Qualitative Reasoning for Reaction Networks with Partial Kinetic Information

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Motivation

B.Subtilis as production machine

surfactine (peptide) overproduction
leucine (metabolite) overproduction

Prediction Tasks

target:	overproduction of metabolites
questions:	which influxes of metabolites to increase?
	which genes to knock out?
approach:	model-based prediction
	qualitative reasoning on reaction networks

Reaction Networks

Modeling

metabolism (easy)
+ regulation (complex)

Example networks

Subti Wiki

Difficulties

partial kinetic information:	how to reason qualitatively?
no formal semantics:	what modeling language to use?

Previous Prediction Approaches

Method	Kinetic information	
Flux balance analysis	none	Almaz'04 Convert'08, ...
Constraint based methods	none except for blocking inhibitors	OptForce'12 OptKnock'13
Elementary mode analysis	none except for blocking inhibitors	Papin'04 Gruchattka'13
Abstract interpretation	mass action law with unknown parameters	Niehren'11

More Precise Kinetic Information

Why?

- Relevant for prediction of knockouts or influx changes.
- Blocking inhibitors are not good.
- Inhibitors do slow reactions down in average in steady states.

Our Approach

- Modeling language for reaction neworks
  - partial kinetic information
  - similarity constraints for kinetic functions
- Qualitative reasoning about such models
  - abstract interpretation
  - abstract unknowns away

Expression Kinetics

Species concentrations

substrates: S
inhibitor: I
activator: A
accelerator: A'

Kinetic function with parameters $k, k_1, k_2 \in \mathbb{R}_+$

$$exp_{k,k_1,k_2}(subs : S, inh : I, act : A, acc : A') = k \cdot S \cdot A \cdot \frac{k_1 + A'}{k_2 + I}$$

Can be generalized to multiple activators and accelerators

Mass-Action Law

$$ma_k(subs : S_1, \ldots, subs : S_n) = k \cdot S_1 \cdot \ldots \cdot S_n$$

Similar Kinetic Functions

This talk

$$\kappa \sim exp \;\Leftrightarrow\; \kappa = exp_{k,k_1,k_2} \quad \text{for some } k, k_1, k_2 \in \mathbb{R}_+$$
$$\kappa \sim ma \;\Leftrightarrow\; \kappa = ma_k \quad \text{for some } k \in \mathbb{R}_+$$

General definition of similarity $\sim$

in our paper!

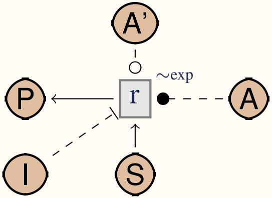
Modeling Language

Reaction networks

- Similar to petri nets.
- Kinetic functions up to similarity.

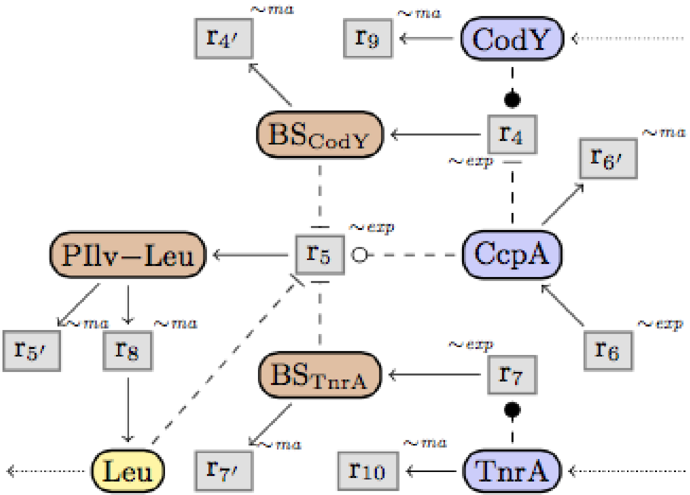
Graphical syntax

P product
I inhibitor



A activator
A' accelerator
S substrate

Example: Regulation of Ilv-Leu Promoter



Prediction Problem

Target

Overproduction of Leucine: $y_{\text{Leu}} = \uparrow$

Which change leads to target?

- which influx must be increased or decreased?

Steady State Semantics

As usual

- Steady state equations can be computed from reaction network.

But

- With variables for unknown kinetic functions.
- Variables are subject to similarity constraints.

Steady-State Equations for PIlv-Leu network

Flux balance equations:

(Leu)	$v_{r_8} = y_{\mathrm{Leu}}$
(CcpA)	$v_{r_6} = v_{r_{6'}}$
(CodY)	$x_{\mathrm{CodY}} = v_{r_9}$
(TnrA)	$x_{\mathrm{TnrA}} = v_{r_{10}}$
($\mathrm{BS}_{\mathrm{CodY}}$)	$v_{r_4} = v_{r_{4'}}$
(PIlv−Leu)	$v_{r_5} = v_{r_{5'}} + v_{r_8}$
($\mathrm{BS}_{\mathrm{TnrA}}$)	$v_{r_7} = v_{r_{7'}}$

Outfluxes:

$$y_{\mathrm{Leu}} = ma^{(8)}(subs\colon z_{\mathrm{Leu}})$$

Reaction rates:

$$v_{r_4} = exp^{(1)}(inh\colon z_{\mathrm{CcpA}}, act\colon z_{\mathrm{CodY}})$$

$$v_{r_{4'}} = ma^{(1)}(subs\colon z_{\mathrm{BS}_{\mathrm{CodY}}})$$

$$v_{r_5} = exp^{(2)}(inh\colon z_{\mathrm{BS}_{\mathrm{CodY}}}, acc\colon z_{\mathrm{CcpA}}, inh\colon z_{\mathrm{Leu}}, inh\colon z_{\mathrm{BS}_{\mathrm{TnrA}}})$$

$$v_{r_{5'}} = ma^{(2)}(subs\colon z_{\mathrm{PIlv-Leu}})$$

$$v_{r_6} = exp^{(3)}()$$

$$v_{r_{6'}} = ma^{(3)}(subs\colon z_{\mathrm{CcpA}})$$

$$v_{r_7} = exp^{(4)}(act\colon z_{\mathrm{TnrA}})$$

$$v_{r_{7'}} = ma^{(4)}(subs\colon z_{\mathrm{BS}_{\mathrm{TnrA}}})$$

$$v_{r_8} = ma^{(5)}(subs\colon z_{\mathrm{PIlv-Leu}})$$

$$v_{r_9} = ma^{(6)}(subs\colon z_{\mathrm{CodY}})$$

$$v_{r_{10}} = ma^{(7)}(subs\colon z_{\mathrm{TnrA}})$$

Simplified Steady-State Equations

$$
\begin{aligned}
v_{r_5} &= v_{r_{5'}} + y_{\mathrm{Leu}} \\
y_{\mathrm{Leu}} &= ma^{(8)}(subs\colon z_{\mathrm{Leu}}) \\
v_{r_4} &= exp^{(1)}(inh\colon z_{\mathrm{CcpA}}, act\colon z_{\mathrm{CodY}}) \\
v_{r_4} &= ma^{(1)}(z_{\mathrm{BS}_{\mathrm{CodY}}}) \\
v_{r_5} &= exp^{(2)}(inh\colon z_{\mathrm{BS}_{\mathrm{CodY}}}, acc\colon z_{\mathrm{CcpA}}, \\
&\qquad inh\colon z_{\mathrm{Leu}}, inh\colon z_{\mathrm{BS}_{\mathrm{TnrA}}}) \\
v_{r_{5'}} &= ma^{(2)}(subs\colon z_{\mathrm{PIlv-Leu}})
\end{aligned}
$$

$$
\begin{aligned}
v_{r_6} &= exp^{(3)}() \\
v_{r_6} &= ma^{(3)}(subs\colon z_{\mathrm{CcpA}}) \\
v_{r_7} &= exp^{(4)}(act\colon z_{\mathrm{TnrA}}) \\
v_{r_7} &= ma^{(4)}(subs\colon z_{\mathrm{BS}_{\mathrm{TnrA}}}) \\
y_{\mathrm{Leu}} &= ma^{(5)}(subs\colon z_{\mathrm{PIlv-Leu}}) \\
x_{\mathrm{CodY}} &= ma^{(6)}(subs\colon z_{\mathrm{CodY}}) \\
x_{\mathrm{TnrA}} &= ma^{(7)}(subs\colon z_{\mathrm{TnrA}})
\end{aligned}
$$

Abstract Interpretation of Steady State Equations

concrete domain	abstract domain
$\mathbb{R}_+$ = real numbers	$\Delta = \{\uparrow, \downarrow, \doteq\}$
$\cdot$	$\cdot^\Delta$
$+$	$+^\Delta$
arithmetic equations	difference constraints
$z_\mathrm{P} = k \cdot z_\mathrm{S} \cdot z_\mathrm{A}$	$z_\mathrm{P} \in z_\mathrm{S} \cdot^\Delta z_\mathrm{A}$

Difference Constraints for PIlv-Leu Network

$$
\begin{array}{lll}
v_{r_5} \in v_{r_{5'}} + y_{Leu} & v_{r_{5'}} = z_{PIlv-Leu} & \\
y_{Leu} = z_{Leu} & v_{r_6} = \doteq & y_{Leu} = z_{PIlv-Leu} \\
v_{r_4} \in z_{CodY} \cdot Inh(z_{CcpA}) & v_{r_6} = z_{CcpA} & x_{CodY} = z_{CodY} \\
v_{r_4} = z_{BS_{CodY}} & v_{r_7} = z_{TnrA} & x_{TnrA} = z_{TnrA} \\
v_{r_5} \in z_{CcpA} \cdot Inh(z_{BS_{CodY}} + z_{Leu} + z_{BS_{TnrA}}) & v_{r_7} = z_{BS_{TnrA}} &
\end{array}
$$

where

$$
\begin{array}{lll}
Inh(\uparrow) = \{\downarrow\} & \uparrow + \uparrow = \{\uparrow\} & \uparrow \cdot \uparrow = \{\uparrow\} \\
Inh(\downarrow) = \{\uparrow\} & \uparrow + \downarrow = \{\uparrow, \downarrow, \doteq\} & \ldots \\
Inh(\doteq) = \{\doteq\} & \ldots &
\end{array}
$$

Solutions of Difference Constraints $\Rightarrow$ Predictions of Influx Changes

$x_{\mathrm{CodY}} = \downarrow \quad \Rightarrow \quad$ decrease influx of CodY

$x_{\mathrm{TnrA}} = \downarrow \quad \Rightarrow \quad$ decrease influx TnrA

Qualitative Reasoning by Constraint Simplification

$$
\begin{array}{llllllll}
(\mathrm{no}_1) & \mathit{Inh}(\doteq) \Rightarrow \doteq & (\mathrm{no}_3) & t \cdot \doteq \Rightarrow t & (\mathrm{ip}) & x + x \Rightarrow x \\
(\mathrm{no}_2) & \mathit{Acc}(\doteq) \Rightarrow \doteq & (\mathrm{no}_4) & \doteq \cdot t \Rightarrow t & (\mathrm{si}) & t \in t' \Rightarrow t = t' \\
(\mathrm{bv}) & \exists x.\ (x = t \wedge \psi) \Rightarrow \psi[t/x] & & & (\mathrm{inh}) & t \in \mathit{Inh}(t + s) \Rightarrow t \in \mathit{Inh}(s)
\end{array}
$$

Simplication of Difference Constraints for PIlv-Leu

Result of exhaustive simplification

$$\uparrow \in \mathit{Inh}(x_{\mathrm{CodY}} + x_{\mathrm{TnrA}})$$

Equivalent to:

$$x_{\mathrm{CodY}} = \downarrow \text{ or } x_{\mathrm{TnrA}} = \downarrow$$

Application (Biotechnology Journal 2015)

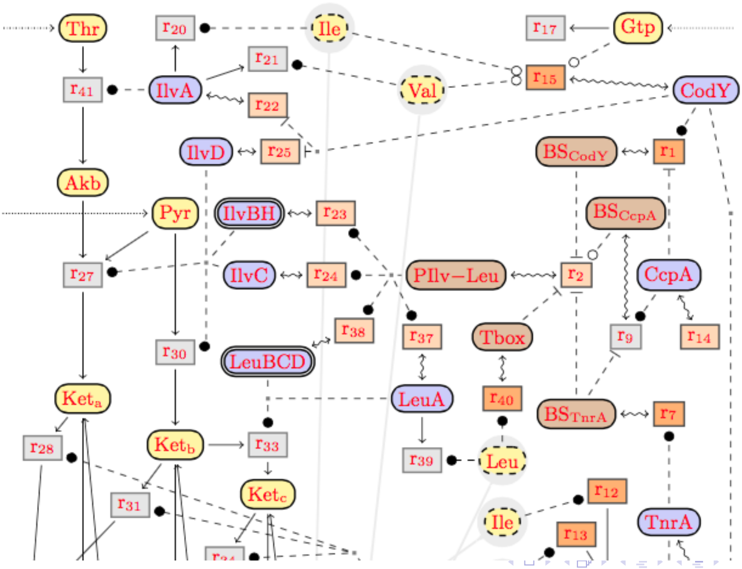
Bigger Leucine model

improving on model in Subti Wiki

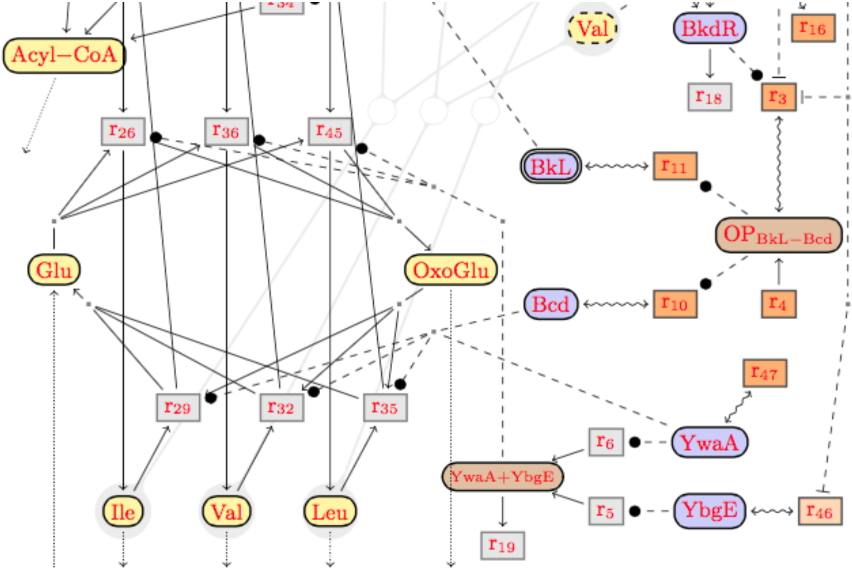
$\geq$ 50 reactions

formal semantics

Big Leucine Network: Upper half



Big Leucine Network: Lower half



Knockout prediction for leucine overproduction

Knockout prediction

21 knockout kandidates
12 predicted for leucine overproduction

Experimental validation

- 6 knockouts verified in wet lab
- all of them increase leucine production!
- CodY knockout optimal for surfactine production: as good as leucine feeding

Future Work

- Other overproduction tasks
- Double knockout prediction
- Quantitative prediction
- Quantitative models