PETRI NETS
FOR SYSTEMS & SYNTHETIC BIOLOGY

in memory of Nadia Busi

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AGENDA

- **MODELLING WITH PETRI NETS**

- **MODEL-BASED SYSTEM ANALYSIS**
  - → qualitative Petri nets
  - → stochastic Petri nets
  - → continuous Petri nets

- **CASE STUDY**
  - → model checking in the three paradigms

- **SUMMARY**
  - → challenges / open questions
LONG-TERM VISION

MEDICAL TREATMENT
LONG-TERM VISION

MEDICAL TREATMENT, APPROACH 1 - TRIAL-AND-ERROR DRUG PRESCRIPTION
LONG-TERM VISION

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MEDICAL TREATMENT, APPROACH 2

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June 2008
LONG-TERM VISION

MEDICAL TREATMENT, APPROACH 2 - MODEL-BASED DRUG PRESCRIPTION
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MEDICAL TREATMENT, APPROACH 2 - MODEL-BASED DRUG PRESCRIPTION
WHAT KIND OF MODEL SHOULD BE USED?
BIO NETWORK REPRESENTATIONS, Ex1

PN & Systems Biology

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\[
\begin{align*}
\frac{d\alpha}{dt} &= -v_1 \\
\frac{d\text{Ste2}}{dt} &= -v_2 + v_3 - v_5 \\
\frac{d\text{Ste2}_{\text{active}}}{dt} &= v_2 - v_3 - v_4 \\
\frac{dS\text{st2}_{\text{active}}}{dt} &= v_46 - v_47 \\
\frac{dG\alpha\beta\gamma}{dt} &= -v_6 + v_9 \\
\frac{dG\alpha\text{GTP}}{dt} &= v_6 - v_7 - v_8 \\
\frac{dG\alpha\text{GDP}}{dt} &= v_7 + v_8 - v_9 \\
\frac{dG\beta\gamma}{dt} &= v_6 - v_9 - v_{10} + v_{11} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32} - v_{42} + v_{43} \\
\frac{d\text{Ste5}}{dt} &= -v_{12} + v_{13} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32} \\
\frac{d\text{Ste11}}{dt} &= -v_{12} + v_{13} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32} \\
\frac{d\text{Ste7}}{dt} &= -v_{14} + v_{15} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32} \\
\frac{d\text{Fus3}}{dt} &= -v_{14} + v_{15} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} - v_{29} + v_{30} + v_{33} \\
\frac{d\text{Ste20}}{dt} &= -v_{18} + v_{19} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32} \\
\end{align*}
\]
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\end{align*}
\]

\[
\begin{align*}
v_1 &= \alpha[t] \cdot \text{Bar}l_{active[t]} \cdot k_1 \\
v_2 &= \text{Ste2}[t] \cdot \alpha[t] \cdot k_2 \\
v_3 &= \text{Ste2}_{active[t]} \cdot k_3 \\
v_4 &= \text{Ste2}_{active[t]} \cdot k_4 \\
v_5 &= \text{Ste2}[t] \cdot k_5 \\
v_6 &= \text{Ste2}_{active[t]} \cdot G\alpha\beta\gamma[t] \cdot k_6 \\
v_7 &= G\alpha\gamma\text{GTP}[t] \cdot k_7 \\
v_8 &= G\alpha\gamma\text{GTP}[t] \cdot Sst2_{active[t]} \cdot k_8 \\
v_9 &= G\alpha\gamma\gamma\text{GDP}[t] \cdot G\beta\gamma[t] \cdot k_9 \\
v_{10} &= G\beta\gamma[t] \cdot C[t] \cdot k_{10} \\
v_{11} &= D[t] \cdot k_{11} \\
v_{12} &= \text{Ste5}[t] \cdot \text{Ste11}[t] \cdot k_{12} \\
v_{13} &= A[t] \cdot k_{13} \\
v_{14} &= \text{Ste7}[t] \cdot \text{Fus3}[t] \cdot k_{14} \\
v_{15} &= B[t] \cdot k_{15} \\
v_{16} &= A[t] \cdot B[t] \cdot k_{16} \\
v_{17} &= C[t] \cdot k_{17} \\
v_{18} &= D[t] \cdot \text{Ste20}[t] \cdot k_{18}
\end{align*}
\]
BIO NETWORKS, SOME PROBLEMS

- knowledge
  - uncertain
  - growing, changing
  - distributed over independent databases, papers, journals, . . . -> PROBLEM 1

- various, mostly ambiguous representations
  - verbose descriptions
  - diverse graphical representations
  - contradictory and/or fuzzy statements -> PROBLEM 2

- network structures
  - tend to grow fast
  - dense, apparently unstructured
  - hard to read -> PROBLEM 3
BIO NETWORKS, SOME PROBLEMS

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

- various, mostly ambiguous representations
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- network structures
  - tend to grow fast
  - dense, apparently unstructured
  - hard to read

- 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
WHAT KIND OF MODEL TO CHOOSE?
Bio Networks

... are networks of (bio-) chemical reactions
**BIO NETWORKS, THREE BASIC PROPERTIES**

- **bipartite - species & reactions**
  
  \[ r: 2 \text{H}_2 + \text{O}_2 \rightarrow 2 \text{H}_2\text{O} \]

- **reactions - sequential, alternative, concurrent**

- **behaviour - stochastic**
The framework illustrates the relationship between qualitative and stochastic elements vs. continuous and timed elements.

- **Qualitative** vs. **Continuous**: Represents the nature of the system, where qualitative models focus on qualitative descriptions, and continuous models focus on quantitative descriptions.
- **Time-Free** vs. **Timed**: Represents the timing aspect, where time-free models do not consider time, and timed models do.

The diagram includes the following approximations:
- **Approximation**
- **Hazard Function, Type (1)**
- **Hazard Function, Type (2)**

The state space is also differentiated as:
- **Discrete State Space**
- **Continuous State Space**

The framework highlights the interplay between these dimensions and their approximations to provide a comprehensive view of system biology.
PETRI NETS -
AN INFORMAL CRASH COURSE
2 NAD$^+$ + 2 H$_2$O $\rightarrow$ 2 NADH + 2 H$^+$ + O$_2$
**PETRI NETS, BASICS - THE STRUCTURE**

- **atomic actions** -> **transitions** -> **chemical reactions**

  \[2 \text{NAD}^+ + 2 \text{H}_2\text{O} \rightarrow 2 \text{NADH} + 2 \text{H}^+ + \text{O}_2\]

- **local conditions** -> **places** -> **chemical compounds**

- **multiplicities** -> **arc weights** -> **stoichiometric relations**

- **condition's state** -> **token(s)** -> **available amount (e.g. mol)**

- **system state** -> **marking** -> **compounds distribution**

- **PN = (P, T, F, m_0), F: ((P x T) U (T x P)) -> N_0, m_0: P -> N_0**
PETRI NETS, BASICS - THE FIRING RULE

- an action may happen, if
  - all preconditions are fulfilled
    (corresponding to the arc weights)

- if an action happens, then
  - tokens are removed from all preconditions
    (corresponding to the arc weights), and
  - tokens are added to all postconditions
    (corresponding to the arc weights)

- action happens (firing of a transition)
  - atomic
  - no time consumption

- TIME-FREE MODEL, WHICH CONSIDERS ALL POSSIBLE TIMING BEHAVIOUR
atomic actions -> transitions -> chemical reactions

2 NAD\(^+\) + 2 H\(_2\)O → 2 NADH + 2 H\(^+\) + O\(_2\)
Typical Basic Structures I

A --> B + C

A --> B, A --> C

A + B --> C

A --> C, B --> C
TYPICAL BASIC STRUCTURES II

A --> B

A <--> B

E
A --> B

E
A <--> B

read arc

macro transition
TYPICAL BASIC STRUCTURES III

- **metabolic networks**
  - $\rightarrow$ *substance flows*

- **signal transduction networks**
  - $\rightarrow$ *signal flows*
# Typical Basic Structures III

- **metabolic networks**
  - \(\rightarrow\) *substance flows*

- **signal transduction networks**
  - \(\rightarrow\) *signal flows*

- **\(\rightarrow\) OPEN / CLOSED SYSTEMS**
**TYPICAL BASIC STRUCTURES IV**

**enzymatic reaction, mass-action approach 1**

A ⇔ A|E → B

r1, r2

r3

MA1
TYPICAL BASIC STRUCTURES IV

enzymatic reaction, mass-action approach 1
reaction-centred view

process-oriented view

gspecies-centred view

logical nodes (fusion nodes)
NET COMPOSITION FROM BUILDING BLOCKS

**DOUBLE PHOSPHOYLATION/DEPHOSPHORYLATION**

**SINGLE MASS-ACTION STEP**

Diagram showing the interactions and pathways of double phosphorylation/dephosphorylation.
NET COMPOSITION FROM BUILDING BLOCKS

DOUBLE PHOSPHOYLATION / DEPHOSPHORYLATION

SINGLE PHOSPHOYLATION / DEPHOSPHORYLATION
r1: A -> B
r1: A \rightarrow B
r2: B \rightarrow C + D
r3: B \rightarrow D + E

-> alternative reactions
r1: A -> B
r2: B -> C + D
r3: B -> D + E
r4: F -> B + a
r6: C + b -> G + c
r7: D + b -> H + c

-> concurrent reactions
r1: A → B
r2: B → C + D
r3: B → D + E
r4: F → B + a
r5: E + H ↔ F
r6: C + b → G + c
r7: D + b → H + c
r8: H ↔ G

- reversible reactions
r1: A -> B  
r2: B -> C + D  
r3: B -> D + E  
r4: F -> B + a  
r5: E + H <-> F  
r6: C + b -> G + c  
r7: D + b -> H + c  
r8: H <-> G

-> reversible reactions  
- hierarchical nodes
r1: A -> B
r2: B -> C + D
r3: B -> D + E
r4: F -> B + a
r5: E + H <-> F
r6: C + b -> G + c
r7: D + b -> H + c
r8: H <-> G
r9: G + b -> K + c + d
r10: H + 28a + 29c -> 29b
r11: d -> 2a
r1: A -> B
r2: B -> C + D
r3: B -> D + E
r4: F -> B + a
r5: E + H <-> F
r6: C + b -> G + c
r7: D + b -> H + c
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r7: D + b -> H + c
r8: H <-> G
r9: G + b -> K + c + d
r10: H + 28a + 29c -> 29b
r11: d -> 2a
PETRI NET ELEMENTS, INTERPRETATIONS

- METABOLIC NETWORKS
  - SIGNAL TRANSDUCTION NETWORKS
  - GENE REGULATORY NETWORKS

- transitions
  - (reversible, stoichiometric) chemical reactions,
  - enzyme-catalysed conversions of metabolites, proteins, . . .
  - complexations / decomplexations, de- / phosphorylations, . . .

- places
  - (primary, secondary) chemical compounds,
  - (various states of) proteins, protein complex, genes, . . .

- tokens
  - molecules, moles, . . .
  - concentration levels, gene expression levels, . . .
    (e.g., high / low = present / not present, or any finite number)
BIOCHEMICAL PETRI NETS, SUMMARY

- biochemical networks
  -> networks of (abstract) chemical reactions

- biochemically interpreted Petri net
  -> partial order sequences of chemical reactions (= elementary actions)
    transforming input into output compounds / signals
    [ respecting the given stoichiometric relations, if any ]
  -> set of all pathways
    from the input to the output compounds / signals
    [ respecting the stoichiometric relations, if any ]

- pathway
  -> self-contained partial order sequence of elementary (re-) actions
BIO PETRI NETS - SOME EXAMPLES
Ex1 - Glycolysis and Pentose Phosphate Pathway

[Reddy 1993]
Ex1 - Glycolysis and Pentose Phosphate Pathway

[Reddy 1993]

[Koch, Heiner 2008]
Ex2 - Carbon Metabolism in Potato Tuber

[Koch; Junker; Heiner 2005]
**Ex3: Apoptosis in Mammalian Cells**

- **Fas Ligand** binds to Fas receptor, activating FADD and Procaspase-8.
- **Bid**, BidC terminal, and **Apoptotic Stimuli** trigger Cytochrome c release from the mitochondrion.
- **Caspase-9** and **Caspase-3** are activated, leading to DNA fragmentation and DFF cleavage.

**[GON 2003]**

- **[HEINER; KOCH; WILL 2004]**

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EX4 - SWITCH CYCLE HALOBACTERIUM SALINARUM

[Marwan; Oesterhelt 1999]
Ex5 - THE RKIP PATHWAY

Mitogens
Growth factors

receptor

Ras

Raf

MEK

ERK

cytoplasmic substrates

Elk
SAP

Gene

...one pathway...
Ex5 - THE RKIP PATHWAY

[Cho et al., CMSB 2003]
Ex5 - The RKIP Pathway, Petri Net

[Gilbert, Heiner 2006]
EX6 - SIGNALLING CASCADE

[LEVCHENKO 2000]
EX6 - SIGNALLING CASCADE

[HEINER, GILBERT, DONALDSON 2008]

[GILBERT, HEINER, LEHRACK 2007]
BIO PETRI NETS, PART II

MODEL-BASED SYSTEM ANALYSIS
MODEL- BASED SYSTEM ANALYSIS

CONSTRUCTION

system

technical system

verification

requirement specification

model

system properties

model properties
MODEL-BASED SYSTEM ANALYSIS

UNDERSTANDING

system

biological system

model

model properties

system properties

known properties

unknown properties

behaviour prediction

validation
QUALITATIVE ANALYSES
TYPICAL PETRI NET QUESTIONS

- How many tokens can reside at most in a given place?
  - $(0, 1, k, \infty)$
  - **BOUNDEDNESS**

- How often can a transition fire?
  - $(0\text{-times, } n\text{-times, } \infty\text{-times})$
  - **LIVENESS**

- How often can a system state be reached?
  - never
  - $n\text{-times}$
  - always reachable again
  - reversible initial state
  - **UNREACHABLE** -> **SAFETY PROPERTIES**
  - **REPRODUCIBLE**
  - **REVERSIBLE (HOME STATE)**
  - **REVERSIBILITY**

GENERAL BEHAVIOURAL PROPERTIES

- **orthogonal**
- **general decidable**
MODEL ANIMATION (?)
DYNAMIC ANALYSES
DYNAMIC ANALYSES
reachability / occurrence graph,
(labelled) state transition graph
CTMC, Kripke structure
REACHABILITY GRAPH CONSTRUCTION

- simple algorithm
- nodes: system states
- arcs: the (single) firing transition
- single step firing rule
Reachability Graph Evaluation

- **Interleaving semantics**
  - \(\rightarrow\) (sequential) finite automaton
  - \(\rightarrow\) concurrency \(==\) enumerating all interleaving sequences

- **Boundedness**
  - \(\rightarrow\) finite graph

- **Reversibility**
  - \(\rightarrow\) one Strongly Connected Component (SCC)

- **Liveness**
  - \(\rightarrow\) every transition contained in all terminal SCC

- **Dead states**
  - \(\rightarrow\) terminal nodes
REACHABILITY GRAPH, EX: MA1, 5 TOKENS
REACHABILITY GRAPH, STATE SPACE COMPLEXITY

- infinite for unbounded nets
- worst-case for finite state spaces [Priese, Wimmel 2003]  
  ... cannot be bounded by a primitive recursive function ...

- proof  -> Petri net computer for a function \( f: \mathbb{N}_0^m \rightarrow \mathbb{N}_0 \)

\[ f \text{ is weakly pn-computable:} \]

\[ \text{EF( out } = f(\text{in) } \& \text{ stop } = 1) \]
Reachability Graph, State Space Complexity

- infinite for unbounded nets

- worst-case for finite state spaces \[\text{[Priese, Wimmel 2003]}\]
  ... cannot be bounded by a primitive recursive function ...

- proof -> Petri net computer for a function \(f: \mathbb{N}_0^m \rightarrow \mathbb{N}_0\)

\[f\text{ is weakly pn-computable:}\]

\[\text{EF( out } = f(\text{in}) \& \text{ stop } = 1)\]

Ackermann function \(a_1\)
Reachability Graph, State Space Complexity

- infinite for unbounded nets

- worst-case for finite state spaces \[\text{[Priese, Wimmel 2003]}\]
  
  \[\ldots \text{cannot be bounded by a primitive recursive function}\ldots\]

- proof  \(\rightarrow\) Petri net computer for a function \(f: \mathbb{N}_0^m \rightarrow \mathbb{N}_0\)

  \(f\) is weakly pn-computable:

  \[\text{EF( out } = f(\text{in}) \& \text{ stop } = 1 \text{ )}\]

  Ackermann function \(a2\)
STATE SPACE COMPLEXITY, CAUSES

n! interleaving sequences
m -> m'

$2^n$ - 2 intermediate states

$(n + k - 1)!$ states
$(n - 1)! k!$

(combination with repetition)
ANALYSIS TECHNIQUES

- static analyses
  - no state space construction
    - structural properties (graph theory)
    - P / T - invariants (linear algebra)

- dynamic analyses
  - total / partial state space construction
  - analysis of general behavioural system properties,
    i.e. boundedness, liveness, reversibility
  - model checking of special behavioural system properties,
    e.g. reachability of a given (sub-) system state (with constraints),
    reproducability of a given (sub-) system state (with constraints)

=> expressed in temporal logics (CTL / LTL),
  as very flexible & powerful query language
TYPICAL PETRI NET QUESTIONS

- How many tokens can reside at most in a given place?
  -> (0, 1, k, oo) -> BOUNDEDNESS

- How often can a transition fire?
  -> (0-times, n-times, oo-times) -> LIVENESS

- How often can a system state be reached?
  -> never -> UNREACHABLE -> SAFETY PROPERTIES
  -> n-times -> REPRODUCIBLE
  -> always reachable again -> REVERSIBLE (HOME STATE)
  -> reversible initial state -> REVERSIBILITY

- Are there behaviourally invariant subnet structures?
  -> token conservation -> P - INVARIANTS
  -> token distribution reproduction -> T - INVARIANTS

- ... and many more -> temporal logics (CTL, LTL)
**ANALYSIS TOOLS**

- **Petri net theory**
  - INA (HU Berlin)
  - TINA (LAAS/CNRS)
  - Charlie

- **model checking**
  - reachability graph
  - lazy state spaces
    - stubborn set reduction
    - symmetry reduction
  - compressed state spaces
    - (BDD, NDD, ..., IDD)
  - Kronecker algebra
  - prefix
  - process automata

**CTL**
- INA, Charlie
- PROD, MARIA

**LTL**
- Charlie
- PROD, MARIA
- PROD (LTL\X)
- bdd-LTL
- idd-LTL
- QQ (LTL\X)
- [Kemper]
- [pd]
ANALYSIS TOOLS

- Petri net theory
  - INA (HU Berlin)
  - TINA (LAAS/CNRS)
  - Charlie

- model checking
  - reachability graph
    - INA, Charlie
  - lazy state spaces
    - stubborn set reduction
      - INA, Charlie
    - symmetry reduction
      - LoLA
  - compressed state spaces
    - bdd-CTL, SMART
    - idd-CTL
  - Kronecker algebra
    - [Kemper]
  - prefix
    - PEP (CTL₀)
  - process automata
    - [pd]
STATIC ANALYSES
STRUCTURAL ANALYSIS, SOME EXAMPLES

- **boundary nodes**
  - input transitions -> not BND
  - input places -> not LIVE
  - LIVE & BND -> no boundary nodes

- **conservative -> BND**

- **Deadlock-Trap Property (DTP)**
  - no structural deadlock -> live
  - ORD & DTP -> no dead states \((\text{Ex6})\)
  - ORD & ES & DTP -> LIVE \((\text{Ex5})\)
  - ORD & EFC & DTP <-> LIVE
INCIDENCE MATRIX C

- a representation of the net structure => stoichiometric matrix

<table>
<thead>
<tr>
<th>P</th>
<th>T</th>
<th>t1</th>
<th>...</th>
<th>tj</th>
<th>...</th>
<th>tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>p1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pi</td>
<td></td>
<td></td>
<td></td>
<td>cij</td>
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<tr>
<td>...</td>
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<tr>
<td>pn</td>
<td></td>
<td></td>
<td></td>
<td>Δtj</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[
c_{ij} = (p_i, t_j) = F(t_j, p_i) - F(p_i, t_j) = \Delta t_j(p_i)
\]

\[
\Delta t_j = \Delta t_j(^*)
\]

- matrix entry \(c_{ij}\): token change in place \(p_i\) by firing of transition \(t_j\)

- matrix column \(\Delta t_j\): vector describing the change of the whole marking by firing of \(t_j\)

- side-conditions are neglected

enzyme-catalysed reaction: \(c_{ij} = 0\)
P-invariants, Basics

Lautenbach, 1973

- P-invariants: integer solutions $y$ of $yC = 0, y \neq 0, y \geq 0$ -> multisets of places

- Minimal P-invariants: there is no P-invariant with a smaller support -> sets of places
  -> $gCD$ of all entries is 1

- Any P-invariant is a non-negative linear combination of minimal ones
  -> multiplication with a positive integer
  -> addition
  -> Division by $gCD$

- Covered by P-Invariants (CPI)
  -> each place belongs to a P-invariant
  -> CPI => BND (sufficient condition)
P-INVARINTEIS, INTERPRETATION

- The firing of any transition has no influence on the weighted sum of tokens on the P-invariant’s places
  - For all t: the effect of the arcs, removing tokens from a P-invariant’s places
    is equal to the effect of the arcs, adding tokens to a P-invariant’s places

- Set of places with
  - A constant weighted sum of tokens for all markings m reachable from m₀
    \[ y_m = y_{m₀} \]
  - Token / compound preservation,
  - Moieties
  - A place belonging to a P-invariant is bounded

- A P-invariant defines a subnet
  - The P-invariant’s places (the support),
    + All their pre- and post-transitions
    + The arcs in between
  - Pre-sets of supports = post-sets of supports
    - Self-contained
THE RKIP PATHWAY, P-INVARIANTS

**P-INV1: MEK**
**P-INV2: RAF-1STAR**
**P-INV3: RP**
**P-INV4: ERK**
**P-INV5: RKIP**
T-INVARIANTS, BASICS

- Lautenbach, 1973 -> Schuster, 1993

- T-invariants
  - integer solutions $x$ of $Cx = 0, x \neq 0, x \geq 0$ -> Parikh vector

- minimal T-invariants
  - there is no T-invariant with a smaller support
  - $gcd$ of all entries is 1

- any T-invariant is a non-negative linear combination of minimal ones
  - multiplication with a positive integer
  - addition
  - Division by $gcd$

- Covered by T-Invariants (CTI)
  - each transition belongs to a T-invariant
  - $BND \& LIVE \Rightarrow CTI$ (necessary condition)
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 occurring one after the other
   -> substance / signal flow
2. relative transition firing rates
   of transitions occurring 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 → 2 B
r2: 2 A → 3 C
- non-trivial T-invariant
  + four trivial ones for reversible reactions
NON-TRIVIAL T-IN Variant, RUN

- realizability check under the constructed marking
- T-invariant’s unfolding to describe its behaviour
  -> partial order structure
- labelled condition / event net
  -> events (boxes)
    - transition occurrences
  -> conditions (circles)
    - involved compounds
- occurrence net
  -> acyclic
  -> no backward branching conditions
  -> infinite
T-invariants may contain any structure

T-invariants generally overlap

-> combinatorial effect brings explosion in the number of min. T-invariants \( 2^4 \)
CONSTRUCTION OF THE INITIAL MARKING

- each P-invariant gets at least one token
  -\> P-invariants are structural deadlocks and traps

- in signal transduction
  -\> exactly 1 token, corresponding to species conservation
  -\> token in least active state

- all (non-trivial) T-invariants get feasible
  -\> to make the net live

- minimal marking
  -\> minimization of the state space

-\> UNIQUE INITIAL MARKING  \<-
validation criterion 1
- all expected structural properties hold
- all expected general behavioural properties hold

validation criterion 2
- CPI (if closed model)
- no minimal P-invariant without biological interpretation

validation criterion 3
- CTI
- no minimal T-invariant without biological interpretation
- no known biological behaviour without corresponding T-invariant

validation criterion 4
- all expected special behavioural properties hold
- temporal-logic properties -> TRUE
QUALITATIVE ANALYSIS, SUMMARY

- construction of initial marking

- subnetwork identification
  - $P$-invariants: token preserving modules (mass conservation)
  - $T$-invariants: state repeating modules (elementary modes)

- network validation
  - structure (topology)
  - initial conditions

- choice of stochastic analysis techniques
NOW WE ARE READY FOR SOPHISTICATED QUANTITATIVE ANALYSES!
STOCHASTIC PETRI NETS

- **transitions**
  - exponentially distributed waiting time
  - state-dependent propensity (hazard) function

- **semantics**
  - Continuous Time Markov Chain (CTMC)

- **CTMC ~ reachability graph + transition rates**
  - all qualitative properties are preserved
  - reversibility -> ergodicity

- **(sufficiently) finite CTMC**
  - analytical = exact CSL model checking

- **(practically) infinite CTMC**
  - simulative = approximative PLTLc model checking
  - approximation: finite number of finite simulation traces
Continuous Petri Nets

- **Continuous places carry continuous tokens**
  > real numbers -> compound concentrations

- **Continuous transitions**
  -> continuous firing / fluxes (if any)
  -> state-dependent rate functions

- **Continuous Petri nets = ODEs**

  \[ v_1 = k_1 \cdot A \cdot E \]
  \[ dA / dt = -v_1 + v_2 \]
  \[ dB / dt = v_3 \]
  \[ dE / dt = -v_1 + v_2 + v_3 \]

  \[ v_2 = k_2 \cdot A \cdot E \]
  \[ v_3 = k_3 \cdot A \cdot E \]

- **Analysis**
  -> all standard ODEs techniques + LTLc model checking
\[
\frac{dm3}{dt} =
\]
\[
\frac{dm_3}{dt} = + r_1 + r_4
\]
\[
\frac{dm_3}{dt} = + r_1 \\
+ r_4 \\
- r_2 \\
- r_3
\]
\[
\frac{dm_3}{dt} = + k_1 \cdot m_1 \cdot m_2 \\
+ r_4 \\
- r_2 \\
- r_3
\]
\[
\frac{dm_3}{dt} = + k_1 \cdot m_1 \cdot m_2 \\
+ k_4 \cdot m_4 \\
- k_2 \cdot m_3 \\
- k_3 \cdot m_3 \cdot m_9
\]
THE QUALITATIVE MODEL BECOMES THE STRUCTURED DESCRIPTION OF THE QUANTITATIVE MODELS!
Bio Petri Nets, Part III
A Case Study

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joint work with David Gilbert, Robin Donaldson
Bioinformatics Research Centre, University of Glasgow

Bertinoro, June, 2008
**Definition:**

A **place/transition Petri net** is a quadruple \( \mathcal{PN} = (P, T, f, m_0) \), where

- \( P, T \) - finite, non empty, disjoint sets (places, transitions)
- \( f : ((P \times T) \cup (T \times P)) \rightarrow \mathbb{N}_0 \) (weighted directed arcs)
- \( m_0 : P \rightarrow \mathbb{N}_0 \) (initial marking)

**Interleaving Semantics:** reachability graph / CTL, LTL
Definition:
A biochemically interpreted stochastic Petri net is a quintuple $\mathcal{SPN}_{Bio} = (P, T, f, \nu, m_0)$, where
- $P, T$ - finite, non empty, disjoint sets (places, transitions)
- $f : ((P \times T) \cup (T \times P)) \rightarrow \mathbb{N}_0$ (weighted directed arcs)
- $m_0 : P \rightarrow \mathbb{N}_0$ (initial marking)
- $\nu : T \rightarrow H$ (stochastic firing rate functions) with
  - $H := \bigcup_{t \in T} \left\{ h_t \mid h_t : \mathbb{N}_0^{\bullet t} \rightarrow \mathbb{R}^+ \right\}$
  - $\nu(t) = h_t$ for all transitions $t \in T$

Semantics: Continuous Time Markov Chain / CSL, PLTLc
Definition:
A biochemically interpreted continuous Petri net is a quintuple \( \mathcal{CPN}_{\text{Bio}} = (P, T, f, \nu, m_0) \), where

- \( P, T \) - finite, non empty, disjoint sets (places, transitions)
- \( f : ((P \times T) \cup (T \times P)) \to \mathbb{R}_0^+ \) (weighted directed arcs)
- \( m_0 : P \to \mathbb{R}_0^+ \) (initial marking)
- \( \nu : T \to H \) (continuous firing rate functions) with
  - \( H := \bigcup_{t \in T} \{ h_t \mid h_t : \mathbb{R}^{|t|} \to \mathbb{R}^+ \} \)
  - \( \nu(t) = h_t \) for all transitions \( t \in T \)

Semantics: ODEs / LTLc
Interpretation of tokens:

- *tokens = molecules, moles*
- *tokens = concentration levels*
Specialised stochastic firing rate function, two examples:

- **molecules semantics**

\[ h_t \equal c_t \cdot \prod_{p \in \cdot t} \left( \frac{m(p)}{f(p, t)} \right) \]  

(1)

- **concentration levels semantics**

\[ h_t \equal k_t \cdot N \cdot \prod_{p \in \cdot t} \left( \frac{m(p)}{N} \right) \]  

(2)
... a typical signalling cascade

modelled in [Levchenko et al. 2000] like this ...
Running Case Study - Origin

[Levchenko et al. 2000], \textit{Supplemental Material : ODEs}

\[
\begin{align*}
    \frac{d\text{Raf}}{dt} &= k_2 \cdot \text{Raf}_\text{RasGTP} + k_6 \cdot \text{RafP}_\text{Phase1} - k_1 \cdot \text{Raf} \cdot \text{RasGTP} \\
    \frac{d\text{RasGTP}}{dt} &= k_2 \cdot \text{Raf}_\text{RasGTP} + k_3 \cdot \text{Raf}_\text{RasGTP} - k_1 \cdot \text{Raf} \cdot \text{RasGTP} \\
    \frac{d\text{Raf}_\text{RasGTP}}{dt} &= k_1 \cdot \text{Raf} \cdot \text{RasGTP} - k_2 \cdot \text{Raf}_\text{RasGTP} - k_3 \cdot \text{Raf}_\text{RasGTP} \\
    \frac{d\text{RafP}}{dt} &= k_3 \cdot \text{Raf}_\text{RasGTP} + k_{12} \cdot \text{MEKP}_\text{RafP} + k_9 \cdot \text{MEK}_\text{RafP} + \\
    & \quad k_5 \cdot \text{RafP}_\text{Phase1} + k_8 \cdot \text{MEK}_\text{RafP} + k_{11} \cdot \text{MEKP}_\text{RafP} - \\
    & \quad k_7 \cdot \text{RafP} \cdot \text{MEK} - k_{10} \cdot \text{MEKP} \cdot \text{RafP} - k_4 \cdot \text{Phase1} \cdot \text{RafP} \\
    \frac{d\text{RafP}_\text{Phase1}}{dt} &= k_4 \cdot \text{Phase1} \cdot \text{RafP} - k_5 \cdot \text{RafP}_\text{Phase1} - k_6 \cdot \text{RafP}_\text{Phase1} \\
    \frac{d\text{MEK}_\text{RafP}}{dt} &= k_7 \cdot \text{RafP} \cdot \text{MEK} - k_8 \cdot \text{MEK}_\text{RafP} - k_9 \cdot \text{MEK}_\text{RafP} \\
    \frac{d\text{MEKP}_\text{RafP}}{dt} &= k_{10} \cdot \text{MEKP} \cdot \text{RafP} - k_{11} \cdot \text{MEKP}_\text{RafP} - k_{12} \cdot \text{MEKP}_\text{RafP} \\
    \frac{d\text{MEKP}_\text{Phase2}}{dt} &= k_{16} \cdot \text{Phase2} \cdot \text{MEKP} - k_{18} \cdot \text{MEKP}_\text{Phase2} - k_{17} \cdot \text{MEKP}_\text{Phase2} \\
    \frac{d\text{MEKPP}_\text{Phase2}}{dt} &= k_{13} \cdot \text{MEKPP} \cdot \text{Phase2} - k_{15} \cdot \text{MEKPP}_\text{Phase2} - k_{14} \cdot \text{MEKPP}_\text{Phase2} \\
    \frac{d\text{ERK}}{dt} &= k_{20} \cdot \text{ERK}_\text{MEKPP} + k_{30} \cdot \text{ERKPP}_\text{Phase3} - k_{19} \cdot \text{MEKPP} \cdot \text{ERK} \\
    \frac{d\text{ERK}_\text{MEKPP}}{dt} &= k_{19} \cdot \text{MEKPP} \cdot \text{ERK} - k_{20} \cdot \text{ERK}_\text{MEKPP} - k_{21} \cdot \text{ERK}_\text{MEKPP} \\
    \frac{d\text{ERKPP}_\text{MEKPP}}{dt} &= k_{22} \cdot \text{MEKPP} \cdot \text{ERK} - k_{24} \cdot \text{ERKPP}_\text{MEKPP} - k_{23} \cdot \text{ERKPP}_\text{MEKPP} \\

\text{etcetera} &= \ldots
\end{align*}
\]
Qualitative Analysis

- **initial marking construction**
  P-invariants

- **subnetwork identification**
  - P-invariants: token preserving modules (*mass conservation*)
  - T-invariants: state repeating modules (*elementary modes*)

- **general behavioural properties**
  - *boundedness*: every place gets finite token number only
  - *liveness*: every transition may happen forever
  - *reversibility*: every state may be reached forever

- **special behavioural properties**
  CTL / LTL model checking
Running Case Study - P-invariants

Raf

RasGTP

Raf_RasGTP

k3

Raf

RafP

k1/k2

k6

RafP_Phase1

Phase1

MEK_RafP

MEKP_RafP

k9

k12

k7/k8

k10/k11

MEK

MEKP

k15

k13/k14

k16/k17

MEKP_Phase2

MEKPP_Phase2

ERK_MEKPP

ERKP_MEKPP

k21

k24

k19/k20

k12/k23

ERK

ERKP

k28/k29

k25/k26

k27

k30

ERKPP_Phase3

ERKP_Phase3

Phase3

k1/k2

k4/k5

k6

k22/k23

k19/k20

k30

k27

k25/k26

k28/k29

k21

k19/k20

k27

k25/k26

k28/k29

k21

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k28/k29

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k19/k20

k27

k25/k26

k28/k29

k21

k19/k20

k27

k25/k26

k28/k29

k21
Running Case Study - P-invariants

- RasGTP
- Raf_RasGTP
- Raf_P
- MEK_RafP
- MEKP_RafP
- MEKP_Phase2
- MEKPP_Phase2
- ERK_MEKPP
- ERKP_MEKPP
- ERKPP_Phase3
- MEK
- Phase1
- Phase2
- Phase3

- k1/k2
- k3
- k4/k5
- k6
- k7/k8
- k9
- k10/k11
- k12
- k13/k14
- k15
- k16/k17
- k18

P-invariant 7

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Running Case Study - initial marking

Raf

RasGTP

MEK

MEKP

ERK

ERKP

ERKPP

Phase1

Phase2

Phase3

k1/k2

k3

k4/k5

k6

k7/k8

k9

k10/k11

k12

k13/k14

k15

k16/k17

k18

k19/k20

k21

k22/k23

k24

k25/k26

k27

k28/k29

k30

k31
Running Case Study - general properties

- **state space**

<table>
<thead>
<tr>
<th>levels</th>
<th>reachability graph number of states</th>
<th>IDD data structure number of nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>118</td>
<td>52</td>
</tr>
<tr>
<td>4</td>
<td>$2.4 \cdot 10^4$</td>
<td>115</td>
</tr>
<tr>
<td>8</td>
<td>$6.1 \cdot 10^6$</td>
<td>269</td>
</tr>
<tr>
<td>80</td>
<td>$5.6 \cdot 10^{18}$</td>
<td>13,472</td>
</tr>
<tr>
<td>120</td>
<td>$1.7 \cdot 10^{21}$</td>
<td>29,347</td>
</tr>
</tbody>
</table>

- Covered by P-invariants (CPI) ⇒ bounded
- Deadlock-Trap Property (DTP) holds ⇒ no dead states
- reachability graph
  - strongly connected ⇒ reversible
  - contains every transition (reaction) ⇒ live
Running Case Study - T-invariants

10 trivial T-invariants

1/O T-invariant
Running Case Study - partial order run of I/O T-invariant

Monika Heiner@tu-cottbus.de  Bio Petri Nets, Part III A Case Study
Running Case Study - partial order run of I/O T-invariant
property Q1:

The signal sequence predicted by the partial order run of the I/O T-invariant is the only possible one; i.e., starting at the initial state, it is necessary to pass through RafP, MEKP, MEKPP and ERKP in order to reach ERKPP.

\[ \neg [ E ( \neg \text{RafP} \ U \ \text{MEKP} ) \lor \\ E ( \neg \text{MEKP} \ U \ \text{MEKPP} ) \lor \\ E ( \neg \text{MEKPP} \ U \ \text{ERKP} ) \lor \\ E ( \neg \text{ERKP} \ U \ \text{ERKPP} ) ] \]
isomorphy of reachability graph and CTMC, thus all qualitative properties still valid

How many levels needed for quantitative evaluation?
- state space(1 levels) = 118 (Boolean interpretation)
- state space(4 levels) = 24,065
- state space(8 levels) = 6,110,643

equivalence check

\[
C_{RafP}(t) = \frac{0.1}{s} \cdot \sum_{i=1}^{4s} (i \cdot P(L_{RafP}(t) = i)) \\
\text{expected value of } L_{RafP}(t)
\]
equivalence check, results, e.g. for MEK:

![Graph showing concentration over time for different models of MEK](image-url)
equivalence check, results, e.g. for RasGTP:

![Graph showing concentration over time for RasGTP with different models: Continuous, Stochastic 4 level, and Stochastic 8 level.](image)
property S1:

What is the probability of the concentration of RafP increasing, when starting in a state where the level is already at L?

$$P_{=?} \left[ ( \text{RafP} = L ) \bigcup_{=100}^{=} ( \text{RafP} > L ) \right] \{ \text{RafP} = L \}$$
property S2:

What is the probability that RafP is the first species to react?

\[ P_{=?} \left[ \left( \left( MEKPP = 0 \right) \land \left( ERKPP = 0 \right) \right) U^{\leq 100} \left( RafP > L \right) \left\{ \left( MEKPP = 0 \right) \land \left( ERKPP = 0 \right) \land \left( RafP = 0 \right) \right\} \right] \]
steady state analysis, results for all 118 ‘good’ states, e.g. for MEK:
steady state analysis for state 1:
**steady state analysis for state 10:**

![Graph showing concentration over time](image)
property C1:

The concentration of RafP rises to a significant level, while the concentrations of MEKPP and ERKPP remain close to zero; i.e. RafP is really the first species to react.

\[(\text{MEKPP} < 0.001) \land (\text{ERKPP} < 0.0002) \supset U (\text{RafP} > 0.06)\]
Qualitative Stochastic Continuous Abstraction Approximation of Hazard function, type (1)

Molecules/Levels
CTL, LTL

Stochastic

Discrete State Space

Continuous

Continuous State Space

Molecules/Levels
Stochastic rates
CSL

Concentrations
Deterministic rates
LTLc

Time-free

Timed, Quantitative

Approximation by Hazard function, type (2)

Abstraction

Framework
model construction, animation, simulation

- Snoopy \((\text{Cottbus})\)

qualitative analysis

- Charlie \((\text{Cottbus})\), INA
- BDD-CTL model checker (Boolean semantics) \((\text{Cottbus})\)
- IDD-CTL model checker (integer semantics) \((\text{Cottbus})\)

stochastic analysis

- analytical model checking : PRISM/CSL
- simulative model checking : MC2(PLTLc) \((\text{Glasgow})\)

continuous analysis

- MATLAB
- BioNessie \((\text{Glasgow})\)
- LTLc model checking : MC2(PLTLc) \((\text{Glasgow})\), BioCham
Finally

end of part III

- all data files and analysis results available at www-dssz.informatik.tu-cottbus.de/examples/levchenko

- laptop demonstration available
BIO PETRI NETS, PART IV

SUMMARY
A BIT OF HISTORY

- Carl Adam Petri, 1962, PhD University of Technology Darmstadt
  - basic ideas introduced

- early 1970’s
  - first papers contributing to Petri net theory

- Petri, 1976
  - application to chemical networks mentioned

- early 1980’s
  - first monographs on Petri net theory

- Reddy, 1993
  - first paper on bio application

- late 1990’s
  - increasing interest for modelling and analysis of bio networks
A Bit of History

C. A. Petri, November 2006
A Bit of History

Essence of Net Theory

1. TWO kinds of world points:
   STATES and TRANSITIONS
   e.g. Substances and Reactions

2. TWO topologies:
   GIVE and TAKE
   e.g. Creation and Annihilation

3. TWO kinds of continuity expressible
   Mathematical continuity ("connected and compact")
   Experienced continuity ("connected")

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June 2008
SUMMARY

- representation of bio networks by Petri nets
  - partial order representation -> better comprehension
  - formal semantics -> sound analysis techniques
  - unifying view -> various abstraction levels

- purposes
  - animation -> to experience the model
  - model validation against consistency criteria -> to increase confidence
  - qualitative / quantitative behaviour prediction -> experiment design, new insights

- step-wise model development
  - qualitative model -> discrete Petri nets
  - discrete quantitative model -> stochastic Petri nets
  - continuous quantitative model -> continuous Petri nets = ODEs
CHALLENGES

- Increasing level number = increasing accuracy
  
  \textit{BUT,} monotonous liveness holds for substructures only!

- Unbounded qualitative model + time = bounded model
  
  \textit{BUT,} that's not always the case!
  
  \rightarrow (structural) criteria for time-dependent boundedness?

- Continuous behaviour = averaged stochastic behaviour
  
  \textit{BUT,} that's not always the case!
  
  \rightarrow stochastic and continuous behaviour may differ; why? when?

- Sharing structure = sharing properties
  
  \textit{BUT,} to which extend?
  
  \rightarrow relation: qualitative & continuous behaviour?
FURTHER READING

- M Heiner, D Gilbert, R Donaldson: 
  Petri Nets for Systems and Synthetic Biology 

- R Breitling, D Gilbert, M Heiner, R Orton: 
  A structured approach for the engineering of biochemical network models, 
  illustrated for signalling pathways; 
  Journal Briefings in Bioinformatics, accepted April 2008.

- D Gilbert, M Heiner, S Rosser, R Fulton, X Gu, M Trybilo: 
  A Case Study in Model-driven Synthetic Biology; 
OUTLOOK

THANKS!
HTTP://WWW-DSSZ.INFORMATIK.TU-COTTBUS.DE