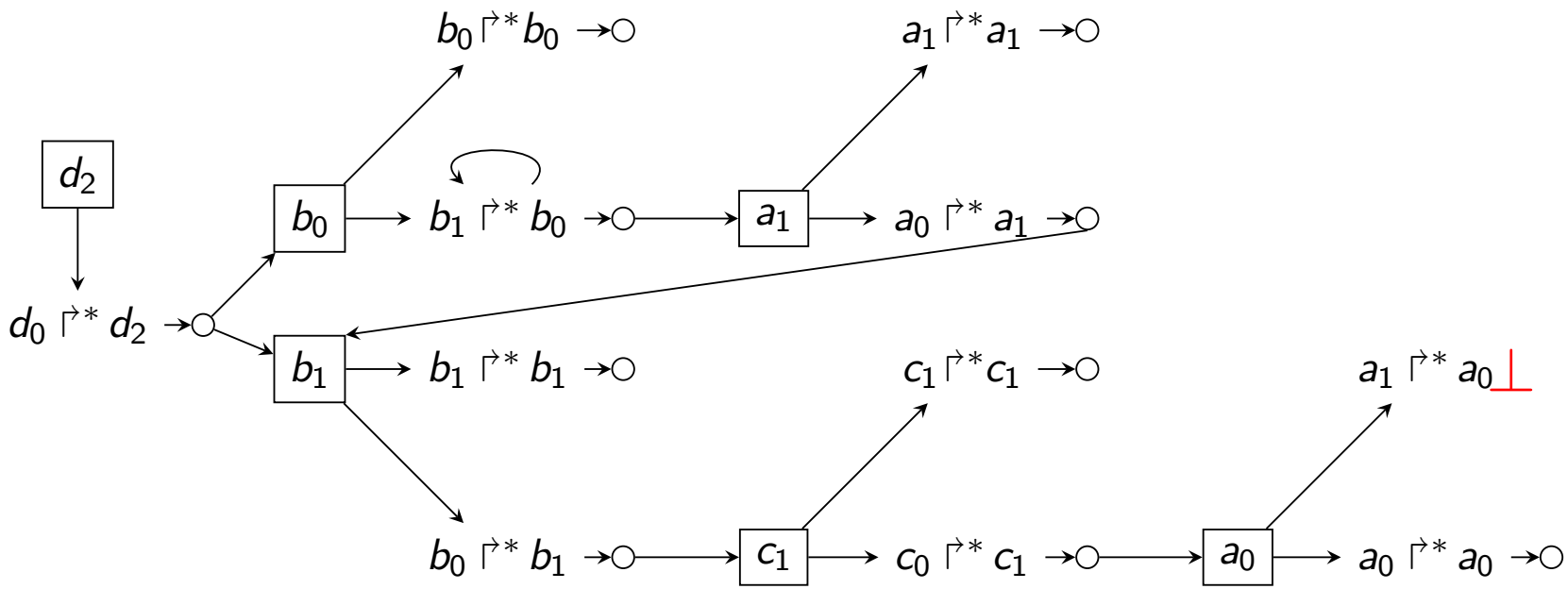

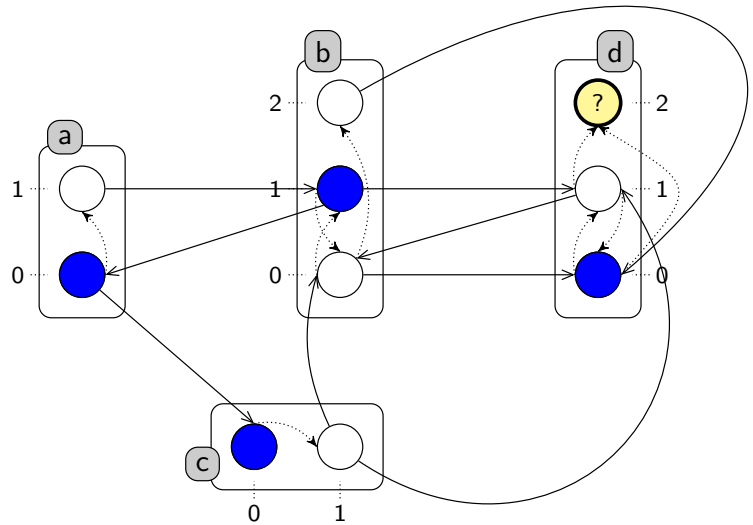

Under-approximation

Sufficient condition P:

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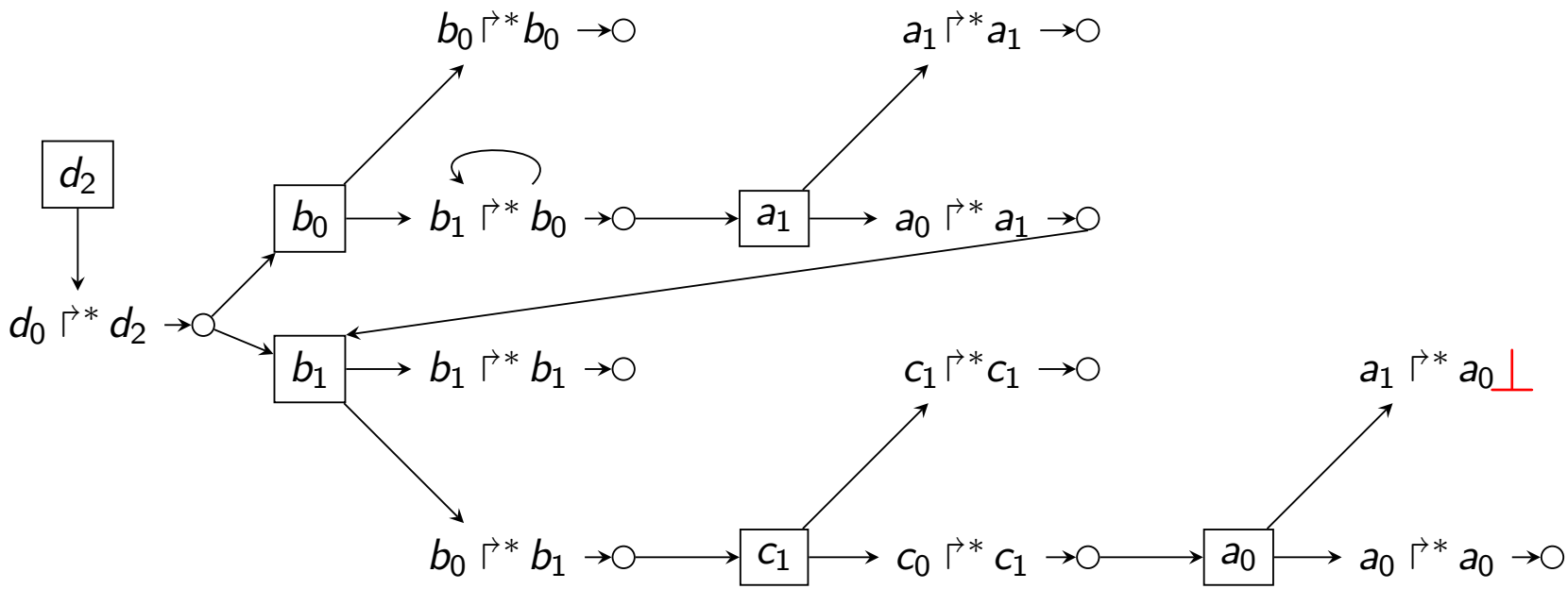
P is false ⇒ Inconclusive



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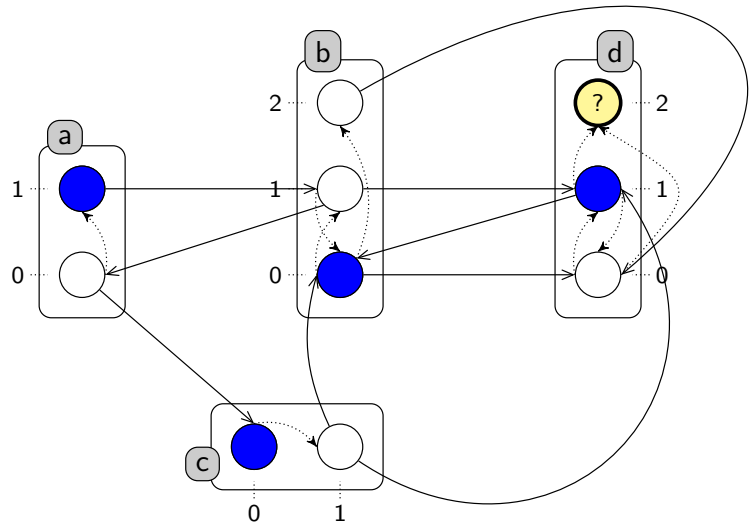
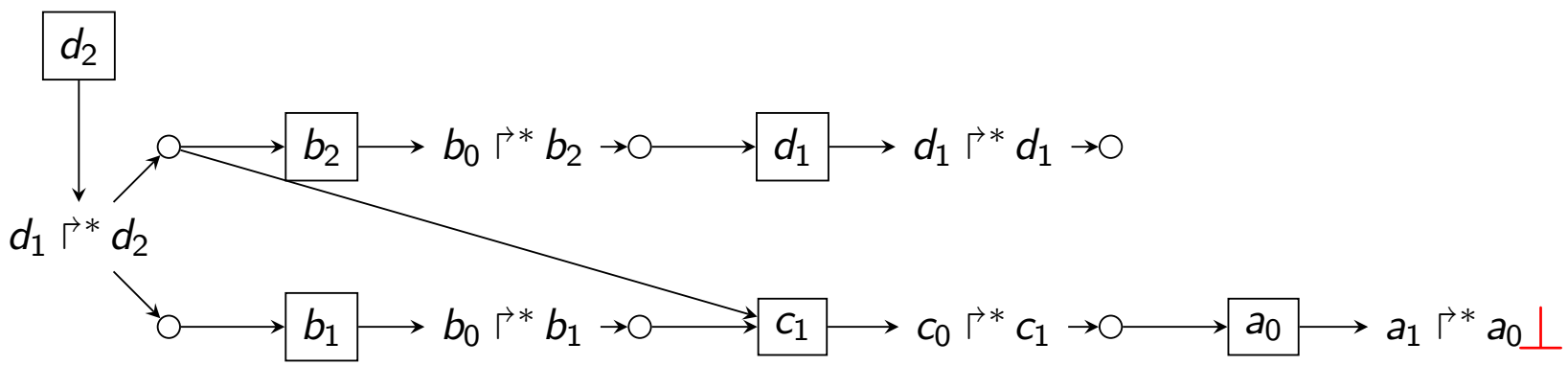
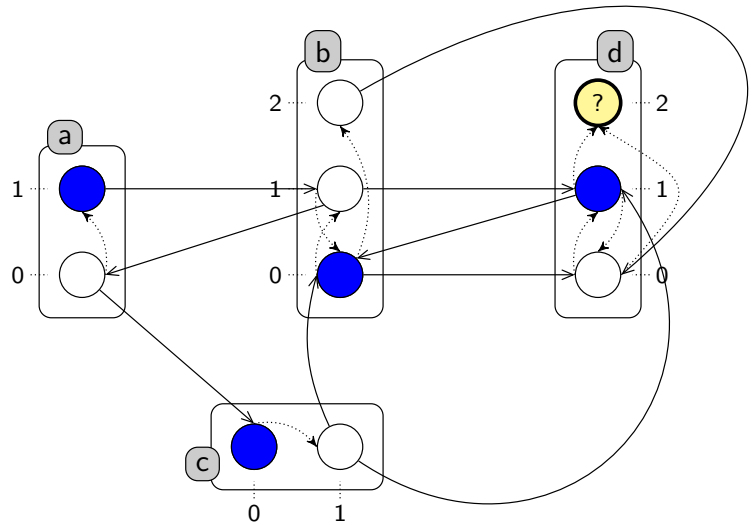


Necessary condition Q:





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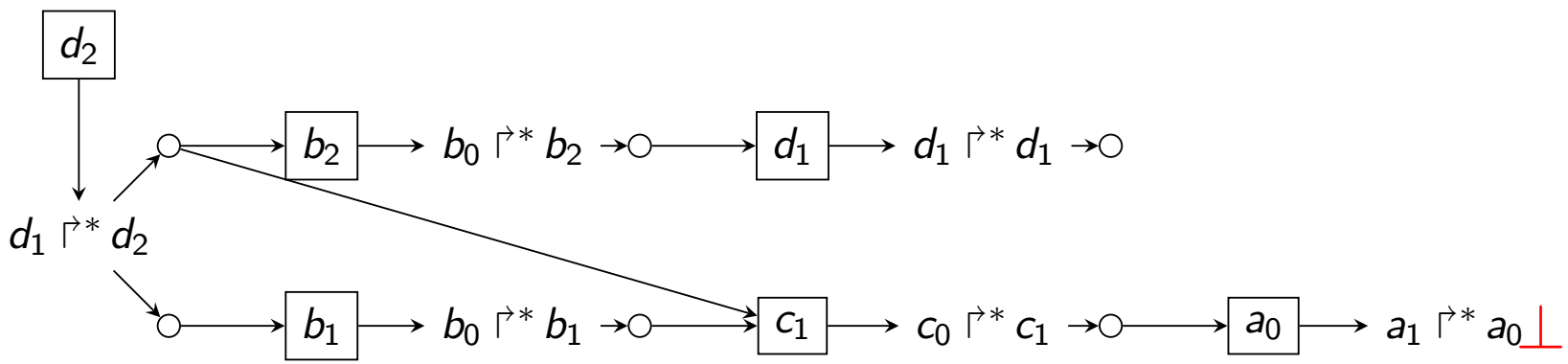

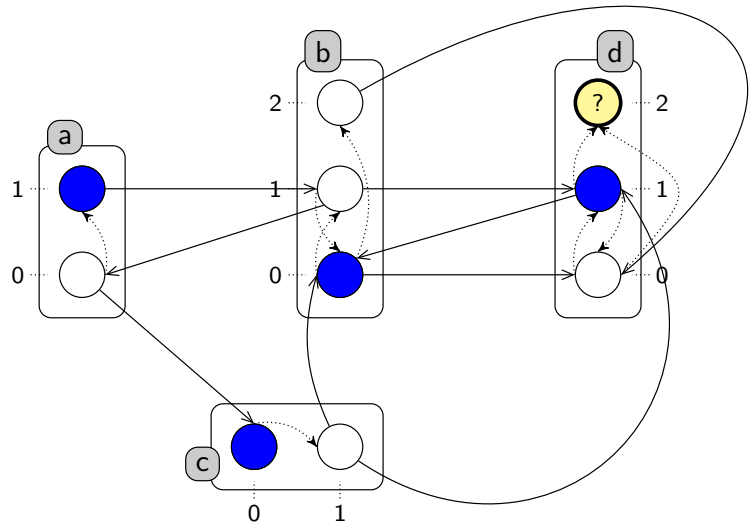

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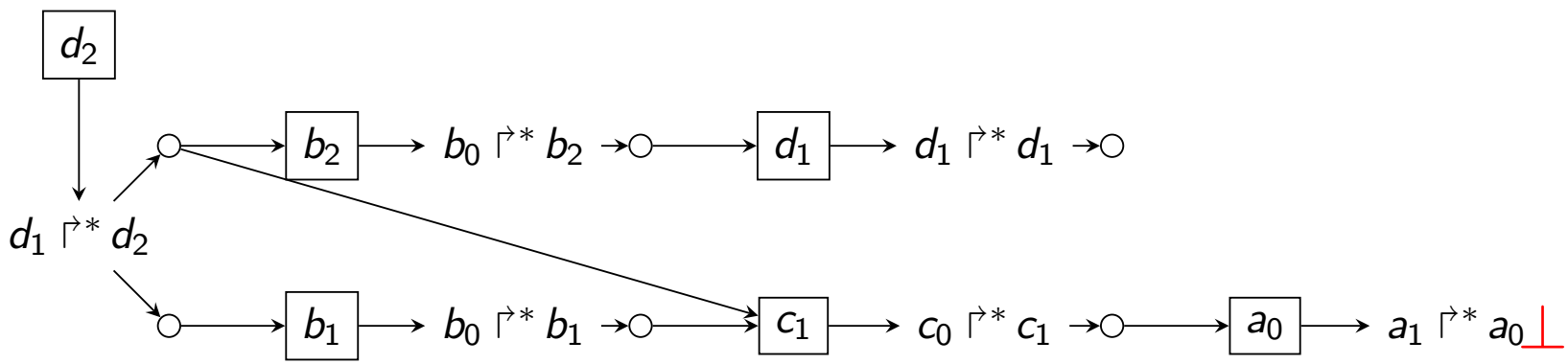

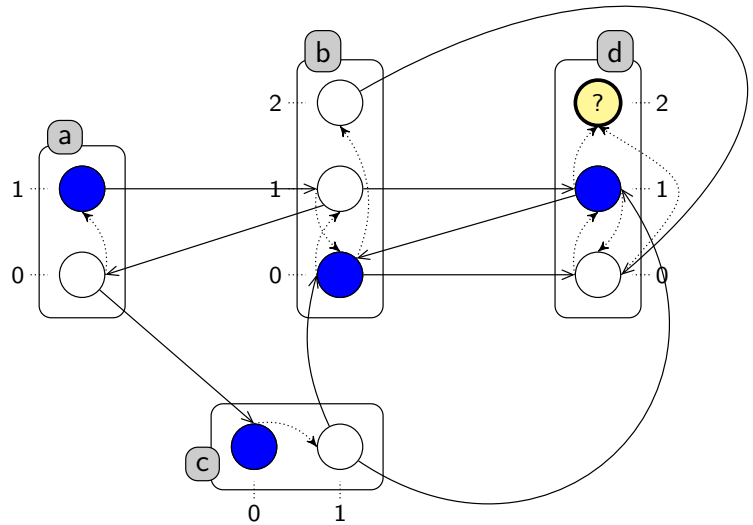

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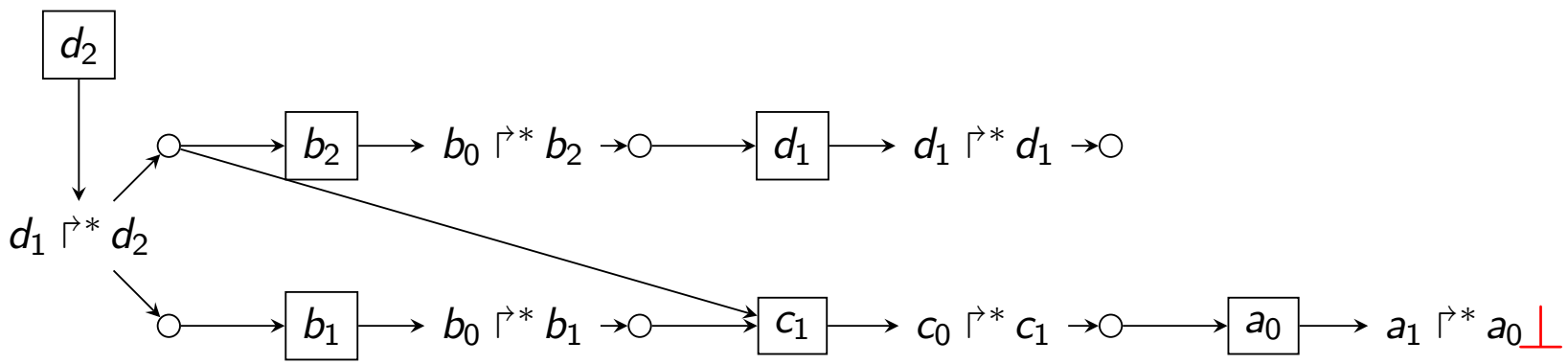

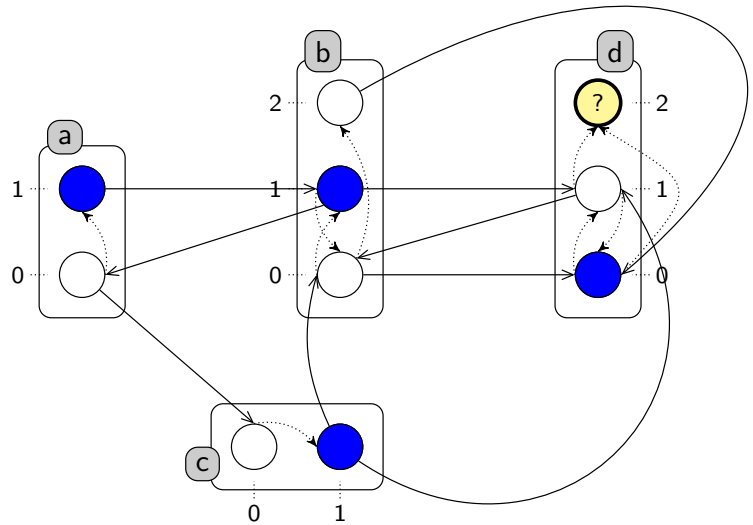

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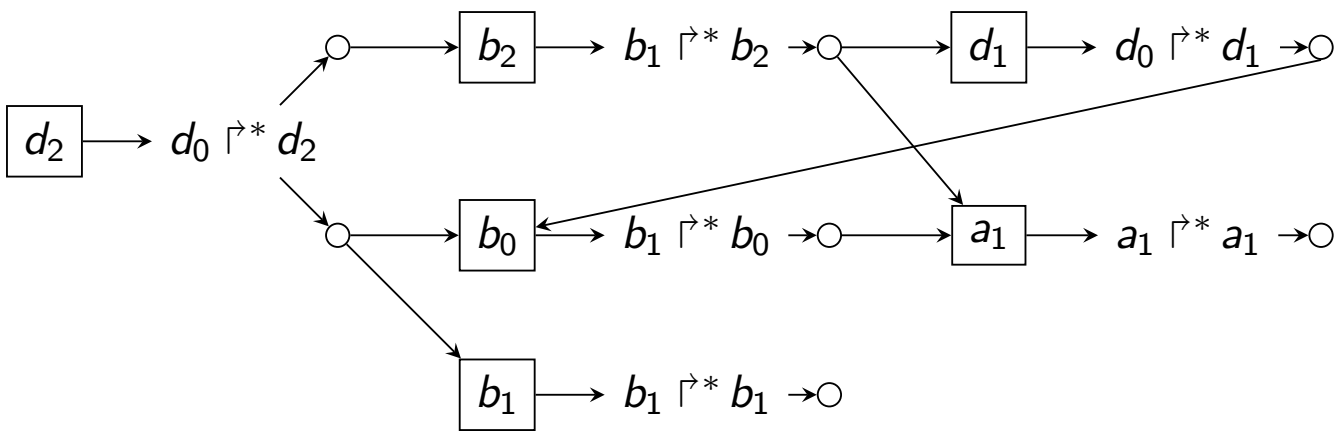

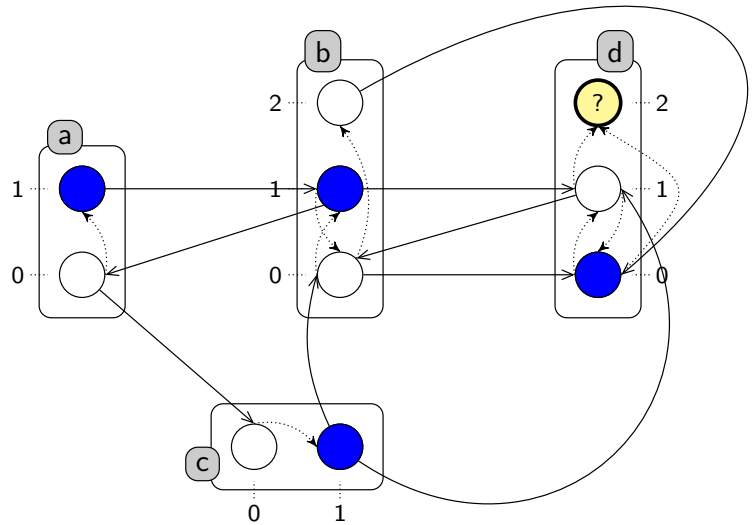

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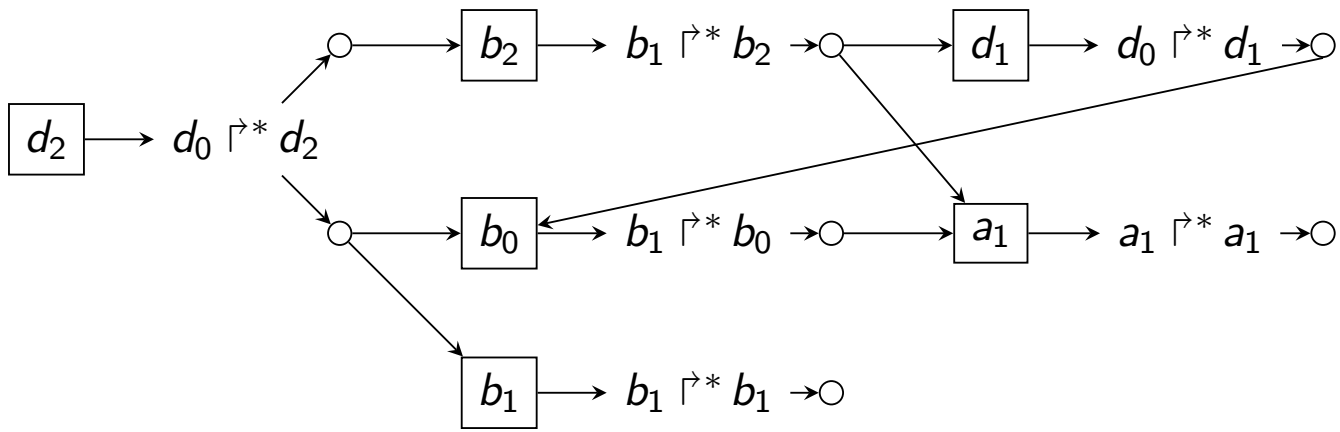


Results

[Paulevé, *Computational Methods in Systems Biology*, 2017]
[Folschette *et al.*, *Theoretical Computer Science*, 2015]

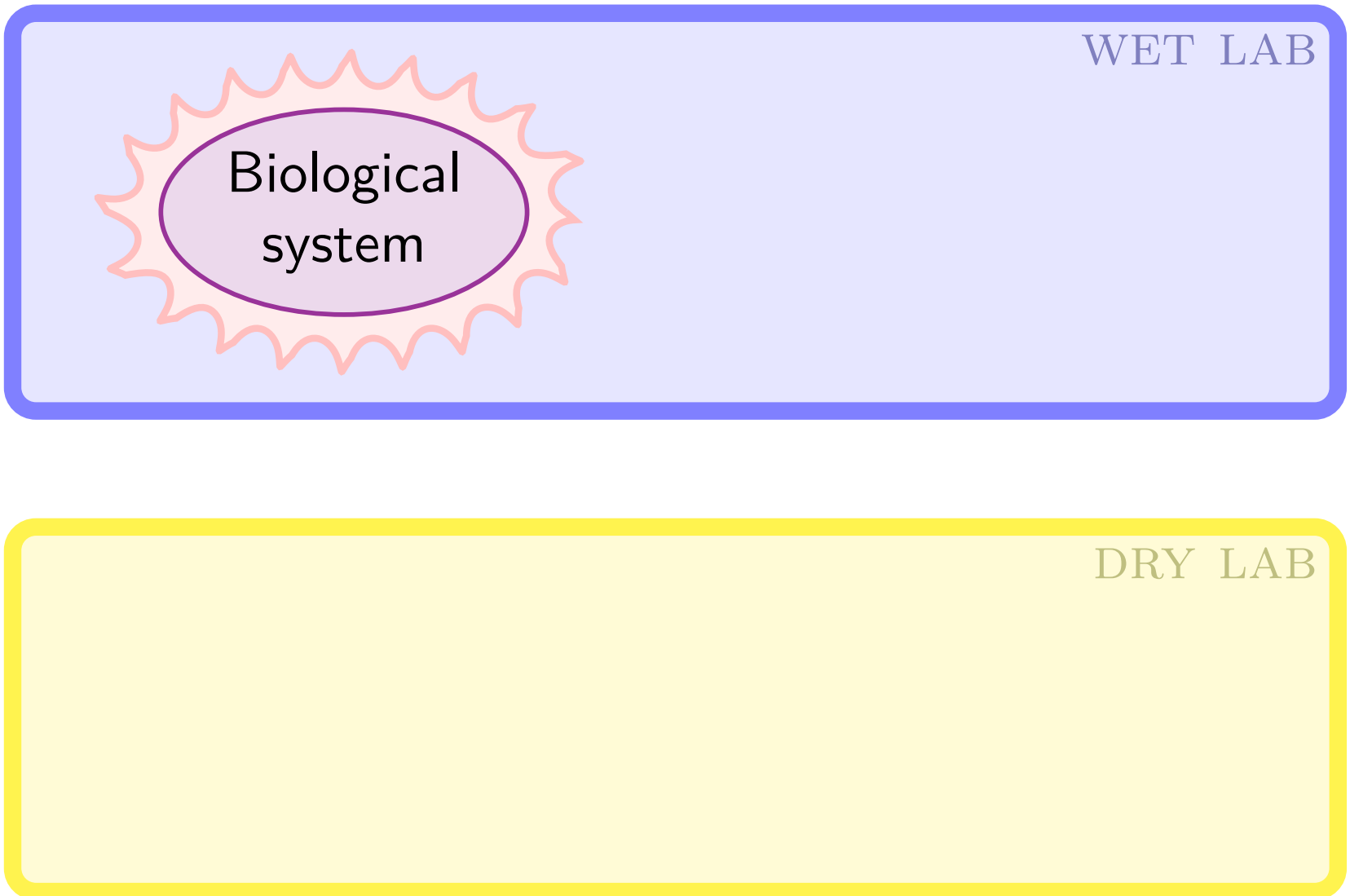
Model	nodes	NuSMV	ITS-reach	Pint
TCell-d	101	2s	0.5s	0.02s
		KO	16min	4.5s
RBE2F	370	KO	KO	0.2s
MAPK	309	KO	KO	48s

- KO = Out of memory
- 100% conclusiveness with Pint (no **Inconclusive**)

NuSMV: [Cimatti *et al.*, *CAV*, 2002. `http://nusmv.fbk.eu/`]
ITS-reach: [Thierry-Mieg, *TACAS*, 2015. `http://ddd.lip6.fr/itstools.php`]
Pint: [Paulevé, *Computational Methods in Systems Biology*, 2017. `http://loicpauleve.name/pint/`]

TCell-d: [Abou-Jaoudé *et al.*, *Frontiers in Bioengineering and Biotechnology*, 2015]
RBE2F: [Rougny, *et al.*, *BMC Systems Biology*, 2016]
MAPK: [Schoeberl *et al.*, *Nature biotechnology*, 2002]

Experiments *in silico*



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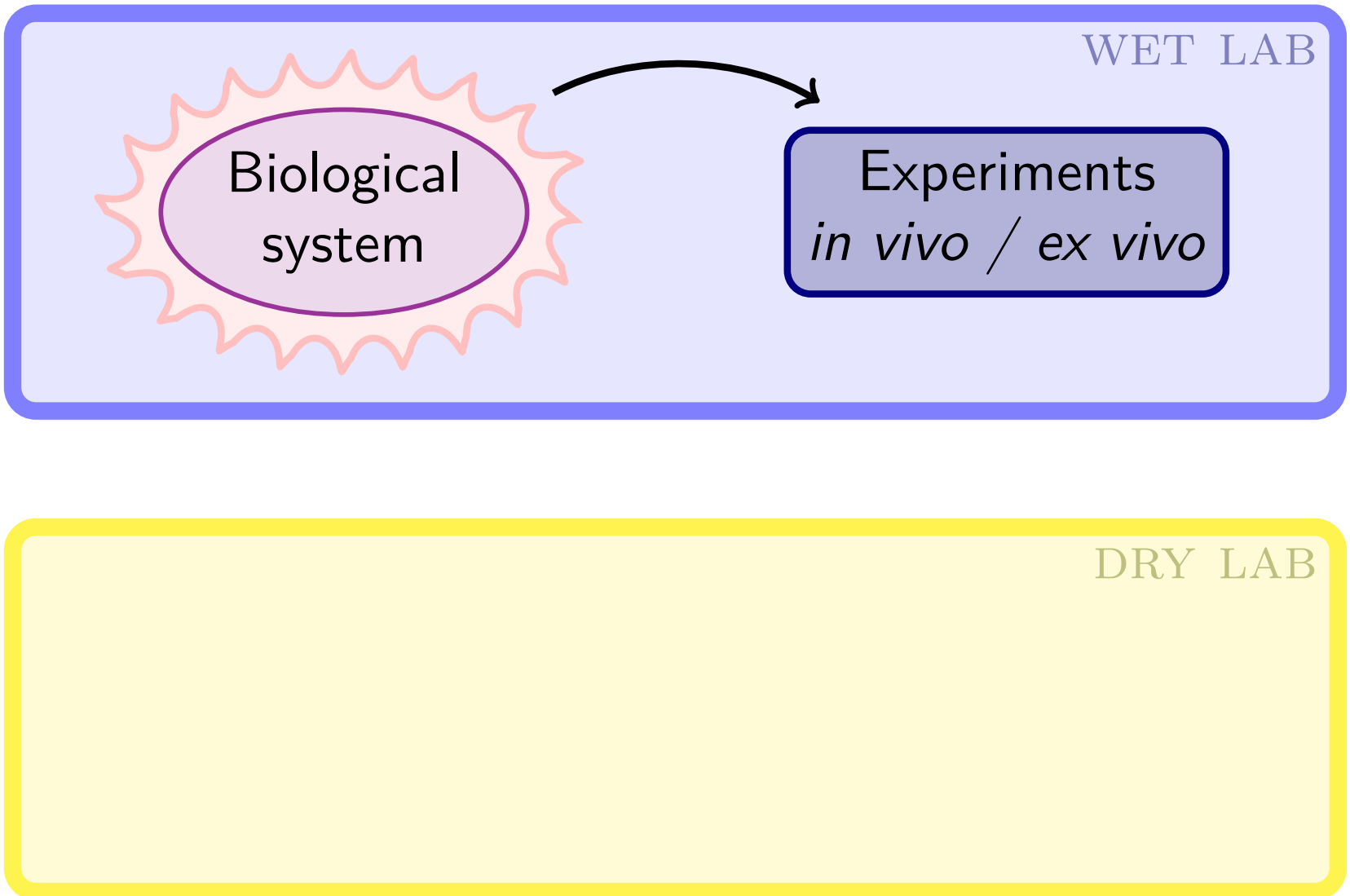

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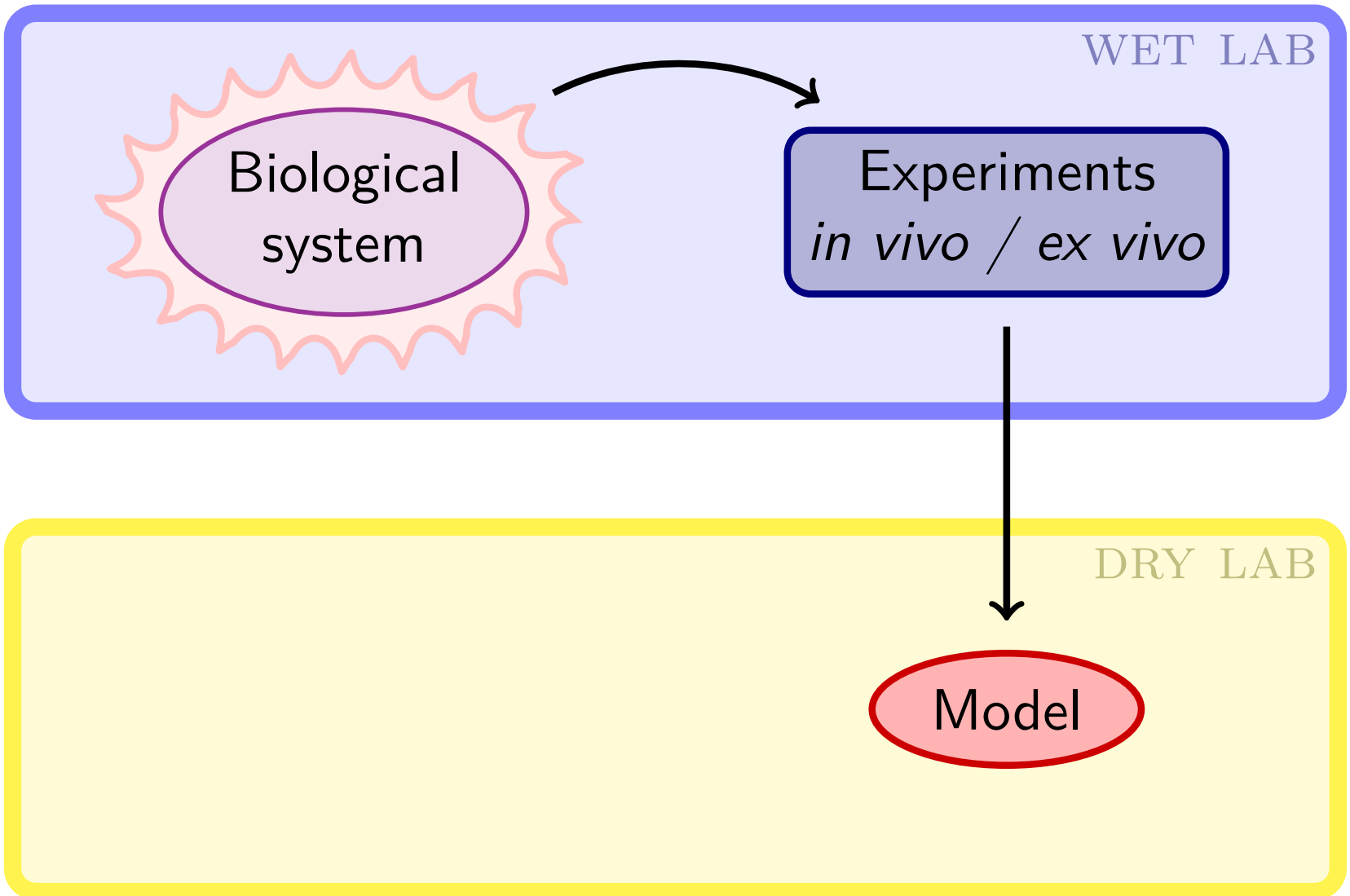

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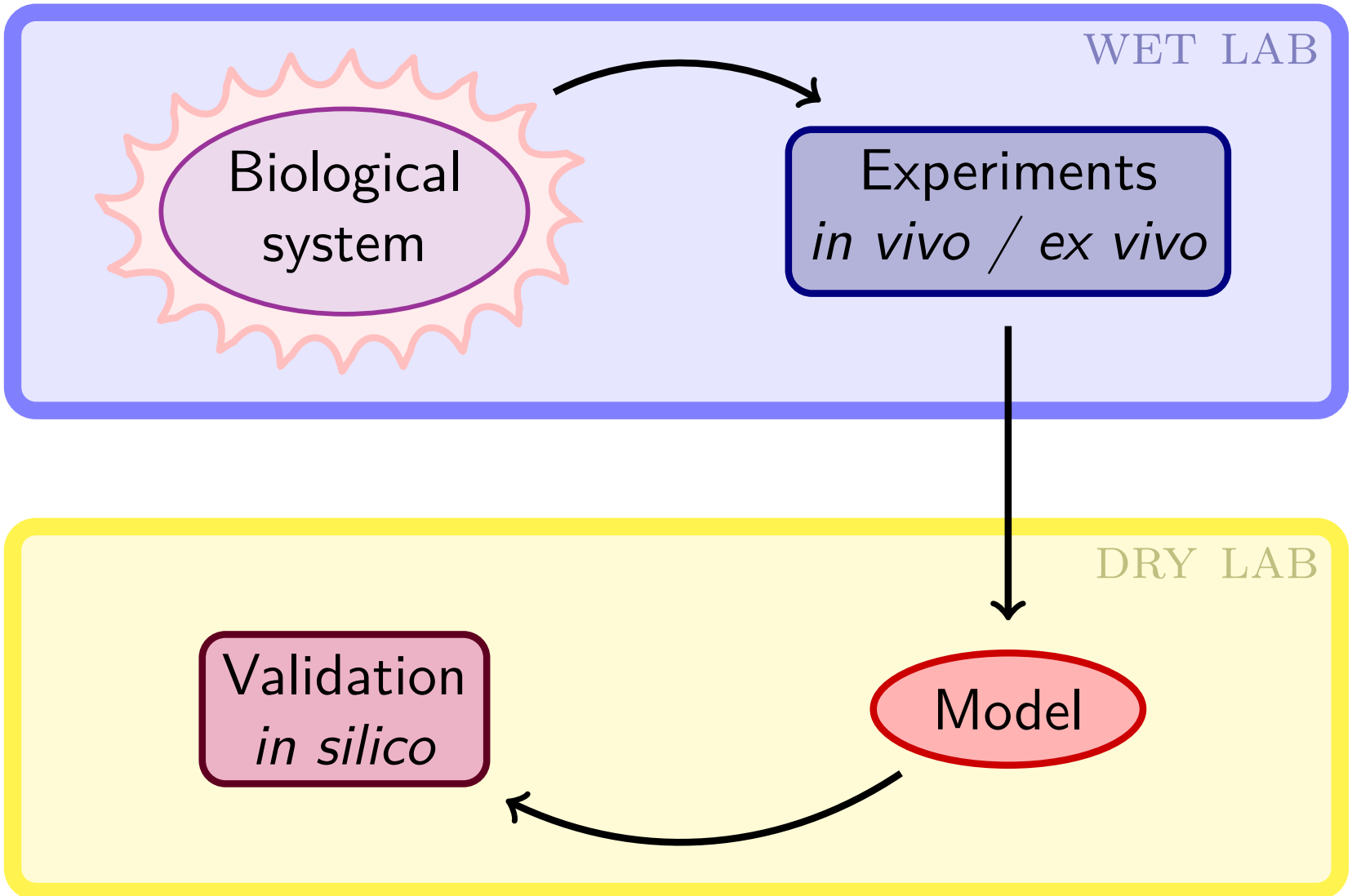

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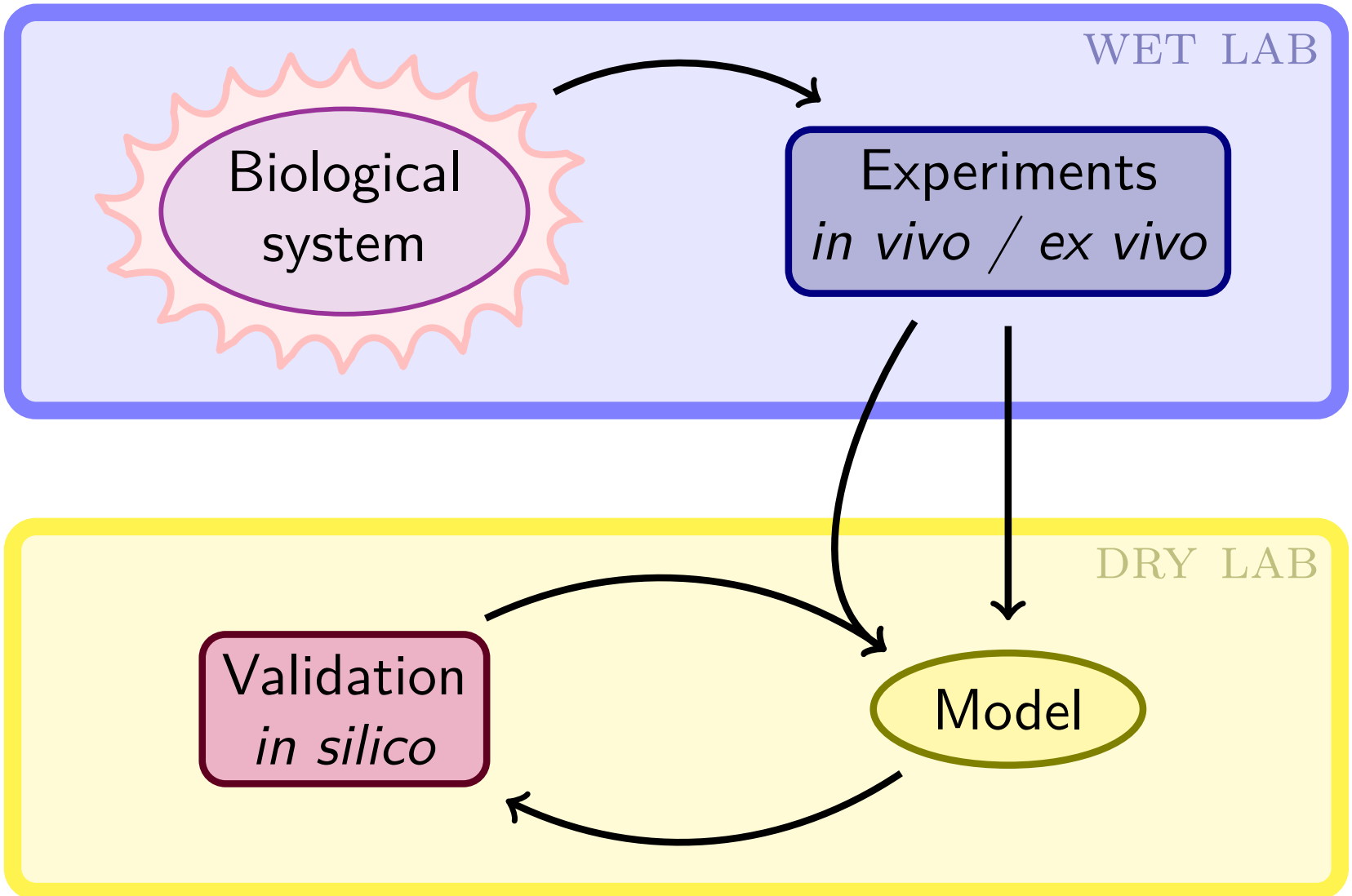

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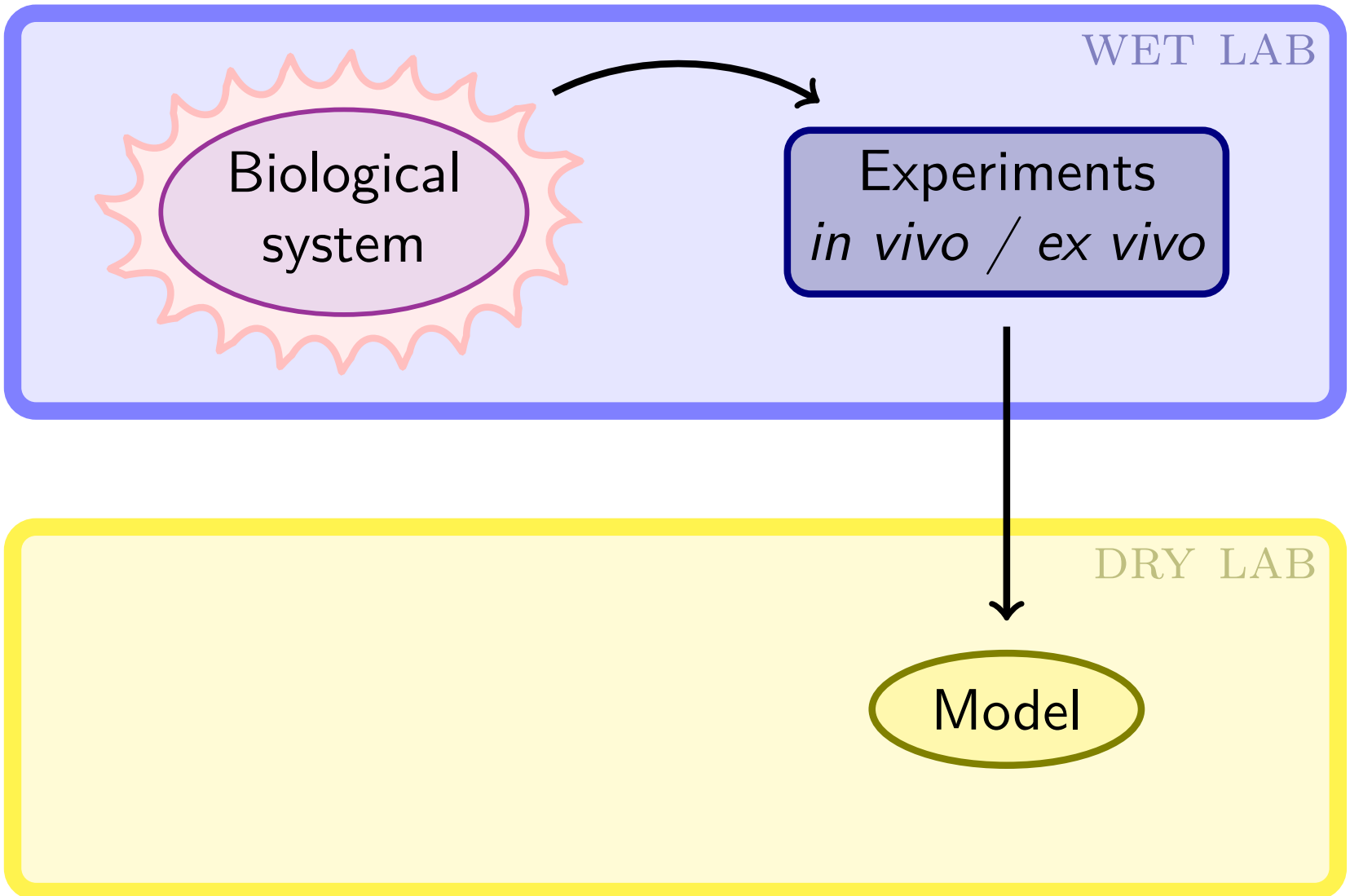

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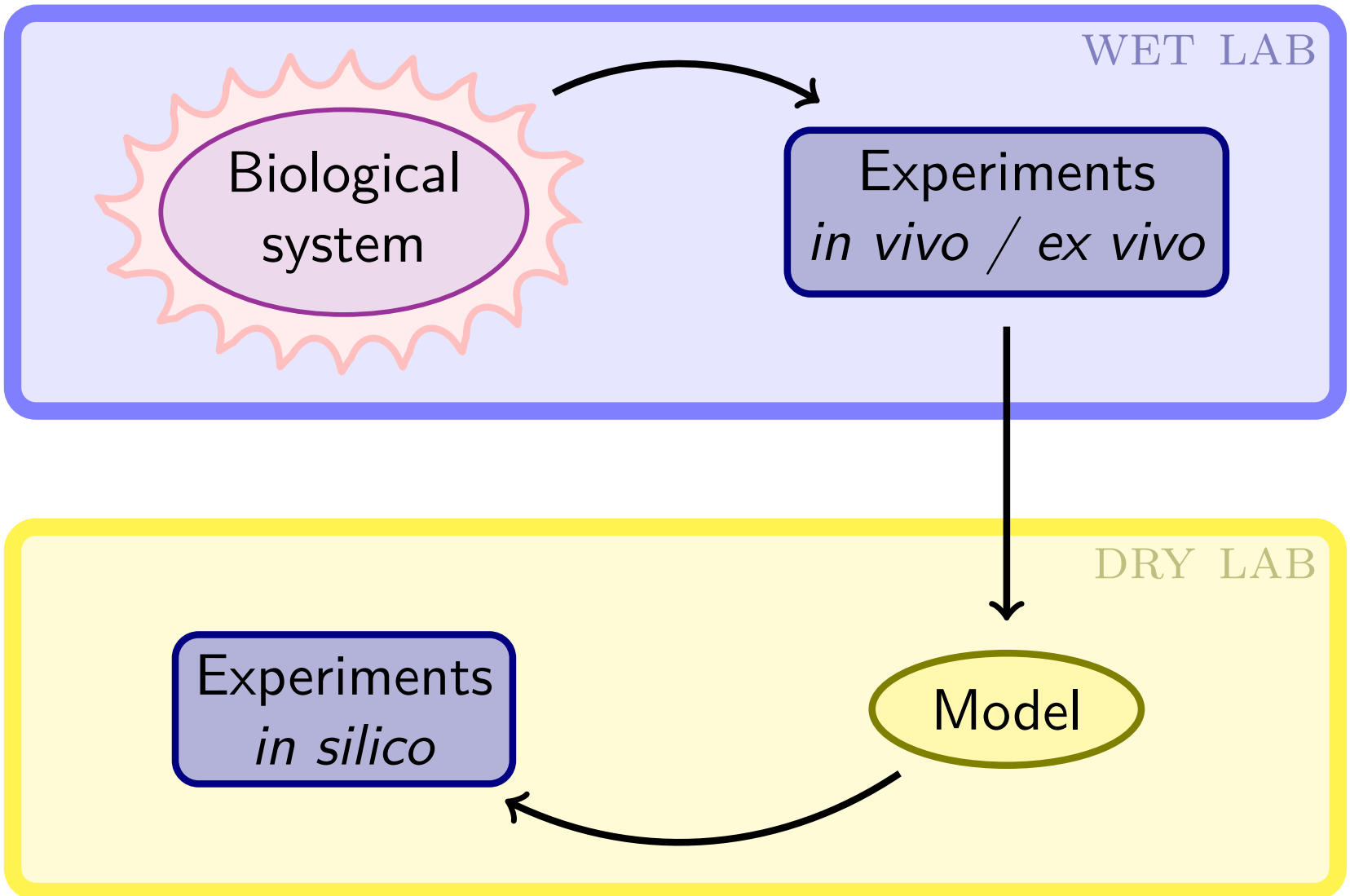

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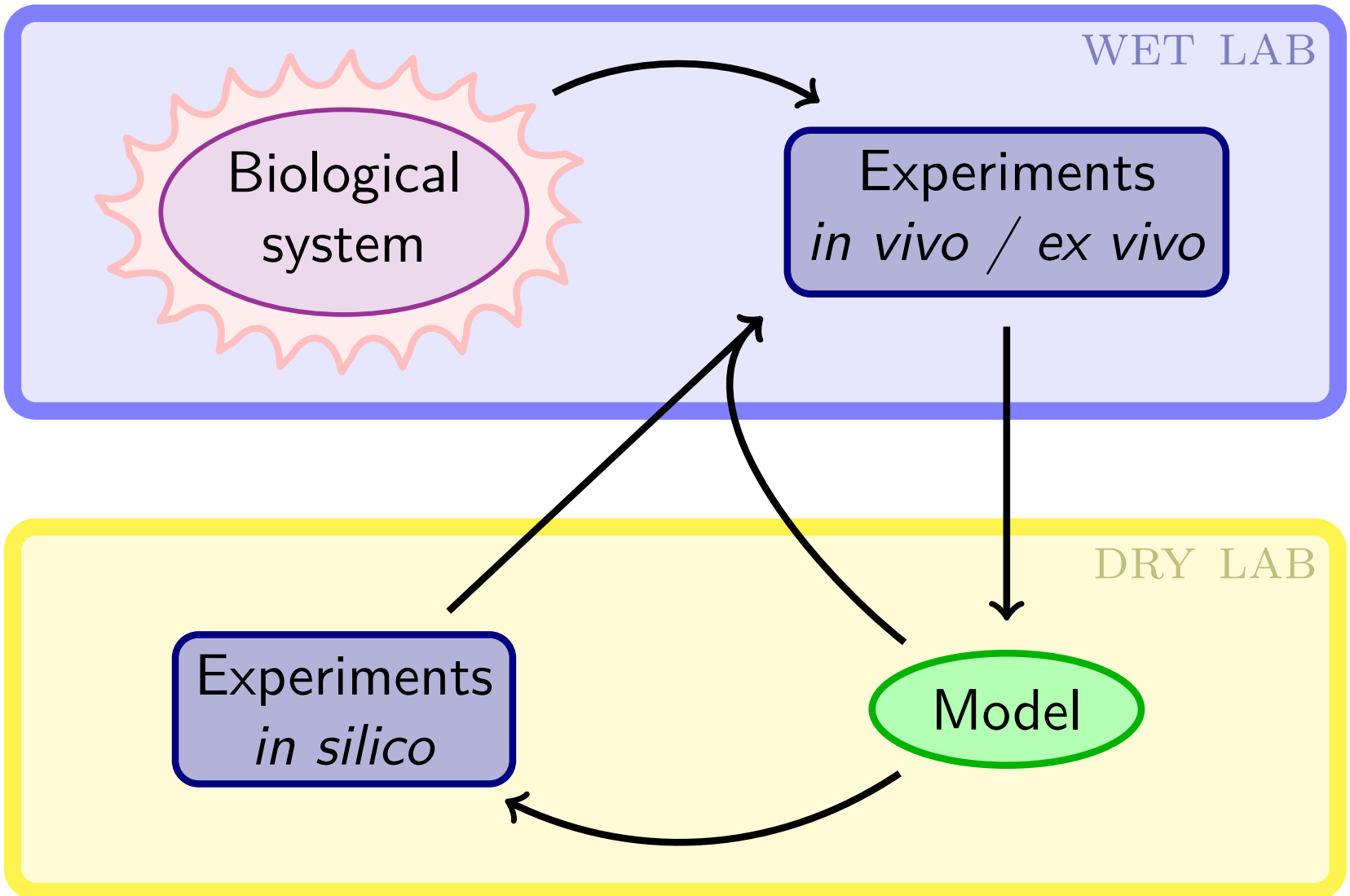

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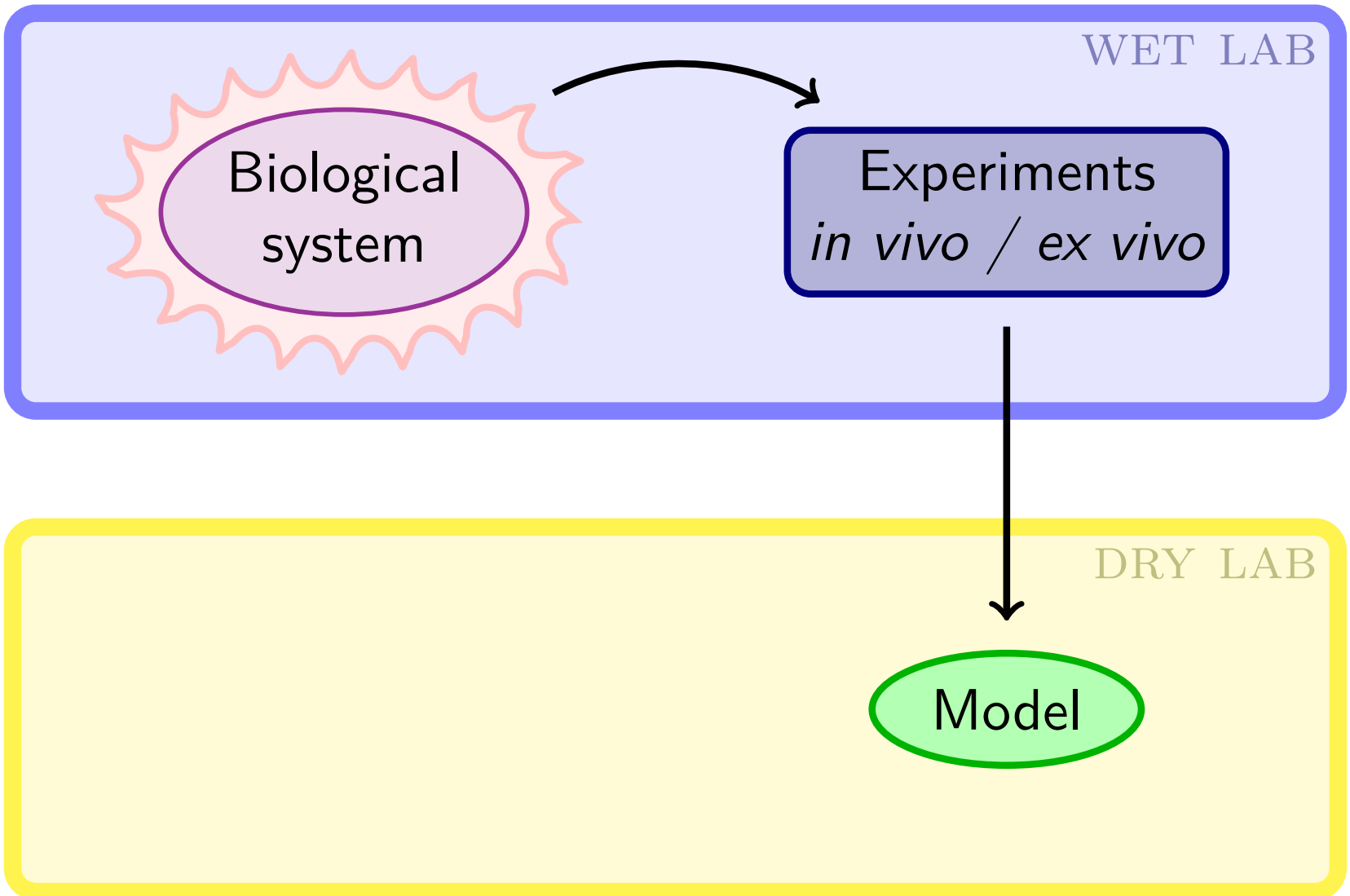

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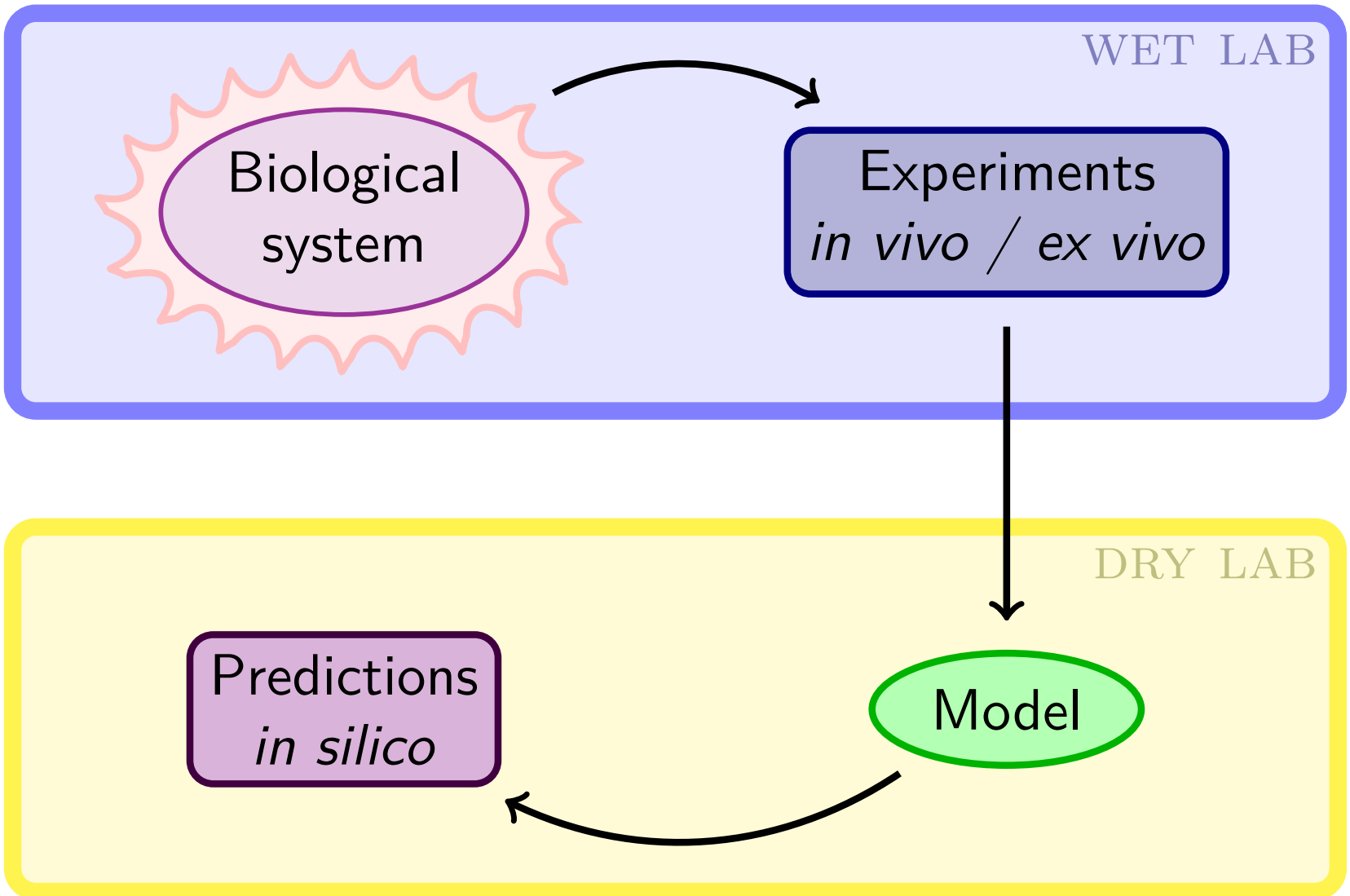

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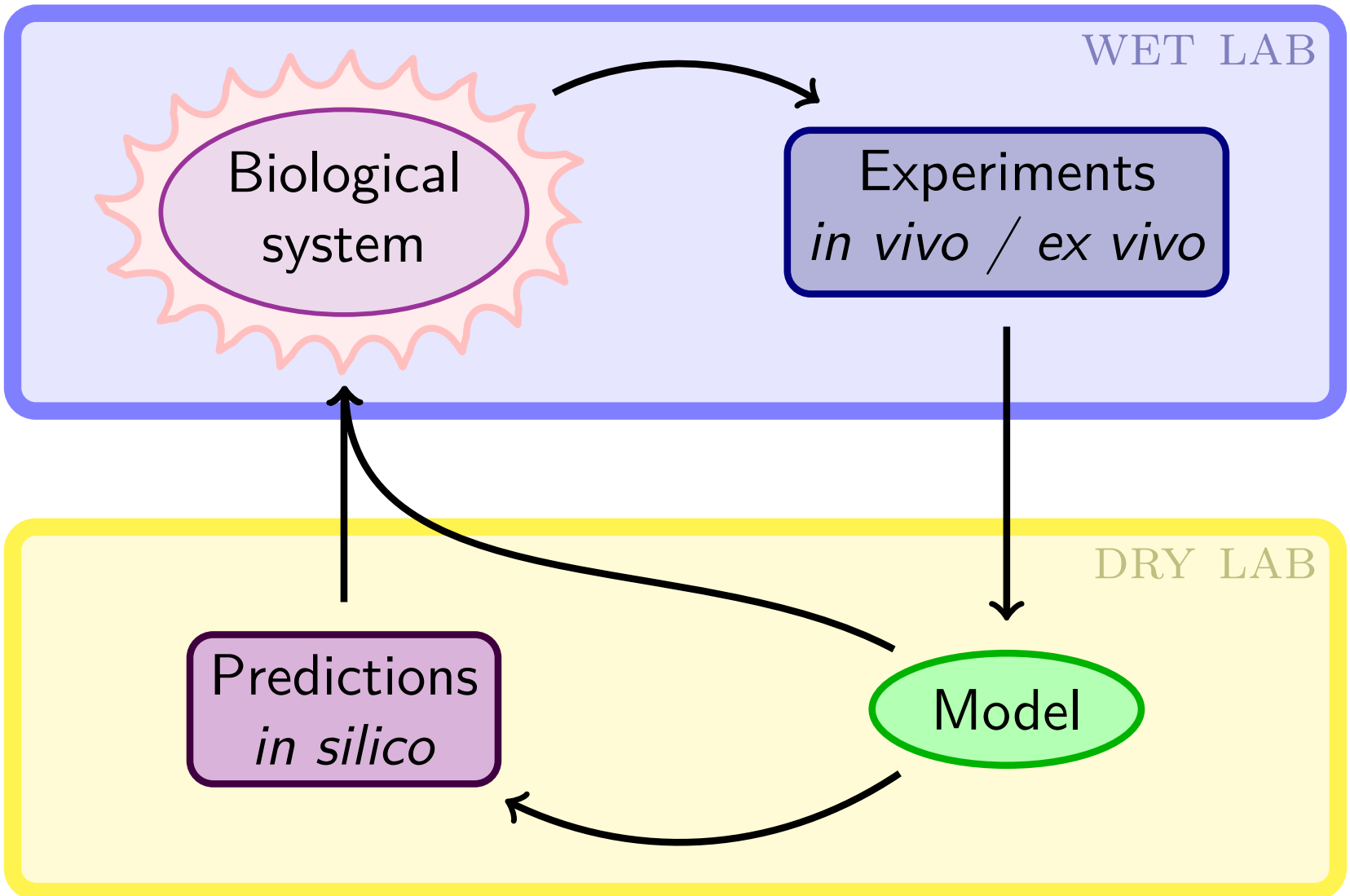
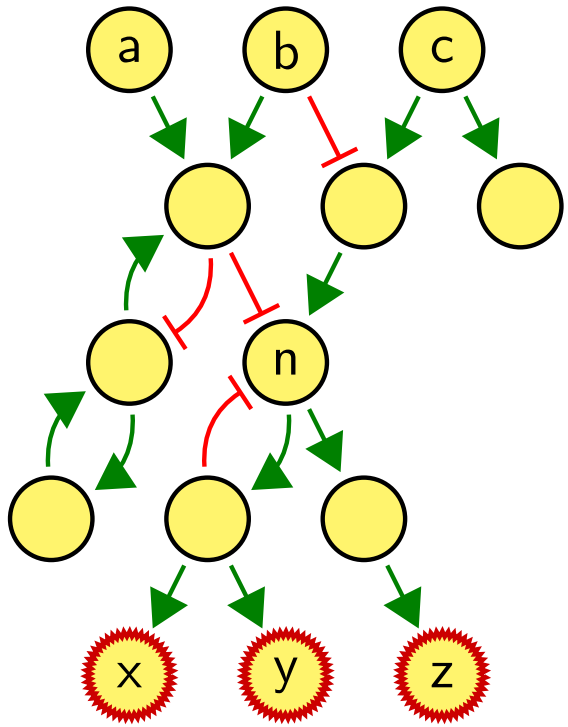


Gene Therapies

Find **therapeutic targets** in complex systems



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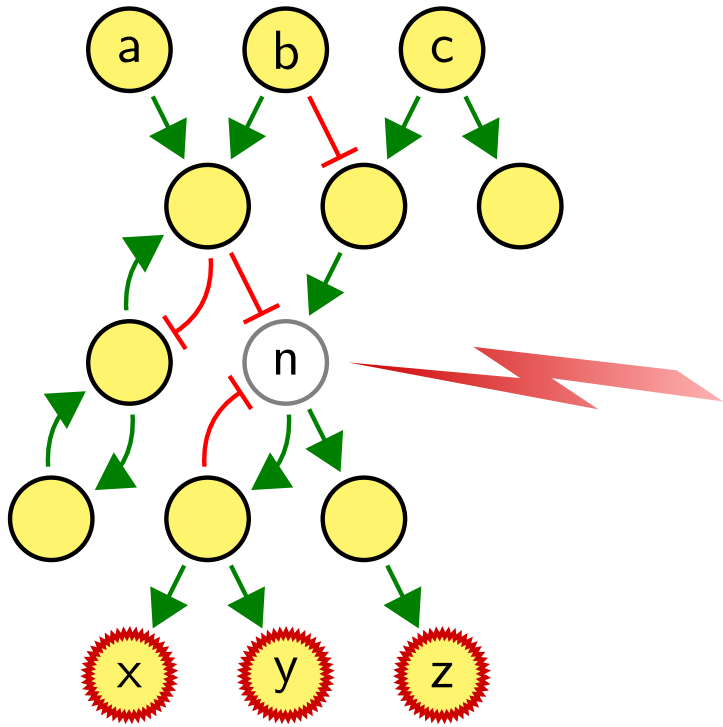

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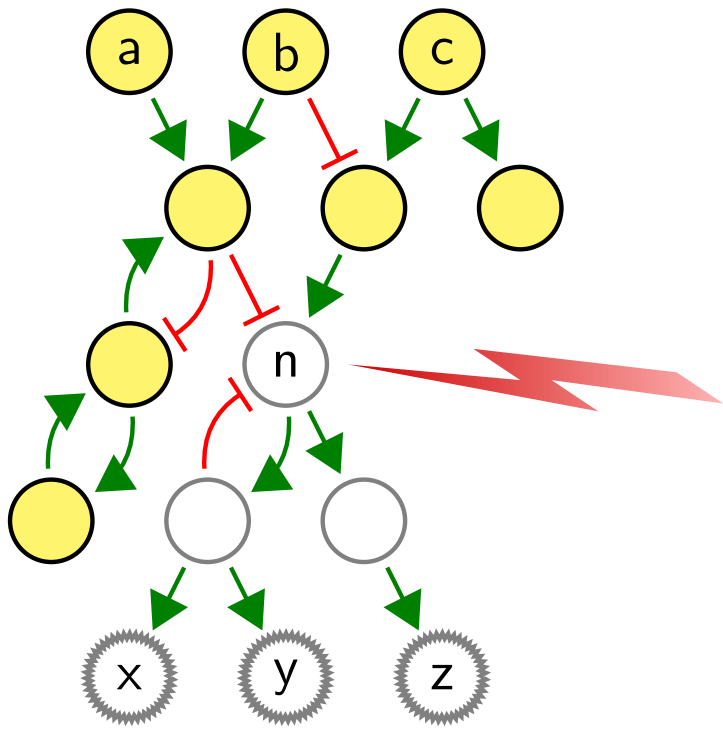
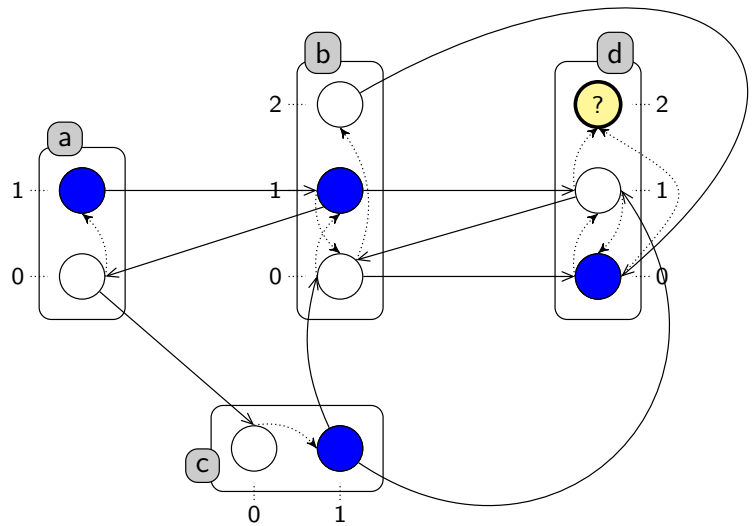


Back to the Over-approximation

[Paulevé *et al.*, *Computer Aided Verification*, 2013]



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There exists a traversal with no cycle

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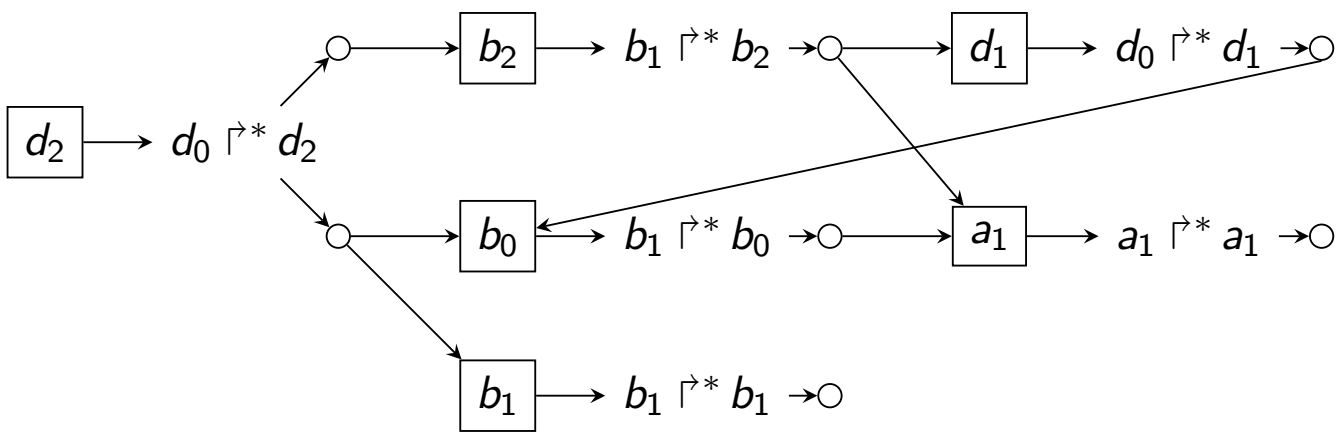

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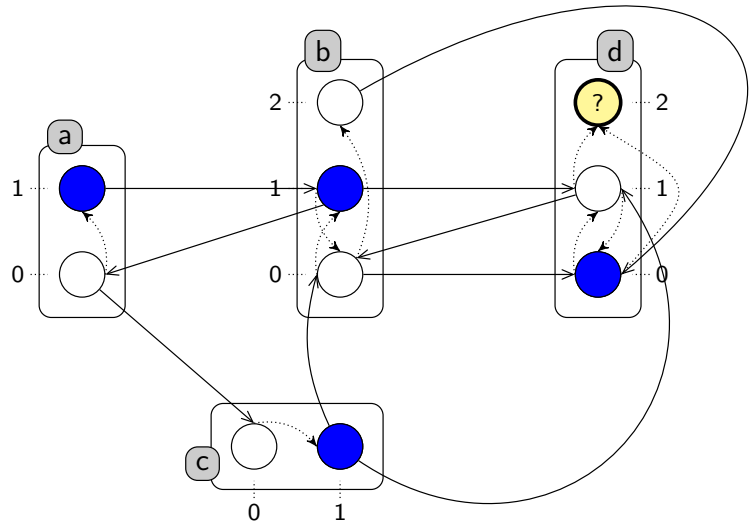

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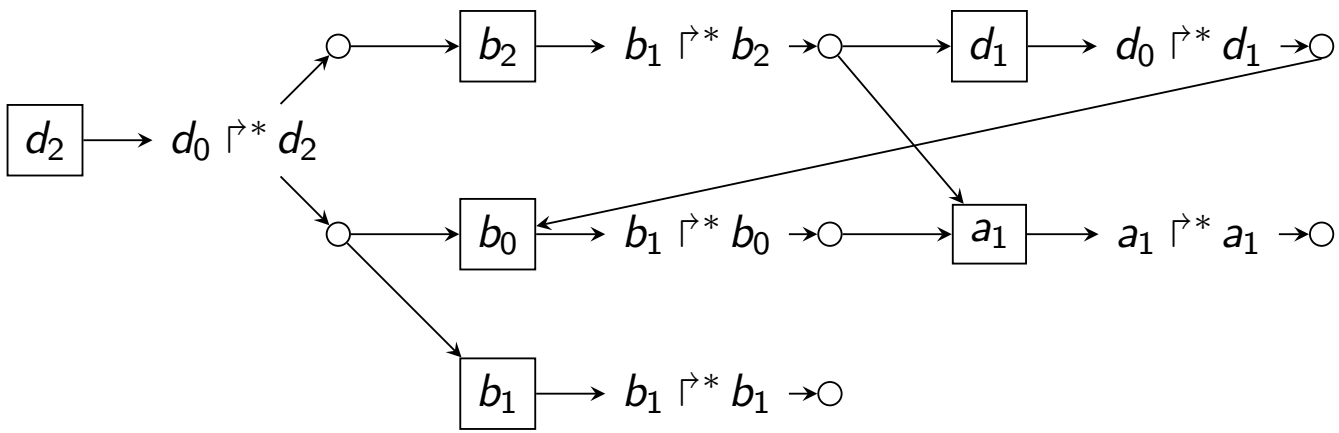
Q is true ⇒ Inconclusive



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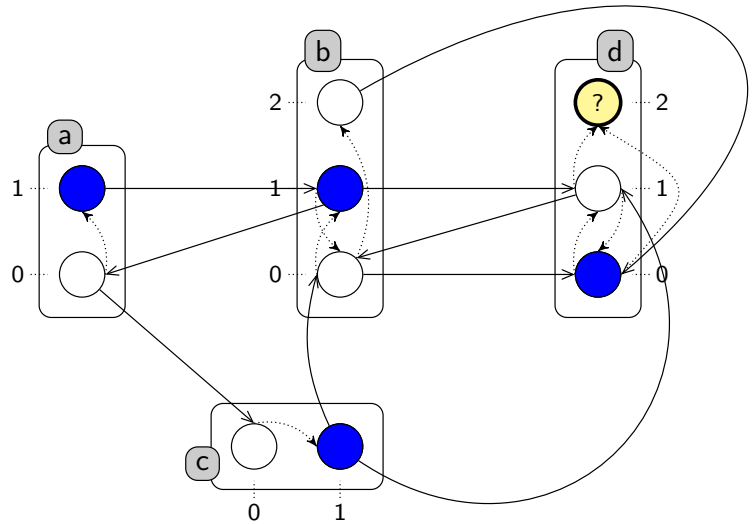

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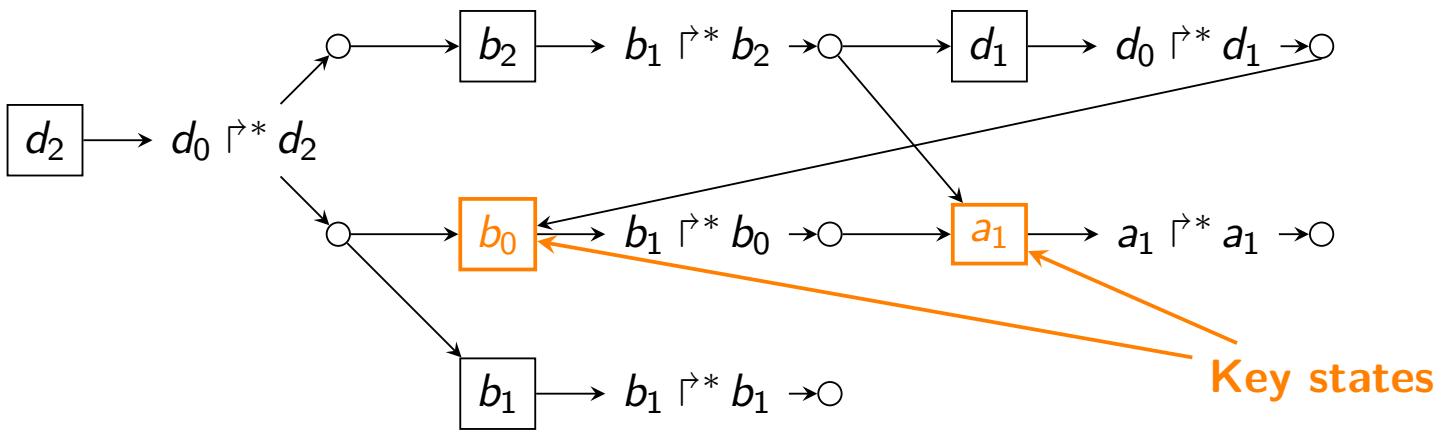

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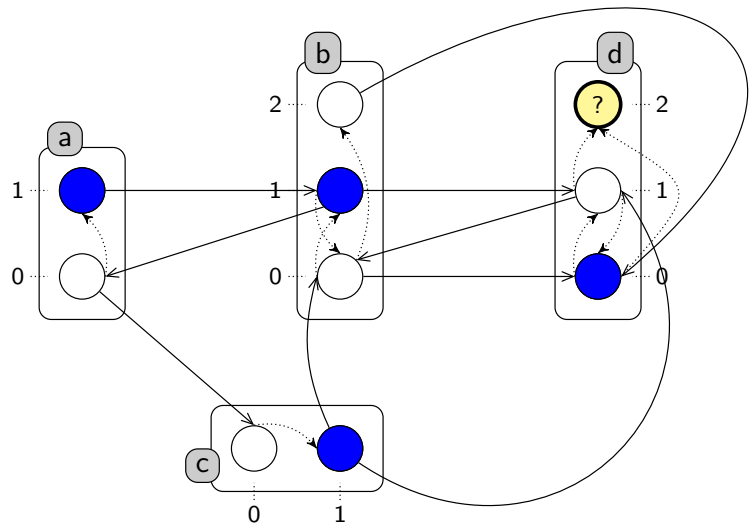

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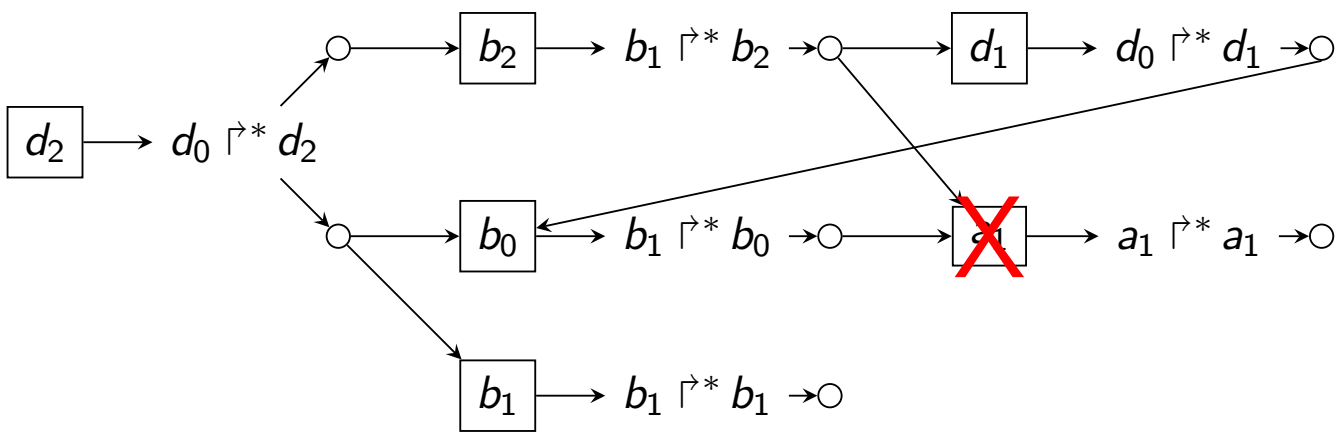

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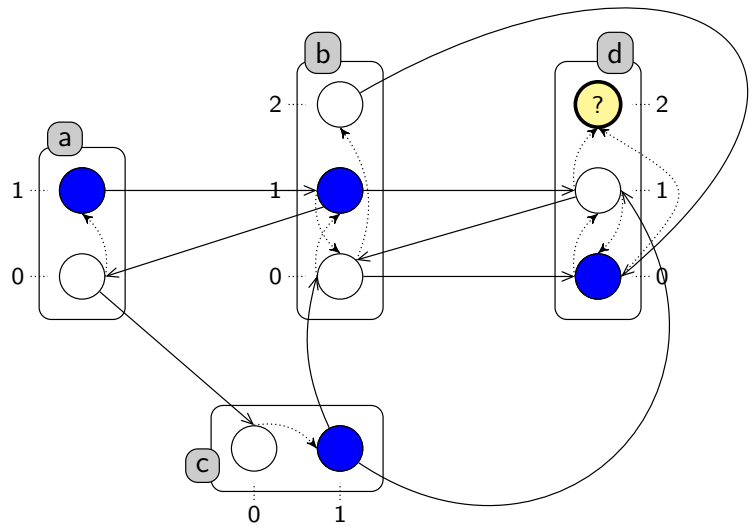

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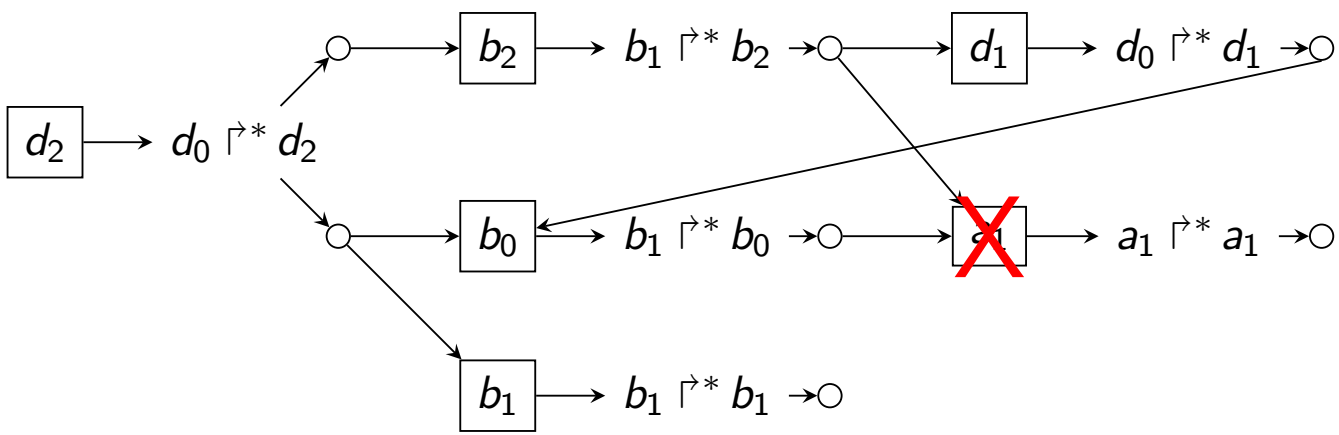

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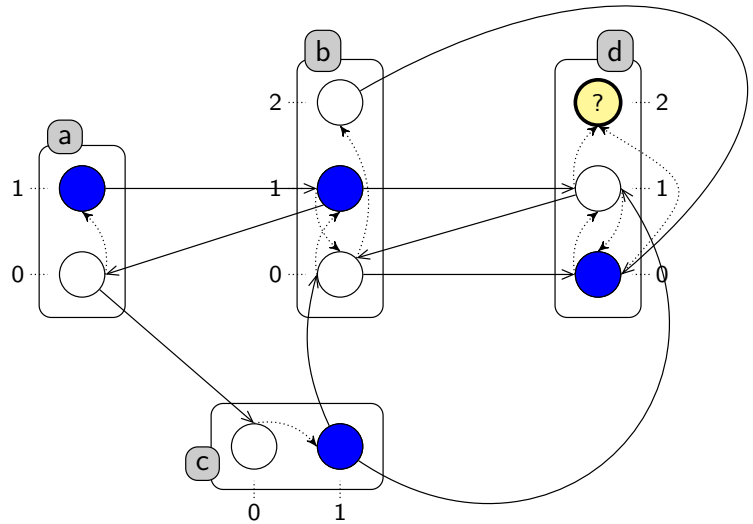

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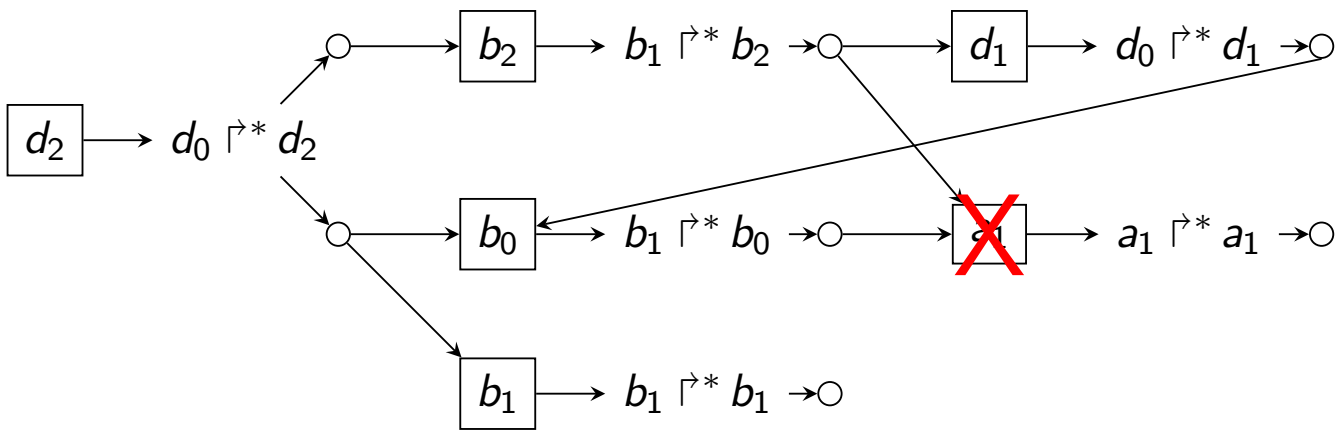
Q is false ⇒ R is false



Summary & Conclusion

Modeling of René Thomas for **Regulatory networks**

$\Rightarrow$ How to study its dynamics?

Dynamic analysis

- Classical approach: state-graph computation
- Precise but computational (exponential)

  $\Rightarrow$ Large models are intractable

Static analysis

- Much faster (polynomial) but lesser range of results
- Reachability analysis:

  $\Rightarrow$ Outperforms classical approaches

  $\Rightarrow$ Very few inconclusive cases

Bibliography

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Thank you