How to learn metabolic regulation rules from time series data?

MERRIN: *MEtabolic Regulation Rules INference from time series data*

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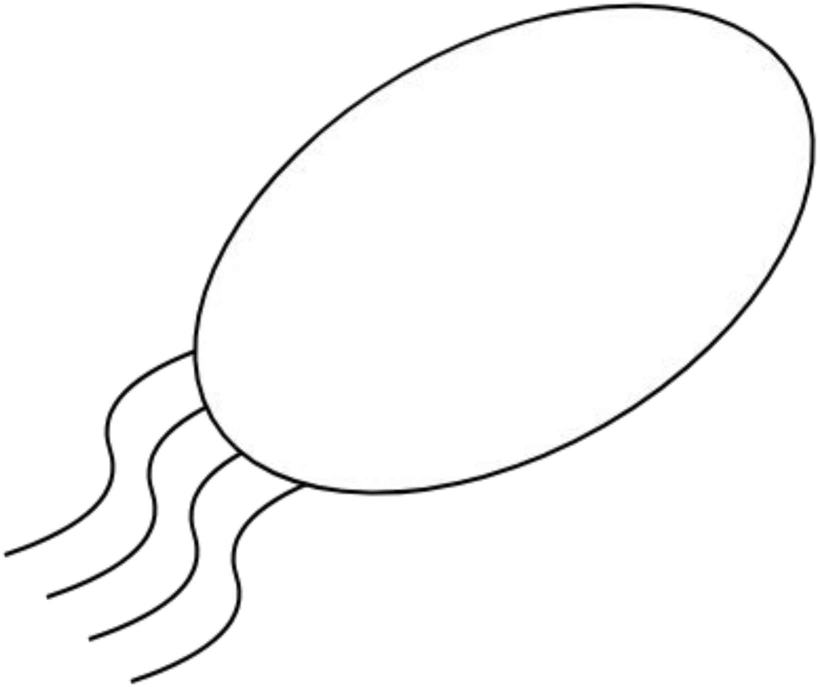
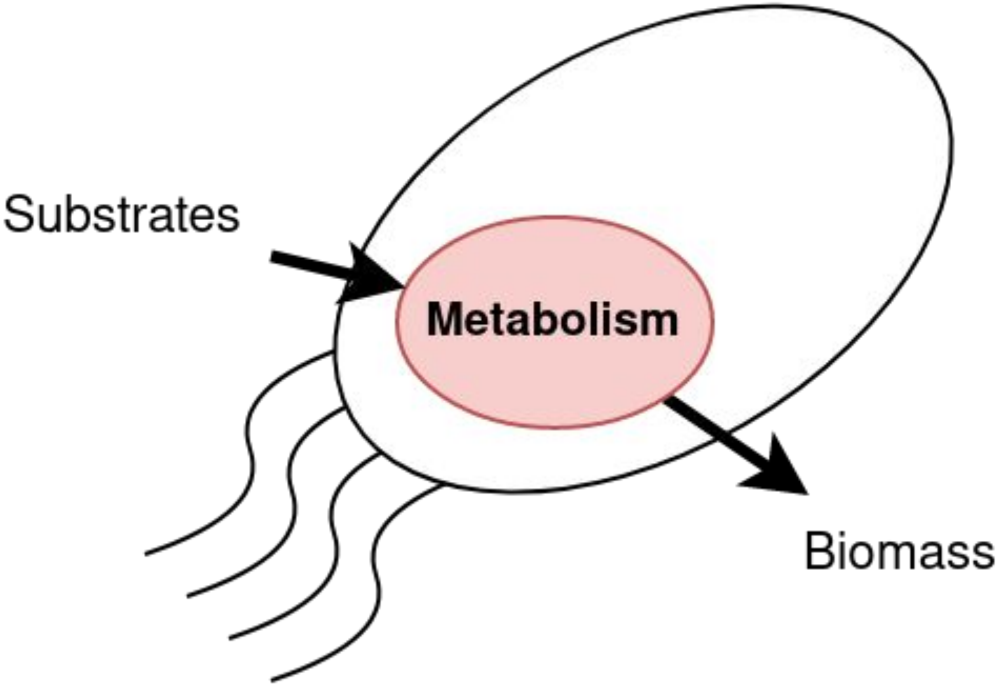
21st February 2023

Cells: hybrid multi-layered structures



Model as two interconnected systems

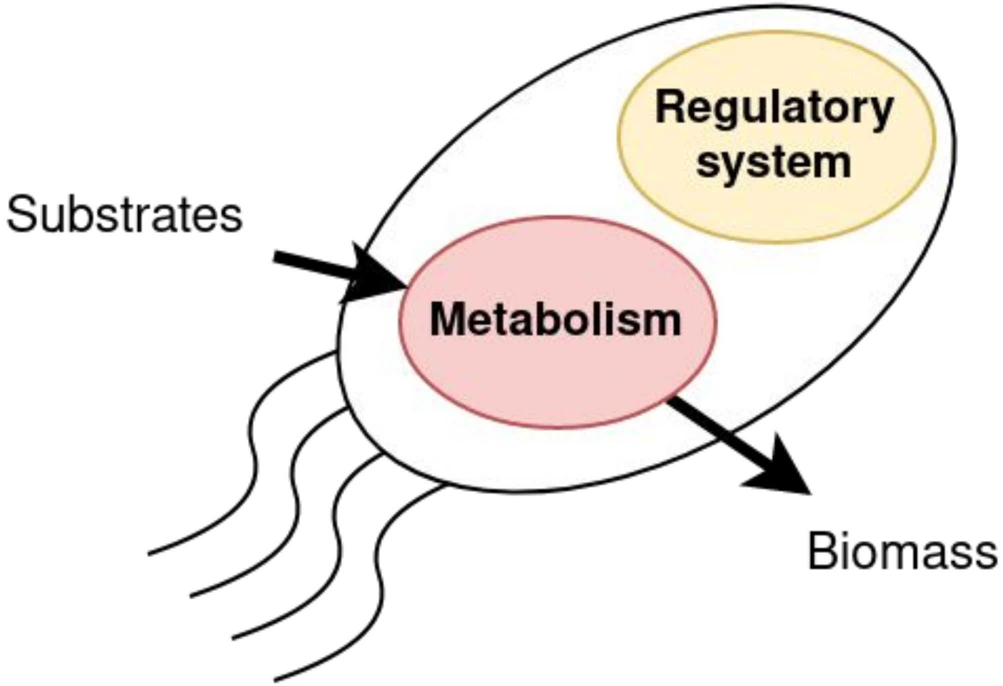
Cells: hybrid multi-layered structures



Model as two interconnected systems

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

Cells: hybrid multi-layered structures



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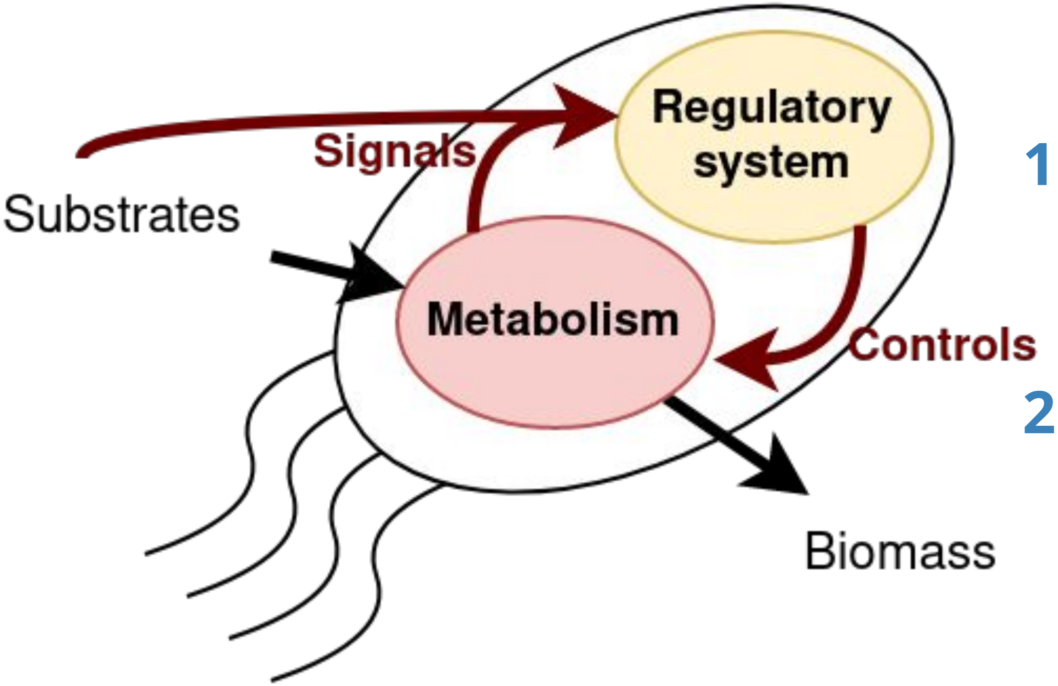
   Chemicals reactions converting substrates to energy and biomass

2. **Regulatory system**
   Rules constraining the metabolism to adapt itself to its environment

Cells: hybrid multi-layered structures



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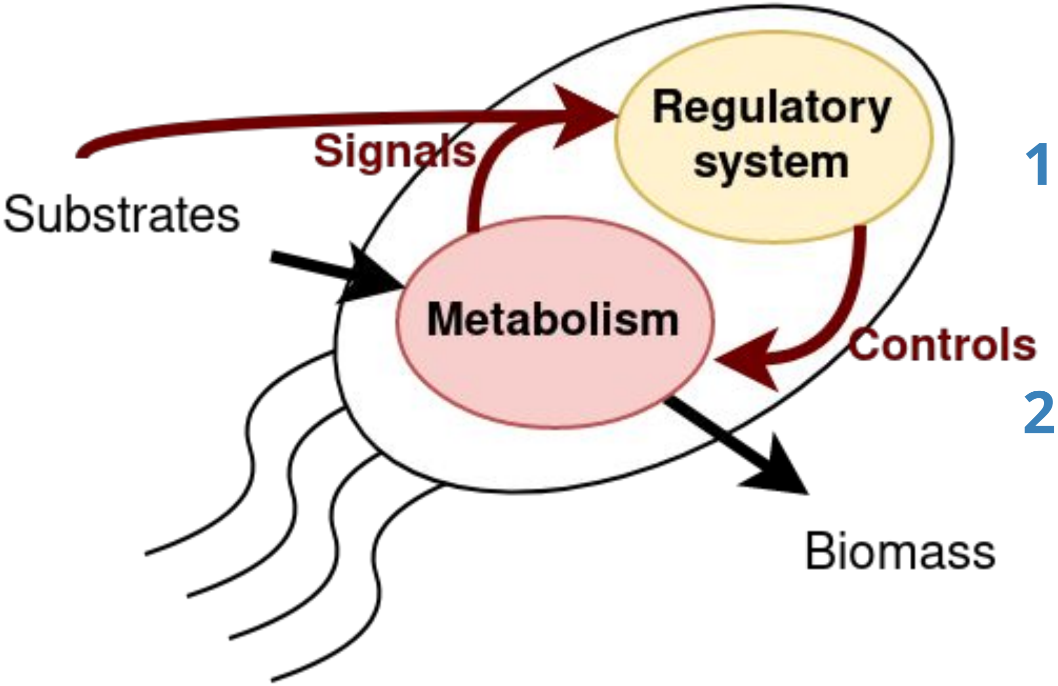


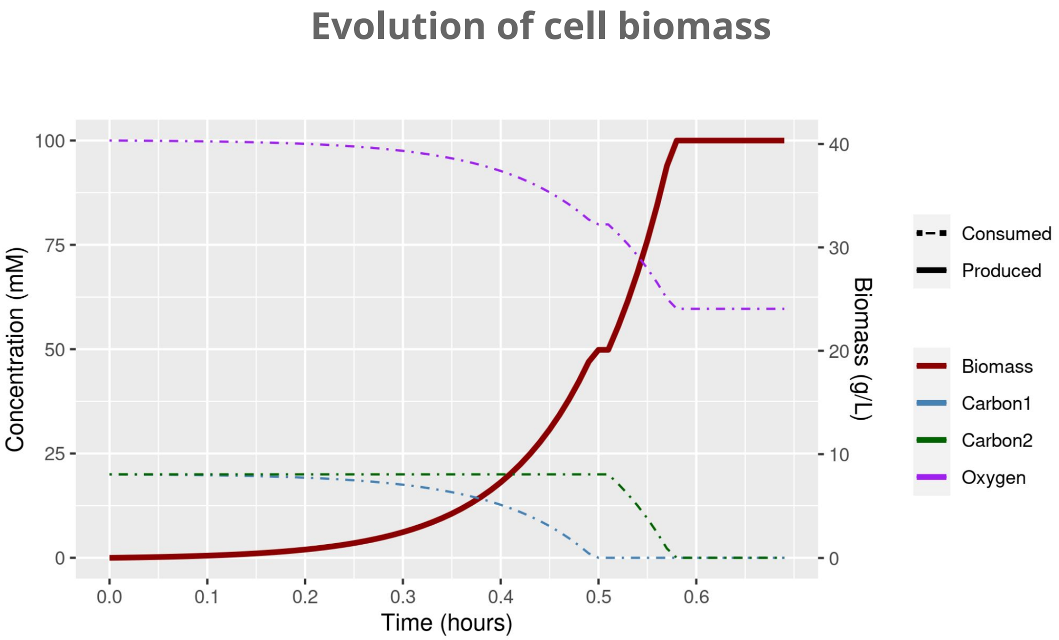
Model as two interconnected systems

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)*

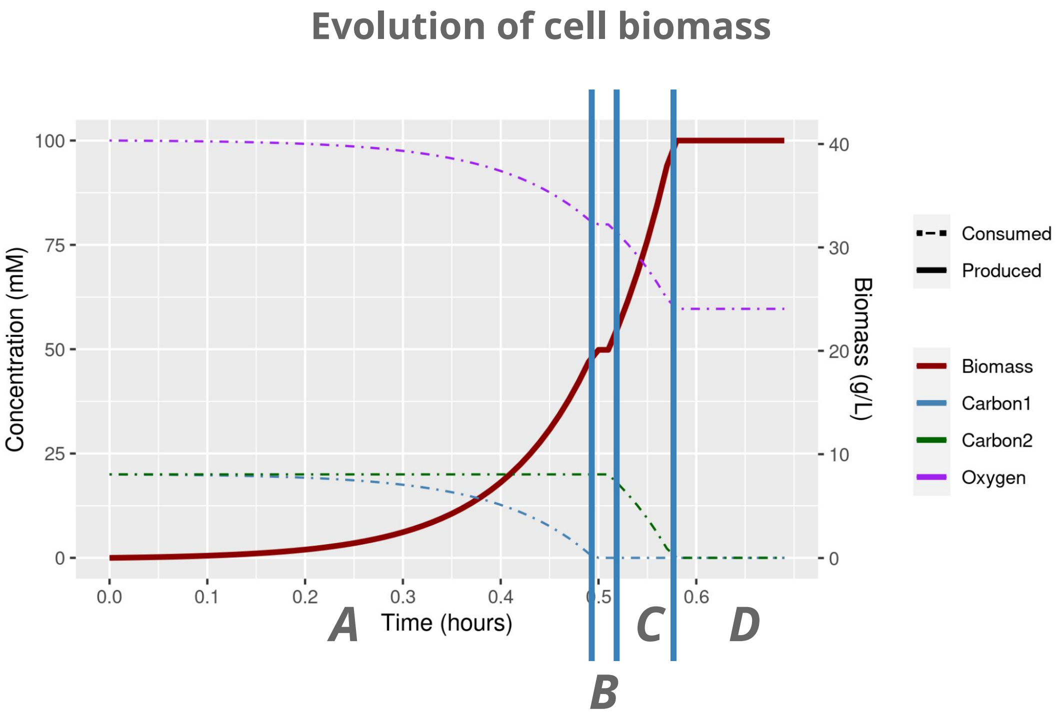


Diauxic shift

→ *Successive growth phases on different mediums*

→ *Control by regulations*

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



Diauxic shift

→ Successive growth phases on different mediums

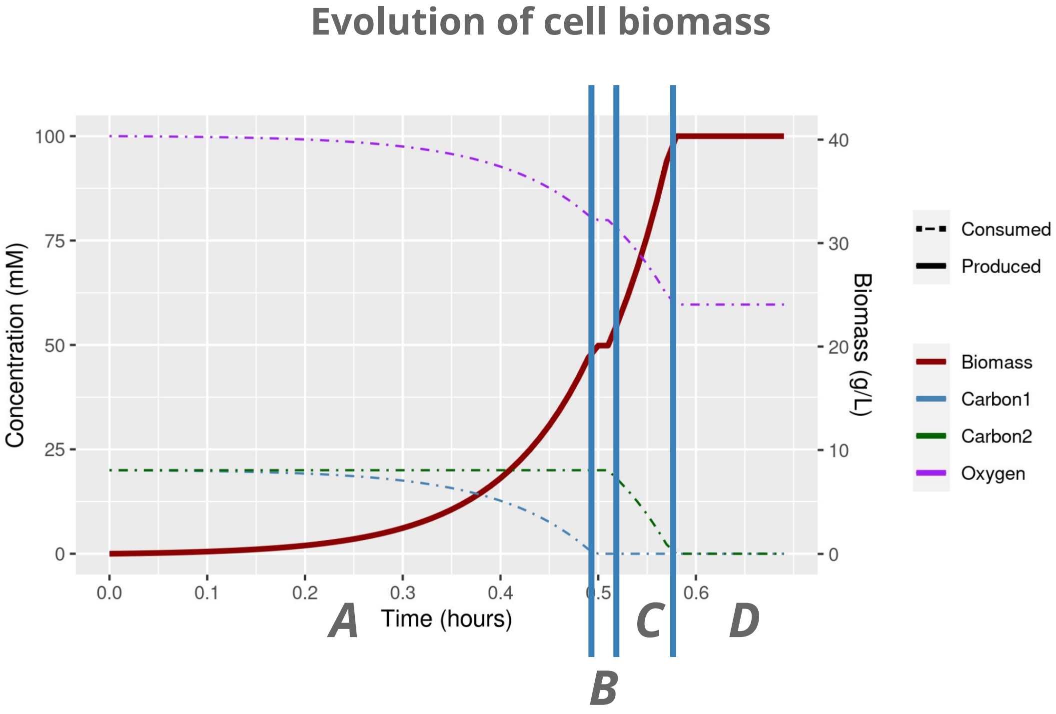
→ Control by regulations

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)*



Diauxic shift

⟶ Successive growth phases on different mediums

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Divided in 4 phases

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

*A ⟶ Growth on **Carbon1 only***

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

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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***

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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:

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

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

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

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$$\underbrace{\begin{array}{l}\text{R8a}=0\\ \text{R8a}=1\\ \text{R8a}=\text{RPh}\end{array}}_{\textit{3 rules}}$$

Regulatory rule of RPh can only depend on:

- activation of Hext

- inhibition of R8a

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

$2 \times 6 \times 3 = 36$ ***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

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

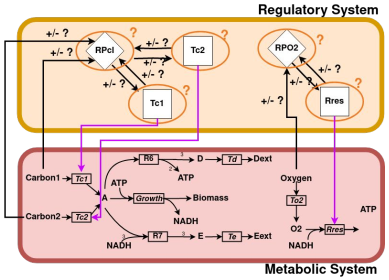
***6 potential Boolean regulatory rules** for these 2 interactions*

Size of the search space

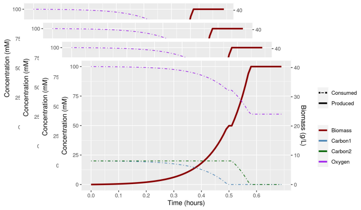
$O(2^{2^n})$ ***in the number*** n ***of interactions***

Problems tackled by MERRIN

Inferring regulatory rules from time series observations



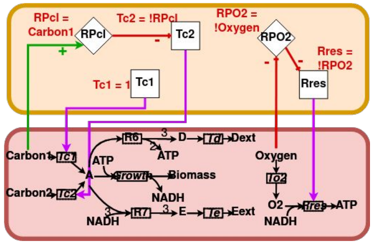
Prior Knowledge Network



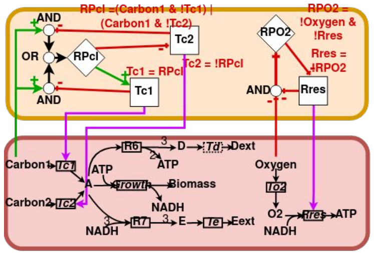
Time series
(kinetics, fluxomics, transcriptomics)

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

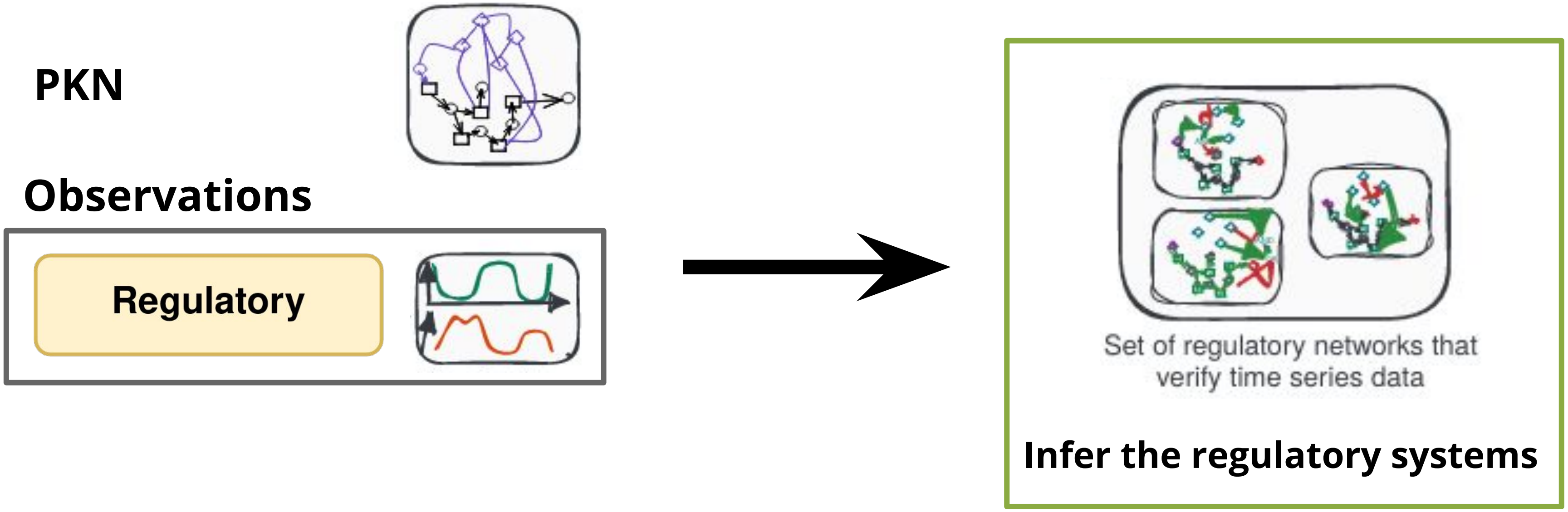
Output:

- **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

Infer the regulatory systems

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

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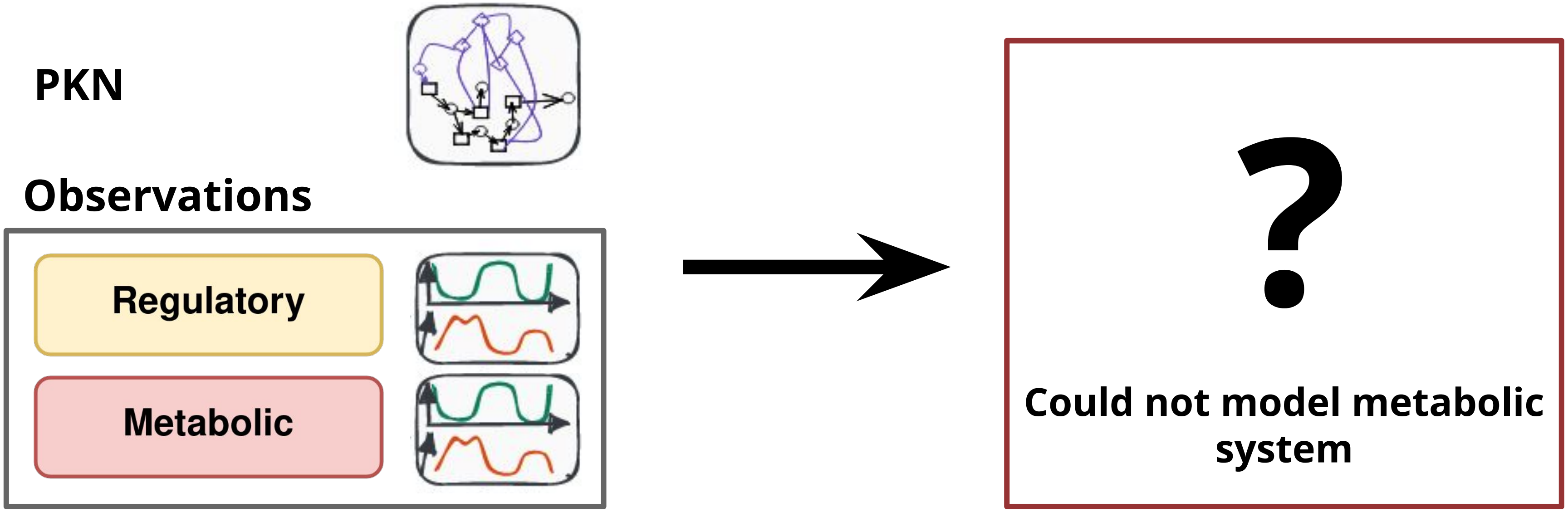

PKN

Observations

Regulatory

Metabolic

?

Could not model metabolic system

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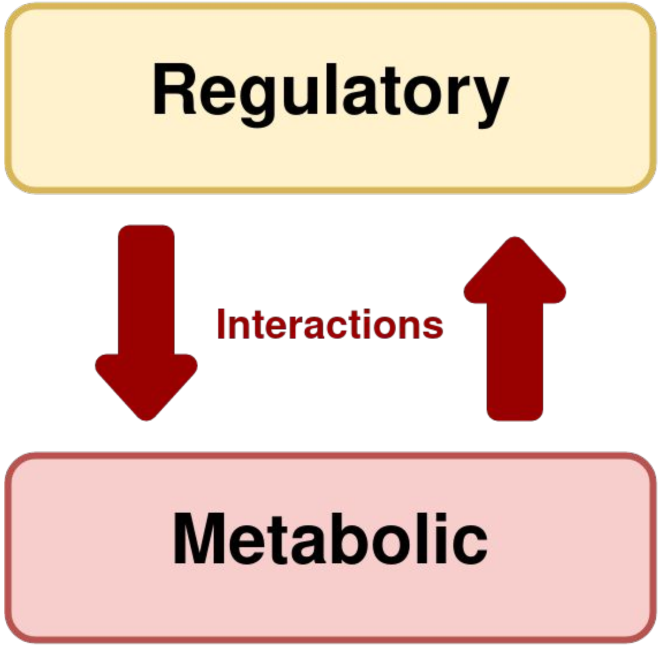
[2] L. Paulevé et al., **Nature Communication**, 2020

Multiplicity of modelling formalisms

Two models with different dynamics



Regulatory

Interactions

Metabolic

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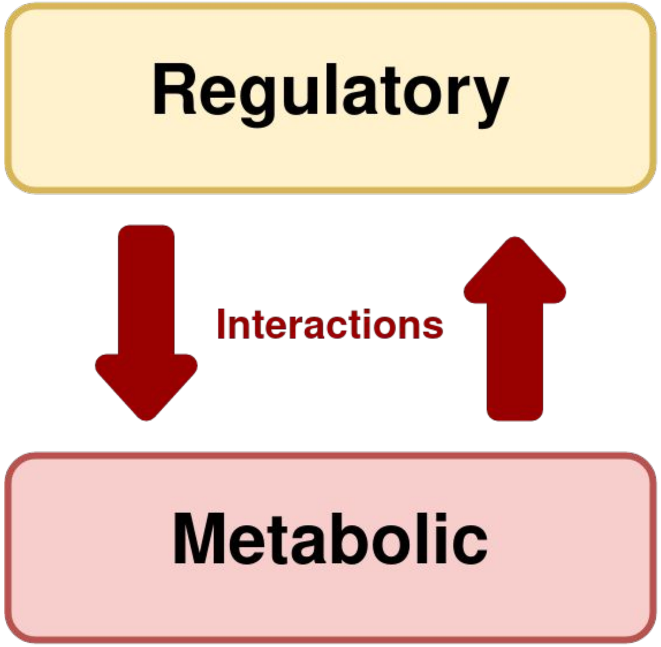
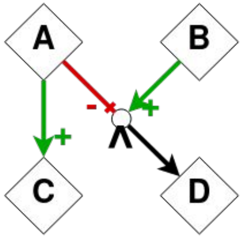


Regulatory

Interactions

Metabolic

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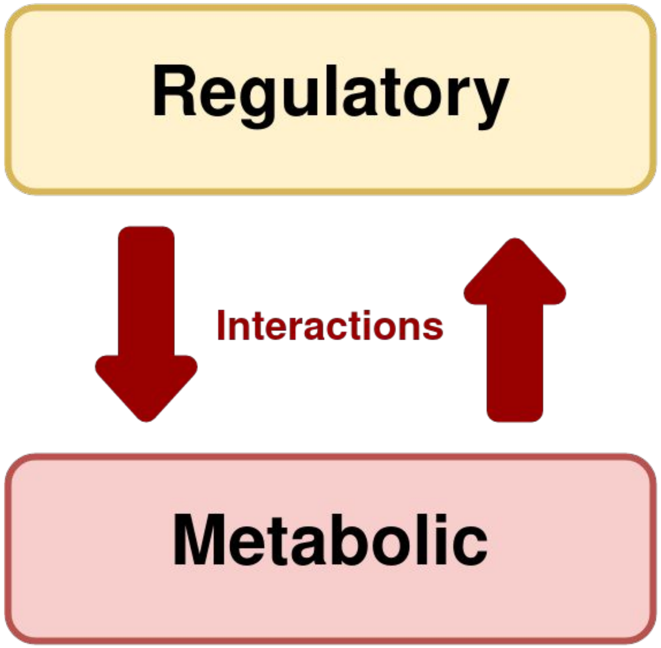
[1] S. Videla et al., **Bioinformatics**, 2016

Multiplicity of modelling formalisms

Two models with different dynamics

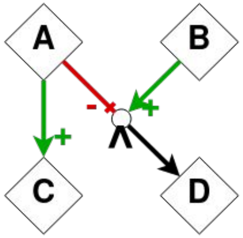


Regulatory

Interactions

Metabolic

Discrete dynamics[1]



Model

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

Logical rules (Boolean network)

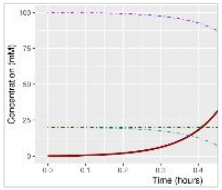
Inputs				Outputs			
x_A	x_B	x_C	x_D	x_A	x_B	x_C	x_D
0	0	x	x	0	0	0	0
0	1	x	x	0	1	0	1
1	0	x	x	1	0	1	0
1	1	x	x	1	1	1	0

Truth table (simulation)

Steady-states approximation[2]

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

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

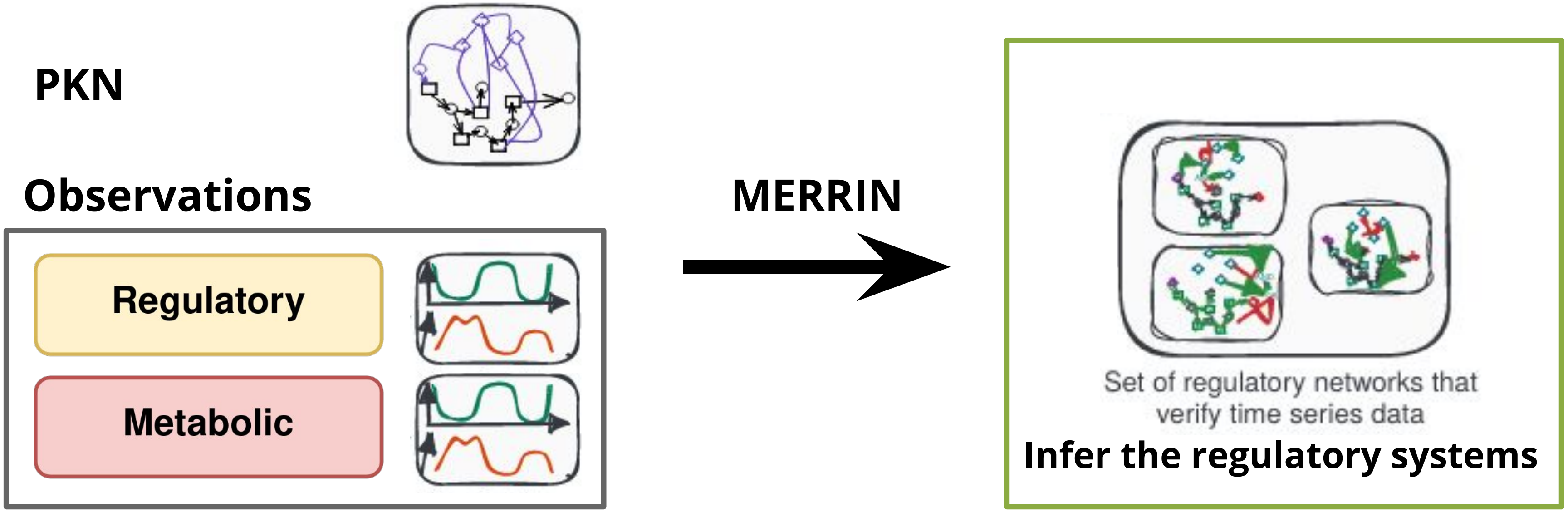
Contribution: MERRIN[1]

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

Set of regulatory networks that verify time series data

Infer the regulatory systems

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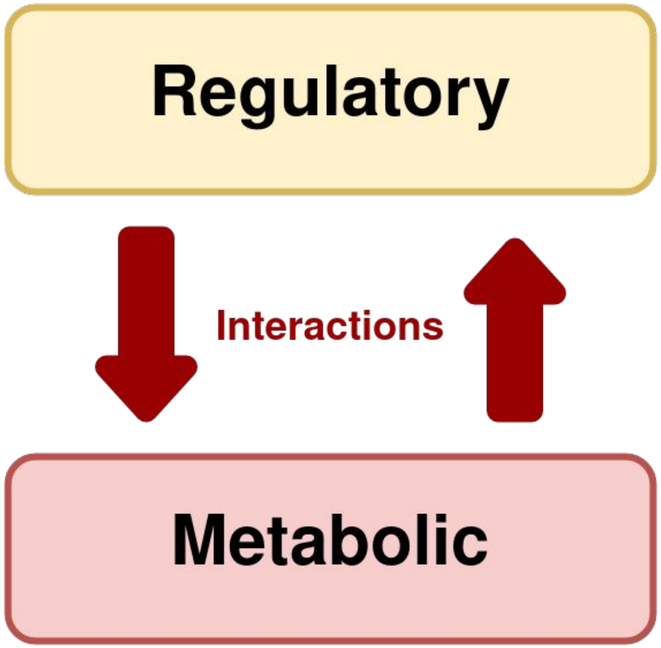
[1] Implementation available on *https://github.com/bioasp/merrin/*

Underlying simulation formalism

Several simulations approaches

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



Regulatory

Interactions

Metabolic

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

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

3. Update the cell environment

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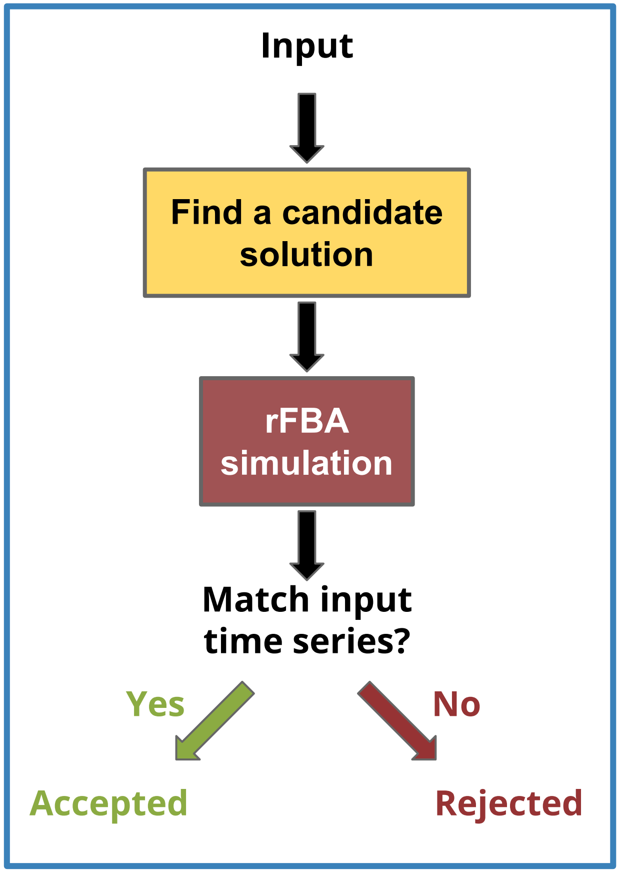
[1] M. W. Covert et al., **Journal of theoretical biology**, 2001

Inferring workflow

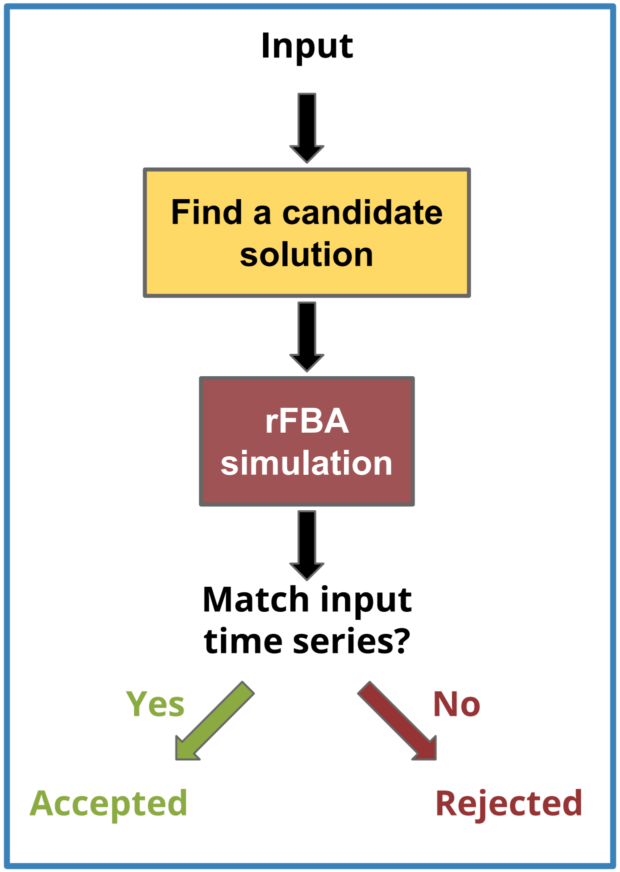
rFBA is directly used in the inferring process



Inferring workflow in a nutshell

Inferring workflow

rFBA is directly used in the inferring process


Input

Find a candidate solution

rFBA simulation

Match input time series?

Yes

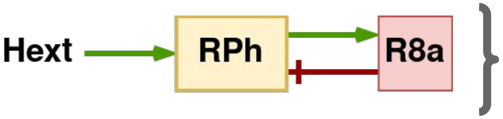
No

Accepted

Rejected

Inferring workflow in a nutshell

No issue for small instance

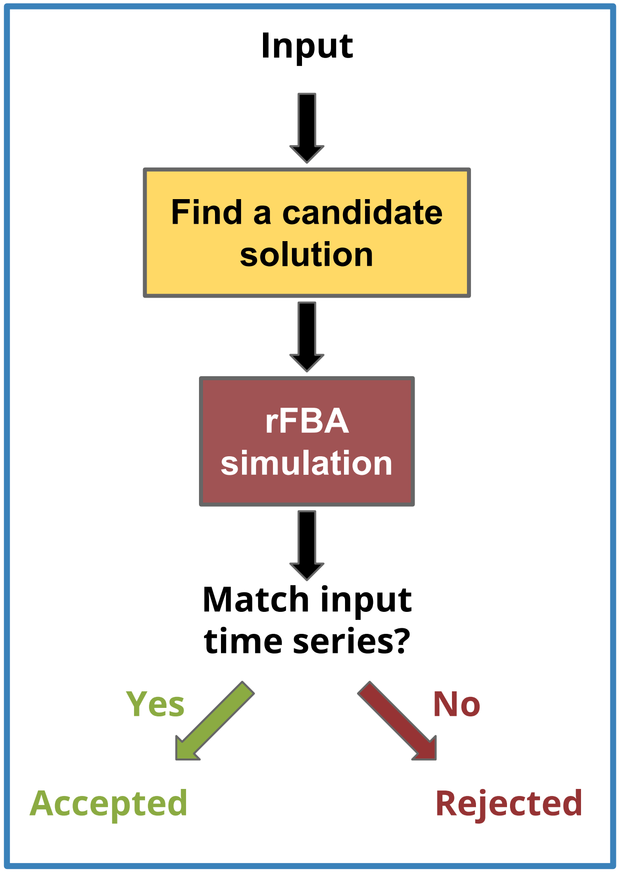


36 compatible regulatory networks

At most 36 rFBA simulations

Inferring workflow

rFBA is directly used in the inferring process



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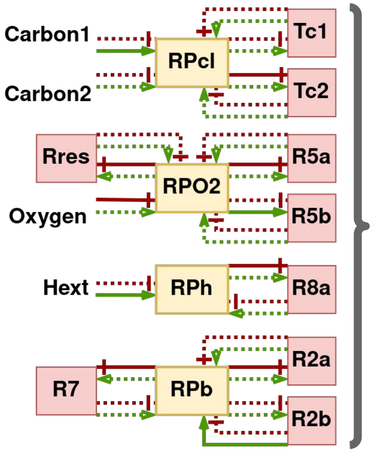
Inferring workflow in a nutshell

No issue for small instance

36 compatible regulatory networks

At most 36 rFBA simulations

Not tractable with bigger ones

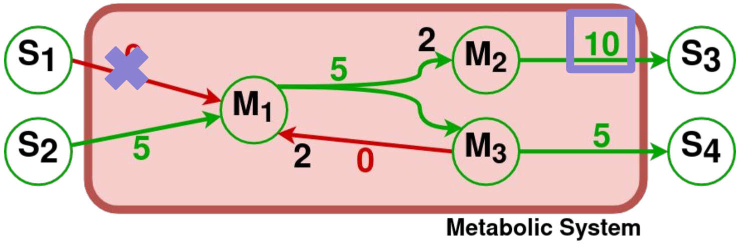


~2.9×10^{12} compatible regulatory networks

Solving ~2.9×10^{12} rFBA simulations is not tractable

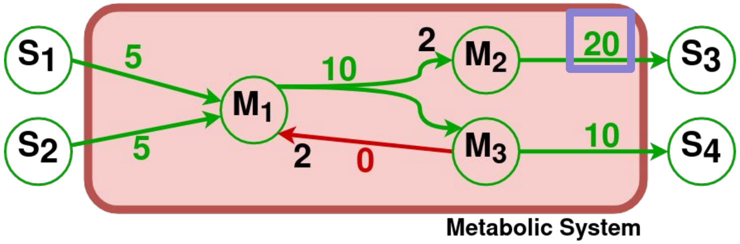
Counter-examples generalisation

Monotone property over network structure



***With** an inhibited reaction*
Less biomass produced

$\leq$

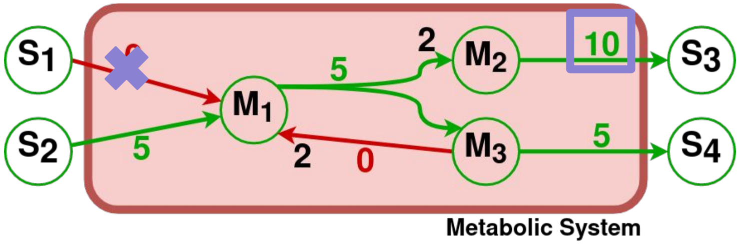


***Without** an inhibited reaction*
More biomass produced

Inhibiting a new reaction can only decrease the biomass production

Counter-examples generalisation

Monotone property over network structure



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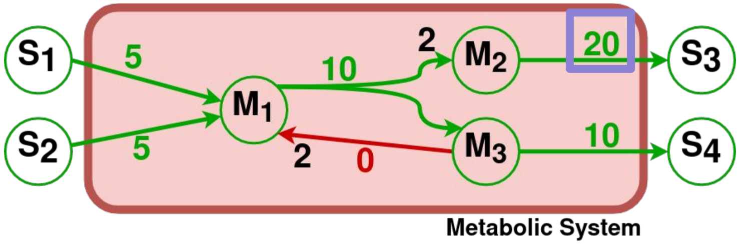


Without *an inhibited reaction*
More biomass produced

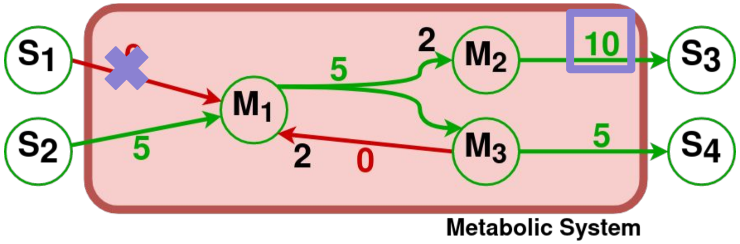
New constraints for candidate solution can be added

- **Too much biomass produced → not enough inhibited reactions**
  All ***subsets of inhibited reactions*** *will produce too much biomass*

Inhibiting a new reaction can only decrease the biomass production

Counter-examples generalisation

Monotone property over network structure



With *an inhibited reaction*
Less biomass produced

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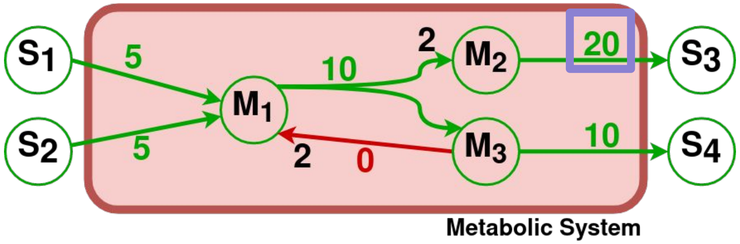


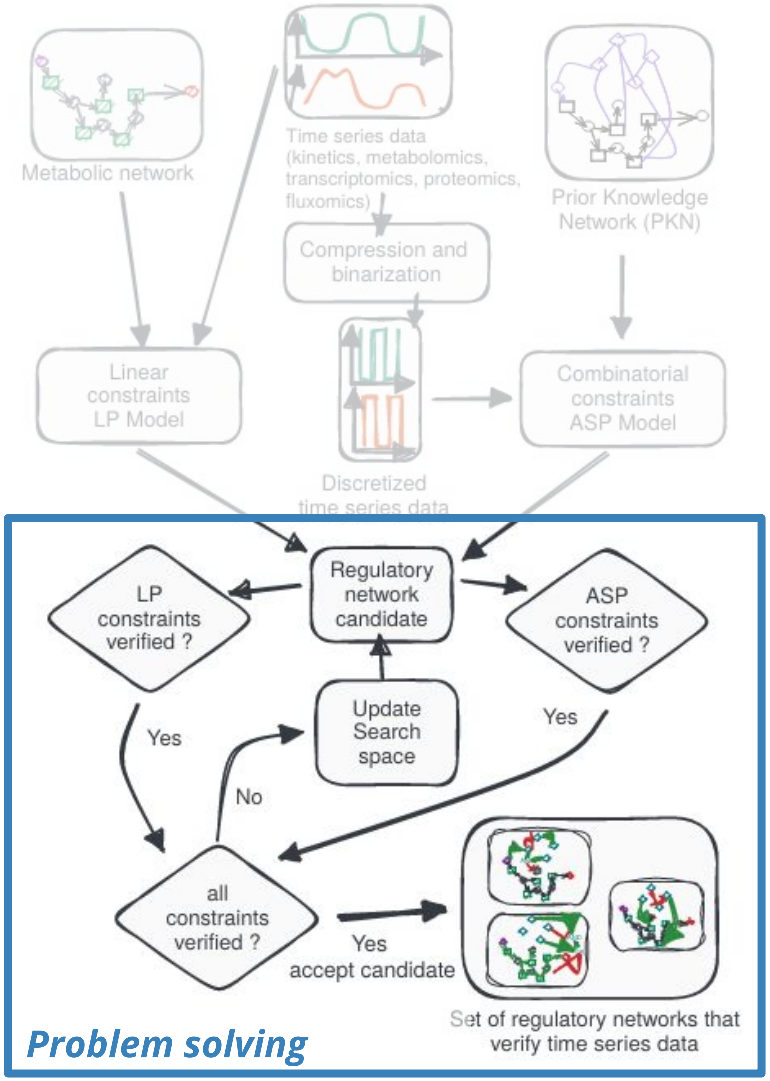
Without *an inhibited reaction*
More biomass produced

New constraints for candidate solution can be added

- **Too much biomass produced → not enough inhibited reactions**
  All ***subsets of inhibited reactions*** *will produce too much biomass*
- **Not enough biomass produced → too much inhibited reactions**
  All ***supersets of inhibited reactions*** *will produce not enough biomass*

Inhibiting a new reaction can only decrease the biomass production

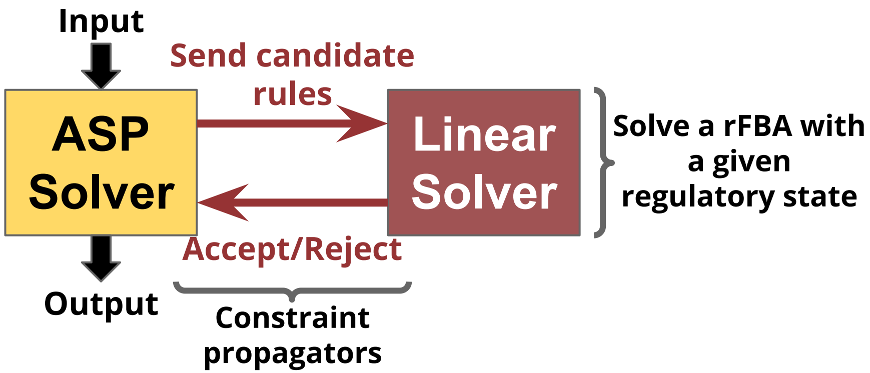
MERRIN’s workflow



Problem solving

Rely on hybrid solving approach

(Answer Set Programming + Linear Programming)



Input

ASP Solver

Send candidate rules

Accept/Reject

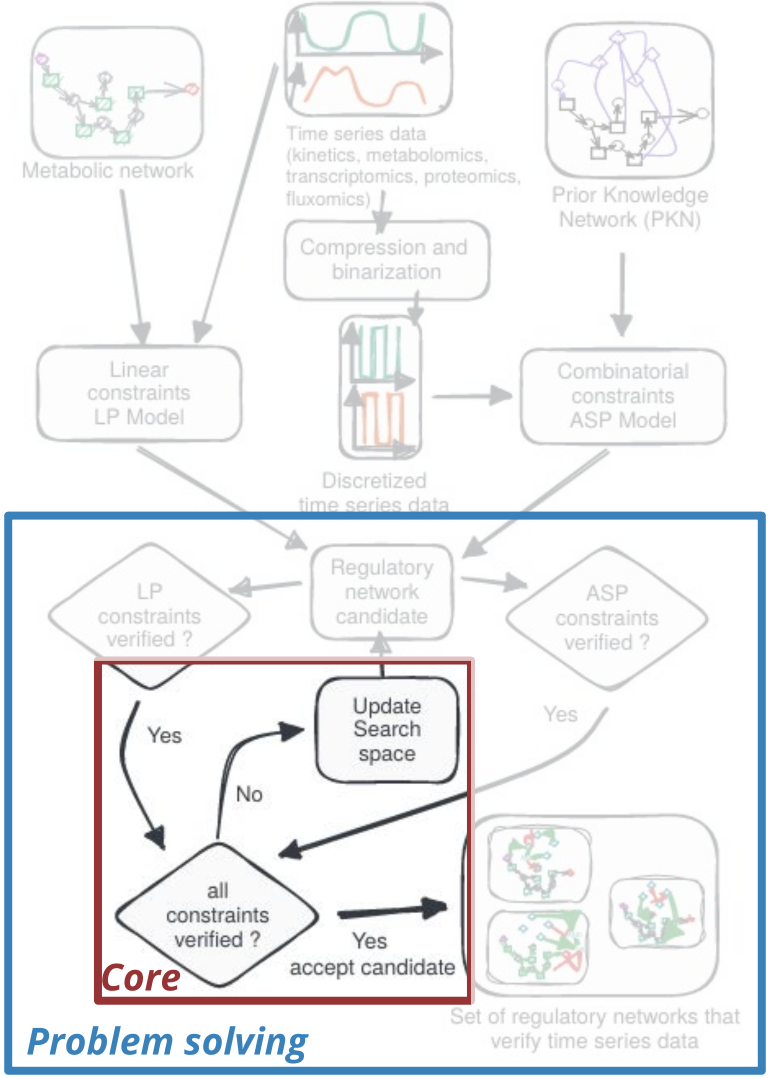
Linear Solver

Solve a rFBA with a given regulatory state

Output

Constraint propagators

MERRIN’s workflow



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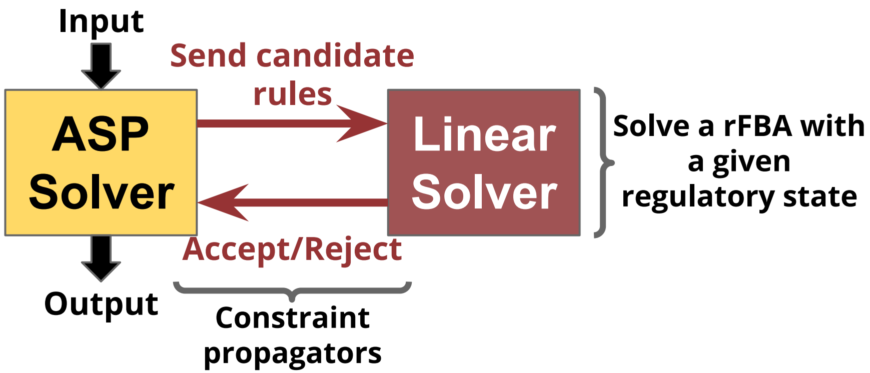
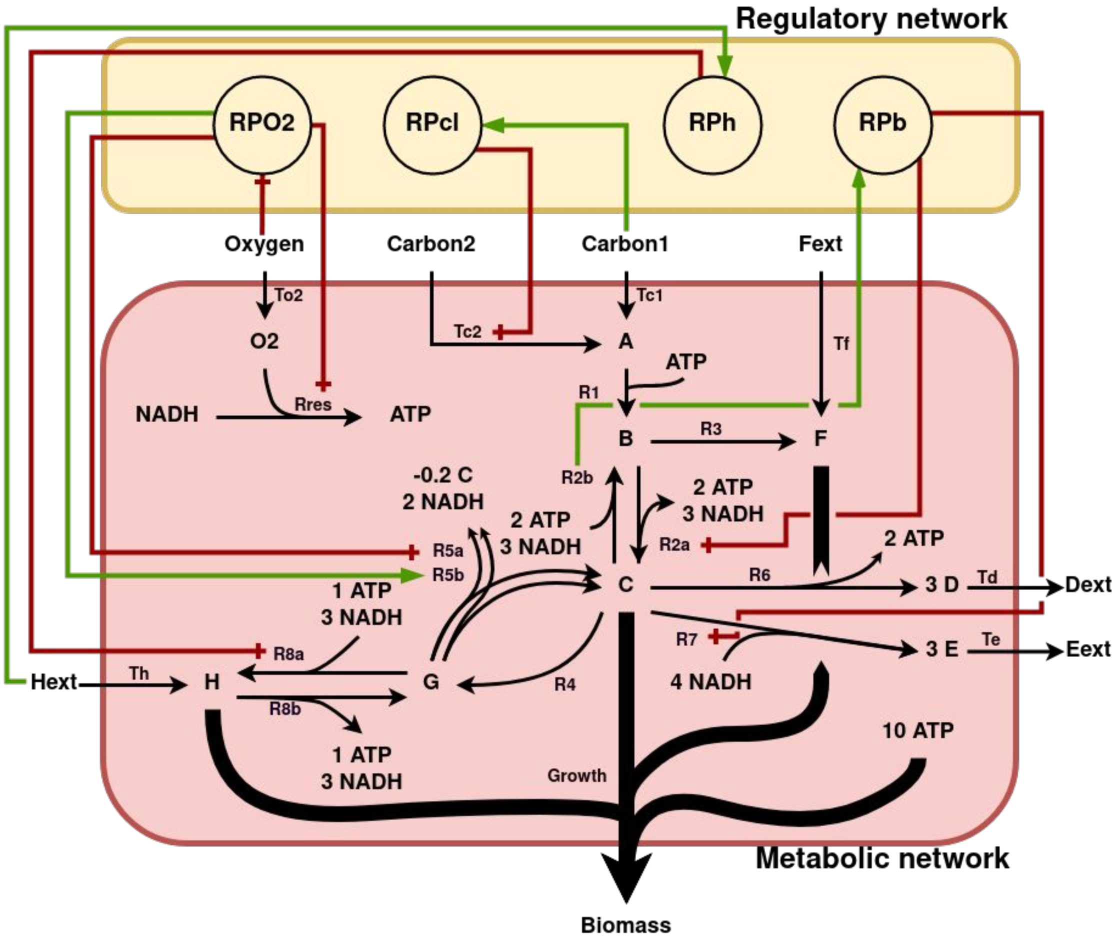


Accelerates the solving process by pre-filtering wrong solutions

→ *Monotone properties on regulatory network structures*
→ *Over-generalisation of counter-examples*

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

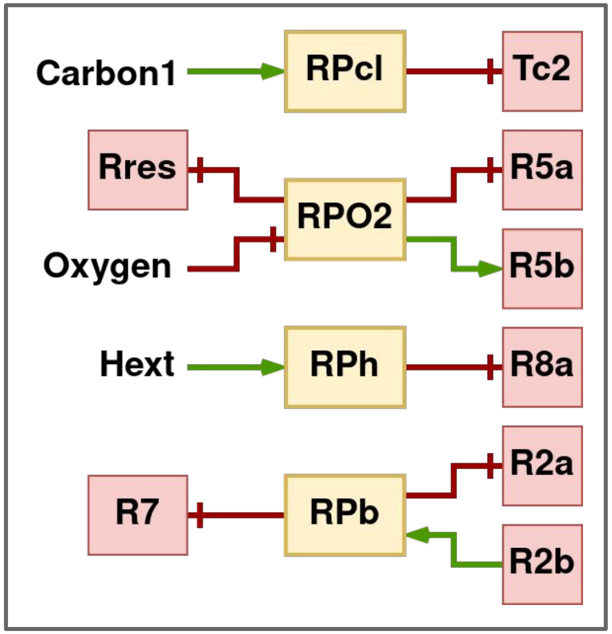


Toy model based on *E.coli*

20 reactions, 4 regulatory proteins,
11 regulations

Model complex behaviours

Diauxic shift, aerobic/anaerobic growth, etc.

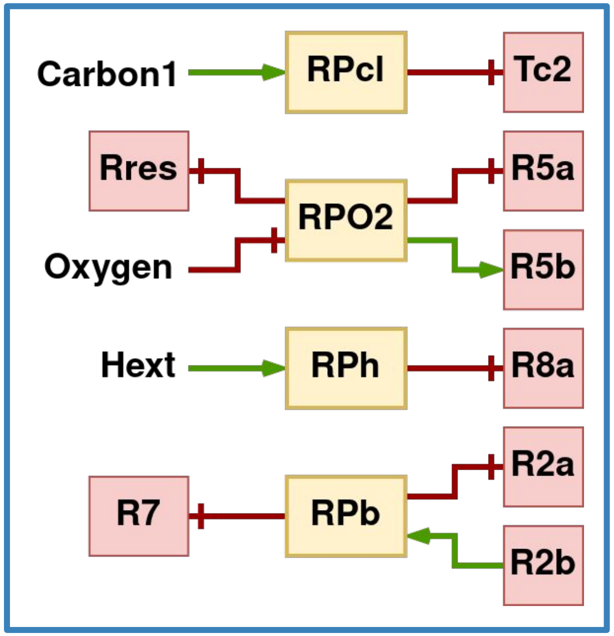


Influence graph

MERRIN inputs

Prior Knowledge Network

Carbon1	*Metabolite*	⊣	*inhibition*
RPcl	*Regulatory protein*	→	*activation*
Tc2	*Reaction*		



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

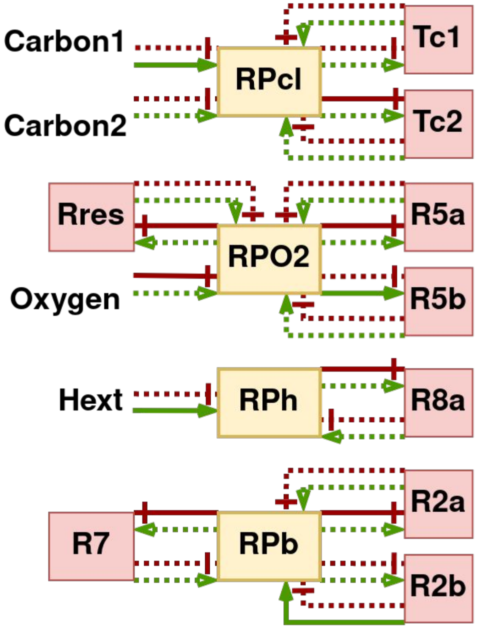
Carbon1 *Metabolite*

RPcl *Regulatory protein*

Tc2 *Reaction*

⊣ *inhibition*

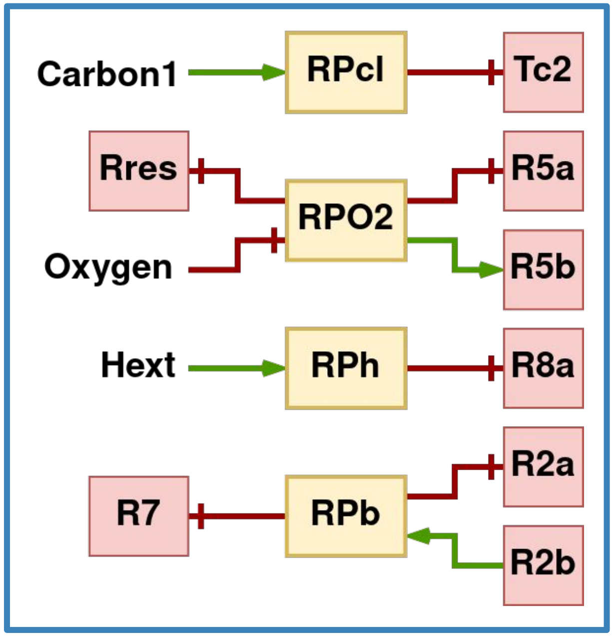
→ *activation*



*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

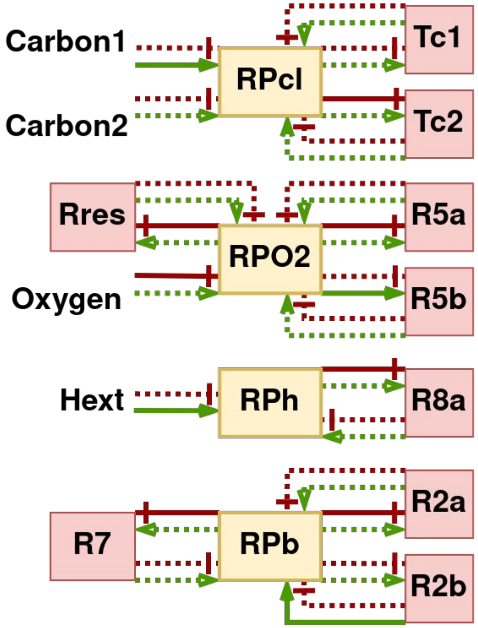
MERRIN inputs

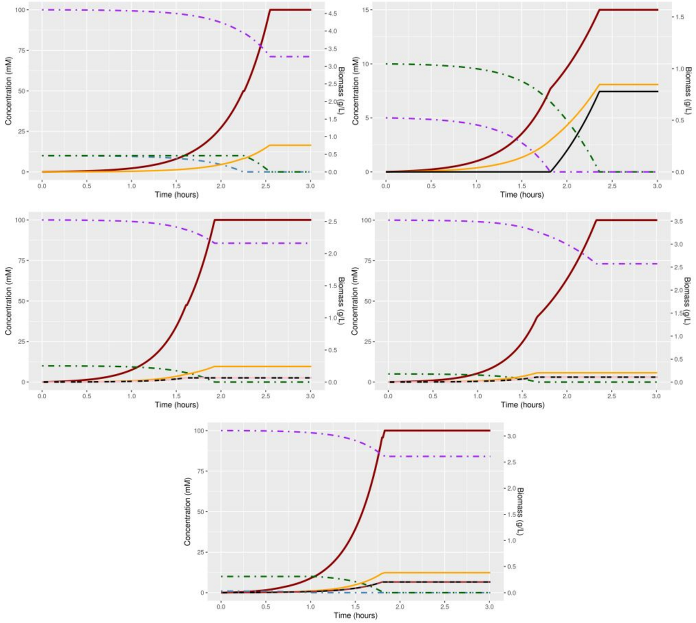
Prior Knowledge Network

Add hypothetical regulations
Remove sign + direction of interactions

5 simulated experiments

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





~2.9x10^{12} potential Boolean networks

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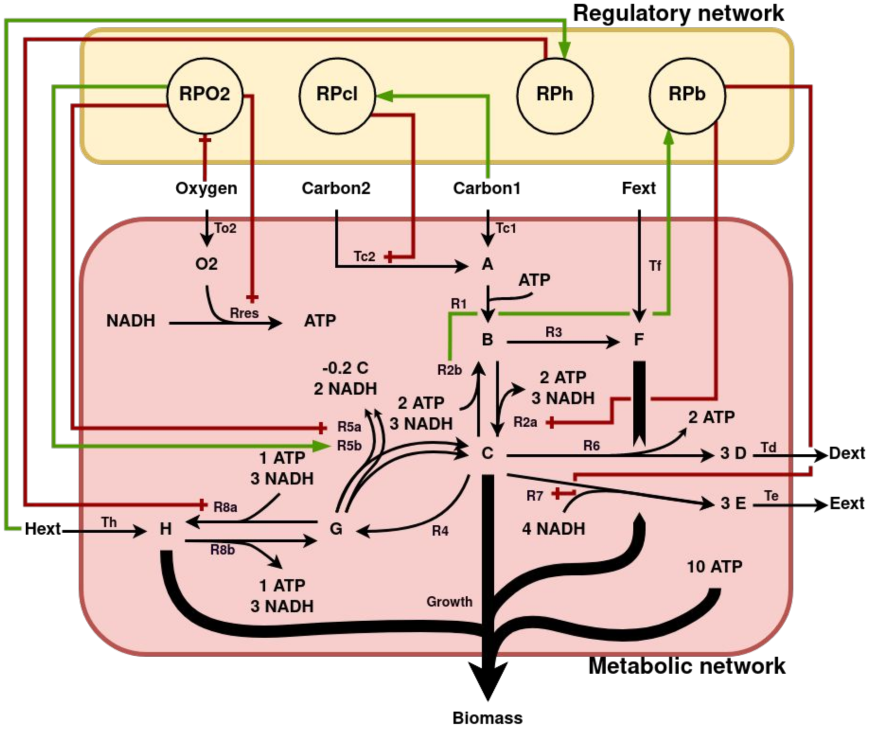
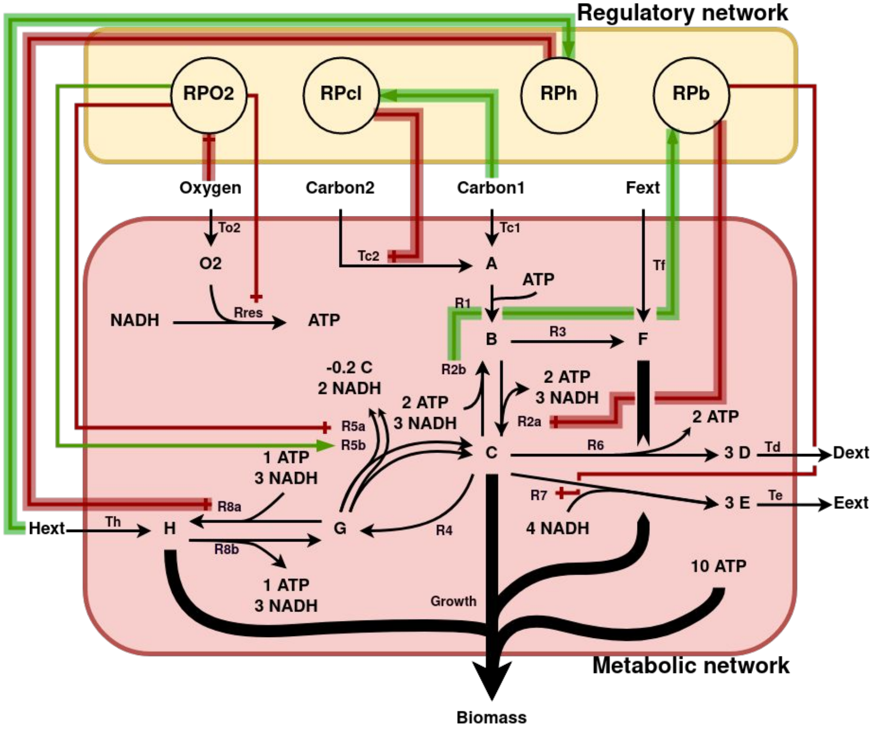
[1] M. W. Covert et al., **Journal of theoretical biology**, 2001

MERRIN validation



Gold standard is in the set of inferred models

MERRIN validation



Focus on smallest BNs

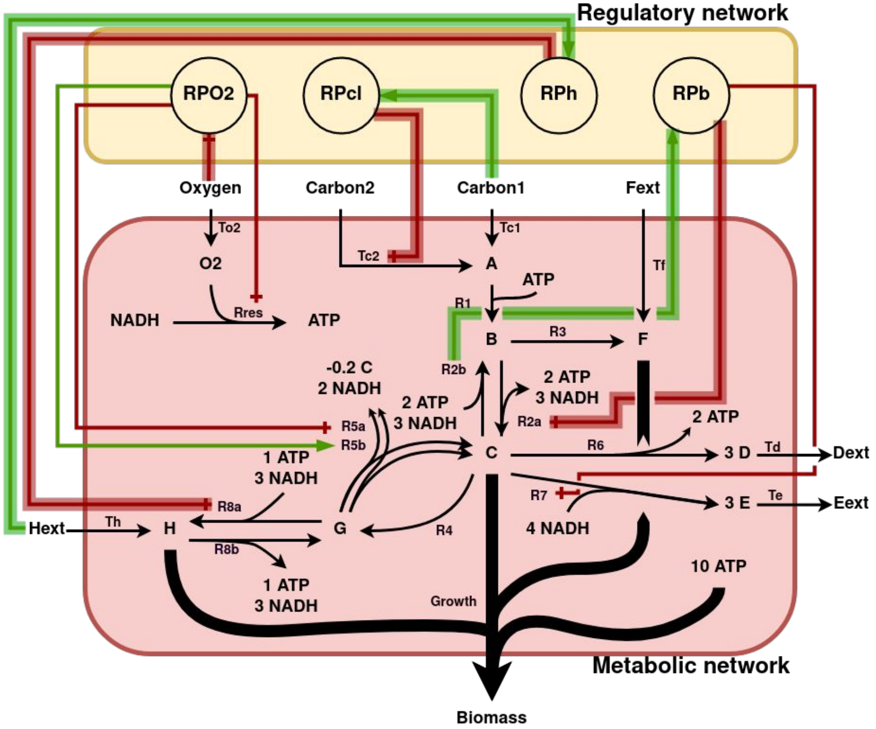
1 smallest BN

Computation time: ~7s

Subset of gold standard BN

Precision: 1.0 / Recall: 0.64

MERRIN validation



Focus on smallest BNs

1 smallest BN

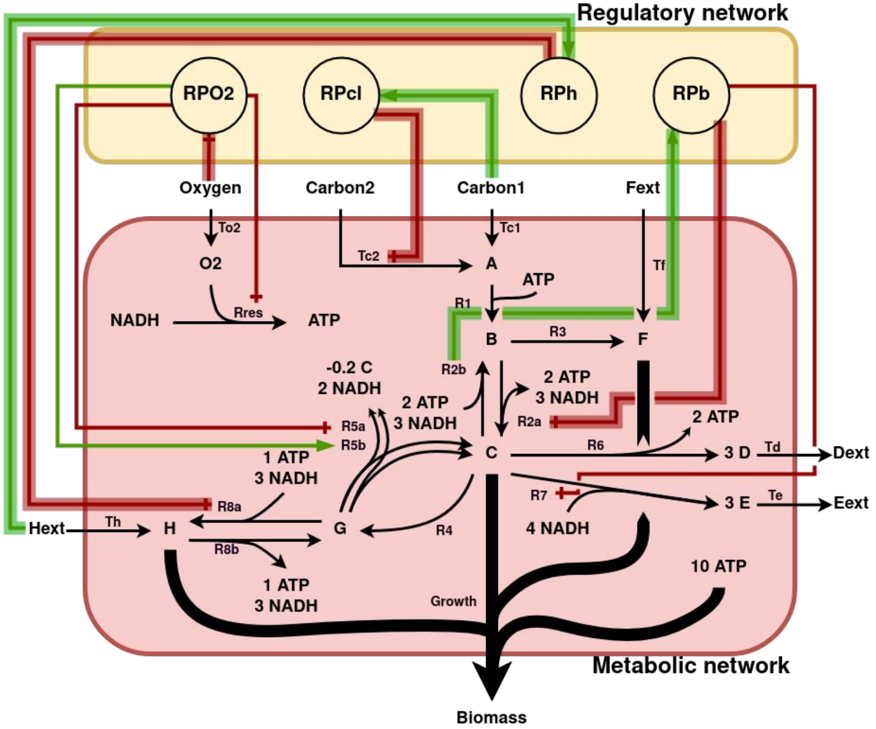
Computation time: ~7s

Subset of gold standard BN

Precision: 1.0 / Recall: 0.64

Did we fail inferring missing regulations ?

MERRIN validation



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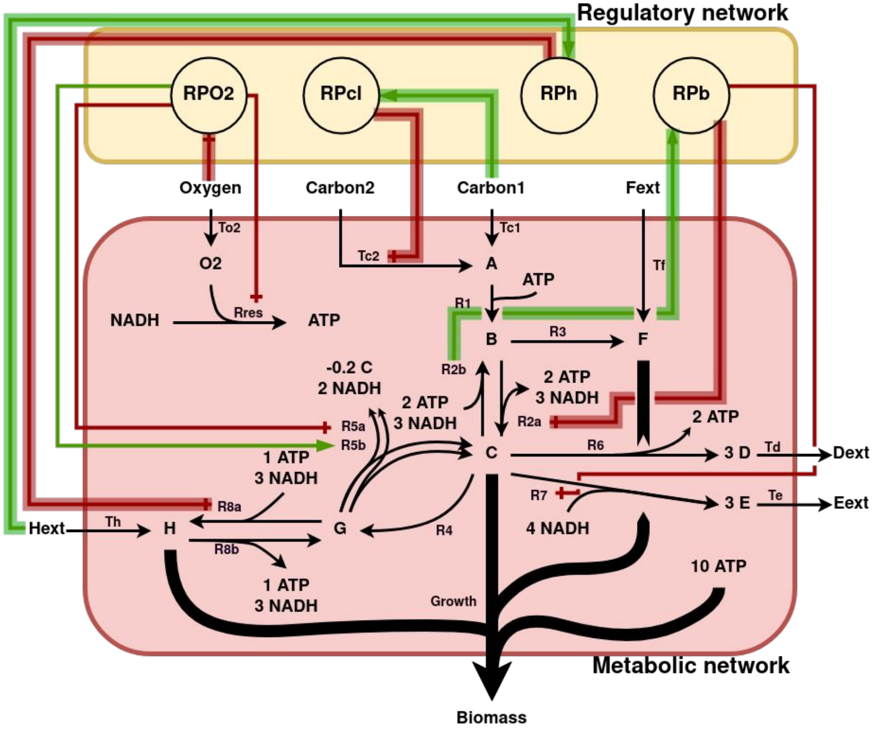
Reproduce exactly the input time series

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

Unrecovered regulations can be explained

Example Rres:

→ Enzyme decay is needed to capture it

→ Does not impact rFBA optimum

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*

Application to 3 classes of measures

Observations of the *regulatory and metabolic* system activities

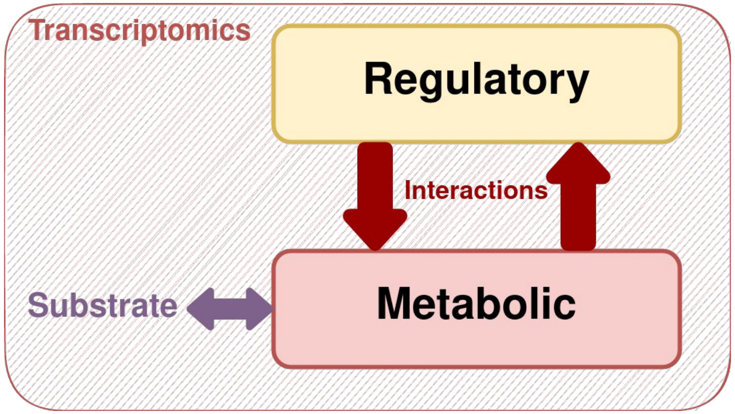
⟶ ***Quantitative*** *and* ***qualitative*** *measurements*

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

3 data types supported by MERRIN:

➔ **Transcriptomics** (qualitative)

Analysis of the RNA transcripts



Application to 3 classes of measures

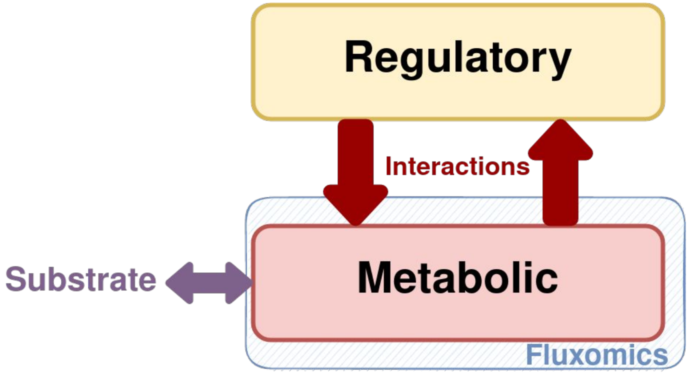
Observations of the *regulatory and metabolic* system activities

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

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

3 data types supported by MERRIN:

- ➔ **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*

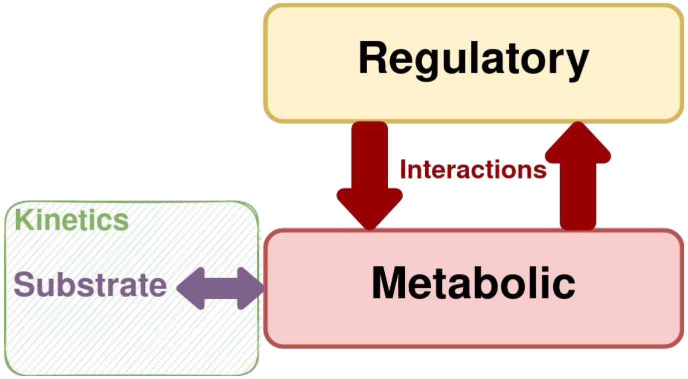
⟶ ***Simulations** mimicking experimental data*

3 data types supported by MERRIN:

➔ **Transcriptomics** (qualitative)
Analysis of the RNA transcripts

➔ **Fluxomics** (quantitative)
Rates of metabolic reactions

➔ **Kinetics** (quantitative)
Substrate concentrations



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:

➔ **Transcriptomics** (qualitative)

Analysis of the RNA transcripts

➔ **Fluxomics** (quantitative)

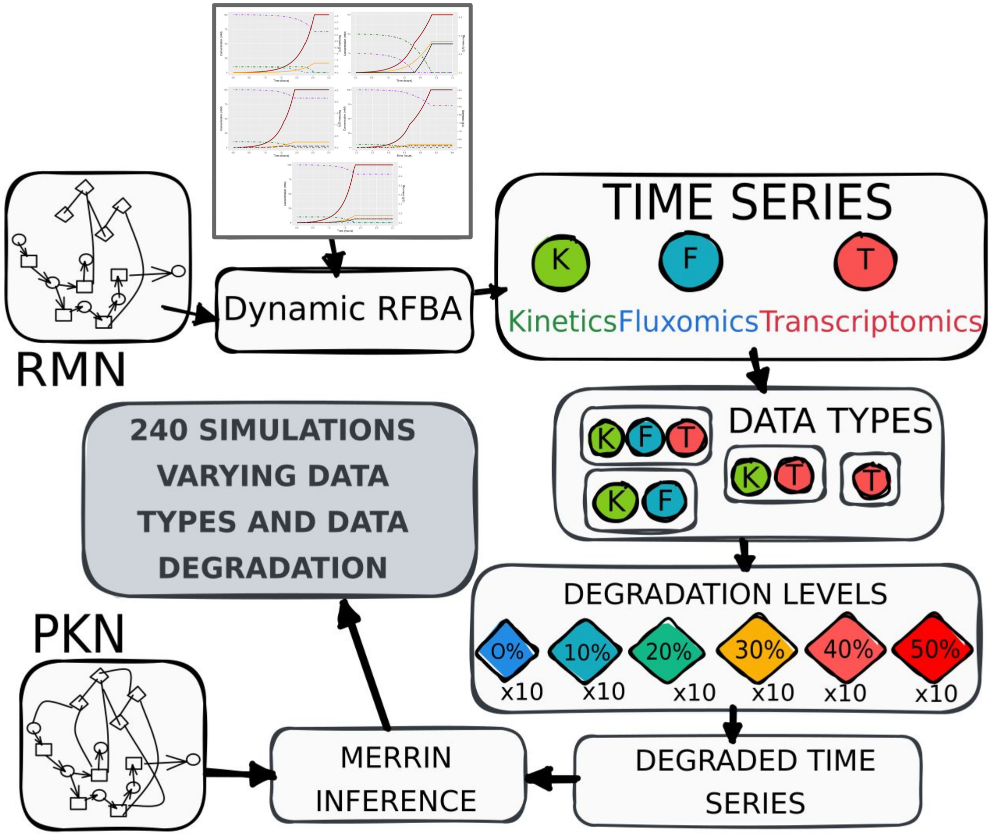
Rates of metabolic reactions

➔ **Kinetics** (quantitative)

Substrate concentrations

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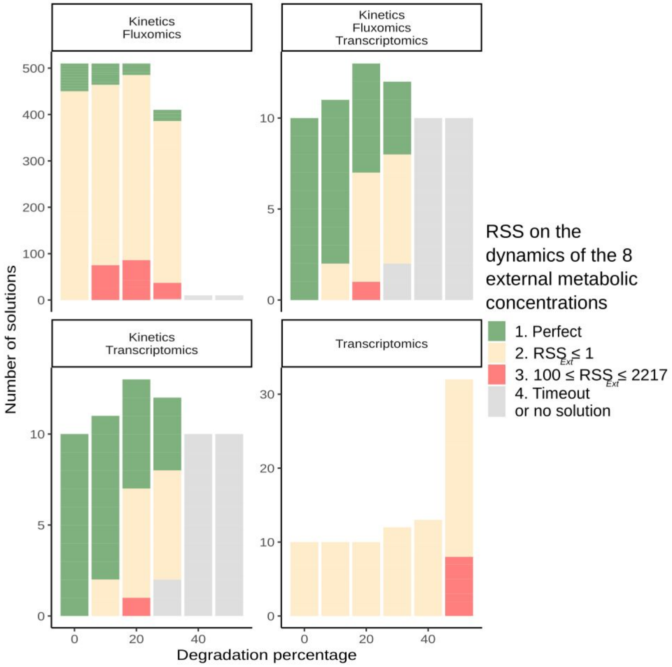
[1] M. W. Covert et al., **Journal of theoretical biology**, 2001

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*

- **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] K. Thuillier et al., **Oxford Bioinformatics**, 2022

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