Possibilities of Petri Net Theory to validate metabolic pathways

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Bertinoro, 14th June 2004
Outline

- Introduction
- Petri net basics
- Analysis possibilities
- Sucrose-to-starch breakdown in the potato tuber
- Simulation of the net
- Conclusions
Introduction

Metabolic Control Analysis - MCA

Metabolic system: connected unit, steady state

MCA is based on solution of systems of differential equations


• **Biochemical systems theory**

• **Flux oriented theory**

**GEPASI**  Mendes, *Comp.Appl.Biosci.* (1993)
Introduction

Graph-Theory

- Hybrid graphs  

- Bond graphs  
  Lefèvre & Barreto, *J.Franklin Inst.* (1985)

- Net-thermodynamics  

- Weighted linear graphs  
  Goldstein & Shevelev, *J.Theor.Biol.* (1985)  

- Meta-nets (with gene expression systems)  

- Bipartite graphs  

- KING (KINetic Graphs)  
Introduction

• Why is a model validation (check model consistency) useful?
  - Before starting a quantitative analysis it should be sure that the model is valid.
  - If the systems become larger with many interactions and regulations it could not be done manually any more.

• How model validation could be performed?
  By qualitative analysis

  Basic dynamic properties: liveness, reversibility, boundedness, dead states, deadlocks, traps,
  Basic structure properties: invariants, robustness, alternative pathways,

Petri net theory provides algorithms and tools to answer these questions.
etri net basics

**Petri nets** (PhD thesis of Carl Adam Petri 1962)
abstract models of information and control data flows, which allow to describe systems and processes at different abstraction levels and in a unique language - developed for systems with causal concurrent processes

**Applications:** business processes, computer communication, automata theory, operating systems, software dependability

**Biological networks:** metabolic networks, signal transduction pathways, gene regulatory networks


(2003)


**Metabolic Petri Net - MPN**

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Petri nets: directed, labelled, bipartite graphs

Nodes:  
(verteces)

places transitions

○ □

passive elements active elements
conditions events
states actions
chemical compounds chemical reactions
metabolites conversions of metabolites catalysed by enzymes
etri net basics

**Arcs:**
(edges)

<table>
<thead>
<tr>
<th>pre-conditions</th>
<th>post-conditions</th>
</tr>
</thead>
</table>

![Diagram](image.png)
etri net basics

**Tokens:** movable objects in discrete units, e.g. units of substances (mole)
- ○ condition is not fulfilled
- ○ condition is (one time) fulfilled
- n condition is n times fulfilled

**Marking:** system state, token distribution, initial marking

**Token flow:** occurring of an event (firing of a transition)
etri net basics

Example: Pentose Phosphate Pathway - one reaction

\[
6\text{-Phosphogluconate} + \text{NADP}^+ \rightarrow \text{Ribose-5-phosphate} + \text{NADPH} + \text{CO}_2
\]
etri net basics

Example: Pentose Phosphate Pathway - sum reaction

\[
G6P + 2 \text{NADP}^+ + H_2O \rightarrow R5P + 2 \text{NADPH} + 2 H^+ + CO_2
\]
etri net basics

Special places:
input: substrates (source, e.g. sucrose)
output: products (sink, e.g. starch)

Special arcs: reading arcs
inhibitor arcs

Additional places & transitions:
logical
hierarchical
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Transitions in MPNs:

Reaction:

- Substrate
- Product
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Transitions in MPNs:

Reaction:

Catalysis:

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Transitions in MPNs:

Reaction:

\[ \text{substrate} \rightarrow \text{product} \]

Catalysis:

\[ \text{substrate} \rightarrow \text{enzyme} \rightarrow \text{product} \]

Auto-catalysis: pro-enzyme

\[ \text{product} = \text{enzyme} \rightarrow \text{pro-enzyme} \]
Model validation

(1) Dynamical (behavioural) properties
(2) Reachability analysis
(3) Structural analysis
(4) Invariant analysis
Dynamic (behavioural) properties

liveness and reversibility

- a net is live, if all its transitions are live in the initial marking

- a net is reversible, if the initial marking can be reached from each possible state

- How often can a transition fire? (0-times, n-times, $\infty$ times)

- infinite systems behaviour, search for dead transitions

- prediction of system deadlocks
dynamic (behavioural) properties

boundedness

- a net is bounded, if there exists a positive integer number k, which represents a maximal number of tokens on each place in all states

- What is the maximal token number for a place? 
  \( (0, 1, k, \infty) \) boundedness \( (k\text{-bounded}) \)

- for bounded nets special algorithms exist
eachability analysis

How many and which system states could be reached? (0, 1, k, ∞)

- the **reachability graph** represents all possible states
- computational problem for large and dense biological networks
- for unbounded networks: computation of the **coverability graph**

- Is a certain system state again and again reachable? **progressiveness**
- Is a certain system state never reachable? **safety**
structural analysis

- aims at discovering net structures to derive conclusions on dynamic properties

Elementary properties:
ordinary: the multiplicity of every arc is equal one
homogeneous: for any place all outgoing edges have the same multiplicity
pure: there is no transition, for which a pre-place is also a post-place (loop-free)
conservative: for each place the sum of input arc weights is equal to the sum of output arc weights – a conservative net is bounded
static conflict-free: there are no transitions with a common pre-place connected, strongly connected: in graph-theoretical sense
structural analysis

structural deadlock:
a set of places that delivers its tokens until a state is reached, where the place set is empty and there is no possibility to get a new token

trap:
the opposite situation that tokens cannot be removed from a place set
nvariant analysis

- properties, which are conserved during the working of the system
- independent of the initial marking
- only the net structure is relevant for their calculation

Are there invariant structures, which are independent from firing of the system?

Place-invariants (P-invariants)
Transition-invariants (T-invariants)
nvariant analysis

incidence matrix \( C = P \times T \)

\[
\begin{pmatrix}
-2 & 1 & 1 \\
1 & -1 & 0 \\
1 & 0 & -1
\end{pmatrix}
\]

place (P-) invariant:
\[
\begin{align*}
x \ C &= 0 \\
-2x_1 + x_2 + x_3 &= 0 \\
x_1 - x_2 &= 0 \\
x_1 - x_3 &= 0
\end{align*}
\]

transition (T-) invariant:
\[
\begin{align*}
C \ y &= 0 \\
-2y_1 + y_2 + y_3 &= 0 \\
y_1 - y_2 &= 0 \\
y_1 - y_3 &= 0
\end{align*}
\]
Minimal semi-positive solutions are of interest with
- all components of the solution vector are \( \geq 0 \)
- basis of the semi-positive solution space such that none solution is contained in another solution, Lautenbach (1973)

The calculation
- of all integer solutions is in P
- of all semi-positive solutions is in P
- of all semi-positive integer solutions is NP-complete, Schrijver (1999)

Extreme Pathways, Schilling et al. (2000)
- minimal basis of semi-positive integer solutions (Hilbert-base)
- subset of T-invariants – biological interpretation?
# Invariant Analysis

<table>
<thead>
<tr>
<th><strong>P-invariants</strong></th>
<th><strong>T-invariants</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- set of places, whose weighted sum of tokens is constant</td>
<td>- set of transitions, whose firing reproduces a given marking</td>
</tr>
<tr>
<td>- covered by P-invariants: sufficient condition for boundedness liveness</td>
<td>- covered by T-invariants: necessary condition for</td>
</tr>
<tr>
<td>- set of metabolites, whose total net concentrations remain unchanged ADP, ATP NADP⁺, NADPH</td>
<td>- minimal set of enzymes which could operate at steady state</td>
</tr>
<tr>
<td></td>
<td>- indicate the presence of cyclic firing sequences</td>
</tr>
<tr>
<td><strong>Elementary modes</strong></td>
<td><strong>Schuster, Hilgetag, Schuster (1993)</strong></td>
</tr>
</tbody>
</table>

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ucrose-to-starch-pathway in potato tuber

- rich in carbohydrates and energy
- a natural source of folate
- full of vitamin C
- low in calories
- good source of niacin, vitamin B6, iodine, thiamine, and minerals
- no cholesterol
- completely fat free

Fernie, Willmitzer, Trethewey (2002)

Research interest: increasing the starch content

Co-operations: Max Planck Institute for Molecular Plant Physiology, Golm
Brandenburg University of Technology at Cottbus

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ucrose-to-starch-pathway in potato tuber

Invertase

sucrose

sucrose-synthase

fructose

UDP-glucose

UDP

fructose-6-P

phospho-glucokinase

phosphogluco-isomerase

UDP-glucose-1-P

phosphogluco-mutase

glucose-1-P

starch

glycolysis

ATP

ADP

hexokinase

ATP

ADP

glucose-6-P

ATP

ADP

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Sucrose-to-starch-pathway in potato tuber

Invertase

Sucrose

Sucrose-synthase

Pi

Sucrose-6-P

UDP-glucose

UDP

Sucrose-phosphate phosphatase

Fructose

Fructose-6-P

Phospho-glucose isomerase

ATP

ADP

Hexokinase

Glucose

Glucose-6-P

Phosphogluco-mutase

ATP

ADP

Starch

Glycolysis

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# ucrose-to-starch-pathway in potato tuber

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>sucrose synthase:</td>
<td>Suc + UDP ↔ UDPglc + Frc</td>
</tr>
<tr>
<td>UDP-glucose Pyrophosphorylase:</td>
<td>UDPglc + PP ↔ G1P + UTP</td>
</tr>
<tr>
<td>phosphoglucomutase:</td>
<td>G6P ↔ G1P</td>
</tr>
<tr>
<td>fructokinase:</td>
<td>Frc + ATP → F6P + ADP</td>
</tr>
<tr>
<td>phosphoglucoisomerase:</td>
<td>G6P ↔ F6P</td>
</tr>
<tr>
<td>hexokinase:</td>
<td>Glc + ATP → G6P + ADP</td>
</tr>
<tr>
<td>invertase:</td>
<td>Suc → Glc + Frc</td>
</tr>
<tr>
<td>sucrose phosphate synthase:</td>
<td>F6P + UDPglc ↔ S6P + UDP</td>
</tr>
<tr>
<td>sucrose phosphate phosphatase:</td>
<td>S6P → Suc + P_i</td>
</tr>
<tr>
<td>glycolysis (b):</td>
<td>F6P + 29 ADP + 28 P_i → 29 ATP</td>
</tr>
<tr>
<td>NDPkinase:</td>
<td>UDP + ATP ↔ UTP + ADP</td>
</tr>
<tr>
<td>sucrose transporter:</td>
<td>eSuc → Suc</td>
</tr>
<tr>
<td>ATP consumption (b):</td>
<td>ATP → ADP + P_i</td>
</tr>
<tr>
<td>starch synthesis:</td>
<td>G6P + ATP → 2P_i + ADP + starch</td>
</tr>
<tr>
<td>adenylate kinase:</td>
<td>ATP + AMP ↔ 2ADP</td>
</tr>
<tr>
<td>pyrophosphatase:</td>
<td>PP → 2 P_i</td>
</tr>
</tbody>
</table>
ucrose-to-starch-pathway in potato tuber

A hierarchical node:

Suc → UDP
R1 → UDPglc
Frc

Interface to the environment:

eSuc → starch
gSuc → rStarch

Tools:

Editing: Ped
Animation: PedVisor
Qualitative analysis: INA

Heiner BTU Cottbus
http://www.informatik.tu-cottbus.de/~wwsdssz/
Starke HU Berlin
http://www.informatik.hu-berlin.de/~starke/ina.html
Qualitative analysis using INA

Elementary properties

| The net is not statically conflict-free. | The net is pure. |
| The net has transitions without pre-place. | The net is not strongly connected. |
| The net is not covered by semipositive P-invariants. | The net is not bounded. |
| The net is not structurally bounded. | The net is not live and safe. |
| The net is not safe. | Transition 18.geSuc has no pre-place. |
| The net has transitions without post-place. | Transition 21.rStarch has no post-place. |
| The net is not ordinary. | The net is not conservative. |
| At least the following transitions are live: 0.SucTrans, 1.Inv, 18.geSuc, | |
| At least the following places are simultaneously unbounded: 0.Suc, 1.eSuc, 2.Glc, 3.Frc, | |
| The net is marked. | The net is not marked with exactly one toke |
| The net is not homogenous. | The net has not a non-blocking multiplicity |
| The net has no nonempty clean trap. | The net has no places without pre-transition |
| The net has no places without post-transition. | Maximal in/out-degree: 6 |
| The net is connected. | |

<table>
<thead>
<tr>
<th>ORD</th>
<th>HOM</th>
<th>NBM</th>
<th>PUR</th>
<th>CSV</th>
<th>SCF</th>
<th>CON</th>
<th>SC</th>
<th>Ft0</th>
<th>tF0</th>
<th>Fp0</th>
<th>pF0</th>
<th>MG</th>
<th>SM</th>
<th>FC</th>
<th>EFC</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

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Qualitative analysis using INA

Structural properties

DTP CPI CTI B SB REV DSt BSt DTr DCF L LV L&S

- liveness could not be decided because the net is unbounded and the reachability graph cannot be calculated
- the coverability graph has more than 4 million states

smaller bounded version: more than $10^{10}$ states of the reachability graph
P-Invariant analysis

The net is not covered by P-invariants.

Following P-invariants were calculated:

1. UDPglc, UTP, UDP
2. ATP, AMP, ADP

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T-Invariant analysis

The net is covered by 19 T-invariants

7 trivials: 1. SPS, SPS_rev, 2. UGPase, UGPASE_rev,
3. SuSy_SuSy_rev, 4. PGM, PGM_rev,
5. NDPkin, NDPkin_rev, 6. AdK, AdK_rev,
7. PGI, PGI_rev

Example:

<table>
<thead>
<tr>
<th></th>
<th>0.sucrose : 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1.invertase : 1</td>
</tr>
<tr>
<td></td>
<td>4.R5 : 1</td>
</tr>
<tr>
<td></td>
<td>9.hexokinase : 1</td>
</tr>
<tr>
<td></td>
<td>10.fructokinase : 1</td>
</tr>
<tr>
<td></td>
<td>18.geSuc : 1</td>
</tr>
<tr>
<td></td>
<td>19.glycolysis : 2</td>
</tr>
<tr>
<td></td>
<td>20.ATP : 56</td>
</tr>
</tbody>
</table>
T-invariant 14

invertase

sucrose transporter

sucrose synthase

hexokinase

fructokinase

phosphoglucoisomerase

glycolysis

phosphoglucomutase

starch synthase

starch

UDP-glucose pyrophosphorylase

UDP-glucose pyrophosphatase

NDP kinase

ATP consumption

ADP

pyrophosphatase

adenylate kinase

AMP

ATP

Pi

Glc

Frc

F6P

G6P

UDP

S6P

UDPglc

Pi

UDP

Suc

geSuc

eSuc

rStarch
T-invariant 14
## T-Invariant analysis

<table>
<thead>
<tr>
<th>Invariant number</th>
<th>sucrose cleavage SuSy Inv</th>
<th>hexoses go into Glyc StaSy</th>
<th>ATP cons</th>
<th>ATP used for cycling Inv SuSy_rev Inv SPS, SPP SuSy SPS, SPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>x</td>
<td>x x x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>9</td>
<td>x</td>
<td>x x x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>10</td>
<td>x</td>
<td>x x x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>11</td>
<td>x</td>
<td>x x x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>12</td>
<td>x</td>
<td>x x x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>13</td>
<td>x</td>
<td>x x x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>14</td>
<td>x</td>
<td>x x x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>15</td>
<td>x</td>
<td>x x x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>16</td>
<td>x</td>
<td>x x x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>17</td>
<td>x</td>
<td>x x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>18</td>
<td>x</td>
<td>x x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>19</td>
<td>x</td>
<td>x x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>
Robustness

Robustness: sensitivity of the system against parameter (fragility) changes (altered enzyme activity, mutations) (Voit, 2000)

Stelling et al., Nature (2002): linear correlation between robustness and the number of elementary modes (T-invariants)

Our suggestion: - enzyme distribution over T-invariants  
- number of alternative paths

Potato net: - fructokinase occurs in all T-invariants  
- there is no enzyme that occurs in only one T-invariant
Conclusions & Outlook

Petri nets provide
(1) a unique description of biological networks
(2) methods for qualitative analysis to check models by the calculation of system properties.
(3) The complexity of biological systems make it necessary to extend Petri net methods.
(4) Automatic interpretation of T-invariants is necessary.
Projects

- **Glycolysis-pentose phosphate pathway in erythrocytes**

- **Apoptosis**

Heiner & Koch, 25th International Conference on Application and Theory of Petri Nets, 21<sup>th</sup> - 25<sup>th</sup> June, Bologna, Italy (2004)

**Ongoing projects:**
1. The whole E.coli pathway  Nina Kramer
2. The whole potato tuber pathway  Nina Kramer

3. Detailed glycolysis with coloured Petri nets in human
   Thomas Runge, BTU Cottbus
4. *G1/S* - phase in mammalian cells  Thomas Kaunath
   (tumour cell lines, Duchenne muscle dystrophy)
Thanks!

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Björn Junker (Max Planck Institute for Molecular Plant Physiology Golm)
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