MODELLING AND ANALYSIS
OF BIOCHEMICAL NETWORKS
WITH TIME PETRI NETS

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FRAMEWORK

bionetworks knowledge

quantitative modelling

quantitative models

animation evaluation/simulation

understanding

model validation

quantitative behavior prediction
FRAMEWORK

bionetworks knowledge

qualitative modelling

quantitative parameters

quantitative modelling

quantitative models

animation evaluation/analysis

invariants

model validation

qualitative behavior prediction

understanding

model checking

understanding

model validation

quantitative behavior prediction
chemical reactions \rightarrow atomic actions \rightarrow Petri net transitions

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

- Chemical reactions → Atomic actions → Petri net transitions

2 \( \text{NAD}^+ + 2 \text{H}_2\text{O} \rightarrow 2 \text{NADH} + 2 \text{H}^+ + \text{O}_2 \)

- Chemical compounds
  - Primary compounds
  - Auxiliary compounds, ubiquitous → fusion nodes
  - Catalyzing compounds

- Petri net places
  - Metabolites
  - E.g. electron carrier
  - Enzymes

input compounds

\[ \text{NAD}^+ \quad 2 \quad \text{r1} \quad 2 \quad \text{NADH} \]

\[ \text{H}_2\text{O} \quad 2 \quad \text{H}^+ \quad 2 \quad \text{O}_2 \]

output compounds

\[ x \quad y \]

\[ A \quad \text{r2} \quad B \quad \text{enzyme} \]
BIONETWORKS, BASICS

- chemical reactions -> atomic actions -> Petri net transitions

2 NAD\(^+\) + 2 H\(_2\)O \rightarrow 2 NADH + 2 H^+ + O\(_2\)

- chemical compounds
  - primary compounds
  - auxiliary compounds, ubiquitous \(\rightarrow\) fusion nodes
  - catalyzing compounds

- stoichiometric relations \(\rightarrow\) Petri net arc multiplicities

- compounds distribution \(\rightarrow\) marking
r1: A -> B
r1: A -> B
r2: B -> C + D
r3: B -> D + E

-> alternative reactions
BIONETWORKS, INTRO

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  
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BIONETWORKS, SUMMARY

- networks of chemical reactions

- biologically interpreted Petri net
  - partial order sequences of chemical reactions
  - transforming input into output compounds
  - respecting the given stoichiometric relations
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- networks of chemical reactions

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

- typical (structural) properties

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BIONETWORKS NEED ENVIRONMENT BEHAVIOR

- to animate the model
  - infinite substance flow
  - deeper insights

- to validate the model
  - consistency criteria

- steady flow
  - input substances
  - output substances

- auxiliary substances
  - as much as necessary

- minimal assumptions
BIONETWORK WITH ENVIRONMENT BEHAVIOR

- **input substances**
  - generating pre-transitions

- **output substances**
  - consuming post-transitions

- **auxiliary substances**
  - both

- **no boundary places, but boundary transitions**

- **transitions without pre-places**
  - live
  - all post-places are unbounded
  - all places simultaneously unbounded (?)
### BIONETWORKS, STEADY STATE BEHAVIOR

- **steady state behavior**
  - all possible flows preserving the given compounds distribution
  - empty marking reproduction
  - elementary modes = minimal T-invariants

- **consistency criteria**
  - pathways analysis
  - CTI
  - no minimal T-invariant without biological interpretation
  - no known biological behaviour without corresponding T-invariant

- **typical properties**

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**how to prove?**
T-INVARIANTS, REFRESHMENT

- Lautenbach, 1973

- T-invariants
  -> integer solutions of \( Cx = 0, x \neq 0, x \geq 0 \)

- 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 integer 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
trivial min. T-invariants (5)

- boundary transitions of auxiliary compounds
  -> (g_a, r_a), (g_b, r_b), (g_c, r_c)

- reversible reactions
  -> (r5, r5_rev), (r8, r8_rev)

non-trivial min. T-invariants (7)

- covering
  boundary transitions of input / output compounds
  -> i/o-T-invariants

- inner cycles
**i/o-T-invariant, example**

<table>
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<tr>
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<td>4</td>
<td>4.r6</td>
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<td>5</td>
<td>5.r7</td>
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<td>9.r9</td>
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<td>12</td>
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<td>13</td>
<td>13.g_A</td>
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<td>14</td>
<td>14.r_K</td>
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<tr>
<td>15</td>
<td>15.g_b</td>
<td>4,</td>
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<td>18</td>
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</tr>
<tr>
<td>20</td>
<td>20.r_a</td>
<td>4</td>
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**sum equation**

\[ A + 4b \rightarrow 2K + 4a + 4c \]
T-IN Variant, Interpretation

- Parikh vector
  - state-reproducing transition sequence (partial order)
    of transitions occurring one after the other
  - relative transition firing rates
    of transitions occurring permanently & concurrently

- relative transition firing rates
  - may be implemented by transition firing times
    - constant
    - interval

- quantitative model
  - qualitative model + firing times reflecting the firing rates
  - time-dependent model

- claim
  - transformation preserves all possible behavior (= minimal T-invariants)
TRANSFORMATION, 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 Y N Y N 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 N
TRANSFORMATION, Ex1

-> properties as time-less net

INA
ORD HOM NBM PUR CSV SCF CON SC Ft0 tF0 Fp0 pF0 MG SM FC EFC ES
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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
TRANSFORMATION, Ex1

-> properties as time 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 Y N N N ? N Y Y Y Y N
TRANSFORMATION, Ex2

-> 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 N Y Y N
-> 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 Y Y N Y Y Y Y N N Y Y Y
CPI CTI B SB REV DSt BST DTr DCF L LV L&S
N Y N N Y N ? N N Y Y N
TRANSFORMATION, Ex2

T-INVARIANTE1
T-INVARIANTE2

-> properties as time net

INA
ORD HOM NBM PUR CSV SCF CON SC Ft0 tF0 Fp0 pF0 MG SM FC EFC ES
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 Y N N N ? N Y Y Y N
transient state
steady state

- **s6** (A,2B,C)
  - t(r2)=3
  - t(cons_C)=1
- **s7** (0,B,C)
  - t(prod_A)=2
  - t(r1)=5
  - t(r2)=2
  - t(cons_B)=2
- **s8** (A,B,3C)
  - t(r1)=3
- **s9** (0,0,2C)
  - t(prod_A)=1
  - t(r1)=1
  - t(r2)=4
  - t(cons_B)=1

Terminal SCC: s5-6 → s11-8

[r1 start, cons_B start, cons_C end]
[prod_A start, r1 start, cons_B start, cons_C end]
[prod_A end, r1 end, cons_B end, cons_C start]
[prod_A end, r2 end, cons_B end, cons_C]
[prod_A start, r2 start, cons_B start, cons_C]
[prod_A start, r2 start, cons_B start, cons_C]

terminal SCC
RG(Ex2), TERMINAL SCC

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

- contains all minimal t-invariants

- timing diagram

- relative transition firing rates

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<th>cons_B</th>
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<td>cons_B</td>
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<td>3</td>
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6 time units
Ex1+ Ex2, SUMMARY

- CTI, but not CPI
- Transient state
  - Initial behaviour to reach steady state
  - Not REV
  - Generally, not DCF
- Steady state behaviour
  - Terminal scc
  - Here, BND
  - Here, DCF

Diagram:
- PN to D/I NET over time
  - PN: not BND, REV, LIVE
  - D/I NET: BND, not REV, LIVE
BUT, WHAT DO WE DO

☐ if the timed model is bounded, but the reachability graph does not fit into memory?

☐ if the timed model is (still) unbounded?
interval time Petri net

initial marking / state

finite transition word $w$

\[
\begin{align*}
 b_1 &\leq a_{11}x_1 + \ldots + a_{1n}x_n \leq c_1 \\
 &\ldots \\
 b_m &\leq a_{m1}x_1 + \ldots + a_{mn}x_n \leq c_m \\
 a_{ij} &\in \{0, 1\}, b_i \in N, c_i \in N \\
 \forall i \forall s \forall k (1 \leq i \leq n \land 1 \leq s \leq k \leq m \land \\
 a_{is} = a_{ik} = 1 \rightarrow \forall j (s \leq j \leq k \rightarrow a_{ij} = 1))
\end{align*}
\]

$w$ is time-dependent realizable / not realizable

$I: T \rightarrow Q_0^+ \times Q_0^+$ and for each $t \in T$ holds $a_t \leq b_t$, where $I(t) = (a_t, b_t)$

$w \in T^*$

if there is an $R$ solution, then there is an $N$ solution.
**Quantitative Analysis, Method I**

Interval time Petri net

Initial marking / state

Finite transition word $w$

$$\begin{align*}
  b_1 &\leq a_{11}x_1 + \cdots + a_{1n}x_n \leq c_1 \\
  \vdots \\
  b_m &\leq a_{m1}x_1 + \cdots + a_{mn}x_n \leq c_m \\
  a_{ij} &\in \{0, 1\}, b_i, c_i \in \mathbb{N}
\end{align*}$$

(SLI)

$w$ is time-dependent realizable / not realizable

$I: T \rightarrow \mathbb{Q}_0^+ \times \mathbb{Q}_0^+$ and for each $t \in T$ holds $a_t \leq b_t$, where $I(t) = (a_t, b_t)$

$w \in T^*$

Polynomial time

If there is an $R$ solution, then there is an $N$ solution.

Linear time
QUANTITATIVE ANALYSIS, METHOD II

interval time Petri net

initial marking / state

finite transition word $w$

$$\min / \max x_1 + \ldots + x_n$$

$\begin{align*}
    b_1 &\leq a_{11} x_1 + \ldots + a_{1n} x_n \leq c_1 \\
    \vdots \\
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\end{align*}$$

(LP)

$I: T \rightarrow Q_0^+ \times Q_0^+$ and for each $t \in T$ holds $a_t \leq b_t$, where $I(t) = (a_t, b_t)$

$w \in T^*$

if there is a solution, then it is an $N$ solution.

$w$ is time-dependent realizable / not realizable

min/max time length of $w$
QUANTITATIVE ANALYSIS, METHOD II

I: $T \rightarrow Q_0^+ \times Q_0^+$ and for each $t \in T$ holds
$a_t \leq b_t$, where $I(t) = (a_t, b_t)$

$$w \in T^*$$

**interval time Petri net**

**initial marking / state**

**finite transition word $w$**

\[
\begin{align*}
\min / \max x_1 + \ldots + x_n & \\
\quad b_1 \leq a_{11}x_1 + \ldots + a_{1n}x_n \leq c_1 & \\
\ldots & \\
\quad b_m \leq a_{m1}x_1 + \ldots + a_{mn}x_n \leq c_m & \\
\quad a_{ij} \in \{0, 1\}, b_i \in \mathbb{N}, c_i \in \mathbb{N} & \\
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\quad a_{is} = a_{ik} = 1 \rightarrow \forall j(s \leq j \leq k \rightarrow a_{ij} = 1)) & \\
\end{align*}
\]

**polynomial time**

if there is a solution, then it is an $N$ solution.

$w$ is time-dependent realizable / not realizable

min/max time length of $w$
QUANTITATIVE ANALYSIS, SUMMARY

❑ structural technique
  - parametric description
  - no state space construction
  - works also for infinite systems

❑ given: full set of the transitions’ time windows
  + transition sequence, esp. a (min.) T-invariant
  - time-dependent realisability
    + in the steady state
  - shortest and longest time length
    - time windows of the recurrent pathways (processes)
  - validation of transformation step
  - measurement approximation

❑ given: partial set of the transitions’ time windows
  + transition sequence, esp. a (min.) T-invariant
  - which time windows guarantee realizability?
CASE STUDY

- carbon metabolism in potato tuber -> 17 P / 25 T
  - stiochiometric relations
  - non-ordinary place/transition net
  - many reversible reactions

- 19 t-invariants
  - 7 trivial ones
  - 12 i/o invariants

- comparison
  - calculated firing rates
  - published kinetic parameters
  - not finished yet

- expected results
  - hints for open experiments to get reliable kinetic parameters
  - validated model of the steady state behavior
CHALLENGES

- extensions
  - read arcs
  - inhibitor arcs !?

- efficient computation of minimal invariants
  - 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 and LP-based time evaluation ?

- model checking
  - relevant properties ?
THANKS!