Hybrid Satisfiability Methods for the Inference of Boolean Regulations Controlling Metabolic Networks

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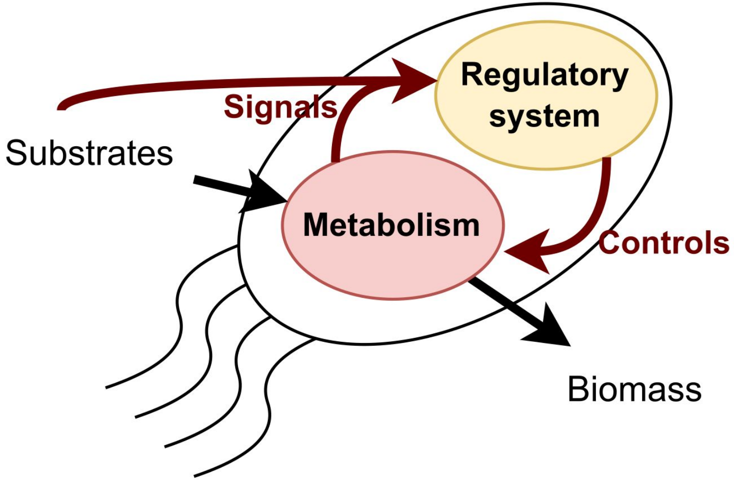
Cells: hybrid multi-scale structures

Composed of thousands of ***interconnected*** *chemical processes*
Occurring at different ***scales***



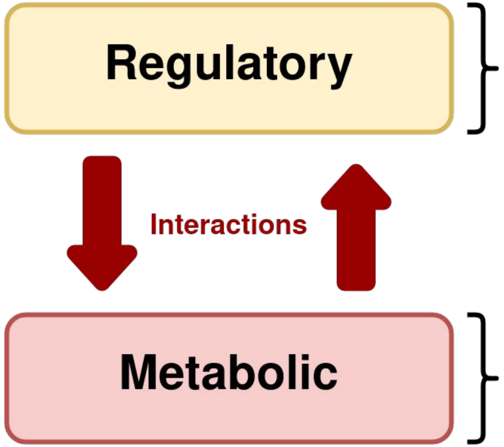
1. **Metabolic scale**
   Chemicals reactions converting substrates to energy and biomass
2. **Regulatory scale**
   Rules constraining the metabolism to adapt itself to its environment

Two scales of interest: metabolic and regulatory

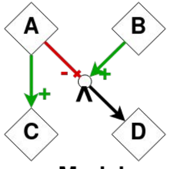
State of the Art:

Multi-scale modeling of cells

Overview of modeling formalisms



Discrete dynamics [Thomas, 1973]



Model

$$
\begin{aligned}
f_A(x_A, x_B, x_C, x_D) &= x_A \\
f_B(x_A, x_B, x_C, x_D) &= x_B \\
f_C(x_A, x_B, x_C, x_D) &= x_A \\
f_D(x_A, x_B, x_C, x_D) &= \neg x_A \wedge x_B
\end{aligned}
$$

Logical rules (Boolean network)

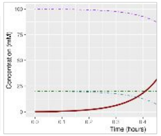
Inputs				Outputs			
x_A	x_B	x_C	x_D	x_A	x_B	x_C	x_D
0	0	x	x	0	0	0	0
0	1	x	x	0	1	0	1
1	0	x	x	1	0	1	0
1	1	x	x	1	1	1	0

Truth table (simulation)

Steady-states approximation [Orth *et al.*, 2010]

$$
\begin{array}{ll}
\text{maximise} & v_{\text{Growth}} \\
\text{such that:} & S \cdot v = 0 \\
 & l_r \cdot x_r \leq v_r \leq u_r \cdot x_r \quad \forall r \in \text{reactions}
\end{array}
$$

Regulatory flux balance analysis (rFBA)



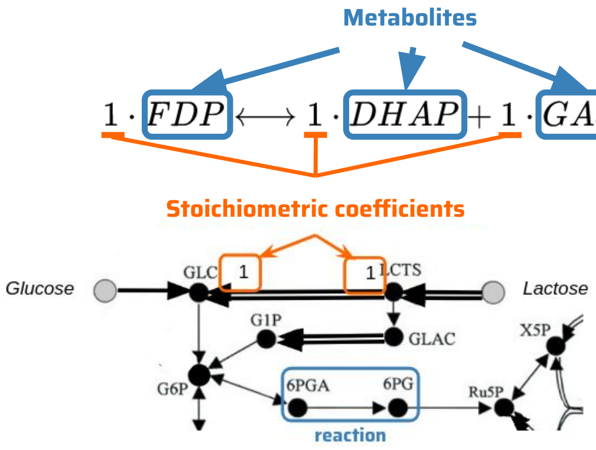
Metabolic traces

Two scales model based on different paradigms and formalisms

Structure

Metabolic scale

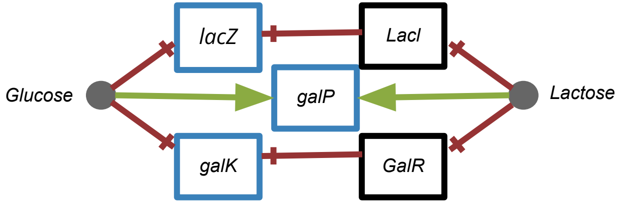
Set of chemical reactions



Metabolic networks

Regulatory scale

Interactions graph



Boolean network [Thomas, 1973]

Logical combination of interactions

$$f_{\mathrm{lacZ}}(x) = \neg x_{\mathrm{Glucose}} \wedge \neg x_{\mathrm{LacI}} \qquad f_{\mathrm{LacI}}(x) = \neg x_{\mathrm{Lactose}}$$

$$f_{\mathrm{galP}}(x) = x_{\mathrm{Glucose}} \vee x_{\mathrm{Lactose}} \qquad f_{\mathrm{galK}}(x) = \neg x_{\mathrm{Glucose}} \wedge \neg x_{\mathrm{GalR}}$$

$$f_{\mathrm{GalR}}(x) = \neg x_{\mathrm{Lactose}}$$

Set of logical rules paired with an directed labeled graph

Inspired by [Covert and Palsson, 2002]

Dynamics

Metabolic scale

Flux balance analysis¹ (FBA) [Orth *et al.*, 2010]

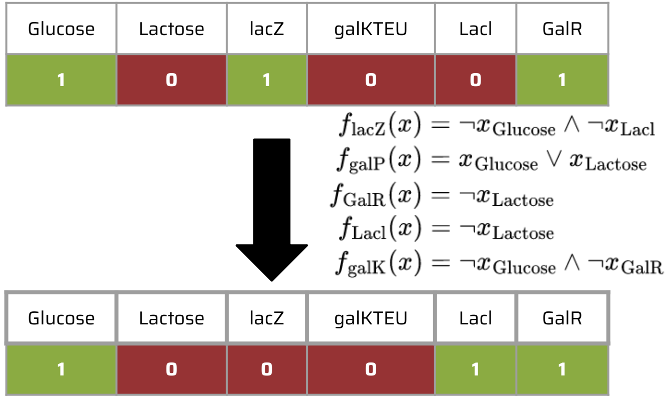
$$\begin{aligned} \text{maximize} \quad & \boxed{v_{\text{Growth}}} \\ \text{such that:} \quad & S \cdot v = 0 \\ & l_r \leq v_r \leq u_r \quad \forall r \in \text{reactions} \end{aligned}$$

Bacteria growth maximization
[Feist and Palsson, 2010]

Based on heuristics: growth optimization + steady-state

Flux-based dynamics

Regulatory scale



Glucose	Lactose	lacZ	galKTEU	LacI	GalR
1	0	1	0	0	1

$$\begin{aligned} f_{\text{lacZ}}(x) &= \neg x_{\text{Glucose}} \wedge \neg x_{\text{LacI}} \\ f_{\text{galP}}(x) &= x_{\text{Glucose}} \vee x_{\text{Lactose}} \\ f_{\text{GalR}}(x) &= \neg x_{\text{Lactose}} \\ f_{\text{LacI}}(x) &= \neg x_{\text{Lactose}} \\ f_{\text{galK}}(x) &= \neg x_{\text{Glucose}} \wedge \neg x_{\text{GalR}} \end{aligned}$$

Glucose	Lactose	lacZ	galKTEU	LacI	GalR
1	0	0	0	1	1

Discrete dynamics [Thomas, 1973]

Various update semantics

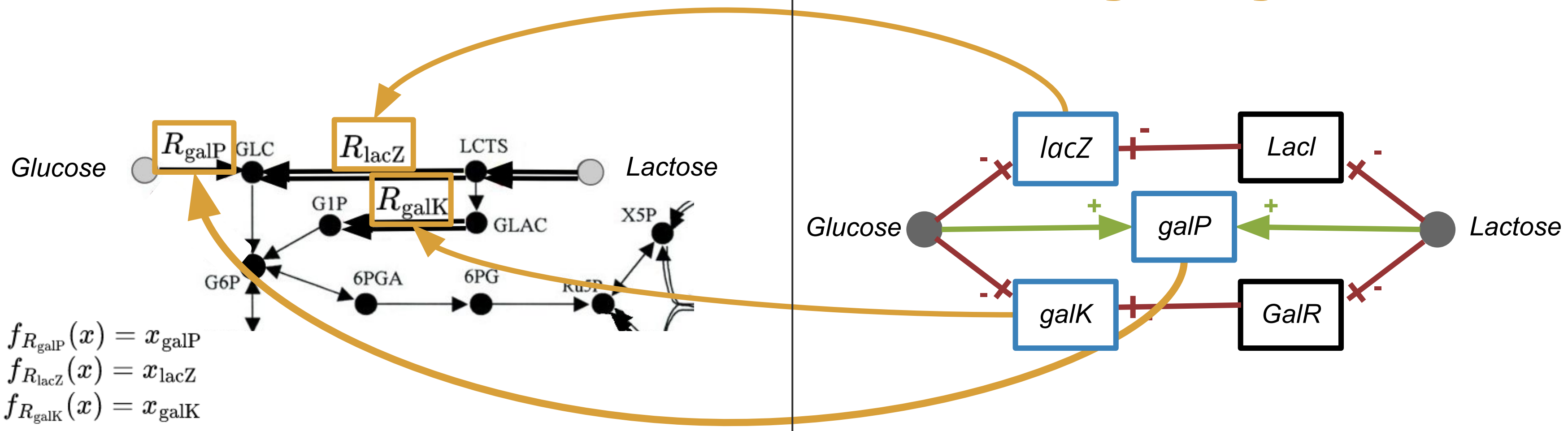
Scale dynamics are based on different paradigms

No straightforward formalism to encompass them

Coupling the scales

Metabolic scale

Regulatory scale



$$f_{R_{\mathrm{galP}}}(x) = x_{\mathrm{galP}}$$
$$f_{R_{\mathrm{lacZ}}}(x) = x_{\mathrm{lacZ}}$$
$$f_{R_{\mathrm{galK}}}(x) = x_{\mathrm{galK}}$$

Regulatory controls:

Regulatory states impact reactions

Metabolic feedback:

Reactions + metabolites impact regulatory states

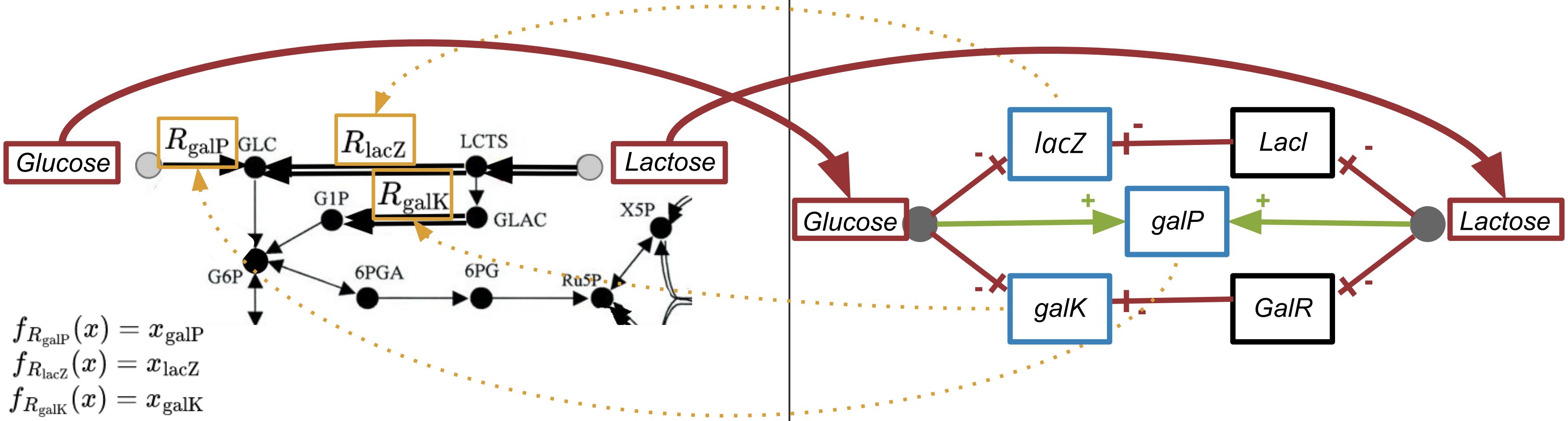
Interconnected scales through regulatory controls and metabolic feedback

Simulating the coupled dynamics through regulatory Flux Balance Analysis (rFBA) **[Covert *et al.*, 2001]**

Coupling the scales

Metabolic scale

Regulatory scale



$$f_{R_{\text{galP}}}(x) = x_{\text{galP}}$$
$$f_{R_{\text{lacZ}}}(x) = x_{\text{lacZ}}$$
$$f_{R_{\text{galK}}}(x) = x_{\text{galK}}$$

Regulatory controls:
Regulatory states impact reactions

Metabolic feedback:
Reactions + metabolites impact regulatory states

Interconnected scales through regulatory controls and metabolic feedback

Simulating the coupled dynamics through regulatory Flux Balance Analysis (rFBA) **[Covert *et al.*, 2001]**

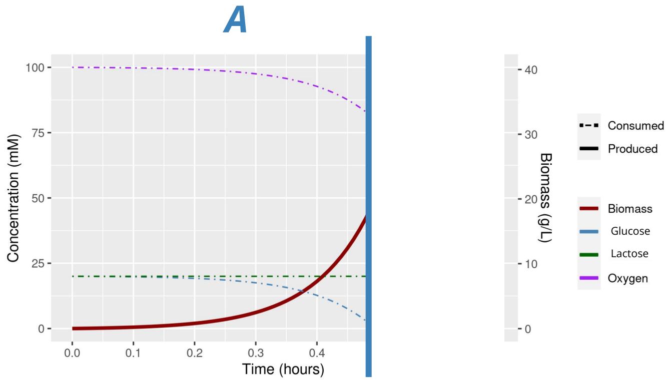
Example of controlled induced behavior: diauxic shift[1]

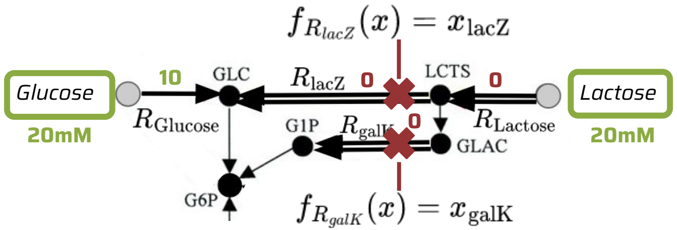
Successives growth phases on different mediums
Controlled by the regulatory scale

needed to import lactose

	Glucose	Lactose	lacZ	galK	LacI	GalR	
A	20mM	20mM	0	0	0	0	Growth on Glucose

rFBA simulation made with FlexFlux **[Marmiesse *et al.*, 2015]**





Phase A: lactose ***could not be imported due to regulatory rules***

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[1] J. Monod, **Annales de l'Institut Pasteur**, 1942

Example of controlled induced behavior: diauxic shift[1]

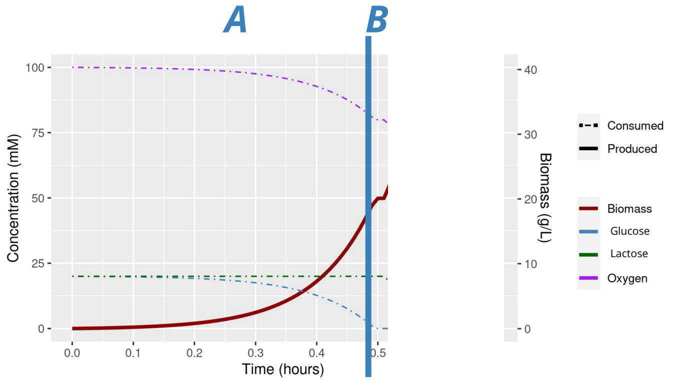
Successives growth phases on different mediums
Controlled by the regulatory scale

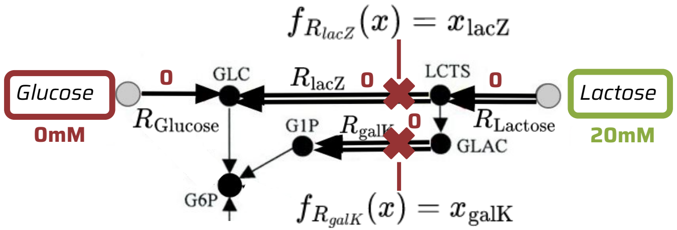
needed to import lactose

	Glucose	Lactose	lacZ	galK	LacI	GalR	
A	20mM	20mM	0	0	0	0	Growth on Glucose
B	0mM	20mM	0	0	0	0	No growth: cannot import Lactose

Metabolic feedback: glucose is depleted

***rFBA simulation made with FlexFlux* [Marmiesse *et al.*, 2015]**





Phase B: ***regulatory mechanisms are slow and need time to react to*** **glucose** ***depletion***

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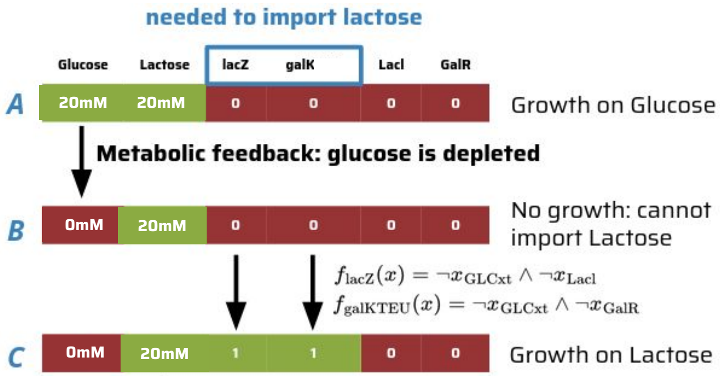
[1] J. Monod, **Annales de l'Institut Pasteur**, 1942

Example of controlled induced behavior: diauxic shift[1]

Successives growth phases on different mediums
Controlled by the regulatory scale



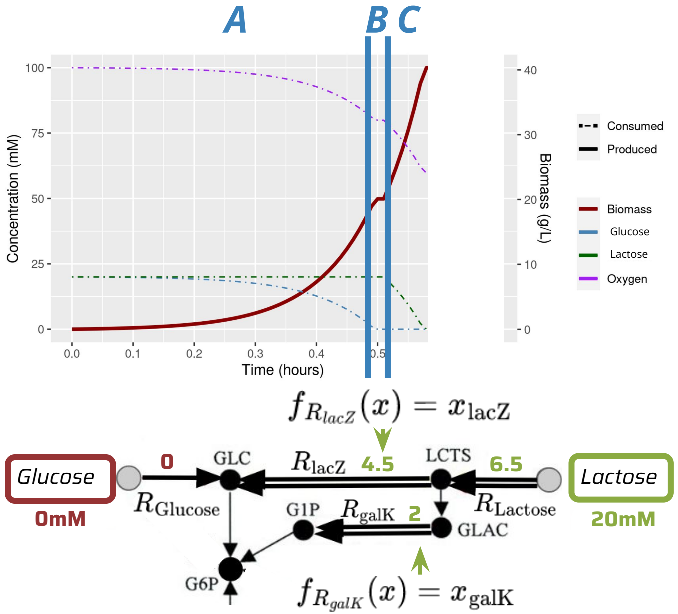
needed to import lactose

	Glucose	Lactose	lacZ	galK	LacI	GalR	
A	20mM	20mM	0	0	0	0	Growth on Glucose
B	0mM	20mM	0	0	0	0	No growth: cannot import Lactose
C	0mM	20mM	1	1	0	0	Growth on Lactose

Metabolic feedback: glucose is depleted

$$f_{\mathrm{lacZ}}(x) = \neg x_{\mathrm{GLCxt}} \wedge \neg x_{\mathrm{LacI}}$$
$$f_{\mathrm{galKTEU}}(x) = \neg x_{\mathrm{GLCxt}} \wedge \neg x_{\mathrm{GalR}}$$

rFBA simulation made with FlexFlux **[Marmiesse *et al.*, 2015]**



$$f_{R_{lacZ}}(x) = x_{\mathrm{lacZ}}$$
$$R_{\mathrm{lacZ}} \quad R_{\mathrm{Glucose}} \quad R_{\mathrm{galK}} \quad R_{\mathrm{Lactose}}$$
$$f_{R_{galK}}(x) = x_{\mathrm{galK}}$$

Phase C: **lacZ** *and* **galKTEU** *states are updated allowing to import lactose*

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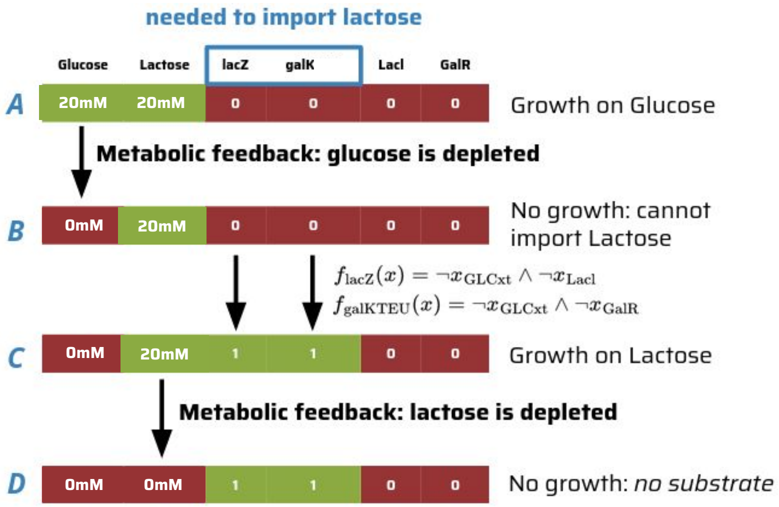
[1] J. Monod, **Annales de l'Institut Pasteur**, 1942

Example of controlled induced behavior: diauxic shift[1]

Successives growth phases on different mediums
Controlled by the regulatory scale



needed to import lactose

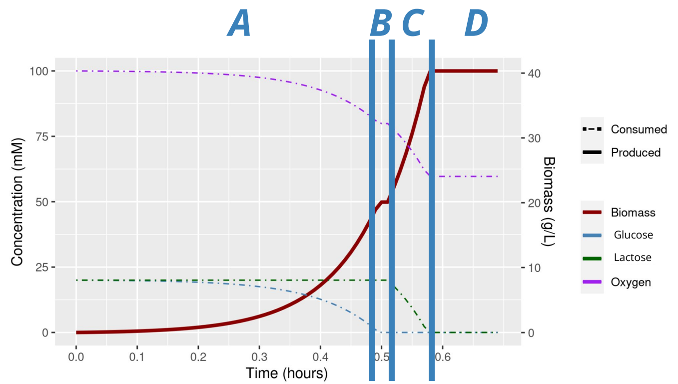
	Glucose	Lactose	lacZ	galK	LacI	GalR	
A	20mM	20mM	0	0	0	0	Growth on Glucose
B	0mM	20mM	0	0	0	0	No growth: cannot import Lactose
C	0mM	20mM	1	1	0	0	Growth on Lactose
D	0mM	0mM	1	1	0	0	No growth: *no substrate*

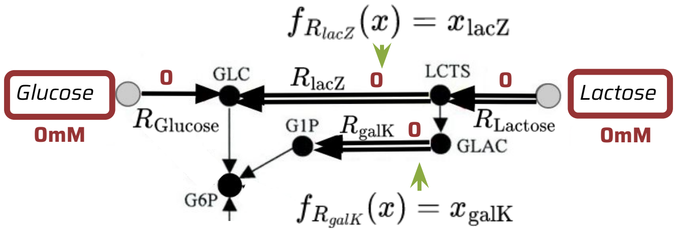
Metabolic feedback: glucose is depleted (A → B)

$$f_{\text{lacZ}}(x) = \neg x_{\text{GLCxt}} \wedge \neg x_{\text{LacI}}$$
$$f_{\text{galKTEU}}(x) = \neg x_{\text{GLCxt}} \wedge \neg x_{\text{GalR}}$$

Metabolic feedback: lactose is depleted (C → D)

rFBA simulation made with FlexFlux **[Marmiesse *et al.*, 2015]**





Phase D: ***no carbon sources to allow growth***

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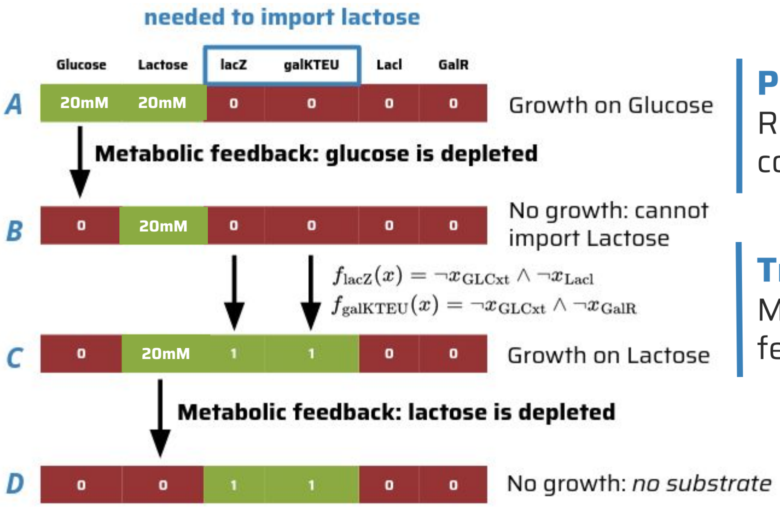
[1] J. Monod, **Annales de l'Institut Pasteur**, 1942

What we learned



needed to import lactose

	Glucose	Lactose	lacZ	galKTEU	LacI	GalR	
A	20mM	20mM	0	0	0	0	Growth on Glucose
B	0	20mM	0	0	0	0	No growth: cannot import Lactose
C	0	20mM	1	1	0	0	Growth on Lactose
D	0	0	1	1	0	0	No growth: *no substrate*

A → B: **Metabolic feedback: glucose is depleted**

B → C:

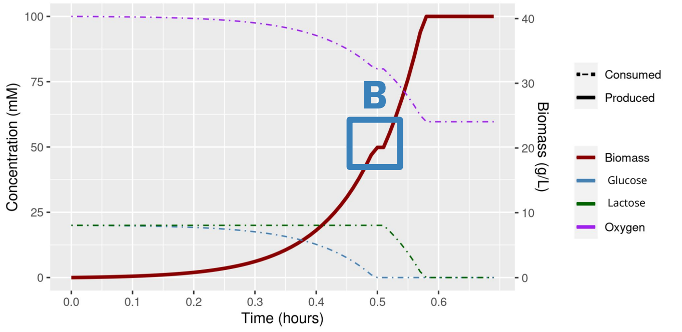
$f_{\text{lacZ}}(x) = \neg x_{\text{GLCxt}} \wedge \neg x_{\text{LacI}}$

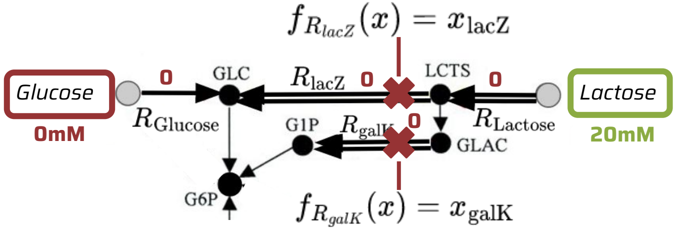
$f_{\text{galKTEU}}(x) = \neg x_{\text{GLCxt}} \wedge \neg x_{\text{GalR}}$

C → D: **Metabolic feedback: lactose is depleted**

Phases A / B: Regulatory controls

Transition B → C: Metabolic feedback





Regulation has impacts on growth

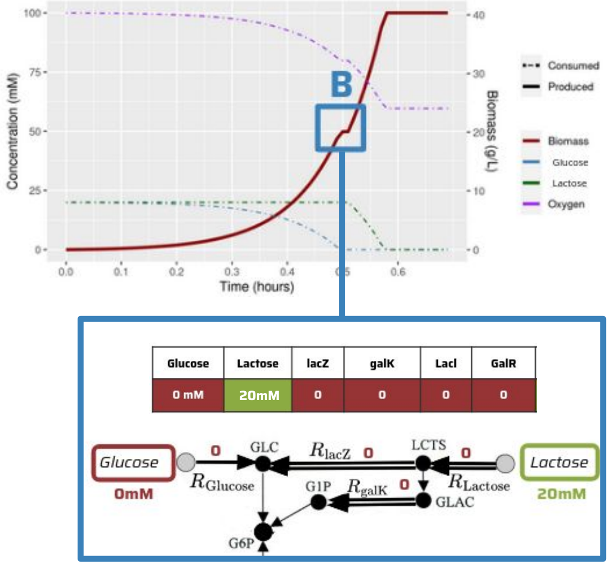
Indirect observation of the regulation on phase B

Regulation impacts on the metabolism are hard to detect

Our initial questions

$$f_{\text{lacZ}}(x) =$$
$$f_{\text{galP}}(x) =$$
$$f_{\text{GalR}}(x) =$$
$$f_{\text{LacI}}(x) =$$
$$f_{\text{galK}}(x) =$$
$$f_{R_{lacZ}}(x) =$$
$$f_{R_{galK}}(x) =$$

???



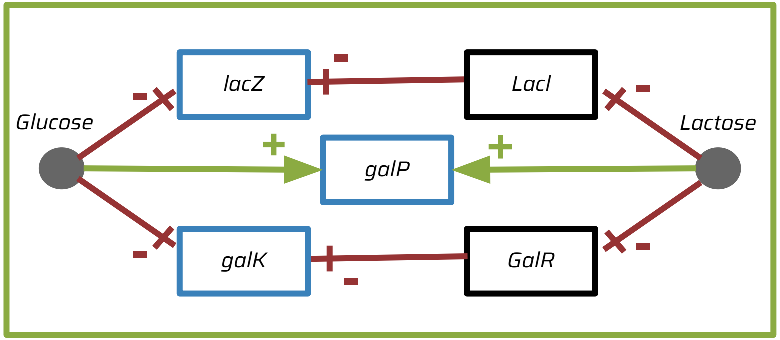
Glucose	Lactose	lacZ	galK	LacI	GalR
0 mM	20mM	0	0	0	0

Can we infer regulatory control of metabolism?
From which kind of data?

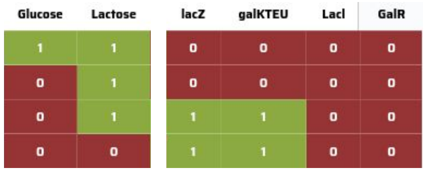
Inference of Boolean networks in the literature: overview

Input:

Interaction graph: define a search space



Observations



Glucose	Lactose	lacZ	galKTEU	LacI	GalR
1	1	0	0	0	0
0	1	0	0	0	0
0	1	1	1	0	0
0	0	1	1	0	0

Gene expression on different experimental conditions

Consider *only* direct impacts of the regulation

Output:

Optimal Boolean networks **in the search space compatible with the observations**

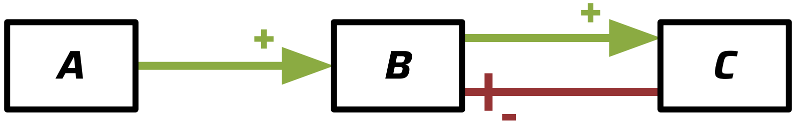
e.g. optimality criteria: network size, observation matching

Only consider regulatory network dynamics
Formalize as combinatorial optimization problems

Search space

Set of Boolean networks compatible with an interaction graph

Example



$$f_A(x) = 0$$
$$f_A(x) = 1$$
$$f_A(x) = x_A$$
$$f_A(x) = \neg x_B$$
$$f_A(x) = x_A \vee \neg x_B$$
$$\underline{f_A(x) = x_A \wedge \neg x_B}$$

6 rules

Regulatory rule of ***B*** can only depends on:

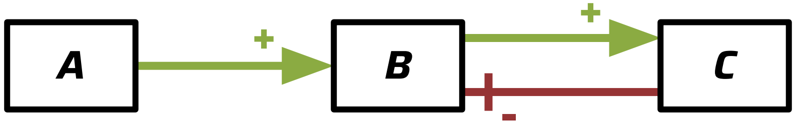
- ➔ constant value 0 or 1
- ➔ activation of *A*
- ➔ inhibition of *C*

Rules are logical combinations of the interactions

Search space

Set of Boolean networks compatible with an interaction graph

Example



$f_A(x) = 0$

$f_A(x) = 1$

2 rules

$f_A(x) = 0$

$f_A(x) = 1$

$f_A(x) = x_A$

$f_A(x) = \neg x_B$

$f_A(x) = x_A \vee \neg x_B$

$f_A(x) = x_A \wedge \neg x_B$

6 rules

$f_C(x) = 0$

$f_C(x) = 1$

$f_C(x) = x_B$

3 rules

Regulatory rule of ***B*** can only depends on:

- ➔ constant value 0 or 1
- ➔ activation of *A*
- ➔ inhibition of *C*

36 Boolean networks compatible
36 = 2 x 6 x 3

Number of compatible Boolean networks is *doubly exponential* in the number of interactions

Outlines

> **Our questions:**
> How to infer Boolean regulatory rules ***that controls metabolic networks*** from observations and ***curated interaction graph***?

1. **Formalization:** of our inference problem as a *combinatorial optimization problem modulo quantified linear constraints (OPT+qLP)*
2. **Solving method:** a generic workflow to address *OPT+qLP*
3. **Benchmark and validation:** application to a benchmark based on *Escherichia coli*

Problem formalization

Published in:

> Thuillier, K., Baroukh, C., Bockmayr, A., Cottret, L., Paulevé, L., and Siegel, A. (2021).
>
> Learning Boolean Controls in Regulated Metabolic Networks: A Case-Study. CMSB 2021.

Formalization of the inference problem

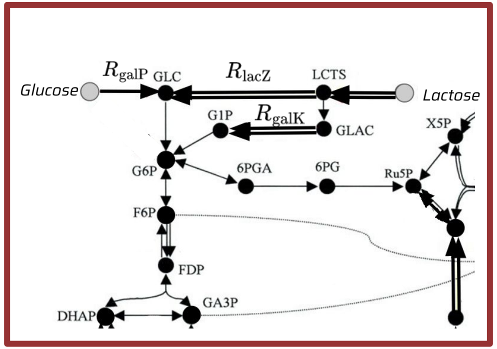
$$
\begin{aligned}
&\text{minimize} \quad f_{\text{obj}}(x) \\
&\text{such that} \\
&\quad \bigwedge_{\alpha} \boxed{c_\alpha(x)} \relbar\joinrel\relbar\joinrel\relbar\joinrel\dashv \; c(x) : \bigvee_i x_i \vee \bigvee_j \neg x_j \\
&\quad \wedge \quad \bigwedge_{\beta} c_\beta(x) \vee \boxed{g_\beta(y) \leq 0} \relbar\joinrel\dashv \; g(y) : \text{linear function} \\
&\quad \wedge \quad \forall z \in \mathbb{R}^p, \left(\bigwedge_{\gamma} c_\gamma(x) \vee g_\gamma(z) \leq 0 \right) \implies \left(\bigwedge_{\zeta} c_\zeta(x) \vee g_\zeta(z) \leq 0 \right) \\
&\text{with } x \in \mathbb{B}^n,\ y \in \mathbb{R}^m
\end{aligned}
$$

Combinatorial optimization problem modulo quantified linear constraints (OPT+qLP)

Inference of Boolean networks controlling the metabolism

Input:

Metabolic network



Metabolic network is an input

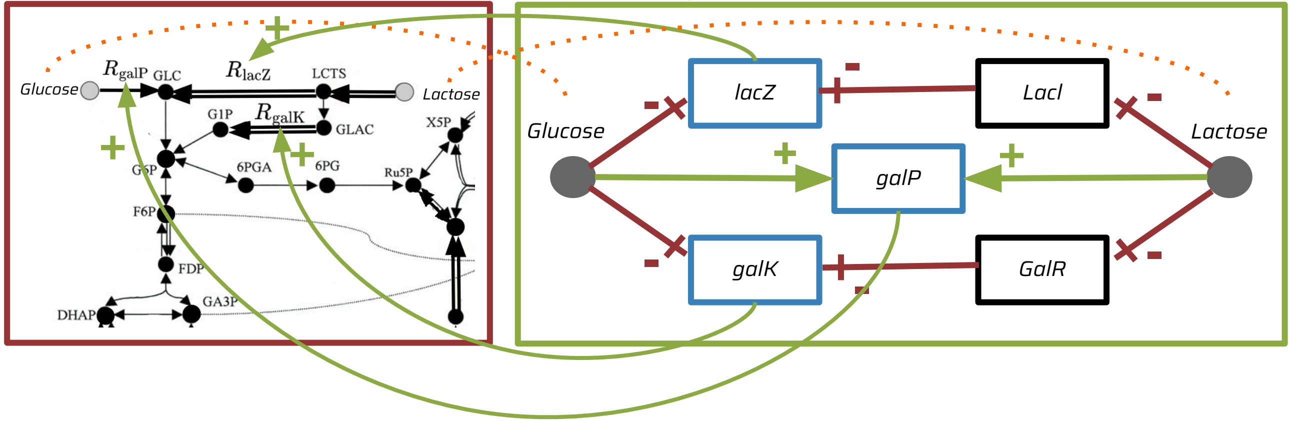
Standard protocol to reconstruct [Thiele *et al.*, 2010]

Public databases with high quality curated metabolic networks e.g. *BiGG* - [King *et al.*, 2015]

Inference of Boolean networks controlling the metabolism

Input:

Metabolic network **Interaction graph:** define a search space



A curated interaction graph

Set of manually selected interactions

Accounting for all the interactions between the regulatory and metabolic scales

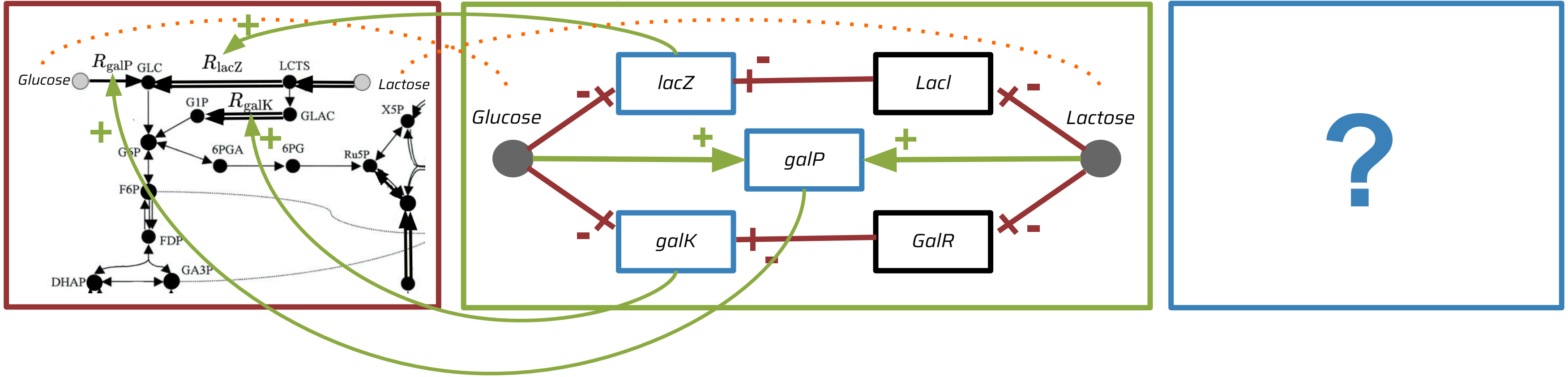
Inference of Boolean networks controlling the metabolism

Input:

Metabolic network

Interaction graph: define a search space

Observations



Time series observations

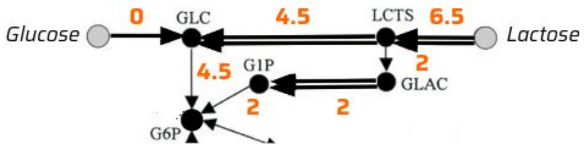
Direct observations:

- ➔ **Transcriptomics** qualitative
  Gene expression data
  Reaction and metabolite state

Glucose	Lactose	lacZ	galK	LacI	GalR	R_lacZ	R_galK
0	1	1	1	0	0	1	1

Indirect observations:

- ➔ **Fluxomics** quantitative
  Rates of reactions activity



- ➔ **Kinetics** quantitative
  Substrate concentrations

Glucose	Lactose	...
0 mM	20 mM	...

+ Growth rate = 1.12

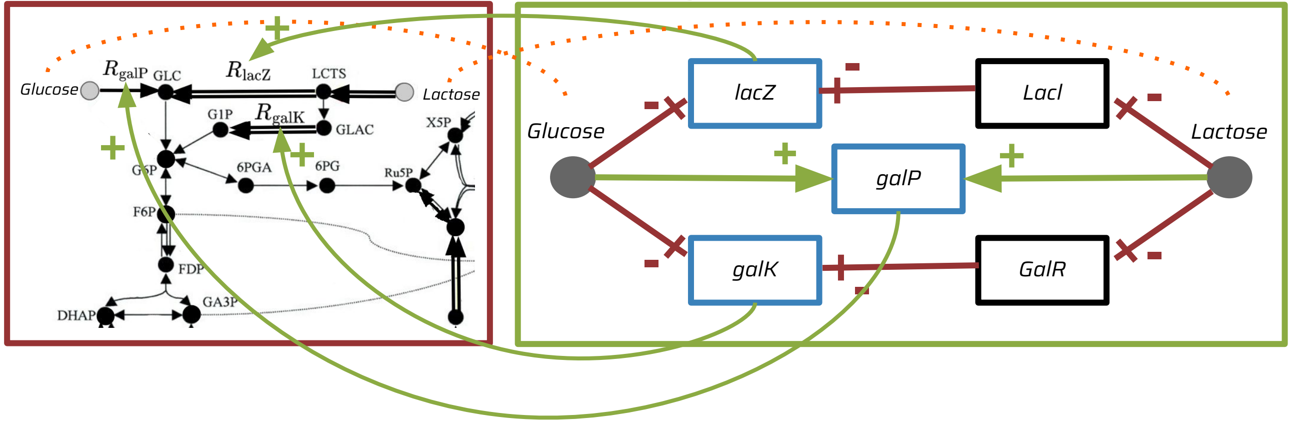
3 data types of interest to infer regulatory rules controlling metabolic networks

Inference of Boolean networks controlling the metabolism

Input:

Metabolic network

Interaction graph: define a search space



***Time series* observations**

Direct observations:
- transcriptomics

Indirect observations:
- kinetics
- fluxomics

Output:

Optimal Boolean networks in the search space with a ***trace compatible with the observations***

General form of the inference problem

$c(x) : \bigvee_i x_i \vee \bigvee_j \neg x_j$

$g(y)$: linear function

$$\text{minimize} \quad f_{\text{obj}}(x)$$

Optimization criteria

such that

$$\bigwedge_\alpha c_\alpha(x)$$

Search space

$$\wedge \quad \bigwedge_\beta c_\beta(x) \vee g_\beta(y) \leq 0$$

$$\wedge \quad \forall z \in \mathbb{R}^p, \left(\bigwedge_\gamma c_\gamma(x) \vee g_\gamma(z) \leq 0 \right) \implies \left(\bigwedge_\zeta c_\zeta(x) \vee g_\zeta(z) \leq 0 \right)$$

with $x \in \mathbb{B}^n$, $y \in \mathbb{R}^m$

Output:

> ***Optimal*** Boolean networks in the search space with a ***trace compatible with the observations***

Regulated metabolic state

1. **Regulatory state**
   Boolean regulatory state of each element

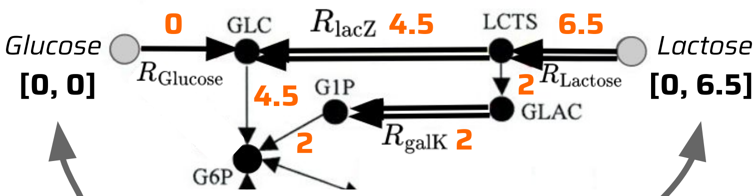
Glucose	Lactose	lacZ	galK	LacI	GalR	R_lacZ	R_galK
0	1	1	1	0	0	1	1

2. **Metabolic state**
   The metabolic activity of each reaction, such that:

$$
\begin{array}{ll}
\text{maximize} & v_{\text{Growth}} \\
\text{such that:} & S \cdot v = 0 \\
 & l_r \leq v_r \leq u_r \quad \forall r \in \text{reactions} \\
 & v_r = 0 \quad \forall r \in \text{inhibited reactions}
\end{array}
$$

Growth rate = 1.12

+



3. **Substrate state**
   External metabolite concentrations

Glucose	Lactose	...
0 mM	20 mM	...

Use to compute thermodynamic bounds

States are composed of 3 layers as for the observations

rFBA states transition

Regulatory flux balance analysis (rFBA) [Covert *et al.*, 2001]

rFBA transition:

1. Update the **regulatory state**
   Synchronous update of the regulatory rules

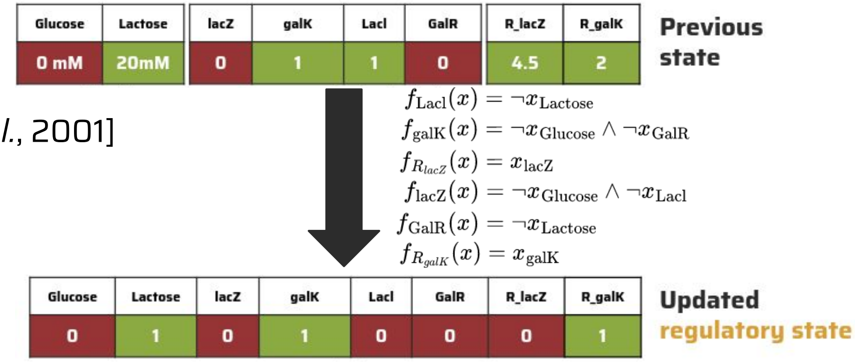
2. Update the **metabolic state**
   Solve the FBA equations:

$$\begin{aligned} &\text{maximize} && v_{\text{Growth}} \\ &\text{such that:} && S \cdot v = 0 \\ & && l_r \leq v_r \leq u_r \quad \forall r \in \text{reactions} \\ & && v_r = 0 \quad \forall r \in \text{inhibited reactions} \end{aligned}$$

3. Update the **substrate state**

> **Successive updates of the regulatory (*discrete*) and metabolic (*linear*) states**

rFBA states transition



Glucose	Lactose	lacZ	galK	LacI	GalR	R_lacZ	R_galK
0 mM	20mM	0	1	1	0	4.5	2

Previous state

$$f_{\text{LacI}}(x) = \neg x_{\text{Lactose}}$$
$$f_{\text{galK}}(x) = \neg x_{\text{Glucose}} \wedge \neg x_{\text{GalR}}$$
$$f_{R_{lacZ}}(x) = x_{\text{lacZ}}$$
$$f_{\text{lacZ}}(x) = \neg x_{\text{Glucose}} \wedge \neg x_{\text{LacI}}$$
$$f_{\text{GalR}}(x) = \neg x_{\text{Lactose}}$$
$$f_{R_{galK}}(x) = x_{\text{galK}}$$

Glucose	Lactose	lacZ	galK	LacI	GalR	R_lacZ	R_galK
0	1	0	1	0	0	0	1

Updated regulatory state

Regulatory flux balance analysis (rFBA) [Covert *et al.*, 2001]

rFBA transition:

1. Update the **regulatory state**
   Synchronous update of the regulatory rules

2. Update the **metabolic state**
   Solve the FBA equations:

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Successive updates of the regulatory (*discrete*) and metabolic (*linear*) states

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Regulatory flux balance analysis (rFBA) [Covert *et al.*, 2001]

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   Synchronous update of the regulatory rules

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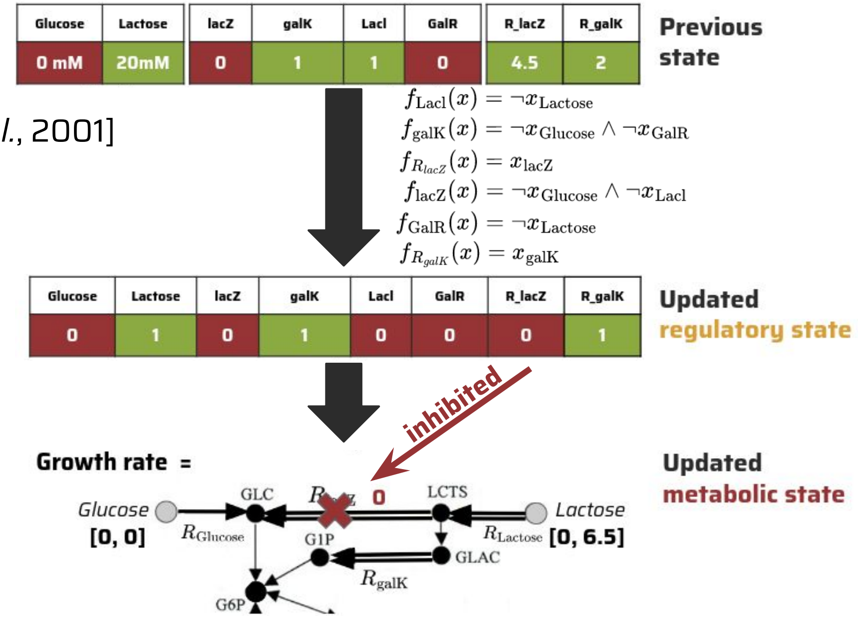
3. Update the **substrate state**



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$$f_{\text{lacZ}}(x) = \neg x_{\text{Glucose}} \wedge \neg x_{\text{LacI}}$$
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Glucose	Lactose	lacZ	galK	LacI	GalR	R_lacZ	R_galK
0	1	0	1	0	0	0	1

Updated regulatory state

Updated metabolic state

Successive updates of the regulatory (*discrete*) and metabolic (*linear*) states

rFBA states transition

Regulatory flux balance analysis (rFBA) [Covert *et al.*, 2001]

rFBA transition:

1. Update the **regulatory state**
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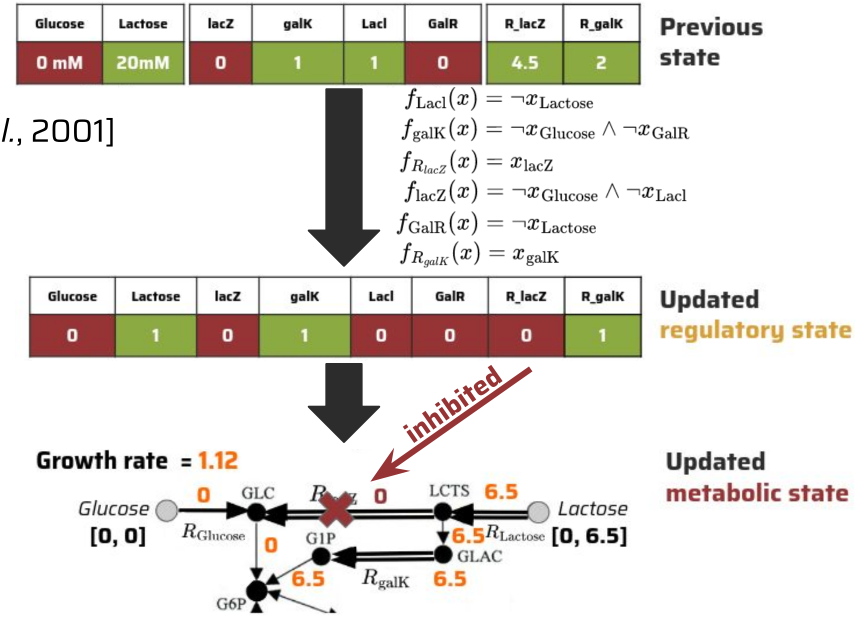
3. Update the **substrate state**



Glucose	Lactose	lacZ	galK	LacI	GalR	R_lacZ	R_galK
0 mM	20mM	0	1	1	0	4.5	2

Previous state

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$$f_{R_{galK}}(x) = x_{\text{galK}}$$

Glucose	Lactose	lacZ	galK	LacI	GalR	R_lacZ	R_galK
0	1	0	1	0	0	0	1

Updated regulatory state

Growth rate = 1.12

Updated metabolic state

Successive updates of the regulatory (*discrete*) and metabolic (*linear*) states

rFBA states transition

Regulatory flux balance analysis (rFBA) [Covert *et al.*, 2001]

rFBA transition:

1. Update the **regulatory state**
   Synchronous update of the regulatory rules

2. Update the **metabolic state**
   Solve the FBA equations:

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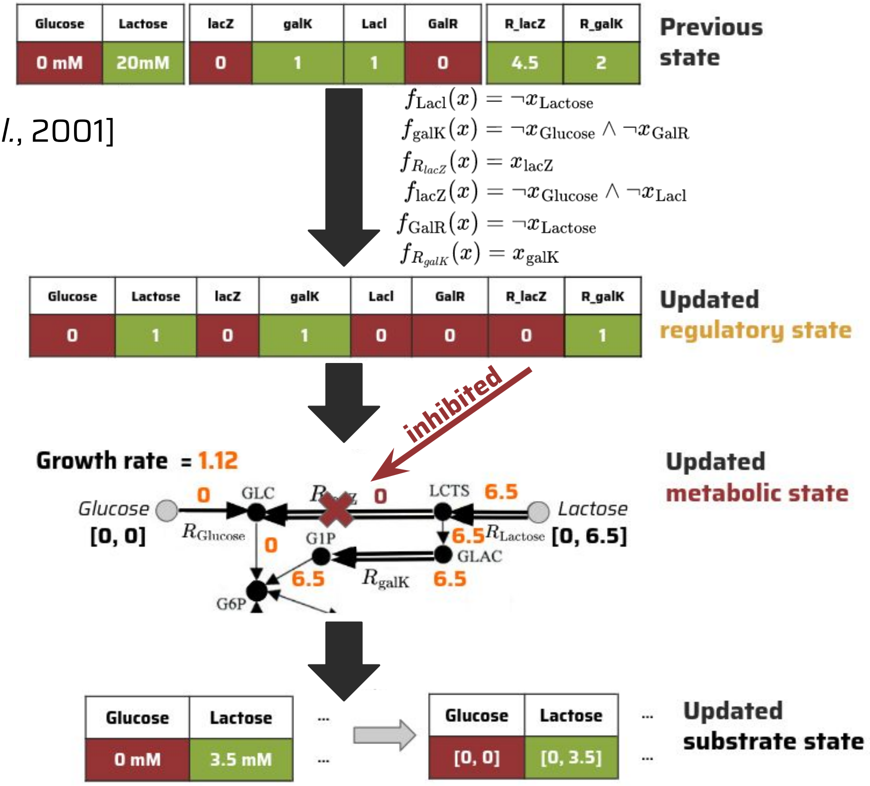
3. Update the **substrate state**



Glucose	Lactose	lacZ	galK	LacI	GalR	R_lacZ	R_galK
0 mM	20mM	0	1	1	0	4.5	2

Previous state

$$f_{\text{LacI}}(x) = \neg x_{\text{Lactose}}$$
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$$f_{\text{lacZ}}(x) = \neg x_{\text{Glucose}} \wedge \neg x_{\text{LacI}}$$
$$f_{\text{GalR}}(x) = \neg x_{\text{Lactose}}$$
$$f_{R_{galK}}(x) = x_{\text{galK}}$$

Glucose	Lactose	lacZ	galK	LacI	GalR	R_lacZ	R_galK
0	1	0	1	0	0	0	1

Updated regulatory state

Growth rate = 1.12

Updated metabolic state

Glucose	Lactose	...
0 mM	3.5 mM	...

Glucose	Lactose	...
[0, 0]	[0, 3.5]	...

Updated substrate state

Successive updates of the regulatory (*discrete*) and metabolic (*linear*) states

General form of the inference problem

$$c(x) : \bigvee_i x_i \vee \bigvee_j \neg x_j$$

$g(y)$: linear function

$$\begin{aligned}
&\text{minimize} \quad f_{\text{obj}}(x) \\
&\text{such that} \\
&\quad \bigwedge_{\alpha} c_\alpha(x) \\
&\quad \wedge \quad \bigwedge_{\beta} c_\beta(x) \vee g_\beta(y) \leq 0 \\
&\quad \wedge \quad \forall z \in \mathbb{R}^p, \left(\bigwedge_{\gamma} c_\gamma(x) \vee g_\gamma(z) \leq 0 \right) \implies \left(\bigwedge_{\zeta} c_\zeta(x) \vee g_\zeta(z) \leq 0 \right) \\
&\text{with } x \in \mathbb{B}^n,\ y \in \mathbb{R}^m
\end{aligned}$$

Optimization criteria

Search space + Regulatory state

Metabolic state

Output:

> ***Optimal*** Boolean networks in the search space with a ***trace compatible with the observations***

Observation compatibility criteria

$c(x) : \bigvee_i x_i \vee \bigvee_j \neg x_j$

$g(y)$: linear function

$$
\begin{aligned}
&\text{minimize} \quad f_{\text{obj}}(x) \\
&\text{such that} \\
&\quad \bigwedge_{\alpha} c_\alpha(x) \\
&\quad \wedge \quad \bigwedge_{\beta} c_\beta(x) \vee g_\beta(y) \leq 0 \\
&\quad \wedge \quad \forall z \in \mathbb{R}^p, \left(\bigwedge_{\gamma} c_\gamma(x) \vee g_\gamma(z) \leq 0 \right) \implies \left(\bigwedge_{\zeta} c_\zeta(x) \vee g_\zeta(z) \leq 0 \right) \\
&\text{with } x \in \mathbb{B}^n,\ y \in \mathbb{R}^m
\end{aligned}
$$

Criteria 1: regulatory state and **substrate state** are equal to **transcriptomics** and **kinetics**

Observation compatibility criteria

$c(x) : \bigvee_i x_i \vee \bigvee_j \neg x_j$

$g(y)$: linear function

$$
\begin{aligned}
&\text{minimize} \quad f_{\text{obj}}(x) \\
&\text{such that} \\
&\quad \bigwedge_\alpha c_\alpha(x)
\end{aligned}
$$

Criteria 1: regulatory state and **substrate state** are equal to **transcriptomics** and **kinetics**

$$\wedge \quad \bigwedge_\beta c_\beta(x) \vee g_\beta(y) \leq 0$$

Criteria 2: reaction activity states in the **metabolic state** and **fluxomics** are identical + **metabolic state** has the same growth rate as **kinetics**

$$\wedge \quad \forall z \in \mathbb{R}^p, \left(\bigwedge_\gamma c_\gamma(x) \vee g_\gamma(z) \leq 0 \right) \implies \left(\bigwedge_\zeta c_\zeta(x) \vee g_\zeta(z) \leq 0 \right)$$

with $x \in \mathbb{B}^n$, $y \in \mathbb{R}^m$

Observation compatibility criteria

$c(x) : \bigvee_i x_i \vee \bigvee_j \neg x_j$

$g(y)$: linear function

$$
\begin{aligned}
&\text{minimize} \quad f_{\text{obj}}(x) \\
&\text{such that} \\
&\quad \bigwedge_{\alpha} c_\alpha(x) \\
&\quad \wedge \quad \bigwedge_{\beta} c_\beta(x) \vee g_\beta(y) \leq 0 \\
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&\text{with } x \in \mathbb{B}^n,\ y \in \mathbb{R}^m
\end{aligned}
$$

Criteria 1: regulatory state and **substrate state** are equal to **transcriptomics** and **kinetics**

Criteria 2: reaction activity states in the **metabolic state** and **fluxomics** are identical + **metabolic state** has the same growth rate as **kinetics**

Criteria 3: all compatible **metabolic states** have a maximum growth less or equal to **kinetics**' growth rate

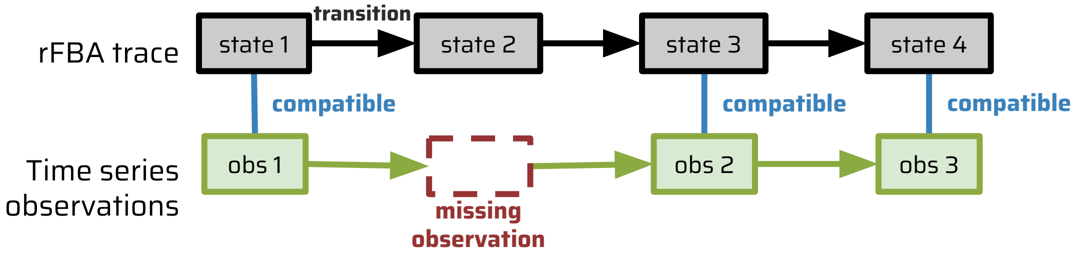
(C3) Based on FBA maximum growth heuristics [Feist and Palsson, 2010]

Regulatory state could not allow a higher growth rate than observed

Compatible Boolean networks

Compatible Boolean networks:

- ➔ Is **in the search space** described by the input interaction graph
- ➔ Has a **rFBA trace compatible with the time series observations**



- ➔ Is **optimal** according to:
  1. **Best fitting:** rFBA traces of minimal length compatible with time series
  2. **Parsimony:** subset minimal Boolean networks

General form of the inference problem

$c(x) : \bigvee_i x_i \vee \bigvee_j \neg x_j$

$g(y)$: linear function

$$\text{minimize} \quad f_{\text{obj}}(x)$$

Optimization criteria

$$\text{such that}$$

$$\bigwedge_{\alpha} c_{\alpha}(x)$$

Search space + Regulatory state + Compatibility - criteria 1

$$\wedge \quad \bigwedge_{\beta} c_{\beta}(x) \vee g_{\beta}(y) \leq 0$$

Metabolic state + Compatibility - criteria 2

$$\wedge \quad \forall z \in \mathbb{R}^{p}, \left(\bigwedge_{\gamma} c_{\gamma}(x) \vee g_{\gamma}(z) \leq 0 \right) \implies \left(\bigwedge_{\zeta} c_{\zeta}(x) \vee g_{\zeta}(z) \leq 0 \right)$$

Compatibility - criteria 3

$$\text{with } x \in \mathbb{B}^{n},\ y \in \mathbb{R}^{m}$$

Problem form:

> **Combinatorial optimization problem modulo quantified linear constraints**

Solving combinatorial optimization problem modulo quantified linear constraints

Published in:

> Thuillier, K., Siegel, A., and Paulevé, L. (2024).
>
> CEGAR-Based Approach for Solving Combinatorial Optimization Modulo Quantified Linear Arithmetics Problems. AAAI 2024.

Combinatorial optimization problem modulo quantified linear constraints - OPT+qLP

$c(x) : \bigvee_i x_i \vee \bigvee_j \neg x_j$

$g(y)$: linear function

$$\begin{aligned}
&\text{minimize} \quad f_{\text{obj}}(x) \\
&\text{such that} \\
&\bigwedge_{\alpha} c_\alpha(x) \\
&\wedge \quad \bigwedge_{\beta} c_\beta(x) \vee g_\beta(y) \leq 0 \\
&\wedge \quad \forall z \in \mathbb{R}^p, \left(\bigwedge_{\gamma} c_\gamma(x) \vee g_\gamma(z) \leq 0 \right) \implies \left(\bigwedge_{\zeta} c_\zeta(x) \vee g_\zeta(z) \leq 0 \right) \\
&\text{with } x \in \mathbb{B}^n,\ y \in \mathbb{R}^m
\end{aligned}$$

How to enumerate solutions of an OPT+qLP problem?

Combinatorial optimization problem modulo quantified linear constraints - OPT+qLP

$c(x) : \bigvee_i x_i \vee \bigvee_j \neg x_j$

$g(y)$: linear function

$$\text{minimize} \quad f_{\text{obj}}(x)$$

such that

$$\bigwedge_{\alpha} c_\alpha(x)$$

OPT problem

MaxSAT,
ASP

$$\wedge \quad \bigwedge_{\beta} c_\beta(x) \vee g_\beta(y) \leq 0$$

$$\wedge \quad \forall z \in \mathbb{R}^p, \left(\bigwedge_{\gamma} c_\gamma(x) \vee g_\gamma(z) \leq 0 \right) \implies \left(\bigwedge_{\zeta} c_\zeta(x) \vee g_\zeta(z) \leq 0 \right)$$

with $x \in \mathbb{B}^n$, $y \in \mathbb{R}^m$

How to enumerate solutions of an OPT+qLP problem?

Combinatorial optimization problem modulo quantified linear constraints - OPT+qLP

$c(x) : \bigvee_i x_i \vee \bigvee_j \neg x_j$

$g(y)$: linear function

$$\text{minimize} \quad f_{\text{obj}}(x)$$

$$\text{such that}$$

$$\bigwedge_\alpha c_\alpha(x)$$

OPT problem

MaxSAT,
ASP

$$\wedge \quad \bigwedge_\beta c_\beta(x) \vee g_\beta(y) \leq 0$$

OPT problem with linear constraints

Conflict driven clause learning (CDCL) methods [Marques-Silva and Sakallah, 1996]

SMT solvers (e.g. z3),
ASP modulo theory (e.g. *Clingo[lpx]*)

$$\wedge \quad \forall z \in \mathbb{R}^p, \left(\bigwedge_\gamma c_\gamma(x) \vee g_\gamma(z) \leq 0 \right) \implies \left(\bigwedge_\zeta c_\zeta(x) \vee g_\zeta(z) \leq 0 \right)$$

$$\text{with } x \in \mathbb{B}^n,\ y \in \mathbb{R}^m$$

How to enumerate solutions of an OPT+qLP problem?

Combinatorial optimization problem modulo quantified linear constraints - OPT+qLP

$c(x): \bigvee_i x_i \vee \bigvee_j \neg x_j$

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\begin{aligned}
&\text{minimize} \quad f_{\text{obj}}(x) \\
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\end{aligned}
$$

OPT problem

MaxSAT,
ASP

OPT problem with linear constraints

Conflict driven clause learning (CDCL) methods [Marques-Silva and Sakallah, 1996]

SMT solvers (e.g. z3),
ASP modulo theory (e.g. *Clingo[lpx]*)

OPT+qLP: OPT problem with one level of quantified linear constraints

Methods mainly rely on:

1. *E-matching* [De Moura and Bjørner, 2007]
2. *Quantifier elimination*
3. *CDCL-based methods*

No solver natively supports linear quantifiers, optimization, and enumeration

Counter-Example Guided Abstraction Refinement – CEGAR[1]

Rely on:

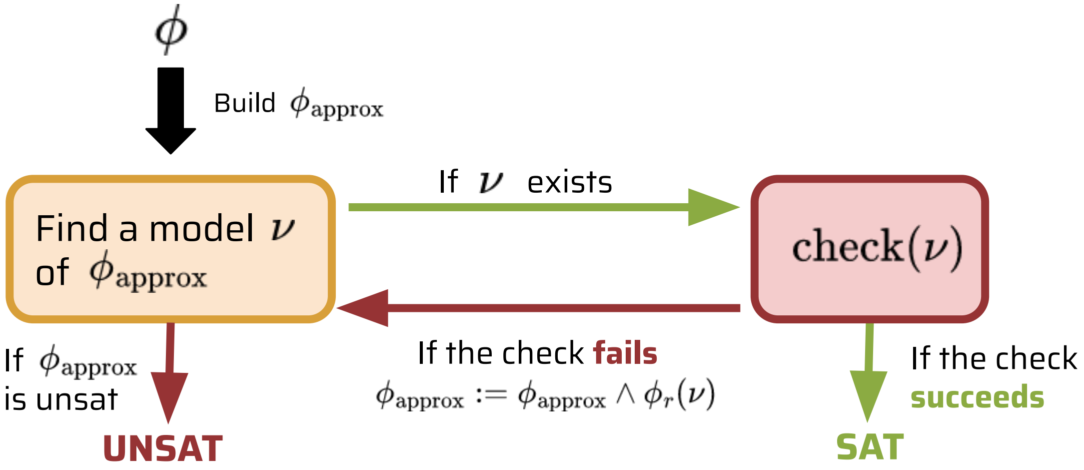
1. An **over-approximation** of the OPT+qLP problem
2. Methods to **check** the validity of an assignment
3. **Refinement functions** to generalize counter-examples

$$\phi \implies \phi_{\text{approx}}$$

$$\text{check}(\nu)$$

$$\phi_r(\nu)$$

Workflow:



Conflict Driven Constraint Learning (*CDCL*)-like solving framework

---

[1] E. Clarke *et al.*, **Journal of the ACM**, 2003

Boolean over-approximation

Replace linear constraints by Boolean variables

True: *linear constraint must hold*

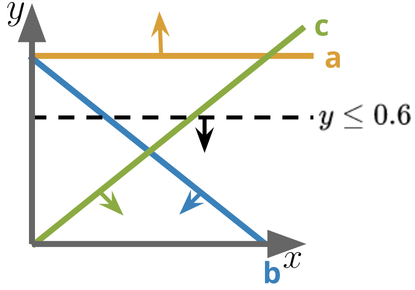
False: *ignored the linear constraint*

$$\frac{\bigwedge_{c\in C} c(x) \quad \wedge \bigwedge_{d\in D} d(x,y) \quad \wedge\ \forall z\in\mathbb{R}^p, \bigwedge_{e\in E} e(x,z) \implies \bigwedge_{h\in H} h(x,z)}{\phi}$$

— Proof in [Thuillier *et al.*, 2024]

Example:

$$\begin{aligned}&\text{minimize} \quad a+b+c\\&\text{such that}\\&\quad (a\vee b\vee c)\\&\quad \wedge\,\forall x,y\in\mathbb{R}, \begin{pmatrix} (y\geq 1\vee\neg a)\\ \wedge \quad (x+y\leq 1\vee\neg b)\\ \wedge \quad (-x+y\leq 0\vee\neg c)\end{pmatrix} \implies y\leq 0.6\\&\text{with } \ a,b,c\in\mathbb{B}\end{aligned}$$



Linear search space

OPT+qLP problems can be approximated by Boolean optimization problems

Boolean over-approximation

Replace linear constraints by Boolean variables

- **True:** *linear constraint must hold*
- **False:** *ignored the linear constraint*

— Proof in [Thuillier *et al.*, 2024]

$$\underbrace{\bigwedge_{c\in C} c(x) \wedge \bigwedge_{d\in D} d(x,y) \wedge \boxed{\forall z\in\mathbb{R}^p,} \bigwedge_{e\in E} e(x,z) \boxed{\implies} \bigwedge_{h\in H} h(x,z)}_{\phi} \implies \underbrace{\bigwedge_{c\in C} c(x) \wedge \bigwedge_{d\in D} \bar{d}(x,\bar{f}_d) \wedge \bigwedge_{e\in E} \bar{e}(x,\bar{f}_e) \boxed{\wedge} \bigwedge_{h\in H} \bar{h}(x,\bar{f}_h)}_{\phi_{\text{approx}}}$$

Example:

$$\begin{aligned}
&\text{minimize} \quad a+b+c\\
&\text{such that}\\
&\quad (a \vee b \vee c)\\
&\quad \wedge \cancel{\boxed{\forall x, y \in \mathbb{R},}} \begin{pmatrix} & (y \geq 1 \vee \neg a)\\ \wedge & (x+y \leq 1 \vee \neg b)\\ \wedge & (-x+y \leq 0 \vee \neg c)\end{pmatrix} \overset{\wedge}{\cancel{\boxed{\implies}}} y \leq 0.6\\
&\text{with} \quad a, b, c \in \mathbb{B}
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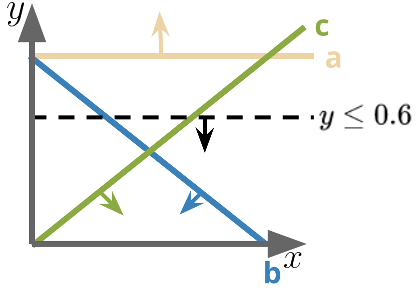
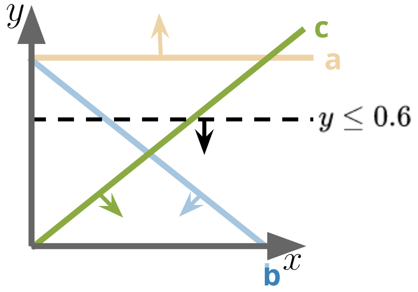


Linear search space for $\{b, c\}$



Linear search space for $\{c\}$

OPT+qLP problems can be approximated by Boolean optimization problems

Checking quantified linear constraints

Given set of quantified linear constraints that must hold:

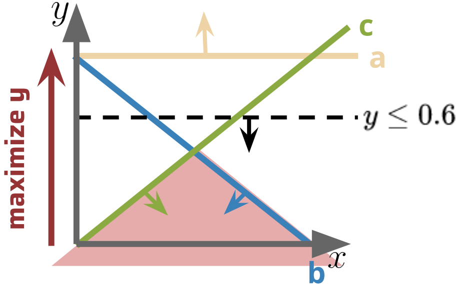
$$\forall y \in \mathbb{R}^p,\ \bigwedge_f f(y) \le 0 \implies g(y) \le 0 \quad \iff \quad \begin{array}{rll} \text{maximize} & g(y) & \le 0 \\ \text{such that:} & f(y) \le 0 & \forall f \\ \text{with} & y \in \mathbb{R}^p & \end{array}$$

Example:

$$\begin{array}{l} \text{minimize} \quad a + b + c \\ \text{such that} \\ \quad (a \lor b \lor c) \\ \quad \land\ \xcancel{\forall x, y \in \mathbb{R},} \left(\begin{array}{rc} & (y \ge 1 \lor \neg a) \\ \land & (x + y \le 1 \lor \neg b) \\ \land & (-x + y \le 0 \lor \neg c) \end{array} \right) \overset{\land}{\xcancel{\implies}} y \le 0.6 \\ \text{with} \ \ a, b, c \in \mathbb{B} \end{array}$$

Valid assignment

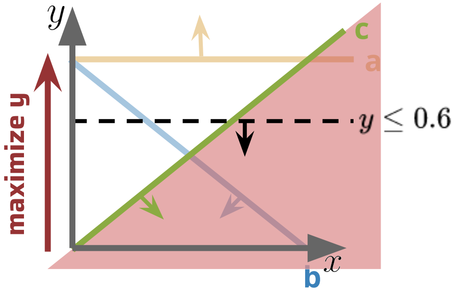
$\max\ y = 0.5$



Linear search space for $\{b, c\}$

Invalid assignment

$\max\ y = \infty$



Linear search space for $\{c\}$

Checking quantified linear constraints = solving a linear optimization problem

Counter-examples generalization

Monotone property:
Adding linear constraints to a linear optimization problem could not increase its maximum

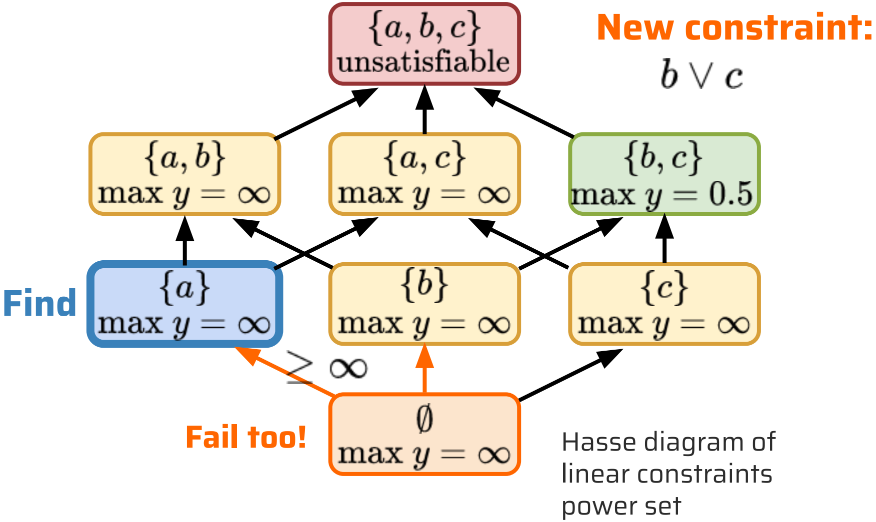
Example:

Objective: maximize y

Constraints

$$a : y \geq 1$$
$$b : x + y \leq 1$$
$$c : -x + y \leq 0$$

Variables: $x, y \in \mathbb{R}$



Hasse diagram of linear constraints power set

Set of linear constraints fails the check $\Longrightarrow$ all its subset will fail too

Further refinements

Optimal core:
A largest superset of linear constraints having a maximum failing the linear check

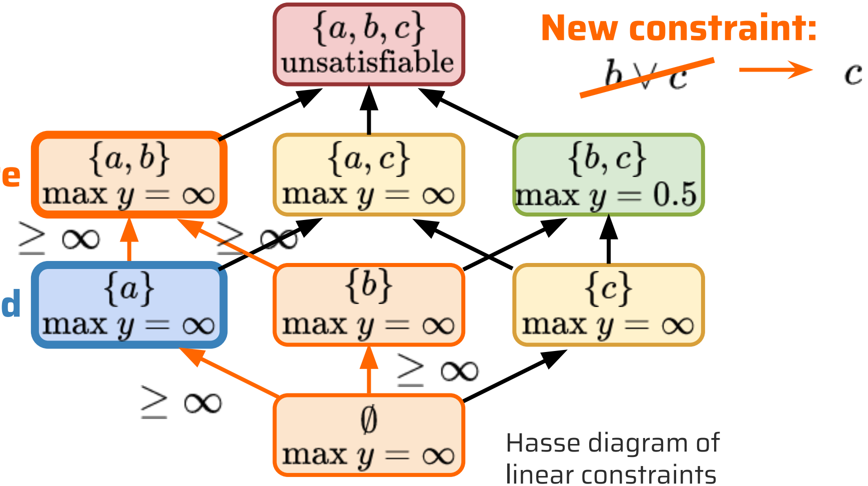
Example:

Objective: $\text{maximize} \quad y$

Constraints

$$a: y \geq 1$$
$$b: x + y \leq 1$$
$$c: -x + y \leq 0$$

Variables: $x, y \in \mathbb{R}$



$\{a, b, c\}$ unsatisfiable

New constraint: $\cancel{b \vee c} \rightarrow c$

Optimal core $\{a, b\}$ $\max y = \infty$

$\{a, c\}$ $\max y = \infty$

$\{b, c\}$ $\max y = 0.5$

Find $\{a\}$ $\max y = \infty$

$\{b\}$ $\max y = \infty$

$\{c\}$ $\max y = \infty$

$\emptyset$ $\max y = \infty$

$\geq \infty$

$\geq \infty$

$\geq \infty$

$\geq \infty$

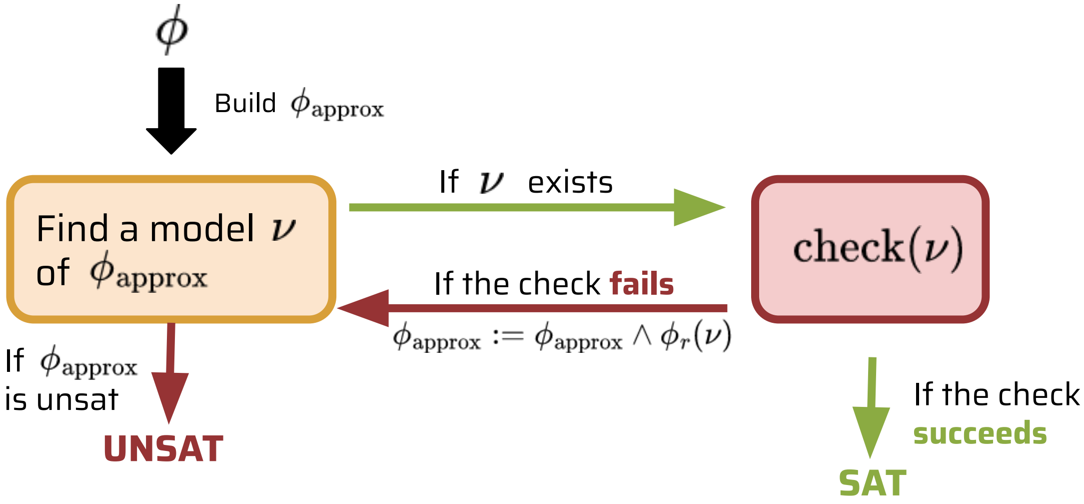
Hasse diagram of linear constraints power set

Similar to unsatisfiable cores but applied to optimum values

Enumerating models with CEGAR

> **Issue:** CEGAR is not efficient to enumerate models
> *Linear checks are made even for surely valid model*

[Brummayer and Biere, 2009]



ϕ

Build ϕ_{approx}

Find a model ν of ϕ_{approx}

If ν exists

$\text{check}(\nu)$

If the check **fails**

$\phi_{\text{approx}} := \phi_{\text{approx}} \wedge \phi_r(\nu)$

If ϕ_{approx} is unsat

UNSAT

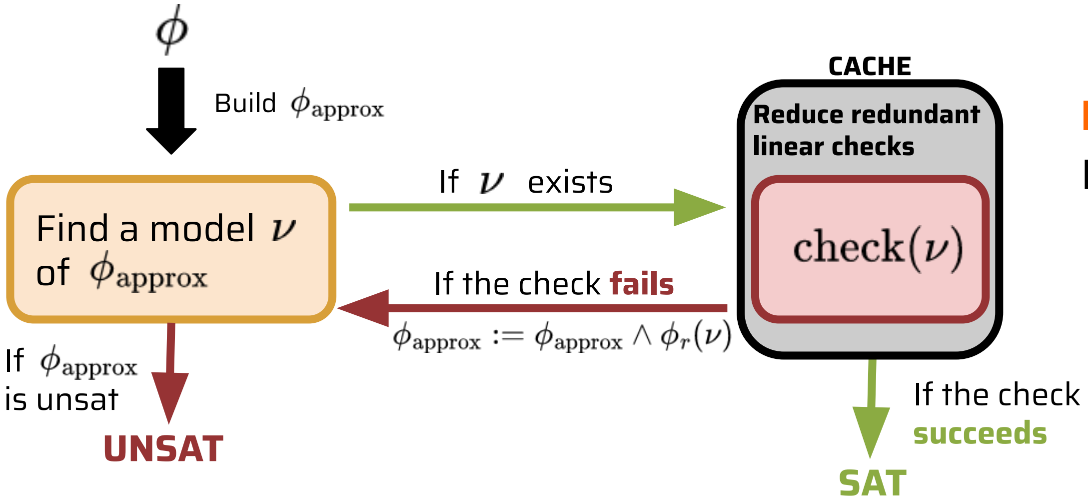
If the check **succeeds**

SAT

Enumerating models with CEGAR

> **Issue:** CEGAR is not efficient to enumerate models
> *Linear checks are made even for surely valid model*

[Brummayer and Biere, 2009]



Rely on the monotone property

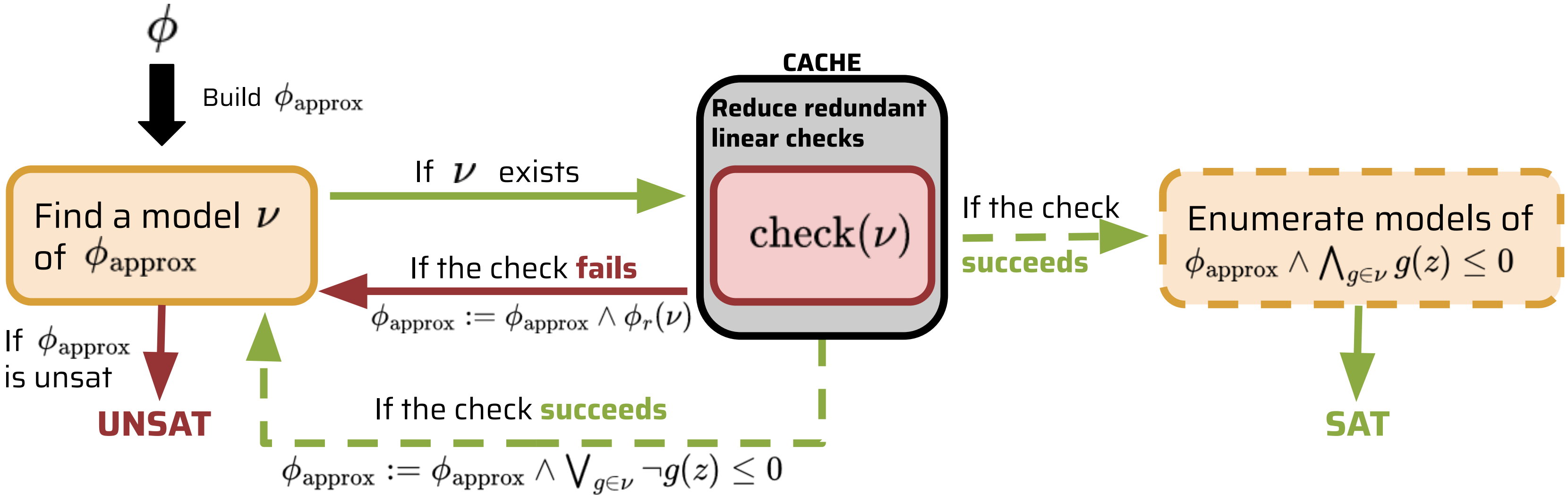
Keep track of:

- Supersets of SAT LP constraints
- Subsets of UNSAT LP constraints

> **Reduce LP solver calls**
> **Still too many redundant operations in the SAT solver**

Enumerating models with CEGAR

Issue: CEGAR is not efficient to enumerate models [Brummayer and Biere, 2009]
Linear checks are made even for surely valid model

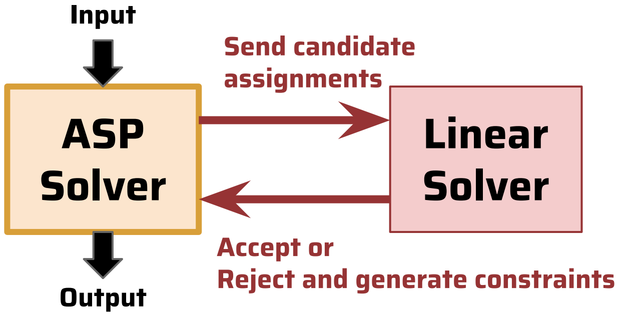


Class of models having the same underlying linear constraints

Implementations

Based on Answer Set Programming (ASP):
Logic programming
Handle optimization and efficient enumeration [Gebser *et al.*, 2011-13]

Linear checks made with generic linear solvers
CBC, GLPK, Gurobi



Tools:

- ➔ **MERRIN:** inference of Boolean network controlling metabolic networks
  Published in Bioinformatics / at ECCB22 [Thuillier *et al.*, 2022] github.com/bioasp/merrin
- ➔ **MerrinASP:** generic solver for OPT+qLP problems
  Published at AAAI24 [Thuillier *et al.*, 2024] github.com/kthuillier/merrinasp
  No enumeration optimization yet

Two implementations of the CEGAR-based workflow based on ASP

Benchmarking

Published in:

> **Thuillier, K., Baroukh, C., Bockmayr, A., Cottret, L., Paulevé, L., and Siegel, A. (2022).**
>
> **MERRIN: MEtabolic Regulation Rule INference from time series data. Bioinformatics.**

Escherichia coli models

Core-carbon metabolism [Covert *et al.*, 2001]

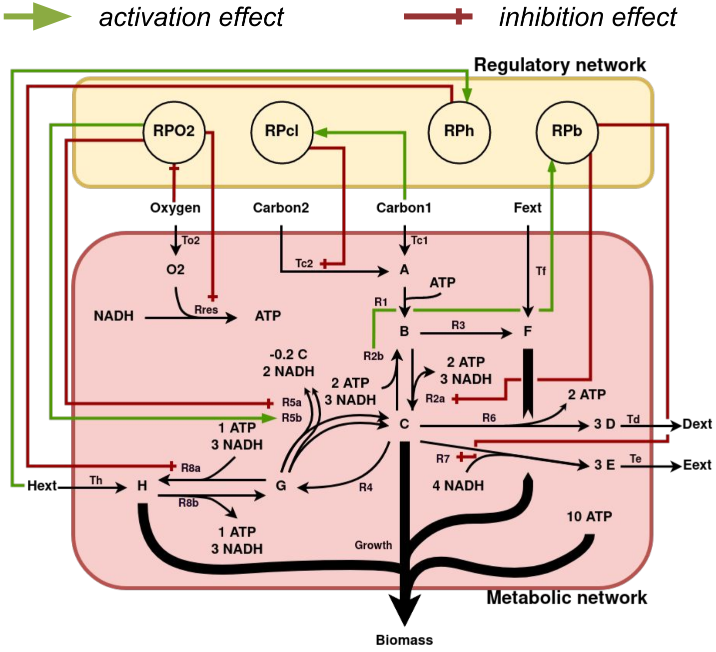
Core-carbon model

- *20 reactions*
- *11 regulatory rules*

***E. coli* core-metabolism [Covert *et al.*, 2002]**

Medium-scale model

- *113 reactions*
- *151 regulatory rules*



Core-carbon metabolism from [Covert *et al.*, 2001]

Time series generation protocol [Thuillier *et al.*, 2022]

From rFBA simulations → noisy time series with different data types

Generation of two synthetic datasets of increasing size based on *e. coli* models

Generated benchmarks

	Instances	Type combinations	Noise range	Repetition	Number of variables		Number of constraints	
					Boolean	Linear	Logic	Linear
Core-carbon	240	F, K, T K, T F T	0% - 50%	10	6.5×10^4	4×10^3	2.7×10^5	1.2×10^4
Medium-scale	60	F, K, T	0%	60	**4×10^9**	**16×10^3**	**18×10^5**	**5×10^4**

x10^5

x4

Core-carbon benchmark:

Impact of observation types and noise rates on the inference

Medium-scale benchmark:

Impact of model size – ensure scalability

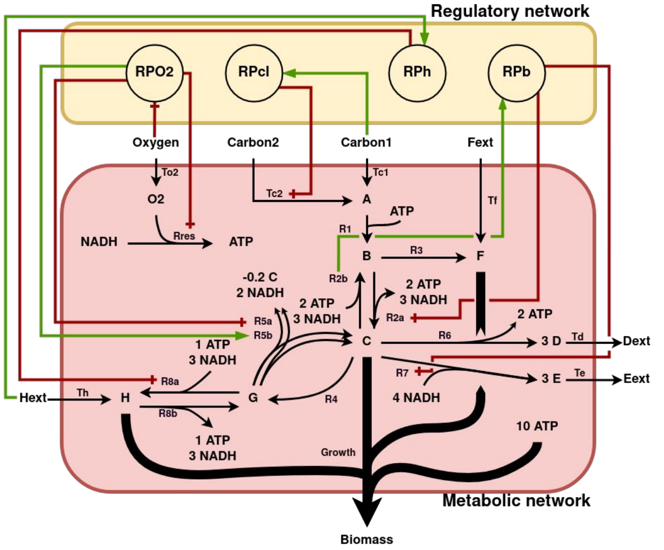
Application on core-carbon model

Complete data

Data types: *Fluxomics, Kinetics, Transcriptomics*
Noise: *0%*

48 Boolean networks
Gold standard is inferred

Computation time: 7s



Gold standard from [Covert *et al.*, 2001]

Gold standard network is inferred from complete observations

Application on core-carbon model

Complete data

Data types: *Fluxomics, Kinetics, Transcriptomics*
Noise: *0%*

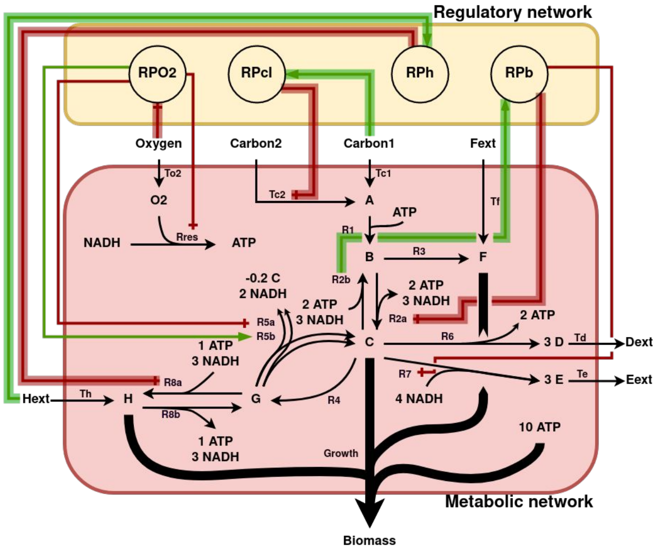
48 Boolean networks

Gold standard is inferred

Computation time: 7s

1 subset minimal Boolean network

- Reproduce exactly the input rFBA time series
  Residual Sum of Squares (RSS): 0
- Smaller than gold standard
  Precision: 1 / Recall: 0.64
  - Not all regulatory rules are retrieved
  - Consistent with [Covert *et al.*, 2001]



Subset minimal model

rFBA formalism does not allow capturing all regulatory process

Perspective: upgrade modeling formalisms to capture missing rules

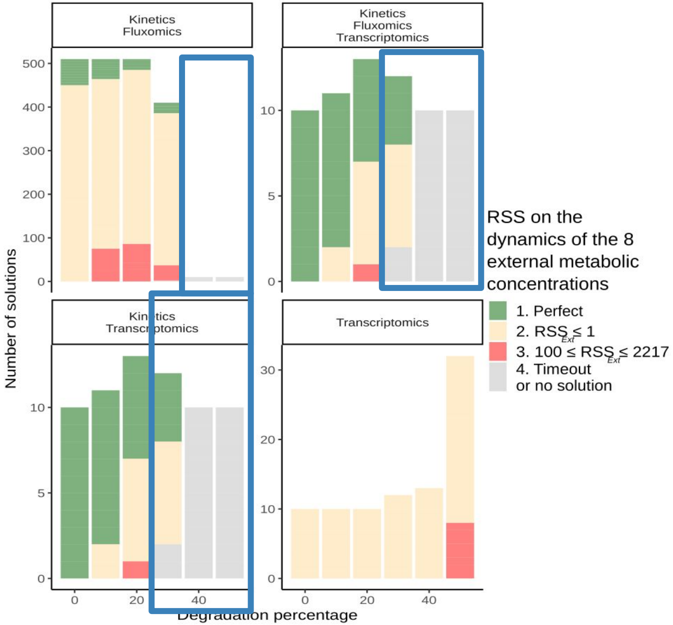
Impact of noise and data types

Benchmark

Data types: *4 combinations*
Noise: *0% - 50%*

Compute subset minimal models for each instance

Unsatisfiable instance due to noise in fluxomics and kinetics



Impact of types combination on *RSS*

MERRIN handles up to 20% of noise

Impact of noise and data types

Benchmark

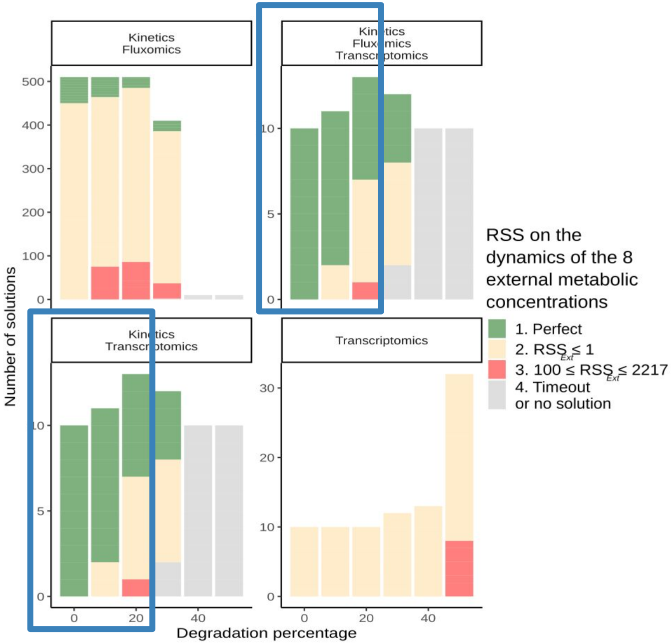
Data types: *4 combinations*
Noise: *0% - 50%*

Compute subset minimal models for each instance
Unsatisfiable instance due to noise in fluxomics and kinetics

Type: transcriptomics + kinetics / noise: 0% - 20%

➔ *RSS < 1*

➔ *Precision: 1 / Recall: 0.64*



Impact of types combination on *RSS*

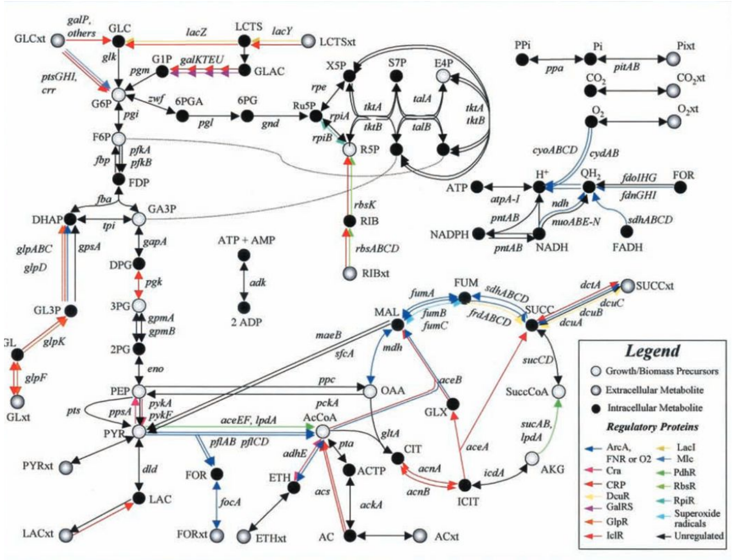
Transcriptomics + kinetics: **sufficient to infer regulations controlling the metabolism**

Application on *E. coli* core-metabolism[1]

Complete data - 3 time series

Data types: *Fluxomics, Kinetics, Transcriptomics*
Noise: *0%*

838 860 800 subset minimal Boolean networks
Computation time: < 8h
All subset minimal models are enumerated



E. coli core-metabolism from [Covert *et al.*, 2002]

MERRIN's method scales to medium-scale models

---

[1] M. W. Covert and B. Ø. Palsson, **Journal of biological chemistry**, 2002

Application on *E. coli* core-metabolism[1]

Complete data - 3 time series

Data types: *Fluxomics, Kinetics, Transcriptomics*
Noise: *0%*

838 860 800 subset minimal Boolean networks

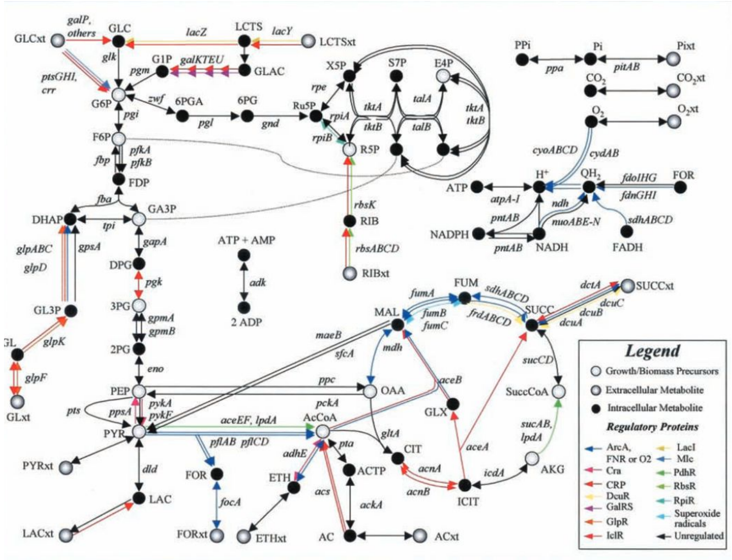
Computation time: < 8h
All subset minimal models are enumerated

Compatibility with time series

Residual Sum of Squares: 0

Smaller than gold standard model

Precision: ~0.87 / Recall: ~0.11



E. coli core-metabolism from [Covert *et al.*, 2002]

Rules may not be inferred due to data incompleteness

---

[1] M. W. Covert and B. Ø. Palsson, **Journal of biological chemistry**, 2002

Conclusion and Research Interest

Conclusion

Initial question: **Can we infer regulatory controls of the metabolism?**

Answer: **Yes! From kinetics and transcriptomics with up to 20% of noise**

Inference problem formalization:

- ➔ No method to infer controls of the metabolism
- ➔ Integrate both scale dynamics: **discrete** + **flux-based**
- ➔ **Combinatorial optimization problem modulo quantified linear constraints (OPT+qLP)**

Solving OPT+qLP problems:

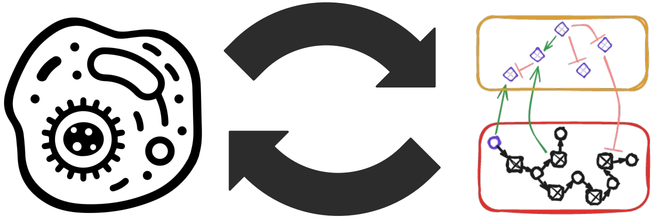
- ➔ Existing hybrid solvers do not handle such problem
- ➔ Developed our **own solving methods**: CEGAR-based + quantifier elimination

Benchmarking:

- ➔ Generate synthetic datasets of 2 *E. coli* models
- ➔ Study the impact of: noise, observations types, and instance size

Research interests

Keywords: knowledge representation, operational research, system biology



Modeling
Knowledge discovery

Explainable
decision-aid tools

1. **Knowledge discovery**

Modeling of living systems
Decision-aid tools (e.g. experiment design)

2. **Constraint programming**

Solving methods + problem formalization
Optimization modulo theory

3. **Explainability in AI**

Formalizing the limits of ML methods
Results reproducibility and explainability

Explainability is essential!

How to maintain models in the long run?

Available regulated metabolic models have been manually curated but are depreciated

Example: Model of Escherichia coli str. K-12 substr. MG1655 [Covert et al., 2004]

- ➔ **Model:** 1.473 regulatory rules / 1.075 reactions
- ➔ **Data:** growth phenotypes for 111 mutant strains over 124 mediums

– ISSUE –
Unable to reproduce
the paper's predictions

Need to develop formal methods for biological models:

Model Checking: how to ensure models and data compatibility?

(Iterative) Model Synthesis: how to update a model with new observations?
e.g. checking SAT problem partitioning[1][2][3] *and parallel solving*[1]

---

[1] Kaufmann *et al.*, **AAAI**, 2016

[2] Tinos *et al.*, **ACM Conference on Foundations of Genetic Algorithms**, 2015

[3] Hyvärinen, *et al.*, **Springer Nature**, 2006

Integrating ML and formal methods

Merging the explainability of formal methods with the flexibility of ML

e.g. refine the problem input knowledge, filter candidate solutions [Réda and Delahaye-Duriez, 2022]

***Example 1: Genetic algorithms used to infer regulatory rules* [Gapo et al., 2020][Liu et al., 2021]**

- ➔ **Infer the interaction graph … *but could not rely on a priori knowledge***
- ➔ **Infer a *lot of spurious interactions***

Can be used to refine our interaction graph with missing interactions

---

Formalizing the scope of use of AI methods

e.g. providing guarantee on the methods' outputs, SAT modulo non-convex constraints

Example 2: Gradient descent for parameter estimation in kinetics equations

- ➔ **Works in some case, but not in others**
- ➔ ***No guarantee that it will reach a global minimum***

Integration in the LMF

Laboratoire
Méthodes
Formelles

Postdoc with: B. Bollig, M. Fuegger, and T. Nowak
CRN modeling of bacteria growth in bioreactors
Formal methods for parameter estimations in kinetics equations

Part of Safe AI through Formal Methods

In the LMF:

Pôle “Modèles”

➔ **Modèle Checking et Synthèse**

Logic/Hybrid programing, SAT solving

Pôle “Interactions”

➔ **Méthodes Formelles pour la Biologie**

Modeling and simulation of living systems

➔ **Méthodes Formelles pour l’IA**

Model synthesis, explainability (SAIF)

+ **Wetlab**

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Thomas Nowak

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Alexander Bockmayr
Ludovic Cottret

… and the cats!

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Appendices

State and observation compatibility

1. **Regulatory state** and **substrate state** are equal to **transcriptomics** and **kinetics**

Transcriptomics:

Glucose	Lactose	lacZ	galK	LacI	GalR	R_lacZ	R_galK
0	1	-	1	0	-	1	1

=

Regulatory state:

Glucose	Lactose	lacZ	galK	LacI	GalR	R_lacZ	R_galK
0	1	1	1	0	0	1	1

Kinetics:

Glucose	Lactose	...
0 mM	20 mM	...

=

Substrate state:

Glucose	Lactose	...
0 mM	20 mM	...

Observations

Regulated metabolic state

Regulatory state matches with input gene expression data

State and observation compatibility

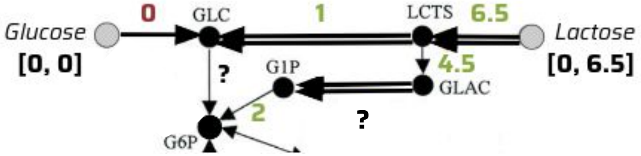
1. **Regulatory state** and **substrate state** are equal to **transcriptomics** and **kinetics**

2. Reaction activity states in the **metabolic state** and **fluxomics** are identical
   \+ **metabolic state** has the same growth rate as **kinetics**

Kinetics:

Growth rate = 1.12

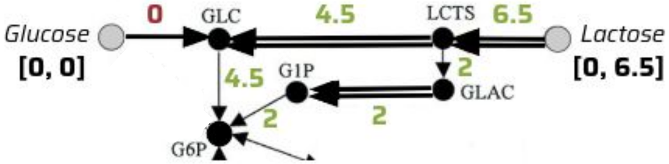
Fluxomics:



Metabolic state:

Growth rate = 1.12

\+



Metabolic state's flux distribution matches with the metabolic observations

State and observation compatibility

1. **Regulatory state** and **substrate state** are equal to **transcriptomics** and **kinetics**

2. Reaction activity states in the **metabolic state** and **fluxomics** are identical
   + metabolic state has the same growth rate as **kinetics**

3. All compatible **metabolic states** have a maximum growth less or equal to **kinetics'** growth rate

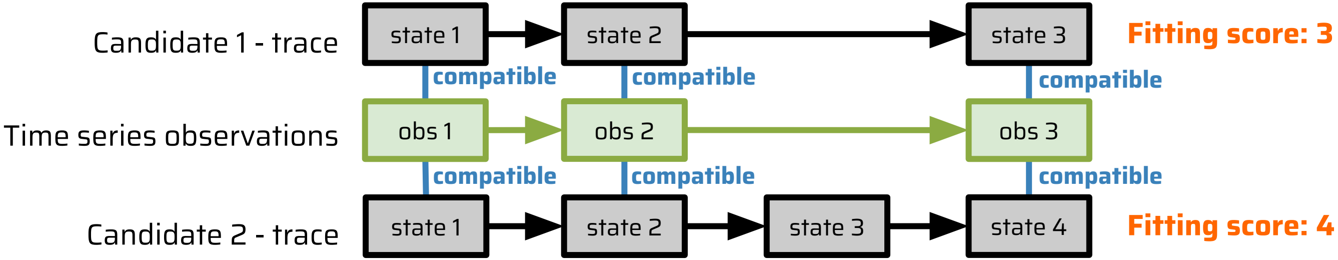
Growth optimization heuristics:
[Feist and Palsson, 2010]

$$
\begin{aligned}
\text{maximize} \quad & v_{\text{Growth}} \leq \text{Growth rate} \\
\text{such that:} \quad & S \cdot v = 0 \\
& l_r \leq v_r \leq u_r \quad \forall r \in \text{reactions} \\
& v_r = 0 \quad \forall r \in \text{inhibited reactions}
\end{aligned}
$$

Regulatory state could not allow a higher growth rate than observed

Optimization criteria

1. **Best fitting:** traces of minimal length compatible with observations



Candidate 1 - trace: state 1 → state 2 → state 3 — Fitting score: 3

compatible

Time series observations: obs 1 → obs 2 → obs 3

compatible

Candidate 2 - trace: state 1 → state 2 → state 3 → state 4 — Fitting score: 4

2. **Parsimony:** subset minimal Boolean networks

$$\begin{aligned} f_{\text{lacZ}}(x) &= \neg x_{\text{GLCxt}} \\ f_{\text{galKTEU}}(x) &= \neg x_{\text{GLCxt}} \end{aligned} \quad \subset \quad \begin{aligned} f_{\text{lacZ}}(x) &= \neg x_{\text{GLCxt}} \\ f_{\text{galKTEU}}(x) &= \neg x_{\text{GLCxt}} \wedge \neg x_{\text{GalR}} \end{aligned} \quad \subset \quad \begin{aligned} f_{\text{lacZ}}(x) &= \neg x_{\text{GLCxt}} \\ f_{\text{galKTEU}}(x) &= \neg x_{\text{GLCxt}} \wedge \neg x_{\text{GalR}} \\ f_{\text{GalR}}(x) &= \neg x_{\text{LCTSxt}} \end{aligned}$$

subset minimal

Combinatorial optimization criteria: minimize trace length, minimize size

Our CEGAR-based workflow in practice

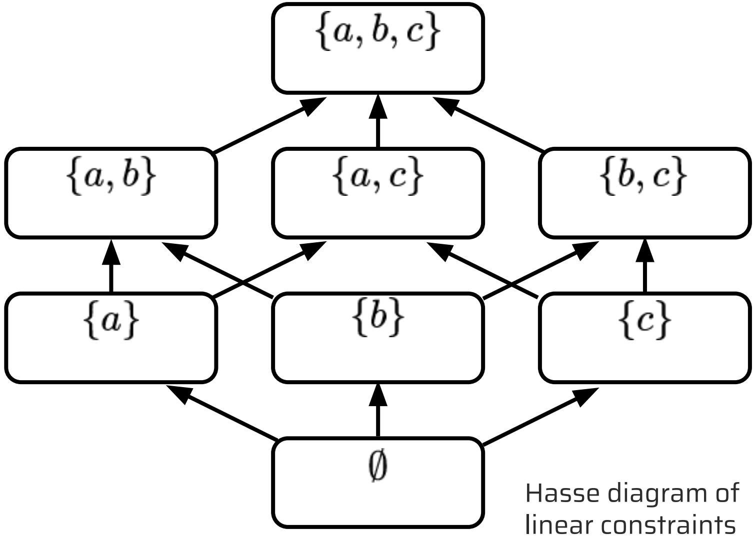
Boolean over-approximation

$$(a \lor b \lor c)$$

$$\land \boxed{\cancel{\forall x, y \in \mathbb{R},}} \begin{pmatrix} & (y \geq 1 \lor \neg a) \\ \land & (x + y \leq 1 \lor \neg b) \\ \land & (-x + y \leq 0 \lor \neg c) \end{pmatrix} \overset{\land}{\boxed{\cancel{\implies}}} y \leq 0.6$$

with $a, b, c \in \mathbb{B}$

Checked Boolean assignments:



Hasse diagram of linear constraints power set

Our CEGAR-based workflow in practice

Boolean over-approximation

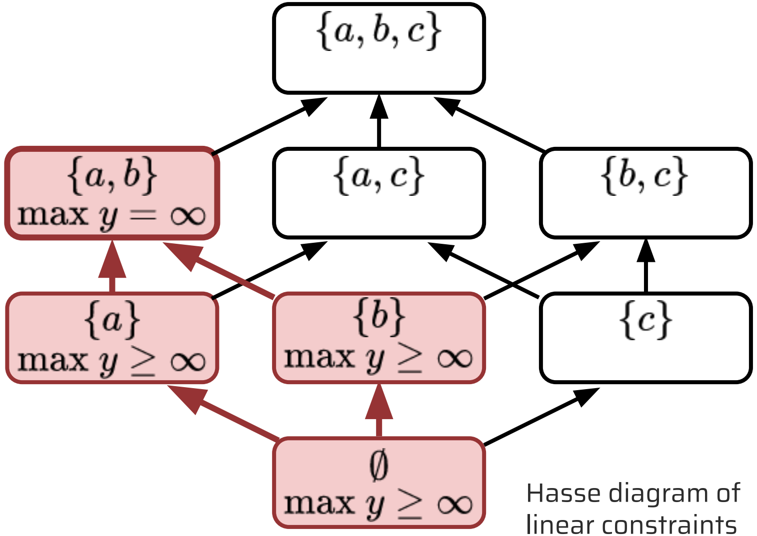
$$(a \vee b \vee c)$$

$$\wedge \cancel{\forall x, y \in \mathbb{R},} \left(\begin{array}{rc} & (y \geq 1 \vee \neg a) \\ \wedge & (x + y \leq 1 \vee \neg b) \\ \wedge & (-x + y \leq 0 \vee \neg c) \end{array} \right) \overset{\wedge}{\cancel{\implies}} y \leq 0.6$$

with $a, b, c \in \mathbb{B}$

Checked Boolean assignments:

1. $\{a, b\} \longrightarrow \max y = \infty$



Hasse diagram of linear constraints power set

All subset of $\{a, b\}$ have $\max y \geq \infty$

Our CEGAR-based workflow in practice

Boolean over-approximation

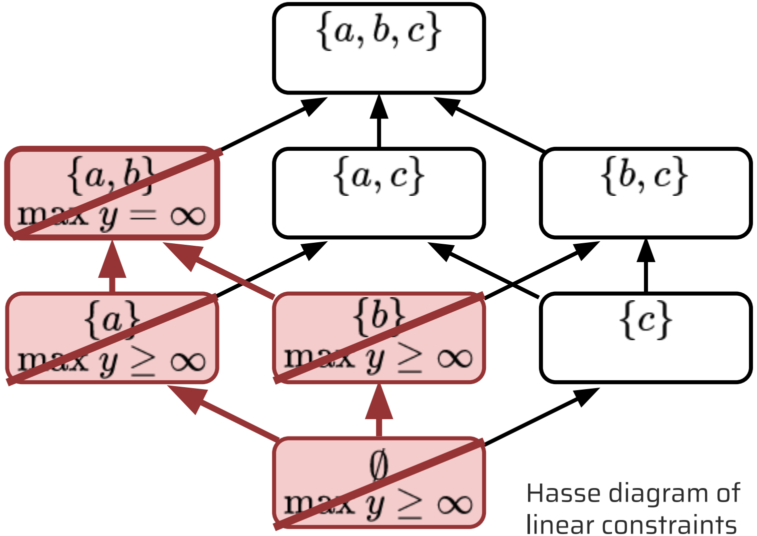
$$(a \lor b \lor c) \land \boldsymbol{(-x + y \leq 0)}$$

$$\land \cancel{\forall x, y \in \mathbb{R},} \begin{pmatrix} & (y \geq 1 \lor \neg a) \\ \land & (x + y \leq 1 \lor \neg b) \\ \land & (-x + y \leq 0 \lor \neg c) \end{pmatrix} \overset{\land}{\cancel{\implies}} y \leq 0.6$$

with $a, b, c \in \mathbb{B}$

Checked Boolean assignments:

1. $\{a, b\} \longrightarrow \max y = \infty$



Hasse diagram of linear constraints power set

All subset of $\{a, b\}$ have $\max y \geq \infty \implies$ Prohibit solutions without $c : -x + y \leq 0$

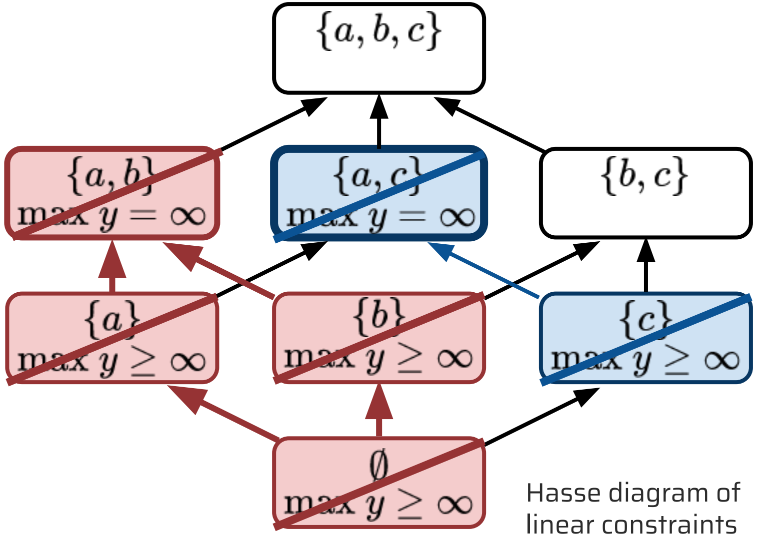
Our CEGAR-based workflow in practice

Boolean over-approximation

$$(a \lor b \lor c) \land (\boldsymbol{-x + y \leq 0}) \land (\boldsymbol{x + y \leq 1})$$

$$\land \cancel{\forall x, y \in \mathbb{R},} \begin{pmatrix} (y \geq 1 \lor \neg a) \\ \land \quad (x + y \leq 1 \lor \neg b) \\ \land \quad (-x + y \leq 0 \lor \neg c) \end{pmatrix} \overset{\land}{\cancel{\Longrightarrow}} y \leq 0.6$$

with $a, b, c \in \mathbb{B}$



Hasse diagram of linear constraints power set

Test Boolean assignments:

1. $\{a, b\} \longrightarrow \max y = \infty$
2. $\{a, c\} \longrightarrow \max y = \infty$

All subset of $\{a, b\}$ **have** $\max y \geq \infty \Longrightarrow$ **Prohibit solutions without** $b : x + y \leq 1$

Our CEGAR-based workflow in practice

Boolean over-approximation

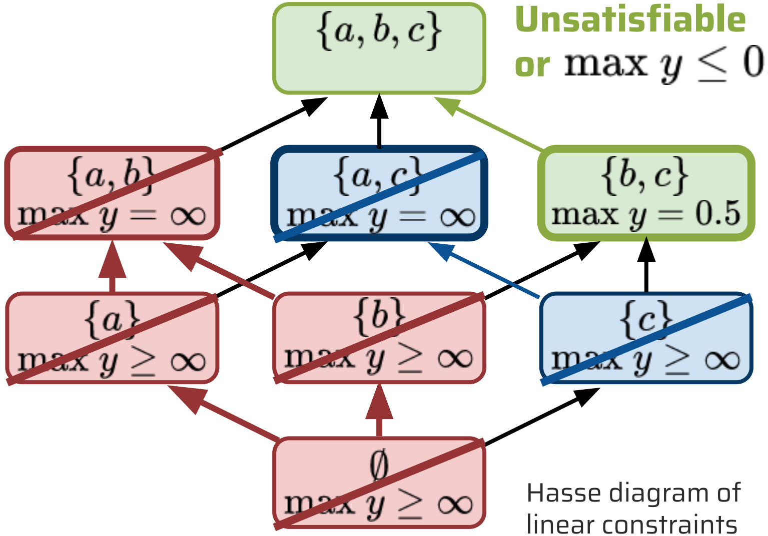
$$(a \lor b \lor c) \land (-x + y \leq 0) \land (x + y \leq 1)$$

$$\land \cancel{\forall x, y \in \mathbb{R},} \begin{pmatrix} & (y \geq 1 \lor \neg a) \\ \land & (x + y \leq 1 \lor \neg b) \\ \land & (-x + y \leq 0 \lor \neg c) \end{pmatrix} \overset{\land}{\cancel{\implies}} y \leq 0.6$$

with $a, b, c \in \mathbb{B}$

Test Boolean assignments:

1. $\{a, b\} \longrightarrow \max y = \infty$
2. $\{a, c\} \longrightarrow \max y = \infty$
3. $\{b, c\} \longrightarrow \max y = 0.5$



Unsatisfiable or $\max y \leq 0.5$

Hasse diagram of linear constraints power set

A valid assignment is found $\implies$ All supersets will be valid too

Quantifier elimination

From **weak duality theorem:**

Universally quantified constraint ***Quantifier-free constraint***

$$\forall z \in \mathbb{R}^p, A \cdot z \leq b \implies c^T \cdot z \leq \lambda \xmapsto[\text{and } A^T \cdot y = c \text{ are satisfiable}]{\text{If } A \cdot z \leq b} \exists y \in \mathbb{R}^{q+}, A^T \cdot y = c \wedge b^T \cdot \leq \lambda$$

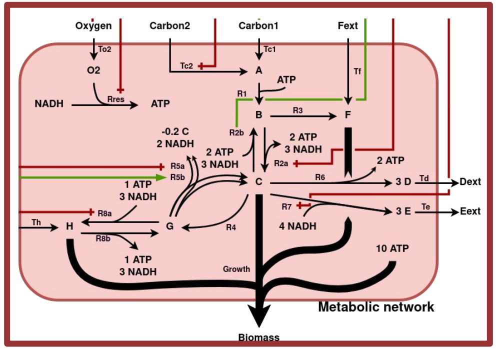
Advantage: usable with any quantifier-free SAT+LP solvers *e.g. clingo[lpx] or z3*

Inconvenient: only an under-approximation in general case

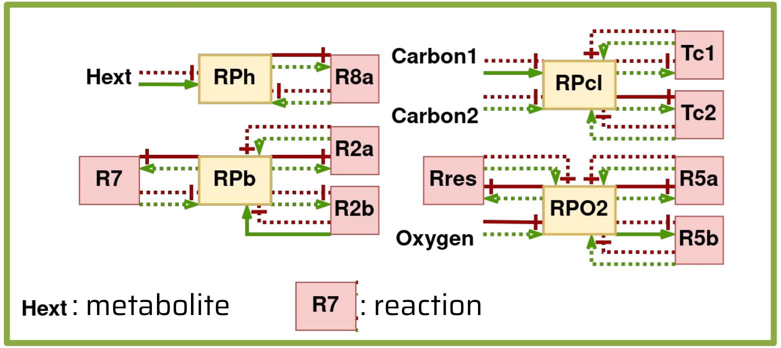
- ➔ Cannot handle: $A \cdot z \leq b$ and $A^T \cdot y = c$ not satisfiable
- ➔ All linear problems that can be built by the SMT solvers must have an optimum

Instance generation: application to core-carbon model

Metabolic network



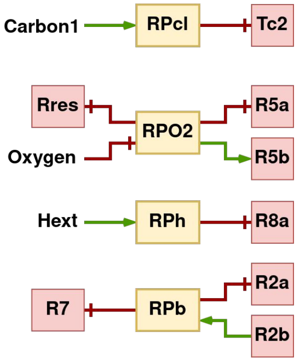
Interaction graph



Observations

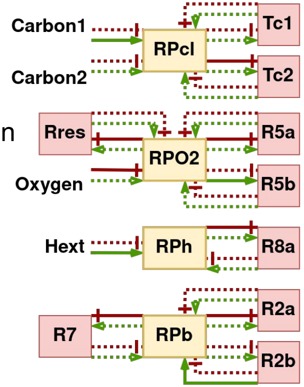
Interaction graph

- ➔ Generate from model’s regulatory networks
- ➔ Add noise: remove interaction signs and directions



Gold standard

- ➔ Remove interactions direction
- ➔ Remove interactions sign
- ➔ Add hypothetical regulation



Interaction graph

Time series generation workflow

Extract data per observation' types:

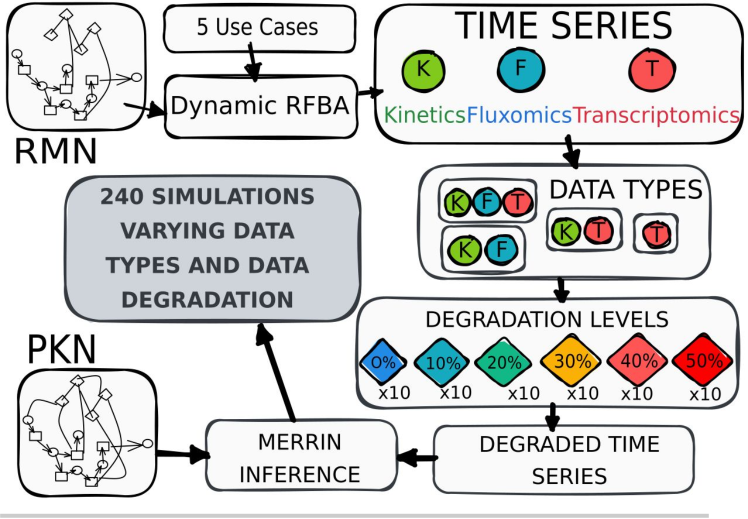
- → **Kinetics:** substrate concentrations
- → **Fluxomics:** reactions fluxes values
- → **Transcriptomics:** binarized observation

Keep simulation timesteps:

- 2 observations per growth phase
- 1 observation per transition

Noise:

- Noise on kinetics and fluxomics values
- Probability to remove an observed values
- Probability to remove a timestep

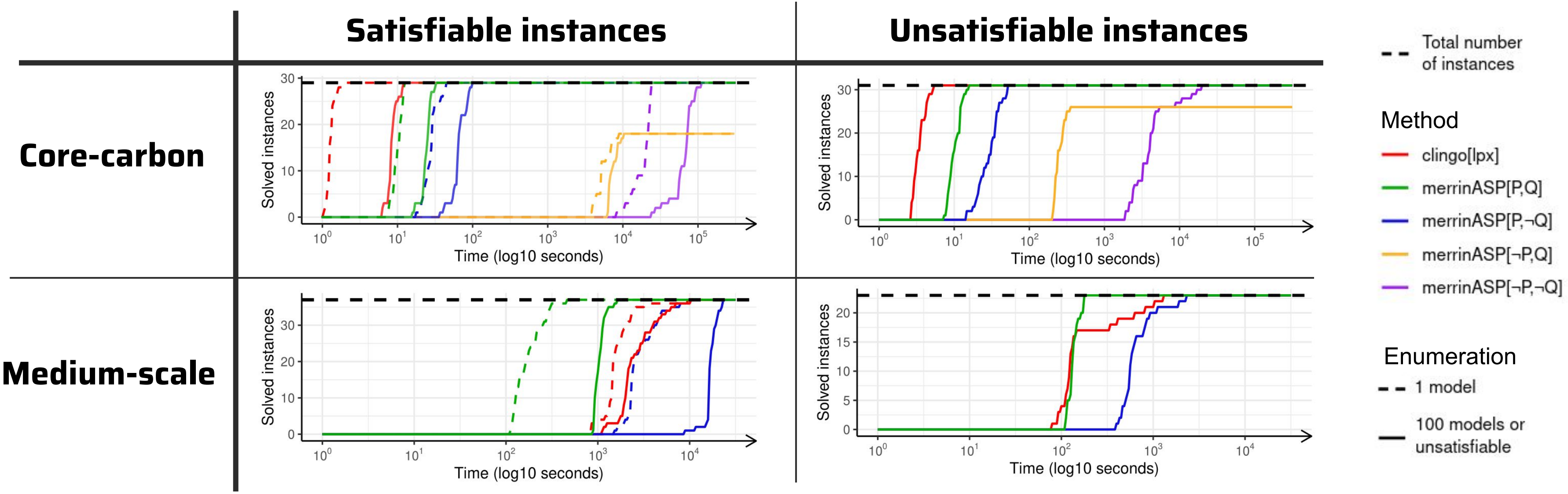


From [Thuillier *et al.*, 2022]

Generate noisy kinetics, fluxomics, and transcriptomics observations from rFBA simulations

MerrinASP - Performance comparison

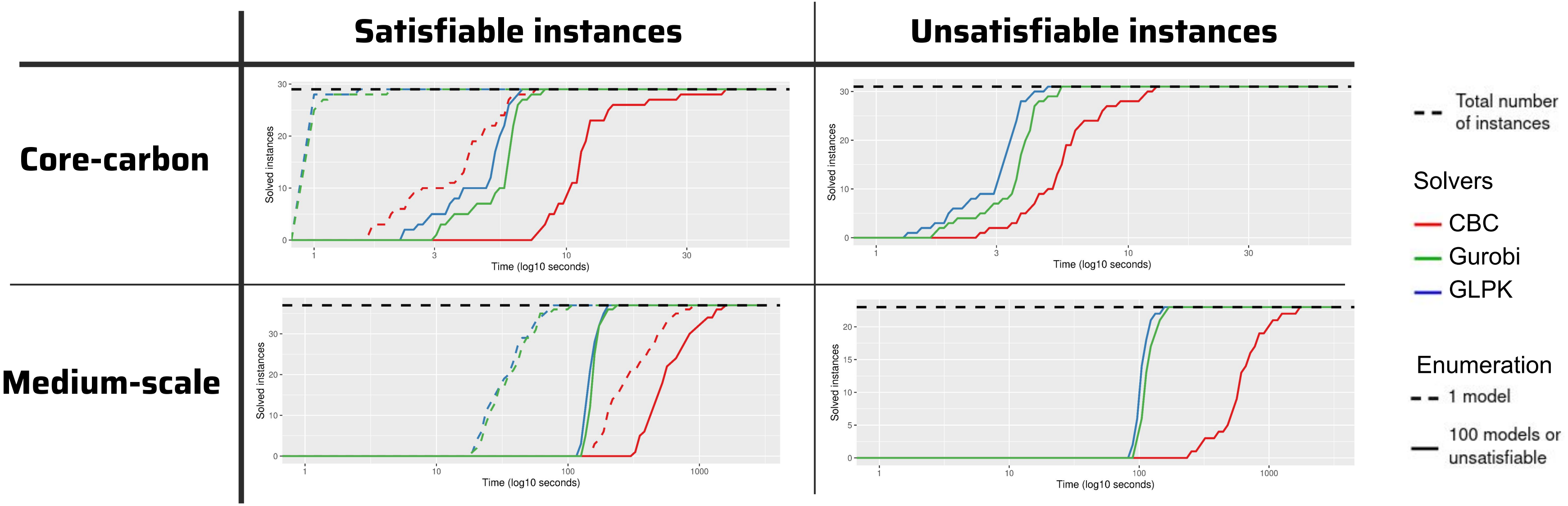
60 instances of the inference problem: transcriptomics, kinetics, and fluxomics
noise from 0% to 50%



CEGAR-based + partition: ~10 times faster than Clingo[lpx] + quantifier elimination

Impact of linear solvers

60 instances of the inference problem: transcriptomics, kinetics, and fluxomics noise from 0% to 50%



Satisfiable instances

Unsatisfiable instances

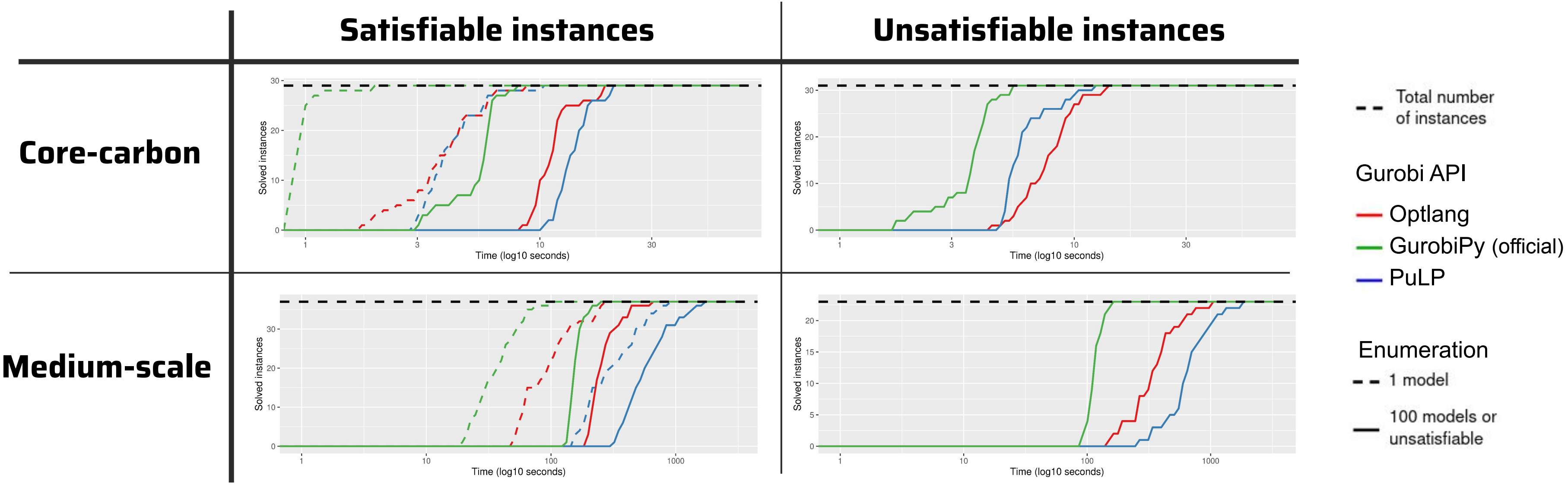
Core-carbon

Medium-scale

Choice of linear solvers impacts MerrinASP performance

Impact of linear solvers’ APIs

60 instances of the inference problem: transcriptomics, kinetics, and fluxomics noise from 0% to 50%



Not all linear solver APIs are adapted to successive solving and constraint updates

MerrinASP - Conflict generation

	Status	Solving methods	Number of calls to linear solvers	Number of refinements
Core-carbon	Satisfiable	Quantifier elimination	937 +/- 111	5 +/- 1
		CEGAR	**501 +/- 41**	6 +/- 1
	Unsatisfiable	Quantifier elimination	669 +/- 221	9 +/- 4
		CEGAR	**252 +/- 54**	9 +/- 4
Medium-scale	Satisfiable	Quantifier elimination	17 957 +/- 5 032	41 +/- 16
		CEGAR	**3 548 +/- 2 184**	21 +/- 11
	Unsatisfiable	Quantifier elimination	7 480 +/- 4 673	17 +/- 8
		CEGAR	**1 155 +/- 307**	13 +/- 3

CEGAR-based method reduce linear solver calls up to a factor of 7

How to maintain models in the long run?

Model checking:

Formally define the semantics of observations

Adapt MERRIN to identify spurious observations

Incremental model synthesis:

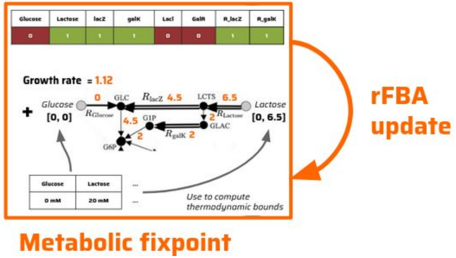
How to update a model to fit to new observations?

Knowledge representation: encoding of past observations

Formal methods: iterative model synthesis formalism

Operational research: scalable OMT solvers (multithreads[1]);
OMT problem partitioning[1,2,3]

For growth phenotype



Glucose	Lactose	lacZ	galK	LacI	GalR	R_lacZ	R_galK
0	1	1	1	0	0	1	1

Glucose	Lactose	...
0 mM	20 mM	...

***For* [Covert et al., 2004]:**

Could not process all observations at the same time → limits of nb of ASP variables

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[1] Kaufmann *et al.*, **AAAI**, 2016

[2] Tinos *et al.*, **ACM Conference on Foundations of Genetic Algorithms**, 2015

[3] Hyvärinen, *et al.*, **Springer Nature**, 2006