Learning Boolean controls in regulated metabolic networks: a case-study

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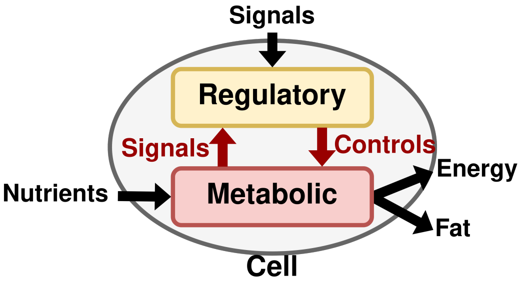
Multilayered structure

Context: Cells modelled as multi-layered structures



Focus

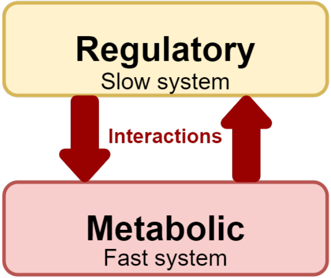
2 classes of processes

- Regulatory system
- Metabolic system

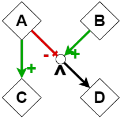
From simulation to learning

Multiplicity of formalisms

2 systems with 2 different dynamics



Discrete dynamics[1]



Model

$f_A(x_A, x_B, x_C, x_D) = x_A$

$f_B(x_A, x_B, x_C, x_D) = x_B$

$f_C(x_A, x_B, x_C, x_D) = x_A$

$f_D(x_A, x_B, x_C, x_D) = \neg x_A \wedge x_B$

Logical rules (Boolean network)

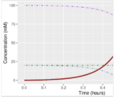
Inputs				Outputs			
x_A	x_B	x_C	x_D	x_A	x_B	x_C	x_D
0	0	x	x	0	0	0	0
0	1	x	x	0	1	0	1
1	0	x	x	1	0	1	0
1	1	x	x	1	1	1	0

Truth table (simulation)

Flow dynamics[2] *(MILP problem)*

$$\begin{aligned} \text{maximise} \quad & x_{\text{Growth}} \\ \text{such that:} \quad & S \cdot x = 0 \\ & l_r \cdot f_r \leq x_r \leq u_r \cdot f_r \qquad \forall r \in \mathcal{R} \end{aligned}$$

Regulatory flux balance analysis (rFBA)



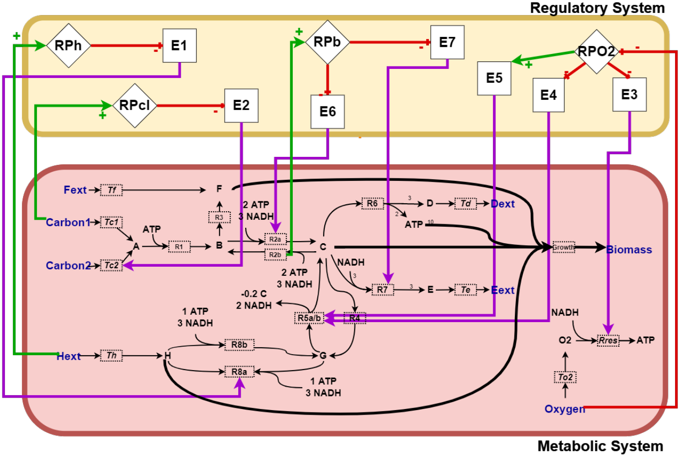
Metabolic traces

Bottleneck: coupling both system dynamics

[1] S. Videla et al., Bioinformatics, 2016

[2] M. W. Covert et al., Journal of theoretical biology, 2001

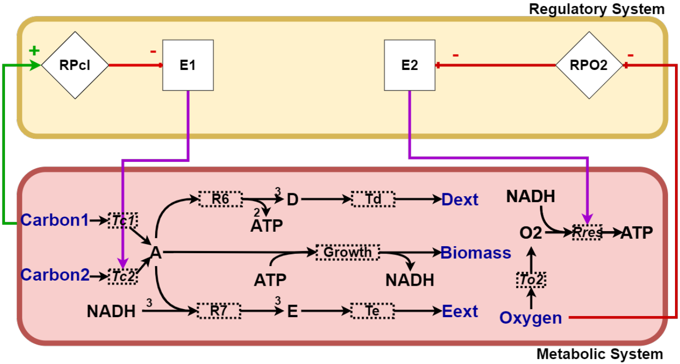
Our case study: Minitoy



Covert's regulated metabolic network of diauxic shift[1]

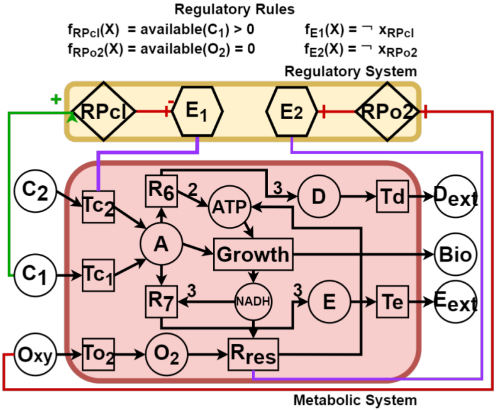
[1] M. W. Covert et al., Journal of theoretical biology, 2001

Our case study: Minitoy



Minitoy: simplified version of Covert's network

Formalism: regulated metabolic networks $\mathcal{N}=(\mathcal{M},\mathcal{R},\mathcal{S},f)$



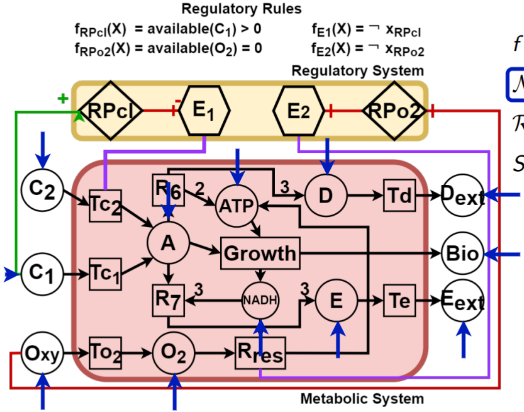
$f : \{0,1\}^n \rightarrow \{0,1\}^n$: regulatory rules

$\mathcal{M}$: metabolites

$\mathcal{R}$: chemical reactions

$\mathcal{S}$: stoichiometric matrix

Formalism: regulated metabolic networks $\mathcal{N}=(\mathcal{M},\mathcal{R},S,f)$



$f : \{0,1\}^n \rightarrow \{0,1\}^n$: regulatory rules

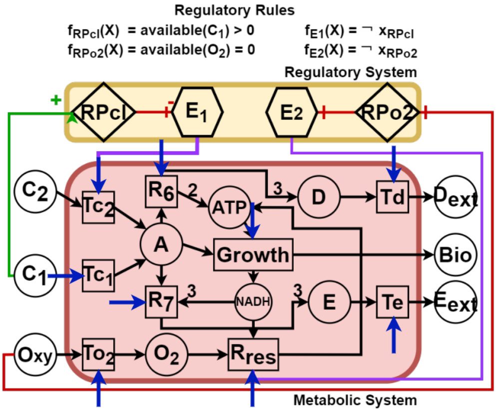
$\mathcal{M}$: metabolites

$\mathcal{R}$: chemical reactions

S: stoichiometric matrix

Metabolites = Chemical components

Formalism: regulated metabolic networks $\mathcal{N}=(\mathcal{M},\mathcal{R},S,f)$



$f : \{0,1\}^n \rightarrow \{0,1\}^n$: regulatory rules

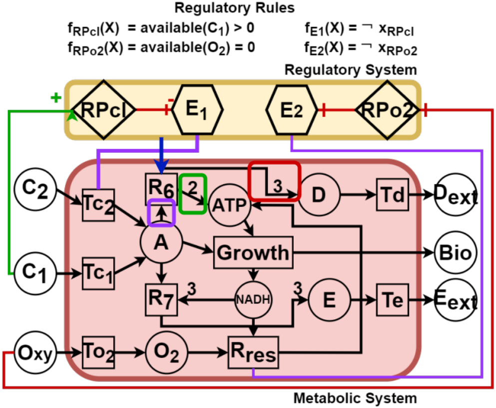
$\mathcal{M}$: metabolites

$\mathcal{R}$: chemical reactions

S: stoichiometric matrix

Chemical reactions are modelled using stoichiometric matrix

Formalism: regulated metabolic networks $\mathcal{N}=(\mathcal{M},\mathcal{R},S,f)$



$f:\{0,1\}^n \rightarrow \{0,1\}^n$: regulatory rules

$\mathcal{M}$: metabolites

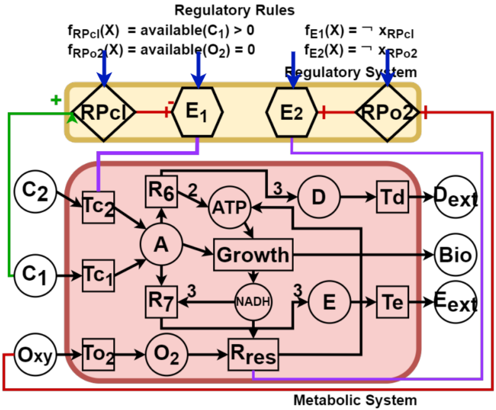
$\mathcal{R}$: chemical reactions

S: stoichiometric matrix

	T_{C1}	T_{C2}	T_{O2}	R_{res}	Growth	R_6	R_7	T_d	T_e
C1	−1	0	0	0	0	0	0	0	0
C2	0	−1	0	0	0	0	0	0	0
Oxy	0	0	−1	0	0	0	0	0	0
O2	0	0	1	−1	0	0	0	0	0
ATP	0	0	0	1	−1	2	0	0	0
NADH	0	0	0	−1	1	0	−3	0	0
A	1	1	0	0	−1	−1	−1	0	0
D	0	0	0	0	0	3	0	−1	0
E	0	0	0	0	0	0	3	0	−1
Dext	0	0	0	0	0	0	0	1	0
Eext	0	0	0	0	0	0	0	0	1
Bio	0	0	0	0	1	0	0	0	0

$$R_6: \quad 1 \cdot A \rightarrow 2 \cdot ATP + 3 \cdot D$$

Formalism: regulated metabolic networks $\mathcal{N}=(\mathcal{M},\mathcal{R},\mathcal{S},f)$



$f : \{0,1\}^n \rightarrow \{0,1\}^n$: regulatory rules

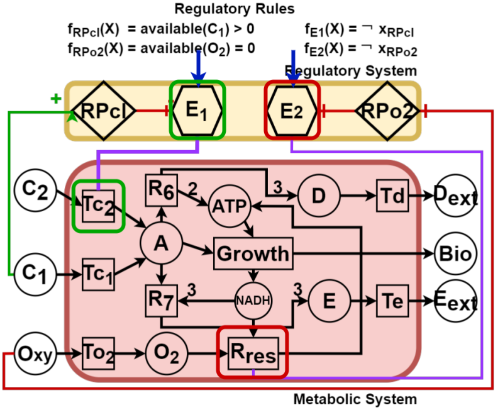
$\mathcal{M}$: metabolites

$\mathcal{R}$: chemical reactions

$\mathcal{S}$: stoichiometric matrix

Regulatory system contains: proteins + enzymes

Formalism: regulated metabolic networks $\mathcal{N}=(\mathcal{M},\mathcal{R},S,f)$



$f : \{0,1\}^n \rightarrow \{0,1\}^n$: regulatory rules

$\mathcal{M}$: metabolites

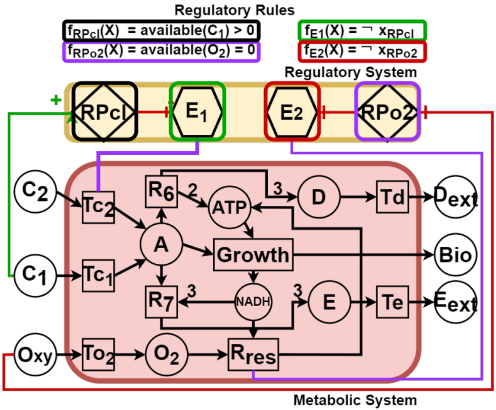
$\mathcal{R}$: chemical reactions

S: stoichiometric matrix

Enzymes control reactions

Regulatory system contains: proteins + enzymes

Formalism: regulated metabolic networks $\mathcal{N}=(\mathcal{M},\mathcal{R},S,f)$



$f : \{0,1\}^n \rightarrow \{0,1\}^n$: regulatory rules

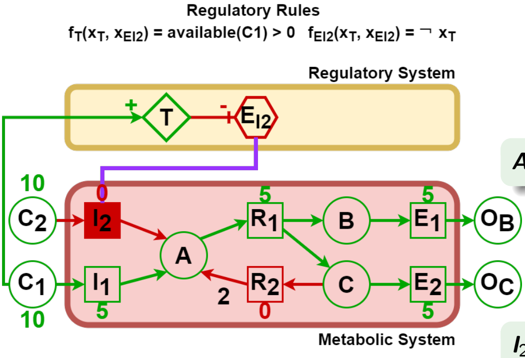
$\mathcal{M}$: metabolites

$\mathcal{R}$: chemical reactions

S: stoichiometric matrix

Regulatory system contains: proteins + enzymes

Formalism: metabolic steady states – MSS: $\boldsymbol{v} \in \mathbb{R}^{|\mathcal{R}|}$



Input and output fluxes are equals

$$\forall m \in \mathcal{M}, \sum_{\substack{r \in \mathcal{R} \\ S_{mr} > 0}} v_r = \sum_{\substack{r \in \mathcal{R} \\ S_{mr} < 0}} v_r \quad (1)$$

A: $v_{I_1} + v_{I_2} + 2 \times v_{R_2} = v_{R_1}$

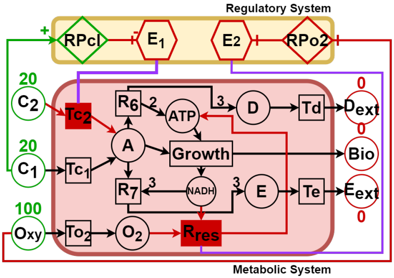
Inhibited reactions have a zero flux

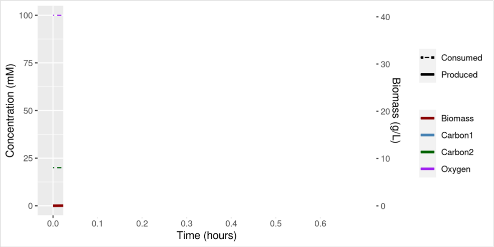
$$\forall r \in \mathcal{R}, x_{E_r} = 0 \implies v_r = 0 \quad (2)$$

I_2: $x_{E_{I_2}} = 0 \implies v_{I_2} = 0$

Steady-state assumption: no components are produced/consumed in excess

Dynamic regulatory flux balance analysis – d-rFBA

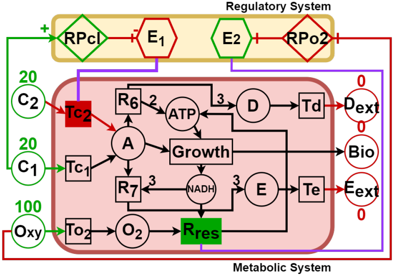


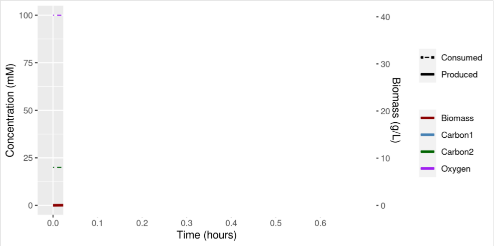


Iterating over the 3 steps:

1. Updating the regulatory system
2. Computing an optimal MSS
3. Updating the input/output

Dynamic regulatory flux balance analysis – d-rFBA





Iterating over the 3 steps:

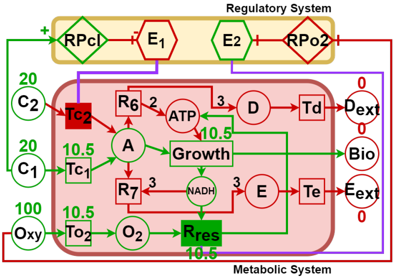
1. Updating the regulatory system
2. Computing an optimal MSS
3. Updating the input/output

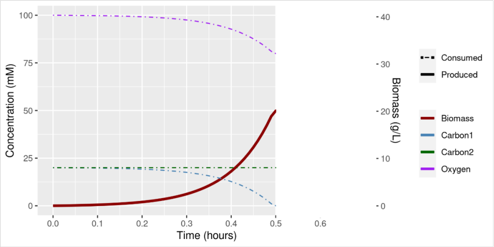
Applying synchronously the Boolean rules on previous regulatory state

$f_{\mathbf{RPcl}}(X) = \mathbf{available}(x_{C1}) > 0 \qquad f_{\mathbf{E1}}(X) = \neg x_{\mathbf{RPcl}}$

$f_{\mathbf{RPo2}}(X) = \mathbf{available}(x_{Oxy}) = 0 \qquad f_{\mathbf{E2}}(X) = \neg x_{\mathbf{RPo2}}$

Dynamic regulatory flux balance analysis – d-rFBA





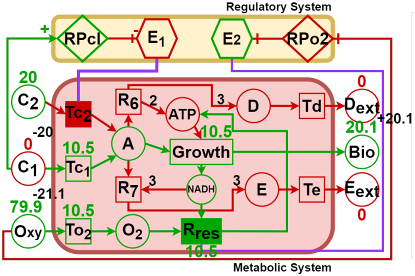
Iterating over the 3 steps:

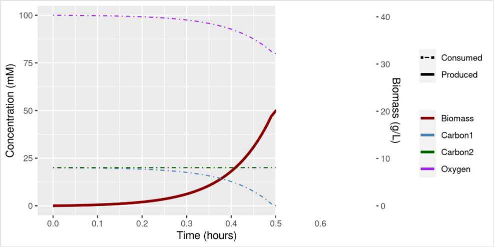
1. Updating the regulatory system
2. Computing an optimal MSS
3. Updating the input/output

Maximising an objective function

$$o(v) = v_{\text{Growth}}$$

Dynamic regulatory flux balance analysis – d-rFBA





Iterating over the 3 steps:

1. Updating the regulatory system
2. Computing an optimal MSS
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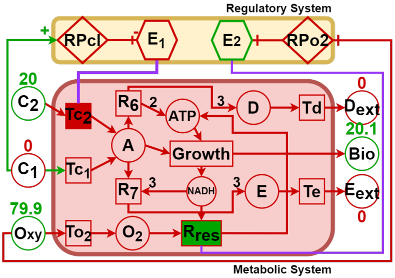
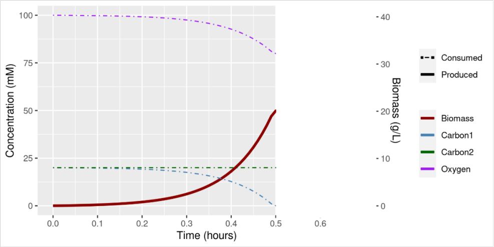
Wait that metabolites are consumed/produced before updating

Dynamic regulatory flux balance analysis – d-rFBA



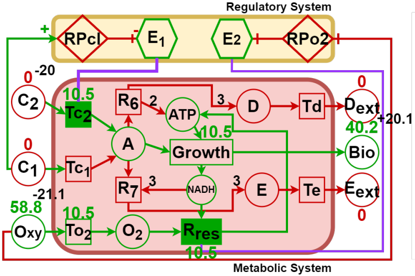


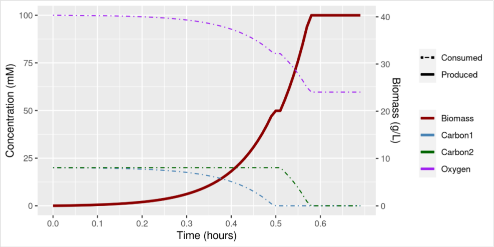
Iterating over the 3 steps:

1. Updating the regulatory system
2. Computing an optimal MSS
3. Updating the input/output

Repeat!

Dynamic regulatory flux balance analysis – d-rFBA





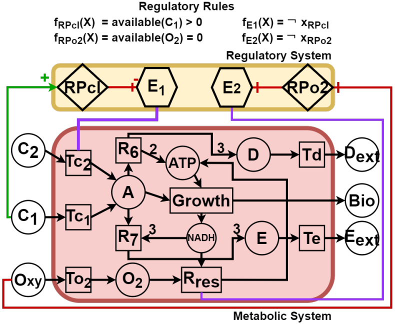
Iterating over the 3 steps:

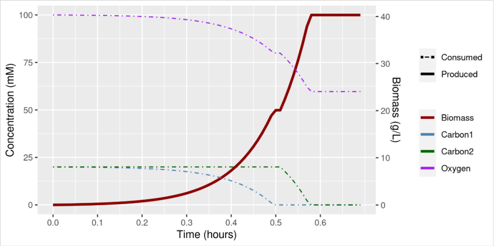
1. Updating the regulatory system
2. Computing an optimal MSS
3. Updating the input/output

Repeat!

d-rFBA: a scalable simulation framework

A coupled model can be simulated as soon as it is built





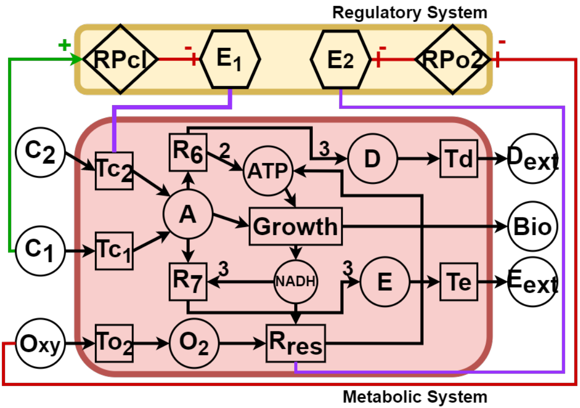
Model	Metabolic		Regulatory	
	Metabolites	Reactions	Regulatory proteins	Regulations
Toy	12	9	2	12
Covert[1]	19	20	4	20
E.coli – genome scale[2]	761	1 075	104	479

No scaling issues

[1] M. W. Covert et al., Journal of theoretical biology, 2001

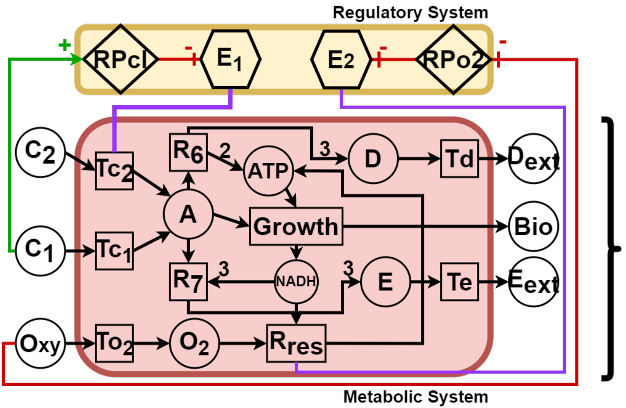
[2] M. W. Covert et al., Nature, 2004

Bottleneck: learning regulation rules of coupled systems



How to build regulatory metabolic networks ?

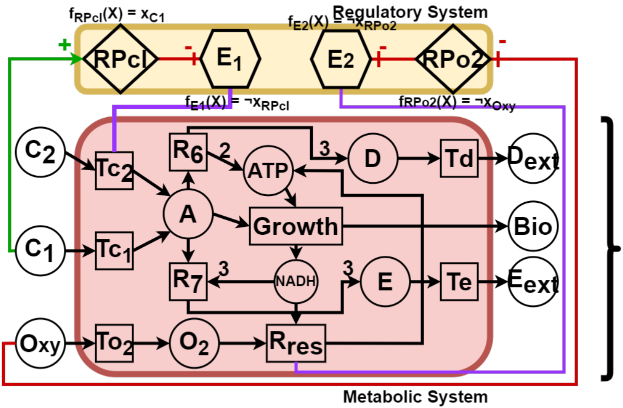
Bottleneck: learning regulation rules of coupled systems



Inferred through genome tagging

How to build regulatory metabolic networks ?

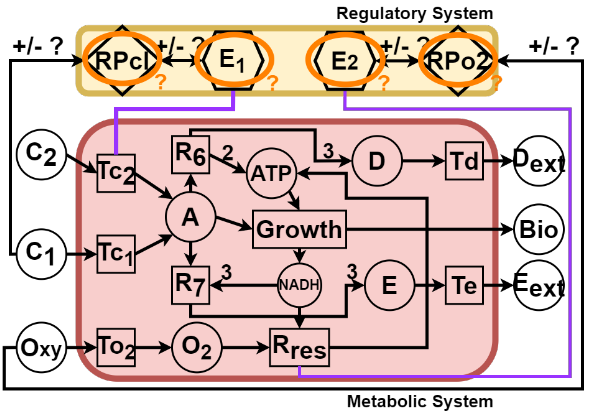
Bottleneck: learning regulation rules of coupled systems



Inferred through genome tagging

How to build regulatory metabolic networks ?

Bottleneck: learning regulation rules of coupled systems

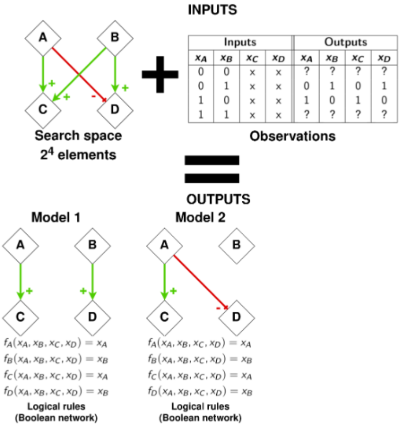


Manually inferred by researchers

Inferred through genome tagging

How to build regulatory metabolic networks ?

Reverse-engineering regulatory rules from observations



Inputs:

1. Observations
2. Domain constraints: finite set of usable interactions

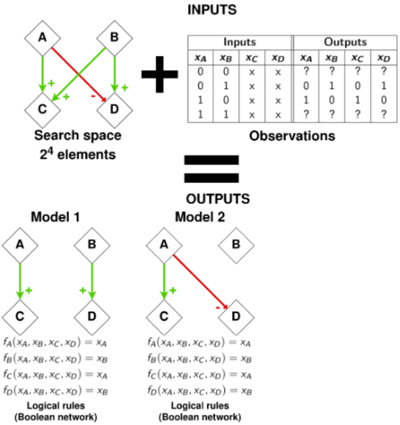
Search space: *all the Boolean networks respecting the domain constraints*

Outputs: sets of logical rules s.t.:

1. in the search domain
2. its simulations match the observations for a given semantics

Size of the search domain in $O(2^{2^n})$ with n number of domain constraints

Reverse-engineering regulatory rules from observations



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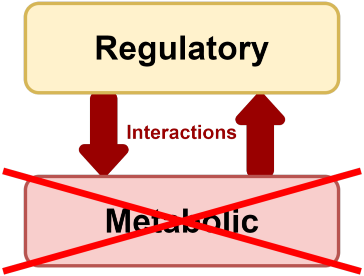
Size of the search domain in $O(2^{2^n})$ with n number of domain constraints

State of the art: learning regulatory systems

State of the art relies on logical programming and combinatorial problem formulation

ASP: declarative programming (1^{st} order logic + SAT-based solvers)



State of the art **only** learns regulatory systems from **Boolean traces of the regulatory system**

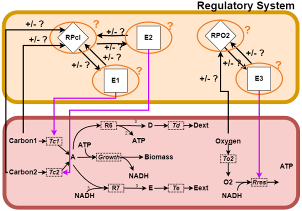
Examples: CASPO[1], CASPOTS[2] or BoNeSis[3]

1 S. Videla et al., Theoretical Computer Science, 2015

2 M. Ostrowski et al., BioSystems, 2016

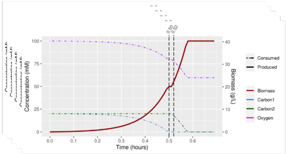
3 L. Paulevé et al., Nature Communications, 2020

Our issue: reverse-engineering coupled system from metabolic traces



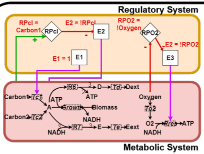
Search domain

+



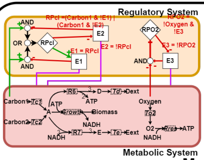
Traces of regulated metabolic network

=



RPcl = Carbon1
RPO2 = ¬Oxygen
E1 = 1
E2 = ¬RPcl
E3 = ¬RPO2

Model 1



RPcl = (Carbon1 ∧ ¬E1) ∨ (Carbon1 ∧ ¬E2)
RPO2 = ¬Oxygen ∧ ¬E3
E1 = RPcl
E2 = ¬RPcl
E3 = ¬RPO2

Model 2

Instance	Search domain size
Minitoy	1 944 320
Covert	2.9×10^{12}

Huge search space
Hybrid problem: combinatorial + linear

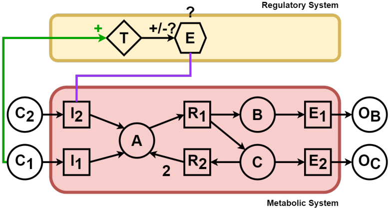
How to learn regulation rules from d-rFBA observations ?

Input metabolites:

$C_1 = C_2 = 5$

Objective function:

$o(v) = v_{E1}$



Observed value:

$V_{E1} = 5$

How to learn regulation rules from d-rFBA observations ?

Input metabolites:

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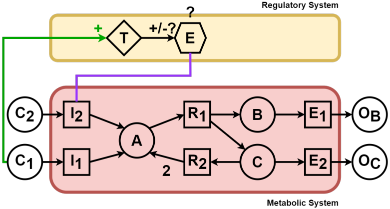
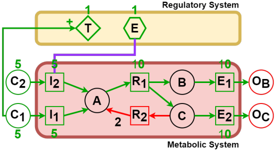
Objective function:

$o(v) = v_{E1}$



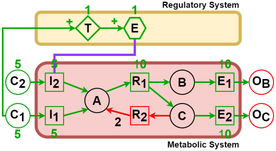
Observed value:

$V_{E1} = 5$



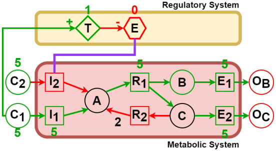
$f_E(x_T, x_E) = 1$

$v_{E1} = 10$



$f_E(x_T, x_E) = x_T$

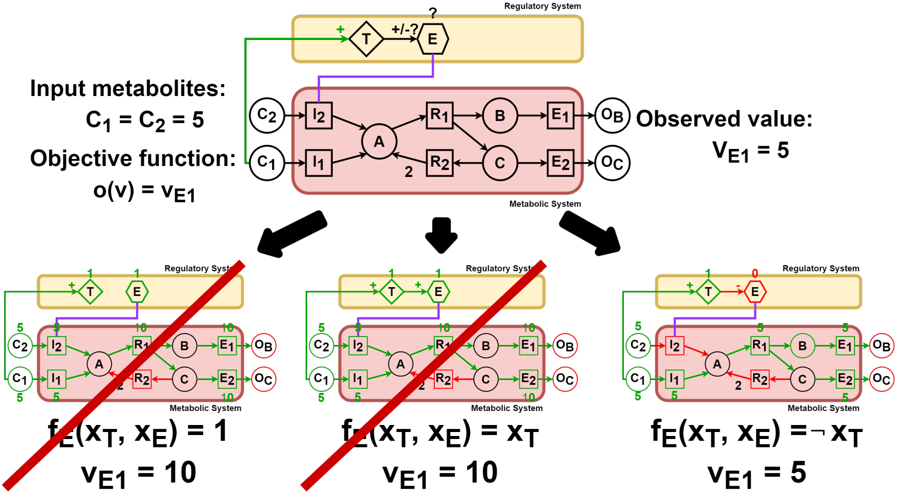
$v_{E1} = 10$



$f_E(x_T, x_E) = \neg x_T$

$v_{E1} = 5$

How to learn regulation rules from d-rFBA observations ?



According to the objective function maximisation assumption
$v_{E1} = 5$ **is observed** $\implies$ **the regulation rules do not allow to have** $v_{E1} > 5$

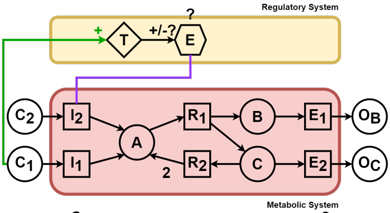
How to learn regulation rules from d-rFBA observations ?

Input metabolites:

$C_1 = C_2 = 5$

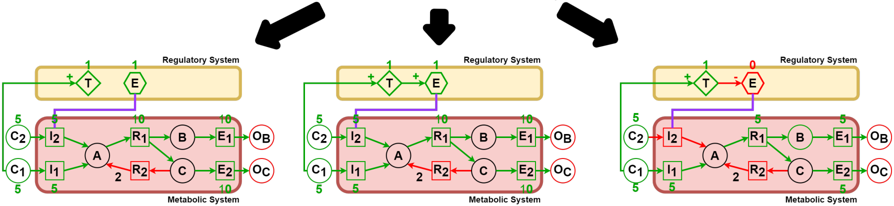
Objective function:

$o(v) = v_{I1}$



Observed value:

$V_{E1} = 5$



$f_E(x_T, x_E) = 1$

$v_{I1} = 5$

$f_E(x_T, x_E) = x_T$

$v_{I1} = 5$

$f_E(x_T, x_E) = \neg x_T$

$v_{I1} = 5$

The choice of objective function is really important

Formalisation of the inference problem – Definition

Inputs: metabolic network $\mathcal{N} = (\mathcal{M}, \mathcal{R}, S, l, u)$ + regulatory proteins $\mathcal{P}$ + search space $\mathbb{F}$ + time series T

Outputs: subset-minimal Boolean networks $f \in \mathbb{F}$ such that:

$$\forall (s, s') \in T, \forall (\hat{v}, \hat{w}, \hat{x}) \in \mathrm{rMSS}(\mathcal{N}, \mathcal{P}, f),$$
$$\hat{w}_{\mathrm{Inp}} = w'_{\mathrm{Inp}} \land \hat{x}_{\mathcal{P}} = x'_{\mathcal{P}} \implies \hat{v}_{\mathrm{Growth}} \leq v'_{\mathrm{Growth}}$$

Hybrid problem: combinatorial + linear

Formalisation of the inference problem – Definition

Inputs: metabolic network $\mathcal{N} = (\mathcal{M}, \mathcal{R}, S, l, u)$ + regulatory proteins $\mathcal{P}$ + search space $\mathbb{F}$ + time series T

Combinatorial part

Outputs: subset-minimal Boolean networks $f \in \mathbb{F}$ such that:

Linear part

$$\forall (s, s') \in T, \forall (\hat{v}, \hat{w}, \hat{x}) \in \text{rMSS}(\mathcal{N}, \mathcal{P}, f),$$
$$\hat{w}_{\text{Inp}} = w'_{\text{Inp}} \wedge \hat{x}_{\mathcal{P}} = x'_{\mathcal{P}} \implies \hat{v}_{\text{Growth}} \leq v'_{\text{Growth}}$$

Hybrid problem: combinatorial + linear

Formalisation of the inference problem – Definition

Inputs: metabolic network $\mathcal{N} = (\mathcal{M}, \mathcal{R}, S, l, u)$ + regulatory proteins $\mathcal{P}$ + search space $\mathbb{F}$ + time series T

Combinatorial part

Outputs: subset-minimal Boolean networks $f \in \mathbb{F}$ such that:

Combinatorial optimisation

Linear part

$$\forall (s, s') \in T, \forall (\hat{v}, \hat{w}, \hat{x}) \in \text{rMSS}(\mathcal{N}, \mathcal{P}, f),$$
$$\hat{w}_{\text{Inp}} = w'_{\text{Inp}} \wedge \hat{x}_{\mathcal{P}} = x'_{\mathcal{P}} \implies \hat{v}_{\text{Growth}} \leq v'_{\text{Growth}}$$

Linear optimisation

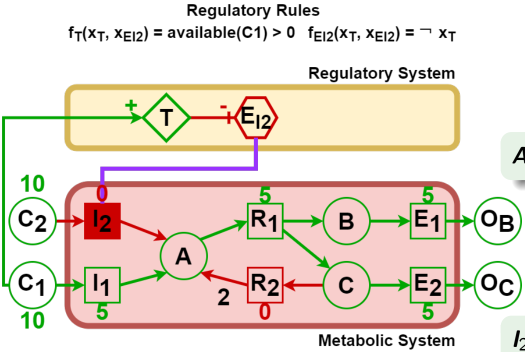
Hybrid problem: combinatorial + linear

Contributions on the inferring of regulatory rules

1. Boolean abstraction of the d-rFBA framework
2. Relaxation of the inferring problem as a combinatorial problem
3. ASP based resolution scheme for the relaxed problem

Solve the inferring problem as a purely combinatorial problem

Metabolic steady states – MSS: $v \in \mathbb{R}^{|\mathcal{R}|}$



Input and output fluxes are equals

$$\forall m \in \mathcal{M}, \sum_{\substack{r \in \mathcal{R} \\ S_{mr} > 0}} v_r = \sum_{\substack{r \in \mathcal{R} \\ S_{mr} < 0}} v_r \quad (1)$$

***A*:** $v_{I_1} + v_{I_2} + 2 \times v_{R_2} = v_{R_1}$

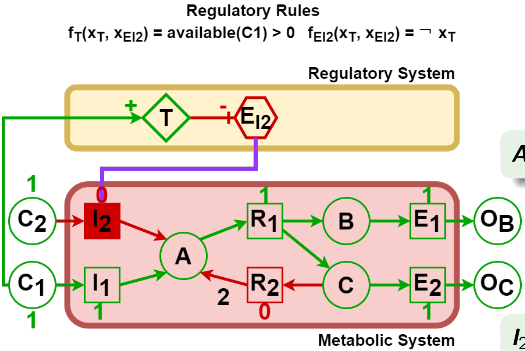
Inhibited reactions have a zero flux

$$\forall r \in \mathcal{R}, x_{E_r} = 0 \implies v_r = 0 \quad (2)$$

***I*₂:** $x_{E_{I_2}} = 0 \implies v_{I_2} = 0$

Abstracting elements according to whether they are present or not

Boolean metabolic steady states – $MSS^{\mathbb{B}}$: $\boldsymbol{v} \in \{0,1\}^{|\mathcal{R}|}$



***m* ∈ $\mathcal{M}$ is produced iff it is consumed**

$$\forall m \in \mathcal{M}, \bigvee_{\substack{r \in \mathcal{R} \\ S_{mr} > 0}} v_r \iff \bigvee_{\substack{r \in \mathcal{R} \\ S_{mr} < 0}} v_r \quad (1)$$

***A*:** $v_{I_1} \vee v_{I_2} \vee v_{R_2} \iff v_{R_1}$

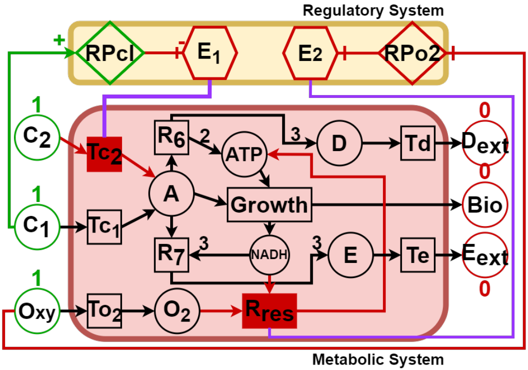
Inhibited reactions have a zero flux

$$\forall r \in \mathcal{R}, x_{E_r} = 0 \implies v_r = 0 \quad (2)$$

***I_2*:** $x_{E_{I_2}} = 0 \implies v_{I_2} = 0$

Abstracting elements according to whether they are present or not

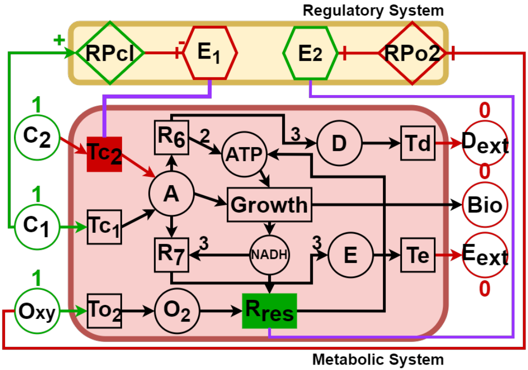
Boolean abstraction of d-rFBA



Iterating over the 3 steps:

1. Updating the regulatory system
2. Computing an optimal $MSS^{\mathbb{B}}$
3. Updating the input/output

Boolean abstraction of d-rFBA



Iterating over the 3 steps:

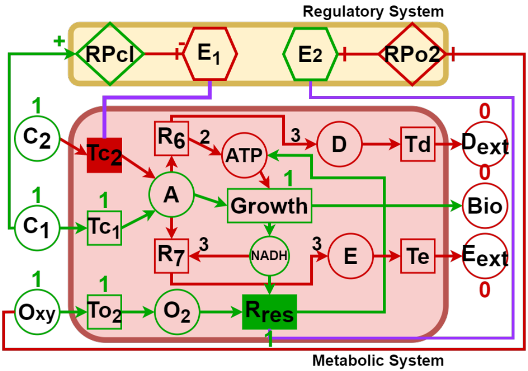
1. Updating the regulatory system
2. Computing an optimal $MSS^{\mathbb{B}}$
3. Updating the input/output

Applying synchronously the Boolean rules on previous regulatory state

$f_{\mathbf{RPcl}}(X')=x'_{C1}$ $\quad f_{\mathbf{E1}}(X')=\neg x'_{\mathbf{RPcl}}$

$f_{\mathbf{RPo2}}(X')=\neg x'_{Oxy}$ $\quad f_{\mathbf{E2}}(X')=\neg x'_{\mathbf{RPo2}}$

Boolean abstraction of d-rFBA



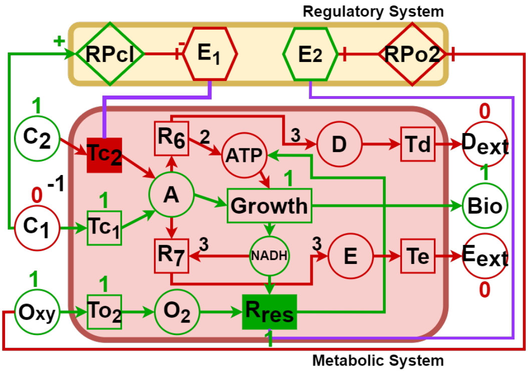
Iterating over the 3 steps:

1. Updating the regulatory system
2. Computing an optimal $MSS^{\mathbb{B}}$
3. Updating the input/output

Maximising an objective function

$$o(v) = x_{\mathbf{Tc1}} + x_{\mathbf{Tc2}} + \mathbf{To2}$$

Boolean abstraction of d-rFBA

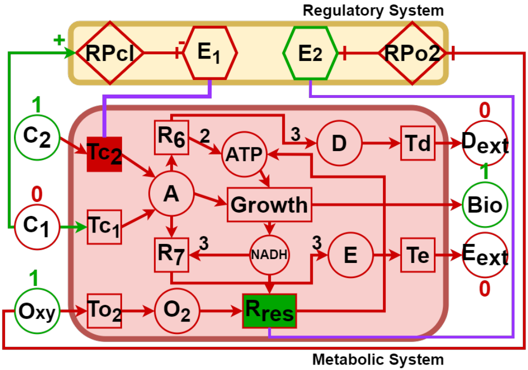


Iterating over the 3 steps:

1. Updating the regulatory system
2. Computing an optimal MSS$^{\mathbb{B}}$
3. Updating the input/output

Wait that metabolites are consumed/produced before updating

Boolean abstraction of d-rFBA

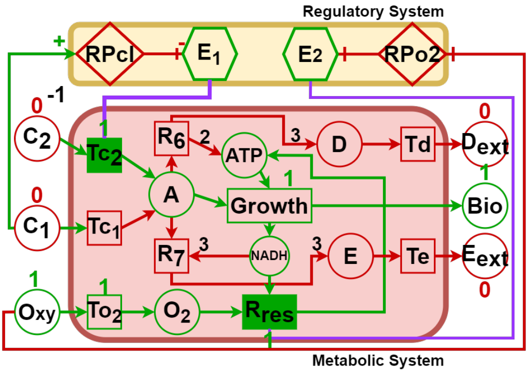


Iterating over the 3 steps:

1. Updating the regulatory system
2. Computing an optimal $MSS^{\mathbb{B}}$
3. Updating the input/output

Repeat!

Boolean abstraction of d-rFBA



Iterating over the 3 steps:

1. Updating the regulatory system
2. Computing an optimal $MSS^{\mathbb{B}}$
3. Updating the input/output

Repeat!

Boolean relaxation of the inference problem – Definition

Inputs: metabolic network $\mathcal{N} = (\mathcal{M}, \mathcal{R}, S, l, u)$ + regulatory protein $\mathcal{P}$ + search space $\mathbb{F}$ + time series $T^{\mathbb{B}}$ + objective function $\hat{o}$

Outputs: all the subset-minimal Boolean networks $f \in \mathbb{F}$ such that:

$$
\begin{aligned}
&\exists f \in \mathbb{F}, \forall (t_1, t_2) \in T^{\mathbb{B}},\\
&\qquad \exists x \in \mathrm{MSS}^{\mathbb{B}}(\mathcal{N}, t_2),\ x \preceq f(t_1),\\
&\qquad \forall y \in \mathrm{MSS}^{\mathbb{B}}(\mathcal{N}, t_2),\ y \not\preceq f(t_1) \lor \hat{o}(y) \leq \hat{o}(x)
\end{aligned}
$$

where $\forall x, y \in \mathbb{B}^n, x \preceq y \iff \forall i \in \{1, \cdots, n\}, x_i \leq y_i$
and $MSS^{\mathbb{B}}(\mathcal{N}, t)$ is the set of admissible metabolic state of $\mathcal{N}$ at time t

2-QBF problem $\Rightarrow$ Σ_2^P-complete[1]

[1] T. Eiter and G. Gottlob, Annals of Mathematics and Artificial Intelligence, 1995

Boolean relaxation of the inference problem – Definition

Inputs: metabolic network $\mathcal{N} = (\mathcal{M}, \mathcal{R}, S, l, u)$ + regulatory protein $\mathcal{P}$ + search space $\mathbb{F}$ + time series $T^{\mathbb{B}}$ + objective function $\hat{o}$

Outputs: all the subset-minimal Boolean networks $f \in \mathbb{F}$ such that:

$$\exists f \in \mathbb{F}, \forall (t_1, t_2) \in T^{\mathbb{B}},$$
$$\exists x \in \mathrm{MSS}^{\mathbb{B}}(\mathcal{N}, t_2),\ x \preceq f(t_1),$$
$$\forall y \in \mathrm{MSS}^{\mathbb{B}}(\mathcal{N}, t_2),\ y \npreceq f(t_1) \vee \hat{o}(y) \leq \hat{o}(x)$$

2-QBF form

$\exists x, \forall y, \Phi(x, y)$

where $\forall x, y \in \mathbb{B}^n,\ x \preceq y \iff \forall i \in \{1, \cdots, n\}, x_i \leq y_i$
and $MSS^{\mathbb{B}}(\mathcal{N}, t)$ is the set of admissible metabolic state of $\mathcal{N}$ at time t

2-QBF problem $\Rightarrow \Sigma_2^{\mathrm{P}}$-complete[1]

[1] T. Eiter and G. Gottlob, Annals of Mathematics and Artificial Intelligence, 1995

Answer Set Programming – ASP

Answer Set Programming – ASP[1]

```
b :- a. # Rule
a :-. # Fact
:- c. # Integrity rule
```

where *a*, *b*, *c* are atoms

Only solution: $\{a, b\}$

- Declarative framework
- Based on first-order logic predicates
  - Rules have the shape: <head> :- <body>
- Use to solve 2-QBF problems
  - Saturation technique[2]

Declarative framework allowing solving combinatorial satisfaction problems

[1] C. Baral, Cambridge University Press, 2003

[2] M. Gesber, Theory and Practice of Logic Programming, 2011

Modelling of the relaxed problem with ASP

```
{clause(N,1..C,L,S): in(L,N,S), maxC(N,C), node(N)}.

:- clause(N,_,L,S), clause(N,_,L,-S).
1 {constant(N,(-1;1)) } 1 :- node(N), not clause(N,_,_,_).
constant(N) :- constant(N,_).

size(N,C,X) :- X = #count{L, S: clause(N,C,L,S)}, clause(N,C,_,_).
:- clause(N,C,_,_), not clause(N,C-1,_,_), C > 1.
:- size(N,C1,X1), size(N,C2,X2), X1 < X2, C1 > C2.
clausediff(N,C1,C2,L) :- clause(N,C1,L,_), not clause(N,C2,L,_), clause(N,C2,_,_),
                         C1 != C2.
mindiff(N,C1,C2,L) :- clausediff(N,C1,C2,L),
                      L <= L': clausediff(N,C1,C2,L'), clause(N,C1,L',_), C1!=C2.
:- size(N,C1,X), size(N,C2,X), C1 > C2,
   mindiff(N,C1,C2,L1), mindiff(N,C2,C1,L2), L1 < L2.
:- size(N,C1,X1), size(N,C2,X2), C1 != C2, X1 <= X2,
   clause(N,C2,L,S): clause(N,C1,L,S).

update(T1,A) :- mode(T1,reg), node(A), not inp(A,_).
mode(T1,reg) :- next(T1,_).

constant(A,-1) :- inp(A,_).
:- constant(A), not inp(A,_).

eval(T,A,C,-1) :- update(T,A), clause(A,C,L,V), read(T,L,-V).
eval(T,A,C,1) :- read(T,L,V): clause(A,C,L,V); update(T,A), clause(A,C,_,_).
eval(T,A,1) :- eval(T,A,C,1), clause(A,C,_,_).
eval(T,A,-1) :- eval(T,A,C,-1): clause(A,C,_,_); update(T,A), clause(A,C,_,_).
eval(T,A,V) :- update(T,A), constant(A,V).

w(T2,A,V) :- inp(A,_), next(_,T2), obs(T2,A,V).
w(T2,A,V) :- next(T1,T2), not inp(A,_), not update(T1,A), v(T1,A,V).
w(T2,A,V) :- next(T1,T2), update(T1,A), eval(T1,A,V).

read(T,A,V) :- next(T,_), not inp(A,_), v(T,A,V).
read(T,A,V) :- next(T,T2), inp(A,_), obs(T2,A,V).

inp(X,R) :- reactant(X,R), not product(X,_).
r(r,A,R) :- reactant(A,R), product(A,_). r(p,A,R) :- product(A,R), reactant(A,_).
varm(A) :- r(_,A,_). varm(A) :- r(_,_,A). varm(A) :- inp(A,_).
time(T1) :- next(T1,_). time(T2) :- next(_,T2).

1 { v(T,A,(1;-1)) } 1 :- time(T), varm(A).
:- obs(T,A,V), v(T,A,-V).
:- time(T), r(S,A,_), v(T,A,1), v(T,R,-1): r(S,A,R).
:- time(T), r(_,A,R), v(T,R,1), v(T,A,-1).
:- time(T), inp(X,R), v(T,X,-1), v(T,R,1).

varx(A) :- node(A), not varm(A).
1{v(T,A,(-1;1))}1 :- varx(A), time(T).
:- varx(A), w(T,A,V), v(T,A,-V).
:- w(T,A,-1), v(T,A,1), node(A).

z(T,A,1);z(T,A,-1) :- time(T), varm(A).

no_rmss(T) :- inp(A,_), v(T,A,V), z(T,A,-V).
no_rmss(T) :- time(T), r(S,A,_), z(T,A,1), z(T,R,-1): r(S,A,R).
no_rmss(T) :- time(T), r(_,A,R), z(T,R,1), z(T,A,-1).
no_rmss(T) :- time(T), inp(X,R), z(T,X,-1), z(T,R,1).

no_rmss(T):- varx(A), w(T,A,V), z(T,A,-V).
n_rmss(T) :- w(T,A,-1), z(T,A,1), node(A).

valid(T) :- time(T), no_rmss(T).
valid(T) :- time(T), score(T,v,V), score(T,o,O), V <= O.

z(T,A,-V) :- time(T), varm(A), z(T,A,V), valid(T).
:- next(_, T), time(T), not valid(T).

#show.
#show clause/4.
```

Boolean network dynamics[1]

Observed Boolean metabolic steady state

Optimal Boolean metabolic steady state

Relaxed problem solved with ASP

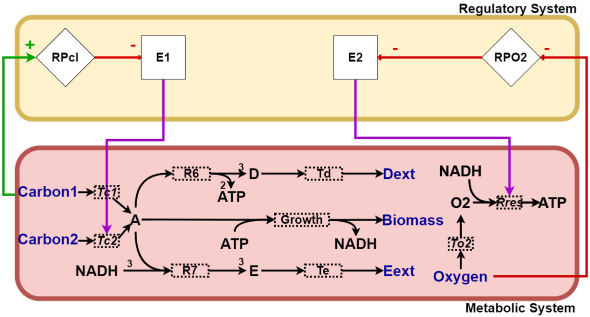
Model composed of 3 parts:

1. Boolean network discrete dynamics[1]
2. Boolean metabolic steady states
3. Computing the optimal Boolean metabolic steady states

– 2-QBF part –

[1] S. Chevalier et al., International Conference on Tools with Artificial Intelligence, 2019

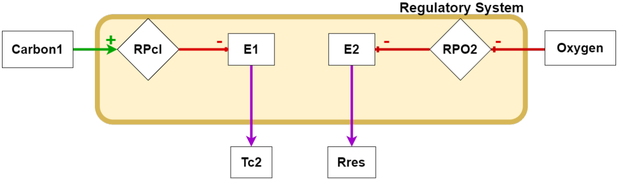
Minitoy: based on Covert's regulated metabolic network[1]



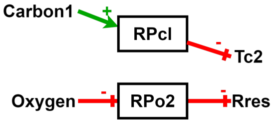
Our case study: Minitoy

[1] M. W. Covert et al., Journal of theoretical biology, 2001

Minitoy: based on Covert's regulated metabolic network[1]



Extended representation

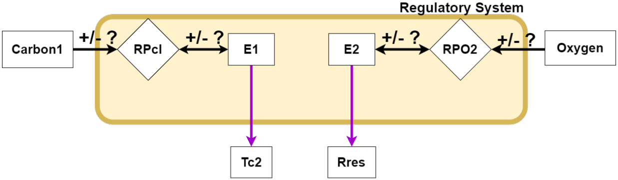


Simplified representation

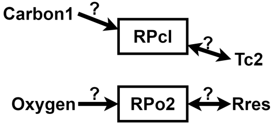
Set of regulations that must be retrieved

[1] M. W. Covert et al., Journal of theoretical biology, 2001

Minitoy: based on Covert's regulated metabolic network[1]



Extended representation

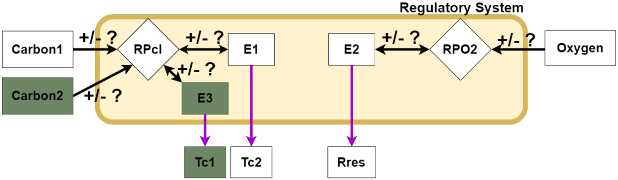


Simplified representation

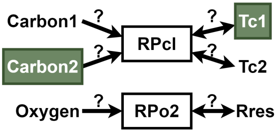
Remove the direction and the sign of each regulation

[1] M. W. Covert et al., Journal of theoretical biology, 2001

Minitoy: based on Covert's regulated metabolic network[1]



Extended representation



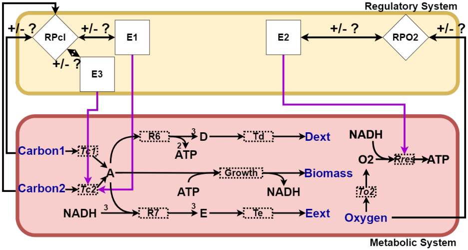
Simplified representation

Extend the search space by adding new domain constraints

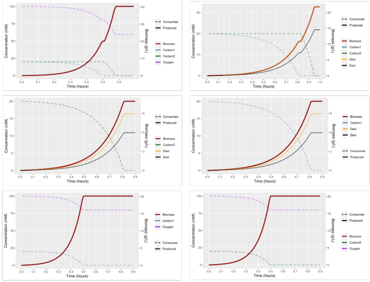
[1] M. W. Covert et al., Journal of theoretical biology, 2001

Instance of the relaxed problem

The 6 input simulations adapt from the litterature[1]



+



Boolean objective function $\hat{o}(v) = \sum_{r \in \text{Inputs}} v_r$

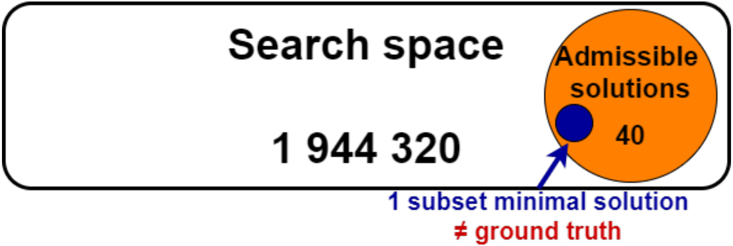
Search space contains 1 944 320 elements

[1] M. W. Covert et al., Journal of theoretical biology, 2001

Exact resolution of the relaxed problem

Exact resolution of the relaxed problem !

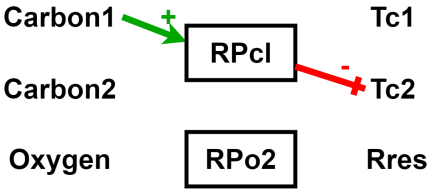
All the solutions have been found and enumerated



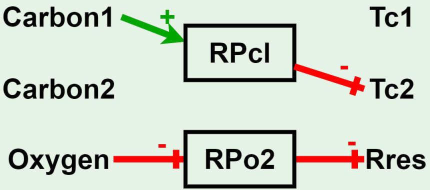
From 1 944 320 elements, 40 has been inferred
1 of which is subset minimal

Subset minimal results of the relaxed problem

40 admissible BNs of which 1 is subset minimal



Subset minimal BN



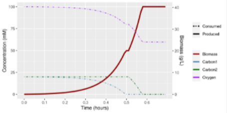
Ground truth BN

Subset minimal model is smaller than the ground truth model
Confirm an assumption made in the litterature[1]

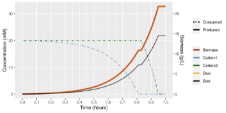
[1] M. W. Covert et al., Journal of theoretical biology, 2001

Results validation with respect to the hybrid problem

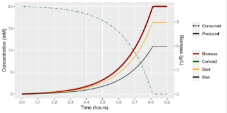
Results are validated by reproducing the input simulations



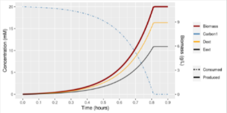
Experiment 1



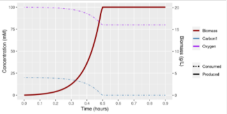
Experiment 2



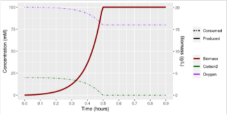
Experiment 3



Experiment 4



Experiment 5



Experiment 6

Subset minimal model allows retrieving the input simulations

Solution to **Hybrid and Combinatorial** problems

Conclusion: inferring regulatory rules from metabolic traces

Advantages:

1. Relaxation of the inferring problem as a combinatorial problem
2. Scale to bigger instances (ex: full Covert's model)
3. Correctly infer ground truth models
   - Find smaller models explaining the input data

Disadvantages:

1. Boolean d-rFBA leads to false negatives/positives results
2. Boolean objective functions are manually defined
   - **Futur works:** explore hybrid solving frameworks as SMT solvers[1]
3. Enzymatic and proteins costs are not considered
   - **Future works:** rely on regulatory dynamic enzyme-cost FBA framework[2]

[1] R. Kaminski et al., arXiv, 2020

[2] L. Liu et al., Journal of Theoretical Biology, 2020

References I

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