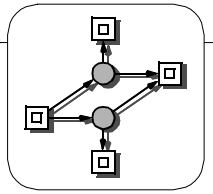

QUALITATIVE MODELLING AND ANALYSIS OF BIOCHEMICAL PATHWAYS WITH PETRI NETS

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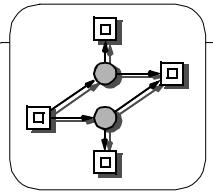
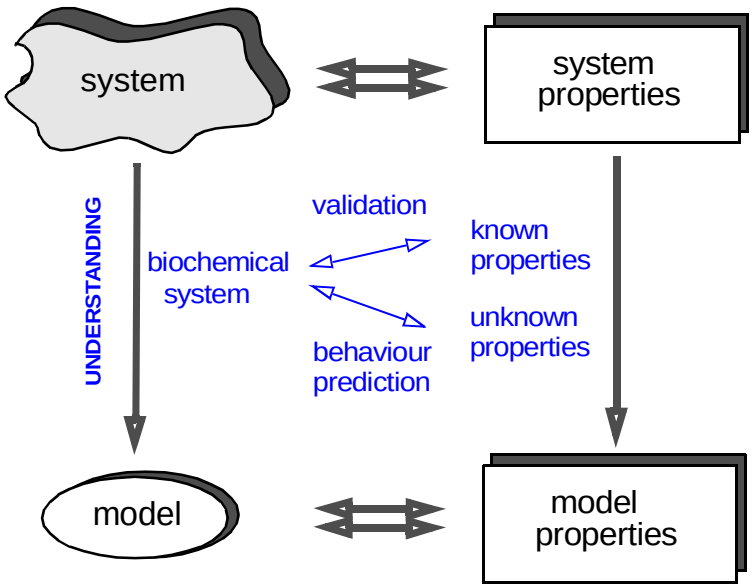


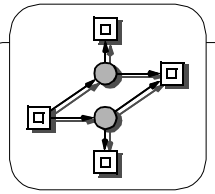
OUTLINE

1.

MOTIVATION

MODEL- BASED SYSTEM ENGINEERING

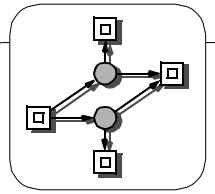




BIOCHEMICAL SYSTEMS, EXAMPLES

- metabolic pathways / networks
  - -> *stoichiometric relations known*
  - -> *concentrations of metabolites often known*
- signal transduction pathways / networks
  - -> *stoichiometric relations unknown*
  - -> *read arcs / test arcs*
  - -> *inhibitor arcs*
- gene regulatory networks
  - -> *stoichiometric relations unknown*
  - -> *mRNA concentrations often known*
  - -> *protein concentrations are hard to be measured*
  - -> *often a mixture of metabolic and signal transduction pathways*

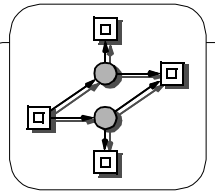
=>> *networks of elementary actions*



BIOCHEMICAL SYSTEMS, SOME PROBLEMS

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

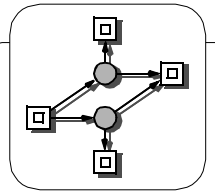
=>> *models of biochemical systems are typically full of assumptions*



REPRESENTATIONS, OBJECTIVES

- readability
  -> *understanding*
- animation
  -> *experience*
- validation
  -> *consistency checks*
- analysis
  -> *behaviour prediction (qualitative / quantitative)*

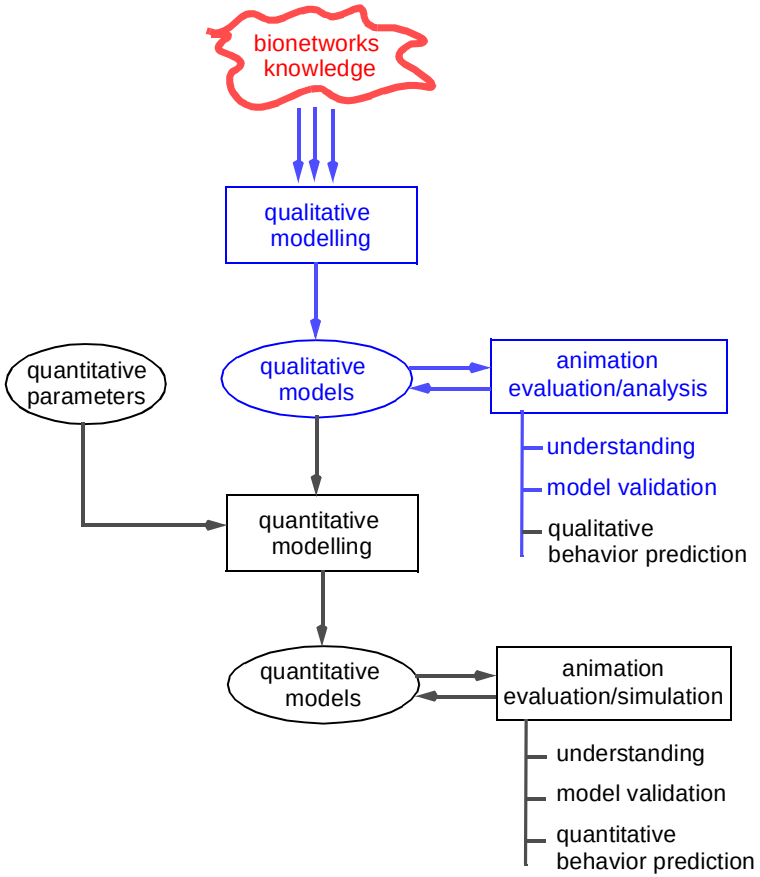
=>> *How many representations do we really need ?*



WHY PETRI NETS ?

- a suitable intermediate representation for
  -> *different languages*
  -> *different stages of certainty*
- modelling power
  -> *partial order semantics*
  -> *applicable on any abstraction level*
  -> *specification of limited resources possible*
- analysis power
  -> *various complementary analysis methods*
  -> *reliable tools*
- integration of qualitative and quantitative analyses
- **BUT:** modelling power <-> analysis power

FRAMEWORK



PETRI NETS, 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 appear*
- Reddy, 1993
  -> *first paper on bio application*
- late 1990's
  -> *increasing interest in applying Petri nets for modelling and analysis of bio networks*

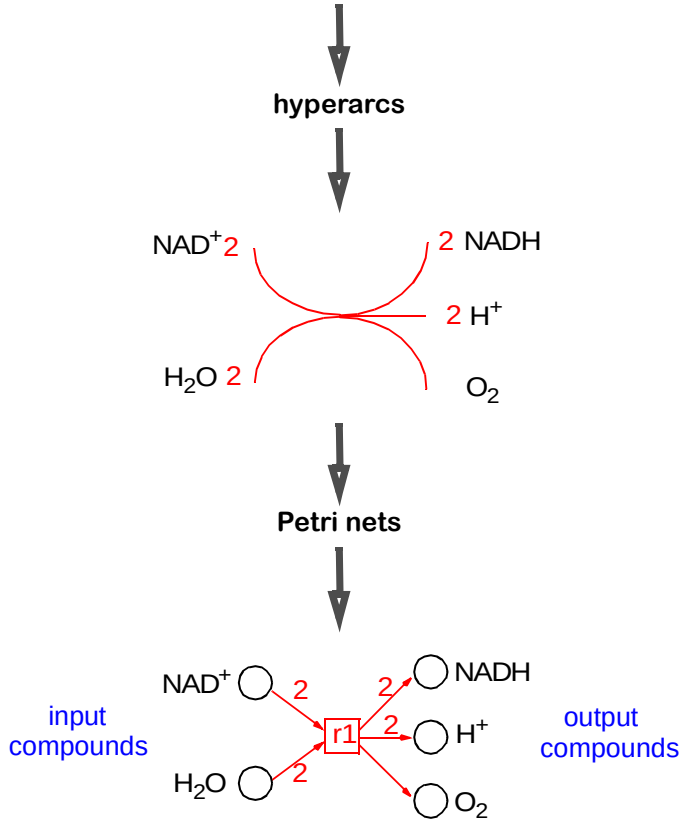
2. INTRODUCTION INTO QUALITATIVE MODELLING

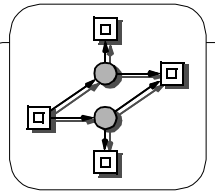
BIOCHEMICAL SYSTEMS, BASIC COMPONENT

- chemical reactions -> atomic actions

EX. LIGHT-INDUCED PHOSPHORYLATION

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

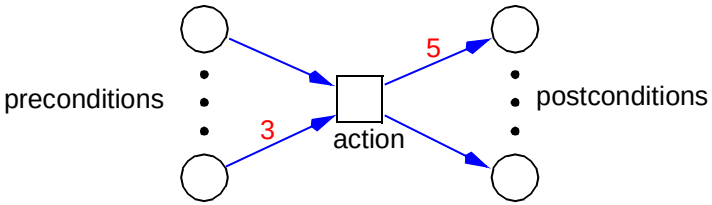




PETRI NETS, STRUCTURE

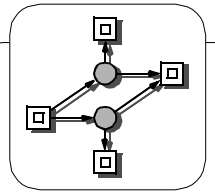
- two types of nodes
  - -> *places*
    "passive elements", conditions, local states, chemical compounds
  - -> *transitions*
    "active elements", events, actions, chemical reactions

- arcs



  - -> *directed*
  - -> *never arcs between nodes of the same type*
  - -> *for any node, arbitrary number of pre-nodes and post-nodes*

- arc inscriptions
  - -> *arc weight / multiplicity*
  - -> *amount of units of the substances involved in the basic (re-) action*

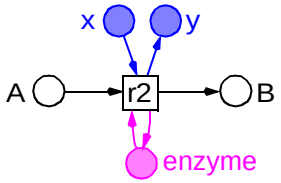


BIO PETRI NETS, PLACES

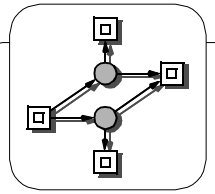
- involved substances / chem. compounds / complexes
- primary compounds
  - -> *metabolites*
  - -> *complexes*
  - -> *. . .*
- input / output compounds
  - -> *special primary compounds*
  - -> *boundary places*

- auxiliary compounds
  - -> *side conditions for reactions*
  - -> *ubiquitous -> fusion nodes*

e. g. electron carrier, phosphate carrier;



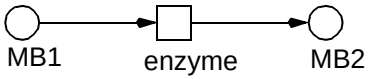
- catalysing compounds
  - -> *enzymes, if any*



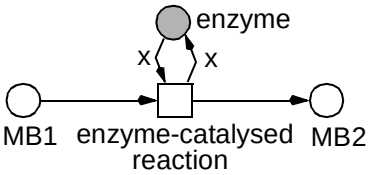
BIO PETRI NETS, TRANSITIONS

- spontaneous reactions
- enzyme-catalysed reactions
  -> *two ways of modelling*

  without enzyme concentration

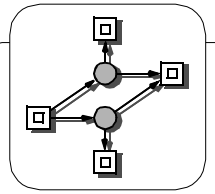


  with enzyme concentration x



  x - amount of enzyme units required by the reaction

- transport steps, if any
  -> *inhomogeneous substance distribution*



PETRI NETS, SYSTEM STATE

- tokens
  -> *moving objects,*
  e. g. units of substances (e. g. Mole), ...

  condition is not fulfilled

  condition is (one times) fulfilled

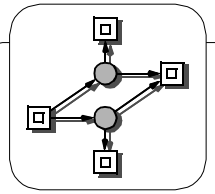
  condition is n times fulfilled

  -> *token amount -*
  amount of available units of a given compound

- marking
  -> *How many tokens are on each place?*

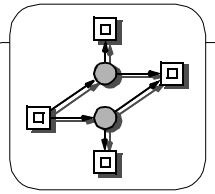
  -> *system state*
  -> *substance distribution*

  -> *initial marking*
  -> *initial substance distribution*

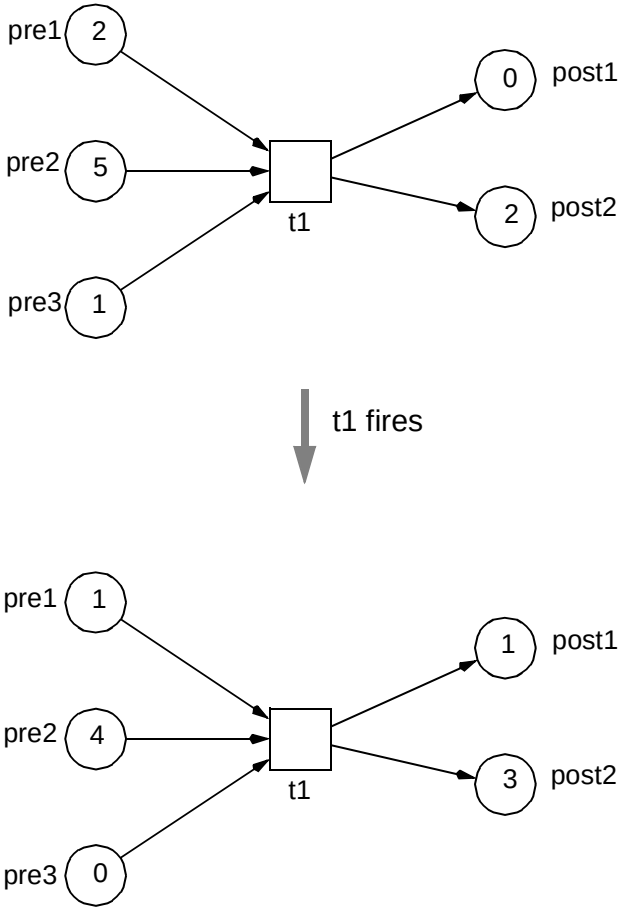


PETRI NETS, BEHAVIOUR

- flow of tokens
  - -> *defined by firing rule*
- an action **may** happen (fire), if
  - -> *all preconditions are fulfilled (corresponding to the arc weights);*
- **if** an action happens (fires), **then**
  - -> *tokens are removed from all preconditions (corresponding to the arc weights), **and***
  - -> *tokens are added to all postconditions (corresponding to the arc weights);*
- an action happens (firing of a transition)
  - -> *atomic*
  - -> *time-less*

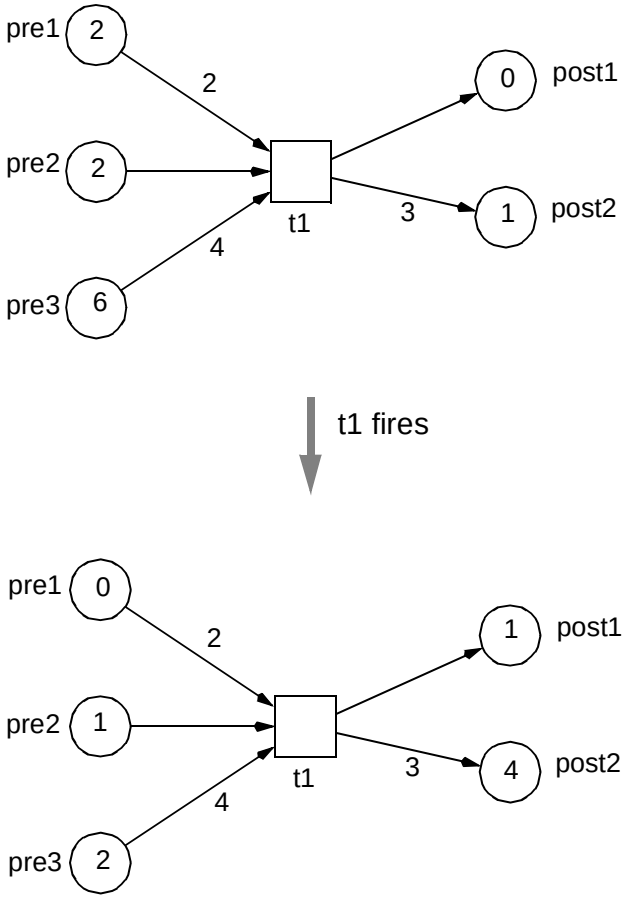


FIRING RULE, EXAMPLE 1



fire1.spped

FIRING RULE, EXAMPLE 2

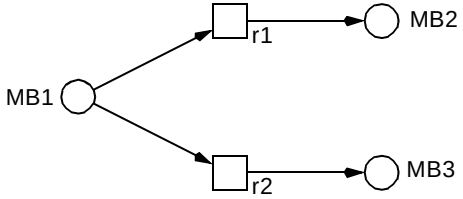


fire2.spped

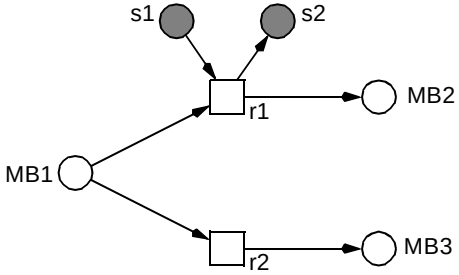
TYPICAL BASIC STRUCTURES 1

- **CHAIN OF REACTIONS**

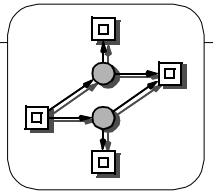
- **(FREE-CHOICE) BRANCHING / CONFLICT**



- **BRANCHING WITH SIDE CONDITION**

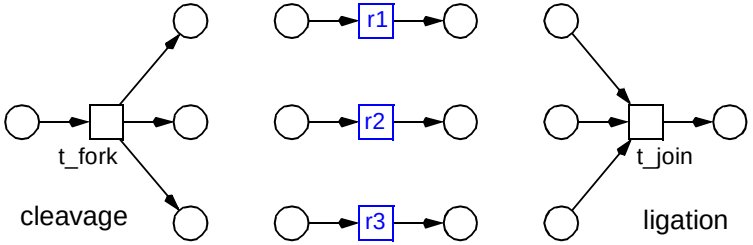


basicStructures1.spped



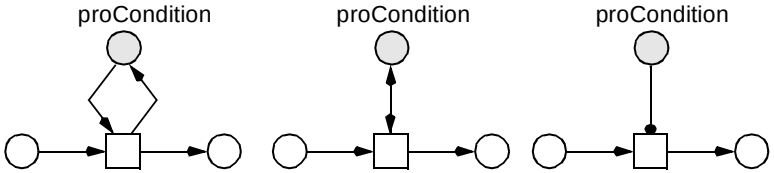
TYPICAL BASIC STRUCTURES 2

❑ CONCURRENCY



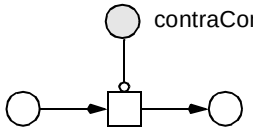
r1, r2, r3 are concurrent = independent

❑ READ ARCS / TEST ARCS

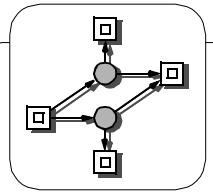


❑ INHIBITOR ARCS

BUT: CAUTION !

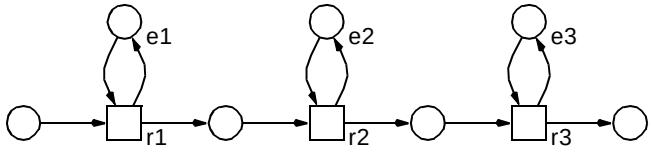


basicStructures2.spped

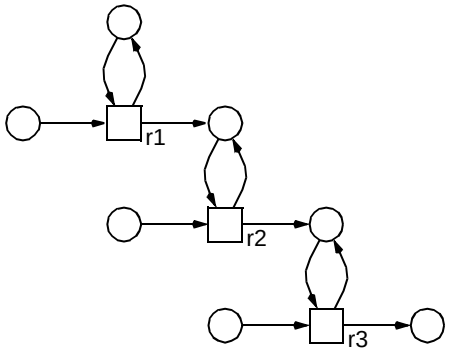


TYPICAL BASIC STRUCTURES 3

❑ METABOLIC PATHWAY

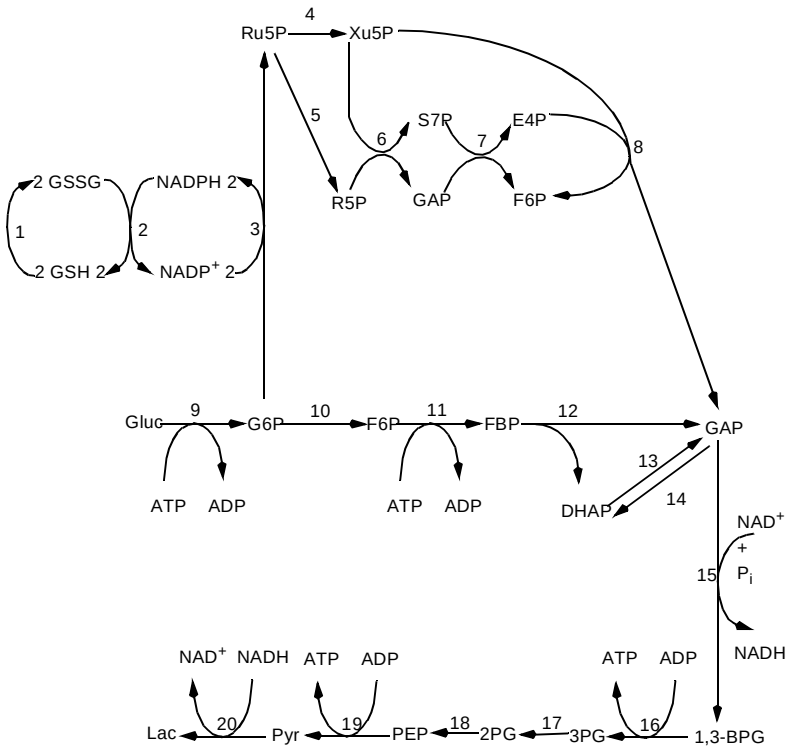


❑ SIGNAL TRANSDUCTION CASCADE



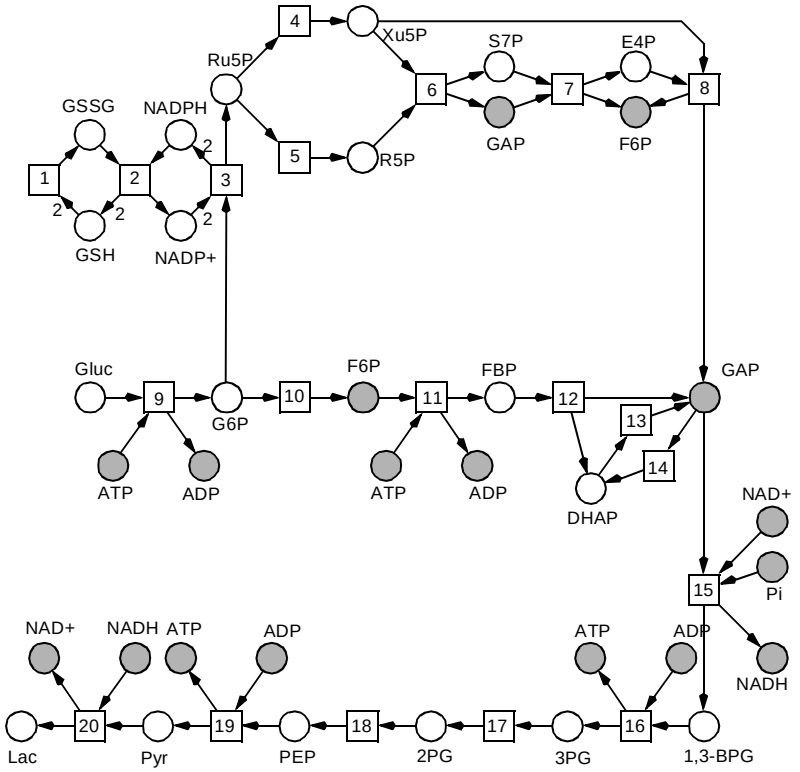
basicStructures3.spped

EXAMPLE 1 [REDDY 1996]

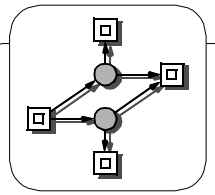


GLYCOLYSIS / PENTOSE PHOSPHATE PATHWAYS IN ERYTHROCYTES

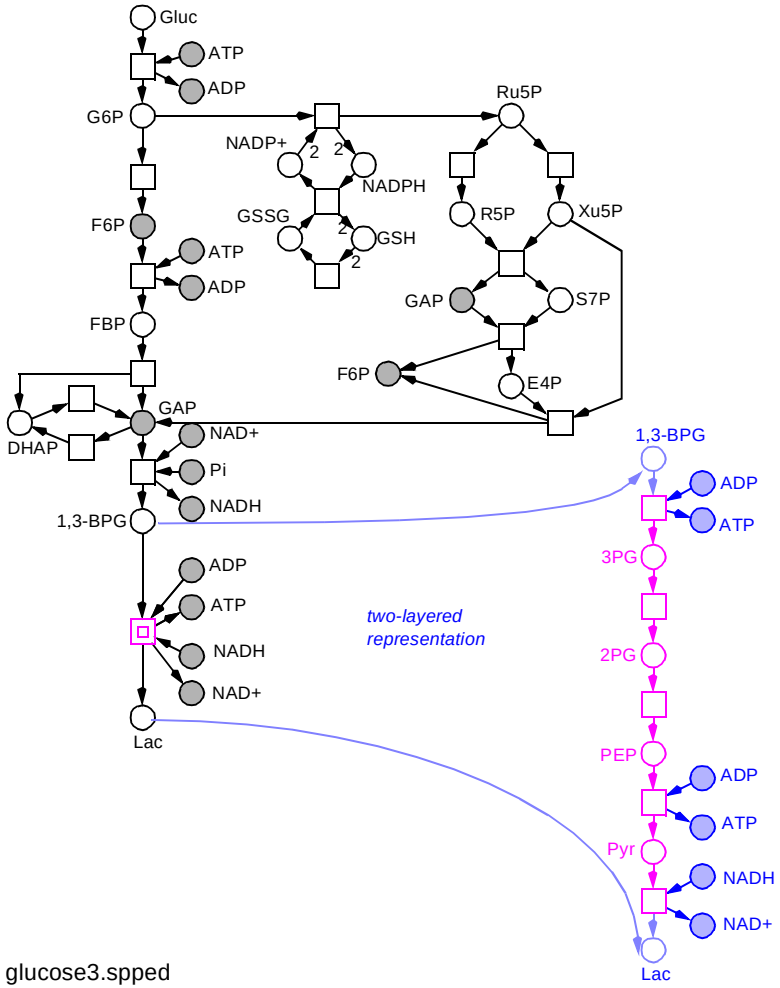
EXAMPLE 1 AS PETRI NET, VERSION 1



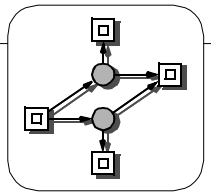
glucose1.spped



EXAMPLE 1 AS PETRI NET, VERSION 3



glucose3.spped

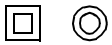


EXTENSIONS, SUMMARY

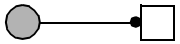
SYNTACTIC SUGAR

- ❑ logical / fusion nodes
  -> *connectors*

- ❑ hierarchical nodes
  -> *different levels of abstraction*

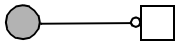


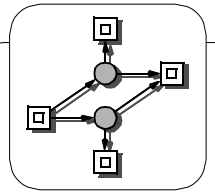
- ❑ read arcs
  -> *pro-conditions*



MODELLING POWER

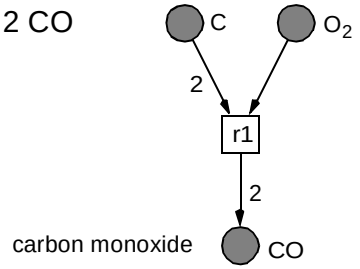
- ❑ inhibitor arcs
  -> *contra-conditions*





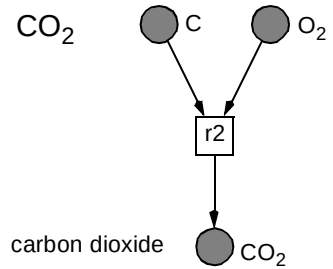
EXAMPLE 2, CARBON OXIDATION, BASIC REACTIONS

r1) $2\ C + O_2$ --> $2\ CO$

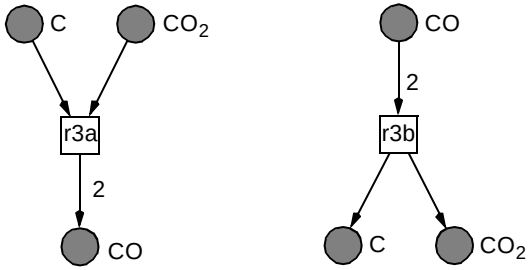


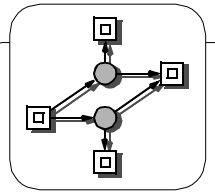
O2 oxygen

r2) $C + O_2$ --> CO_2



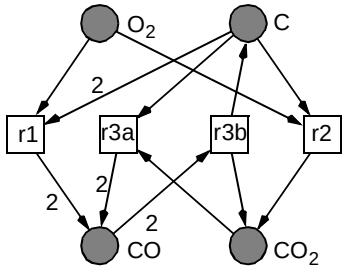
r3) $C + CO_2$ <--> $2\ CO$





EXAMPLE 2, COMPOSITION

BASIC MODEL

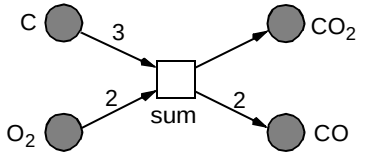


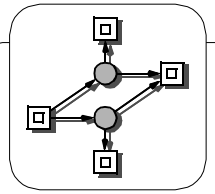
SYSTEM'S TOTAL EQUATION

1) $2\ C + O_2 \rightarrow 2\ CO$
2) $C + O_2 \rightarrow CO_2$
3) $C + CO_2 \leftrightarrow 2\ CO$

$3\ C + 2\ O_2 \rightarrow 2\ CO + CO_2$

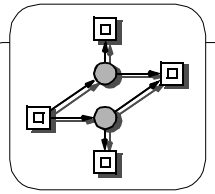
MODEL OF THE SYSTEM'S TOTAL EQUATION





NETWORKS NEED ENVIRONMENT BEHAVIOUR

- to animate the model
  - -> *infinite substance flow*
  - -> *deeper insights*
- to validate the model
  - -> *consistency criteria*
- steady flow
  - -> *input compounds*
  - -> *output compounds*
- auxiliary *compounds*
  - -> *as much as necessary*
- minimal assumptions



ENVIRONMENT BEHAVIOUR, THREE STYLES

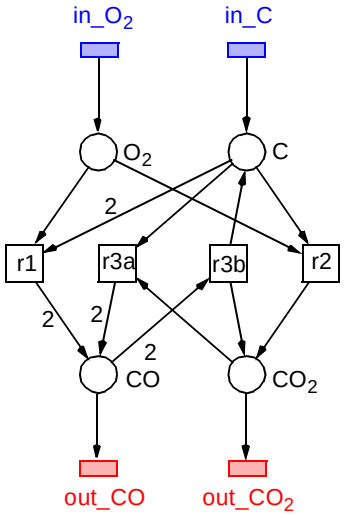
- style 1 -> validation criterion 1
  - -> *weak assumptions*
  - -> *infinite flow into/out the network*
- style 2 -> validation criterion 2
  - -> *firm assumptions*
  - -> *infinite many primary compounds*
  - -> *finite, but sufficient reservoir of auxiliary compounds*
- style 3 -> validation criterion 3
  - -> *strong assumptions*
  - -> *finite, but sufficient reservoir of auxiliary compounds*
  - -> *quantitative relations of input / output compounds*
  - -> *finite reservoir of primary compounds*

INCREASING STRENGTH

ENVIRONMENT BEHAVIOUR, STYLE 1

- no assumptions about quantitative relations of input / output compounds
- input *compounds*
  -> *generating pre-transitions*
- output *compounds*
  -> *consuming post-transitions*
- auxiliary *compounds*
  -> *generating pre-transitions*
  -> *consuming post-transitions*
  -> *infinite reservoir*
- no boundary places, but boundary transitions

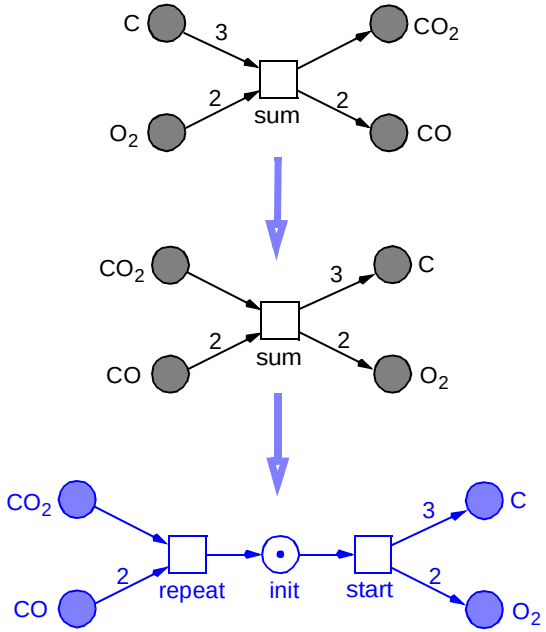
CARBON OXIDATION, SYSTEM MODEL, STYLE 1



carbon1.spped

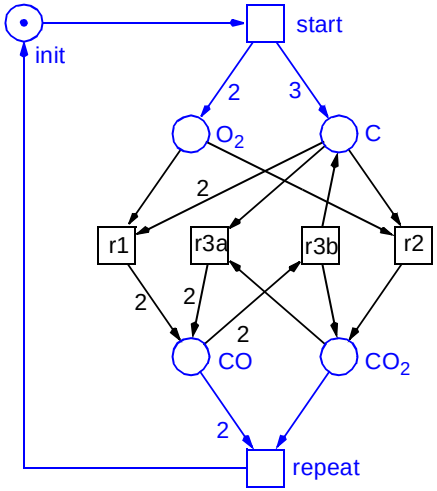
ENVIRONMENT BEHAVIOUR, STYLE 3

- strong assumptions about quantitative relations of input / output compounds
- 'inverse' total equation



- there are no boundary nodes

CARBON OXIDATION, SYSTEM MODEL, STYLE 3



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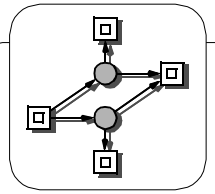
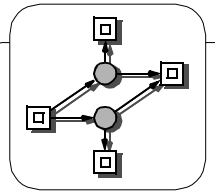


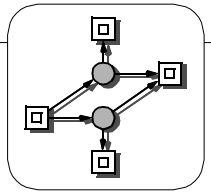
ENVIRONMENT BEHAVIOUR, STYLE 2

- mixture of style 1 and style 3
- no assumptions about quantitative relations of input / output compounds
- input *compounds*
  -> *generating pre-transitions*
- output *compounds*
  -> *consuming post-transitions*
- auxiliary *compounds*
  -> *finite, but sufficient reservoir*
  -> *no boundary pre- / post-transitions*
- boundary transitions only for input / output compounds

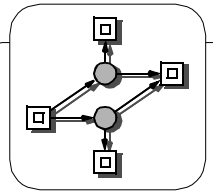


BIOCHEMICAL PETRI NETS, SUMMARY

- biochemical networks
  -> *networks of (abstract) chemical reactions*
- biochemically interpreted Petri net
  -> *partial order sequences of chemical reactions*
  - transforming input into output compounds
  - respecting the given stoichiometric relations
  -> *set of all pathways*
  from the input to the output compounds
  respecting the stoichiometric relations
- pathway
  -> *self-contained partial order sequence of elementary (re-) actions*
- basic assumption
  -> *steady state behaviour*



3. INTRODUCTION INTO QUALITATIVE ANALYSIS



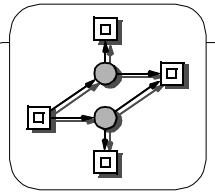
QUALITATIVE PROPERTIES

behavioural properties

- general semantic properties
  - *boundedness*
  - *liveness*
  - *reversibility*
- special semantic properties
  - *safety properties*
  - *progress properties*

structural properties

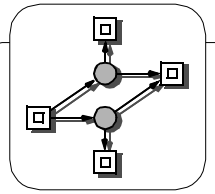
- especially valuable:
  local(ly decidable) structural properties
- certain combinations of structural properties
  allow conclusions on
  behavioural properties



PETRI NET PROPERTIES, OVERVIEW / INA

1. SIMPLE STRUCTURAL PROPERTIES

ORD	ordinary *(1-multiplicity of all arcs)*
HOM	homogeneous *(all output arcs of a given place have the same multiplicity)*
NBM	non-blocking multiplicity *(for each place applies: MIN multiplicity of input arcs >= MAX multiplicity of output arcs)*
PUR	pure *(no side conditions)*
CSV	conservative *(any firing preserves token amount)*
SCF	static conflict free
CON	connected
SC	strongly connected
Ft0	there is a transition without pre-place
tF0	there is a transition without post-place
Fp0	there is a place without pre-transition
pF0	there is a place without post-transition
MG	marked graph *(synchronization graph)*
SM	state machine
FC	free choice net
EFC	extended free choice net
ES	extended simple net



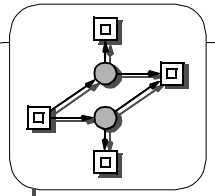
PETRI NET PROPERTIES, OVERVIEW / INA

2. MORE EXPENSIVE STRUCTURAL PROPERTIES

DTP	deadlock trap property
SMC	state machine coverable *(covered with SM components)*
SMD	state machine decomposable *(covered with SCSM components)*
SMA	state machine allocatable
CPI	covered with place invariants
CTI	covered with transition invariants
SB	structurally bounded

3. BEHAVIOURAL PROPERTIES

B	bounded
REV	reversible *(the initial state m_0 can be reached again from all reachable states: home state)*
DSt	dead states *(a state where no transition is enabled)*
BSt	bad states *(a state where a fact is enabled)*
DTr	dead transitions *(at the initial state)*
DCF	dynamically conflict free
L	live
LV	live, excepted transitions dead at the initial marking
L&S	live & safe *(1-bounded)*

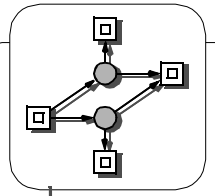


BEHAVIOURAL NET PROPERTIES, OVERVIEW

- MARKABILITY of places
  - markable *(place liveness)*
  - k-bounded *(1-bounded / safe)*
  - unbounded
- LIVENESS of transitions
  - zero times firing *(m_0-dead)*
  - finite times firing *(dead, non-live)*
  - infinite times firing, probably *(live)*
  - infinite times firing, definitely *(livelock free)*
- REACHABILITY of states
  - dead states
  - reproducibility
  - reversibility *(m_0 - home state)*
  - bad states *(facts)*
  - user-specified states
- NET INVARIANTS
  - transition invariants
  - place invariants
- temporal relationship of logic formulae
  - safety properties
  - progress properties

general semantic properties

special semantic properties



QUALITATIVE ANALYSIS METHODS, OVERVIEW

- NET REDUCTION
- STRUCTURAL PROPERTIES
- LINEAR PROGRAMMING
  - place / transition invariants
  - state equation
  - trap equation
- STATE SPACE ANALYSIS
  - (complete) reachability graph
  - compressed state spaces
    - BDDs, NDDs, ..., xDDs
    - Kronecker products
  - reduced state spaces
    - coverability graph
    - symmetry
    - stubborn sets
  - branching process

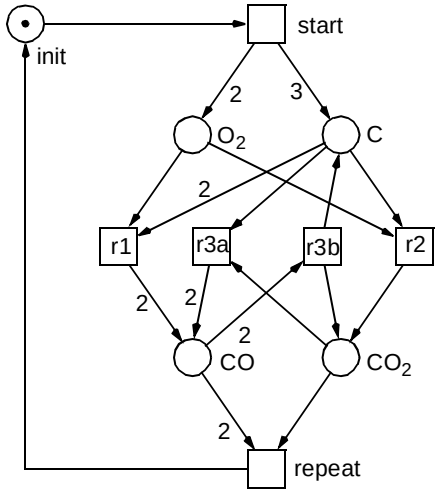
static analysis

dynamic analysis

(model checking)

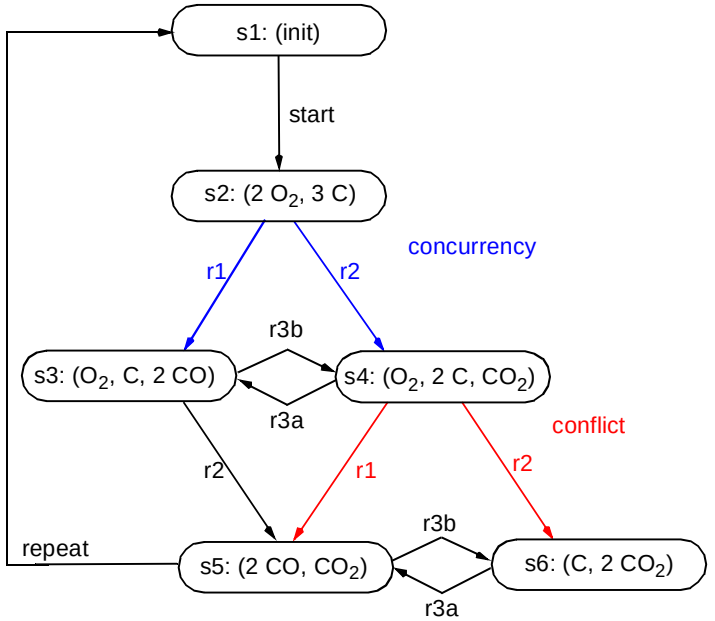
REACHABILITY GRAPH (RG)

- nodes - system states
- arcs - the (single) firing transition
- example - carbon oxidation, environment style 3

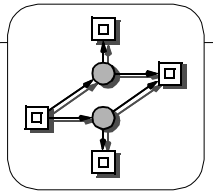


carbon2.ssped

RG (CARBON OXIDATION)

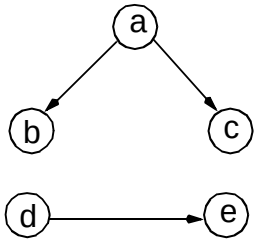


-> *interleaving description of the whole system behaviour*

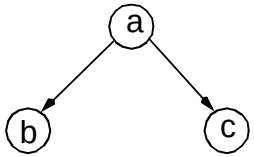


STRONGLY CONNECTED GRAPH

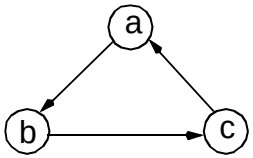
- basic graph properties
  -> applies also for general (monochromatic) graphs
- needs directed graphs
  undirected graphs:
  connected =
  strongly connected
- for each pair of nodes x, y holds:
  there exists a path
  from x to y
  -> path(x, y);
- path(x, y):
  sequence of arcs
  starting at x and
  ending at y;
- general importance
  ex:
  system of one-way streets;
  question:
  is every place (intersection) from any place reachable?



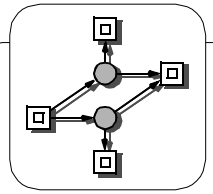
not connected



connected

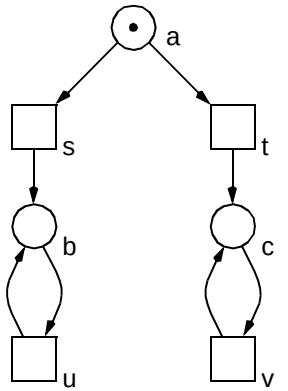


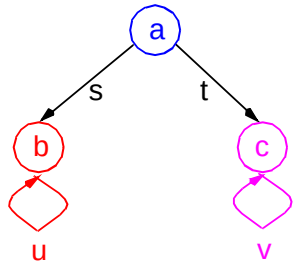
strongly connected

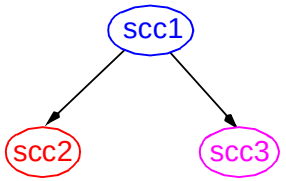


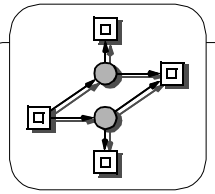
EXAMPLE: RG AND THREE BASIC PN PROPERTIES

- no concurrency
  -> rg(pn) == pn
- rg - finite
  -> bounded pn
- rg - not sc
  -> pn not reversible
- no dead states,
  but liveness?
- condensed rg
  node - sc component (scc)
  scc:
  maximal set of sc nodes;
  a terminal scc
  -> *possible terminal system behaviour*
  -> *must contain all transitions in a live pn*
- not all terminal scc
  contain all transitions
  -> the pn is not live



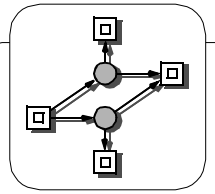




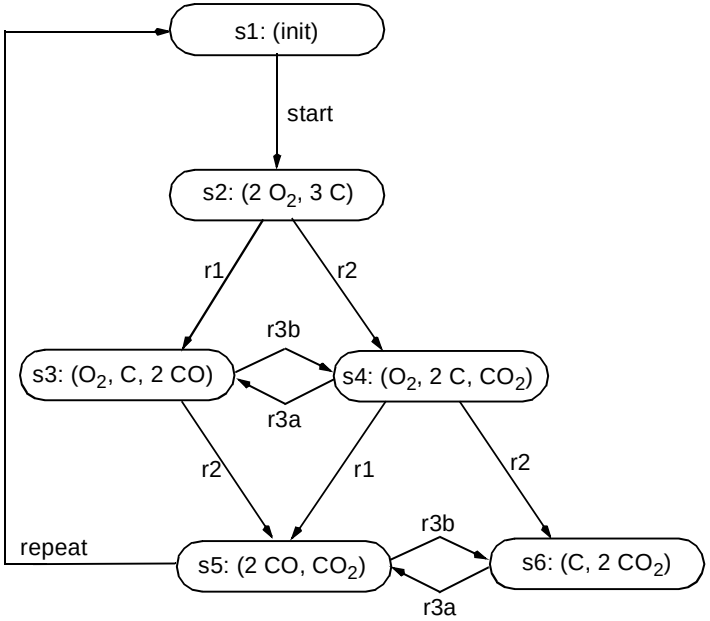


BASIC PROPERTIES & RG, SUMMARY

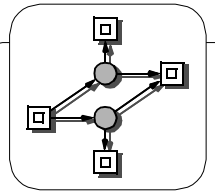
- How many tokens may reside at most in a given place . . .
  -> (0, 1, k, oo) ?
    -> boundedness
    -> rg is finite
- How often may a transition fire . . .
  -> (0-times, n-times, oo-times) ?
    -> liveness
    -> every terminal scc contains all transitions
- Is the initial system state . . .
  -> always reachable again ?
    -> reversibility
    -> rg is sc (consists of one scc)



RG(CARBON OXIDATION), EVALUATION



- RG is finite
  -> *BND*
- 1 Strongly Connected Component (SCC)
  -> *REV*
- the only SCC contains all transitions
  -> *LIVE*



REACHABILITY GRAPH, CONSTRUCTION ALGORITHM

PROCEDURE rg (**IN** Net *pn*, **IN** Marking *m0*,
OUT MSet *nodes*, **OUT** ArcSet *arcs*);

MSet $U = \{m0\}$, // unprocessed markings
$N = \emptyset$; // rg nodes
ArcSet $E = \emptyset$; // rg arcs (pre, post, t)
Marking m'; // successor marking
Transition t;

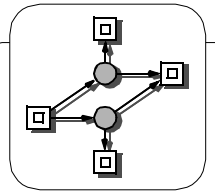
WHILE $U \neq \emptyset$ **DO**
choose one $m \in U$;
$U = U - \{m\}$; $N = N \cup \{m\}$;

FOR ALL t enabled at m **DO**
$m' = m + \Delta t$;
IF $m' \notin N \cup U$ // new marking
THEN $U = U \cup \{m'\}$
ENDIF;
$E = E \cup \{(m, m', t)\}$
ENDFOR

ENDWHILE;

nodes = *N*; *arcs* = *E*;

ENDPROC rg.



REACHABILITY GRAPH, OBSERVATIONS

- **unbounded** Petri net
  -> *the RG is **infinite***
  bounded Petri net
  -> *the RG is **finite***

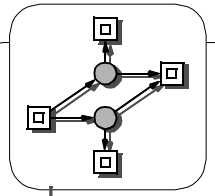
- simple construction algorithm
  -> *single step firing rule*

- concurrency
  -> *enumeration of all interleaving sequences*

- branching arcs in the RG
  -> *conflict* ***OR***
  -> *concurrency*

- RG tend to be very large
  -> *automatic evaluation necessary*

- worst case: over-exponential growth
  -> *alternative analyses techniques ?*



QUALITATIVE ANALYSIS METHODS, OVERVIEW

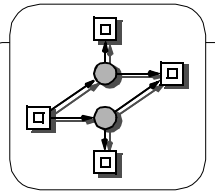
- NET REDUCTION
- STRUCTURAL PROPERTIES
- LINEAR PROGRAMMING
  - place / transition invariants
  - state equation
  - trap equation

} static analysis

- STATE SPACE ANALYSIS
  - (complete) reachability graph
  - compressed state spaces
    - BDDs, NDDs, ..., xDDs
    - Kronecker products
  - reduced state spaces
    - coverability graph
    - symmetry
    - stubborn sets
  - branching process

} dynamic analysis

(model checking)



INCIDENCE MATRIX C - A REPRESENTATION OF THE NET STRUCTURE

P+T \ P+T	p1 card(P)	t1 card(T)
p1 card(P)	ϕ	- PRE
t1 card(T)	+ POST	ϕ

$\downarrow$ $POST^T$ - PRE

$C =$

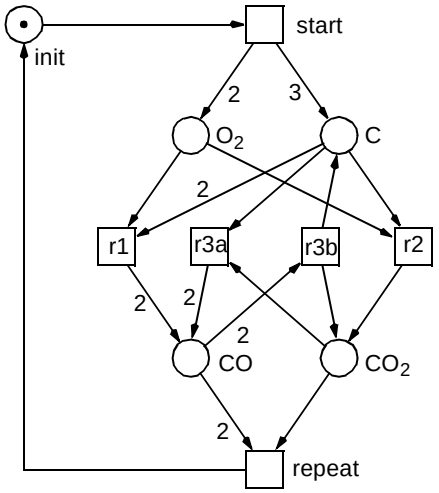
P \ T	t1 . . .	tj	. . . tm
p1 : :			
pi		cij	
: pn			

cij = (pi, tj) = F(tj,pi) - F(pi, tj) = Δ tj(pi)

-> token change in place pi by firing of transition tj

-> stoichiometric matrix

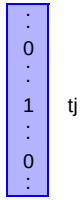
CARBON/BND, INCIDENCE MATRIX



P \ T	r1	r2	r3a	r3b	start	repeat
O_2	-1	-1	0	0	+2	0
C	-2	-1	-1	+1	+3	0
CO	+2	0	2	-2	0	-2
CO_2	0	+1	-1	+1	0	-1
init	0	0	0	0	-1	+1

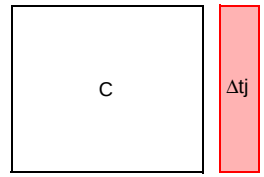
STATE EQUATION

PARIKH VECTOR
parikh(tj)



incidence matrix C

P \ T	t1	**tj**	tm
p1			
pi		**Δtj**	
pn			

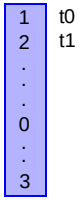


Δtj - vector describing the change of the whole marking by firing of tj

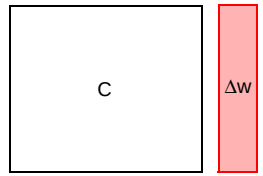
Let the word $w \in T^*$ be a sequence of firing transitions.

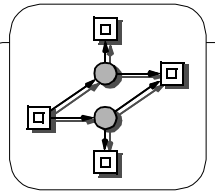
parikh(w) - transition vector, whereby the position j gives the amount of tj in w

PARIKH VECTOR
parikh(w)



The change on the marking Δw by firing a transition sequence w can be computed by multiplying the incidence matrix C with the Parikh vector parikh(w) of that transition sequence.

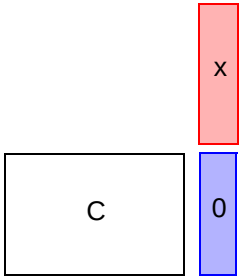




T-INVARIANTS

- Lautenbach, 1973

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



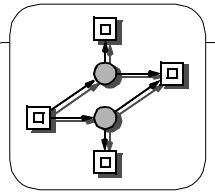
- **minimal** T-invariants
  - -> *there is no T-invariant with a smaller support*
  - -> *greatest common divisor (gcd) of all entries is 1*

- support
  - -> *set of transitions belonging to the T-invariant*

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

$$kx = \sum_i a_i x_i$$

- Covered by T-Invariants (CTI)
  - -> *each transition belongs to a T-invariant*
  - -> *if a bounded net is live, then it is CTI*



T-INVARIANTS, INTERPRETATION

- T-invariants = (multi-) sets of transitions
  - -> *zero effect on marking*
  - -> *reproducing a marking / system state*
  - -> *steady state substance flows*
  - -> *elementary modes, Schuster 1993*

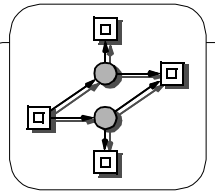
- the T-invariant corresponds to cycles in the RG, if the T-invariant is realizable

- in the RG, concurrency of transitions is described by all transitions' interleaving sequences

- if there are concurrent transitions in a realizable T-invariant, then there is a RG cycle for each interleaving sequence
  -> *T-inv3*, *T-inv4*

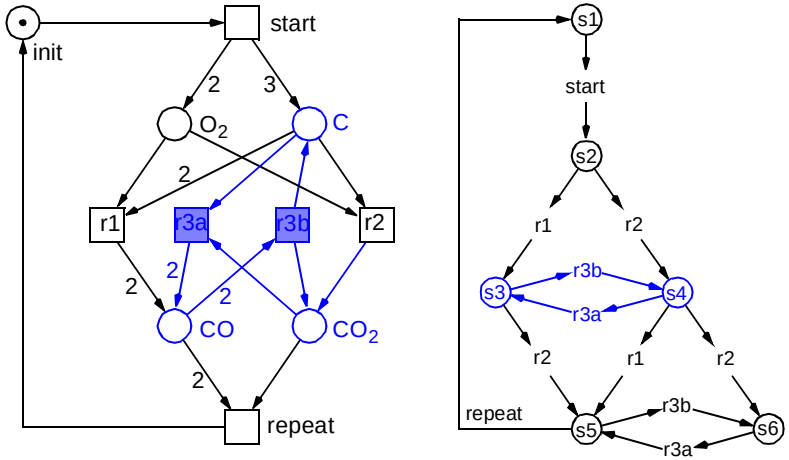
- pre-sets of supports = post-sets of supports

- a T-invariant defines a subnet
  - -> *the T-invariant's transitions (the support),*
    + all their pre- and post-places
    + the arcs in between

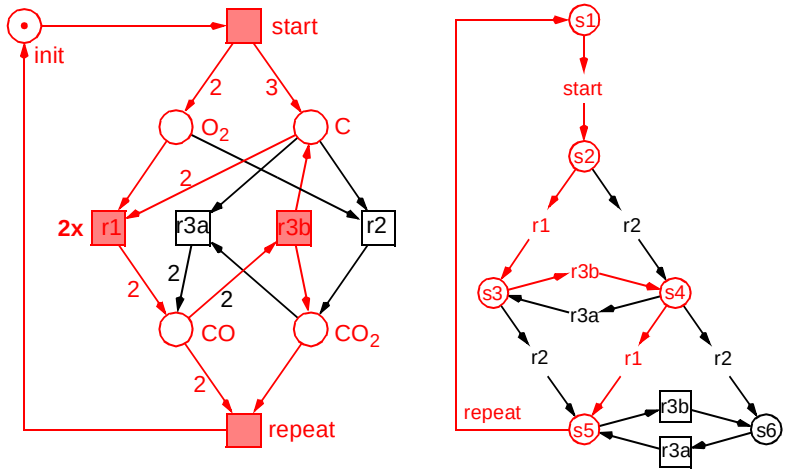


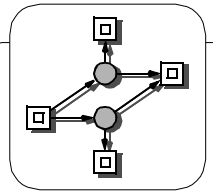
CARBON/BND, T-INVARIANTS 1, 2

T-inv1 = (r3a, r3b) -> inner cycle



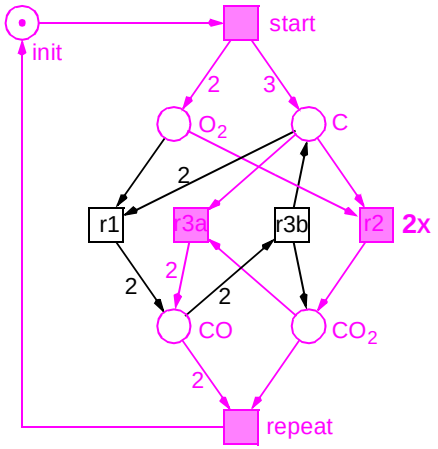
T-inv2 = (start, 2 r1, r3b, repeat) -> input/output cycle

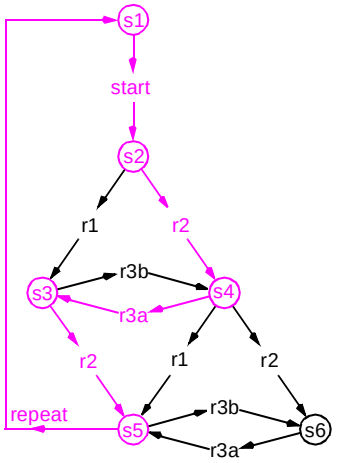


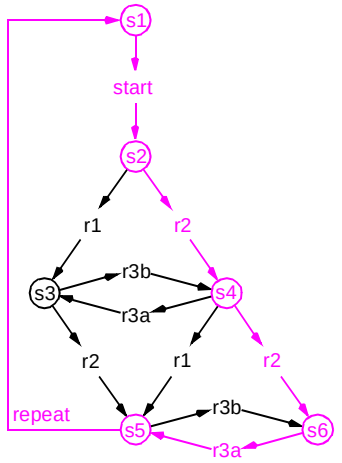


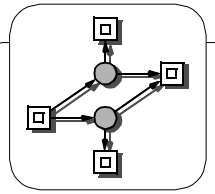
CARBON/BND, T-INVARIANTS 3

T-inv3 = (start, 2 r2, r3a, repeat) → start — r2 < r2 / r3a > repeat



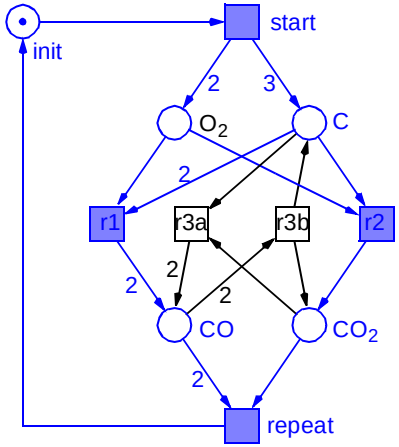


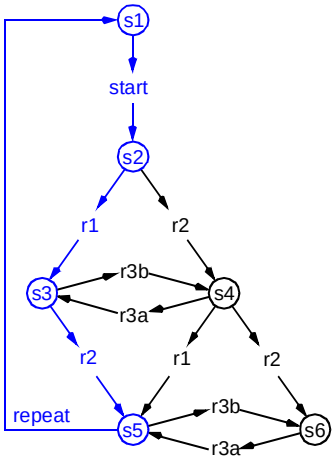


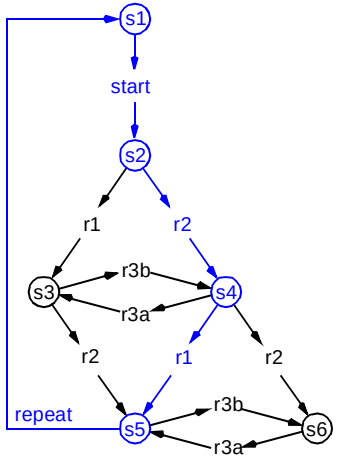


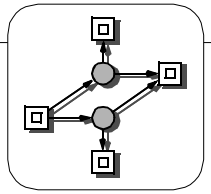
CARBON/BND, T-INVARIANTS 4

T-inv4 = (start, r1, r2, repeat) → start < r1 r2 > repeat









CARBON/UNBOUNDED, T-INVARIANTS, INTERPRETATION

- steady state = constant token distribution

- preservation of
  a given system state under continuous firing
  requires
  - -> *relative transition firing rates = T-invariant's entries*
  - -> *ex T-inv2: a given state is preserved, if in_C and out_CO fire twice as often as in_O_2 and r1;*

- the in- / out-components of the T-invariant
  - -> *sum equation of the T-invariants remaining transitions*

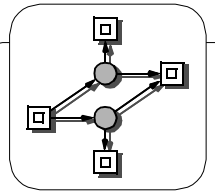
  T-inv1: --
  -> *inner cycle*

  T-inv2: O_2 + 2 C -> 2 CO
  -> *stoichiometric equation of r1*

  T-inv3: C + O_2 -> CO_2
  -> *stoichiometric equation of r2*

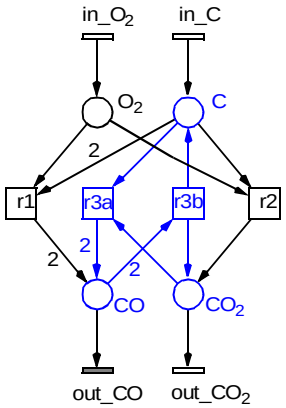
  T-inv4: 2 C + O_2 -> 2 CO
  -> *sum of the stoichiometric equations of r2, r3a*

  T-inv5: C + O2 -> CO2
  -> *sum of the stoichiometric equations of r1, r3b*

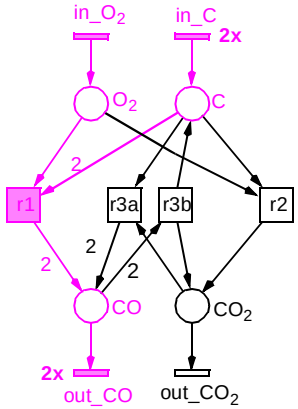


CARBON/UNBOUNDED, T-INVARIANTS 1 - 3

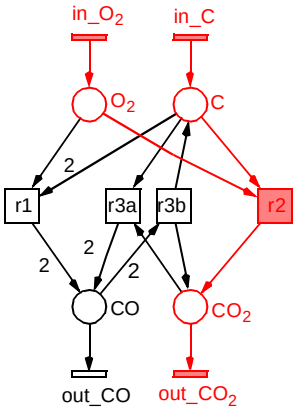
T-inv1 = (r3a, r3b)

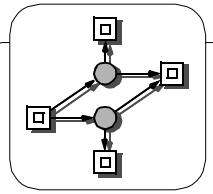


T-inv2 = (in_O_2 , 2 in_C, r1, 2 out_CO)



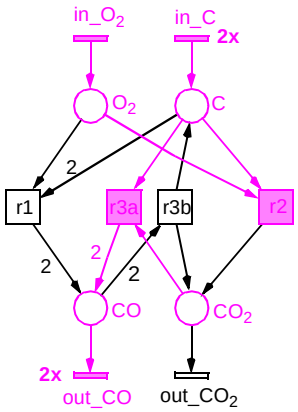
T-inv3 = (in_O_2, in_C, r2, out_CO_2)



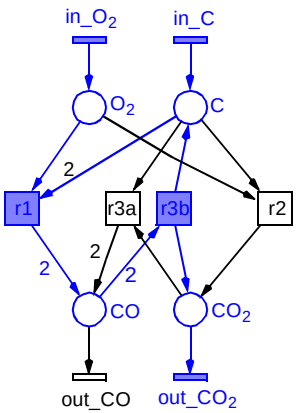


CARBON/UNBOUNDED, T-INVARIANTS 4, 5

T-inv4 = (in_O_2, 2 in_C, r2, r3a, 2 out_CO)



T-inv5 = (in_O_2, in_C, r1, r3b, out_CO_2)



T-INVARIANTS, SUMMARY

TWO INTERPRETATIONS

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

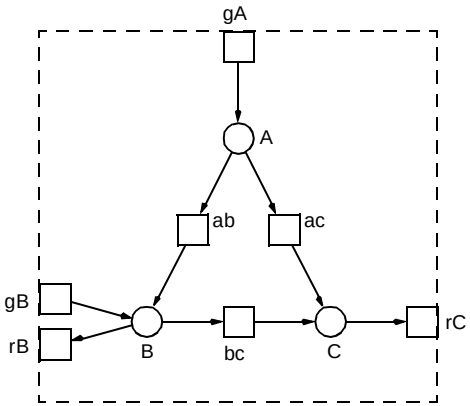
BASIC TYPES IN BIO NETWORKS

- trivial minimal T-invariants
  - -> *boundary transitions of auxiliary compounds*
  - -> *reversible reactions*
- non-trivial minimal T-invariants
  - -> *i/o-T-invariants*

    covering boundary transitions of input / output compounds
  - -> *inner cycles*

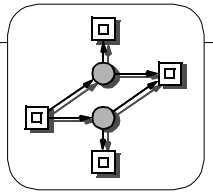
PATHWAY ANALYSIS

- substances involved
  - -> *input substance A*
  - -> *output substance C*
  - -> *auxiliary substance B*



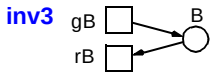
papin2003.spped

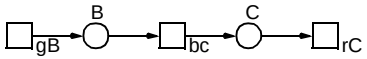
- steady state substance flows
  - -> *T-invariants*
- all flow behaviour under the steady state assumption
  - -> *non-negative linear combination of minimal T-invariants*



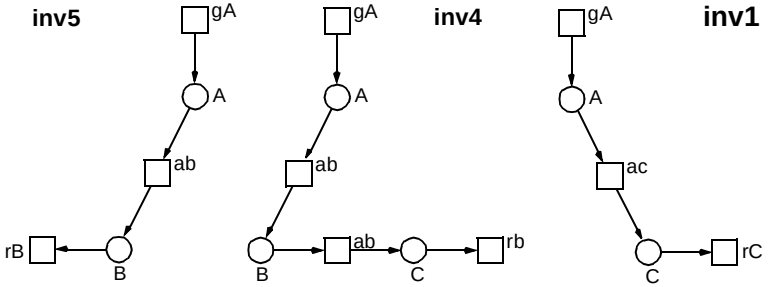
T-INVARIANTS AND EXTREME PATHWAYS

Schilling, 2000



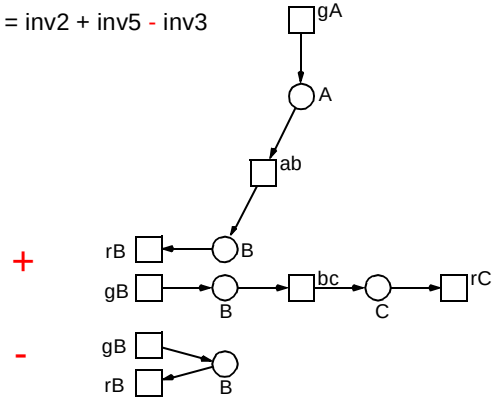


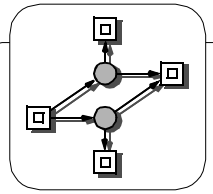
no elementary mode



no extreme pathway

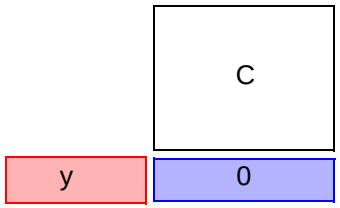
inv4 = inv2 + inv5 - inv3





P-INVARIANTS

- Lautenbach, 1973



- P-invariants
  - -> *integer solutions y of* $yC = 0, y \neq 0, y \geq 0$
  - -> *exponential complexity*
- **minimal** P-invariants
  - -> *there is no P-invariant with a smaller support*
  - -> *gcd of all entries is 1*
- support
  - -> *set of places belonging to the P-invariant*
- any P-invariant is a non-negative linear combination of minimal ones
  - -> *multiplication with a positive integer*
  - -> *addition*
  - -> *division by gcd*

$$ky = \sum_i a_i y_i$$

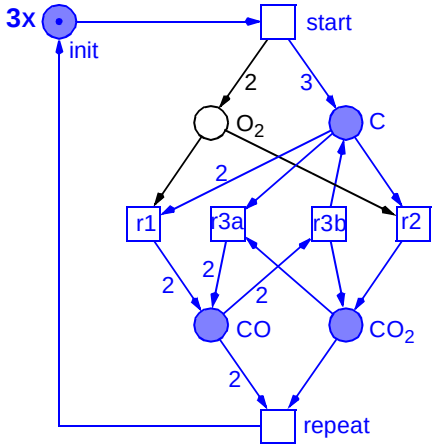
- Covered by P-Invariants (CPI)
  - -> *each place belongs to a P-invariant*
  - -> *sufficient condition for BND*

P-INVARIANTS, INTERPRETATION

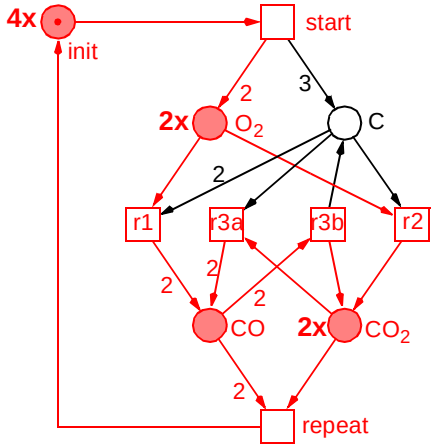
- set of places with
  - -> *a constant weighted sum of tokens*
    $ym = ym_0$
    for all from m_0 reachable markings m
  - -> *token / compound preservation*
- a place belonging to a P-invariant is bounded
  - -> *CPI - sufficient condition for BND*
- the firing of any transition has no influence on the weighted sum of tokens on the P-invariant's places
  - -> *for all transition t:*
    the effect of the arcs,
    removing tokens from a P-invariant's place
    is equal to the effect of the arcs,
    adding tokens to a P-invariant's place
- pre-sets of supports = post-sets of supports
- a P-invariant defines a subnet
  - -> *the P-invariant's places (the support),*
    + all their pre- and post-transitions
    + the arcs in between

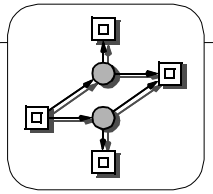
CARBON/BND, P-INVARIANTS

P-inv1 = (3 init, C, CO, CO_2) -> carbon preservation



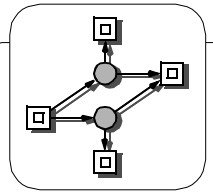
P-inv2 = (4 init, 2 O_2, CO, 2 CO_2) -> oxygen preservation





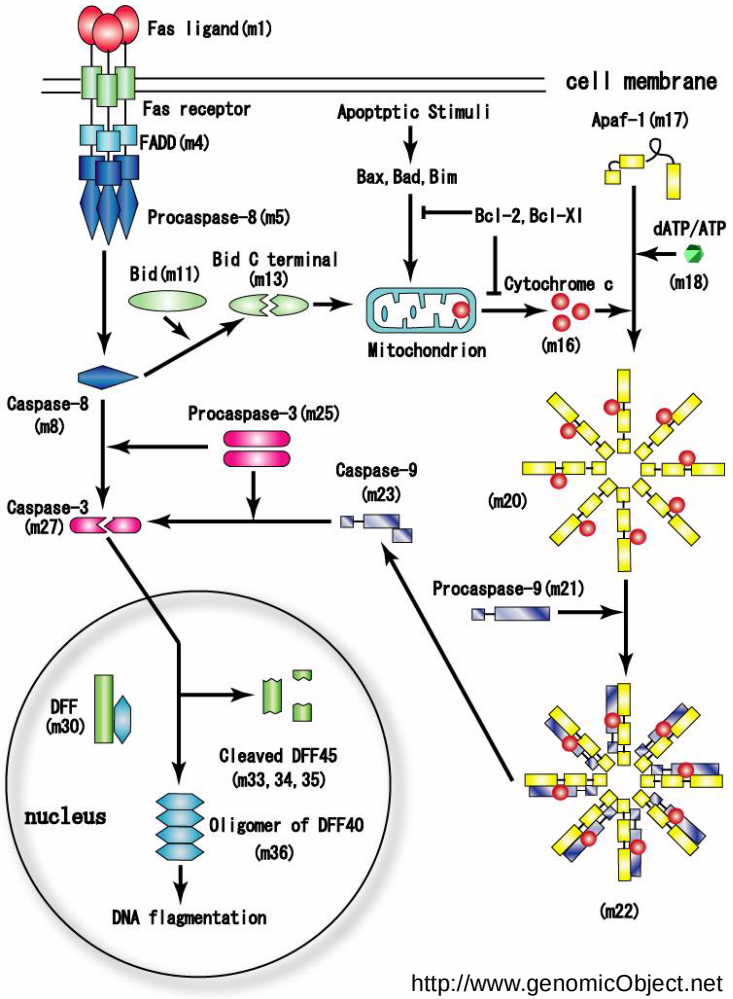
ANALYSIS, SUMMARY

- validation criterion 1
  - -> *CTI,*
    stronger - covered by i/o T-invariants
  - -> *no minimal T-invariant*
    without biological interpretation
  - -> *no known biological behaviour*
    without corresponding T-invariant
- validation criterion 2
  - -> *no minimal P-invariant*
    without biological interpretation (?)
- validation criterion 3
  - -> *CPI*
  - -> *all expected temporal-logic properties -> TRUE*
    (not discussed here)



4.
CASE STUDIES

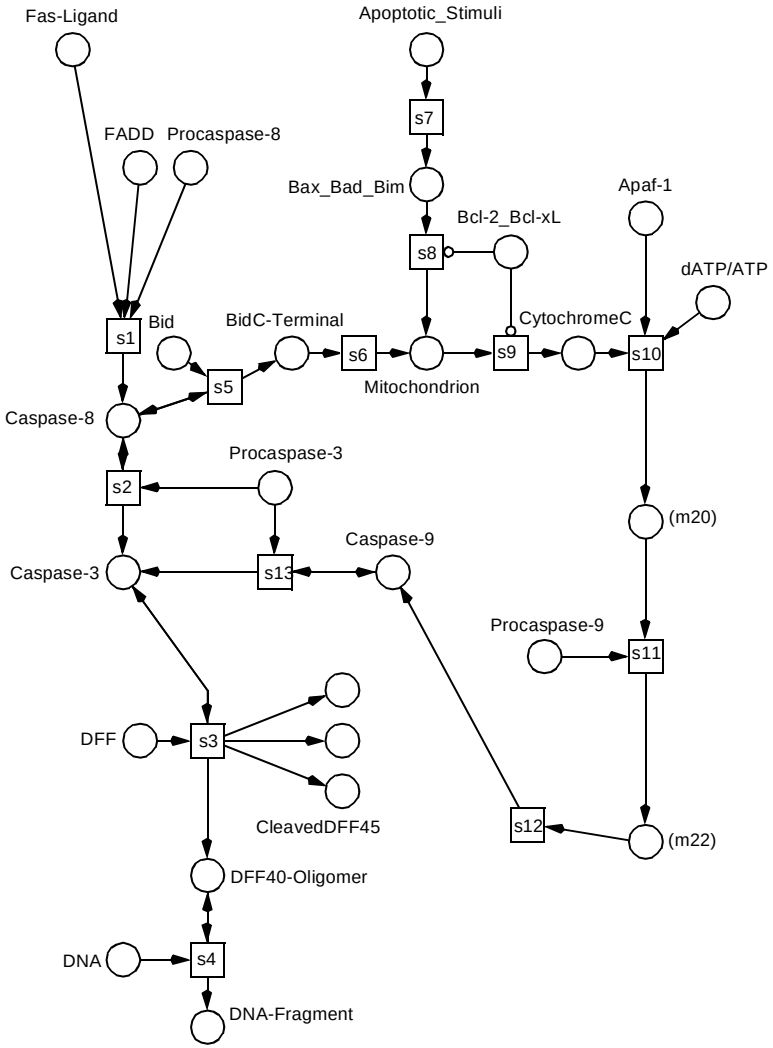
CASE STUDY 1 - APOPTOSIS, THREE BASIC PATHWAYS



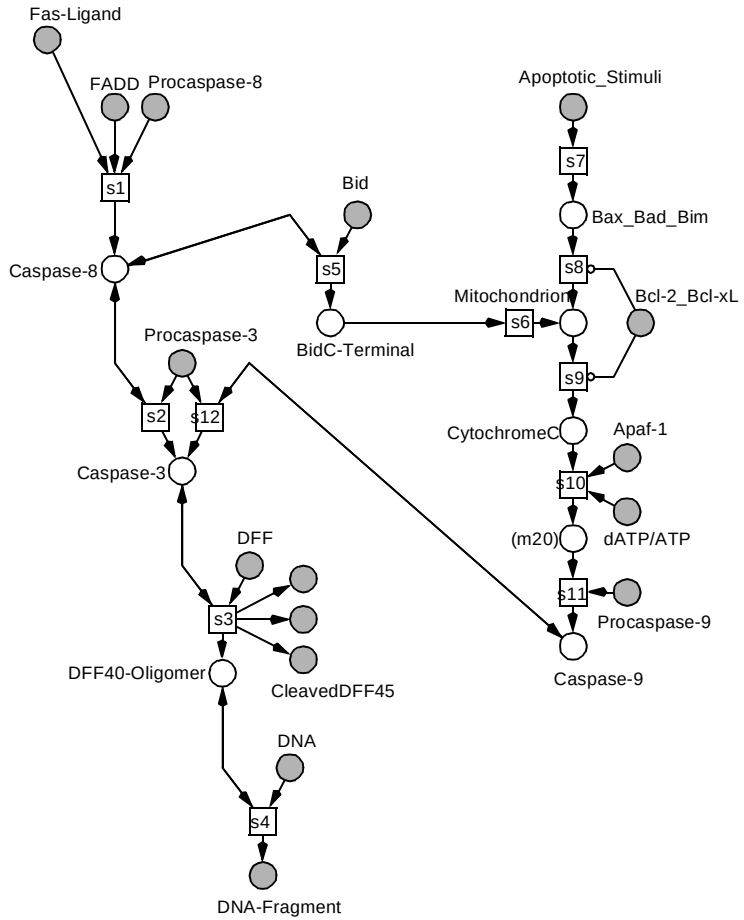
http://www.genomicObject.net

APOPTOSIS IN MAMMALIAN CELLS

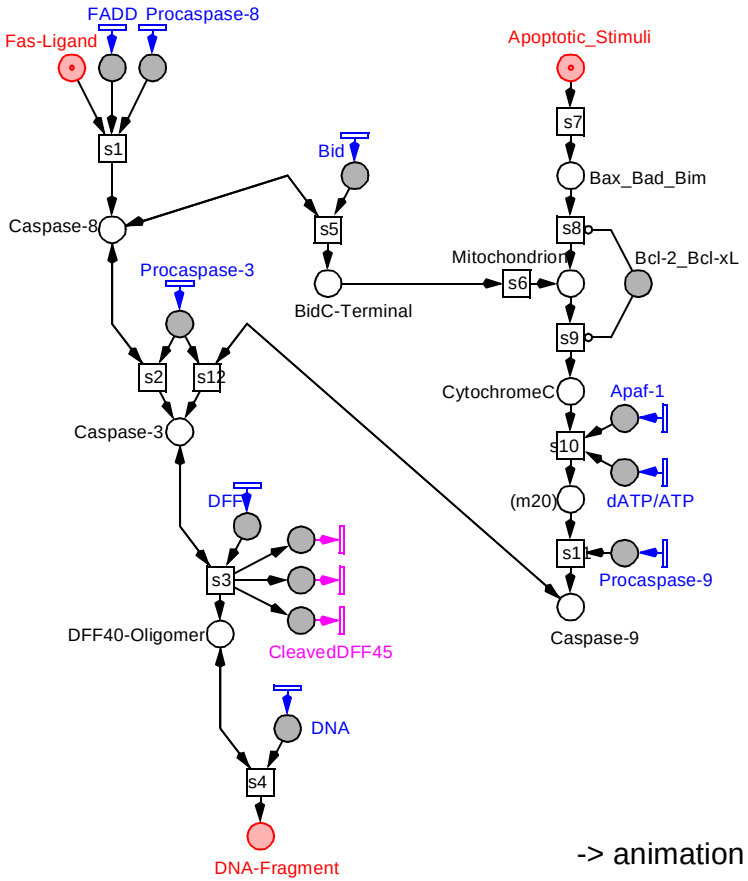
FAS1



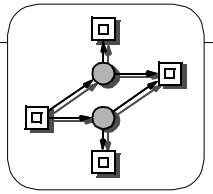
FAS2 = FAS1



FAS3

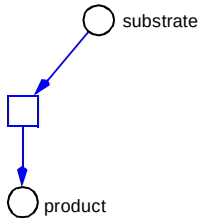


-> animation

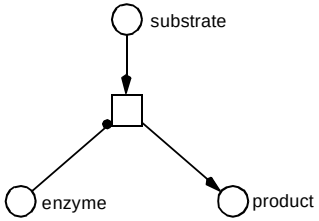


REFINEMENT: AUTOCATALYSIS

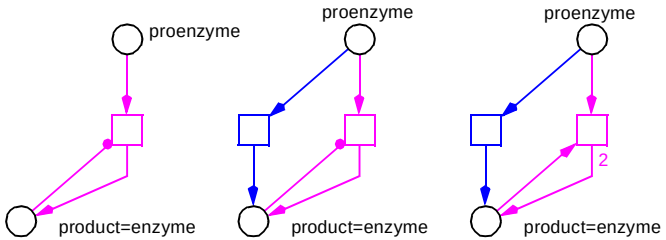
- REACTION

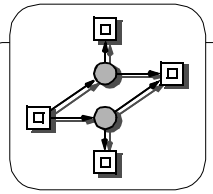


- CATALYSIS

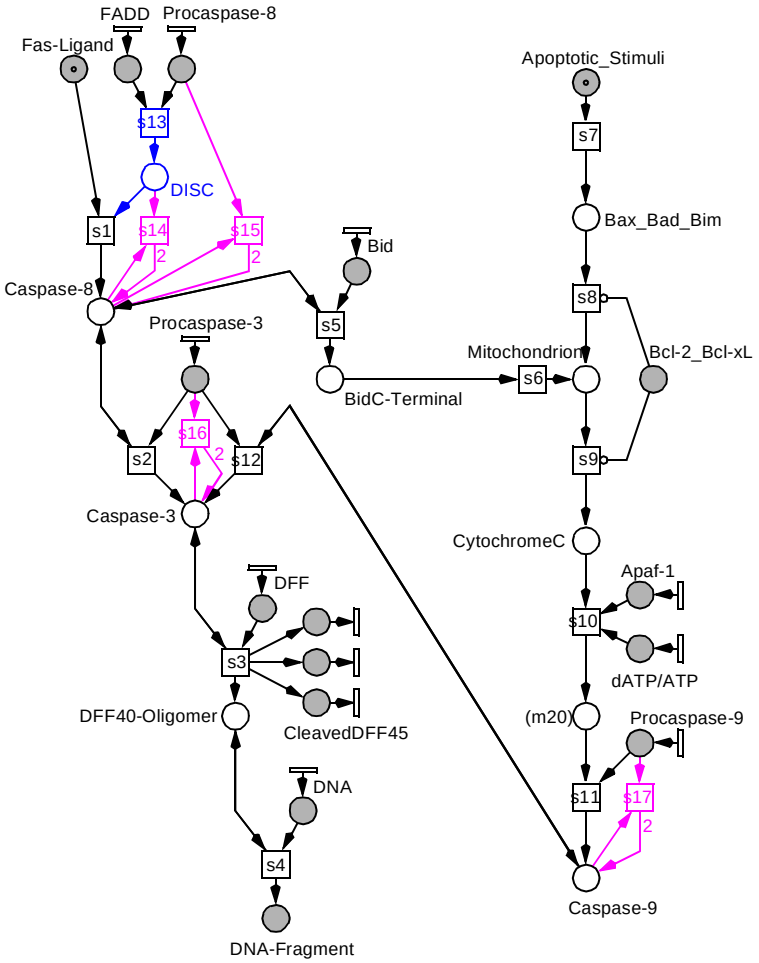


- AUTOCATALYSIS

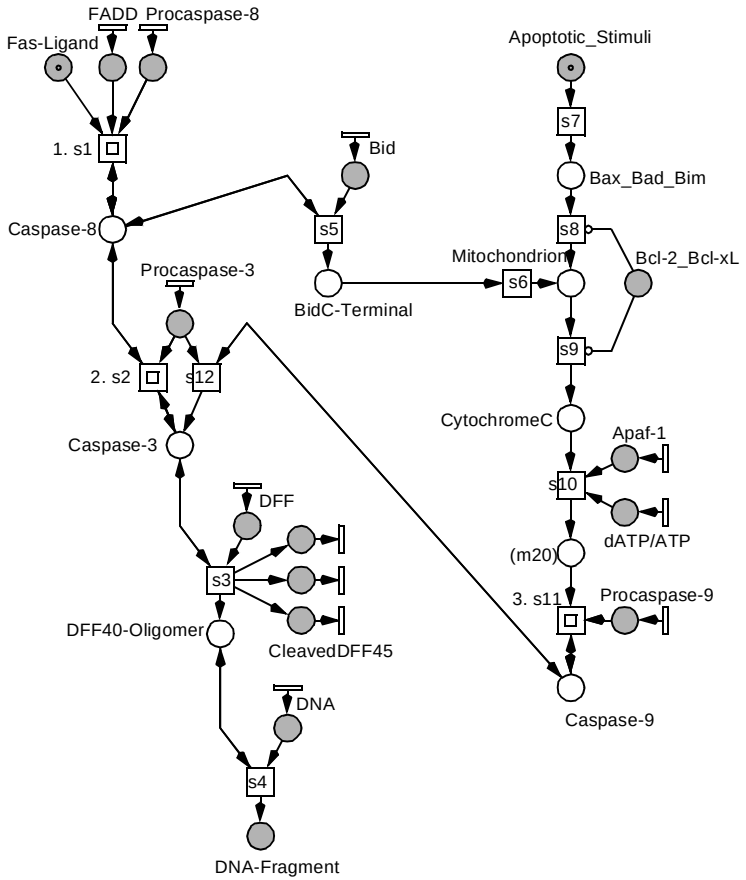




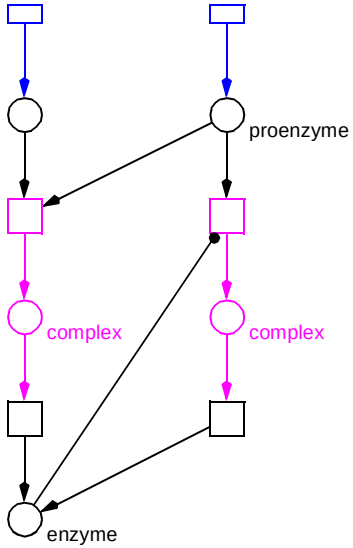
FAS4A



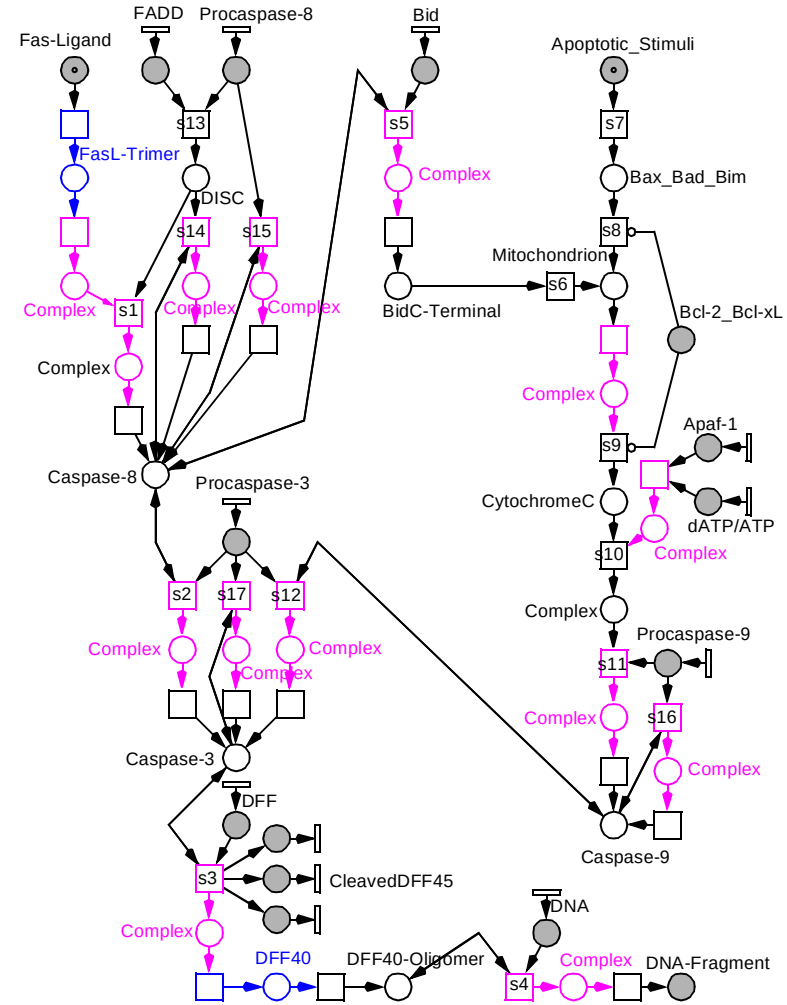
FAS4B ≈ FAS3



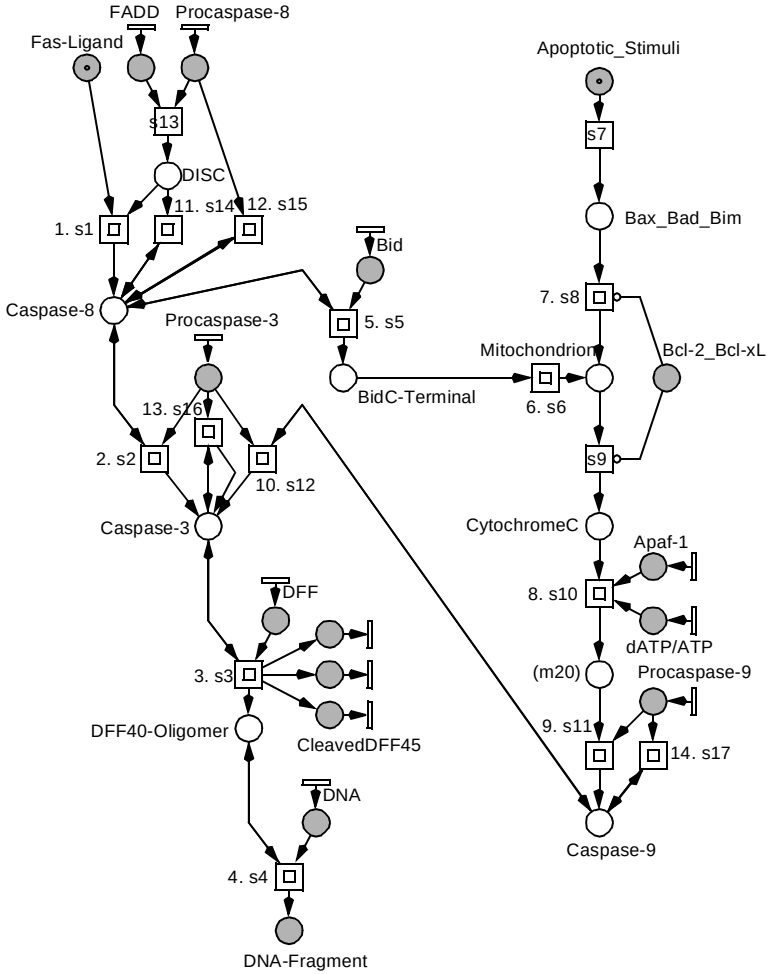
REFINEMENT: INTERMEDIATE COMPLEXES



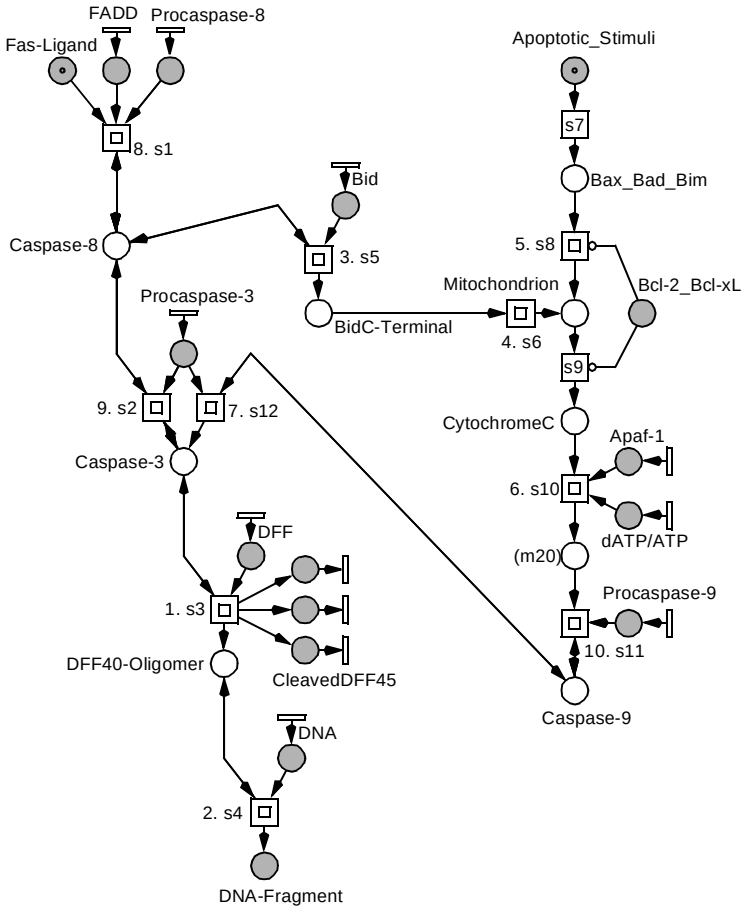
FAS5A



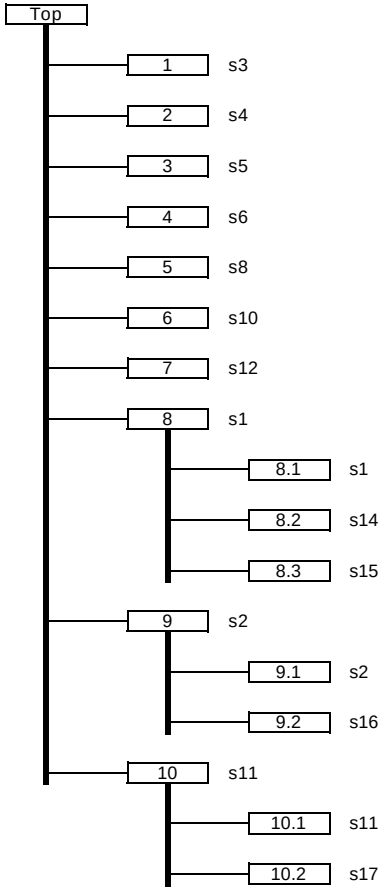
FAS5B
≈ FAS4A



FAS5C ≈ FAS3



HIERARCHY TREE FAS5C



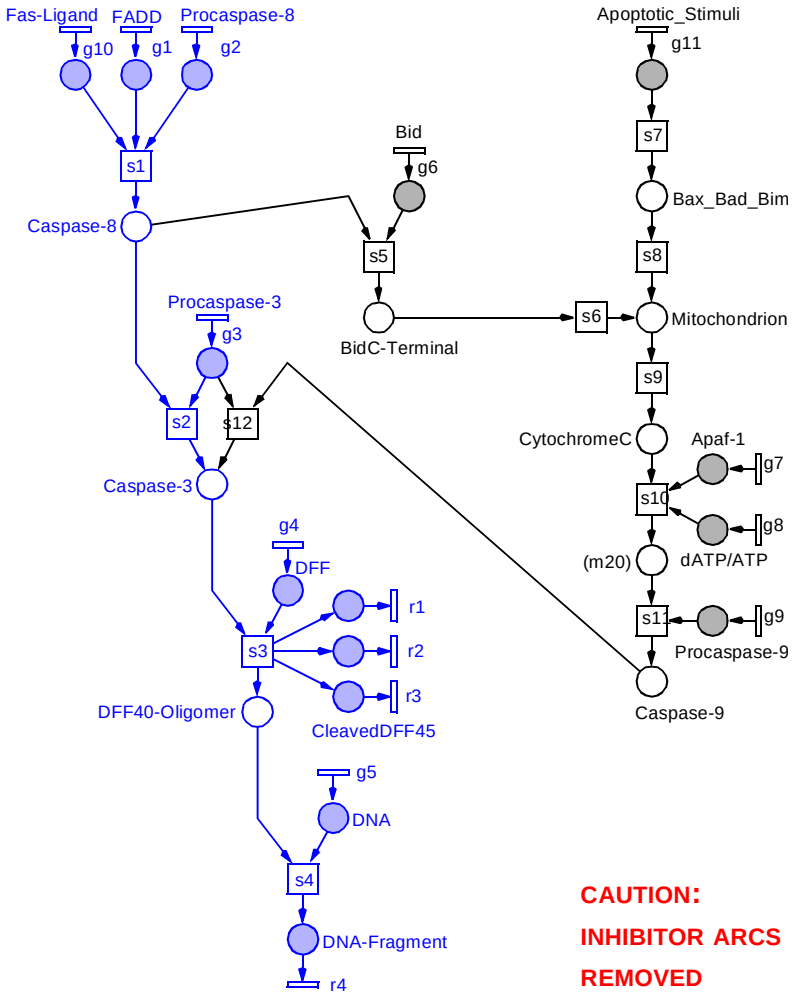
STEP-WISE MODELLING

1. literal scheme transformation — Fas1
2. layout improvement — Fas2
   -> use of syntactic sugar
3. adding environment behaviour — Fas3
   -> animation
4. adding autocatalysis — Fas4a
   -> hierarchic Petri net — Fas4b
5. adding intermediate complexes — Fas5a
   -> refined hierarchies — Fas5b
   Fas5c

-->> EXPLOIDING SYNTACTIC SUGAR

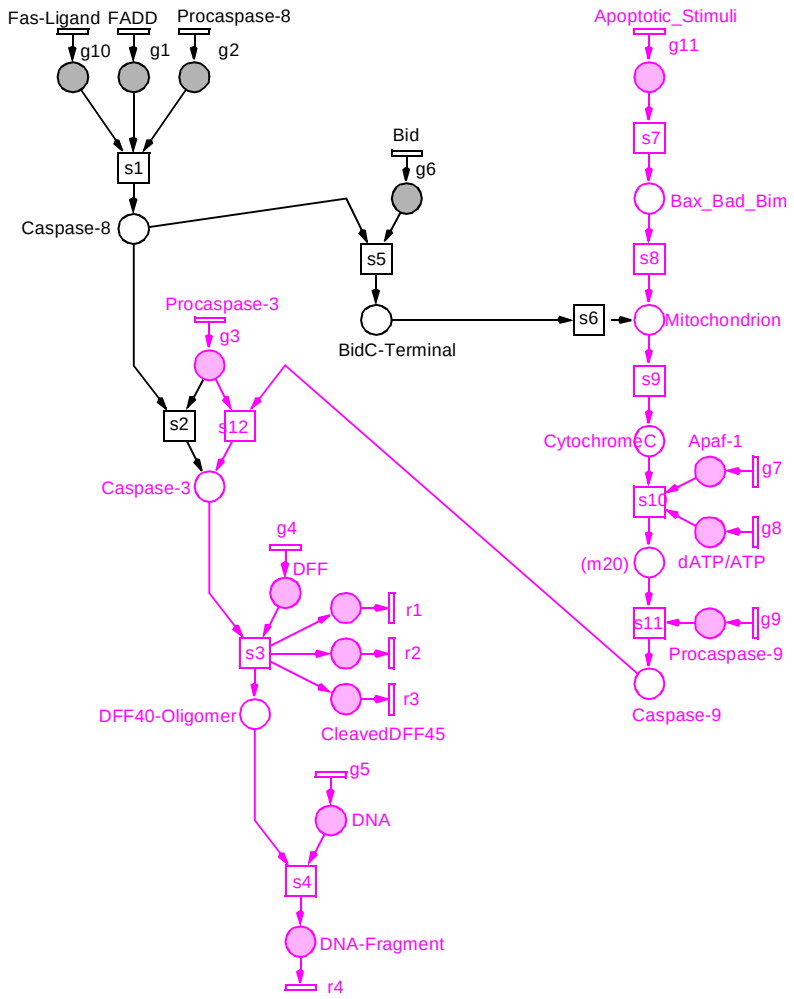
6. abstraction for analysis — Fas6

ANALYSIS OF FAS6, T- INVARIANT 1, DEATH-RECEPTOR PATHWAY

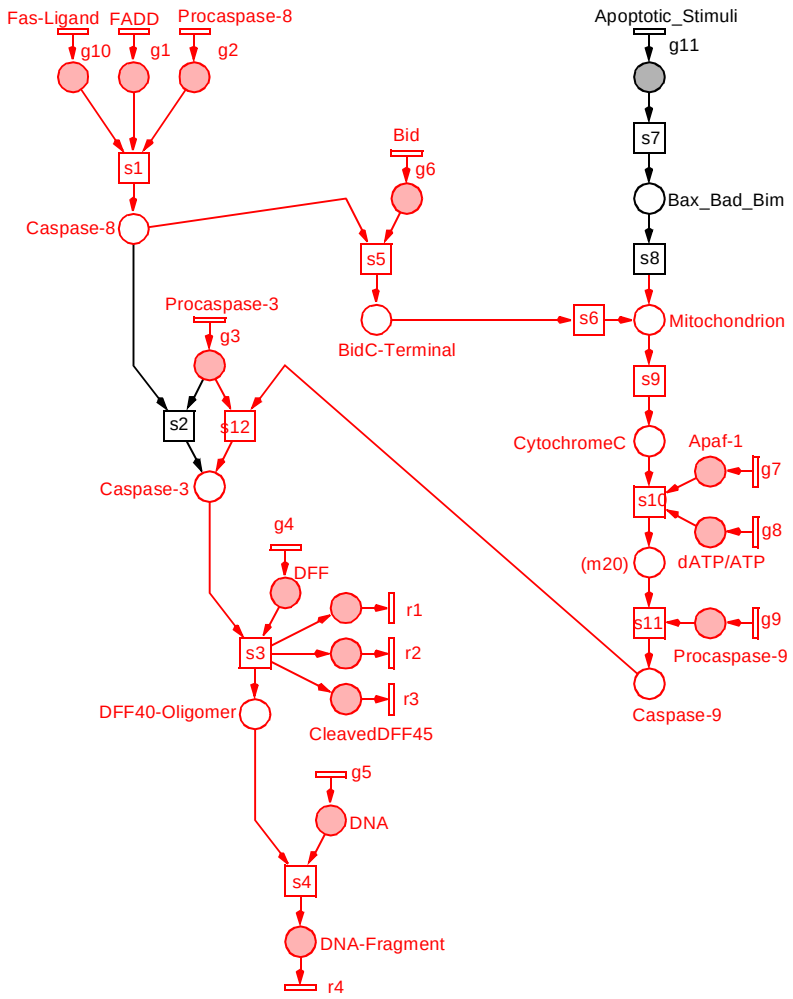


CAUTION:
INHIBITOR ARCS REMOVED

ANALYSIS OF FAS6, T- INVARIANT 2, MITOCHONDRIAL PATHWAY



ANALYSIS OF FAS6, T- INVARIANT 3, CROSS-TALK BY BID



APOPTOSIS, ANALYSES SUMMARY

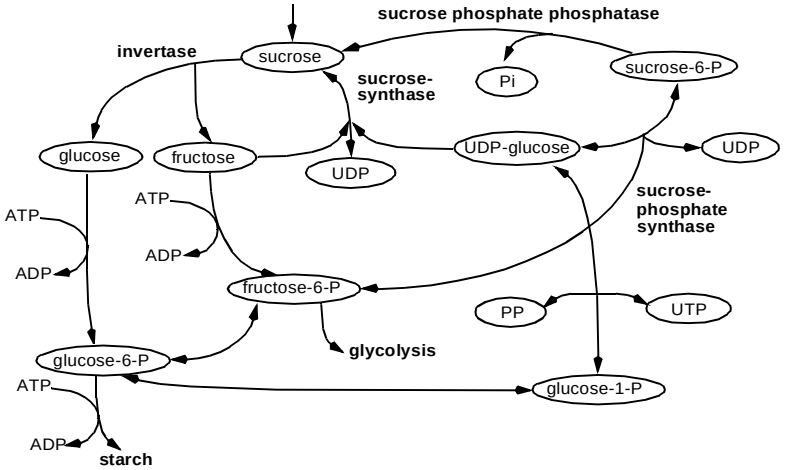
- environment style 1
- size of the net - 24 places / 27 transitions
- many read arcs, resolved for analysis
- no P-invariants
- three minimal positive i/o T-invariants
  - -> *three basic behaviours*
  - -> *any net behaviour = non-negative linear combination of them*
- covered by i/o T-invariants
  - -> *no idle parts*
- reproducible empty marking (guess)
  - -> *cyclic behaviour possible (reversibility)*
- INA result vector

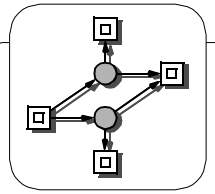
ORD	HOM	NBM	PUR	CSV	SCF	CON	SC	Ft0	tF0	Fp0	pF0	MG	SM	FC	EFC	ES
Y	Y	Y	Y	N	N	Y	N	Y	Y	N	N	N	N	N	N	N

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

CASE STUDY 2 - POTATO TUBER

- central carbon metabolism in potato tubers
  - -> *stoichiometric relations known*
  - -> *non-ordinary place/transition net*
  - -> *many reversible reactions*
- schematic overview





STOICHIOMETRIC EQUATIONS

R1. SuSy: *sucrose synthase*
Suc + UDP <--> UDPglc + Frc

R2. UGPase: *UDPglucose pyrophosphorylase*
UDPglc + PP <--> G1P + UTP

R3. PGM: *phosphoglucomutase*
G6P <--> G1P

R4. FK: *fructokinase*
Frc + ATP --> F6P + ADP

R5. PGI: *phosphoglucose isomerase*
G6P <--> F6P

R6. HK: *hexokinase*
Glc + ATP --> G6P +ADP

R7. Inv: *invertase*
Suc --> Glc + Frc

R8. Glyc(b): *glycolysis*
F6P + 29 ADP + 28 P_i --> 29 ATP

R9. SPS: *sucrose phospahate synthase*
F6P + UDPglc <--> S6P + UDP

R10. SPP: *sucrose phosphate phosphatase*
S6P --> Suc + P_i

R11. NDPkin: *NDP kinase*
UDP + ATP <--> UTP +ADP

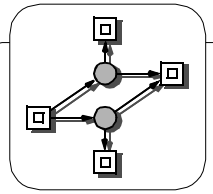
R12. SucTrans: *sucrose transporter*
eSuc --> Suc

R13. ATPcons(b): *ATP consumption*
ATP --> ADP + P_i

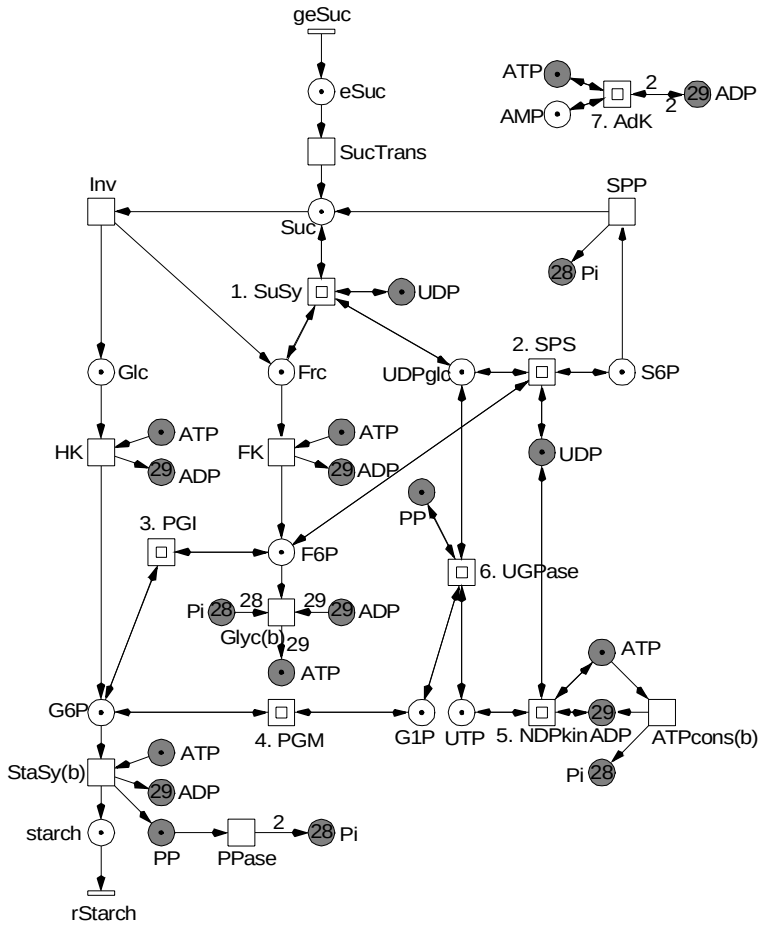
R14. StaSy(b): *starch synthesis*
G6P + ATP --> starch + ADP + PP

R15. AdK: *adenylate kinase*
ATP + AMP <--> 2 ADP

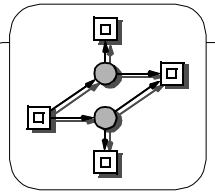
R16. PPase: *pyrophosphatase*
PP --> 2 P_i



POTATO TUBER, PETRI NET



potato.spped

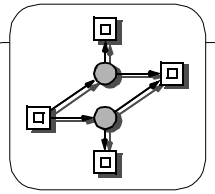


POTATO TUBER, ANALYSES SUMMARY 1

- environment style 2
- size of the net - 17 places / 25 transitions
- three P-invariants, but not CPI
  - -> *PI-1: UDPglc, UTP, UDP.*
    uridine preservation
  - -> *PI-2: ATP, AMP, ADP.*
    adenosine preservation
  - -> *PI-3: G6P, F6P, G1P, UTP, ATP(2), ADP, S6P, Pi, PP(2).*
    preservation of phosphorylated metabolites
- P-invariants need sufficient tokens at initial marking to make the net live
  - -> *how to calculate ?*
- INA result vector

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	Y	Y	N	N	N	N	N	N	N

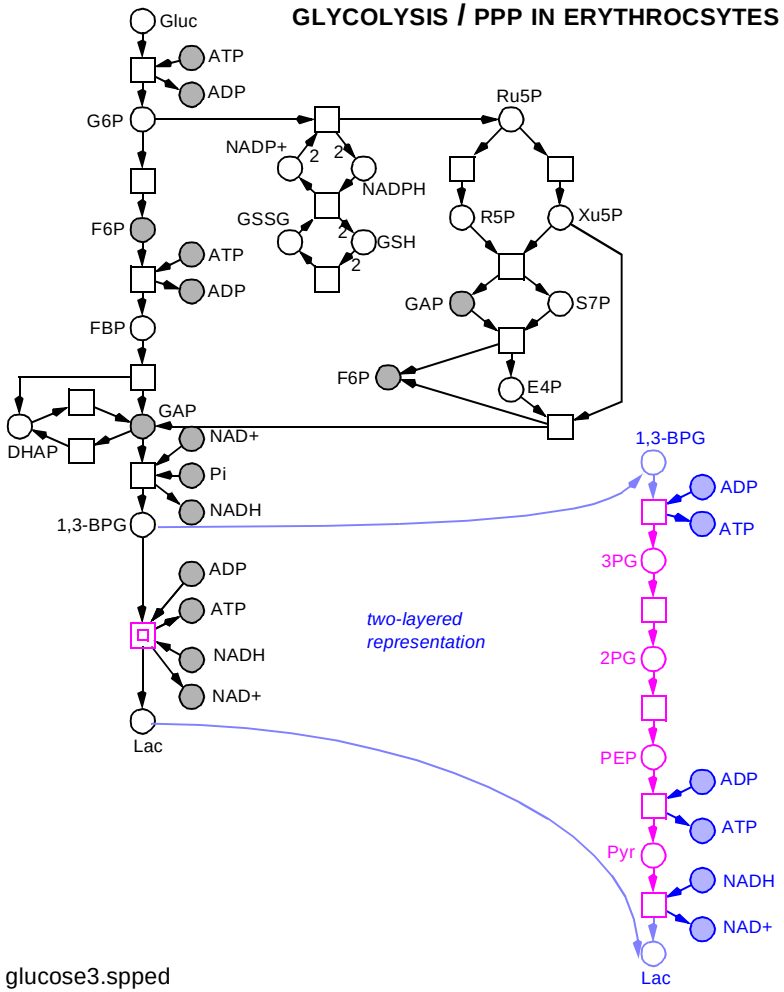
DTP	CPI	CTI	B	SB	REV	DSt	BSt	DTr	DCF	L	LV	L&S
?	N	**Y**	**N**	N	**?**	?	?	?	?	**?**	?	N



POTATO TUBER, ANALYSES SUMMARY 2

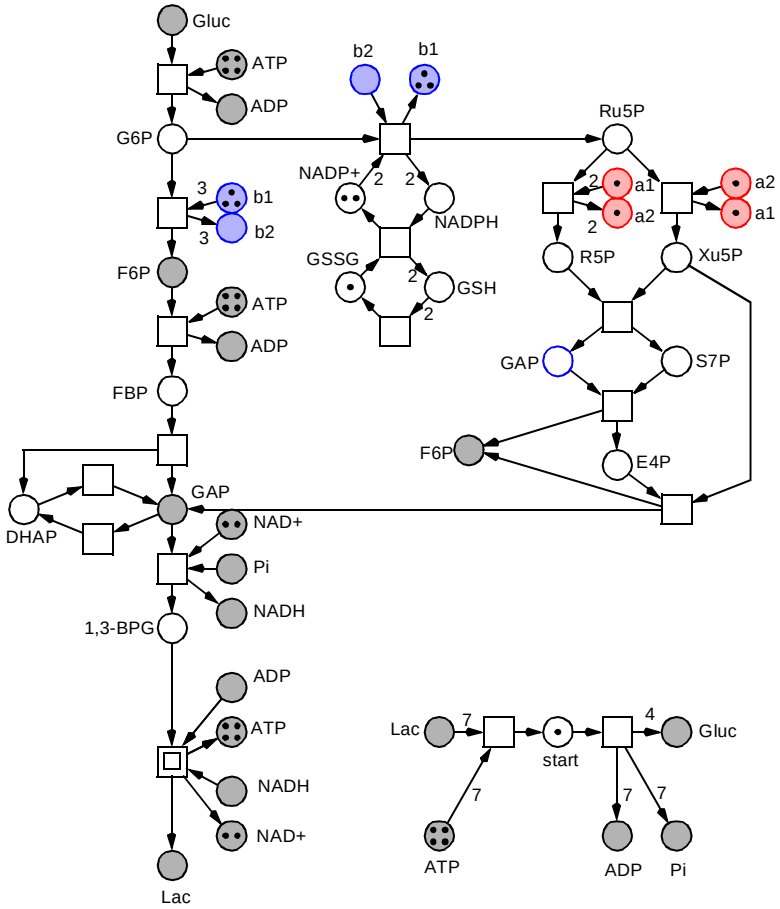
- covered by i/o T-invariants
- 19 T-invariants
  - -> *7 trivial ones (reversible reactions)*
  - -> *12 i/o invariants*
  - -> *no inner cycles*
- 3 i/o T-invariants with
  sucrose cleavage by sucrose synthase
  - -> *e.g. TI-8: geSuc, SucTrans, SuSy(29), UGPase, PGM_rev, FK(29), Glyc(b), StaSy(b), rStarch, SPS(28), SPP(28), NDPkin_rev.*
- 9 i/o T-Invariants with sucrose cleavage by invertase
  - -> *e.g. TI-11: geSuc, SucTrans, Inv(14), UGPase_rev(13), PGM(13), HK(14), FK, Glyc(b), StaSy(b), rStarch, SuSy_rev(13), NDPkin(13), PPase(14).*
- AdK and SPS_rev
  - -> *do not occur in a no-trivial T-invariant*
  - -> *removing AdK and ◁ or SPS_rev has no influence on the system behaviour*

CASE STUDY 3 - GLYCOLYSIS, VERSION 3

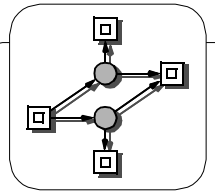


glucose3.spped

GLYCOLYSIS, VERSION 4



glukose4.spped

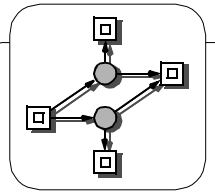


ASSUMPTIONS IN VERSION 4

- the two appearances of GAP
  can be separated
  (no logical / fusion nodes)

- the branching probabilities
  at the conflicts of
  G6P and Ru5P
  are known and
  may be characterized by the relations
  G6P - 3 :1
  Ru5P - 2 :1

  -> *STEADY STATE:*
  all intermediates have to be balanced with respect to inputs and outputs



GLYCOLYSIS, ANALYSES SUMMARY

- environment style 3

- size of the net - 32 places, 22 transitions

- CPI
  -> BND
  -> *39 P-invariants*
  -> *interpretation ?*

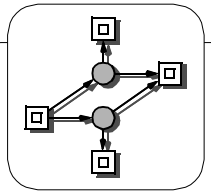
- CTI
  -> *1 trivial T-invariant*
  -> *1 i/o invariant*

- size of the reachability graph - 14.862
  -> *live*
  -> *reversible*

- INA result vector

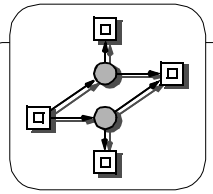
```
ORD HOM NBM PUR CSV SCF CON SC  Ft0 tF0 Fp0 pF0 MG  SM FC EFC ES
N   Y   N   Y   N   N   Y   Y   N   N   N   N   N   N  N  N   Y

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



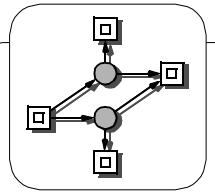
5.

SUMMARY AND OUTLOOK



BIO PETRI NETS, WHAT FOR ?

- unifying view
  - -> *different biochemical systems*
- step-wise model construction
  of graphical (= visual) models for
  - -> *animation*
  - -> *validation*
  - -> *(qualitative) analysis*
  - -> *(quantitative) simulation*
- integration of
  - -> *model validation*
  - -> *behaviour prediction*
- one all-purpose model
  - -> *animation model*
  - -> *“qualitative model = animation model”*
  - -> *“quantitative model =*
    qualitative model
    + quantitative parameter”

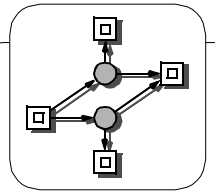


MODEL CLASSES

PETRI NETS

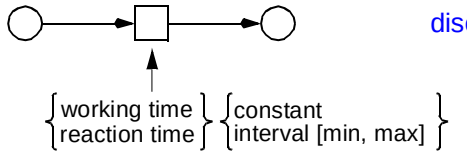
- PLACE/TRANSITION PETRI NET (COLOURED PN)
  - validation by Petri net theory
  - validation/prediction by model checking
- TIME-DEPENDENT PN
  - DISCRETE *) PETRI NET
    - worst-case evaluation
  - CONTINUOUS PETRI NET
    - behaviour prediction
  - STOCHASTIC PETRI NET
    - reliability prediction

*) DISCRETELY TREATABLE

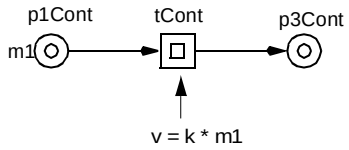


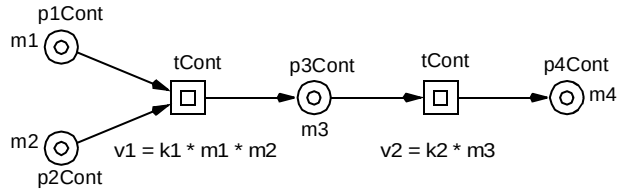
INTEGRATION OF QUANTITATIVE ANALYSES

- CONTINUOUS, BUT DISCRETELY TREATABLE TIME



- CONTINUOUS TIME



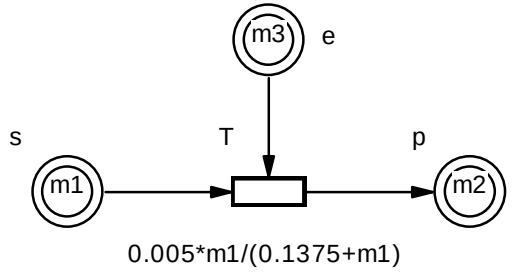


system of differential equations

$$\begin{aligned} d[p1Cont]/dt &= d[p2Cont]/dt = -v1 \\ d[p4Cont]/dt &= v2 \\ d[p3Cont]/dt &= v1 - v2 \end{aligned}$$

-> SELF-MODIFYING PETRI NETS

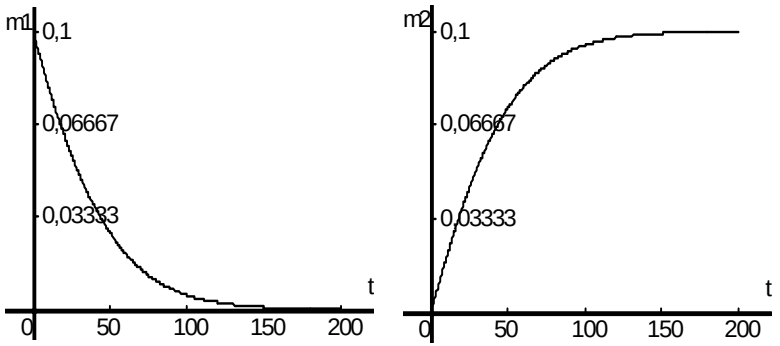
MICHAELIS-MENTEN REACTION *[GENOMIC OBJECT NET]*



Vmax = 0.005 (maximal reaction rate)

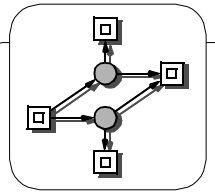
Km = 0.1375 (Michaelis constant)

d[s]/dt = d[p]/dt = Vmax*[s] / (Km+[s])



CASE STUDIES

- apoptosis in mammalian cells
- detailed glycolysis/pentose phosphate pathways in all human cells
- blood clotting in human (hemostasis versus fibrinolysis)
- lipoprotein metabolism (liver) in human
- G1/S - phase in mammalian cells
- detailed central carbon metabolism in potato tubers
- central carbon metabolism in *E.coli*



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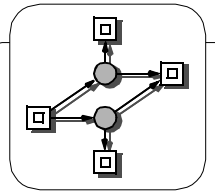

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