MODEL CHECKING OF BIOCHEMICAL NETWORKS USING PETRI NETS

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MODEL-BASED SYSTEM ANALYSIS

CONSTRUCTION

technical system

verification

requirement specification

model

system

system properties

model properties
MODEL- BASED SYSTEM ANALYSIS

UNDERSTANDING

system

biological system

model

validation

behaviour prediction

system properties

known properties

unknown properties

model properties
## Temporal Logics, Overview

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<th>Interleaving</th>
<th>Partial Order</th>
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<td><strong>Runs</strong></td>
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<td>Linear (LTL)</td>
<td>(no conflict, no concurrency)</td>
<td>(no conflict, but concurrency)</td>
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<td></td>
<td>Manna &amp; Pnueli, Kröger, <em>jsp 2001</em></td>
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<td>DSSZ/LTL</td>
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<td>Branching (CTL)</td>
<td><strong>Reachability graph</strong></td>
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<td>(conflict &amp; concurrency not distinguishable)</td>
<td>(conflicts &amp; concurrency)</td>
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<tr>
<td></td>
<td>Emmerson, Clarke</td>
<td>McMillan, Esparza, <em>pd 2001</em></td>
</tr>
<tr>
<td></td>
<td>PROD/MARIA, INA, DSSZ/CTL</td>
<td>PEP</td>
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*Tools: ?*
<table>
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<tr>
<th>technique</th>
<th>CTL</th>
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<td>INA</td>
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<td>stubborn set reduced reachability graph</td>
<td>LoLA</td>
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<td>LoLA (symmetric formulas)</td>
<td>?</td>
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<td>BDD, NDD, ..., xDD</td>
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<td>Kronecker algebra</td>
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<td>?</td>
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<td>process automata</td>
<td>[pd]</td>
<td>?</td>
</tr>
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</table>
Temporal Logics, Basics

- Extension of classical (propositional) logics by temporal operators

- Atomic propositions
  - Elementary statements, having - in a given state - a well-defined truth value
  - E.g. mutex, for 1-bounded pn
  - E.g. buffer = 2, buffer > 2, else

- Constants
  - True, FALSE

- Classical Boolean operators
  - Negation !  Conjunction *
  - Disjunction +  Implication ->

- Temporal operators
  - To refer to the sequence of states
### CTL Operators, Interleaving Semantics

<table>
<thead>
<tr>
<th></th>
<th>next f</th>
<th>finally f</th>
<th>globally f</th>
<th>f1 until f2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ax</strong> on all branches</td>
<td><img src="Ax_diagram.png" alt="Diagram" /></td>
<td><img src="Af_diagram.png" alt="Diagram" /></td>
<td><img src="Ag_diagram.png" alt="Diagram" /></td>
<td><img src="Au_diagram.png" alt="Diagram" /></td>
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<tr>
<td><strong>Ex</strong> on some branch</td>
<td><img src="Ex_diagram.png" alt="Diagram" /></td>
<td><img src="Ef_diagram.png" alt="Diagram" /></td>
<td><img src="Eg_diagram.png" alt="Diagram" /></td>
<td><img src="Eu_diagram.png" alt="Diagram" /></td>
</tr>
</tbody>
</table>
MODEL CHECKING

- is a technique for verifying finite-state concurrent systems
  
  Clarke, E. M. Jr.; Grumberg, O.; Peled, D. A.:
  Model Checking;
  MIT Press 2001

- finite state systems = steady state systems = bounded pn

- model checking of unbounded systems
  
  -> CTL undecidable
  -> LTL decidable, but no tools (not yet ?)
  -> unboundedness + inhibitors = undecidability

- how to get bounded bionetworks?
TWO APPROACHES

❑ approach 1: qualitative model
  -> model assumptions of environment behaviour: -> strong assumptions
  quantitative relations of input / output compounds
  -> control of conflicting alternative pathways

❑ approach 2: quantitative model = time-dependent model
  -> model assumptions of environment behaviour: -> weak assumptions
  infinite flow into/out the network
  -> relative transition firing rates
  -> control of conflicting alternative pathways

❑ claim
  -> transformation preserves all possible behaviour (= minimal T-invariants)
**Approach 1**

- **Additional model component**
  
  \[
  aA + bB + cC \rightarrow dD + eE
  \]

- **Precondition**
  
  \(\rightarrow\) equal sum equation for all pathways

---

**network sum equation**

```
\[
\text{network: } aA + bB + cC \rightarrow dD + eE
\]
```
**APPROACH 1**

- **additional model component, refinement**

- **precondition**

  - controlled conflicts between pathways with unequal sum equations
APPROACH 1, Ex

- example - apoptosis
  -> Matsuno et al.

- signal-transduction pathway

http://www.genomicObject.net
- example - apoptosis  
  -> Matsuno et al.

- signal-transduction pathway

- inhibitor arcs

http://www.genomicObject.net
example - apoptosis

network model

inhibitor arcs

three pathways = min. T-invariants
example - apoptosis

network model

environment, style 1
- three pathways = min. T-invariants

T-invariant 1
- Fas-induced
- 'death-receptor' pathway
example - apoptosis

network model

environment, style 1
- three pathways
  = min. T-invariants

T-invariant 2
- apoptotic-stimuli-induced
- 'mitochondrial' pathway
example - apoptosis

network model

environment, style 1

three pathways

min. T-invariants

T-invariant 3

cross-talk by Bid

pathway
example - apoptosis

environment model

pathway 1 / 3
- overlap at the beginning
- then branch
- controlled by places choice1 / choice2

all pathways share the same ending
- only one repeat transition
APPROACH 1, Ex

- example - apoptosis

- network model, adapted

- system model
  - network model
  - environment model

- system model
  - 1-bounded
  - live

- ready for model checking
property 1

if inhibitor substance \textit{Bcl\textunderscore2\textunderscoreBcl\textunderscoreXL} is present,
then the progress of the cross-talk pathway is stopped at \textit{Mitochondrion}
\[
AG ( Bcl\textunderscore2\textunderscoreBcl\textunderscoreXL \ast Mitochondrion \rightarrow AX (Mitochondrion) ) ;
\]

property 2

if inhibitor substance \textit{Bcl\textunderscore2\textunderscoreBcl\textunderscoreXL} is present,
then the progress of the cross-talk pathway is stopped at \textit{Mitochondrion}
until the inhibitor substance disappears
\[
AG ( Bcl\textunderscore2\textunderscoreBcl\textunderscoreXL \ast Mitochondrion \rightarrow A (Mitochondrion U ! Bcl\textunderscore2\textunderscoreBcl\textunderscoreXL) ) ;
\]

property 3

if inhibitor substance \textit{Bcl\textunderscore2\textunderscoreBcl\textunderscoreXL} is not present,
then the progress of the cross-talk pathway is not stopped at \textit{Mitochondrion}
\[
AG ( !Bcl\textunderscore2\textunderscoreBcl\textunderscoreXL \ast Mitochondrion \rightarrow EX (CytochromeC) ) ;
\]
-> properties as time-less net

INA
ORD HOM NBM PUR CSV SCF CON SC Ft0 tF0 Fp0 pF0 MG SM FC EFC ES
N Y N Y N Y Y N Y Y N N Y N Y Y Y
CPI CTI B SB REV DSt BSt DTr DCF L LV L&S
N Y N N Y N ? N Y Y Y Y N
APPROACH 2, Ex1

-> properties as time-less net

INA
ORD HOM NBM PUR CSV SCF CON SC Ft0 tF0 Fp0 pF0 MG SM FC EFC ES
N Y N Y N Y Y N Y Y N N Y N Y Y Y
CPI CTI B SB REV DSt BSt DTr DCF L LV L&S
N Y N Y N Y N ? N Y Y Y N

prod_A  prod_B
   ↑      ↑
  1-2  2-1  T-INVARIANTE

r1

↓

cons_C  cons_D
1-3  3-1
APPRAOH 2, Ex1

-> properties as duration net

INA
ORD HOM NBM PUR CSV SCF CON SC Ft0 tF0 Fp0 pF0 MG SM FC EFC ES
N Y N Y N Y Y N Y N Y N Y Y Y
CPI CTI B SB REV DSt BSt DTr DCF L LV L&S
N Y Y N N N ? N Y Y Y N

T-INVARIANTE

size (RG (d-net)) = 8 nodes
T-INVARIANTE 1
T-INVARIANTE 2

-> properties as duration net

size (RG (d-net)) = 11 nodes
transient state
steady state

\begin{itemize}
\item $S_6(A,2B,C)$
\item $t(r_2)=3$
\item $t(cons_C)=1$
\item $S_7(0,B,C)$
\item $t(prod_A)=2$
\item $t(r_1)=5$
\item $t(r_2)=2$
\item $t(cons_B)=2$
\item $S_8(A,B,3C)$
\item $t(r_1)=3$
\item $S_9(0,0,2C)$
\item $t(prod_A)=1$
\item $t(r_1)=1$
\item $t(r_2)=4$
\item $t(cons_B)=1$
\end{itemize}

$t(r_1)=1$
$t(r_2)=4$
$t(cons_B)=1$
$t(cons_C)=1$

prod_A start
r1 start
cons_B start, cons_C end

prod_A end
r1 end
cons_B end, cons_C

prod_A start
r2 start
cons_B end, cons_C

prod_A end
r2 end
cons_B end, cons_C

prod_A start
r2 start
cons_B start, cons_C

prod_A end
r2 end
cons_B start, cons_C

terminal SCC
Ex2, Terminal SCC

- contains all transitions
  - always running
  - start / end at different time points

- contains all minimal t-invariants

- relative transition firing rates

  prod_A : 1 + 1
  r1 : 1  r2 : 1
  cons_B : 2  cons_C : 3

Timing diagram:

- prod_A
- r1
- r2
- cons_B
- cons_C

6 time units
APPROACH 2, SUMMARY

- CTI, but not CPI

- transient state
  - initial behaviour to reach steady state
  - not REV
  - generally, not DCF

- steady state behaviour
  - terminal scc
  - BND
  - here, DCF
BIONETWORKS, VALIDATION

- validation criterion 1
  - CTI
  - no minimal T-invariant without biological interpretation
  - no known biological behaviour without corresponding T-invariant

- validation criterion 2
  - P-invariants - groups of compounds with conservation property
  - no minimal P-invariant without biological interpretation

- validation criterion 3
  - CPI
  - all expected temporal-logic properties  -> TRUE
CHALLENGES

❑ extensions
  -> read arcs
  -> inhibitor arcs !?

❑ efficient computation of minimal invariants
  -> exponential complexity
  -> compositional / step-wise refinement approach ?

❑ analysis of bounded, but not safe non-ordinary nets with inhibitor arcs
  -> huge state spaces, beyond exponential growth (?)
  -> smaller, bounded version of case study 2 \( \geq 10^{10} \) states \( (IDD-based \ mc \ tool) \)

❑ analysis of unbounded nets
  -> besides T-invariant analysis ?

❑ model checking
  -> relevant properties ?
SUMMARY

- representation of bionetworks by Petri nets
  - unifying view
  - animation
  - model validation against consistency criteria
  - qualitative/quantitative behaviour prediction

- steady state behaviour
  - qualitative model
  - quantitative model

- many challenging questions for analysis techniques
THANKS!