# MODELLING AND ANALYSIS OF BIOCHEMICAL NETWORKS WITH TIME PETRI NETS

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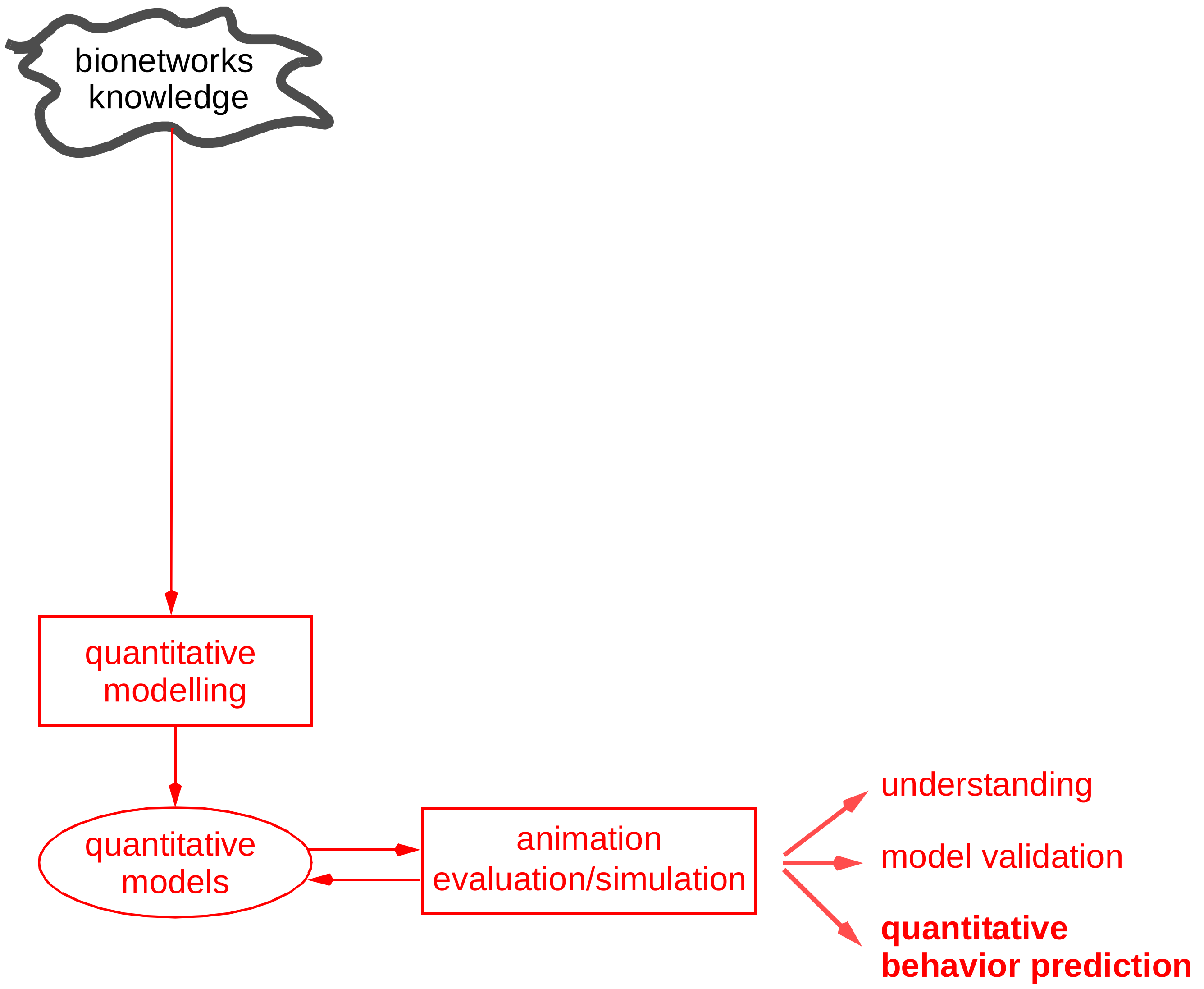
# FRAMEWORK


bionetworks knowledge
quantitative modelling
quantitative models
animation evaluation/simulation
understanding
model validation
quantitative behavior prediction

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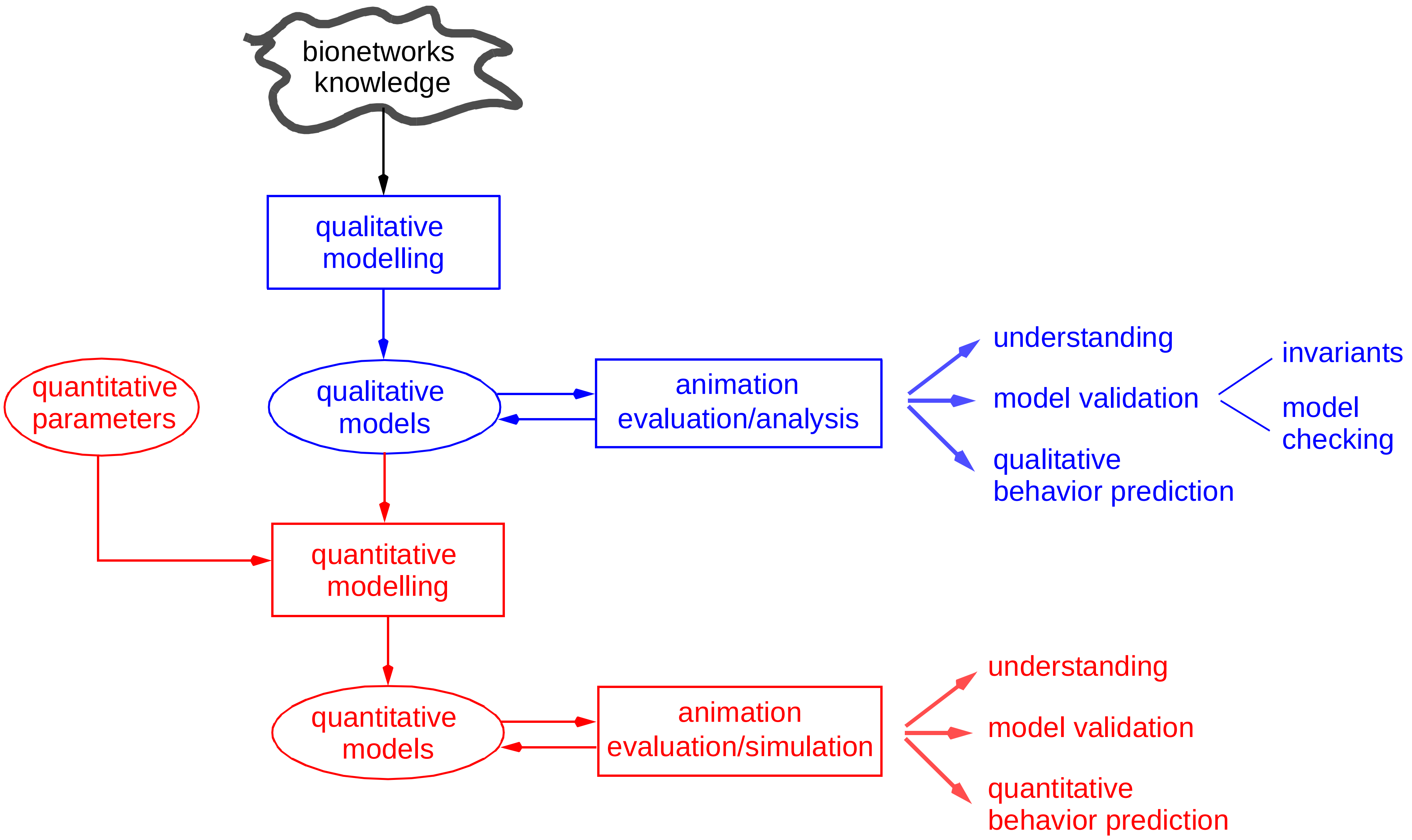
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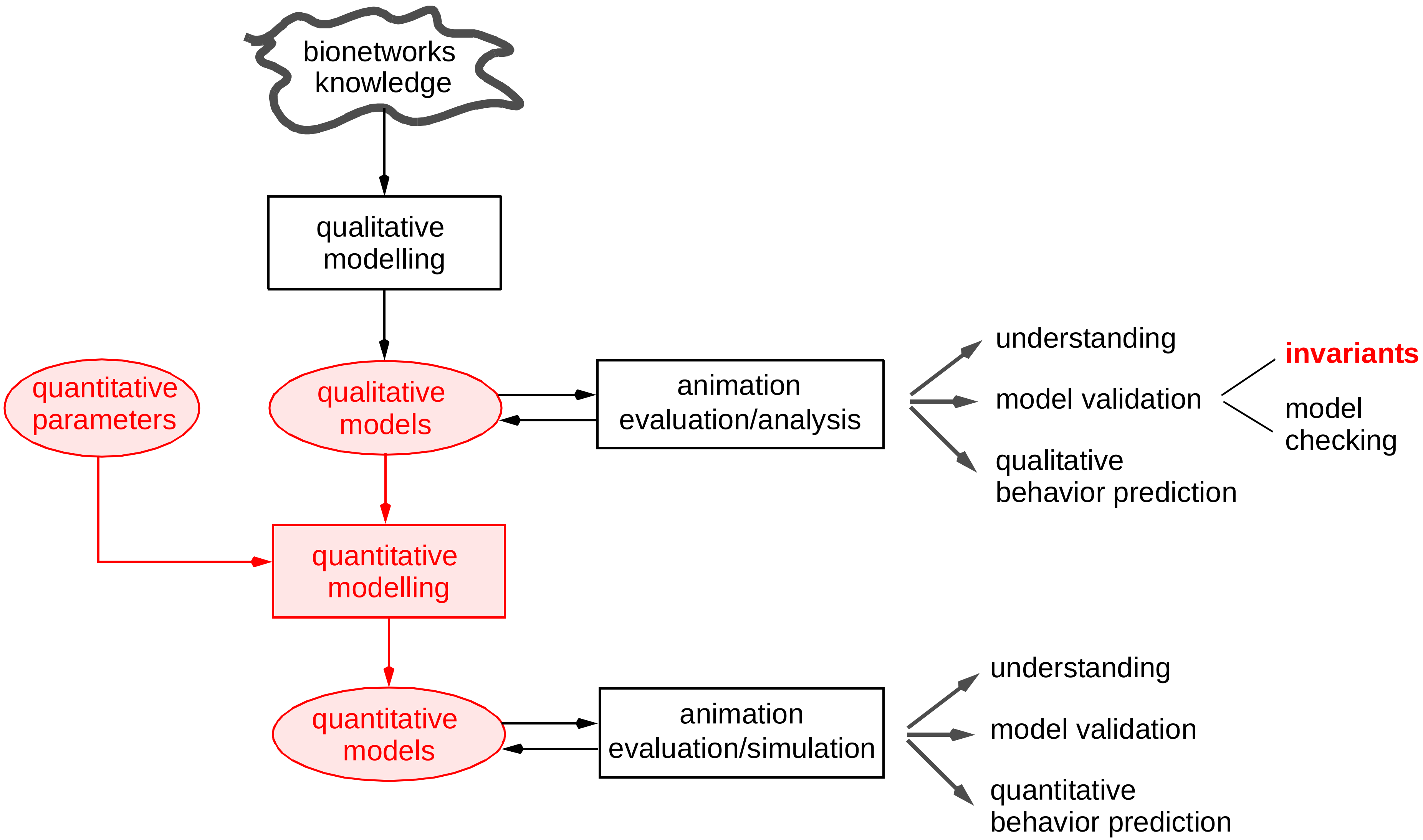
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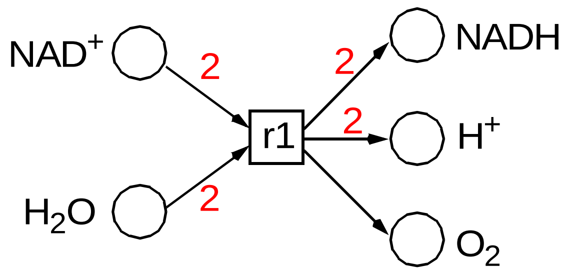
- **chemical reactions -> atomic actions -> Petri net transitions**

$$2\ NAD^+ + 2\ H_2O \rightarrow 2\ NADH + 2\ H^+ + O_2$$

input compounds




output compounds

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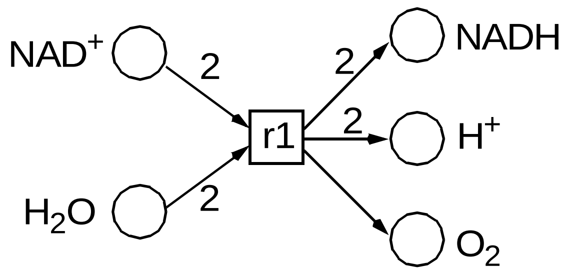
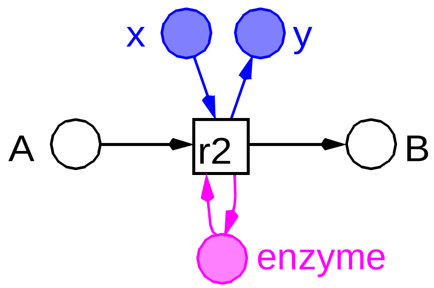



output compounds

- **chemical compounds -> Petri net places**
  - *primary compounds* - *metabolites*
  - *auxiliary compounds, ubiquitous -> fusion nodes* - *e. g. electron carrier*
  - *catalyzing compounds* - *enzymes*



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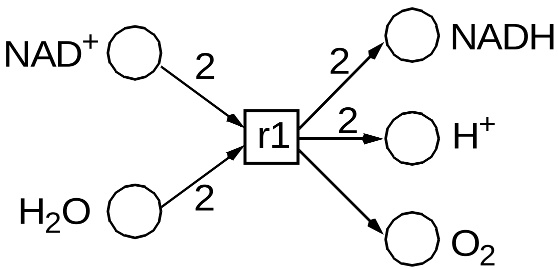


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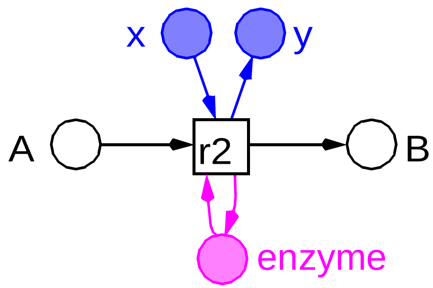
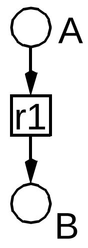



- **stoichiometric relations -> Petri net arc multiplicities**

- **compounds distribution -> marking**

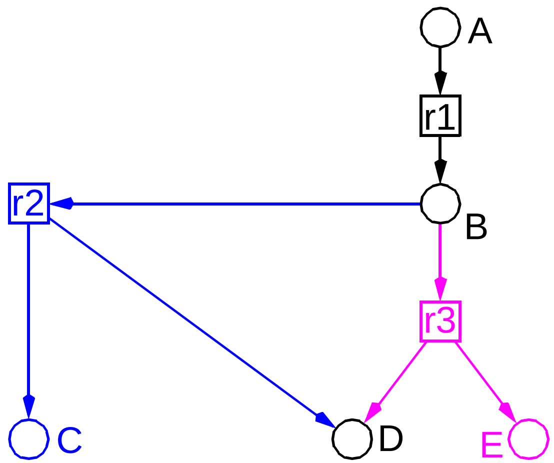
r1: A -> B



r1: A -> B

r2: B -> C + D

r3: B -> D + E




*-> alternative reactions*

r1: A -> B

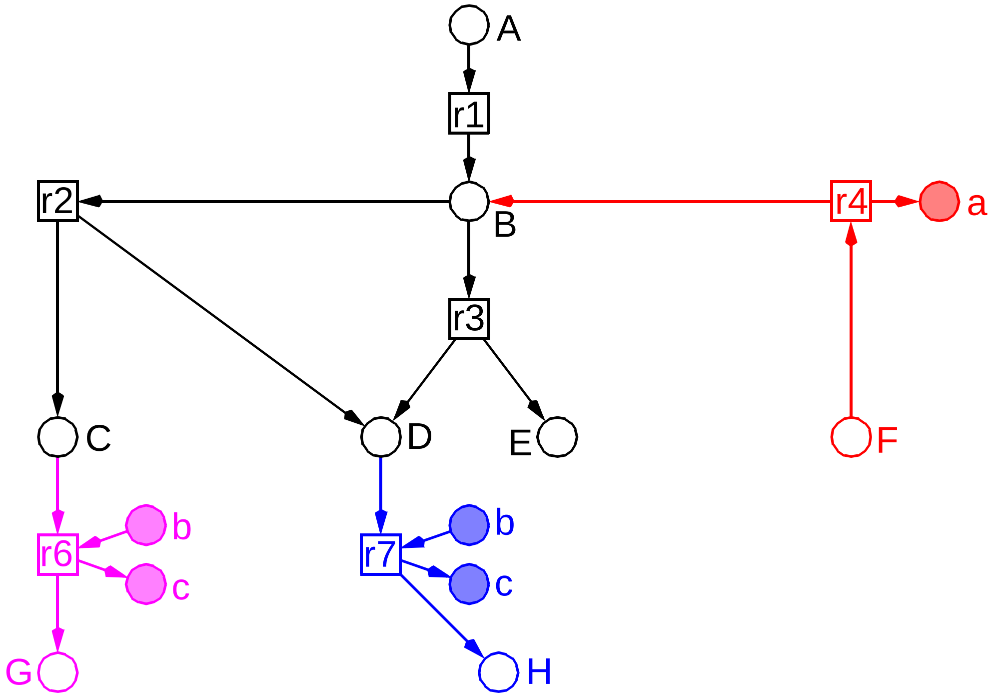
r2: B -> C + D

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

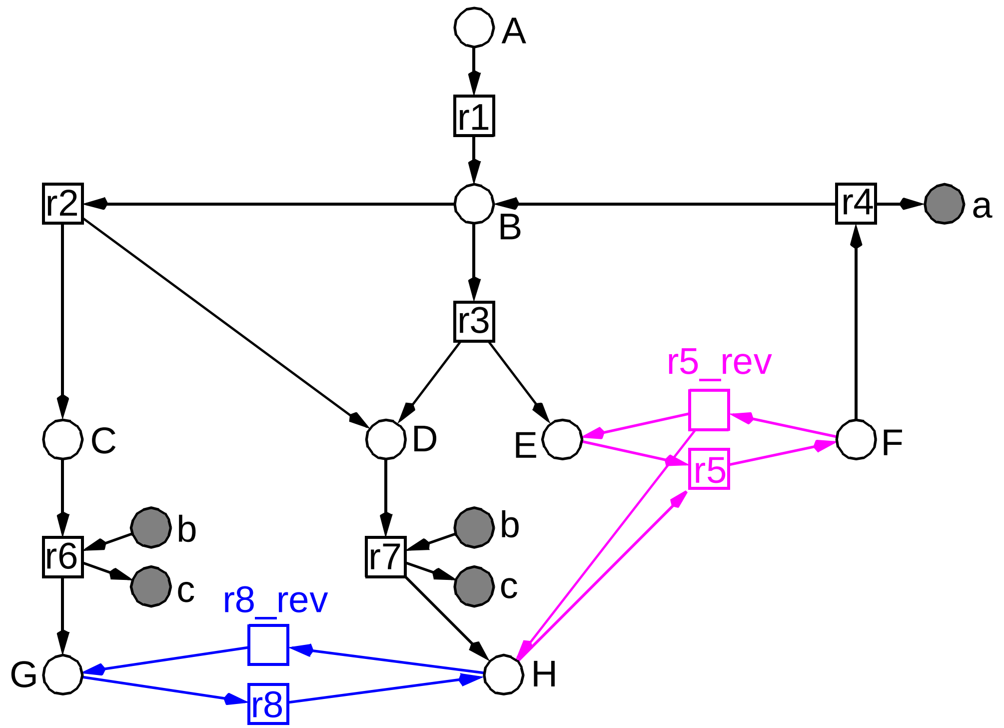
r4: F -> B + a

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

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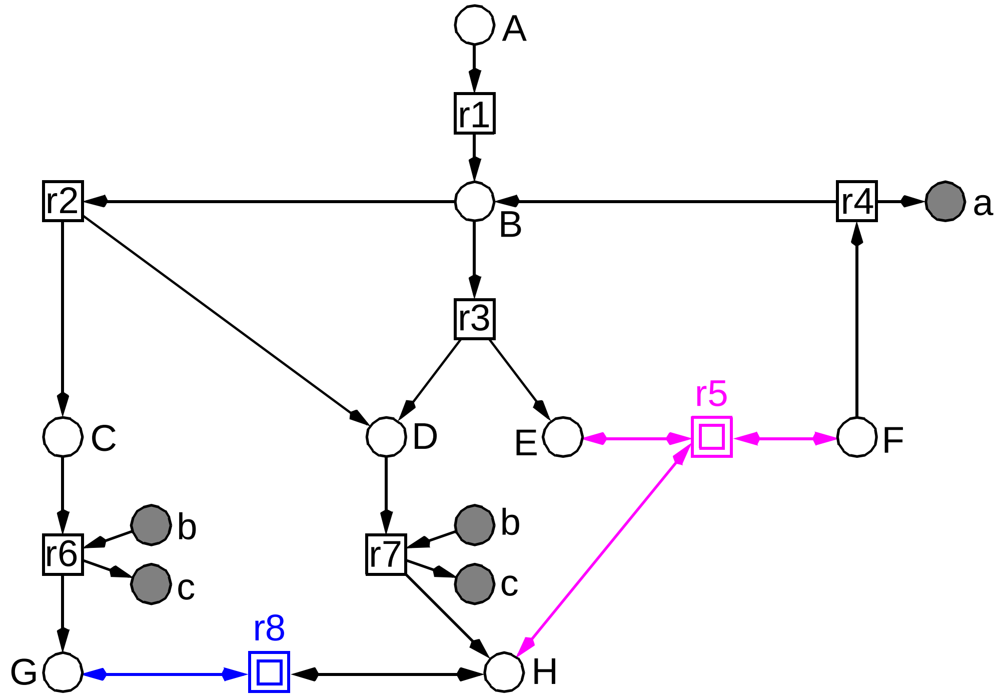



*-> reversible reactions*
*- hierarchical nodes*

r1: A -> B

r2: B -> C + D

r3: B -> D + E

r4: F -> B + a

r5: E + H <-> F

r6: C + b -> G + c

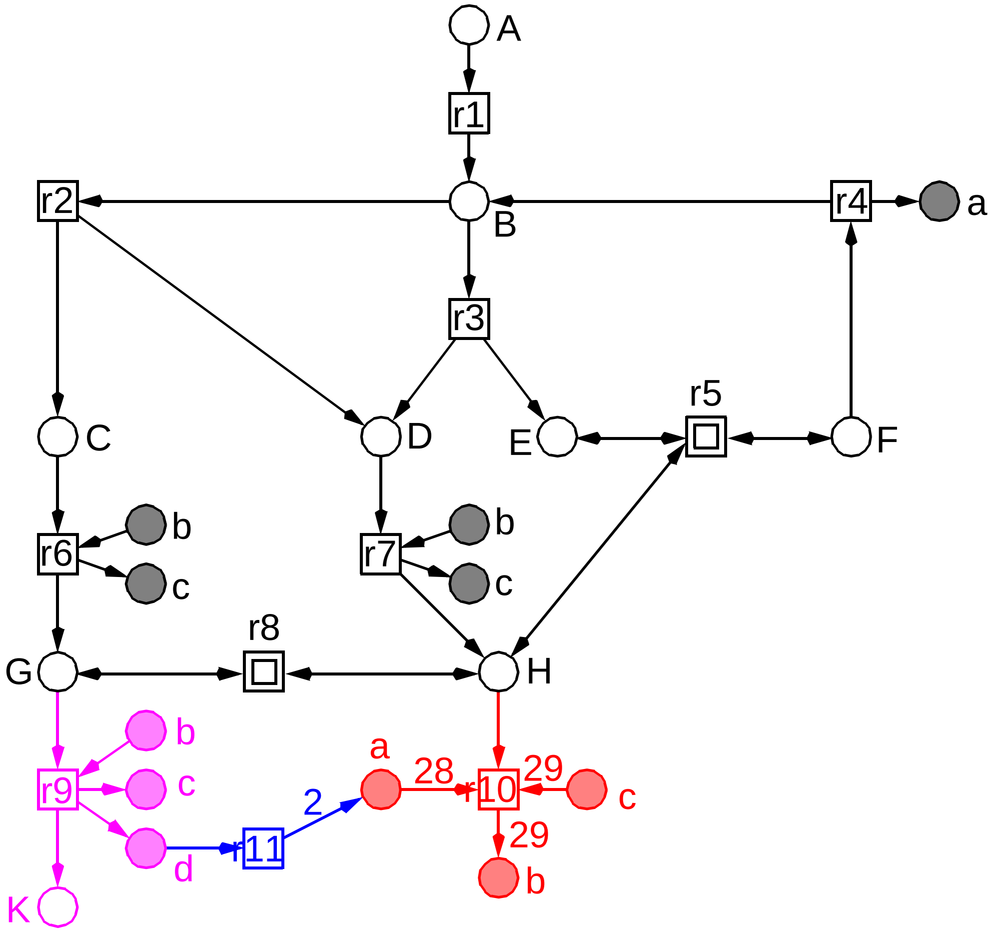
r7: D + b -> H + c

r8: H <-> G

r9: G + b -> K + c + d

r10: H + 28a + 29c -> 29b

r11: d -> 2a



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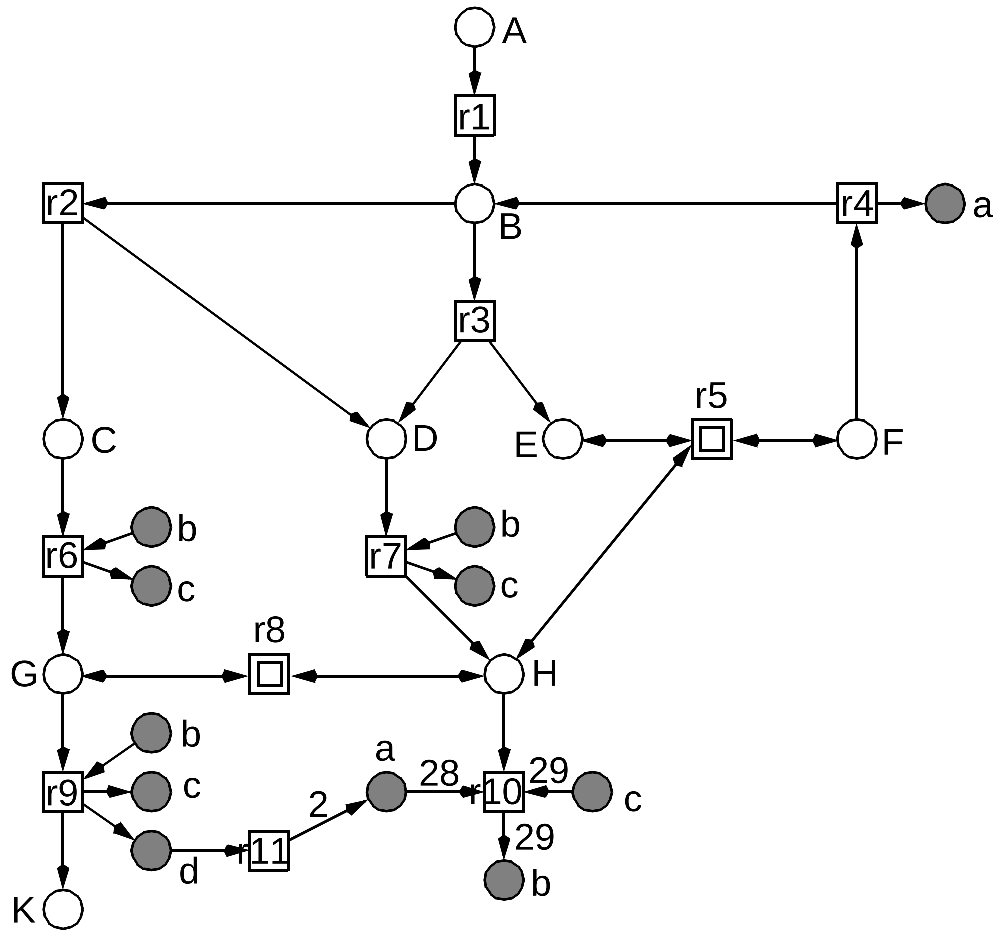

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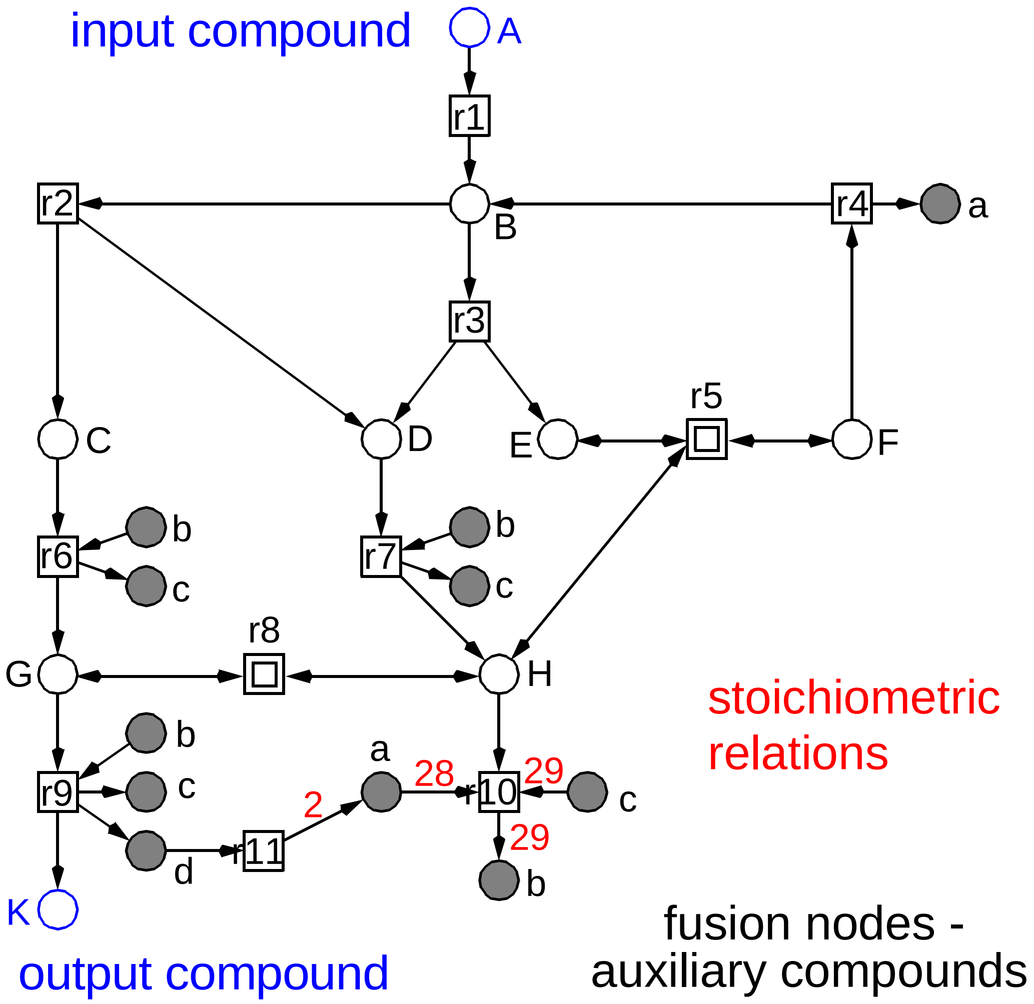

# BIONETWORKS, SUMMARY

- **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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  - -> *partial order sequences of chemical reactions*
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- **network structure**
  - -> *dense, apparently unstructured*
  - -> *hard to read*
  - -> *tend to grow fast*
- **typical (structural) properties**

INA

| ORD | HOM | NBM | PUR | CSV | SCF | CON | SC | Ft0 | tF0 | Fp0 | pF0 | MG | SM | FC | EFC | ES |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | N | N | Y | N | N | Y | N | N | N | Y | Y | N | N | N | N | N |

| DTP | CPI | CTI | B | SB | REV | DSt | BSt | DTr | DCF | L | LV | L&S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | N | N | Y | Y | ? | ? | ? | ? | ? | N | ? | N |

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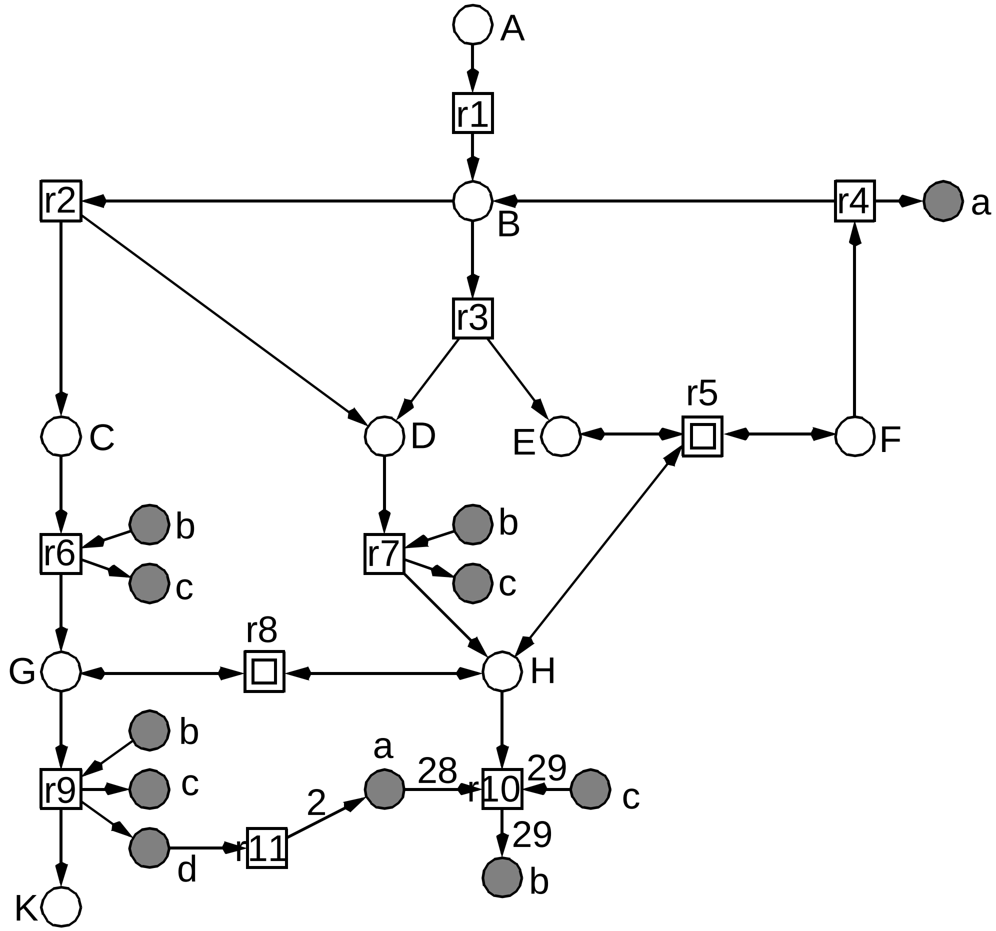
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | N | N | Y | Y | ? | ? | ? | ? | ? | N | ? | N |

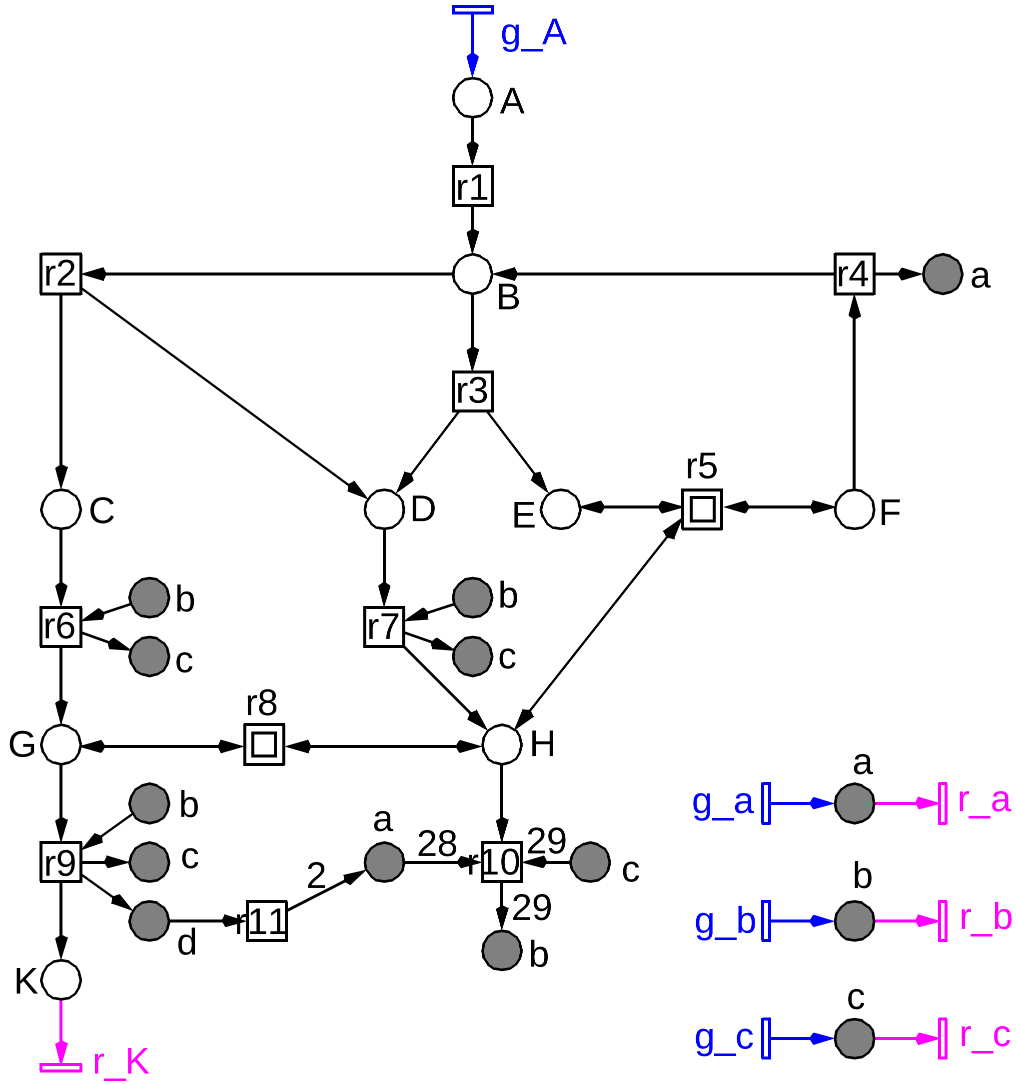
# BIONETWORKS NEED ENVIRONMENT BEHAVIOR

- **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 (?)*



- **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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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | N | N | Y | N | N | Y | N | Y | Y | N | N | N | N | N | N | N |

| DTP | CPI | CTI | B | SB | REV | DSt | BSt | DTr | DCF | L | LV | L&S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ? | N | Y | N | N | ? | N | ? | n | n | y | y | N |

how to prove ?

# T-INVARIANTS, REFRESHMENT

- **Lautenbach, 1973**

- **T-invariants** -> *multisets of transitions*
  - -> *integer solutions of* $Cx = 0, x \neq 0, x \geq 0$

- **minimal T-invariants**
  - -> *there is no T-invariant with a smaller support* -> *sets of transitions*
  - -> *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)

-> CTI

- boundary transitions of auxiliary compounds

  *-> (g_a, r_a), (g_b, r_b), (g_c, r_c)*

- reversible reactions

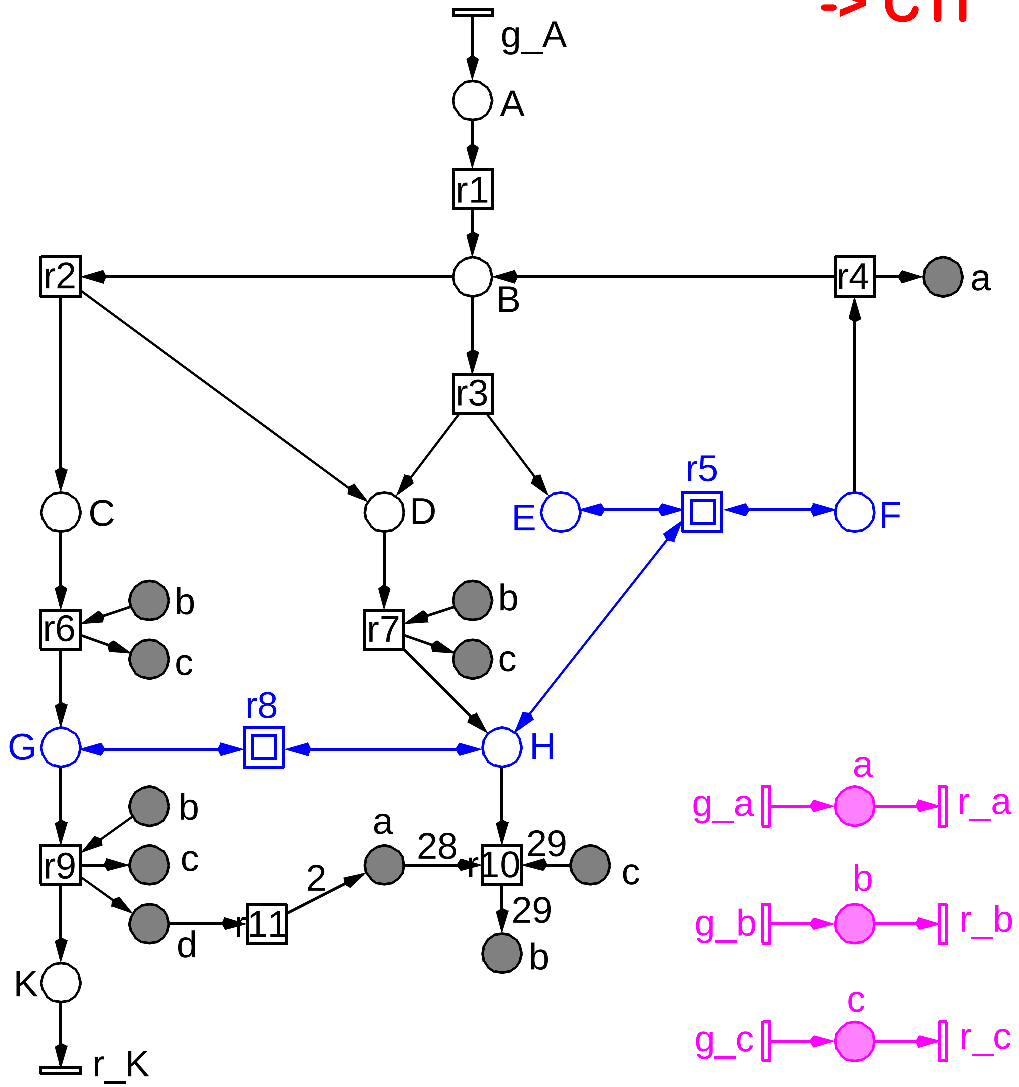
  *-> (r5, r5_rev), (r8, r8_rev)*

## non-trivial min. T-invariants (7)

- covering boundary transitions of input / output compounds

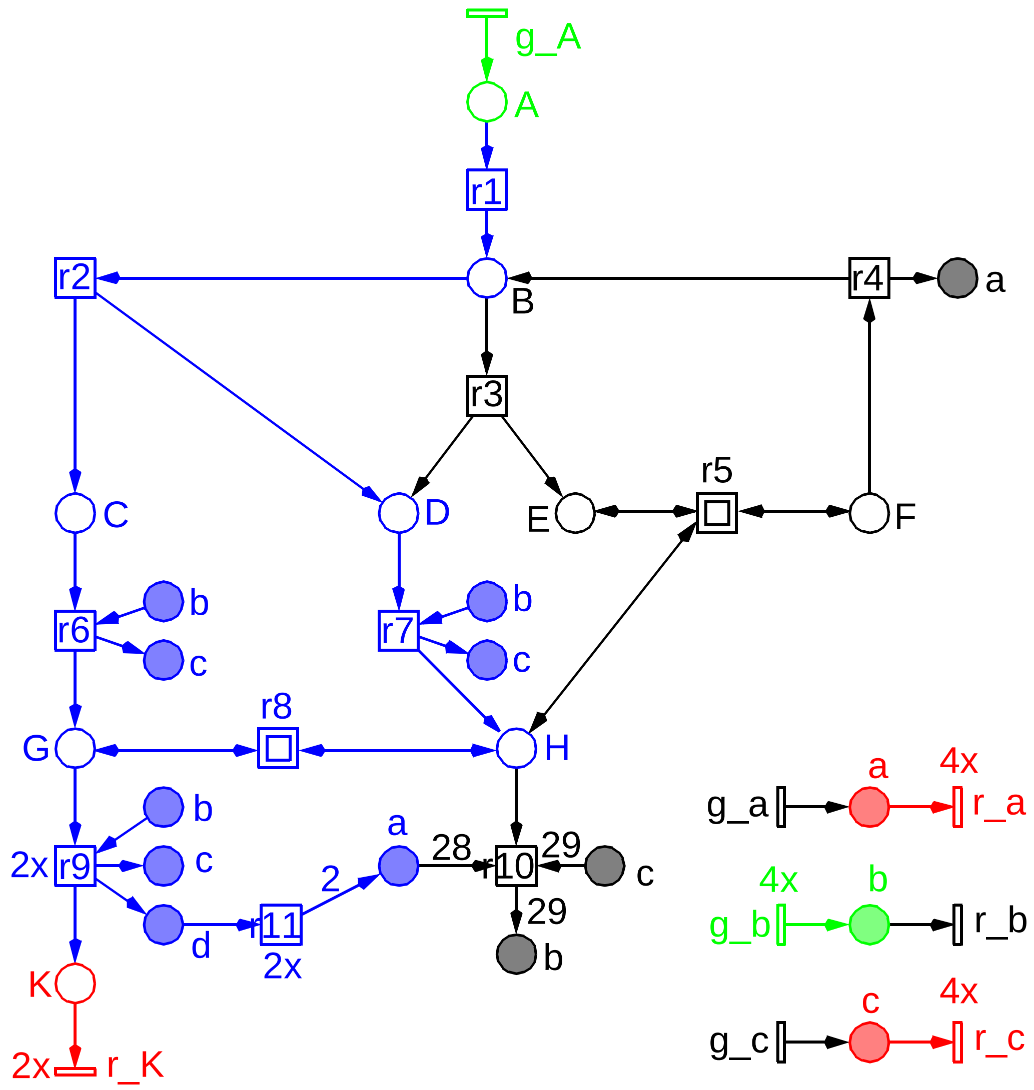
  -> *i/o-T-invariants*

- inner cycles



## ❑ i/o-T-invariant, example

*12 | 0.r1 : 1*
*| 1.r2 : 1,*
*| 3.r8_rev : 1,*
*| 4.r6 : 1,*
*| 5.r7 : 1,*
*| 9.r9 : 2,*
*| 12.r11 : 2,*
*| 13.g_A : 1,*
*| 14.r_K : 2,*
*| 15.g_b : 4,*
*| 18.r_c : 4,*
*| 20.r_a : 4*




## ❑ sum equation

*A + 4b -> 2K +4a + 4c*

# T-INVARIANT, INTERPRETATION

- **Parikh vector**

  -> *state-reproducing transition sequence (partial order)*
  *of transitions occuring one after the other*

  -> *relative transition firing rates*
  *of transitions occuring permanently & concurrently*

- **relative transition firing rates**

  -> *may be implemented by transition firing times*

  - *constant*
  - *interval*

- **quantitative model**

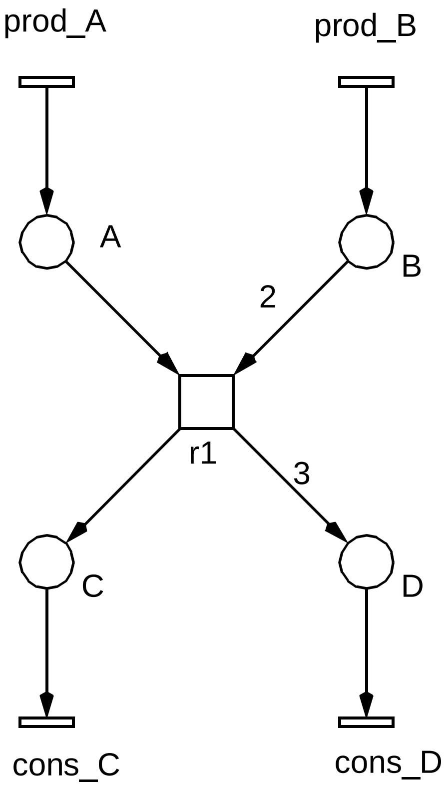
  -> *qualitative model + firing times reflecting the firing rates*

  -> *time-dependent model*

- **claim**

  -> *transformation preserves all possible behavior (= minimal T-invariants)*

# TRANSFORMATION, EX1




-> properties as time-less net

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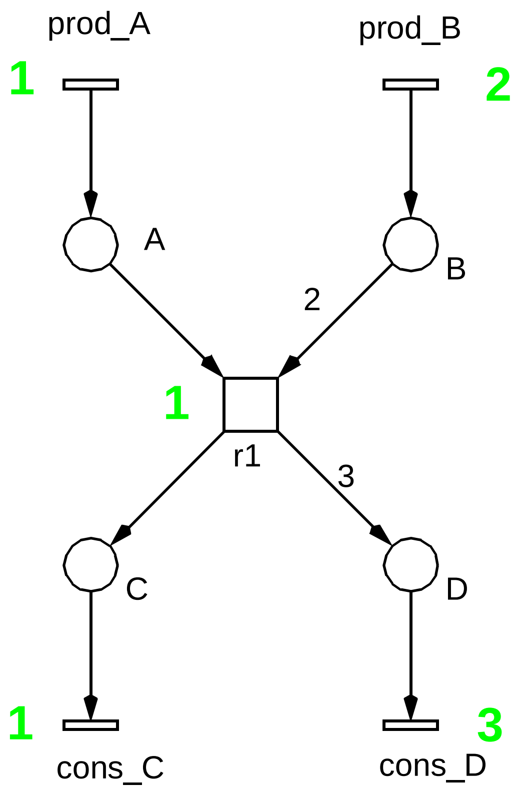
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | Y | N | Y | N | Y | Y | N | Y | Y | N | N | Y | N | Y | Y | Y |

| CPI | CTI | B | SB | REV | DSt | BSt | DTr | DCF | L | LV | L&S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| N | Y | N | N | Y | N | ? | N | Y | Y | Y | N |




**T-INVARIANTE**

-> properties as time-less net

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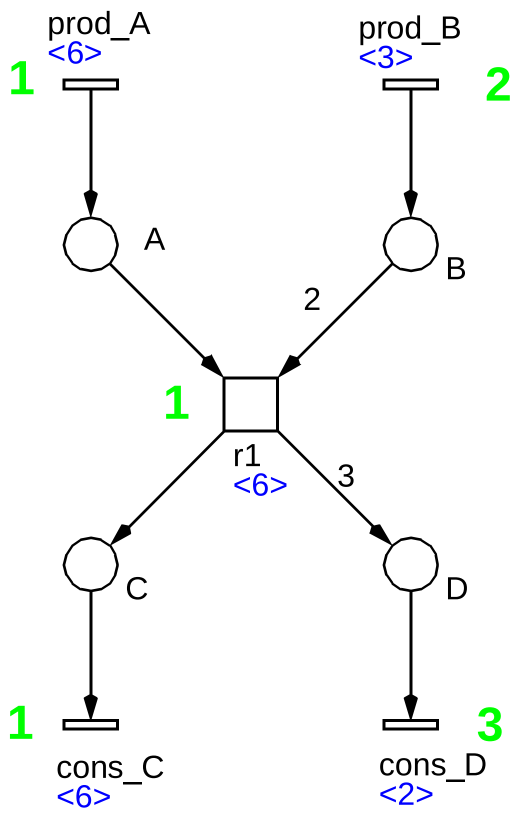
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | Y | N | Y | N | Y | Y | N | Y | Y | N | N | Y | N | Y | Y | Y |

| CPI | CTI | B | SB | REV | DSt | BSt | DTr | DCF | L | LV | L&S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| N | Y | N | N | Y | N | ? | N | Y | Y | Y | N |




**T-INVARIANTE**

-> properties as time net

INA

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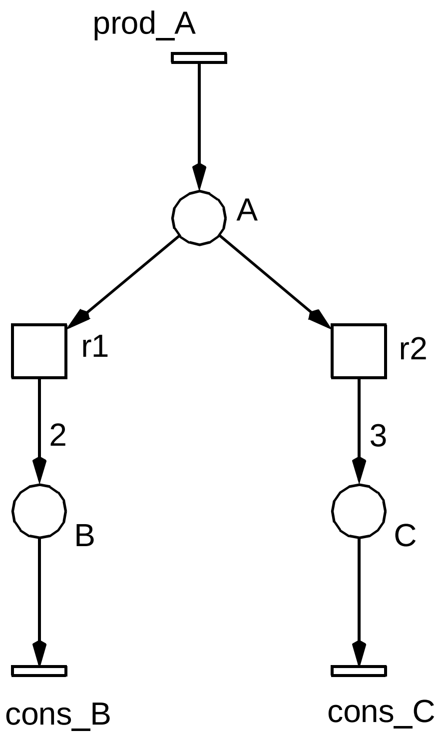
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | Y | N | Y | N | Y | Y | N | Y | Y | N | N | Y | N | Y | Y | Y |

| CPI | CTI | B | SB | REV | DSt | BSt | DTr | DCF | L | LV | L&S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| N | Y | Y | N | N | N | ? | N | Y | Y | Y | 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 |

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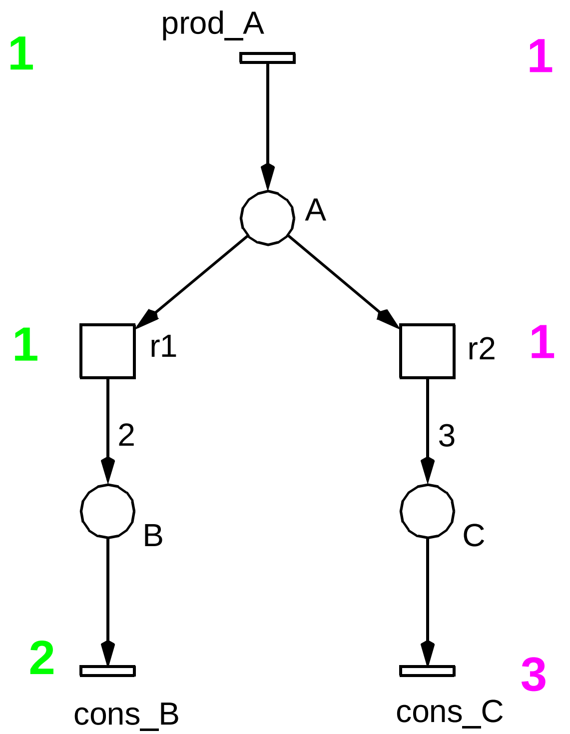


T-INVARIANTE1

T-INVARIANTE2

-> properties as time-less net

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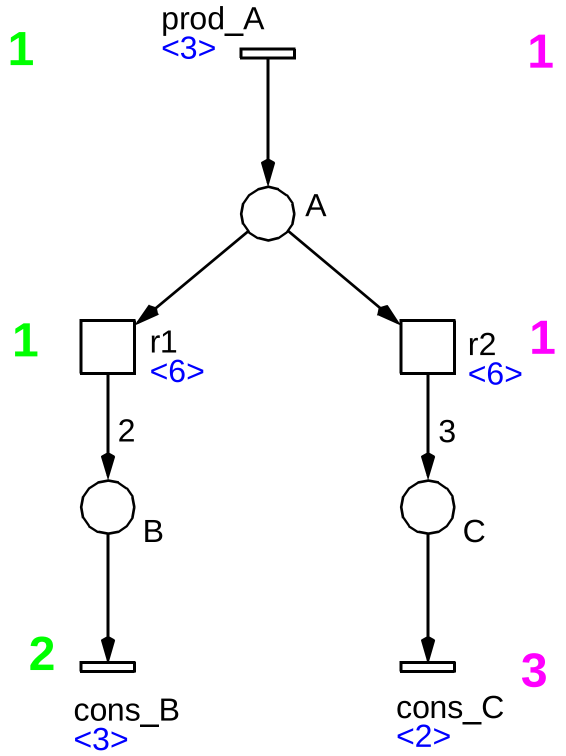
| ORD | HOM | NBM | PUR | CSV | SCF | CON | SC | Ft0 | tF0 | Fp0 | pF0 | MG | SM | FC | EFC | ES |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | Y | N | Y | N | Y | Y | N | Y | Y | N | N | Y | N | Y | Y | Y |

| CPI | CTI | B | SB | REV | DSt | BSt | DTr | DCF | L | LV | L&S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| N | Y | N | N | Y | N | ? | N | N | Y | Y | N |




**T-INVARIANTE1**

**T-INVARIANTE2**

-> properties as time net

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | Y | N | Y | N | Y | Y | N | Y | Y | N | N | Y | N | Y | Y | Y |

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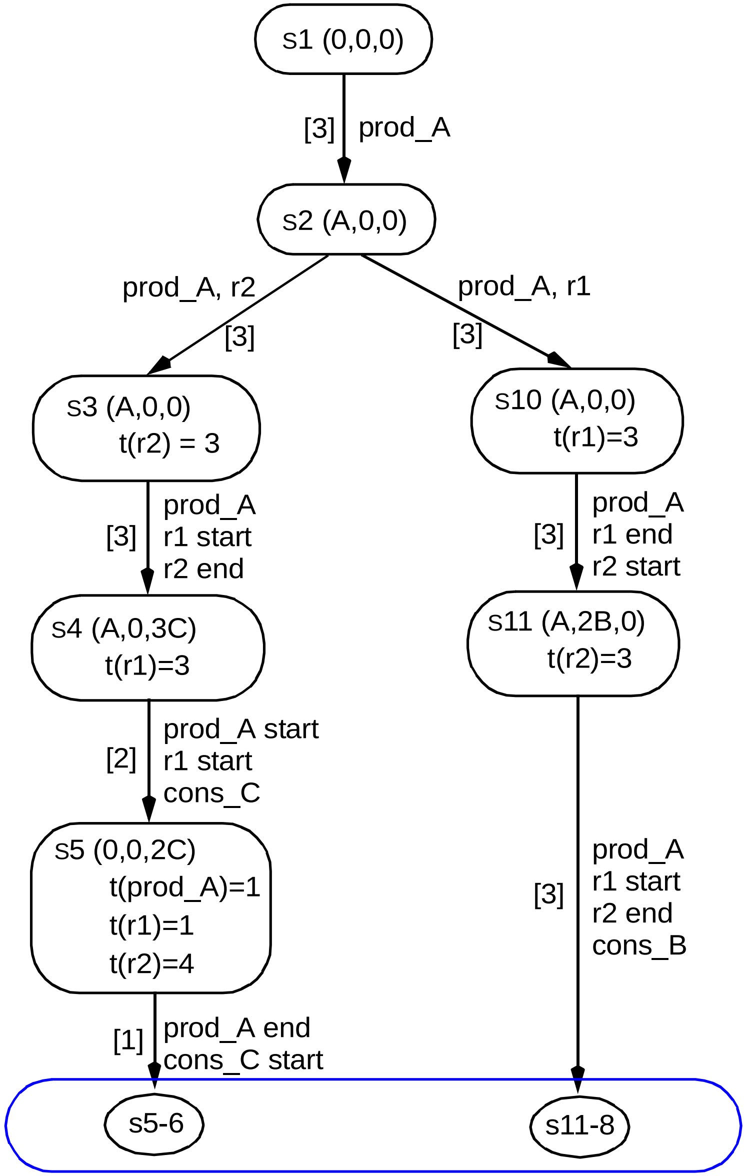
|---|---|---|---|---|---|---|---|---|---|---|---|
| N | Y | Y | N | N | N | ? | N | Y | Y | Y | N |

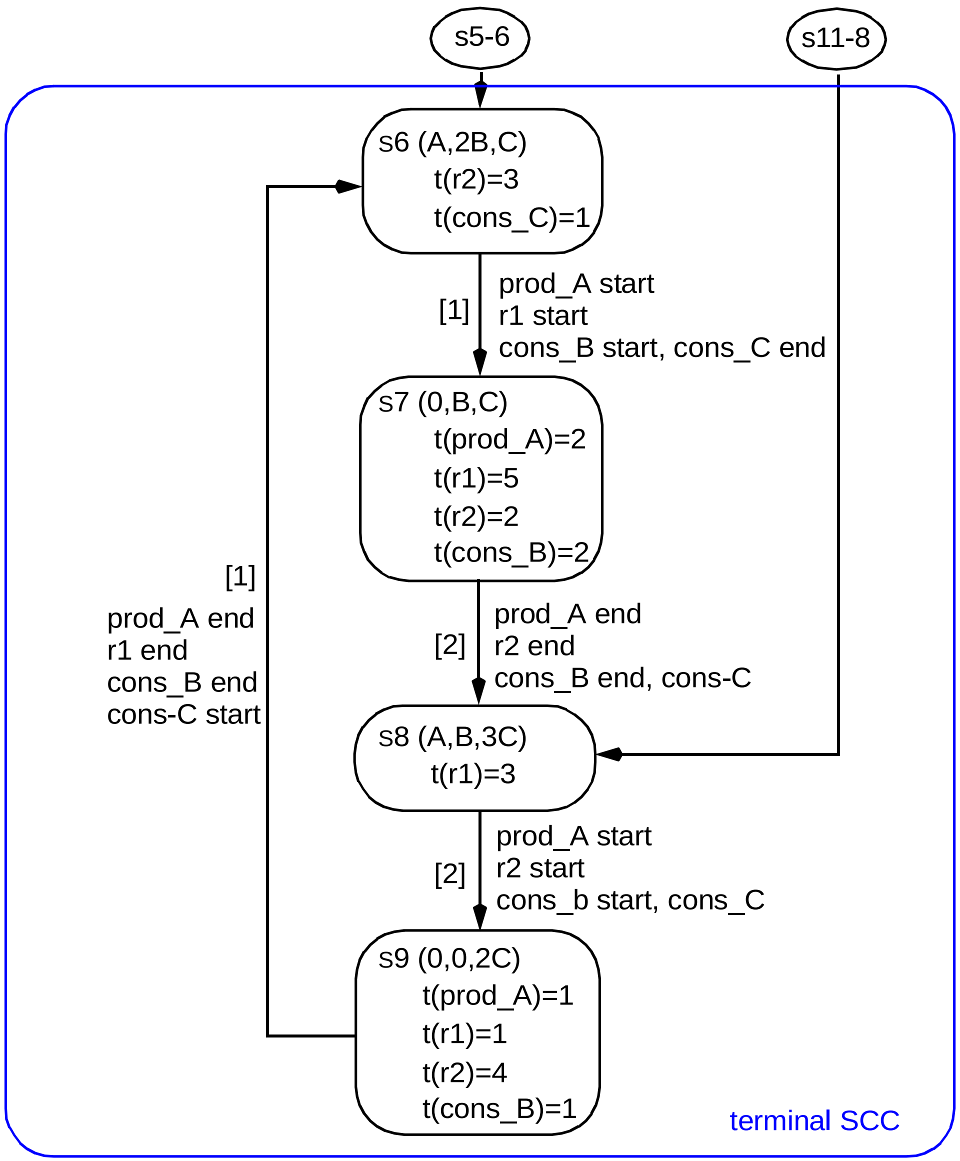
# RG(Ex2), Part 1

- transient state



# RG(Ex2), Part 2

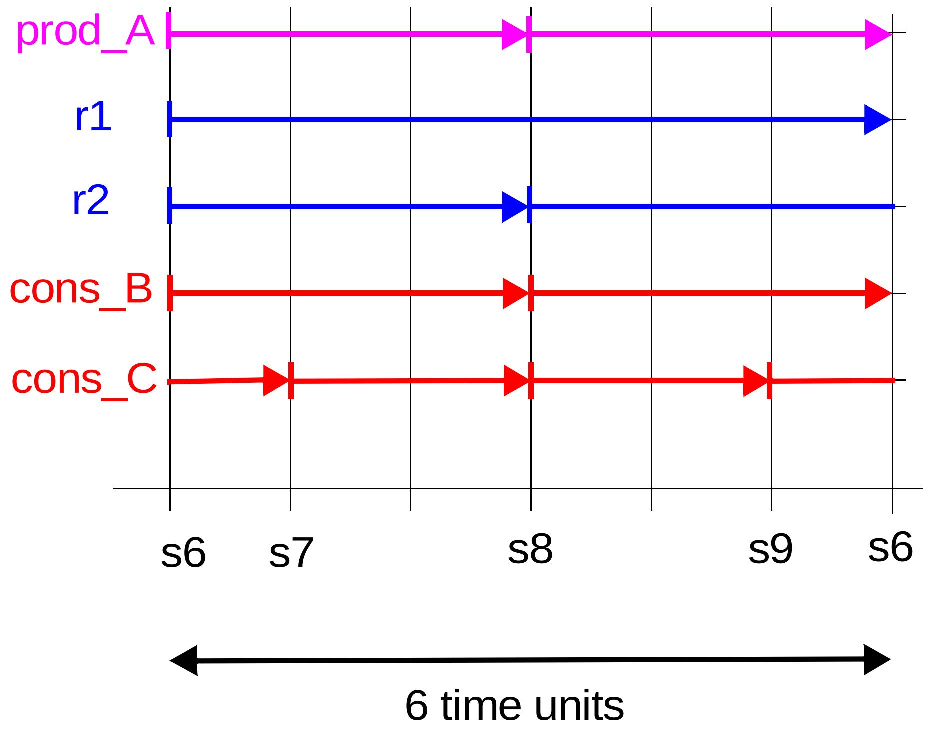
- steady state



# 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**

| | | | | | |
|---|---|---|---|---|---|
| **prod_A** | : | 1 | + | | 1 |
| **r1** | : | 1 | **r2** | : | 1 |
| **cons_B** | : | 2 | **cons_C** | : | 3 |



# 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*

| **PN** | time → | **D/I NET** |
|---|---|---|
| not BND<br>REV<br>LIVE | | BND<br>not REV<br>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 ?

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# QUANTITATIVE ANALYSIS, METHOD I

interval time Petri net — $I: T \rightarrow \mathbf{Q_0}^+ \times \mathbf{Q_0}^+$ and for each $t \in T$ holds $a_t \leq b_t$, where $I(t) = (a_t, b_t)$

initial marking / state

finite transition word *w* — $w \in T^*$

$$
\text{(SLI)} \begin{cases}
b_1 \leq a_{11}x_1 + \ldots + a_{1n}x_n \leq c_1 \\
\ldots \\
b_m \leq a_{m1}x_1 + \ldots + a_{mn}x_n \leq c_m \\
a_{ij} \in \{0, 1\},\ b_i \in \mathbf{N},\ c_i \in \mathbf{N} \\
\forall i \forall s \forall k (1 \leq i \leq n \wedge 1 \leq s \leq k \leq m \wedge \\
a_{is} = a_{ik} = 1 \rightarrow \forall j (s \leq j \leq k \rightarrow a_{ij} = 1))
\end{cases}
$$

if there is an $\boldsymbol{R}$ solution, then there is an $\boldsymbol{N}$ solution.

*w* is time-dependent realizable / not realizable

# Quantitative Analysis, Method I

interval time Petri net — $I: T \rightarrow \mathbf{Q_0}^+ \times \mathbf{Q_0}^+$ and for each $t \in T$ holds $a_t \leq b_t$, where $I(t) = (a_t, b_t)$

initial marking / state

finite transition word *w* — $w \in T^*$

(SLI)

$$
\begin{cases}
b_1 \leq a_{11}x_1 + \ldots + a_{1n}x_n \leq c_1 \\
\ldots \\
b_m \leq a_{m1}x_1 + \ldots + a_{mn}x_n \leq c_m \\
a_{ij} \in \{0, 1\},\ b_i \in \mathbf{N},\ c_i \in \mathbf{N} \\
\forall i \forall s \forall k (1 \leq i \leq n \wedge 1 \leq s \leq k \leq m \wedge \\
a_{is} = a_{ik} = 1 \rightarrow \forall j (s \leq j \leq k \rightarrow a_{ij} = 1))
\end{cases}
$$

polynomial time ↑

if there is an $\mathbf{R}$ solution,
then there is an $\mathbf{N}$ solution.

↓ linear time

*w* is time-dependent realizable / not realizable

# QUANTITATIVE ANALYSIS, METHOD II

interval time Petri net — $I: T \rightarrow \mathbf{Q_0}^+ \times \mathbf{Q_0}^+$ and for each $t \in T$ holds $a_t \leq b_t$, where $I(t) = (a_t, b_t)$

initial marking / state

finite transition word $w$ — $w \in T^*$

(LP)

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

$$\begin{cases} b_1 \leq a_{11}x_1 + \ldots + a_{1n}x_n \leq c_1 \\ \ldots \\ b_m \leq a_{m1}x_1 + \ldots + a_{mn}x_n \leq c_m \\ a_{ij} \in \{0, 1\}, b_i \in \mathbf{N}, c_i \in \mathbf{N} \\ \forall i \forall s \forall k (1 \leq i \leq n \wedge 1 \leq s \leq k \leq m \wedge \\ a_{is} = a_{ik} = 1 \rightarrow \forall j ( s \leq j \leq k \rightarrow a_{ij} = 1 )) \end{cases}$$

if there is a solution, then it is an $\mathbf{N}$ solution.

$w$ is time-dependent realizable / not realizable

min/max time length of $w$

# Quantitative Analysis, Method II

interval time Petri net — $I: T \rightarrow \mathbf{Q_0}^+ \times \mathbf{Q_0}^+$ and for each $t \in T$ holds $a_t \leq b_t$, where $I(t) = (a_t, b_t)$

initial marking / state

finite transition word *w* — $w \in T^*$

(LP)

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

$$\begin{cases} b_1 \leq a_{11}x_1 + \ldots + a_{1n}x_n \leq c_1 \\ \ldots \\ b_m \leq a_{m1}x_1 + \ldots + a_{mn}x_n \leq c_m \\ a_{ij} \in \{0, 1\}, b_i \in \mathbf{N}, c_i \in \mathbf{N} \\ \forall i \forall s \forall k (1 \leq i \leq n \wedge 1 \leq s \leq k \leq m \wedge \\ a_{is} = a_{ik} = 1 \rightarrow \forall j ( s \leq j \leq k \rightarrow a_{ij} = 1 )) \end{cases}$$

polynomial time ↑ if there is a solution, then it is an $\mathbf{N}$ 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* -> *validation of transformation step*
  - -> *shortest and longest time length* -> *measurement approximation*
    - -> *time windows of the recurrent pathways (processes)*

- **given: partial set of the transitions' time windows**
  **+ transition sequence, esp. a (min.) T-invariant**
  - -> *which time windows guarantee realizability ?*

- **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*

- **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 !