BIOCHEMICALLY INTERPRETED PETRI NETS
- SOME OPEN PROBLEMS -

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OUTLINE

- **BACKGROUND**

- **HOW STRUCTURAL ANALYSIS TECHNIQUES CAN CONTRIBUTE**
  - modularization approach
    - connected ADT sets = flow equivalent server component?
  - identify core network

- **LATEST NEWS**
  - our tool box: Snoopy, Charlie, Marcie
  - colored framework
  - Generalized Hybrid Petri Nets
BACKGROUND
MODEL- BASED SYSTEM ANALYSIS
MODEL-BASED SYSTEM ANALYSIS

system \leftrightarrow \text{model}

system properties \leftrightarrow \text{model properties}
MODEL- BASED SYSTEM ANALYSIS

CONSTRUCTION

system

technical system

verification

model

requirement specification

system properties

model properties
MODELLING = FORMAL KNOWLEDGE REPRESENTATION

natural biosystem → wetlab experiments → observed behaviour → model → predicted behaviour → model-based experiment design → wetlab experiments → formalizing understanding
MODELLING = FORMAL KNOWLEDGE REPRESENTATION

natural biosystem → wetlab experiments → observed behaviour → formalizing understanding

predicted behaviour → model → model-based experiment design

model validation

MODEL VALIDATION = CONFIDENCE INCREASE
MODELS IN SYNTHETIC BIOLOGY

MODELLING = BLUEPRINT FOR SYSTEM CONSTRUCTION

RELIABLE AND ROBUST ENGINEERING REQUIRES VERIFIED MODELS
WHAT KIND OF MODEL SHOULD BE USED?
BIO NETWORKS, SOME PROBLEMS
Network Representations, Ex2

\[ \frac{d\alpha}{dt} = -v_1 \]
\[ \frac{d\text{Ste2}}{dt} = -v_2 + v_3 - v_5 \]
\[ \frac{d\text{Ste2}_{\text{active}}}{dt} = v_2 - v_3 - v_4 \]
\[ \frac{d\text{Sst2}_{\text{active}}}{dt} = v_{46} - v_{47} \]
\[ \frac{dG\alpha\beta\gamma}{dt} = -v_6 + v_9 \]
\[ \frac{dG\alpha\text{GTP}}{dt} = v_6 - v_7 - v_8 \]
\[ \frac{dG\alpha\text{GDP}}{dt} = v_7 + v_8 - v_9 \]
\[ \frac{dG\beta\gamma}{dt} = v_6 - v_9 - v_{10} + v_{11} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32} \]
\[ - v_{42} + v_{43} \]
\[ \frac{d\text{Ste5}}{dt} = -v_{12} + v_{13} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32} \]
\[ \frac{d\text{Ste11}}{dt} = -v_{12} + v_{13} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32} \]
\[ \frac{d\text{Ste7}}{dt} = -v_{14} + v_{15} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32} \]
\[ \frac{d\text{Fus3}}{dt} = -v_{14} + v_{15} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} - v_{29} \]
\[ + v_{30} + v_{33} \]
\[ \frac{d\text{Ste20}}{dt} = -v_{18} + v_{19} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32} \]

\[ v_1 = \alpha[t] \cdot \text{Bar1}_{\text{active}}[t] \cdot k_1 \]
\[ v_2 = \text{Ste2}[t] \cdot \alpha[t] \cdot k_2 \]
\[ v_3 = \text{Ste2}_{\text{active}}[t] \cdot k_3 \]
\[ v_4 = \text{Ste2}_{\text{active}}[t] \cdot k_4 \]
\[ v_5 = \text{Ste2}[t] \cdot k_5 \]
\[ v_6 = \text{Ste2}_{\text{active}}[t] \cdot G\alpha\beta\gamma[t] \cdot k_6 \]
\[ v_7 = G\alpha\text{GTP}[t] \cdot k_7 \]
\[ v_8 = G\alpha\text{GTP}[t] \cdot \text{Sst2}_{\text{active}}[t] \cdot k_8 \]
\[ v_9 = G\alpha\text{GDP}[t] \cdot G\beta\gamma[t] \cdot k_9 \]
\[ v_{10} = G\beta\gamma[t] \cdot C[t] \cdot k_{10} \]
\[ v_{11} = D[t] \cdot k_{11} \]
\[ v_{12} = \text{Ste5}[t] \cdot \text{Ste11}[t] \cdot k_{12} \]
\[ v_{13} = A[t] \cdot k_{13} \]
\[ v_{14} = \text{Ste7}[t] \cdot \text{Fus3}[t] \cdot k_{14} \]
\[ v_{15} = B[t] \cdot k_{15} \]
\[ v_{16} = A[t] \cdot B[t] \cdot k_{16} \]
\[ v_{17} = C[t] \cdot k_{17} \]
\[ v_{18} = D[t] \cdot \text{Ste20}[t] \cdot k_{18} \]
BIO NETWORKS, SOME PROBLEMS

- knowledge
  - uncertain
  - growing, changing
  - distributed over independent data bases, papers, journals, . . .

- various, mostly ambiguous representations
  - verbose descriptions
  - diverse graphical representations
  - contradictory and / or fuzzy statements

- network structure
  - tend to grow fast
  - dense, apparently unstructured
  - hard to read

- PROBLEM 1
- PROBLEM 2
- PROBLEM 3
BIO NETWORKS, SOME PROBLEMS

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  -> tend to grow fast
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-> MODELS ARE FULL OF ASSUMPTIONS <-
**Bio Network Representations Should Be**

- **Readable**
  - fault avoidance
  - informal = cartoon-like representations?

- **Analysable**
  - formal = mathematical representations

- **Executable**
  - to experience the model

- **Unifying Power**
  - high-level description for various analysis approaches
... ARE NETWORKS OF BIOCHEMICAL REACTIONS ...

NATURALLY EXPRESSIBLE AS PETRI NETS

2 NAD$^+$ + 2 H$_2$O -> 2 NADH + 2 H$^+$ + O$_2$
SUMMARY, FRAMEWORK

time-free

RG
CTL, LTL

QUALITATIVE

approximation
hazard function, type (1)

approximation
hazard function, type (2)

STOCHASTIC
CTMC
CSDL, PLTLc

discrete state space

CONTINUOUS
ODEs
PLTLc

continuous state space

timed, quantitative

abstraction

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BIO PETRI NETS - SOME EXAMPLES
EX1 - SIGNALLING CASCADE

RasGTP

Raf → RafP

Phosphatase1

MEK → MEKP

MEKPP

Phosphatase2

ERK → ERKP

ERKPP

Phosphatase3
EX1 - SIGNALLING CASCADE
positive feedback

TF + S  ↔  TF|S

PhzMS

PCA → PYO

tf
Ex2 - BIOSENSOR

1. TF expression
2. TF degradation
3. TFS association
4. TFS disassociation
5. TFS degradation
6. Reporter expression
7. Reporter degradation
8. Response production
9. Response degradation

Positive Feedback

TF

PB

TFS

Reporter

Precursor

Signal
Ex3 - Switch Cycle Halobacterium Salinarum

[Marwan; Oesterhelt 1999]
Ex5 - ANGIOGENESIS

[... Balbo ... 2009]
MOULARIZATION
BY T-INVARIANTS
r1: \( A \rightarrow 2B \)
r2: \( 2A \rightarrow 3C \)
r1: $A \rightarrow 2B$
r2: $2A \rightarrow 3C$
**T-invariants, Ex1**

\[ r1: \quad A \rightarrow 2B \]
\[ r2: \quad 2A \rightarrow 3C \]
INCIDENCE MATRIX C, Ex1

PN & Systems Biology

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INCI DENCE M AT RIX C, Ex1
**T-invariants, Basics**

- Lautenbach, 1973 -> Schuster, 1993
- **T-invariants**
  -> integer solutions $x$
  \[ Cx = 0, \ x \neq 0, \ x \geq 0 \]
- minimal T-invariants
  -> there is no T-invariant with a smaller support
  -> gcd of all non-zero entries is 1
- any T-invariant is a non-negative linear combination of minimal ones
  -> multiplication with a positive integer
  \[ kx = \sum_i a_i x_i \]
  -> addition
  -> division by a common divisor
- **Covered by T-Invariants (CTI)**
  -> each transition belongs to a T-invariant
T-INVARIANTS, INTERPRETATIONS

- T-invariants = (multi-) sets of transitions = Parikh vector
  - zero effect on marking
  - reproducing a marking / system state

- two interpretations
  1. partially ordered transition sequence
     of transitions occuring one after the other
     - substance / signal flow
  2. relative transition firing rates
     of transitions occuring permanently & concurrently
     - steady state behaviour

- a minimal T-invariant defines a connected subnet
  - the T-invariant’s transitions (the support),
    + all their pre- and post-places
    + the arcs in between
  - pre-set of support = post-set of support
**T-INVARIANTS, Ex1**

\[ r1: \ A \rightarrow 2\ B \]
\[ r2: \ 2\ A \rightarrow 3\ C \]
**T-INVARIENTS, Ex1**

r1: $A \rightarrow 2B$

r2: $2A \rightarrow 3C$

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**T-INVARIENT 1**

**T-INVARIENT 2**
- T-invariants may contain any structure

- T-invariants generally overlap

  \[ \rightarrow \text{combinatorial effect brings \textit{explosion} in the number of min. T-invariants} \quad (2^4) \]
**Abstract Dependent Transition Sets (ADT-Sets)**

- Let $X$ denote a set of (all / non-trivial) minimal $T$-invariants $x$ of a given PN.

- **dependency relation:**
  Two transitions $i, j$ depend on each other,
  if they always appear together in all minimal $T$-invariants $x$, i.e.
  \[
  \forall x \in X: \ i \in \text{supp}(x) \iff j \in \text{supp}(x)
  \]

- **equivalence relation** in the transition set, leading to a partition of $T$
  - reflexive
  - symmetric
  - transitive

- **the equivalence classes $A$ represent maximal ADT-sets**
  \[
  \forall x \in X: A \subseteq \text{supp}(x) \lor A \cap \text{supp}(x) = \emptyset
  \]
ADT-SETS, Ex1

\[ r1: \quad A \rightarrow 2\ B \]
\[ r2: \quad 2\ A \rightarrow 3\ C \]
r1: \( A \rightarrow 2 \ B \)
r2: \( 2 \ A \rightarrow 3 \ C \)

**T-INARIANT 1**

**T-INARIANT 2**
ADT-SETS, Ex2

\[ r1: \ A \rightarrow 2 \ B \]
\[ r2: 2 \ A \rightarrow 3 \ B \]
ADT-SETS, Ex2

\[ r1: \quad A \rightarrow 2 \, B \]
\[ r2: \quad 2 \, A \rightarrow 3 \, B \]
ADT-sets, Ex2

\[ r1: \ A \rightarrow 2 \ B \]
\[ r2: \ 2 \ A \rightarrow 3 \ B \]


**ADT-sets, Interpretation**

- **maximal ADT-sets**
  - disjunctive subnets
  - not necessarily connected

- **minimal T-invariants**
  - overlapping subnets
  - connected

- **interpretation**
  - structural decomposition into rather small subnets
  - smallest biologically meaningful functional units
  - building blocks

- **variations**
  - with / without trivial T-invariants
  - whole / partial set of T-invariants

- **classification of all transitions based on the T-invariants’ support**

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ADT-SETS, APPLICATIONS

- maximal ADT-sets
  \(-\) not necessarily connected

- further decomposition into connected ADT-sets
  \(-\) possibly according to primary compounds, only,
  \(i.e.\) neglecting connections by auxiliary compounds
  \(-\) non-maximal ADT-sets

- coarse network structure, definition
  \(-\) macro transitions \(-\) abstract from connected ADT-sets
  \(-\) places \(-\) interface between functional units

- coarse network structure, what for?
  \(-\) set of \(T\)-invariants gets structured
  \(-\) better understanding of the net behaviour

- flow equivalent server component ?
BIO PETRI NETS,
SOME EXAMPLES
Ex1 - Glycolysis and Pentose Phosphate Pathway

[Reddy 1993]
Ex1 - Glycolysis and Pentose Phosphate Pathway
Ex1 - Glycolysis and Pentose Phosphate Pathway

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Ex1 - Glycolysis and Pentose Phosphate Pathway
Ex2: Apoptosis in Mammalian Cells

[GON 2003]
Ex2: Apoptosis in Mammalian Cells

[GON 2003]

[Heiner; Koch; Will 2004]
Ex2: Apoptosis in Mammalian Cells
Ex2: Apoptosis in Mammalian Cells
Ex3 - Carbon Metabolism in Potato Tuber

[Koch; Junker; Heiner 2005]

ADT-sets without trivial T-invariants
Ex3 - Carbon Metabolism in Potato Tuber

[*Koch; Junker; Heiner 2005]*

*ADT-sets without trivial T-invariants*
ADT-SETS, Interpretations

- “promote hierarchical thinking & unbiased modularization”
- structured representation of invariants
  -> may contribute to a better understandability

- coarse network structure identifies sensitive net parts
  -> the knock-off of interface places affects several ADT-sets

- efficient design of wetlab experiments
  -> minimal sets of observation points providing coverage of the whole network
  (one for each ADT-set)

- support of dedicated layout algorithms

  “can include non-obvious groups of reactions and differ from groupings
  of reactions based on a visual inspection of the network topology”
  
  (Papin, Reed, Palsson 2004)
ADT-sets, **Summary**

**PROS**

- algorithmically defined
- static analysis technique (state space not constructed), works also for unbounded models

**CONS**

- may be computational expensive
- to avoid computation of all (T-) invariants:

\[
C x = 0, \ x \neq 0, \ x \geq 0, \quad x(i) = 0, \ x(j) \neq 0, \ \forall i, j \in T
\]

-- especially helpful for analyzing bio Petri nets --

**related work (T-invariants)**

- MCT-sets (Sackmann, Heiner, Koch 2006)
- (A)DT-sets (Winder 2006)
- partially correlated reaction sets (Papin, Reed, Palsson 2004)
- Flux coupling analysis (Burgard 2004)
CORE NETWORK IDENTIFICATION
(a) (b)

HIF (steady state values)

Oxygen

(b) + k19

(c) + k4, k21

(d) + k16

(e) + k13

k5, k6, k29, k30
HOW TO GENERALIZE?
LATEST NEWS
OUR TOOL BOX

- **Snoopy**
  -> in collaboration with Wolfgang Marwan, Magdeburg

- **Charlie**
  -> inspired by INA
  -> analysis tasks done by threads

- **MARCIE**
  -> **Model checking** and **Reachability analysis** done **efficiently** of qualitative and stochastic models
  -> **Interval Decision Diagrams**
  -> **CTL model checking**
  -> “matrix free” transient and steady state analysis
  -> full CSL model checking + rewards
  -> distributed simulative PLTL model checking (pre-beta-version)
COLOURED FRAMEWORK

COLOURED QUALITATIVE

RG
CTL, LTL

time-free

timed, quantitative

COLOURED STOCHASTIC

CTMC
CSL, PLTLc

COLOURED CONTINUOUS

ODEs
PLTLc

approximation hazard function, type (1)
approximation hazard function, type (2)
discrete state space
continuous state space
COLOURED PETRI NETS, APPLICATIONS

- get multiple copies of patterns
  -> Halo model, new order of net sizes

- differentiate between submodels within a master net
  -> T-invariants
  -> generated models in conformance with wet-lab data
  -> mutants
  -> algorithmic folding

- encode locality
  -> Ca channel models
  -> agents on a grid
  -> ...

- dynamic membrane systems

...
Ex - C. Elegance

[Li et al. 2009]
[Bonzanni et al. 2009]

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GENERALIZED HYBRID PETRI NETS (GHPN)

- Extended Generalized Stochastic Petri Nets (XSPN)
  - `discrete places`
  - `discrete transitions: stochastic, immediate, deterministically delayed, scheduled`
  - `special arcs: read, inhibitor, equal, reset`

- Continuous Petri Nets (CPN)
  - `continuous places`
  - `continuous transitions`
  - `special arcs: read, inhibitor`

- GHPN = XSPN + CPN

- hybrid simulation engine

- dynamic partitioning
THANKS!

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