MODEL CHECKING OF BIOCHEMICAL NETWORKS USING PETRI NETS

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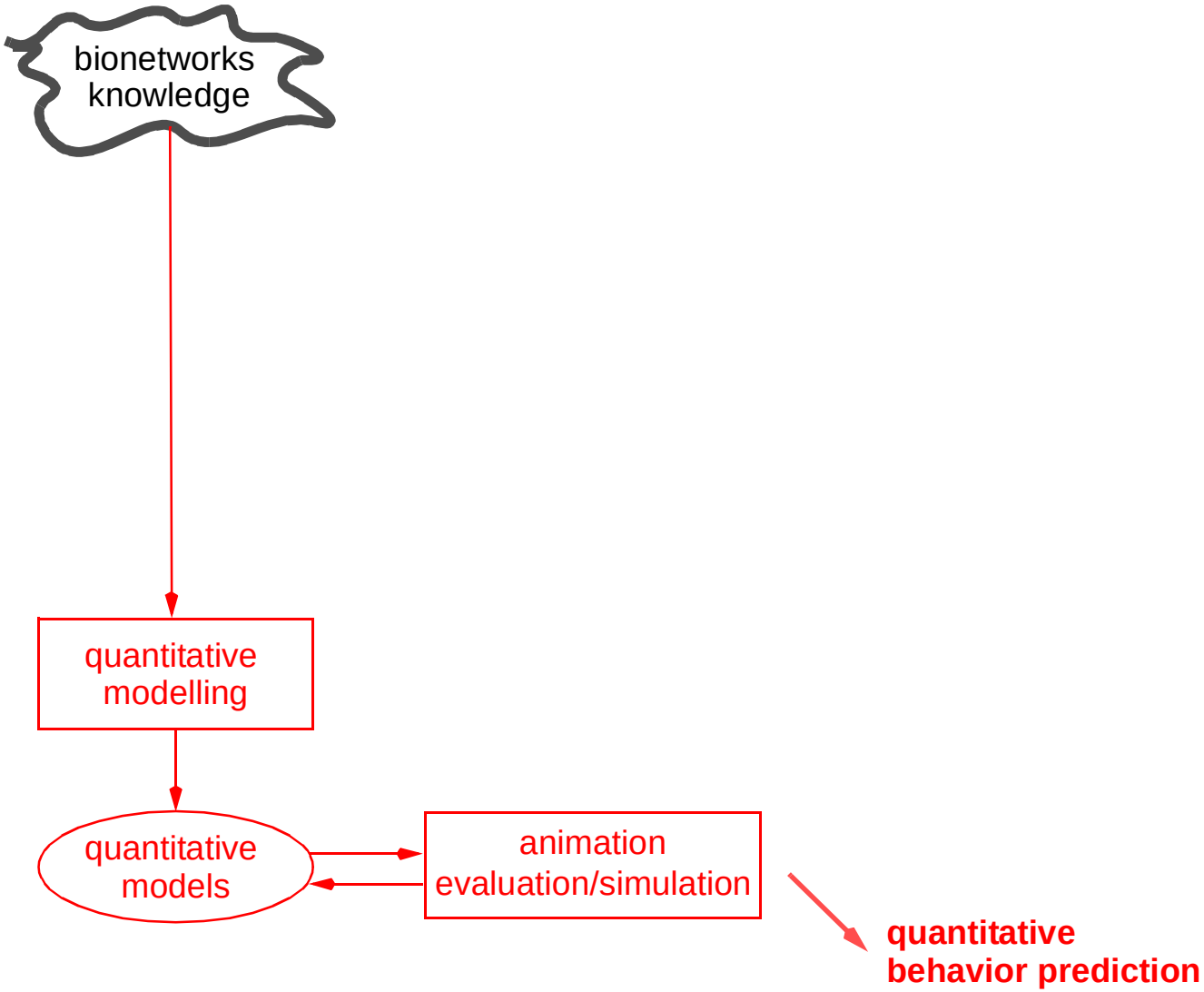
Dep. of CS

BIONETWORKS, SOME PROBLEMS

- **knowledge** ***-> PROBLEM 1***
  - -> *uncertain*
  - -> *growing, changing*
  - -> *distributed over independent data bases, papers, journals, . . .*
- **various, mostly ambiguous representations** ***-> PROBLEM 2***
  - -> *verbose descriptions*
  - -> *diverse graphical representations*
  - -> *contradictory and / or fuzzy statements*
- **network structure** ***-> PROBLEM 3***
  - -> *tend to grow fast*
  - -> *dense, apparently unstructured*
  - -> *hard to read*

- - >> models are full of ASSUMPTIONS << - -

FRAMEWORK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quantitative modelling
quantitative models
animation evaluation/simulation
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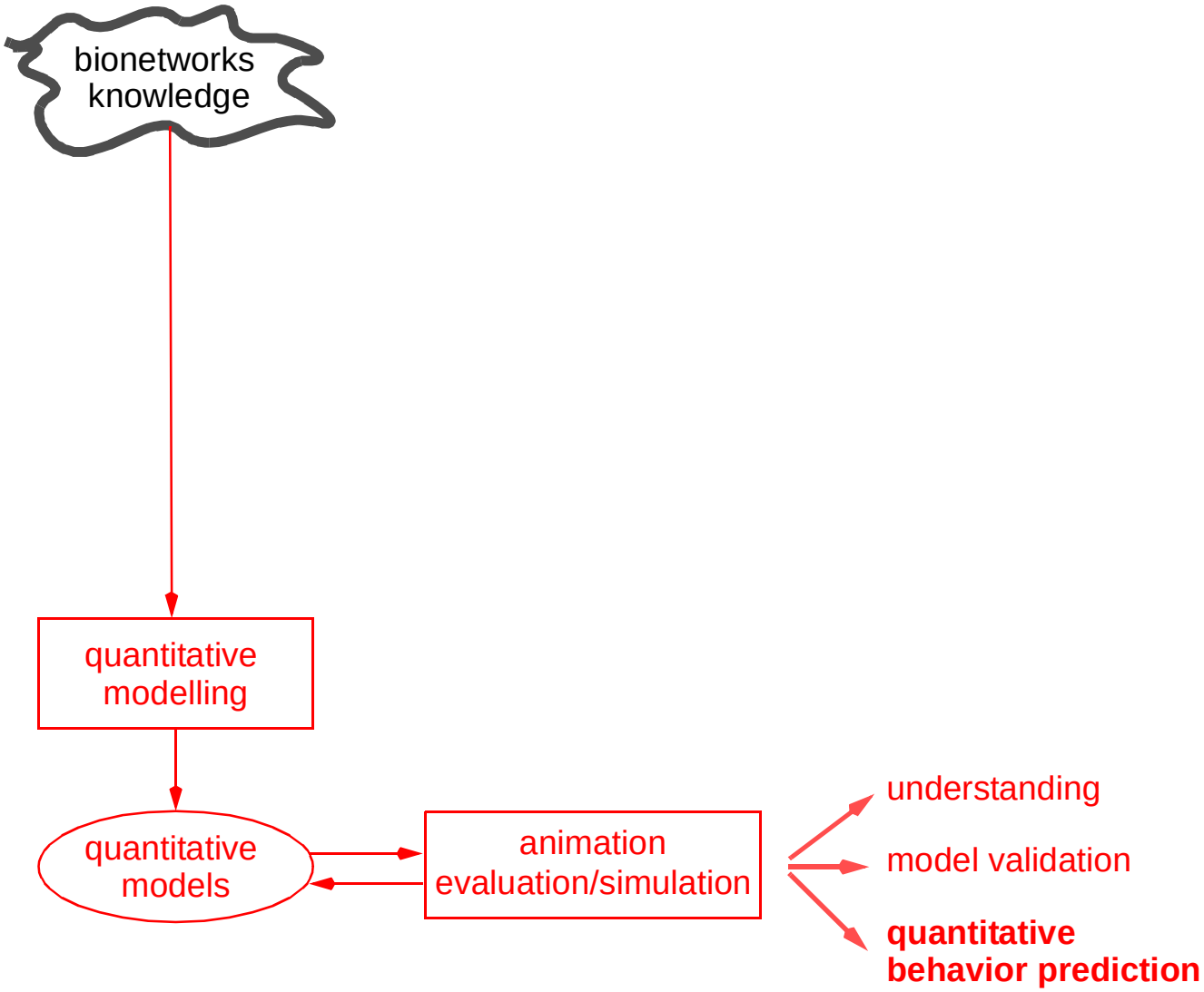
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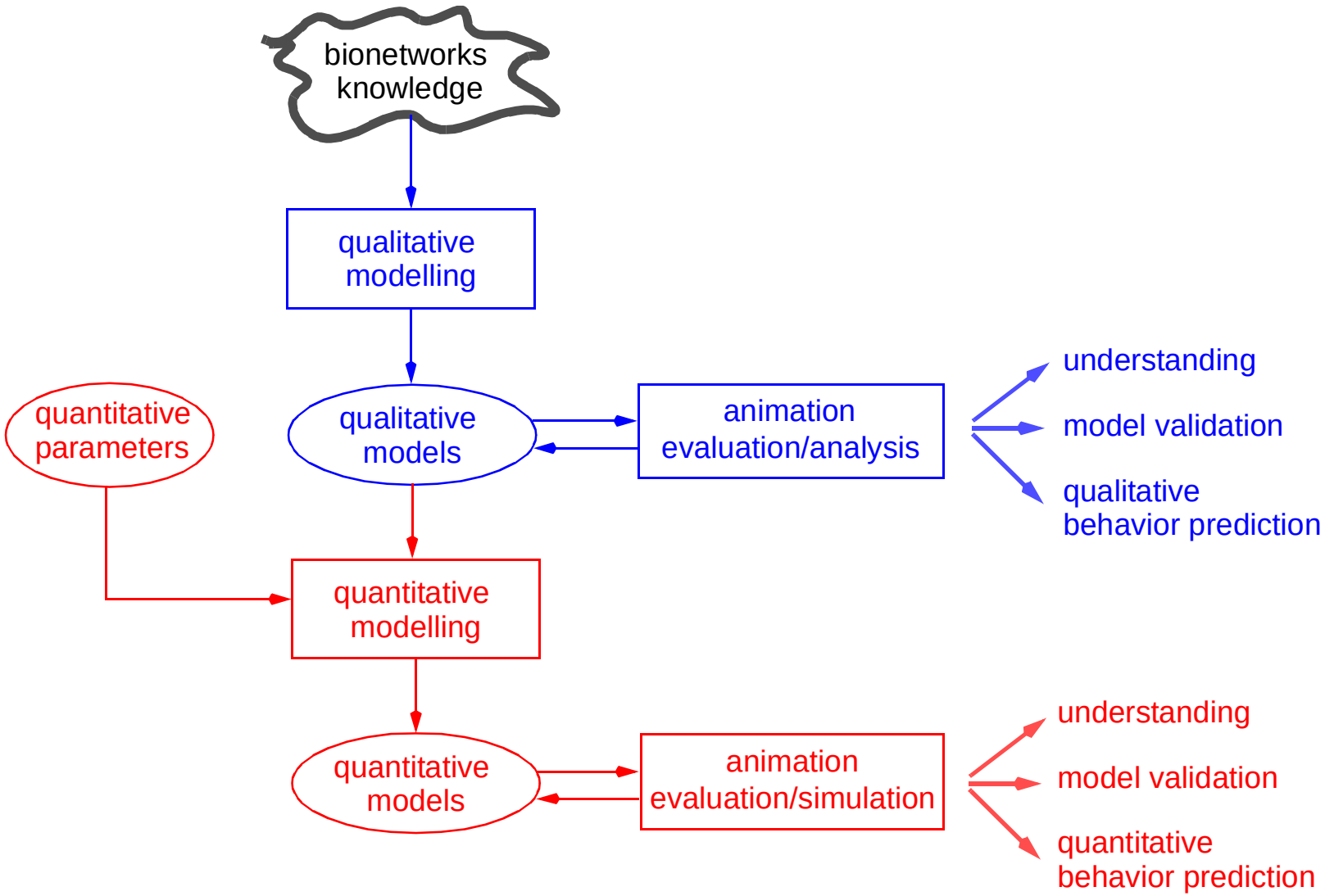
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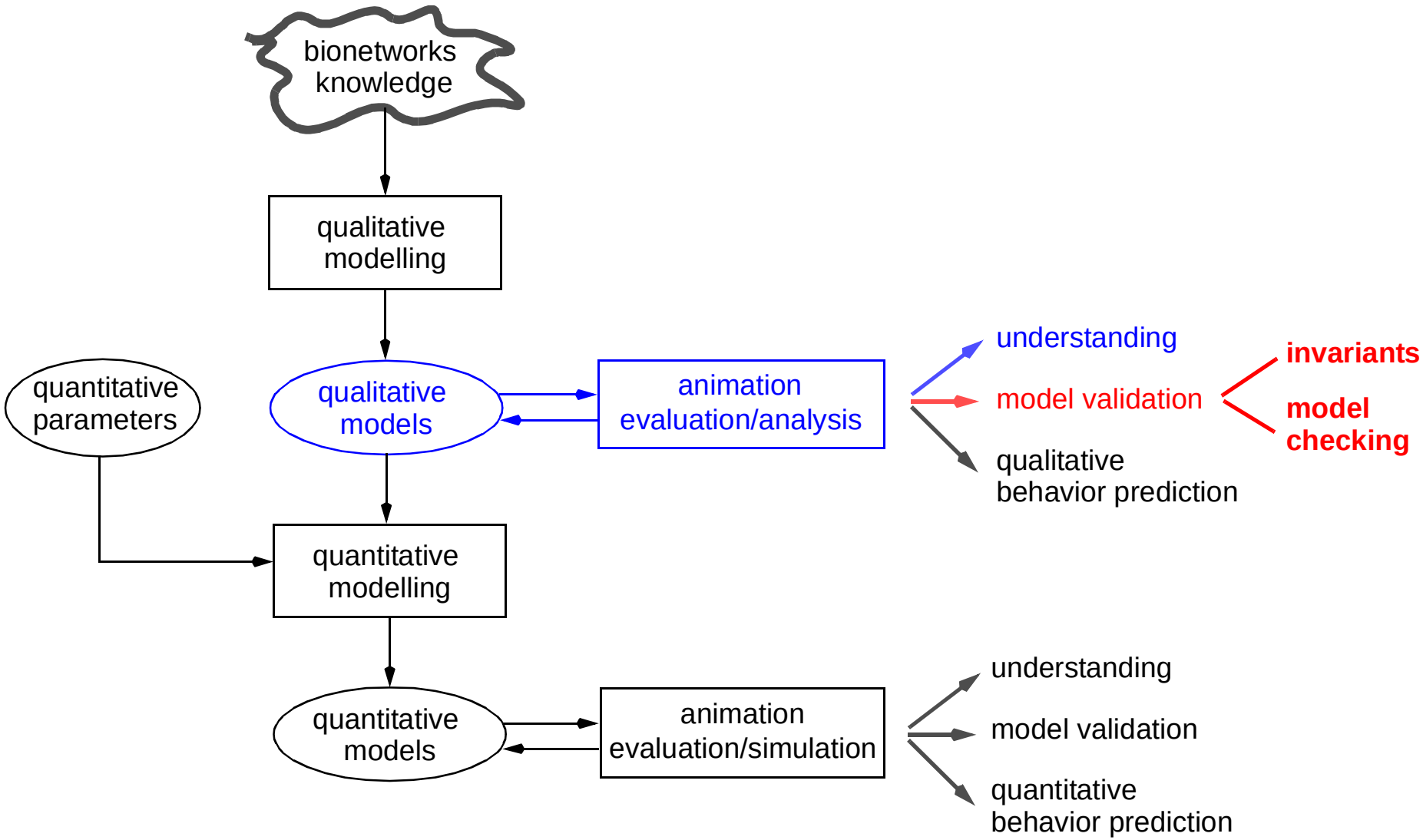
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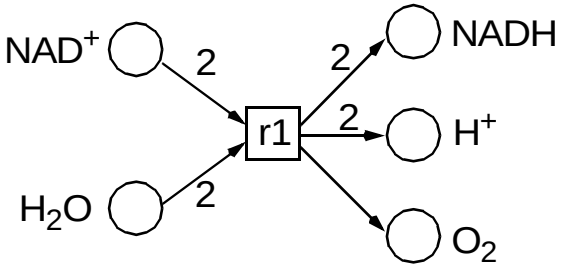
- **chemical reactions -> atomic actions -> Petri net transitions**

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

input compounds



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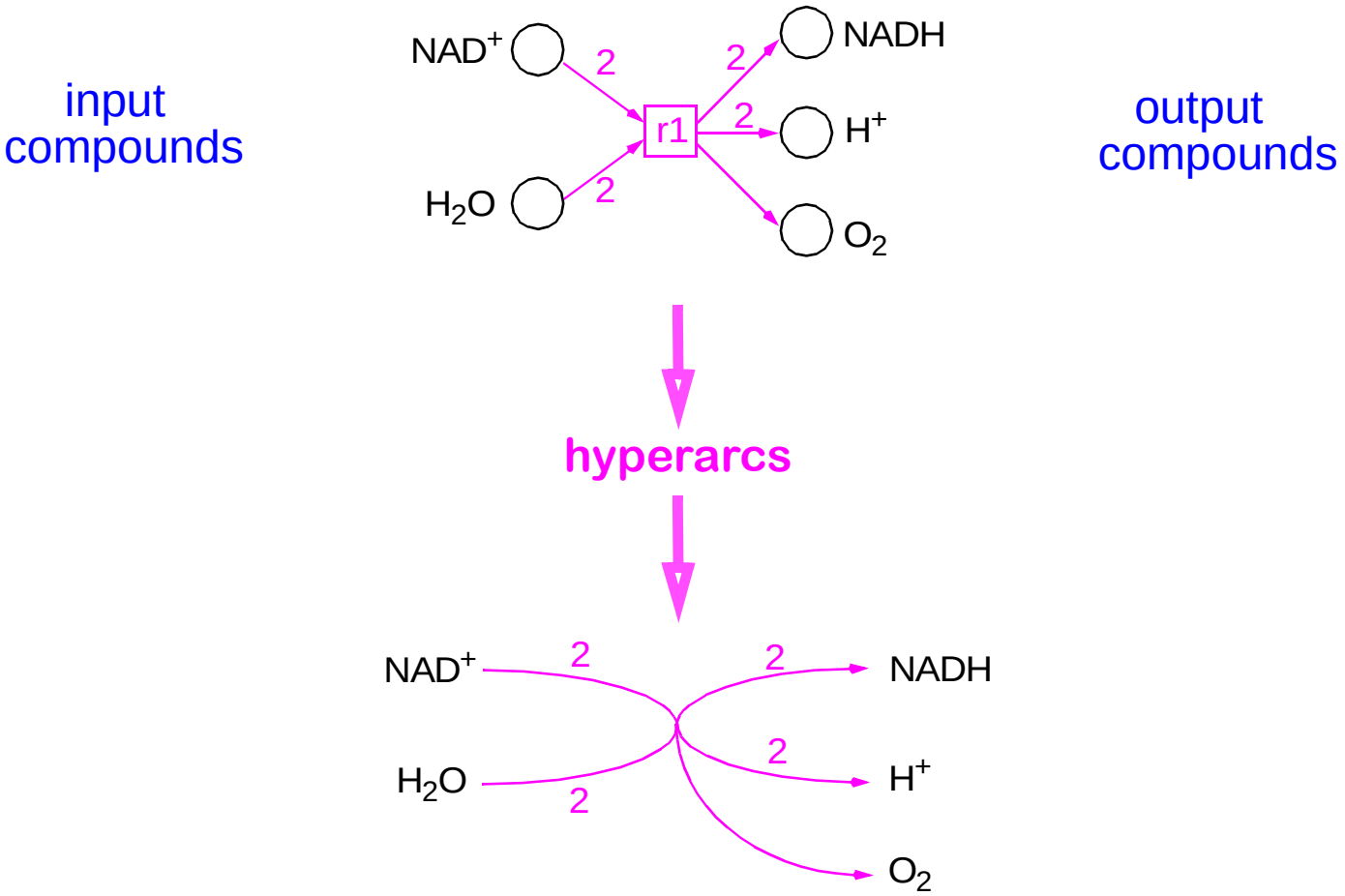

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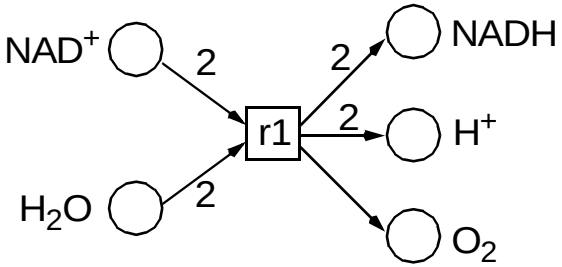

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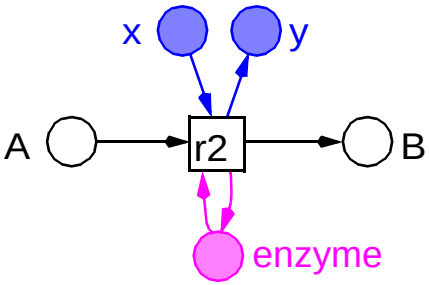
❑ **chemical compounds -> Petri net places**

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



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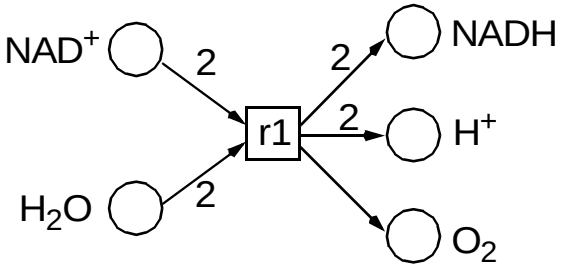

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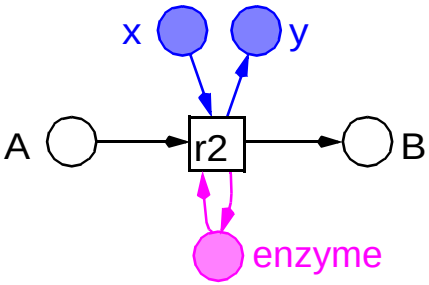

- **stoichiometric relations -> Petri net arc multiplicities**
- **compounds distribution -> marking -> tokens residing in places**

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

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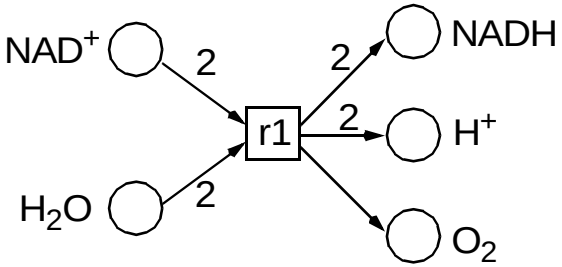

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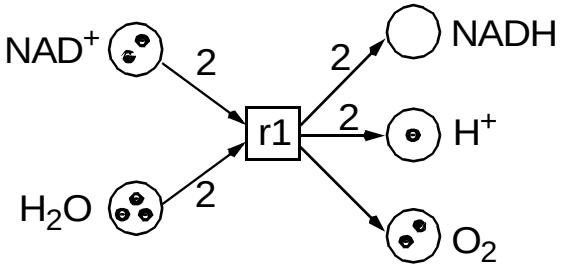

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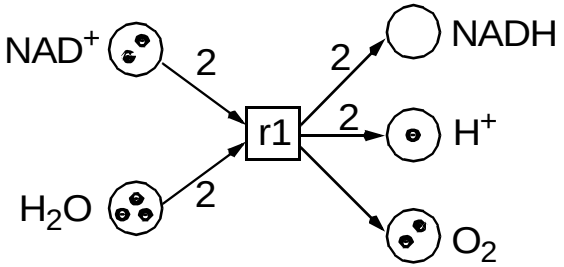
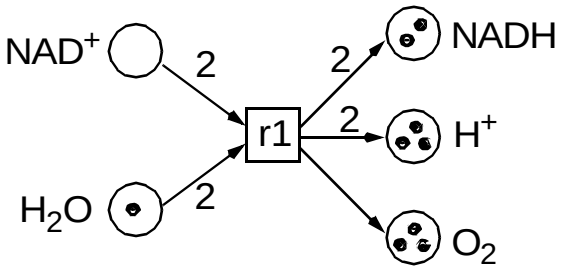


output compounds

FIRING



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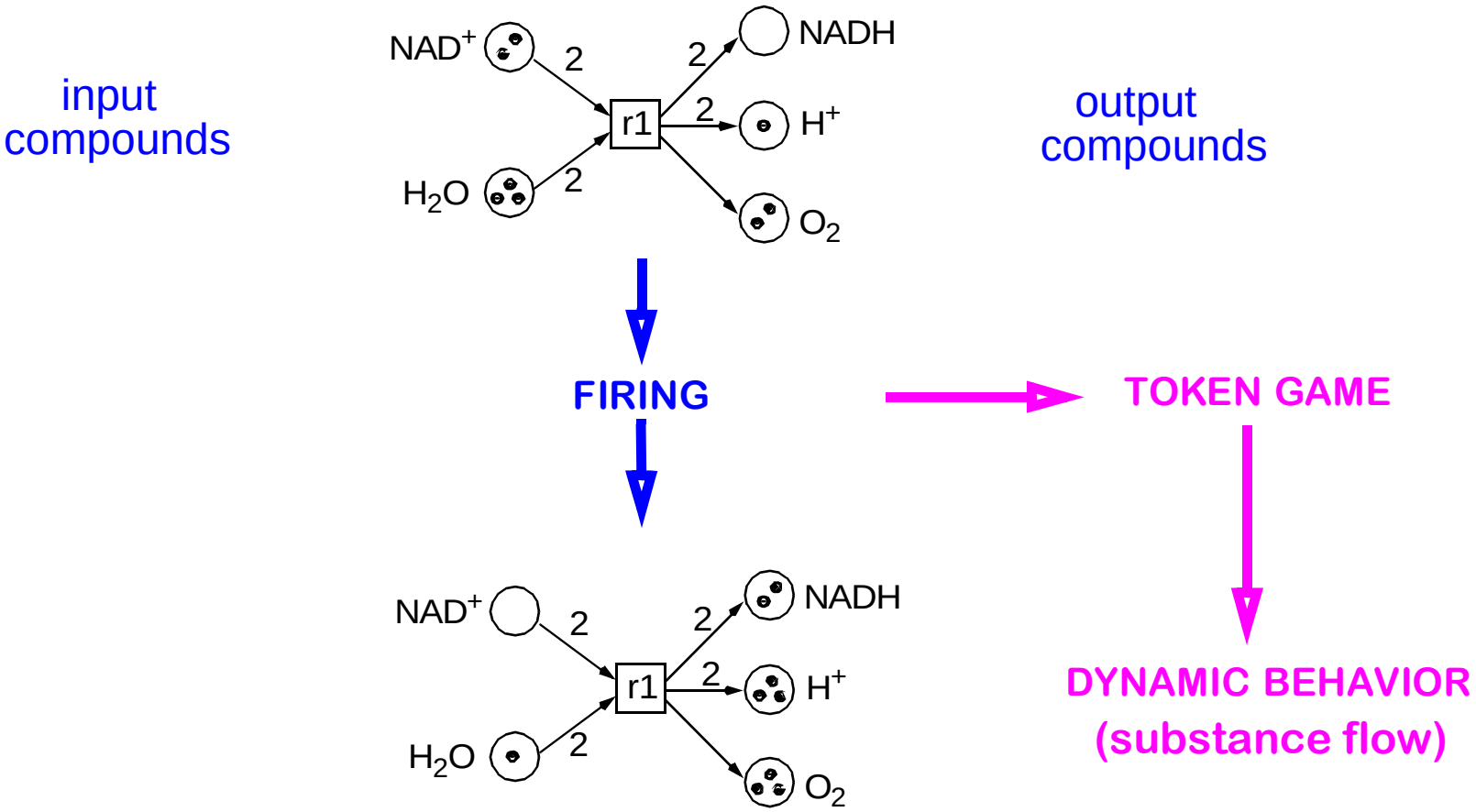
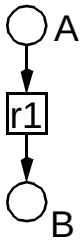


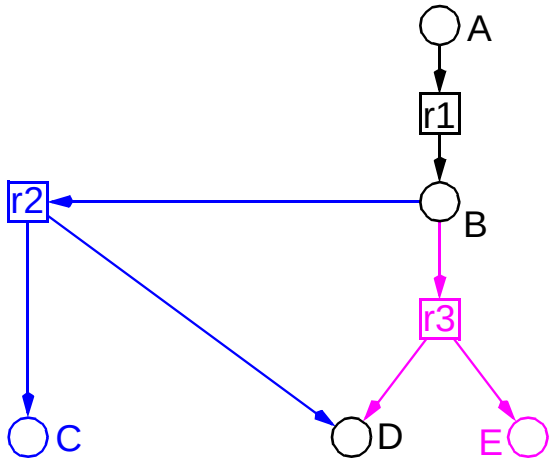
r1: A -> B



r1: A -> B

r2: B -> C + D

r3: B -> D + E



-> alternative reactions

r1: A -> B

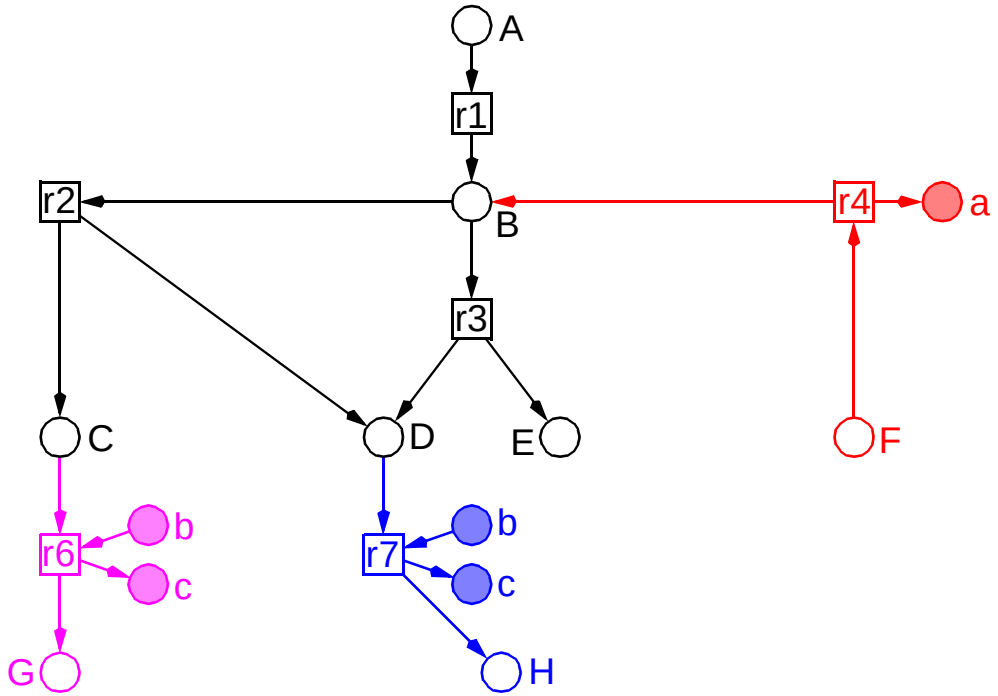
r2: B -> C + D

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

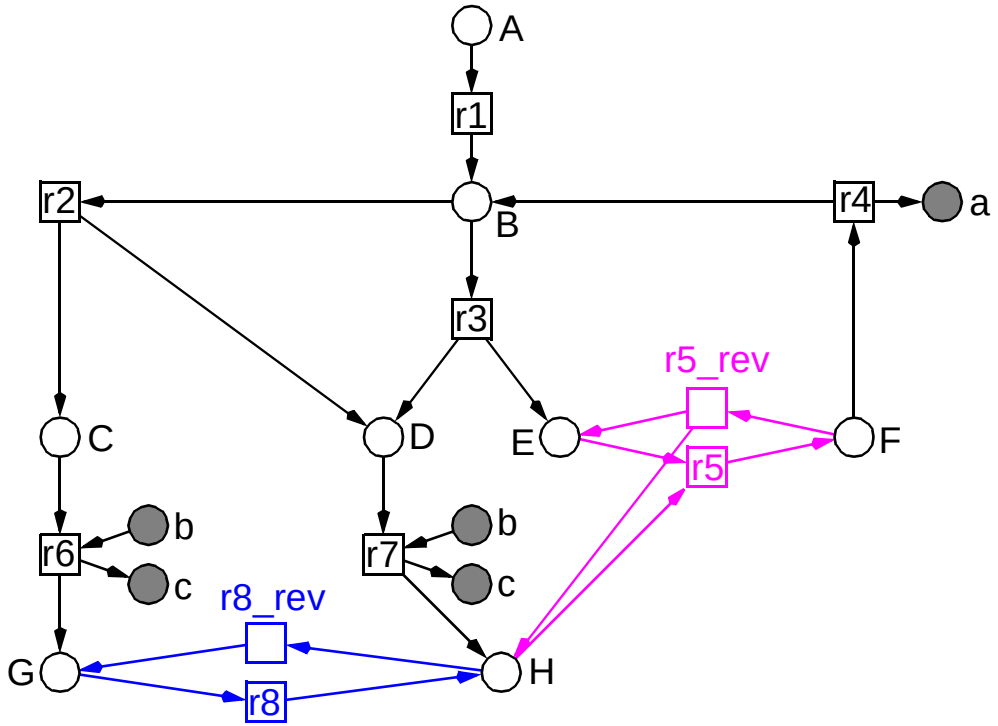
r4: F -> B + a

r5: E + H <-> F

r6: C + b -> G + c

r7: D + b -> H + c

r8: H <-> G



-> reversible reactions

r1: A -> B

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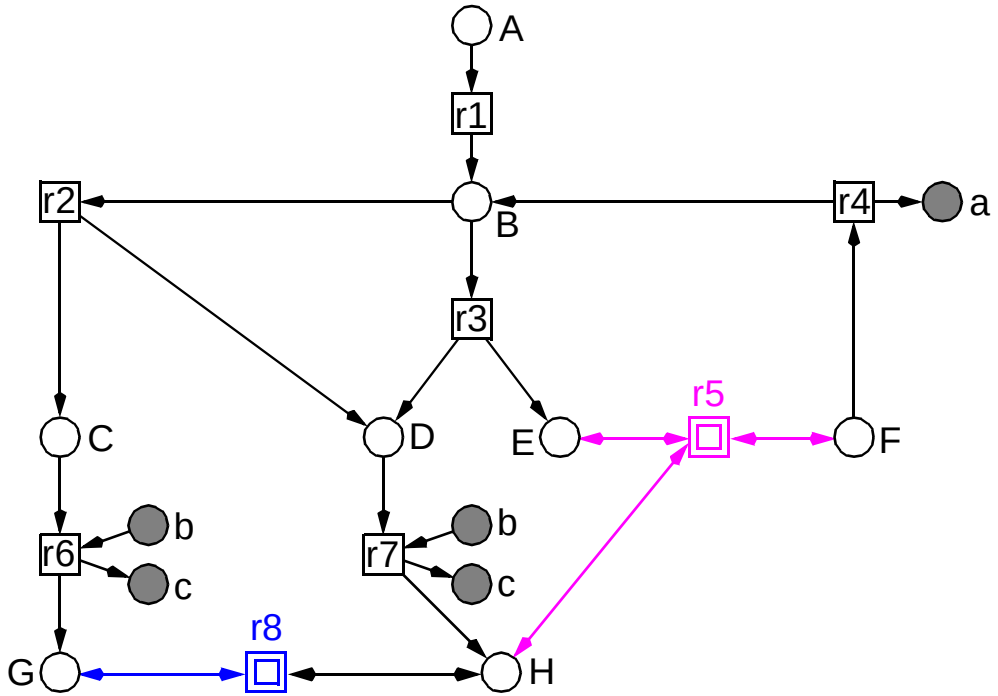


-> reversible reactions

- 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

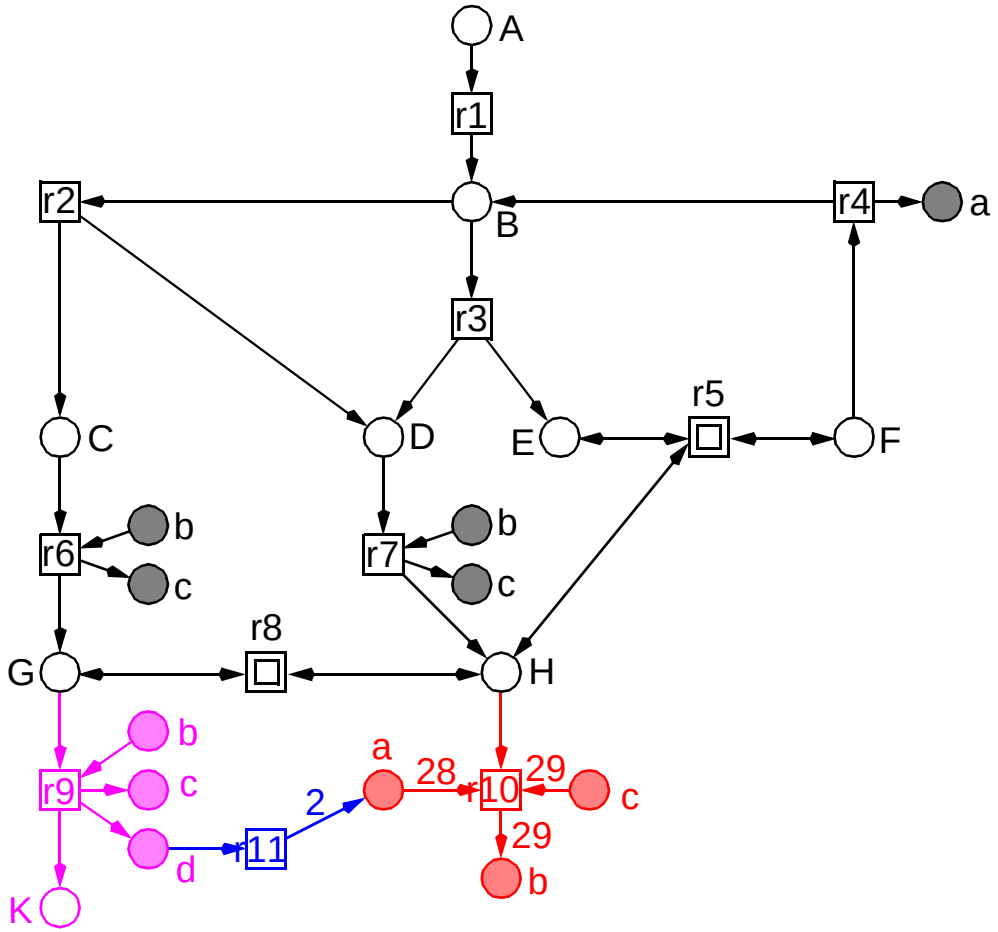
r7: D + b -> H + c

r8: H <-> G

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

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

r11: d -> 2a



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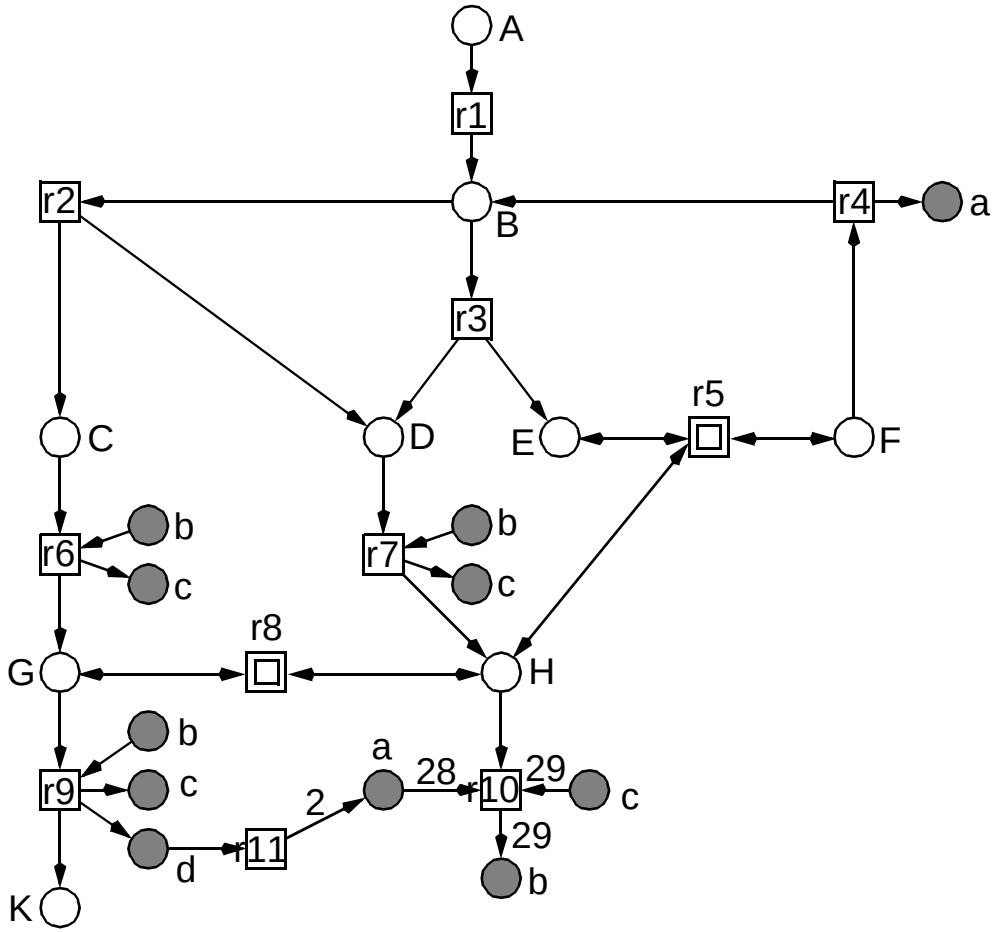

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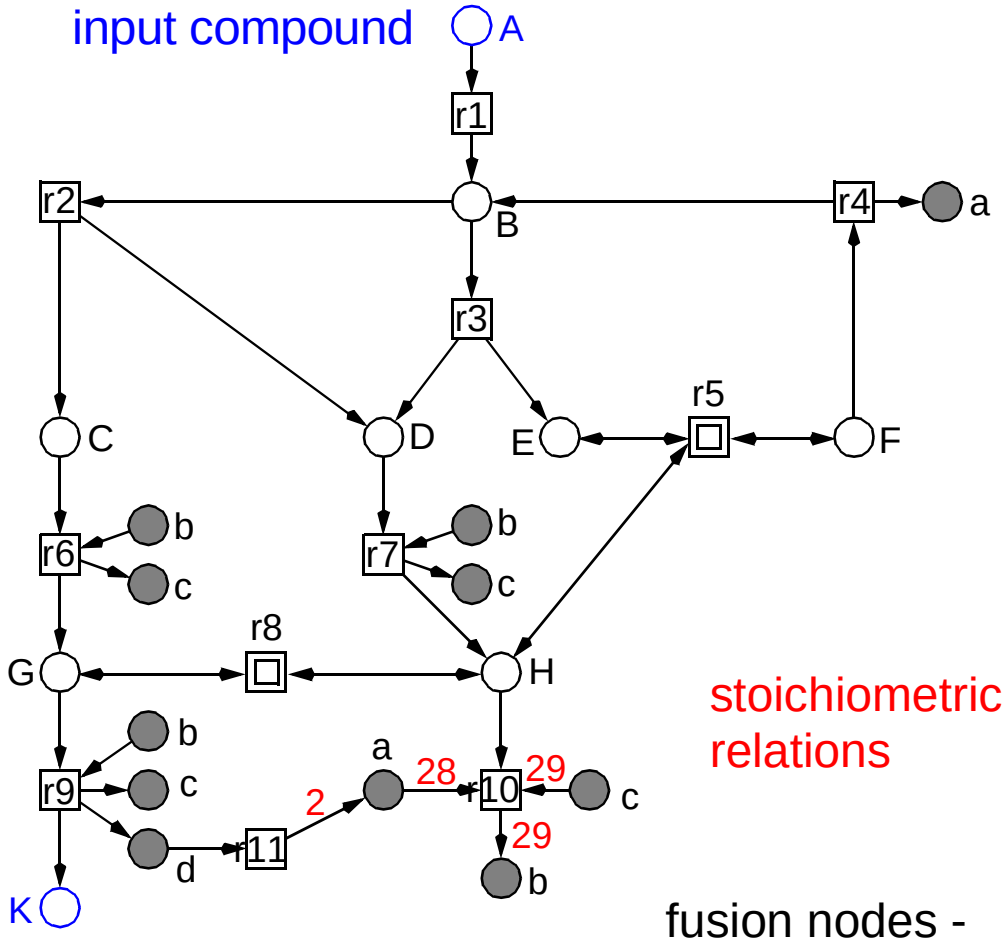

- **networks of chemical reactions**

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

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- typical (structural) properties

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

- networks need environment behavior !!

ENVIRONMENT BEHAVIOUR, THREE STYLES

- **STYLE 1**
  - -> *weak assumptions*
  - -> *infinite flow into/out the network*

- **STYLE 2**
  - -> *firm assumptions*
  - -> *infinite many primary compounds*
  - -> *finite, but sufficient reservoir of auxiliary compounds*

- **STYLE 3**
  - -> *strong assumptions*
  - -> *finite, but sufficient reservoir of auxiliary compounds*
  - -> *quantitative relations of input/output compounds*
    - *finite reservoir of primary compounds*

INCREASING STRENGTH

ENVIRONMENT BEHAVIOUR, THREE STYLES

- **STYLE 1** -> **VALIDATION CRITERION 1**
  - -> *weak assumptions*
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- **STYLE 2** -> **VALIDATION CRITERION 2**
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  - -> *infinite many primary compounds*
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- **STYLE 3** -> **VALIDATION CRITERION 3**
  - -> *strong assumptions*
  - -> *finite, but sufficient reservoir of auxiliary compounds*
  - -> *quantitative relations of input/output compounds*
    - *finite reservoir of primary compounds*

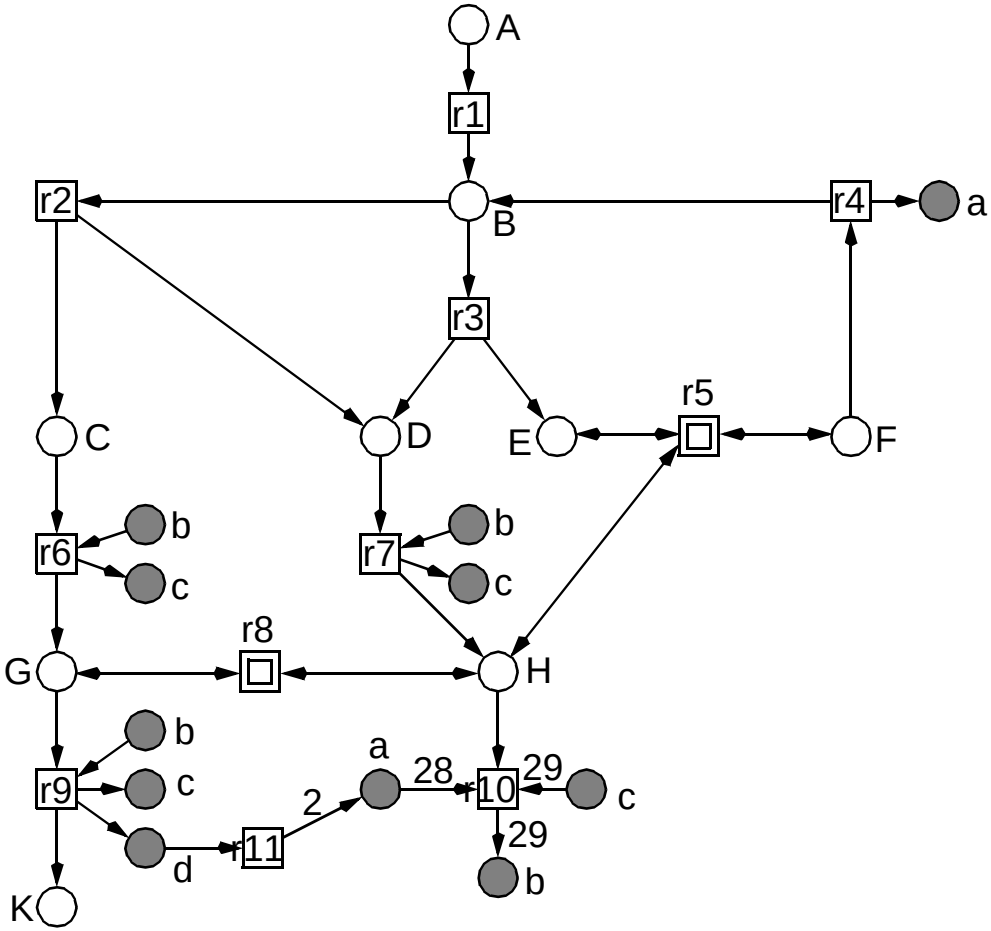
INCREASING STRENGTH

- **validation criteria ?**
  - -> *objective*
  - -> *reproducible*
  - -> *computable*

- **steady state behaviour**
  - -> *all flows preserving the given compounds distribution*

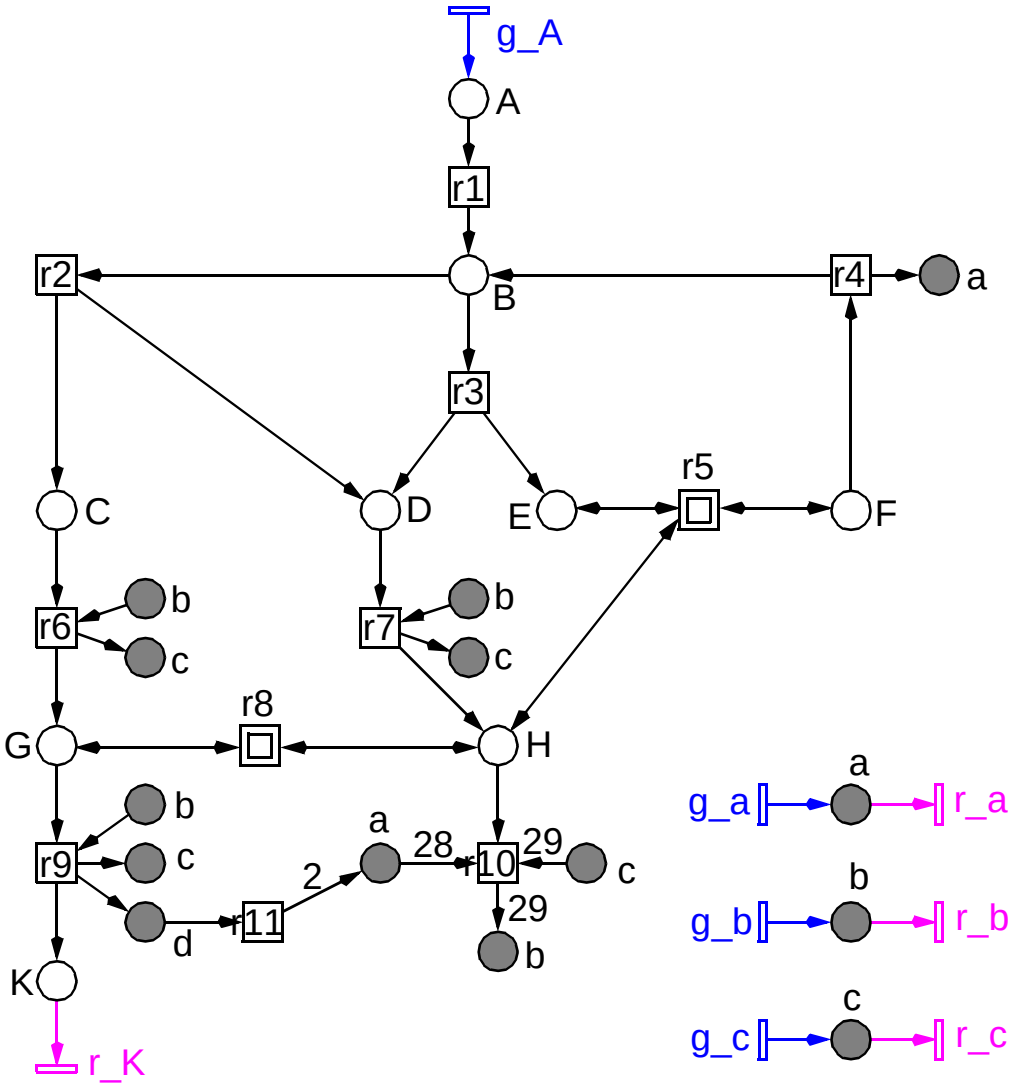
ENVIRONMENT BEHAVIOUR, STYLE 1

- **weak assumtions**
- **to animate the model**
  - -> *deeper insights*
  - -> *infinite substance flow*
- **to validate the model**



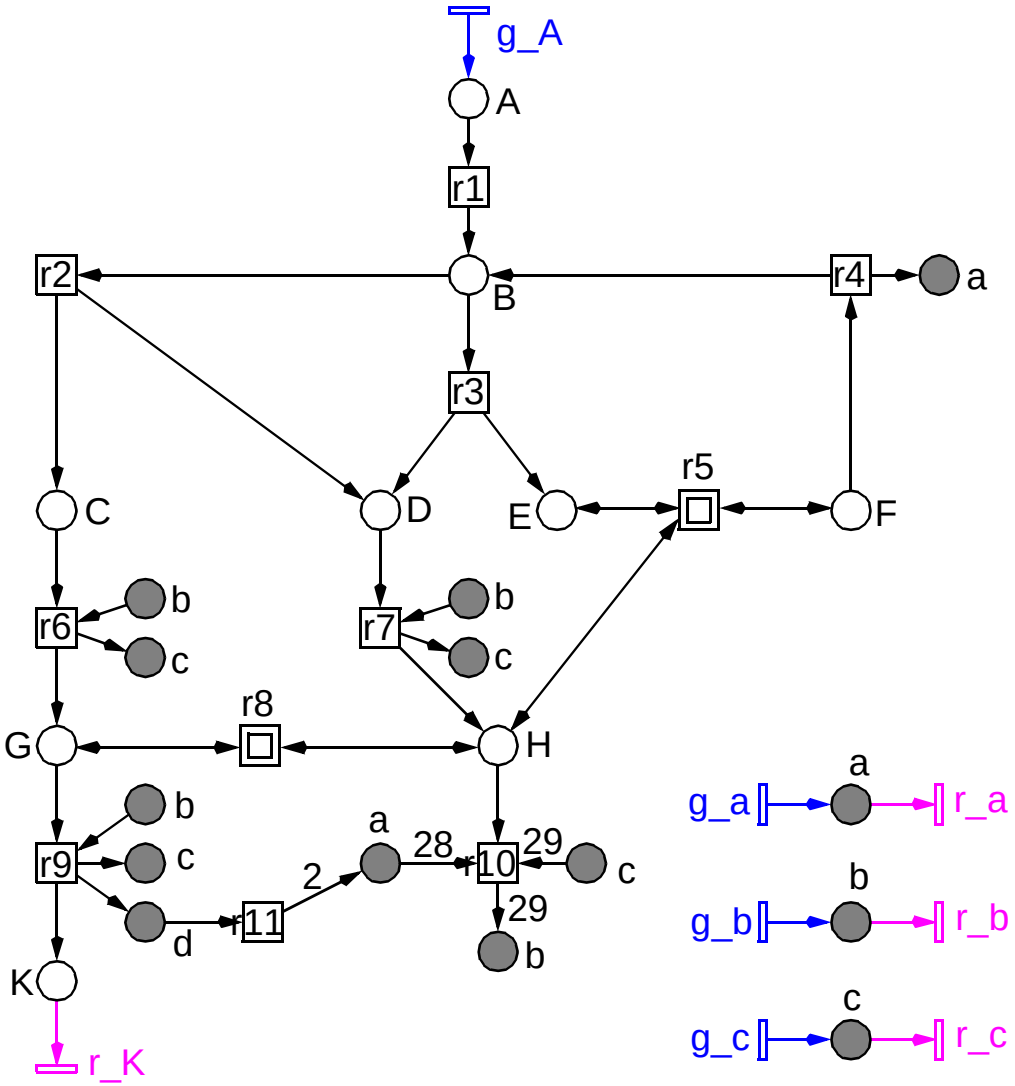
ENVIRONMENT BEHAVIOUR, STYLE 1

- **weak assumptions**
- **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*



ENVIRONMENT BEHAVIOUR, STYLE 1

- **weak assumptions**
- **initial marking**
  -> *empty marking*
- **steady state behaviour**
  -> *empty marking reproduction*
- **(cause effect) pathway**
  -> *minimal flow preserving given compounds distribution*
  -> *elementary modes*
  = *minimal T-invariants*



T-INVARIANTS, BASICS

- **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 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*

T-INVARIANTS, BASICS

- **Lautenbach, 1973**
- **T-invariants** -> *multisets of transitions*
  -> *integer solutions of* $Cx = 0, x \neq 0, x \geq 0$ -> *exponential complexity*
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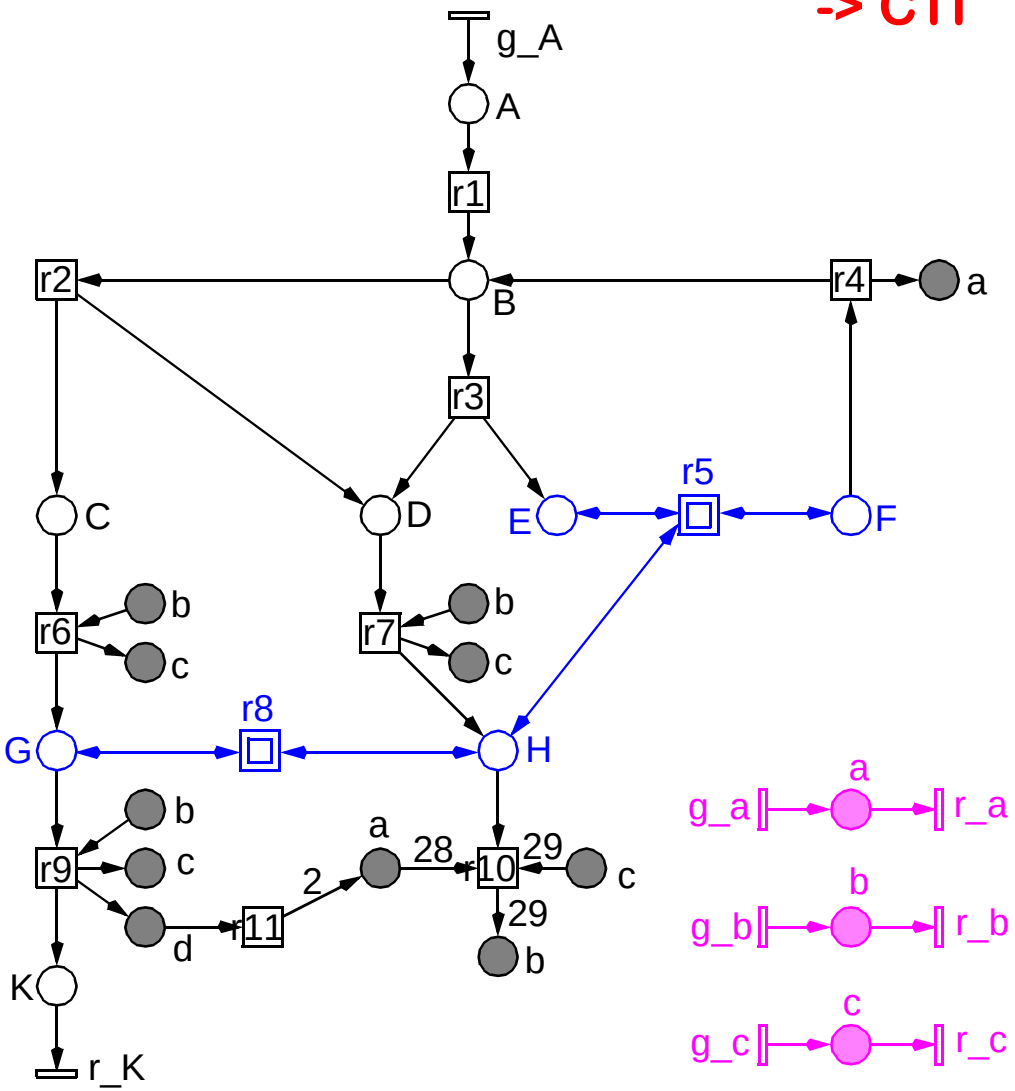
trivial min. T-invariants (5)

-> 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

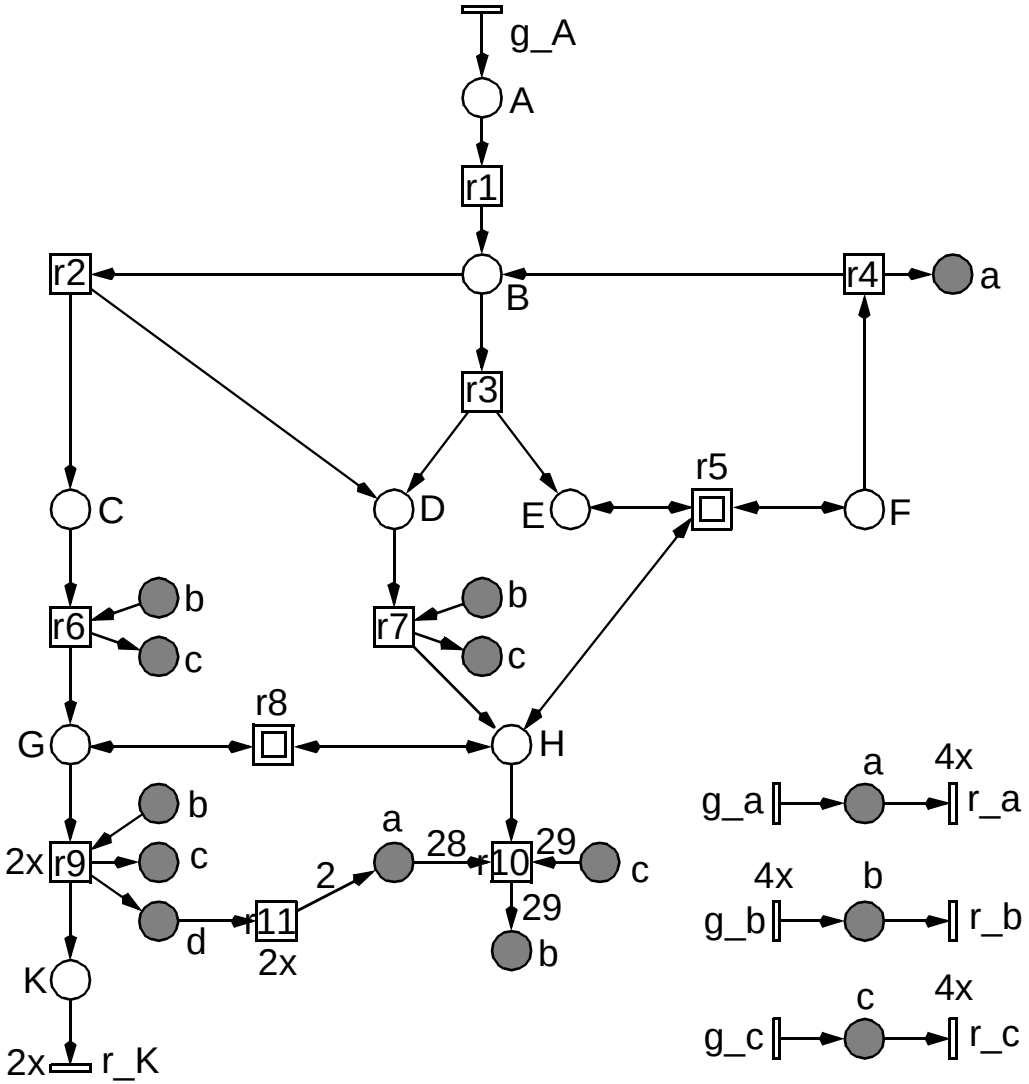


- **i/o-T-invariant, example**

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



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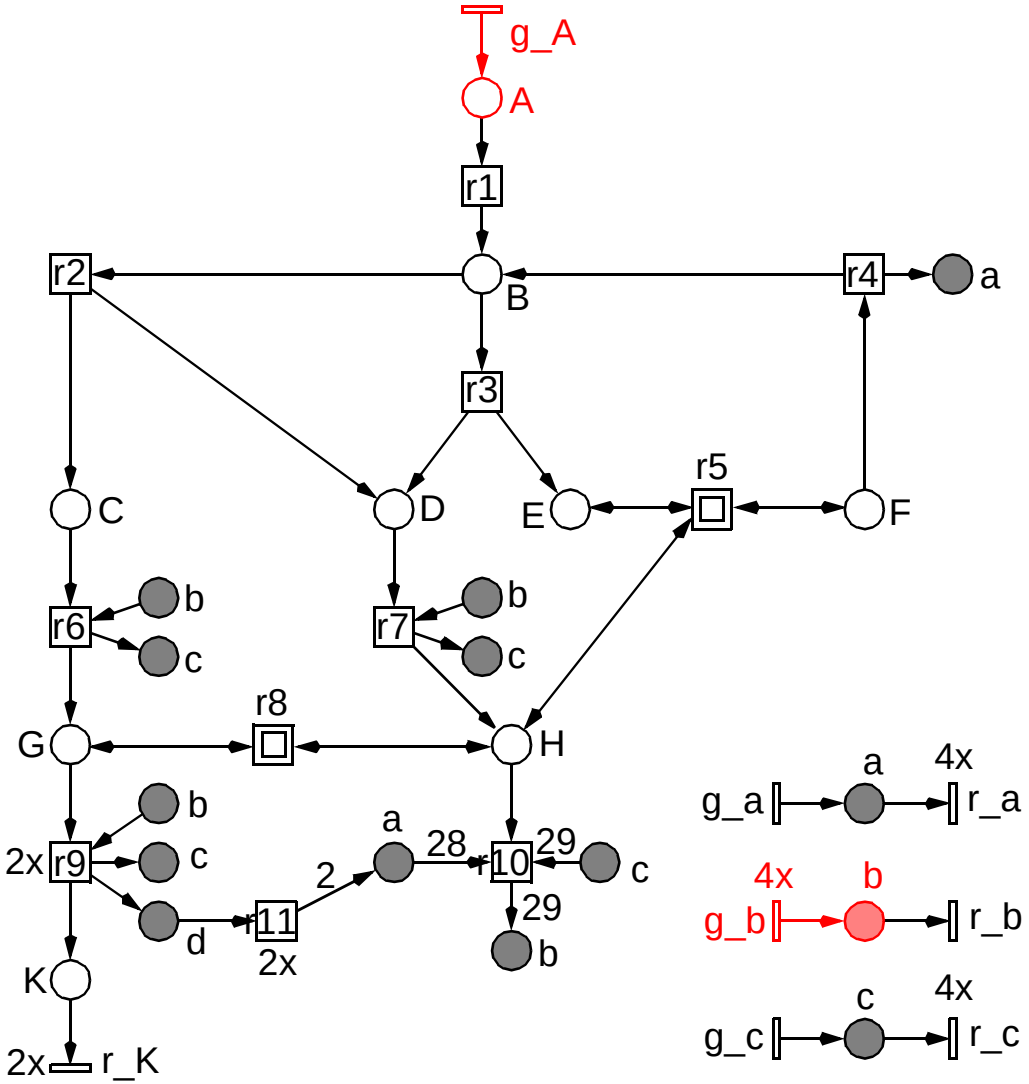

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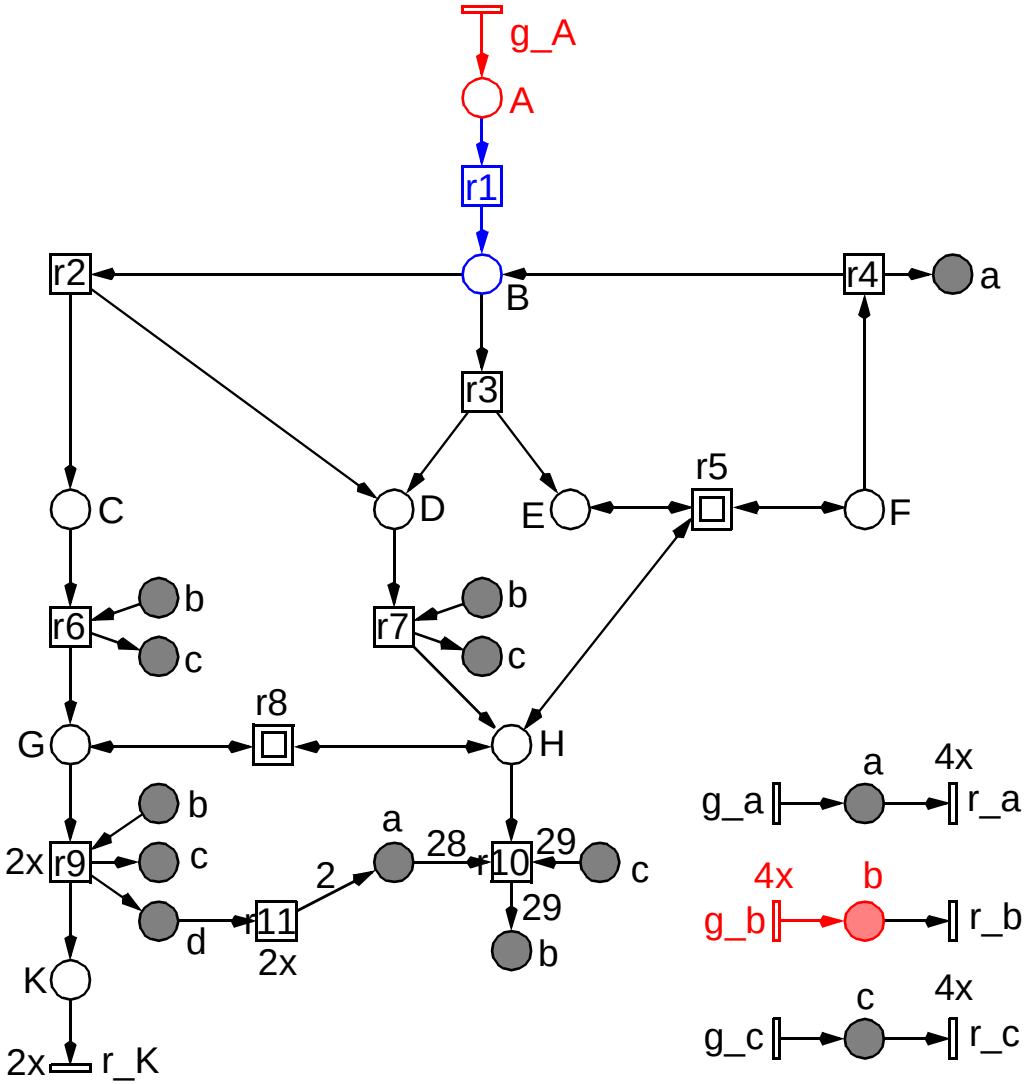

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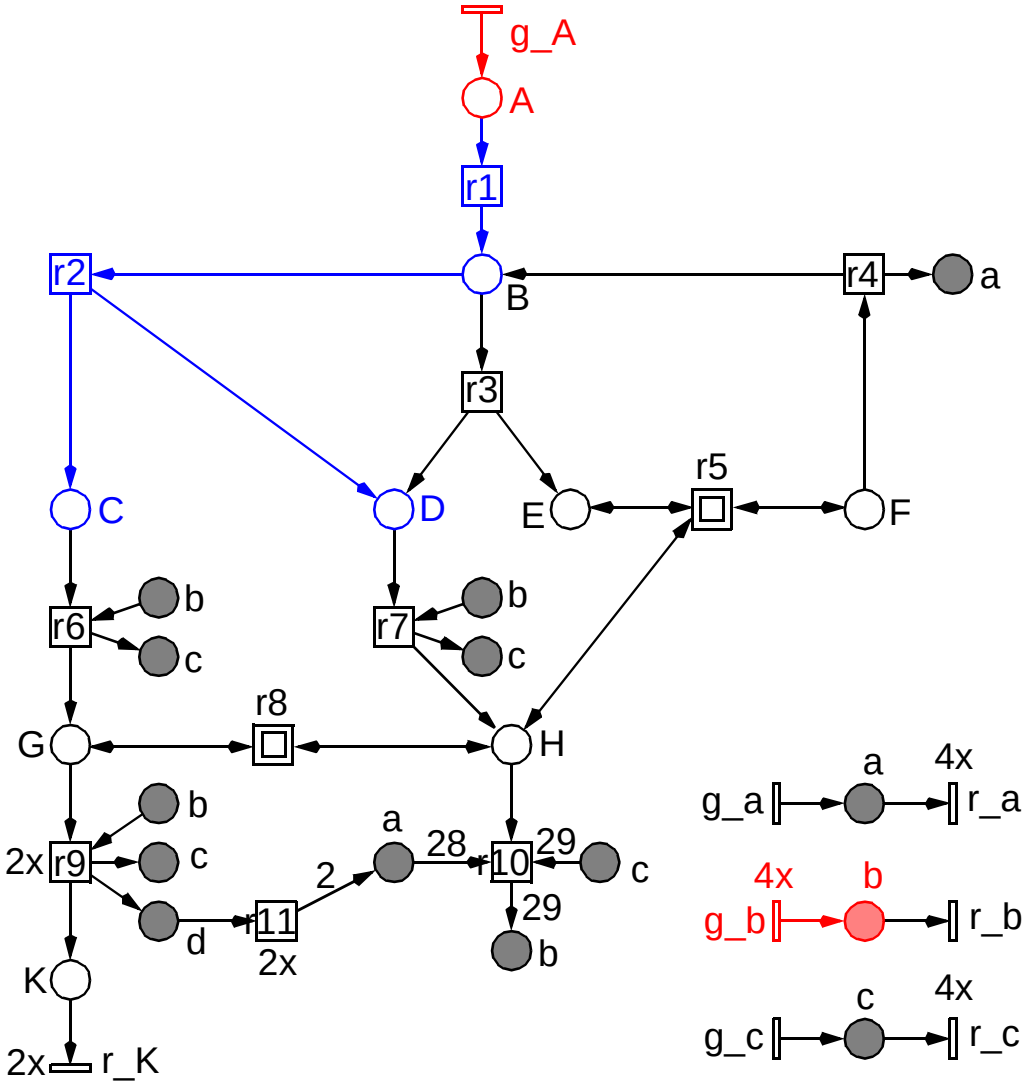

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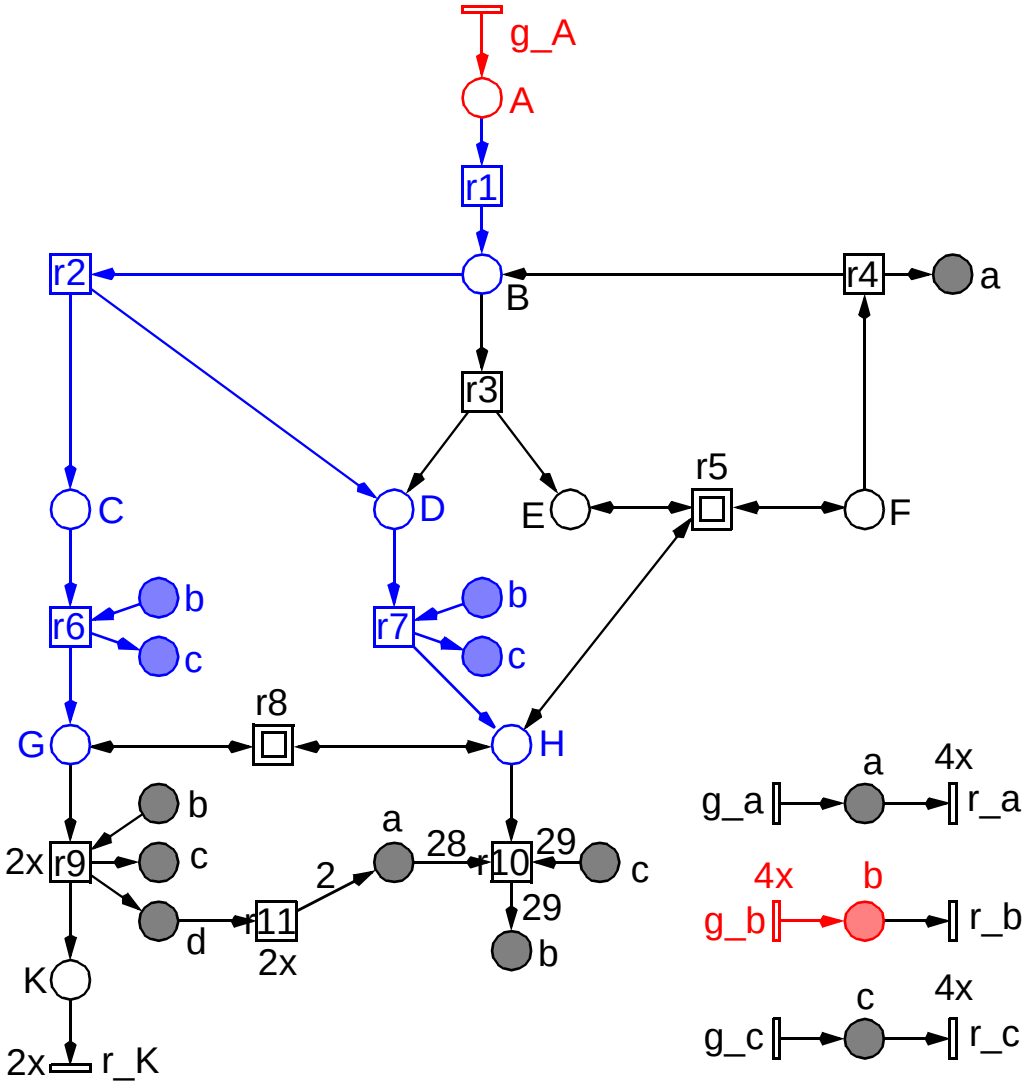
	12.r11	*:*	*2,*
	13.g_A	*:*	*1,*
	14.r_K	*:*	*2,*
	15.g_b	*:*	*4,*
	18.r_c	*:*	*4,*
	20.r_a	*:*	*4*

- **sum equation**

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

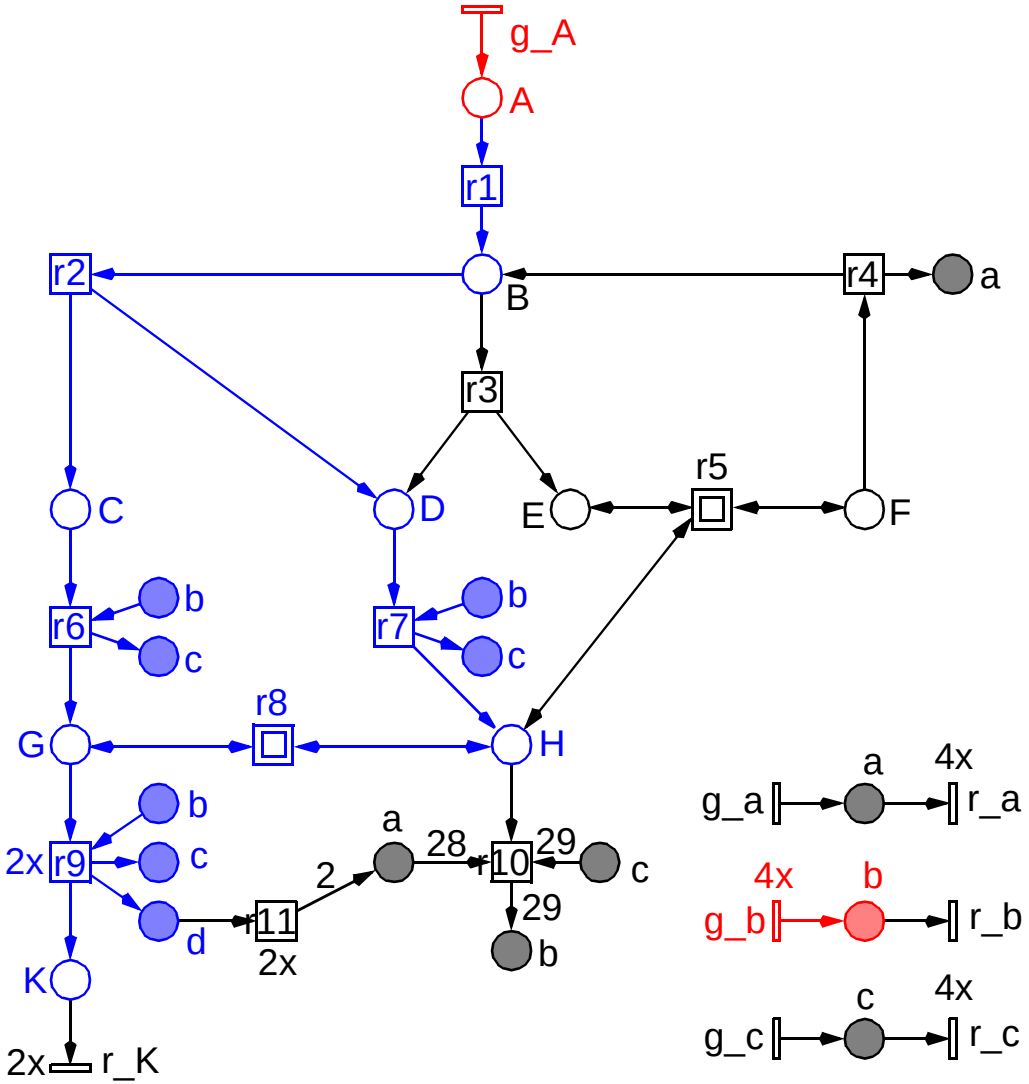


- **i/o-T-invariant, example**

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

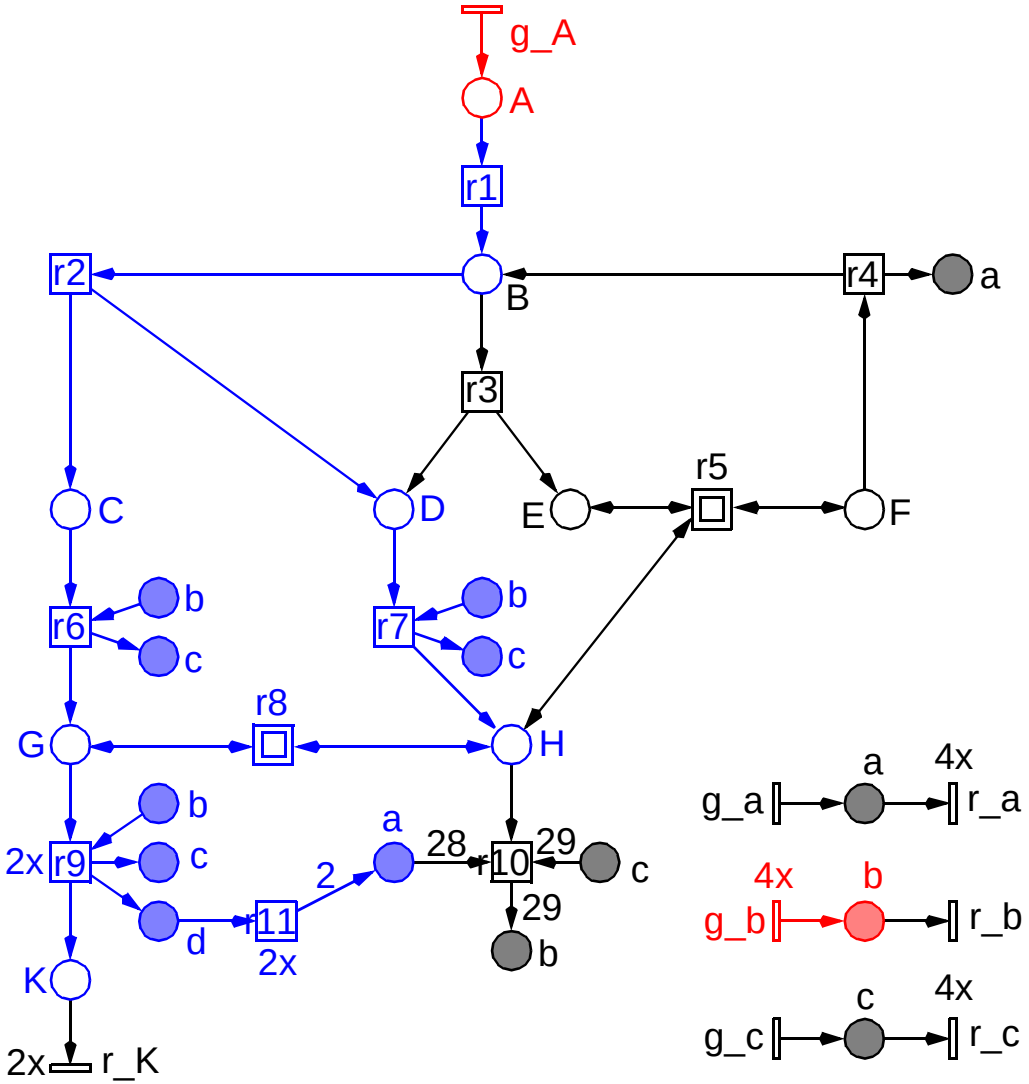


- **i/o-T-invariant, example**

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



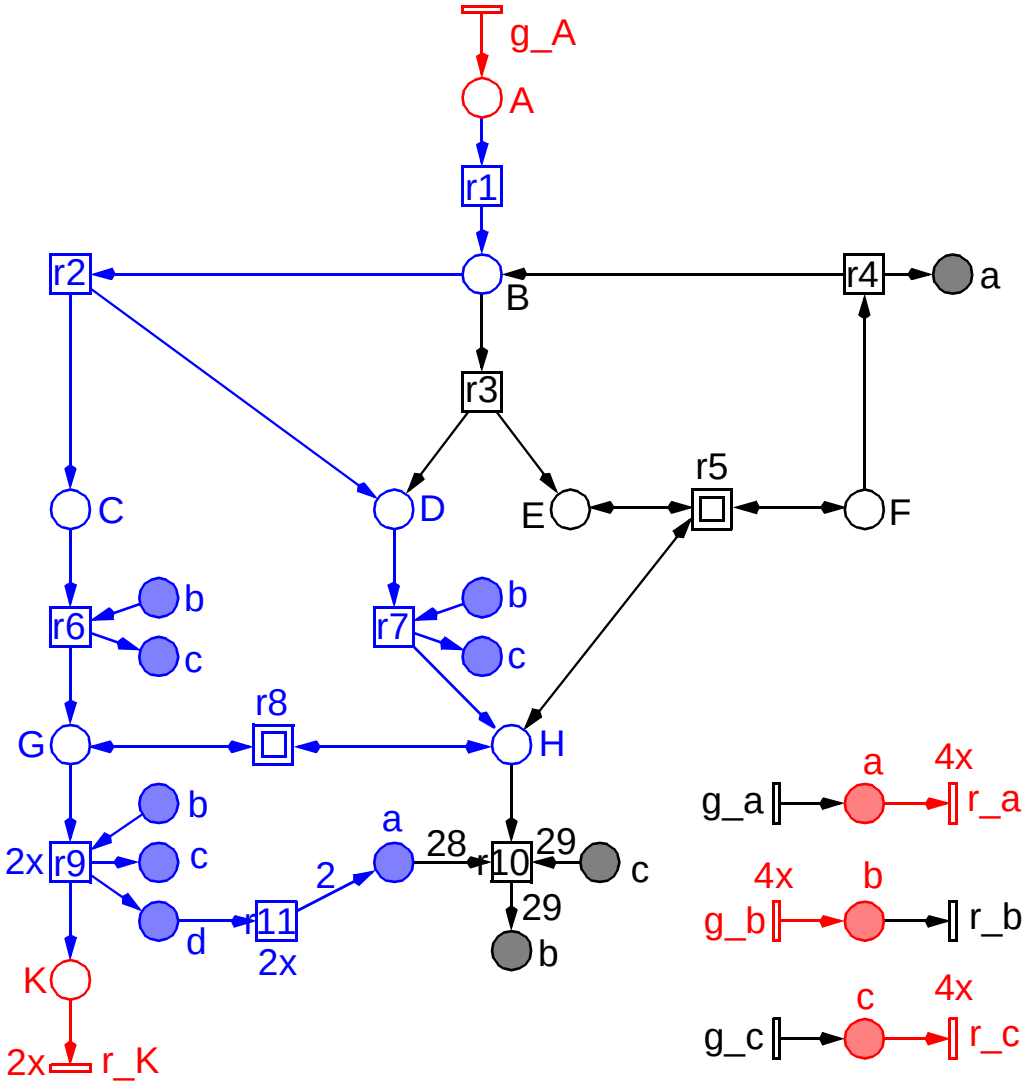
BIONETWORK, I/O-T-INVARIANT

- **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**

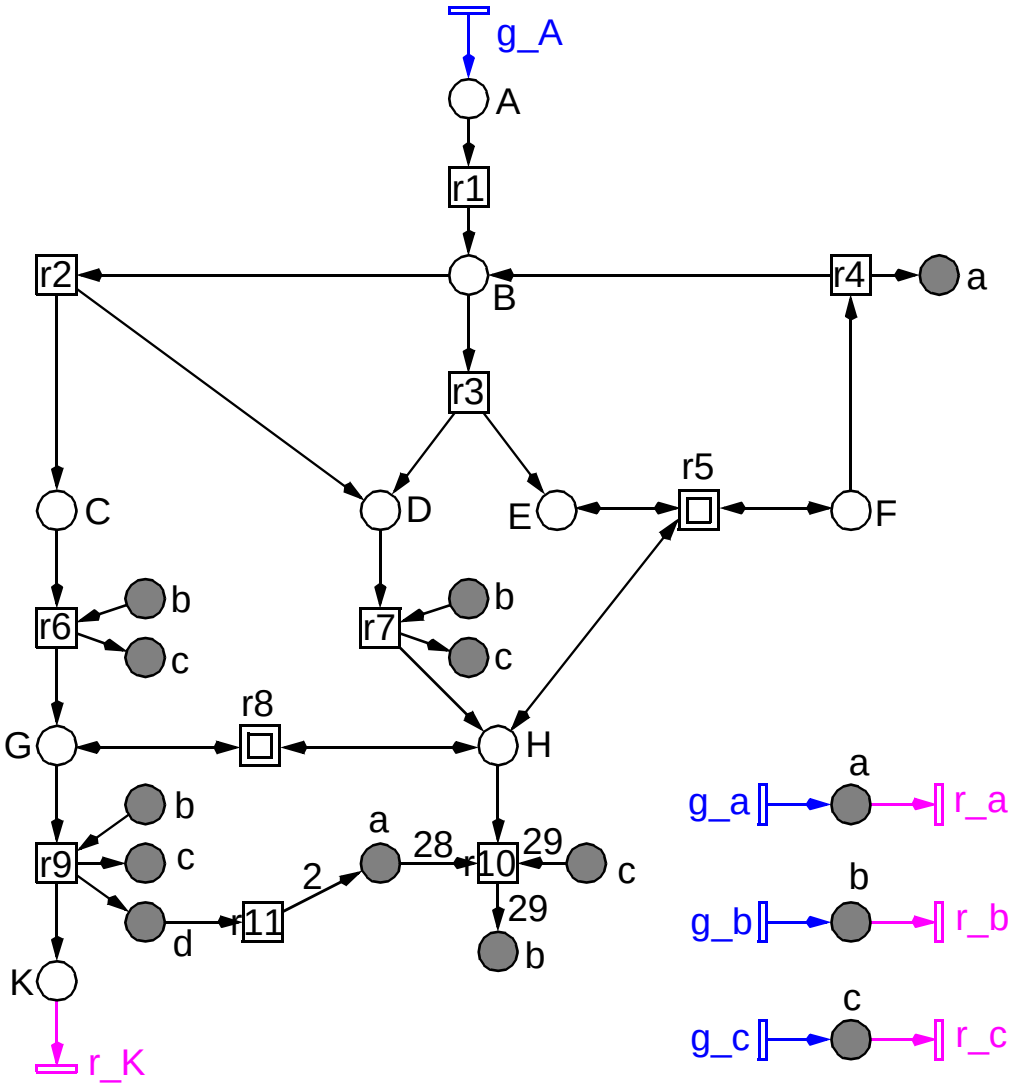
A + 4b -> 2K +4a + 4c



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

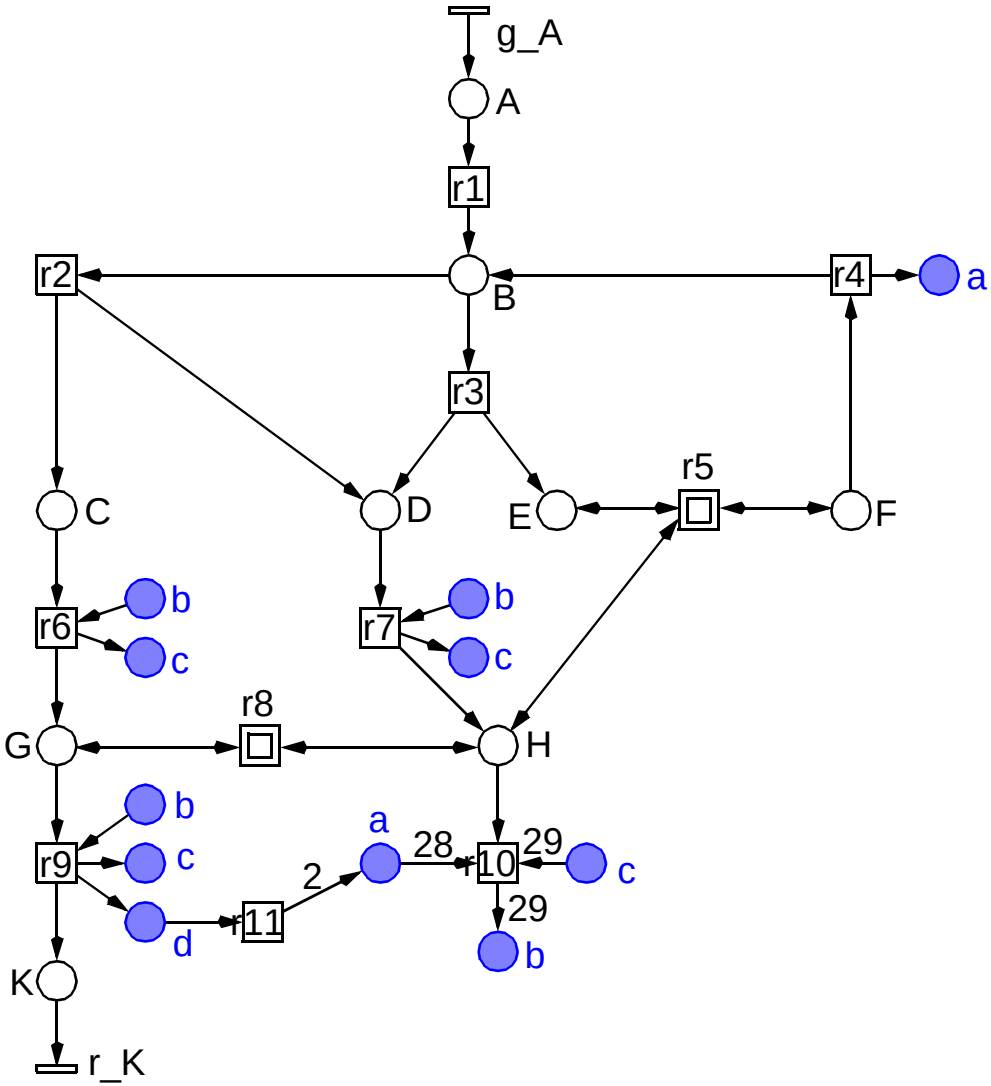
ENVIRONMENT BEHAVIOUR, STYLE 1 -> 2

- **firm -> weak assumptions**
- **input substances**
  -> *generating pre-transitions*
- **output substances**
  -> *consuming post-transitions*



ENVIRONMENT BEHAVIOUR, STYLE 2

- **firm assumptions**
- **input substances**
  -> *generating pre-transitions*
- **output substances**
  -> *consuming post-transitions*
- **auxiliary substances**
  -> *finite reservoir*
  -> *P-invariants*
  (= traps = co-traps)



P-INVARIANTS, BASICS

- **Lautenbach, 1973**
- **P-invariants**
  -> *integer solutions of* $yC = 0, y \neq 0, y \geq 0$
  -> *sets of places with* $my = m_0 y, \forall m \in RG(m_0)$
- **minimal P-invariants**
  -> *there is no P-invariant with a smaller support*
  -> *gcD of all entries is 1*

token conservation

- **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*

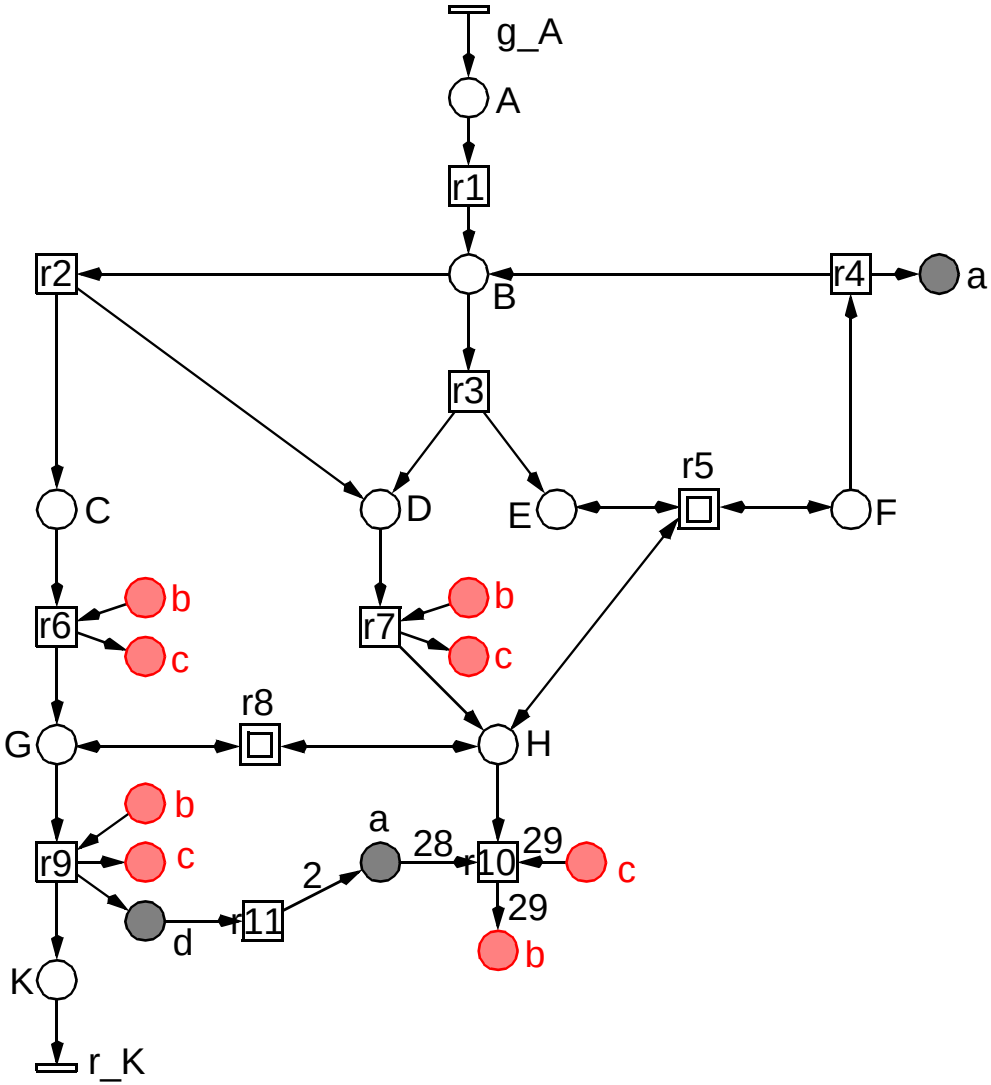
ENVIRONMENT BEHAVIOUR, STYLE 2

- **P-invariants**

  -> *substance conservation*

- **P-invariants,examples**

  1 | 7.b : 1,
  | 8.c : 1



ENVIRONMENT BEHAVIOUR, STYLE 2

- **P-invariants**

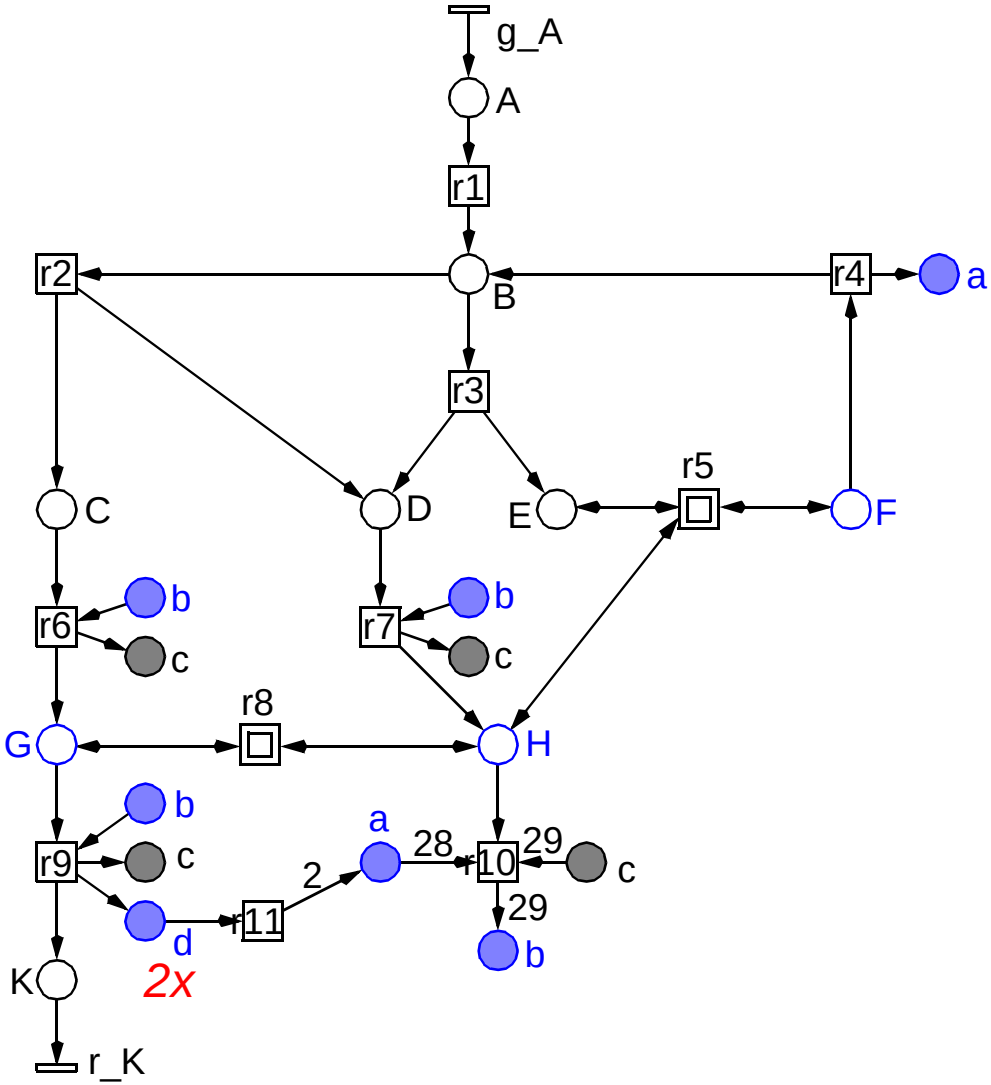
  -> *substance conservation*

- **P-invariants,examples**

  1 | 7.b : 1,
  | 8.c : 1

  2 | 5.G : 1,
  | 6.H : 1,
  | 7.b : 1,
  | 9.F : 1,
  | 10.a : 1,
  | 11.d : 2

- **Which (minimal) initial token distribution makes the net live ?**



- **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*

ENVIRONMENT BEHAVIOUR, STYLE 3

- **strong assumptions**
  - -> *quantitative relations of input/output compounds*
  - -> *finite reservoir of auxiliary compounds*

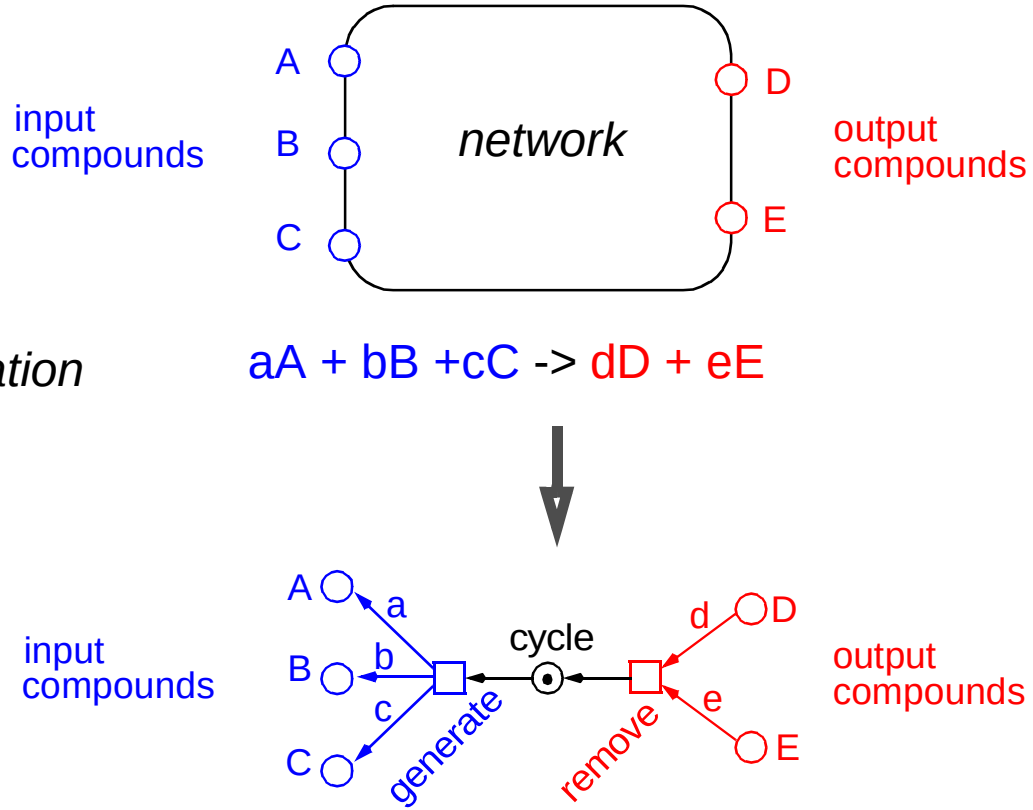
- **typically expected properties**

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

- **boundedness (finite state space)**
  - -> *model checking of specific properties*
    - *- CTL (undecidable for unbounded nets)*
    - *- LTL (decidable, but no tools, not yet ?)*
  - -> *preserving all possible behavior (= minimal T-invariants)*

ENVIRONMENT BEHAVIOUR, STYLE 3

- **additional model component**

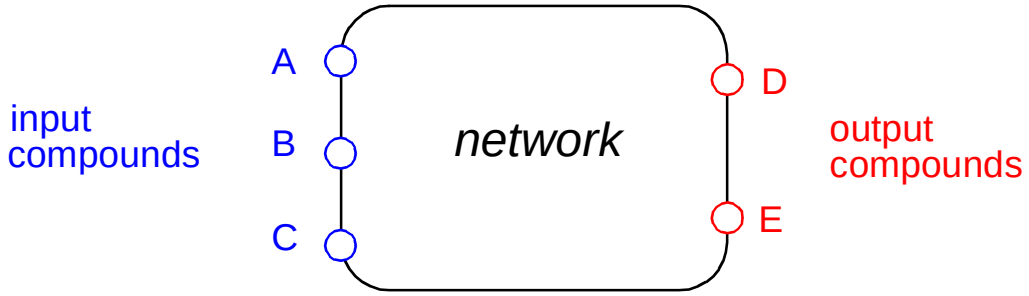


- **precondition**

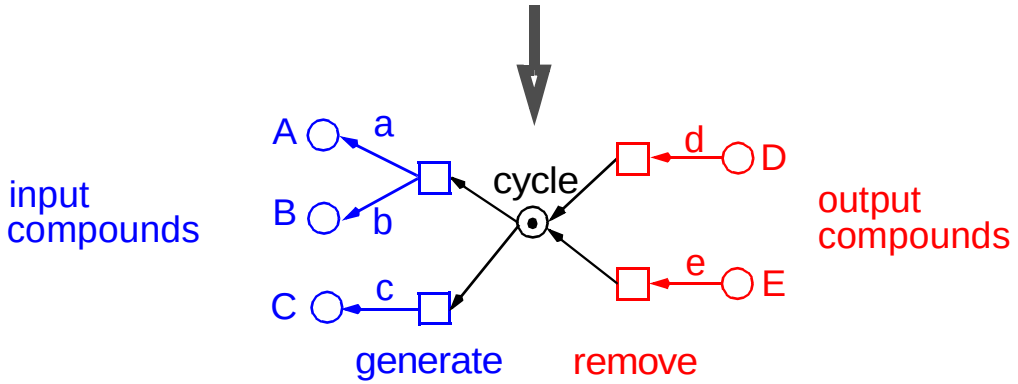
  -> *equal sum equation for all pathways*

ENVIRONMENT BEHAVIOUR, STYLE 3

- **additional model component, refinement**



pathway1, sum equation aA + bB -> dD
pathway2, sum equation cC -> eE

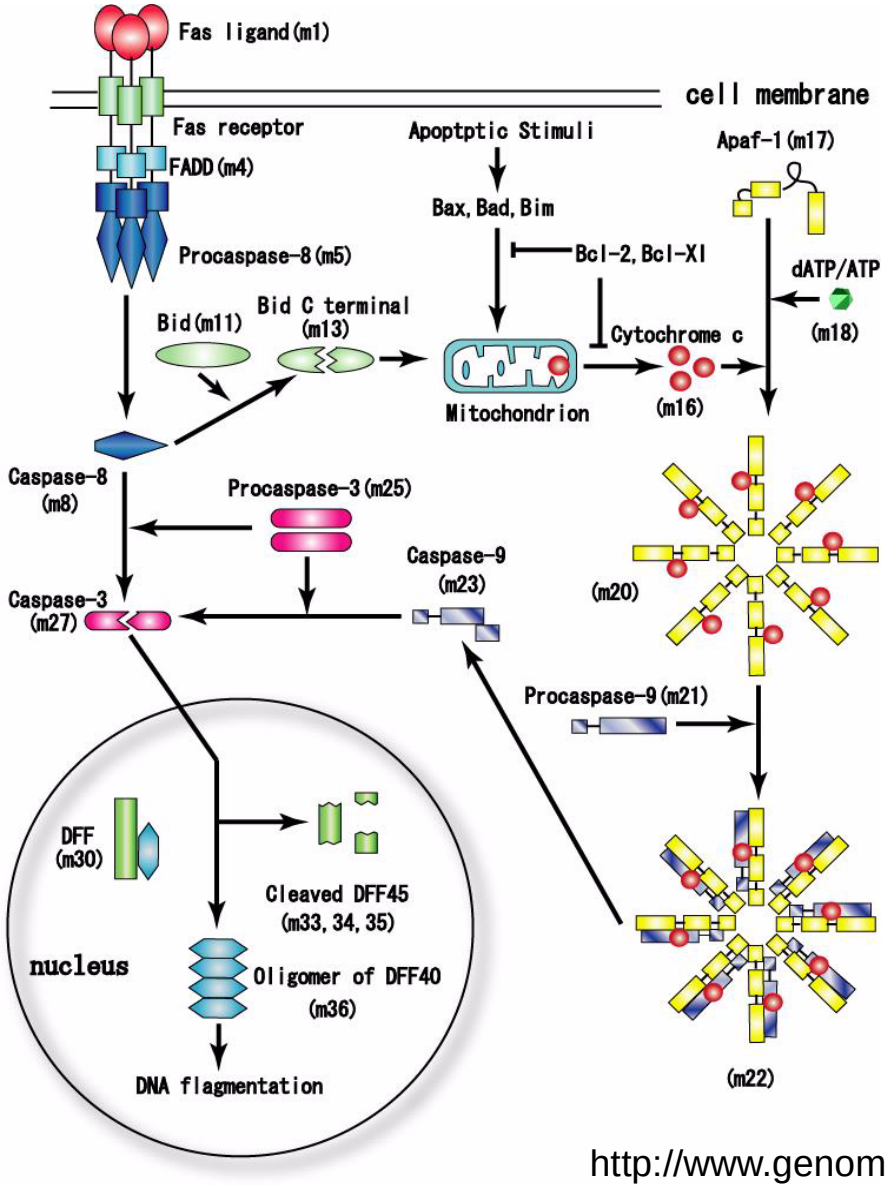


- **precondition**

  -> *controlled conflicts between pathways with unequal sum equations*

ENVIRONMENT BEHAVIOUR, STYLE 3

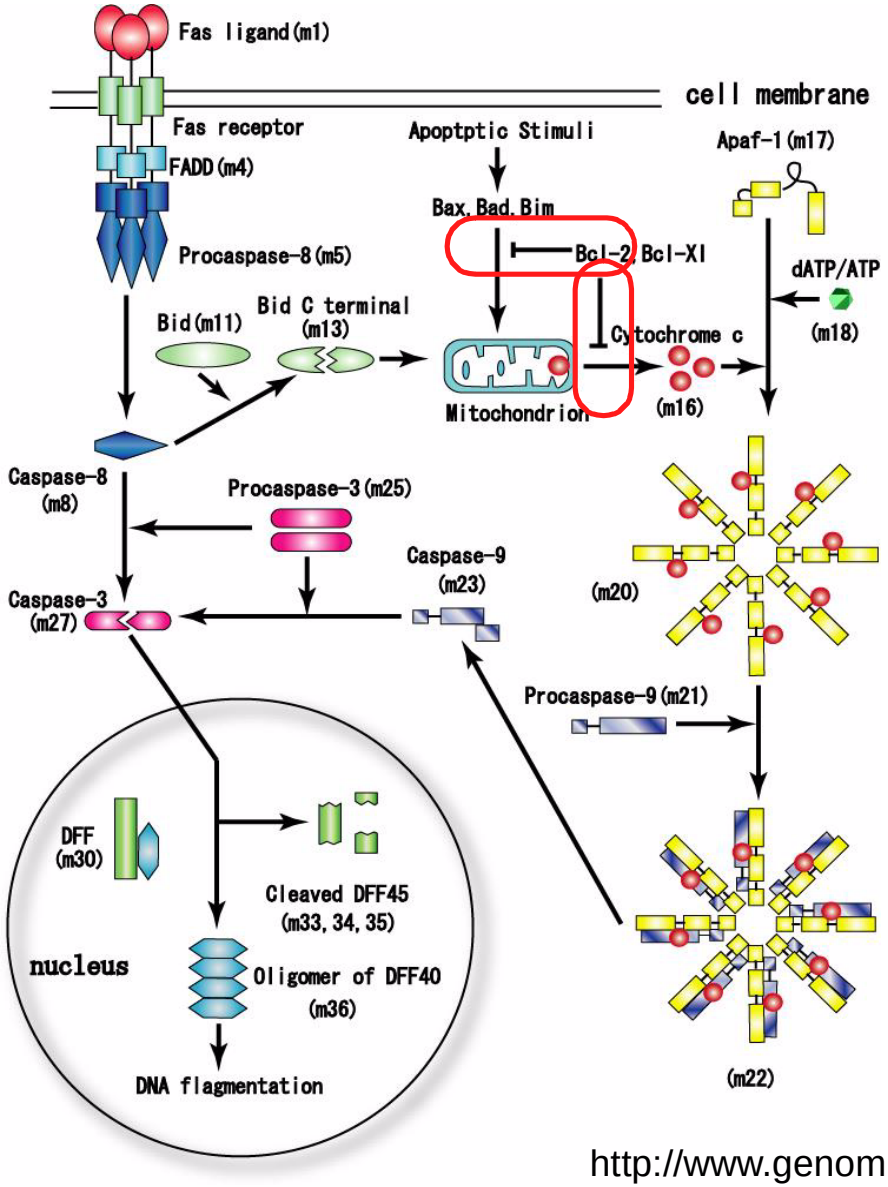
- **example - apoptosis**
  -> Matsuno et al.
- **signal-transduction pathway**



http://www.genomicObject.net

ENVIRONMENT BEHAVIOUR, STYLE 3

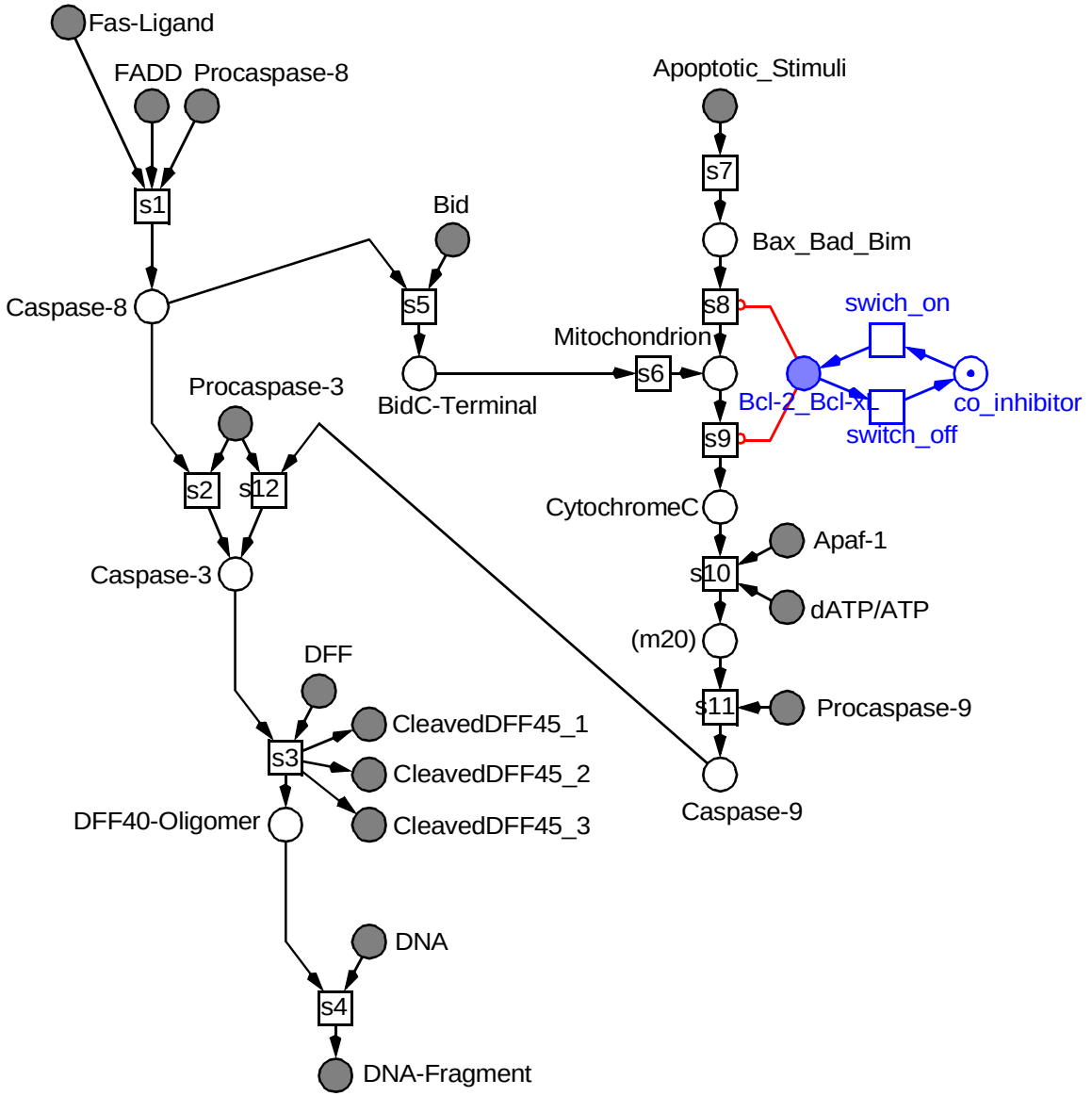
- **example - apoptosis**
  -> Matsuno et al.
- **signal-transduction pathway**
- **inhibitor arcs**



http://www.genomicObject.net

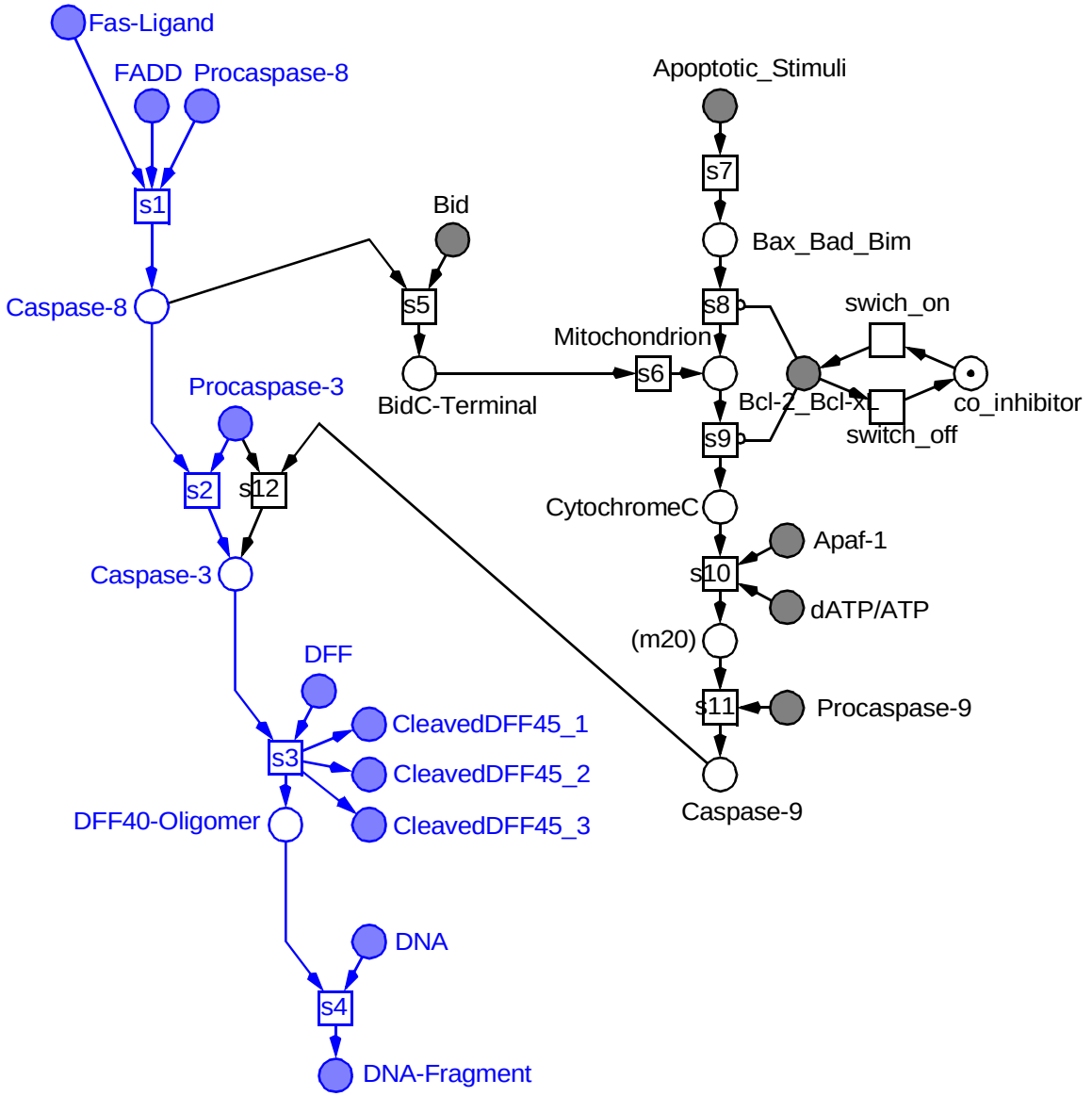
ENVIRONMENT BEHAVIOUR, STYLE 3

- example - apoptosis
- network model
- inhibitor arcs
- three pathways
  = min. T-invariants



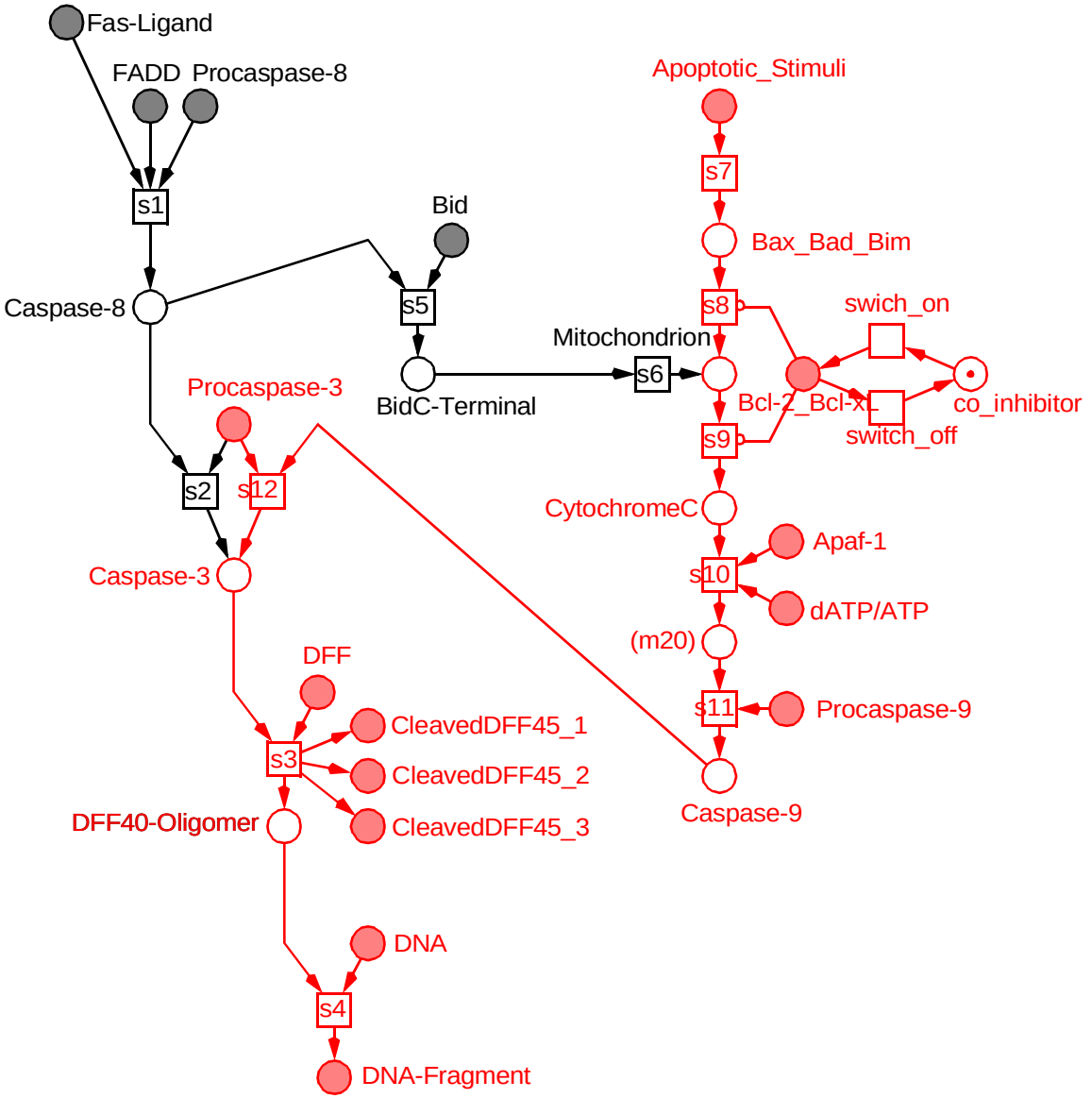
ENVIRONMENT BEHAVIOUR, STYLE 3

- **example - apoptosis**
- **network model**
- **environment, style 1**
  - -> *three pathways = min. T-invariants*
- **T-invariant 1**
  - -> *Fas-induced*
  - -> *'death-receptor' pathway*



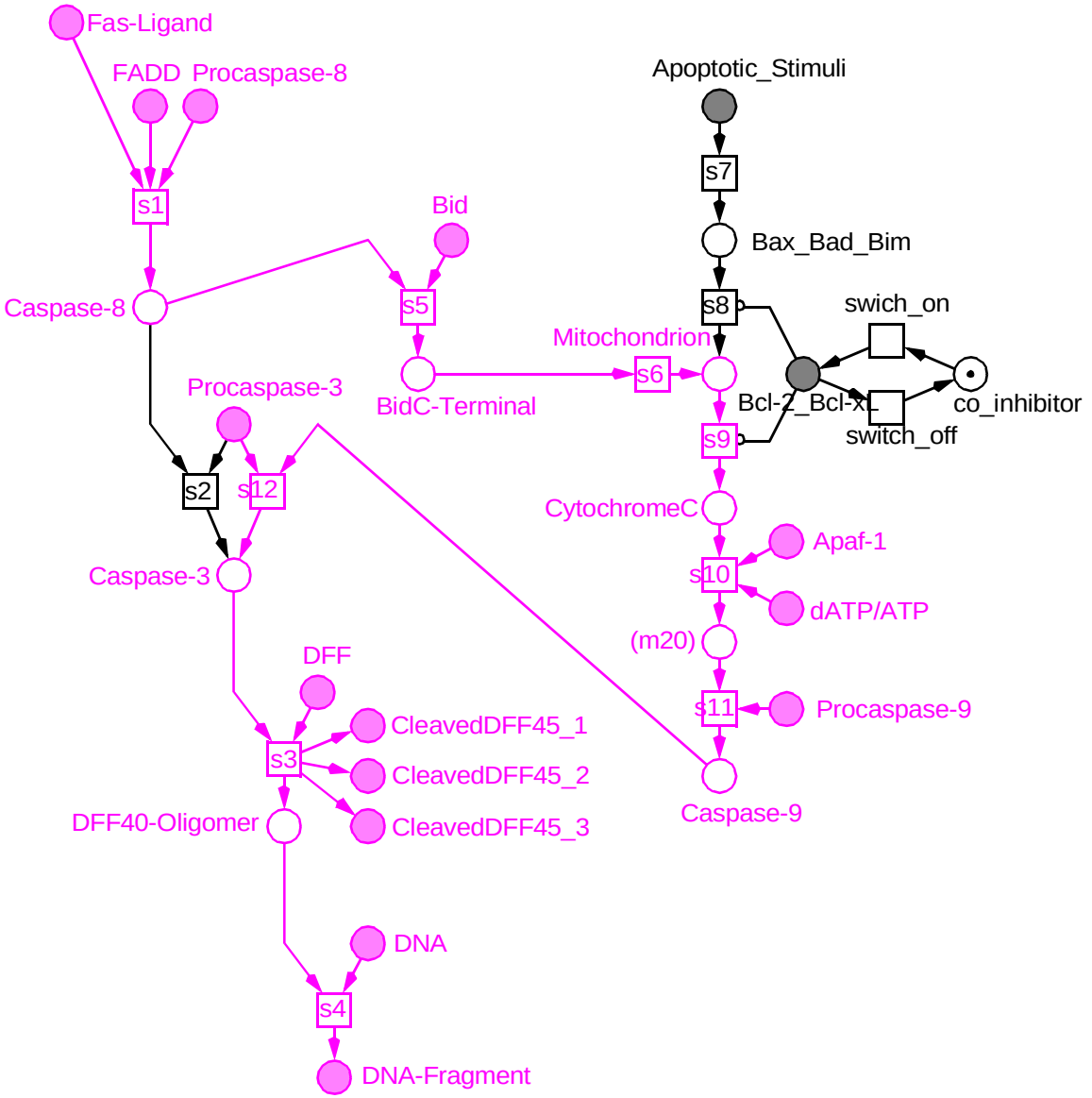
ENVIRONMENT BEHAVIOUR, STYLE 3

- **example - apoptosis**
- **network model**
- **environment, style 1**
  - -> *three pathways = min. T-invariants*
- **T-invariant 2**
  - -> *apoptotic-stimuli-induced*
  - -> *'mitochondrial' pathway*



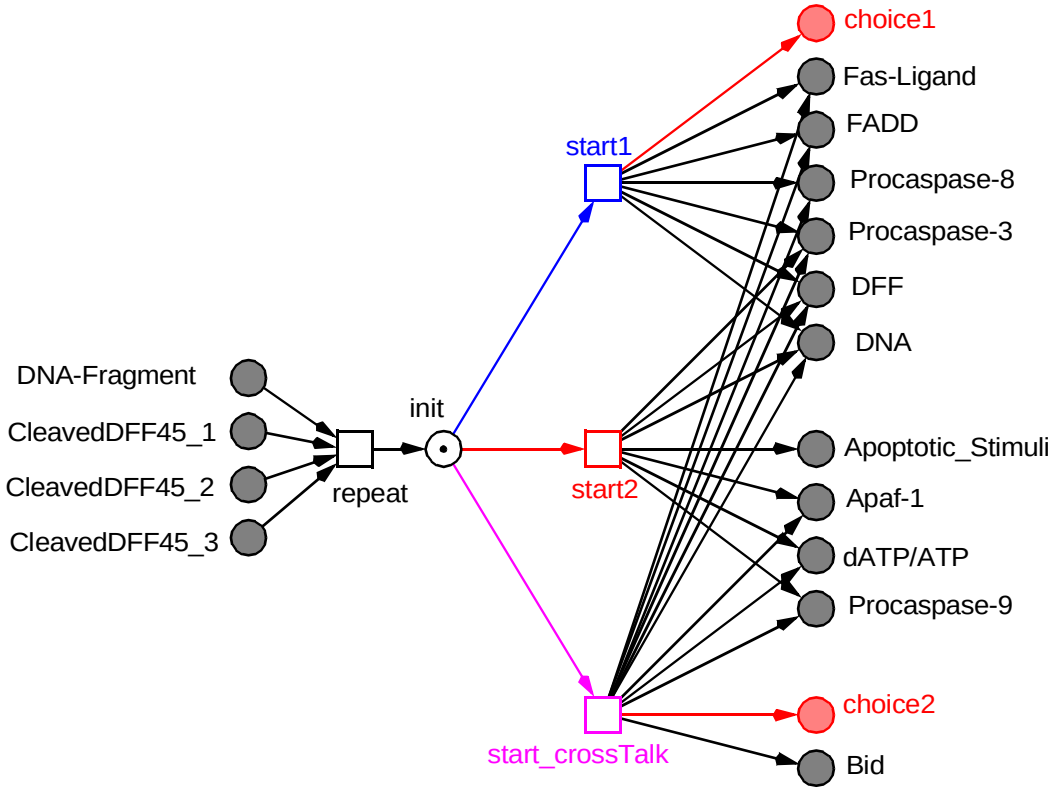
ENVIRONMENT BEHAVIOUR, STYLE 3

- **example - apoptosis**
- **network model**
- **environment, style 1**
  -> *three pathways*
  = min. T-invariants
- **T-invariant 3**
  -> *'cross-talk by Bid' pathway*



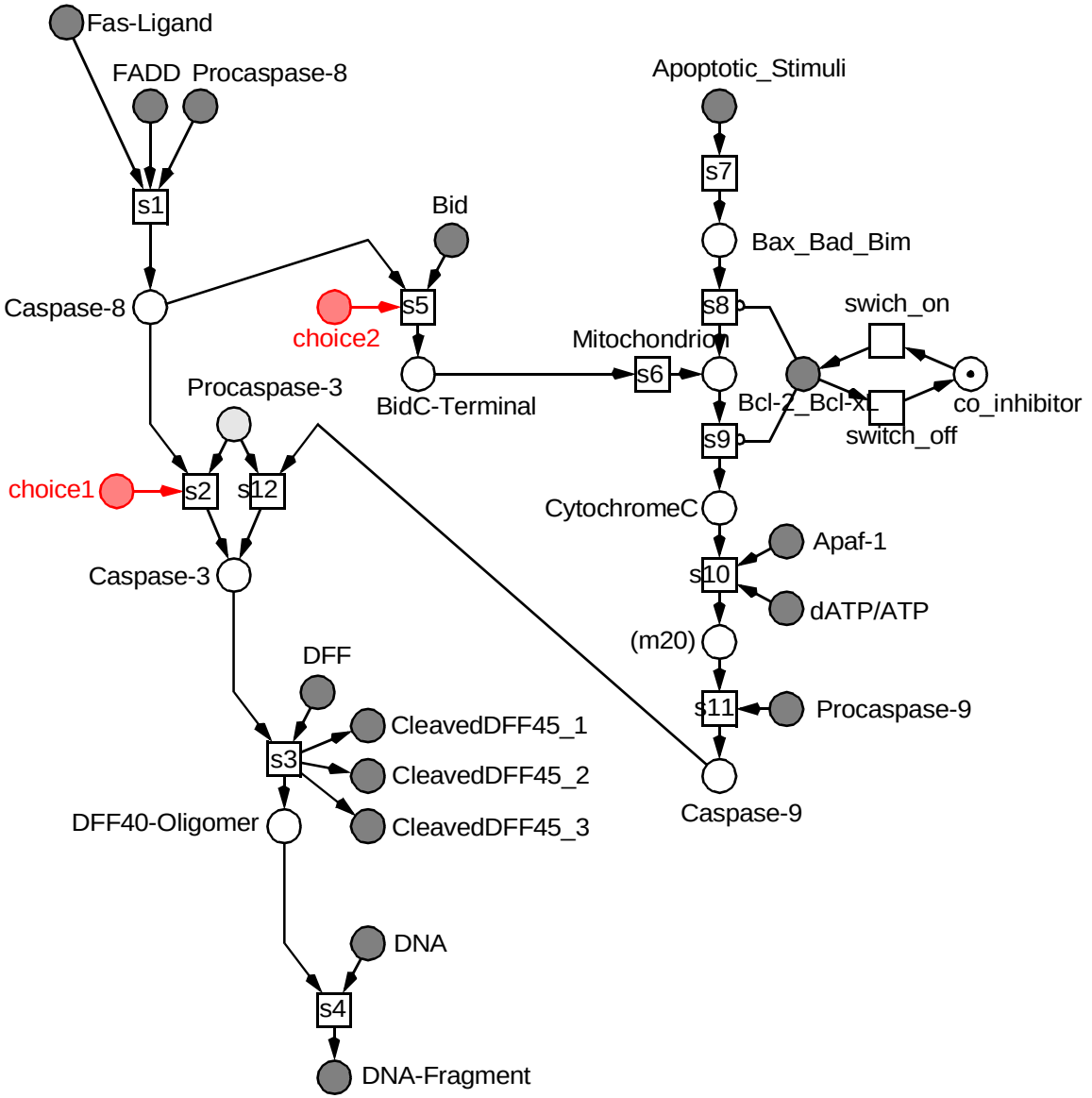
ENVIRONMENT BEHAVIOUR, STYLE 3

- **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*

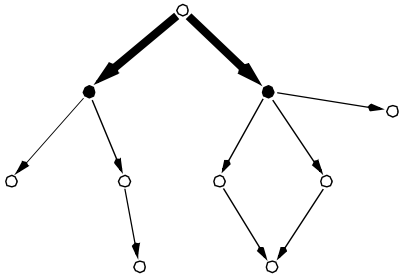
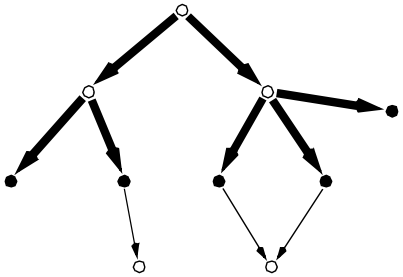
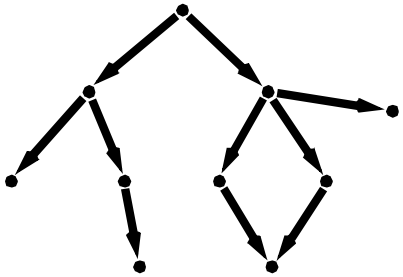
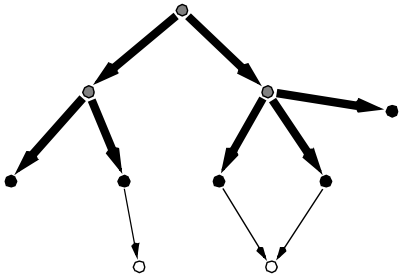
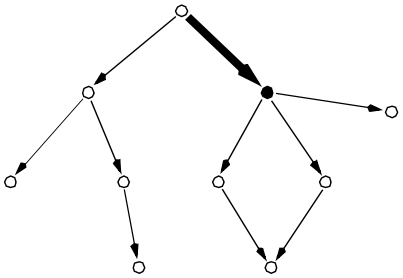
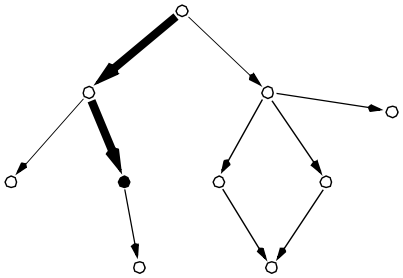
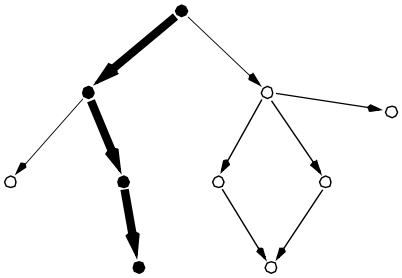
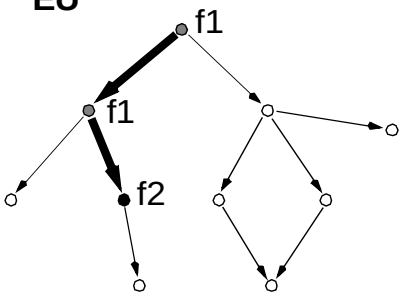


ENVIRONMENT BEHAVIOUR, STYLE 3

- **example - apoptosis**
- **network model, adapted**
- **system model**
  - -> *network model*
  - -> *environment model*
- **system model**
  - -> *1-bounded*
  - -> *live*
- **ready for model checking**



CTL OPERATORS, INTERLEAVING SEMANTICS

	next f	finally f	globally f	f1 until f2
on all branches	AX 	AF 	AG 	AU 
on some branch	EX 	EF 	EG 	EU 

- **property 1**

  if inhibitor substance *Bcl_2_Bcl_xL* **is present,**
  then the progress of the cross-talk pathway is stopped at *Mitochondrion*

  *AG (Bcl_2_Bcl_xL * Mitochondrion -> AX (Mitochondrion));*

- **property 2**

  if inhibitor substance *Bcl_2_Bcl_xL* **is present,**
  then the progress of the cross-talk pathway is stopped at *Mitochondrion*
  until the inhibitor substance disappears

  *AG (Bcl_2_Bcl_xL * Mitochondrion -> A (Mitochondrion U ! Bcl_2_Bcl_xL));*

- **property 3**

  if inhibitor substance *Bcl_2_Bcl_xL* **is not present,**
  then the progress of the cross-talk pathway is not stopped at *Mitochondrion*

  *AG (!Bcl_2_Bcl_xL * Mitochondrion -> EX (CytochromeC));*

- **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*
- **qualitative model -> steady state behavior**
- **three styles of environment description**
  - -> *style 1* -> *T -invariants <-> pathways, CTI*
  - -> *style 2* -> *P-invariants <-> substance conservation*
  - -> *style 3* -> *CPI, model checking of CTL - properties*
- **many challenging questions for analysis techniques**

THANKS !