How to learn metabolic regulation rules from time series data?

__MERRIN:__ MEtabolic Regulation Rules INference from time series data

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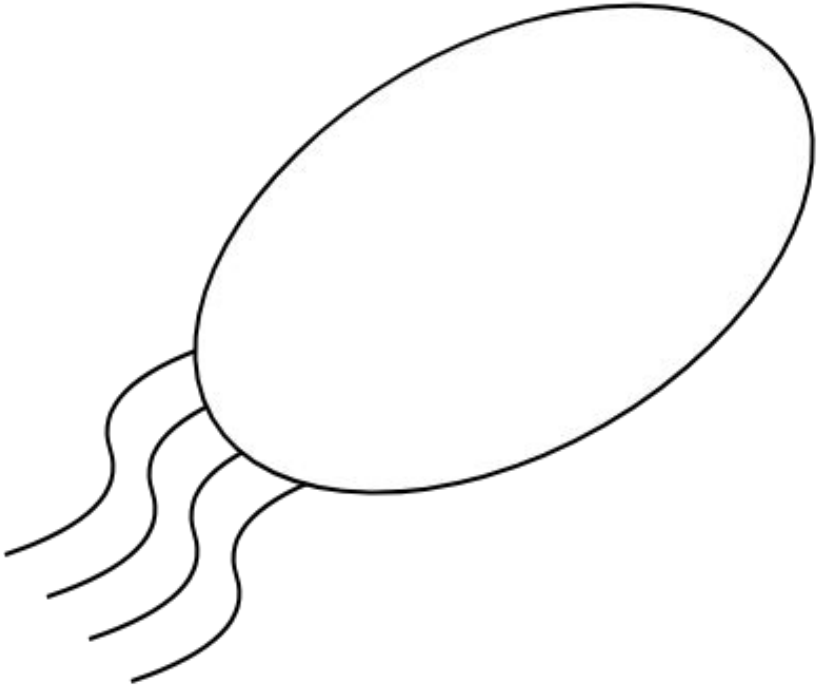
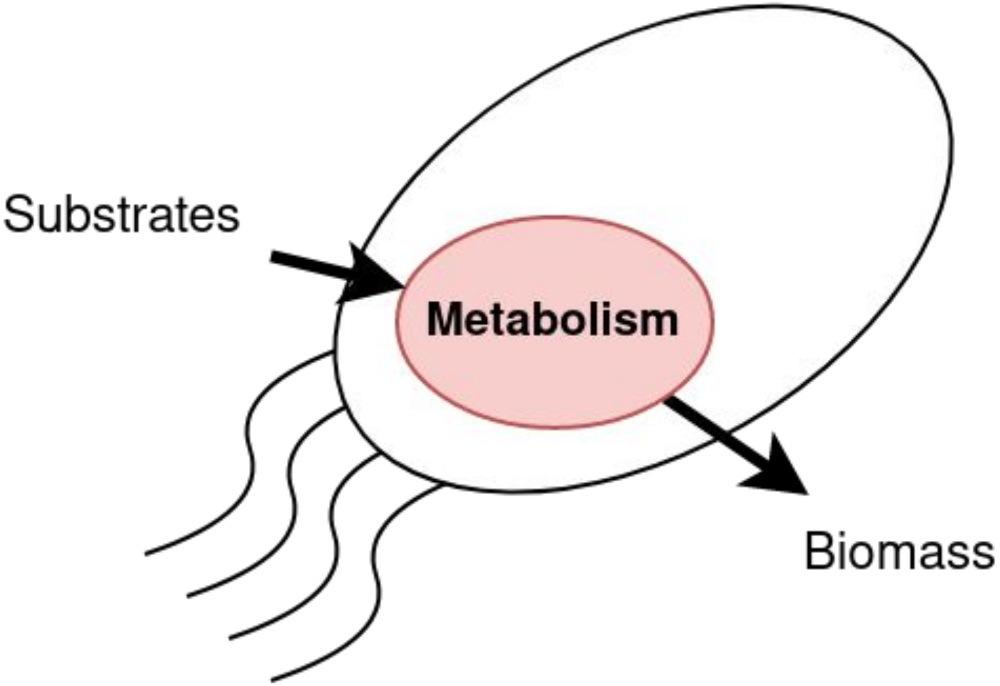
19th September 2022

Cells: hybrid multi-layered structures



Model as two interconnected systems

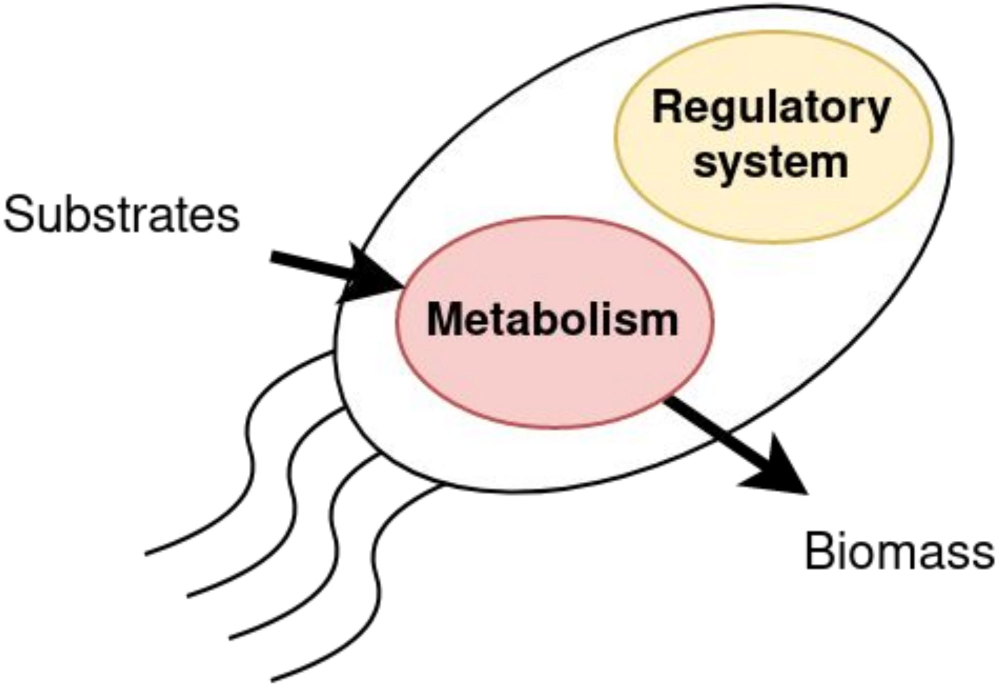
Cells: hybrid multi-layered structures



Model as two interconnected systems

1. **Metabolic system**
   Chemicals reactions converting substrates to energy and biomass

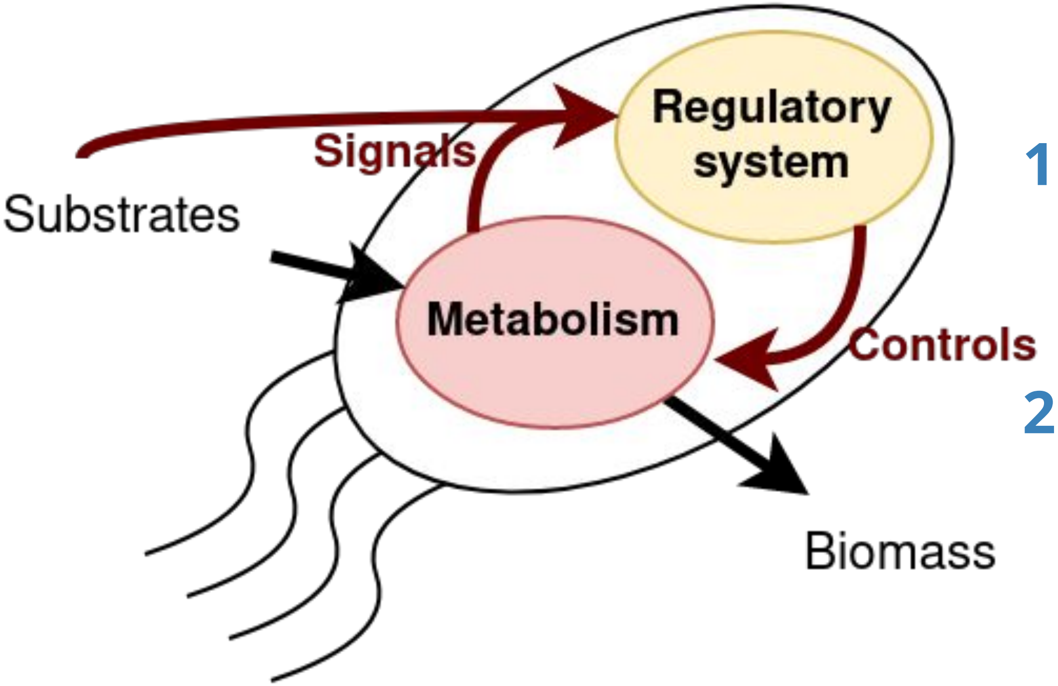
Cells: hybrid multi-layered structures



Model as two interconnected systems

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

Cells: hybrid multi-layered structures



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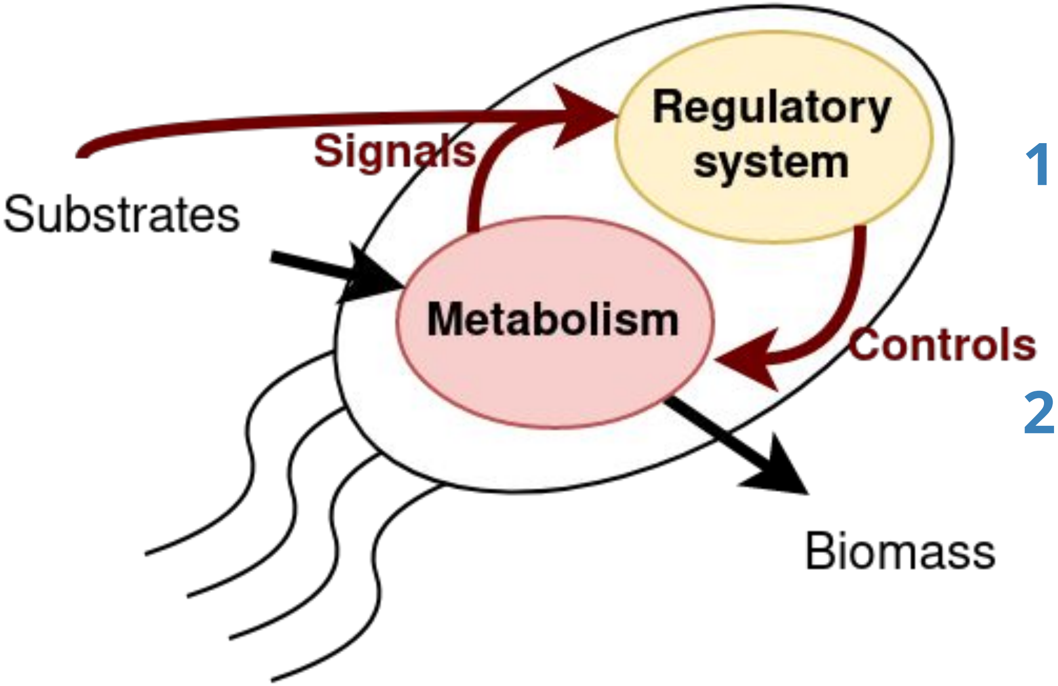


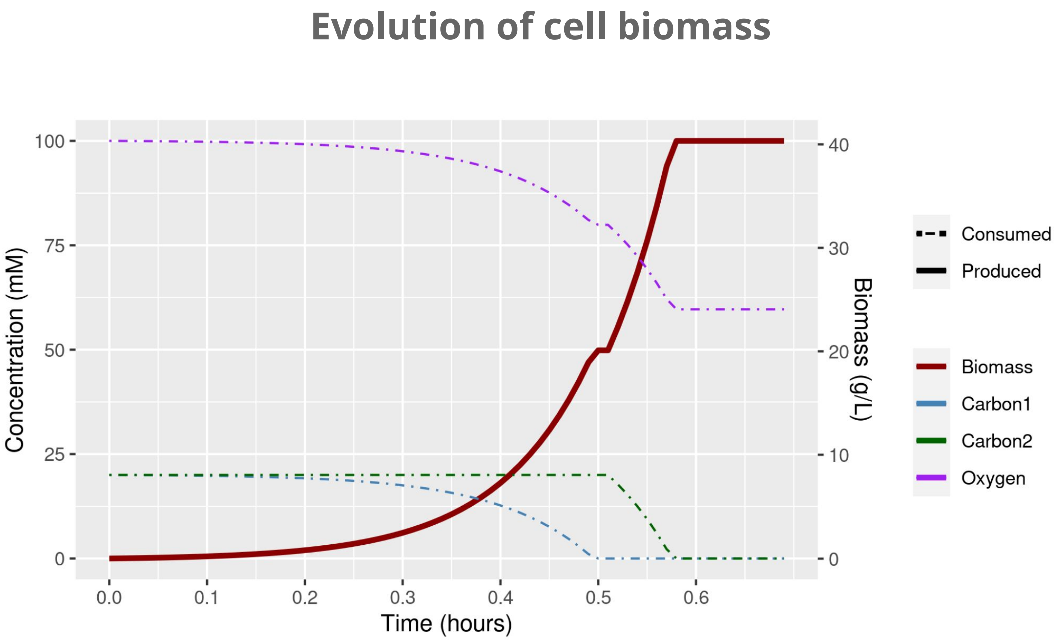
Model as two interconnected systems

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

Objective:

Inferring the **regulatory system** from time series observations of the cells *(metabolism and regulation)*

Example: diauxic shift *(Monod et al., 1953)*

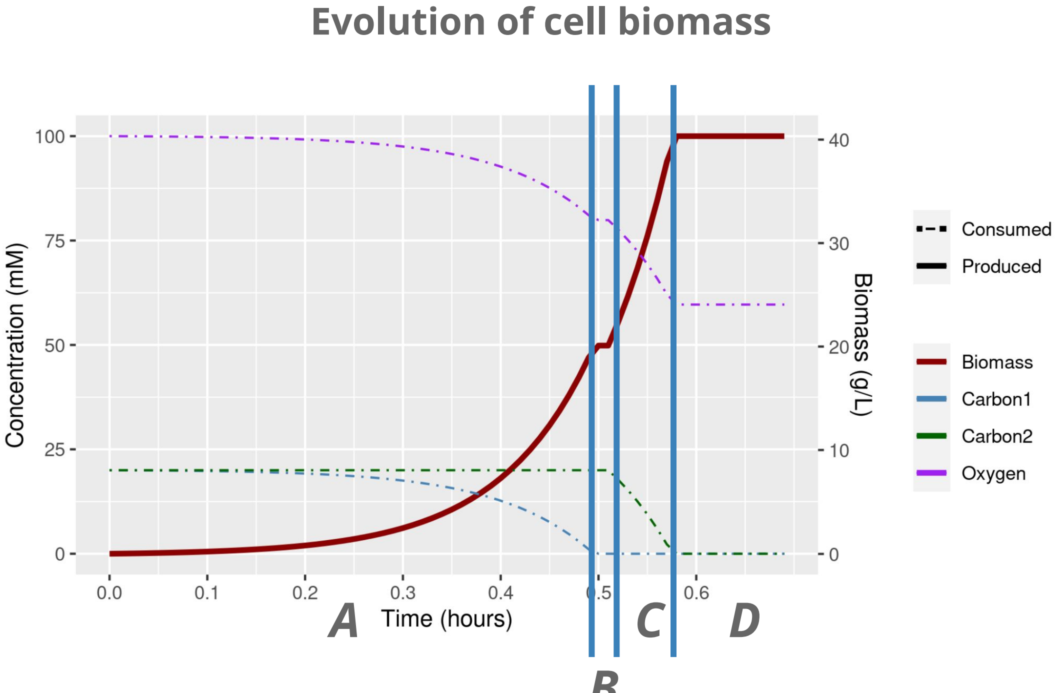


Diauxic shift

→ *Successive growth phases on different mediums*

→ *Control by regulations*

Example: diauxic shift *(Monod et al., 1953)*



Diauxic shift

⟶ Successive growth phases on different mediums

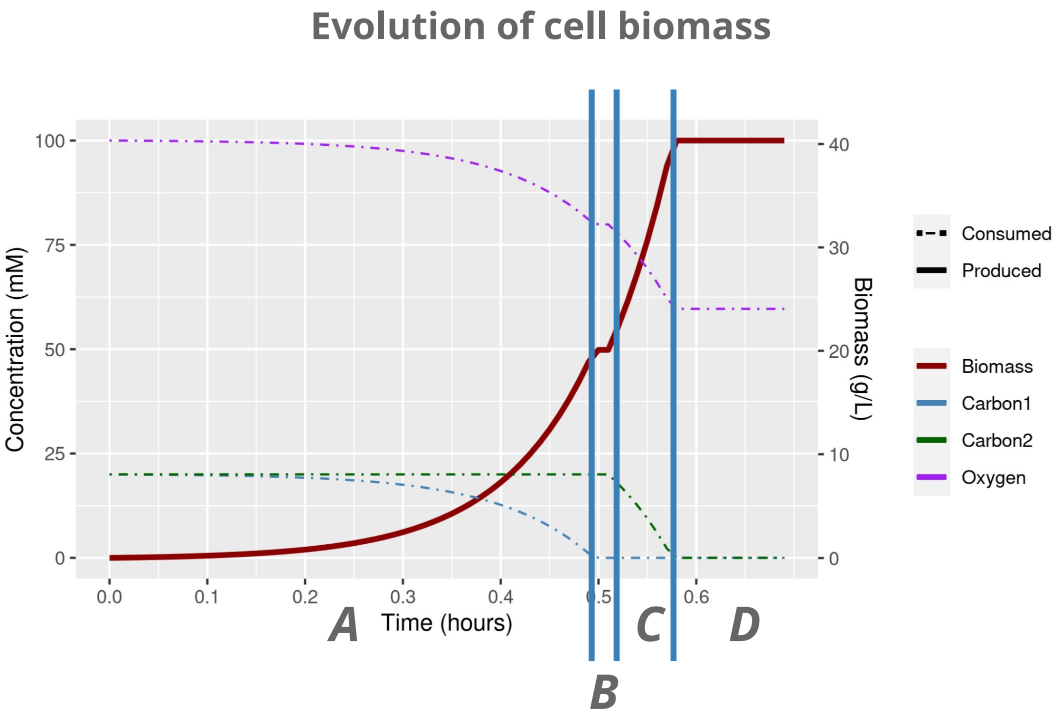
⟶ Control by regulations

Divided in 4 phases

Characterize by different qualitative behaviours (e.g. growth medium)

A *⟶ Growth on* ***Carbon1 only***
B *⟶* ***No growth*** *due to regulations*
C *⟶ Growth on* ***Carbon2 only***
D *⟶* ***No growth****, no growth medium*

Example: diauxic shift *(Monod et al., 1953)*



Evolution of cell biomass

Diauxic shift

⟶ *Successive growth phases on different mediums*

⟶ *Control by regulations*

Divided in 4 phases

Characterize by different qualitative behaviours (e.g. growth medium)

*A ⟶ Growth on **Carbon1 only***

*B ⟶ **No growth** due to regulations*

*C ⟶ Growth on **Carbon2 only***

*D ⟶ **No growth**, no growth medium*

Objective:

Learning all the regulatory rules explaining the cell behaviour

Taking knowledge into account

Only specific interactions (*activations*, *inhibitions*) between proteins and enzymes can be used in Boolean regulatory rules

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Prior Knowledge Network:

Set of authorised interactions: activation and inhibition effects

Example:

Regulatory rule of RPh can only depend on:

- ***activation of Hext***
- ***inhibition of R8a***

Taking knowledge into account

Only specific interactions (*activations*, *inhibitions*) between proteins and enzymes can be used in Boolean regulatory rules

Prior Knowledge Network:

Set of authorised interactions: activation and inhibition effects

Example:

$$\underbrace{\begin{aligned} \mathrm{RPh} &= 0 \\ \mathrm{RPh} &= 1 \\ \mathrm{RPh} &= \mathrm{Hext} \\ \mathrm{RPh} &= \neg\mathrm{R8a} \\ \mathrm{RPh} &= \mathrm{Hext} \wedge \neg\mathrm{R8a} \\ \mathrm{RPh} &= \mathrm{Hext} \vee \neg\mathrm{R8a} \end{aligned}}_{\textit{6 rules}}$$

Regulatory rule of RPh can only depend on:
- activation of Hext
- inhibition of R8a

***6 potential Boolean regulatory rules** for these 2 interactions*

Taking knowledge into account

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Prior Knowledge Network:

Set of authorised interactions: activation and inhibition effects

Example:

$\text{Hext} = 0$
$\text{Hext} = 1$

2 rules

$\text{RPh} = 0$
$\text{RPh} = 1$
$\text{RPh} = \text{Hext}$
$\text{RPh} = \neg\text{R8a}$
$\text{RPh} = \text{Hext} \wedge \neg\text{R8a}$
$\text{RPh} = \text{Hext} \vee \neg\text{R8a}$

6 rules

$\text{R8a} = 0$
$\text{R8a} = 1$
$\text{R8a} = \text{RPh}$

3 rules

Regulatory rule of RPh can only depend on:
- ***activation of Hext***
- ***inhibition of R8a***

***6 potential Boolean regulatory rules** for these 2 interactions*

$2 \times 6 \times 3 = 36$ ***compatible regulatory networks***

Taking knowledge into account

Only specific interactions (*activations*, *inhibitions*) between proteins and enzymes can be used in Boolean regulatory rules

Prior Knowledge Network:

Set of authorised interactions: activation and inhibition effects

Example:

$$\underbrace{\begin{array}{l}\mathrm{Hext}=0\\ \mathrm{Hext}=1\end{array}}_{\textit{2 rules}}\qquad \underbrace{\begin{array}{l}\mathrm{RPh}=0\\ \mathrm{RPh}=1\\ \mathrm{RPh}=\mathrm{Hext}\\ \mathrm{RPh}=\neg\mathrm{R8a}\\ \mathrm{RPh}=\mathrm{Hext}\wedge\neg\mathrm{R8a}\\ \mathrm{RPh}=\mathrm{Hext}\vee\neg\mathrm{R8a}\end{array}}_{\textit{6 rules}}\qquad \underbrace{\begin{array}{l}\mathrm{R8a}=0\\ \mathrm{R8a}=1\\ \mathrm{R8a}=\mathrm{RPh}\end{array}}_{\textit{3 rules}}$$

Regulatory rule of RPh can only depend on:
- ***activation of Hext***
- ***inhibition of R8a***

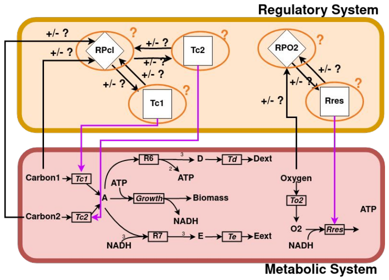
6 potential Boolean regulatory rules *for these 2 interactions*

Size of the search space

$O(2^{2^n})$ ***in the number*** n ***of interactions***

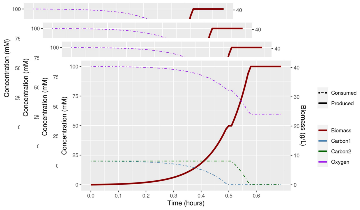
Problems tackled by MERRIN

Inferring regulatory rules from time series observations



Prior Knowledge Network

+



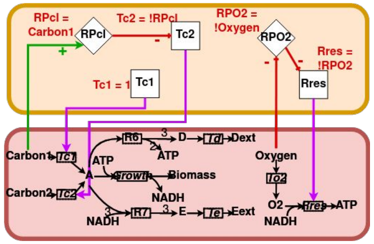
Time series
(kinetics, fluxomics, transcriptomics)

=?

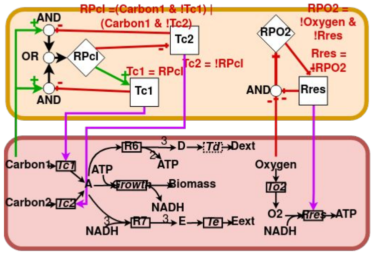
Input:

➔ **Prior Knowledge Network (PKN)**
Set of admissible interactions between components of the regulatory network

➔ **Time series data**
Kinetics, fluxomics and/or transcriptomics



Solution n°1



Solution n°2

Output:

➔ **Set of consistent regulatory networks**
Respecting the admissible interactions
Allowing to reproduce the input time series

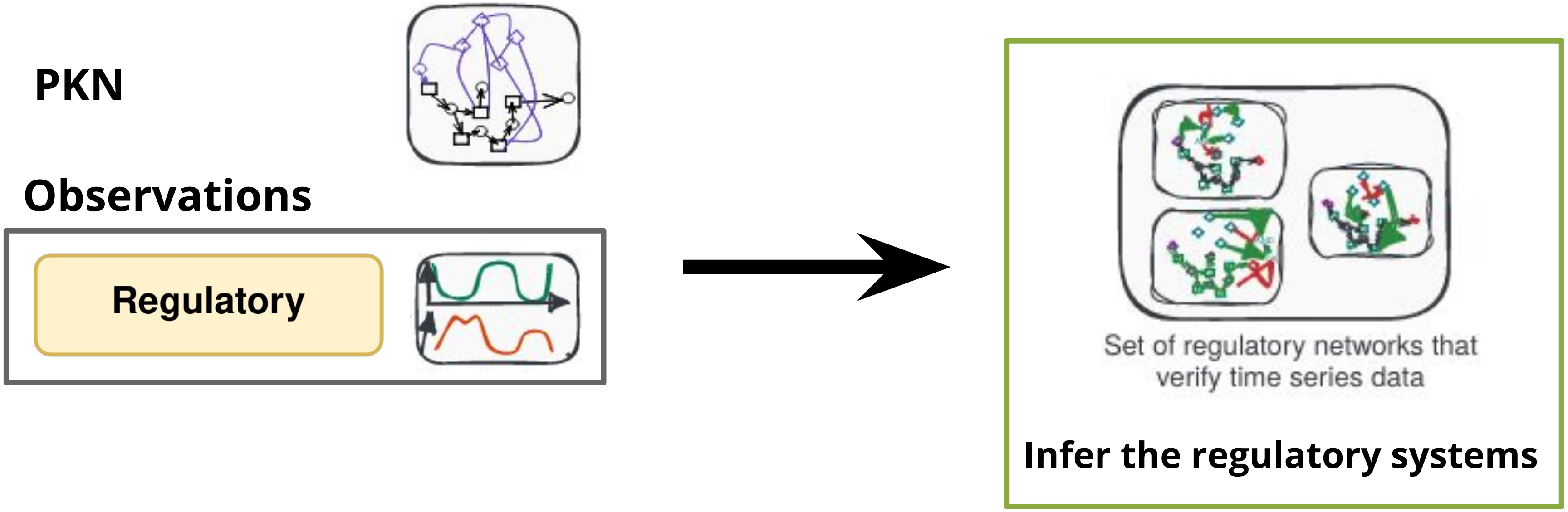
State of the art: learning regulatory rules

Several inferring approaches[1,2]

⟶ Based on **constraint programming**

⟶ **Discrete modelling** of the regulatory system dynamics

⟶ **Observations of the regulatory system + PKN**



PKN

Observations

Regulatory

Set of regulatory networks that verify time series data

Infer the regulatory systems

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[1] M. Ostrowski et al., **BioSystems**, 2016 – *CASPOTS*

[2] L. Paulevé et al., **Nature Communication**, 2020

State of the art: learning regulatory rules

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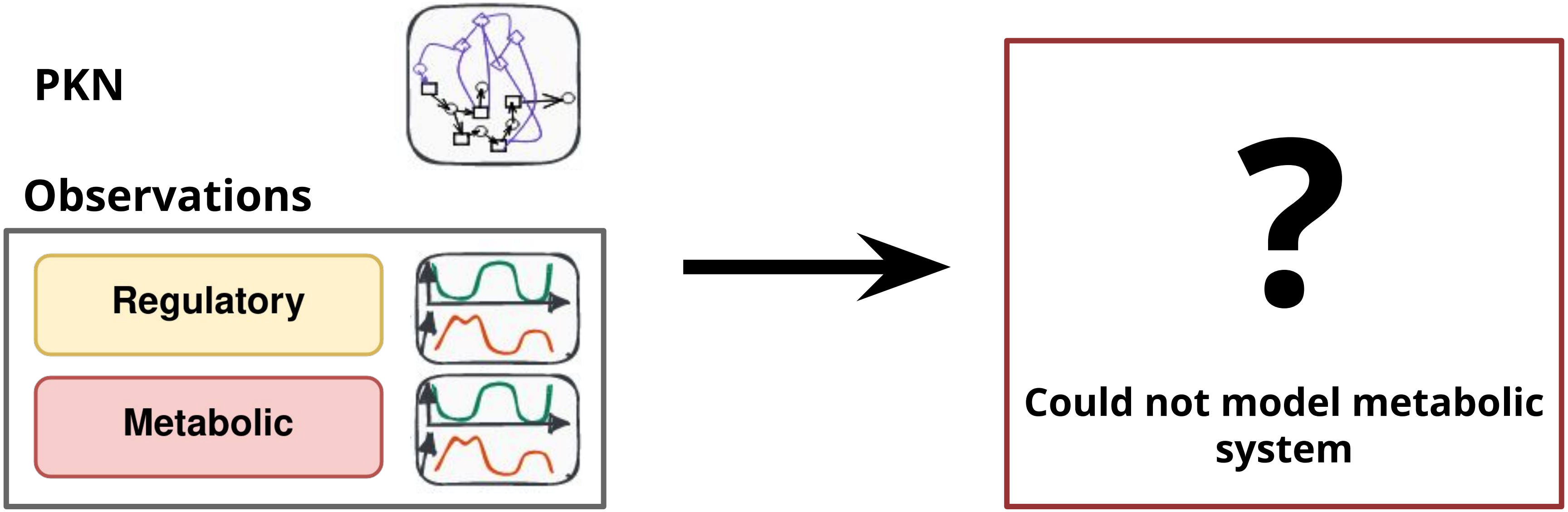

PKN

Observations

Regulatory

Metabolic

?

Could not model metabolic system

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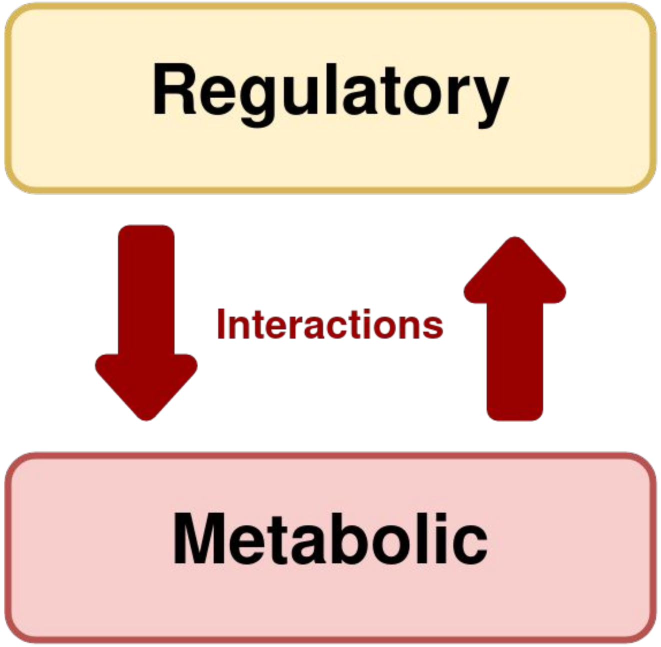
[1] M. Ostrowski et al., **BioSystems**, 2016 – *CASPOTS*
[2] L. Paulevé et al., **Nature Communication**, 2020

Multiplicity of modelling formalisms

Two models with different dynamics



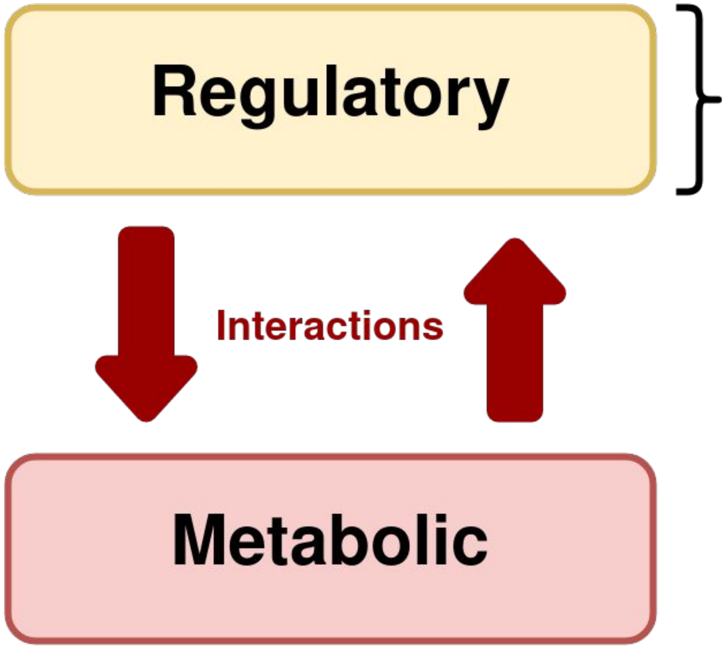
Regulatory

Interactions

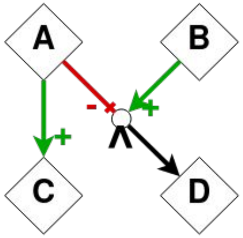
Metabolic

Multiplicity of modelling formalisms

Two models with 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)

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

Multiplicity of modelling formalisms

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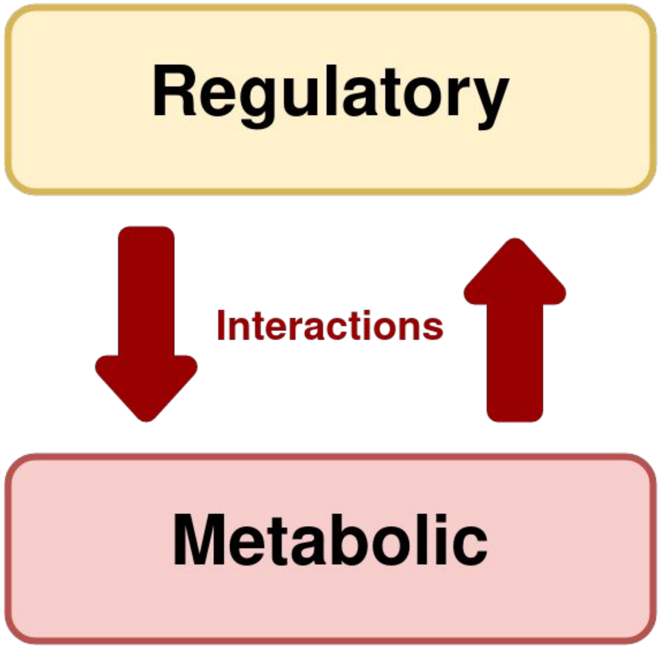

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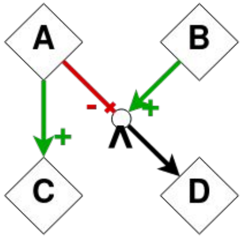

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Logical rules
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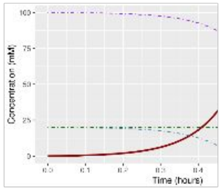
Inputs				Outputs			
x_A	x_B	x_C	x_D	x_A	x_B	x_C	x_D
0	0	x	x	0	0	0	0
0	1	x	x	0	1	0	1
1	0	x	x	1	0	1	0
1	1	x	x	1	1	1	0

Truth table (simulation)

Steady-states approximation[2]

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

Dynamic flux balance analysis (dFBA)



Metabolic traces

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

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

Contribution: MERRIN[1]

Input:

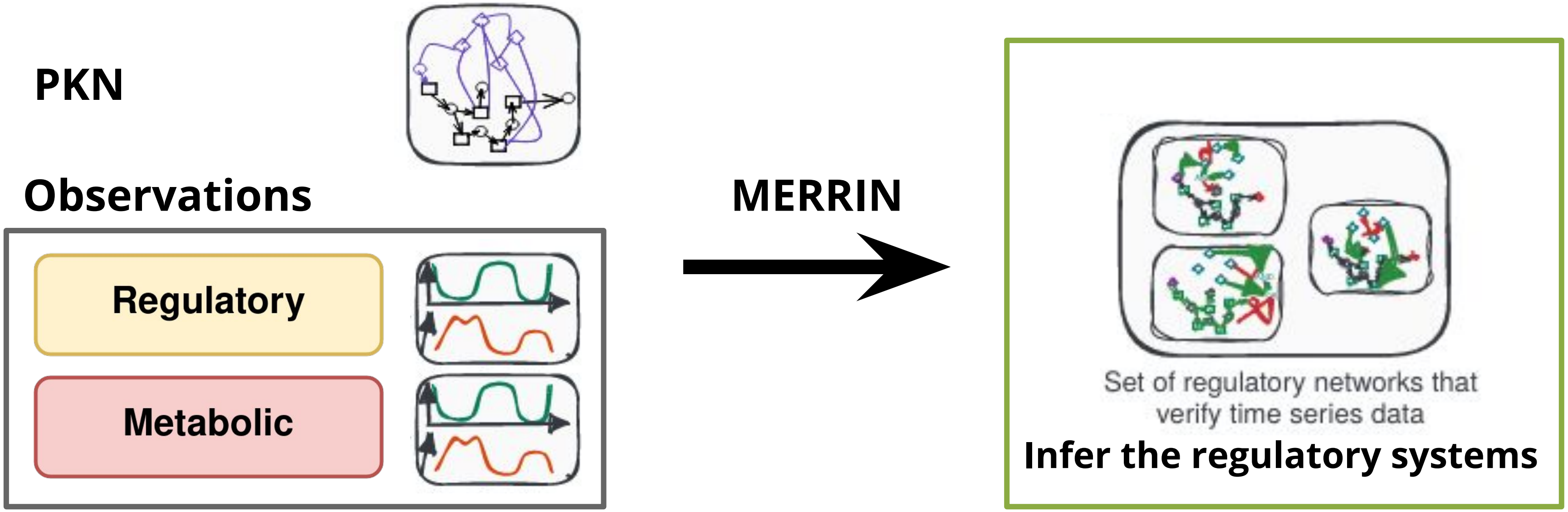
⟶ *Metabolic network,*

⟶ *Prior Knowledge Network (PKN),*

⟶ *Time series data*

Output:

All regulatory networks s.t:
⟶ ***consistent with the PKN***
⟶ *and **time series data***



PKN

Observations

Regulatory

Metabolic

MERRIN

Infer the regulatory systems

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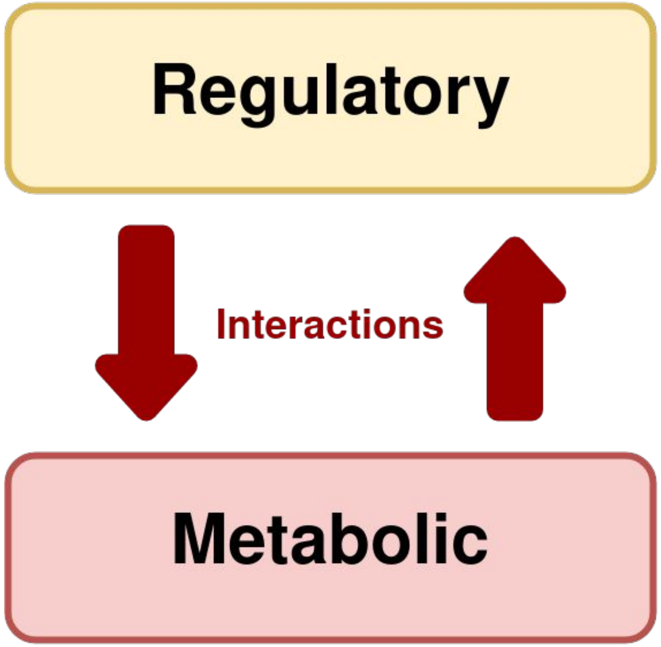
[1] Implementation available on *https://github.com/bioasp/merrin/*

Underlying simulation formalism

Several simulations approaches

Based on ***regulatory Flux Balance Analysis***[1] *(dynamic + regulations)*



rFBA timestep:

1. Update the **regulatory system**

   1 synchronous update of the Boolean network

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

2. Update the **metabolic system**

   Solve FBA — LP problem

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

3. Update the cell environment

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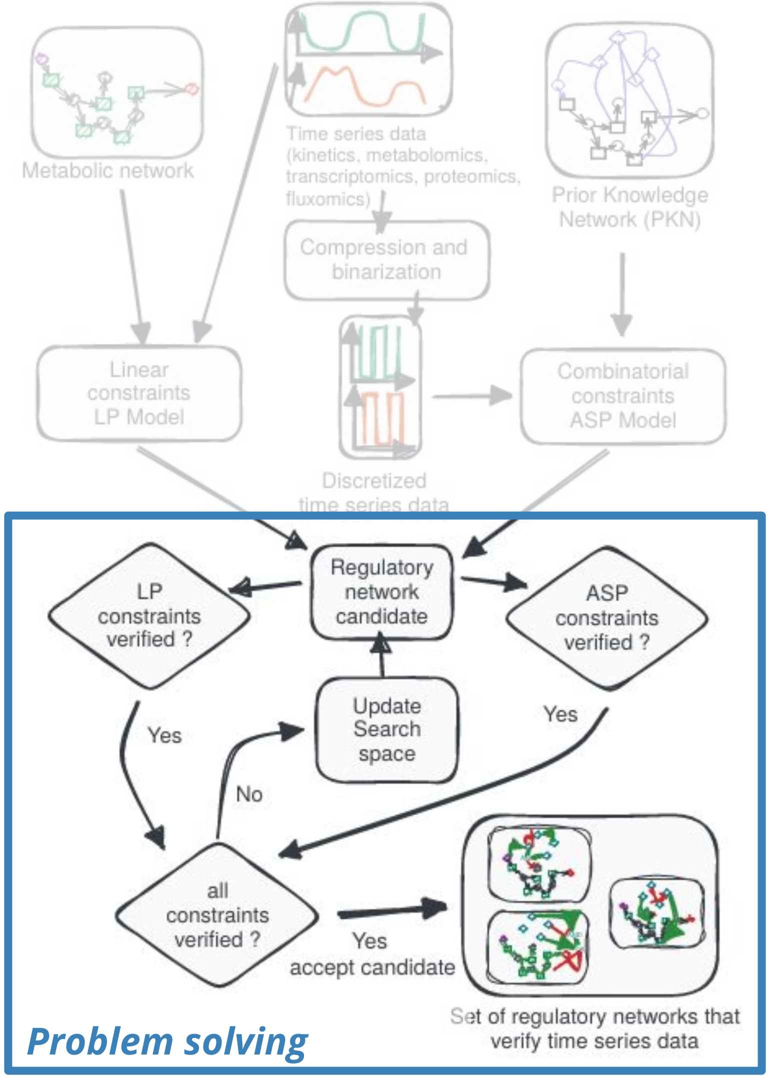
[1] M. W. Covert et al., **Journal of theoretical biology**, 2001

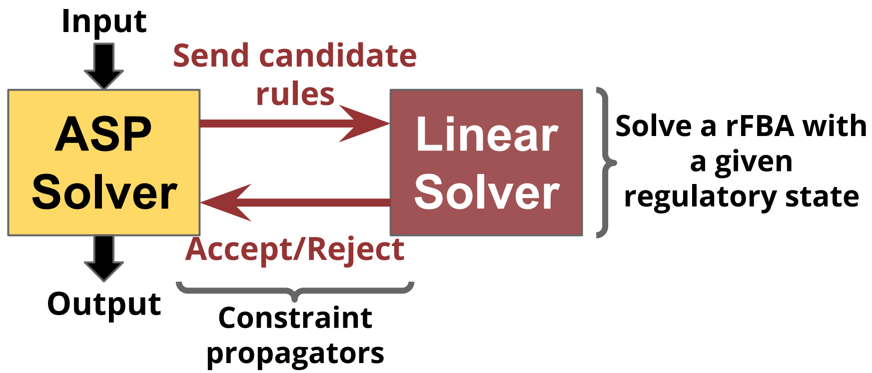
MERRIN's workflow



Problem solving

Rely on hybrid solving approach

(Answer Set Programming + Linear Programming)



Input

Send candidate rules

ASP Solver

Linear Solver

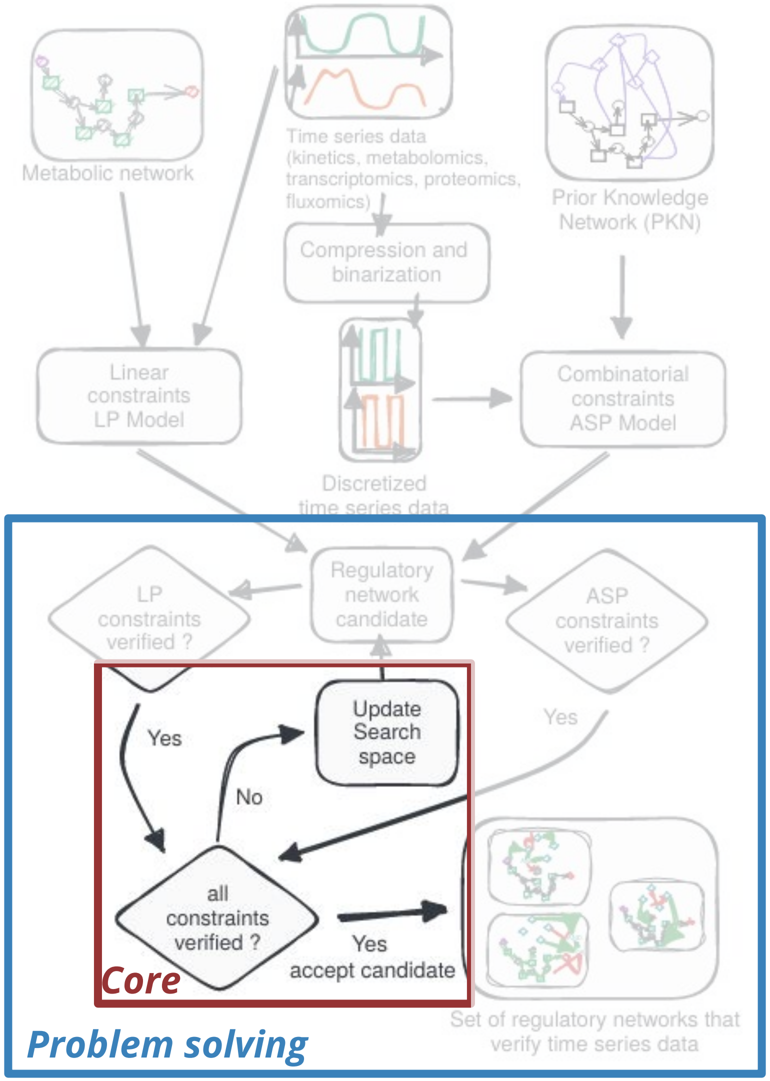
Solve a rFBA with a given regulatory state

Accept/Reject

Output

Constraint propagators

MERRIN’s workflow



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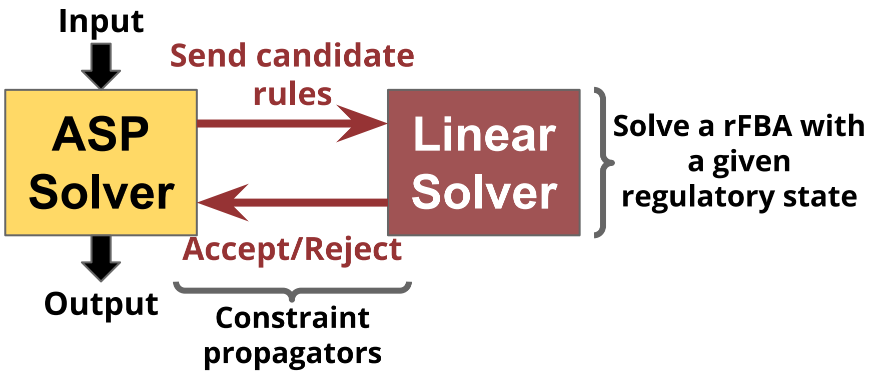


Input

Send candidate rules

ASP Solver

Linear Solver

Solve a rFBA with a given regulatory state

Accept/Reject

Output

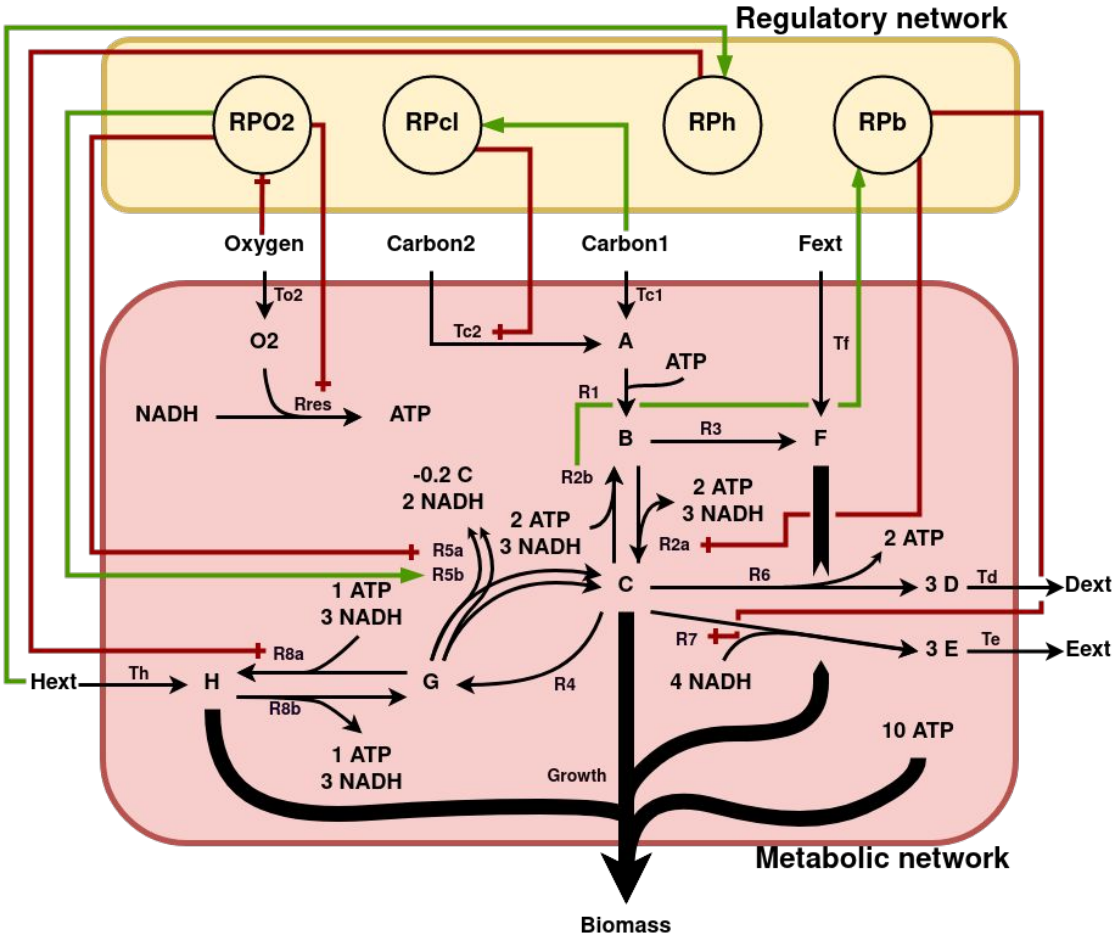
Constraint propagators

Accelerates the solving process by pre-filtering wrong solutions

→ ***Monotonic properties on regulatory network structures***

→ ***Over-generalisation of counter-examples***

Gold standard instance *(Covert et al, 2001)*



Toy model based on *E.coli*

20 reactions, 4 regulatory proteins, 11 regulations

Model complex behaviours

Diauxic shift, aerobic/anaerobic growth, etc.

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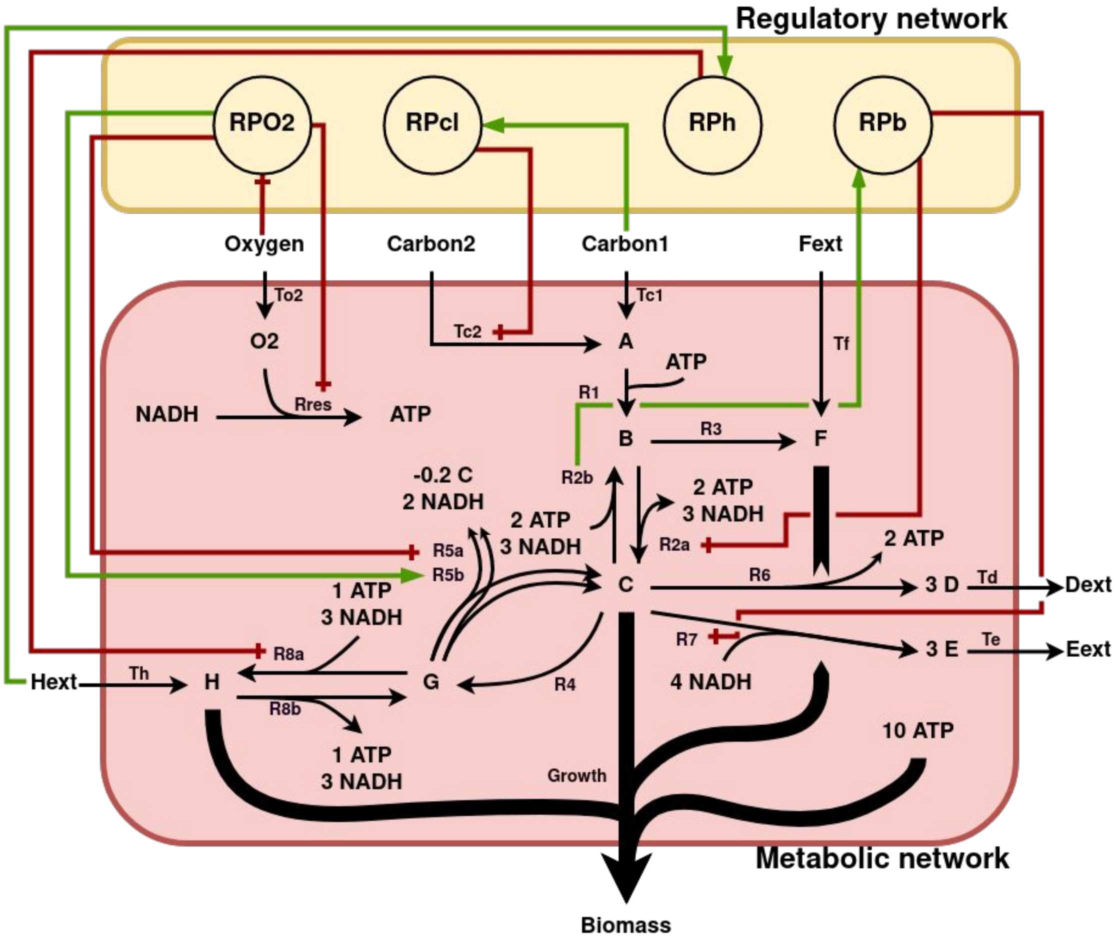
[1] M. W. Covert et al., **Journal of theoretical biology**, 2001

Gold standard instance *(Covert et al, 2001)*



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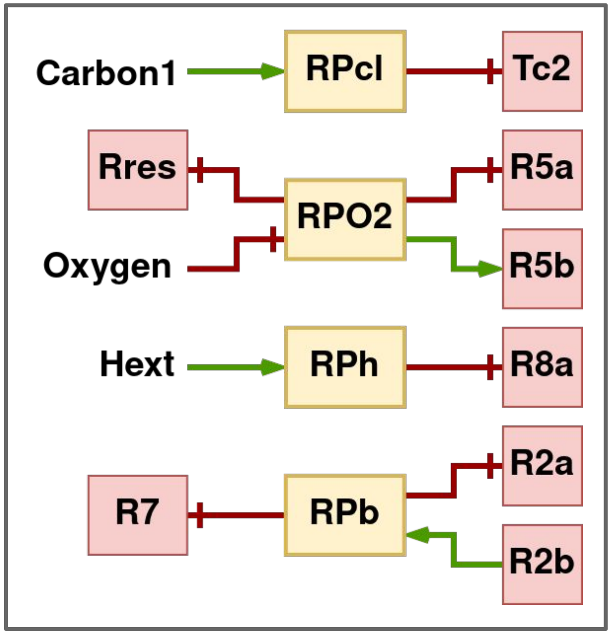
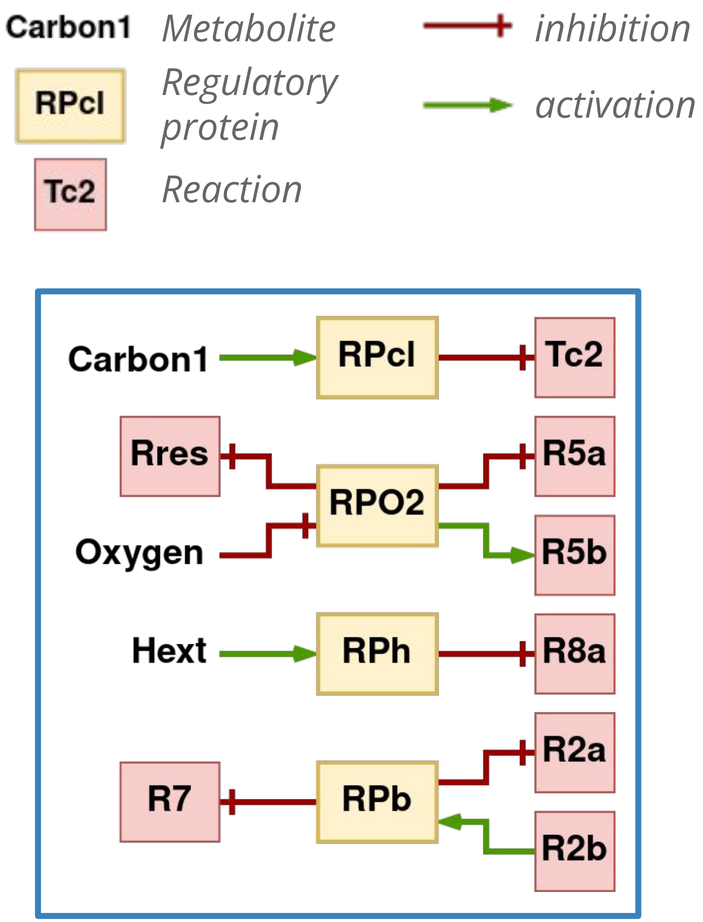


Influence graph

MERRIN inputs

Prior Knowledge Network



Gold standard influence graph

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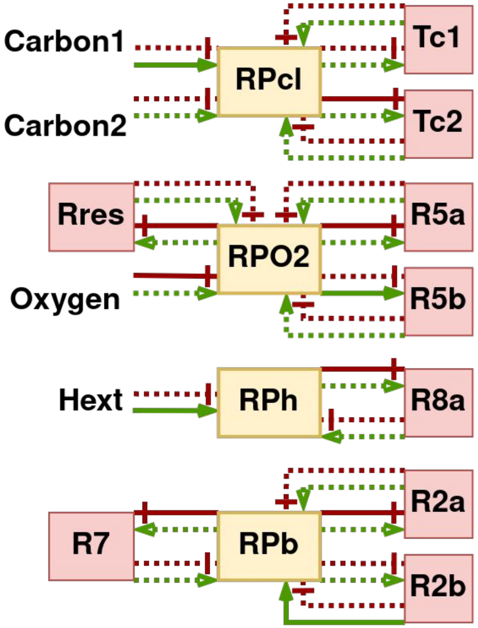
[1] M. W. Covert et al., **Journal of theoretical biology**, 2001

MERRIN inputs

Prior Knowledge Network

Prior Knowledge Network

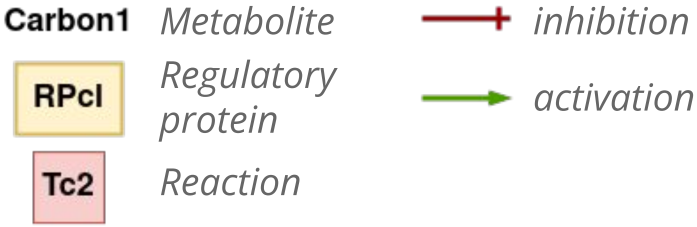
Set of admissible interactions
Superset of Gold Standard influence graph

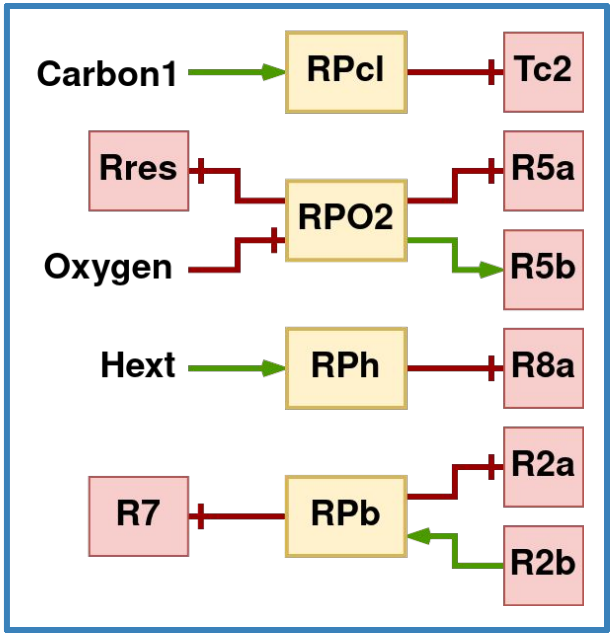


Remove interaction ***signs***
Remove interaction ***directions***

Add hypothetical regulations
(e.g. RPcl and Tc1)

~2.9x10^{12} potential
Boolean networks





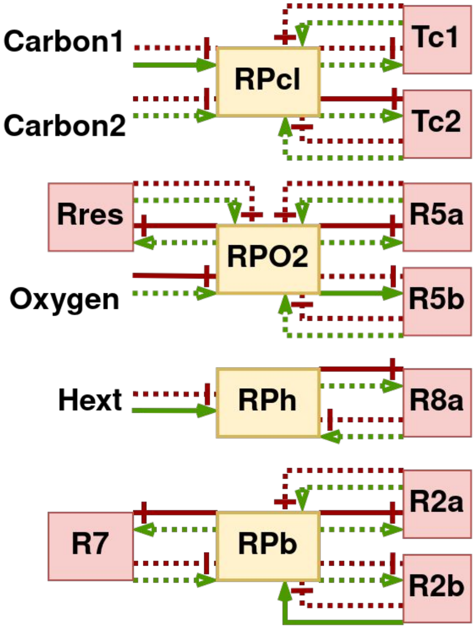
Gold standard influence graph

Instance generation

MERRIN inputs

Prior Knowledge Network

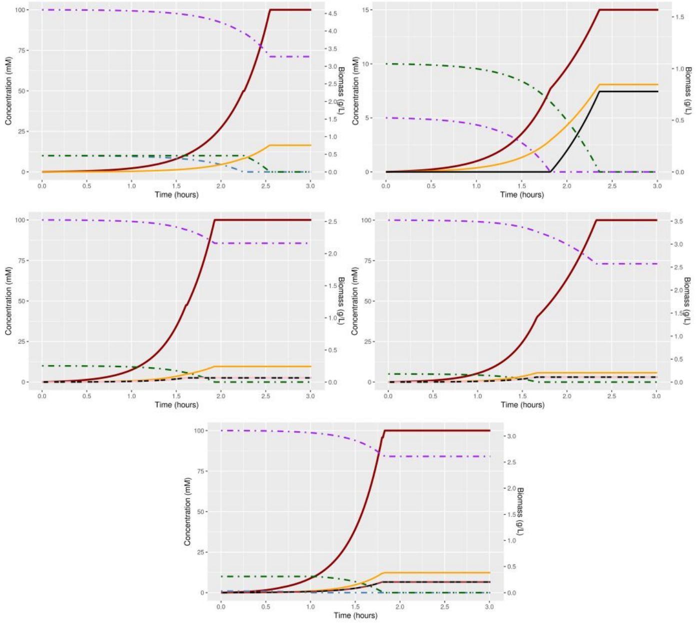
Add hypothetical regulations
Remove sign + direction of interactions



~2.9x10^{12} potential Boolean networks

5 simulated experiments

Provided by literature[1]
d-rFBA - made with FlexFlux



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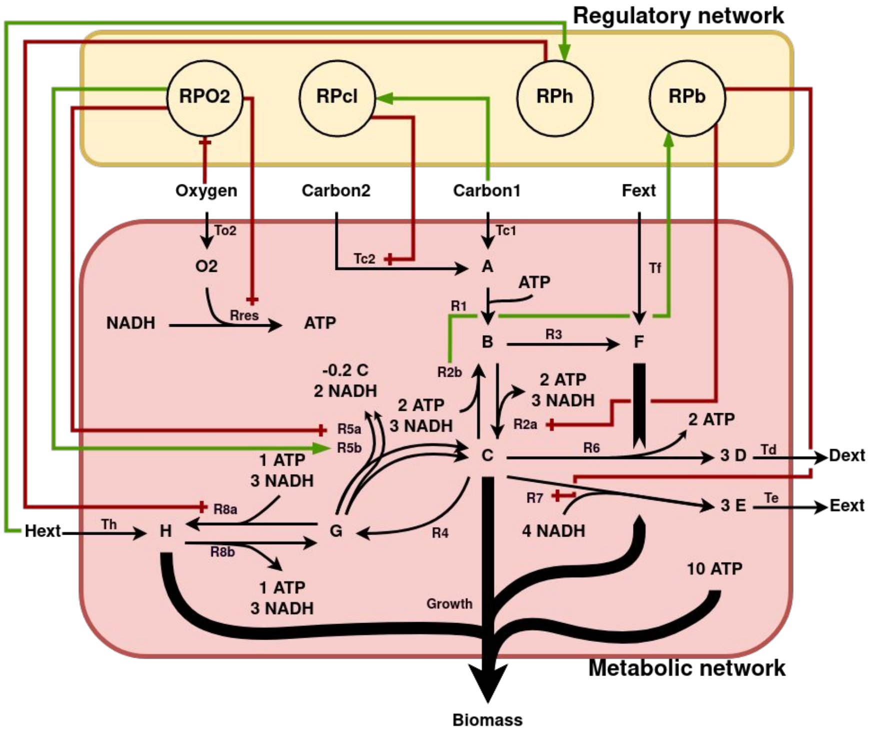
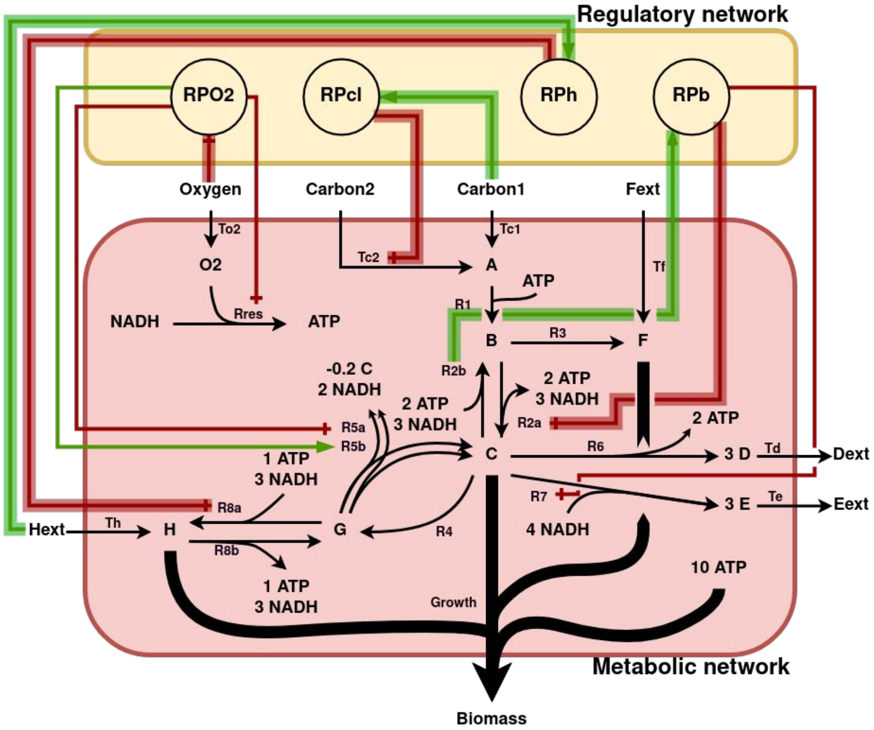
[1] M. W. Covert et al., **Journal of theoretical biology**, 2001

MERRIN validation



Gold standard is in the set of inferred models

MERRIN validation



Focus on smallest BNs

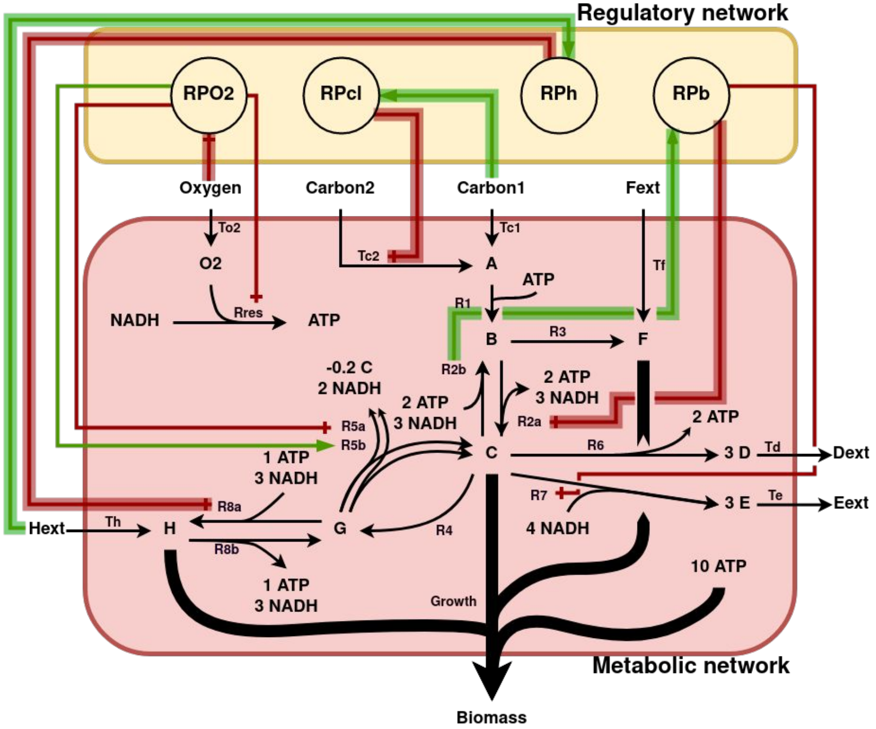
1 smallest BN

Computation time: ~7s

Subset of gold standard BN

Precision: 1.0 / Recall: 0.64

MERRIN validation



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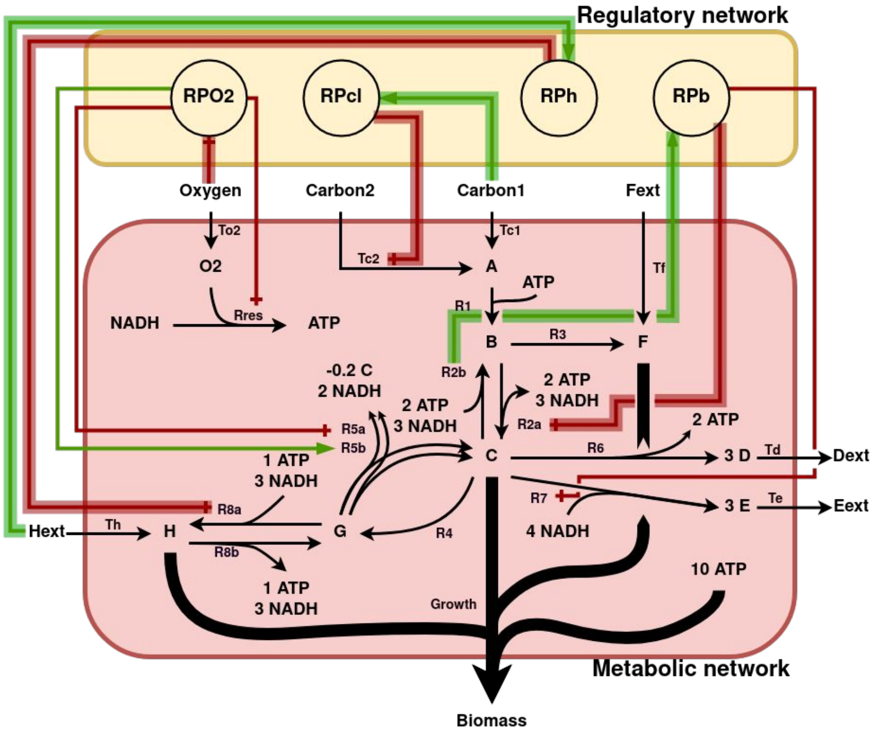
Did we fail inferring missing regulations ?

MERRIN validation



Focus on smallest BNs

1 smallest BN

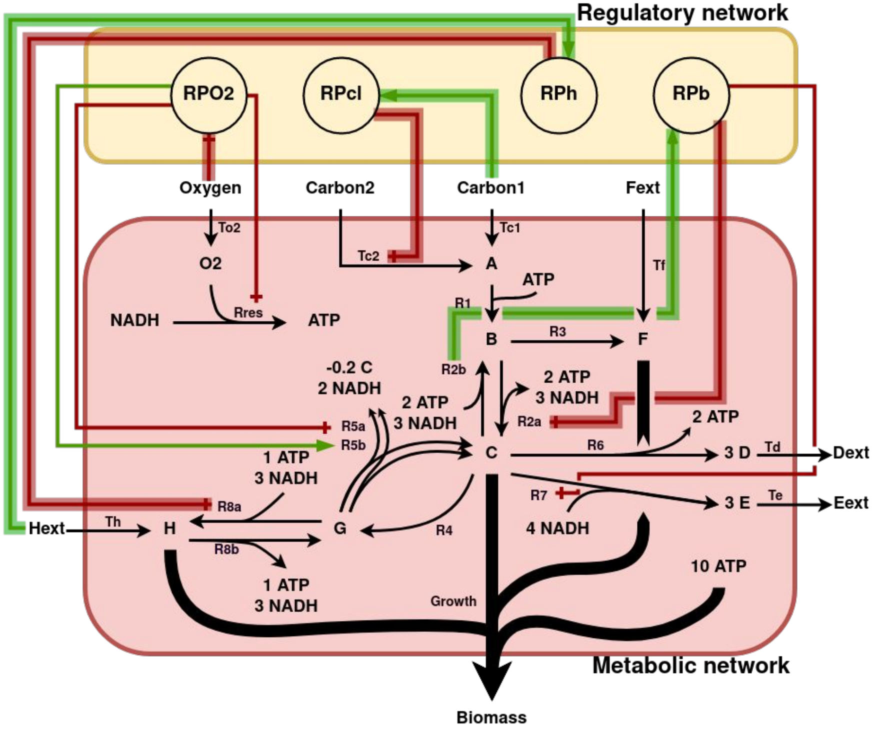
Computation time: ~7s

Subset of gold standard BN

Precision: 1.0 / Recall: 0.64

Reproduce exactly the input time series

MERRIN validation



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Unrecovered regulations can be explained

Example Rres:

→ Enzyme decay is needed to capture it

→ Does not impact rFBA optimum

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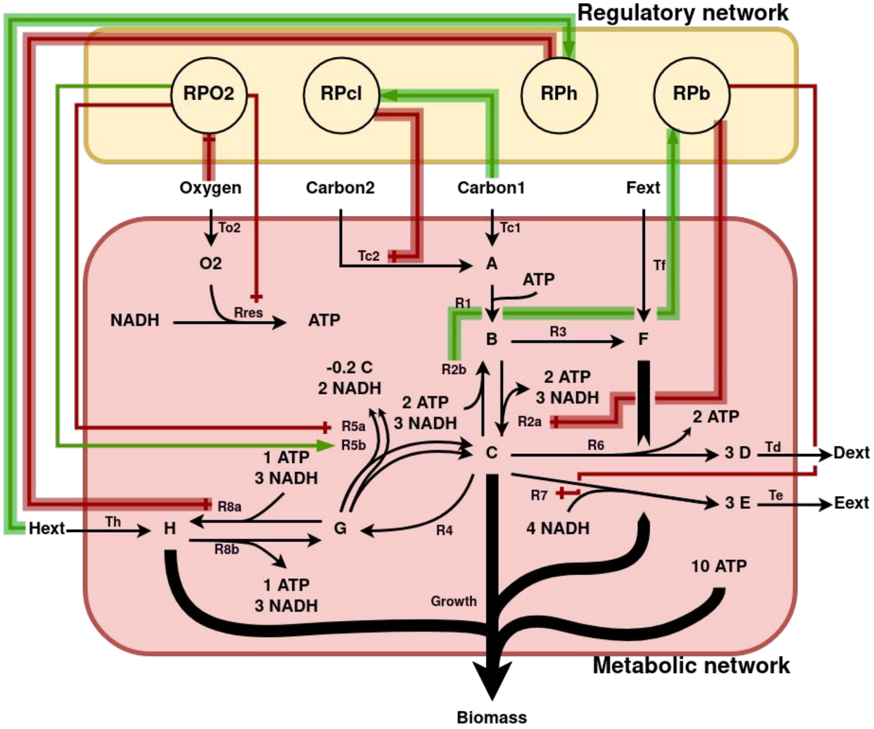

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More parsimonious model than gold standard

Application to 3 classes of measures

Observations of the *regulatory and metabolic* system activities

⟶ ***Quantitative*** *and* ***qualitative*** *measurements*

⟶ ***Simulations*** *mimicking experimental data*

3 data types supported by MERRIN:

Application to 3 classes of measures

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⟶ ***Quantitative*** *and* ***qualitative*** *measurements*

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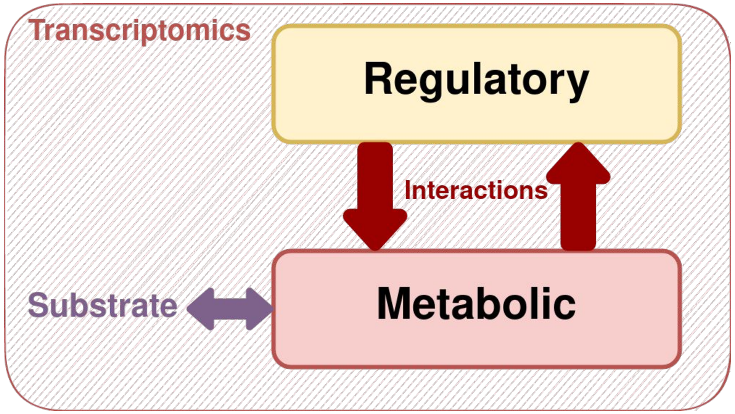
3 data types supported by MERRIN:

➔ **Transcriptomics** (qualitative)

Analysis of the RNA transcripts



Application to 3 classes of measures

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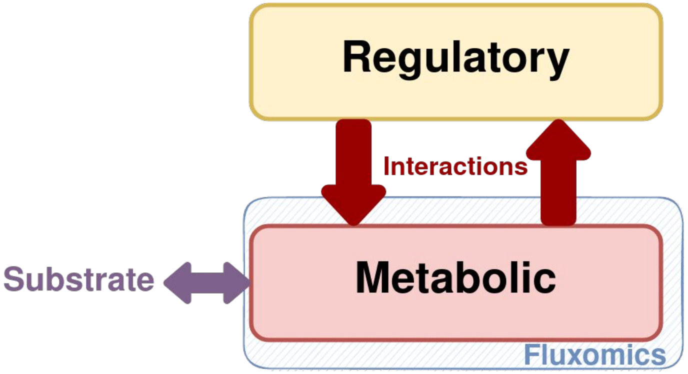
3 data types supported by MERRIN:

- ➔ **Transcriptomics** (qualitative)
  Analysis of the RNA transcripts
- ➔ **Fluxomics** (quantitative)
  Rates of metabolic reactions



Application to 3 classes of measures

Observations of the *regulatory and metabolic* system activities

⟶ ***Quantitative** and **qualitative** measurements*

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3 data types supported by MERRIN:

- ➔ **Transcriptomics** (qualitative)
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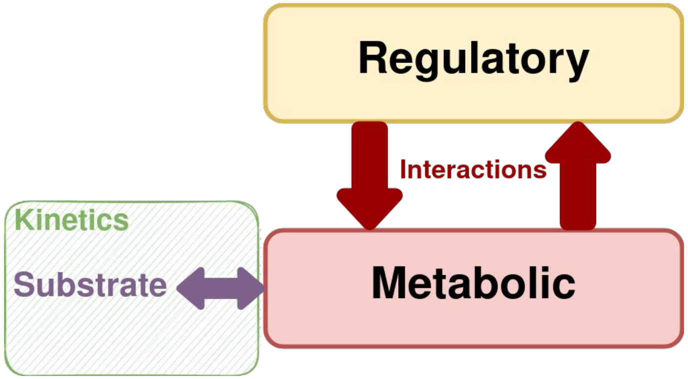
- ➔ **Fluxomics** (quantitative)
  Rates of metabolic reactions
- ➔ **Kinetics** (quantitative)
  Substrate concentrations



Application to 3 classes of measures

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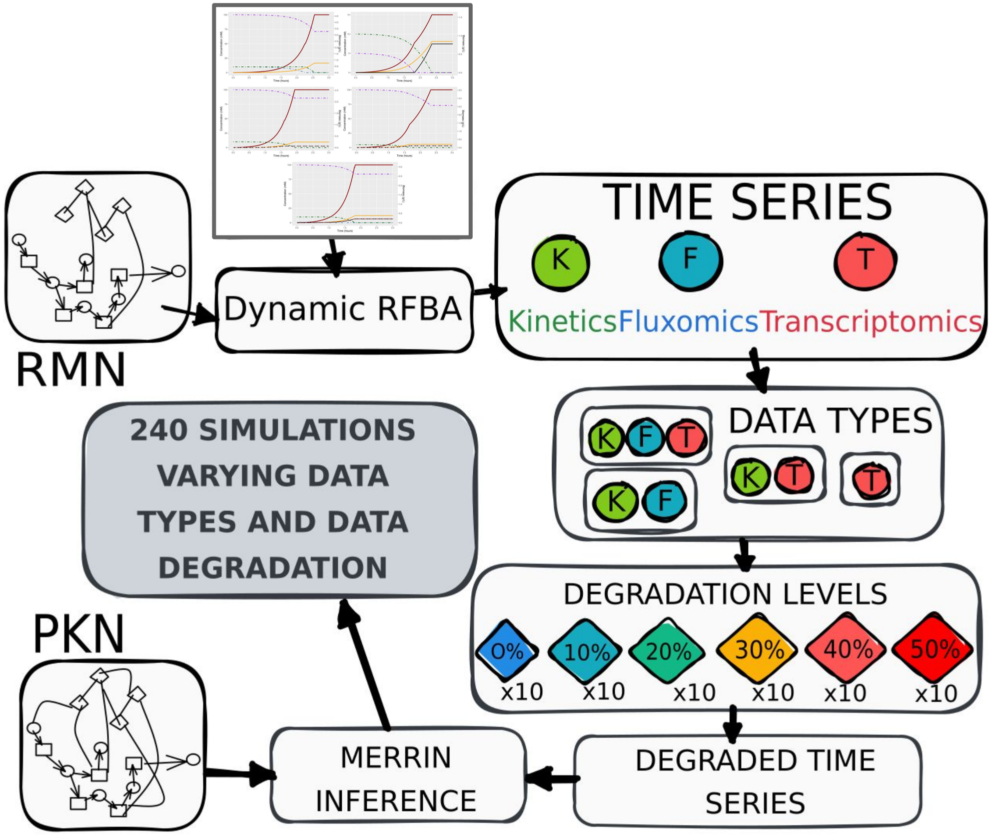
3 data types supported by MERRIN:

➔	**Transcriptomics** *Analysis of the RNA transcripts*	(qualitative)
➔	**Fluxomics** *Rates of metabolic reactions*	(quantitative)
➔	**Kinetics** *Substrate concentrations*	(quantitative)

MERRIN supports any combination of these 3 data types

Benchmark generation



5 simulations[1]:

- ➔ *Kinetics, fluxomics and transcriptomics*

4 data type combinations:

- ➔ *Kinetics, fluxomics, transcriptomics*
- ➔ *Kinetics, fluxomics*
- ➔ *Kinetics, transcriptomics*
- ➔ *Transcriptomics*

6 noise rates:

- ➔ *From **0% to 50%***
- ➔ *Missing observations and measures*

Benchmark of 240 instances

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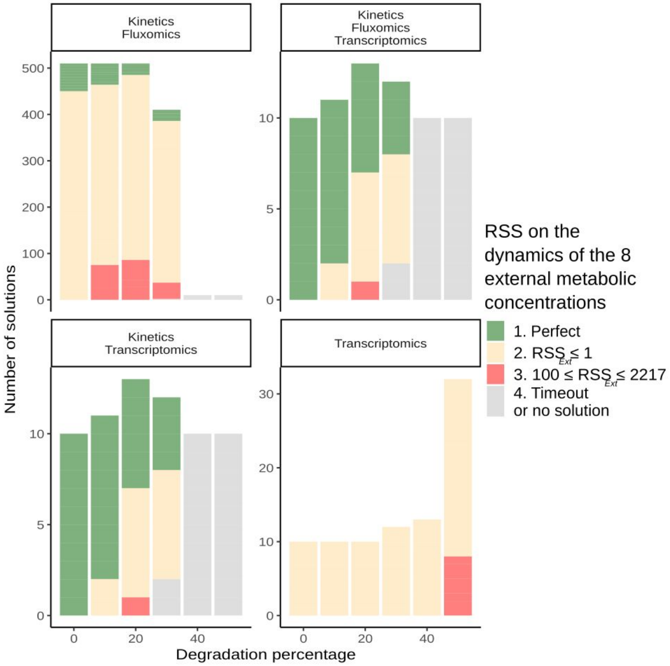
[1] M. W. Covert et al., **Journal of theoretical biology**, 2001

MERRIN robustness testing

Simulation reproducibility

Residual Sum of Squares – RSS



Average computation time ~25s

- **Robustness testing on**

  → ***noises:*** *0% – 50%*

  → ***data types:*** *KFT, KF, KT, T*

MERRIN robustness testing

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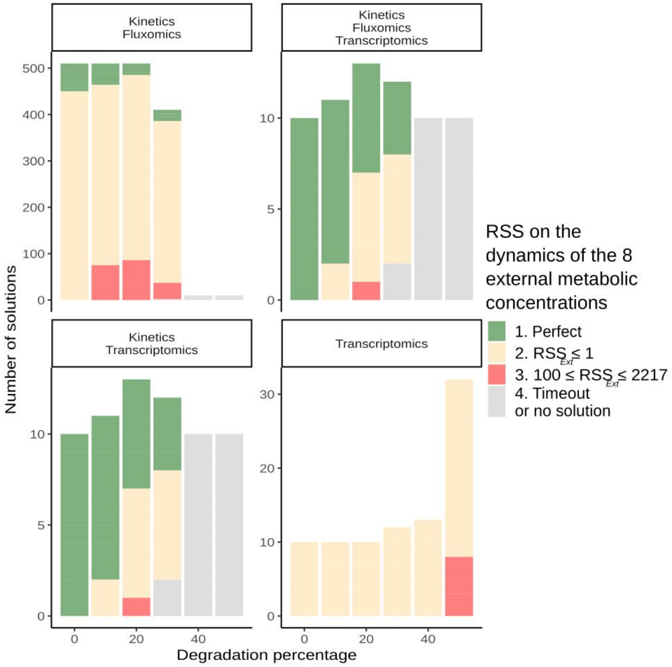

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  - ***Simulation reproducibility***

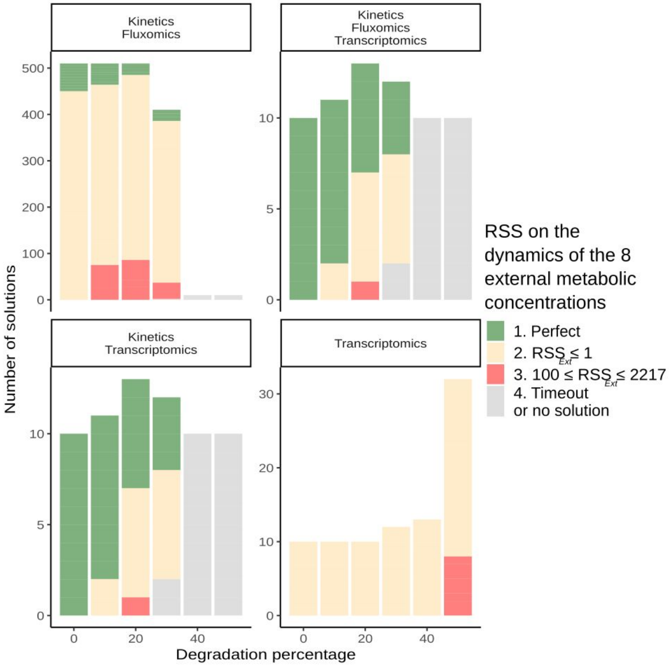
    Residual Sum of Square — RSS
  - ***Regulatory network structure***

    Precision and Recall

MERRIN robustness testing

Simulation reproducibility

Residual Sum of Squares – RSS



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- **Robustness testing on**

  ⟶ ***noises:*** *0% – 50%*

  ⟶ ***data types:*** *KFT, KF, KT, T*

- **2 evaluation metrics**
  - ○ ***Simulation reproducibility***

    Residual Sum of Square — RSS

    ➔ ***RSS < 1***
  - ○ ***Regulatory network structure***

    Precision and Recall

    ➔ ***Precision = 1 / Recall = 0.64***

MERRIN optimal inputs

At least **transcriptomic** and **kinetic** data
With a **noise ≤ 20%**

Conclusion

Contribution

- **MERRIN[1]: inferring regulatory rules from time series**
  ⟶ ***Hybrid (ASP + LP) resolution***
- **Validation and robustness testing**
  ⟶ *Finding a **more parsimonious model** than the gold standard*
  ⟶ *Impact of **noise** and **data type** on inferred regulations*

Perspectives

- **Scalability to genome-scale metabolic networks**
  ⟶ ***rFBA scales well**, the **PKN size should be the issue***
  ⟶ *Next step: validation on **E.coli core medium-scale** model*
- **Study unrecovered regulations**
  ⟶ *Impact of experiments and data types on the inferred regulations*
  ⟶ *Extend simulation formalisms with **enzyme amounts** and **synthesis** — r-deFBA[2]*

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[1] Implementation available on *https://github.com/bioasp/merrin/*

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