PETRI NETS FOR SYSTEMS & SYNTHETIC BIOLOGY

in memory of Nadia Busi

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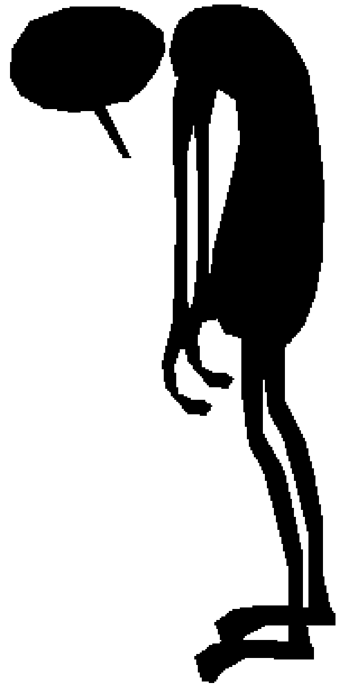
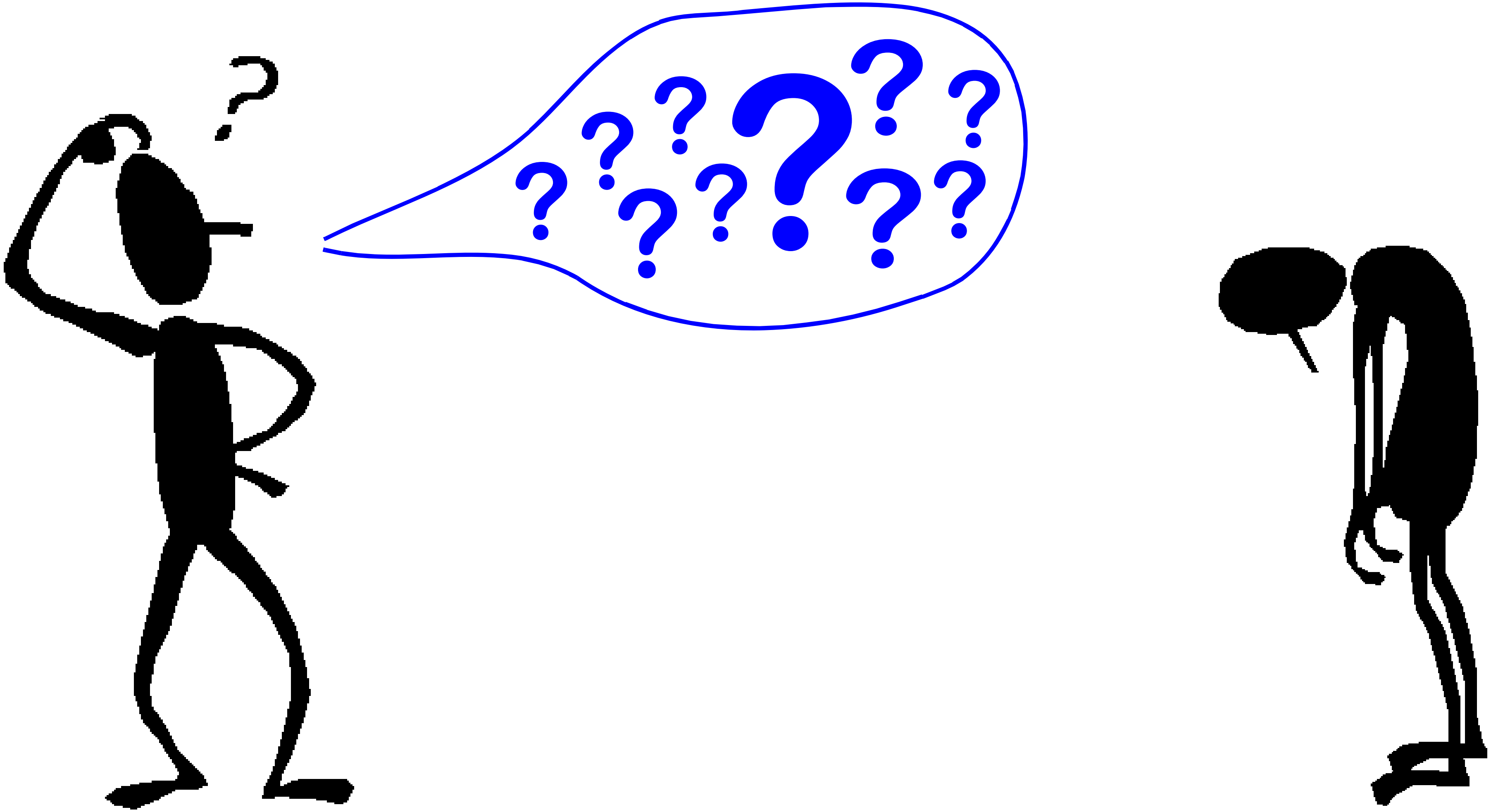
AGENDA

- **MODELLING WITH PETRI NETS**
- **MODEL-BASED SYSTEM ANALYSIS**
  - .-.-> *qualitative Petri nets*
  - .-.-> *stochastic Petri nets*
  - .-.-> *continuous Petri nets*
- **CASE STUDY**
  -> *model checking in the three paradigms*
- **SUMMARY**
  -> *challenges / open questions*

MEDICAL TREATMENT



MEDICAL TREATMENT, APPROACH 1- TRIAL-AND-ERROR DRUG PRESCRIPTION



MEDICAL TREATMENT, APPROACH 1- TRIAL-AND-ERROR DRUG PRESCRIPTION

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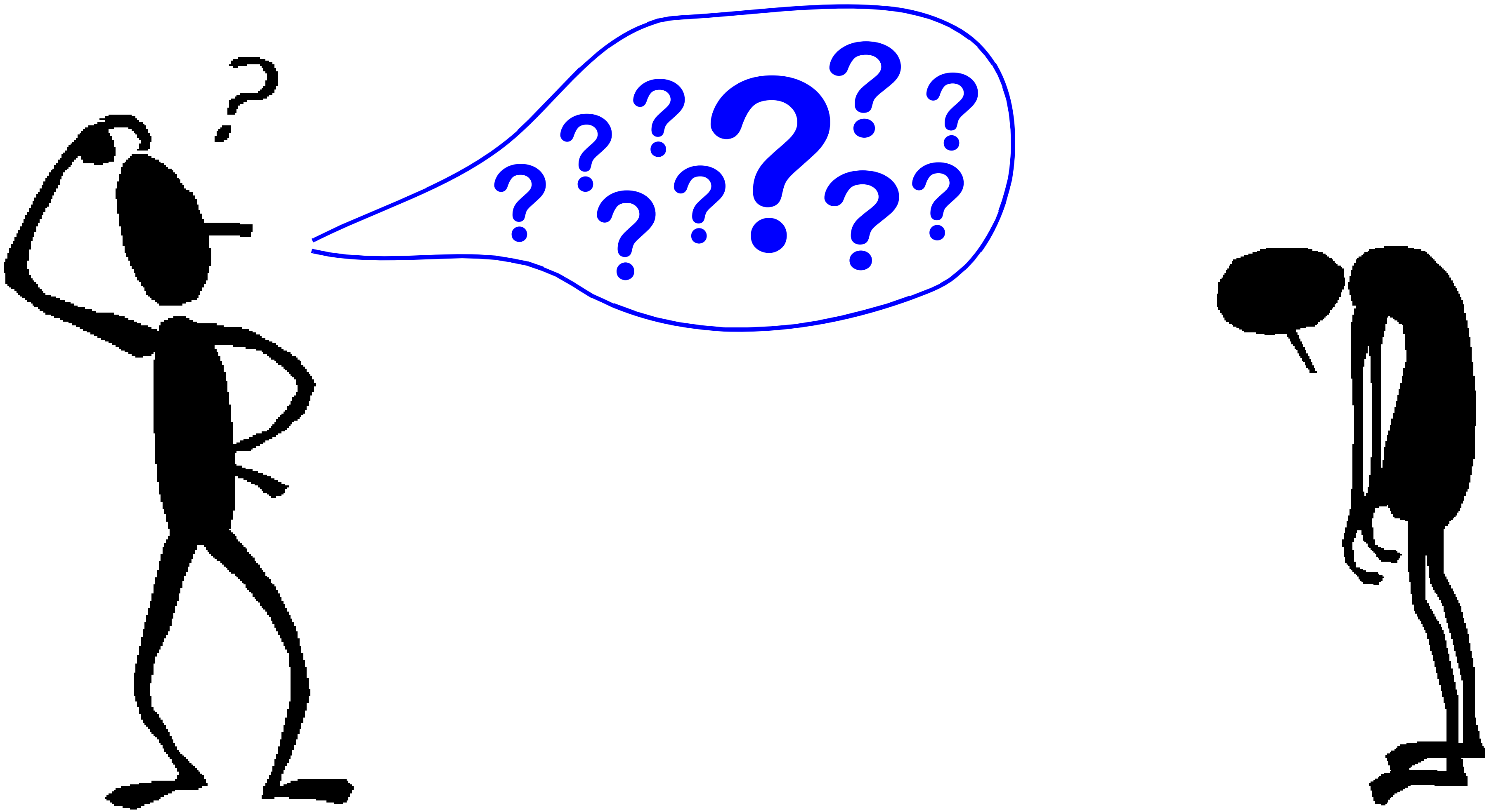
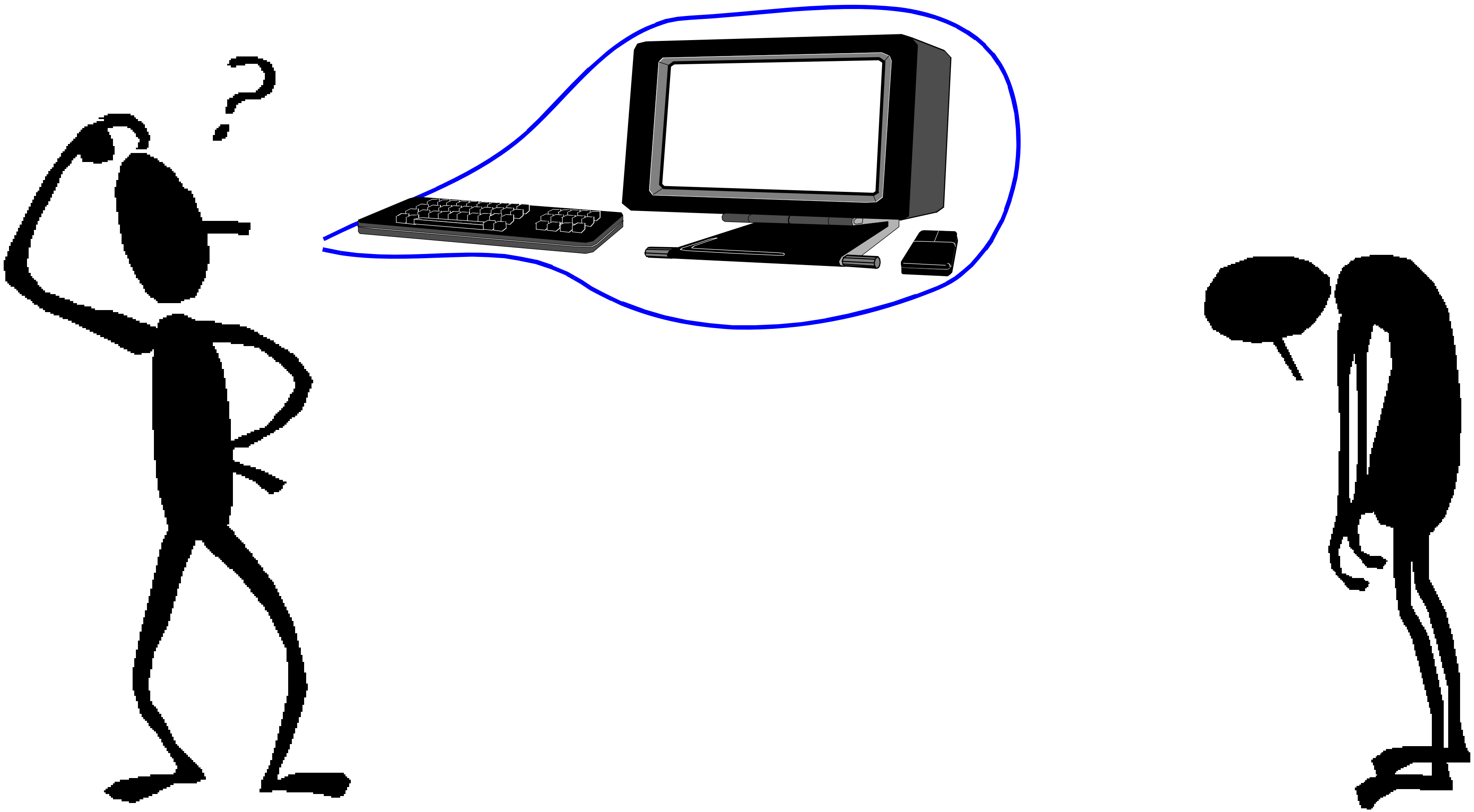
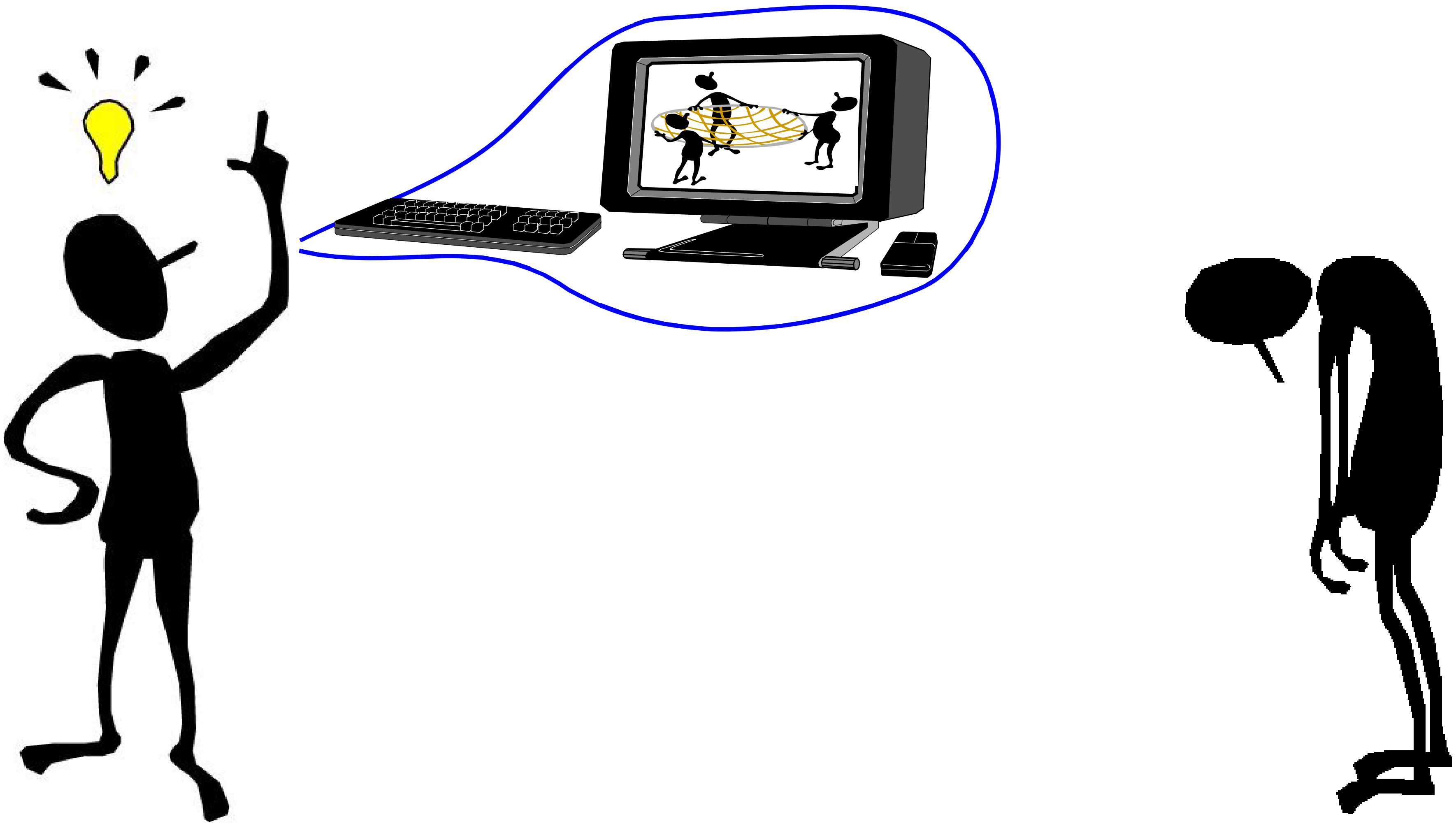
MEDICAL TREATMENT, APPROACH 2



MEDICAL TREATMENT, APPROACH 2 - MODEL-BASED DRUG PRESCRIPTION



MEDICAL TREATMENT, APPROACH 2 - MODEL-BASED DRUG PRESCRIPTION



MEDICAL TREATMENT, APPROACH 2 - MODEL-BASED DRUG PRESCRIPTION

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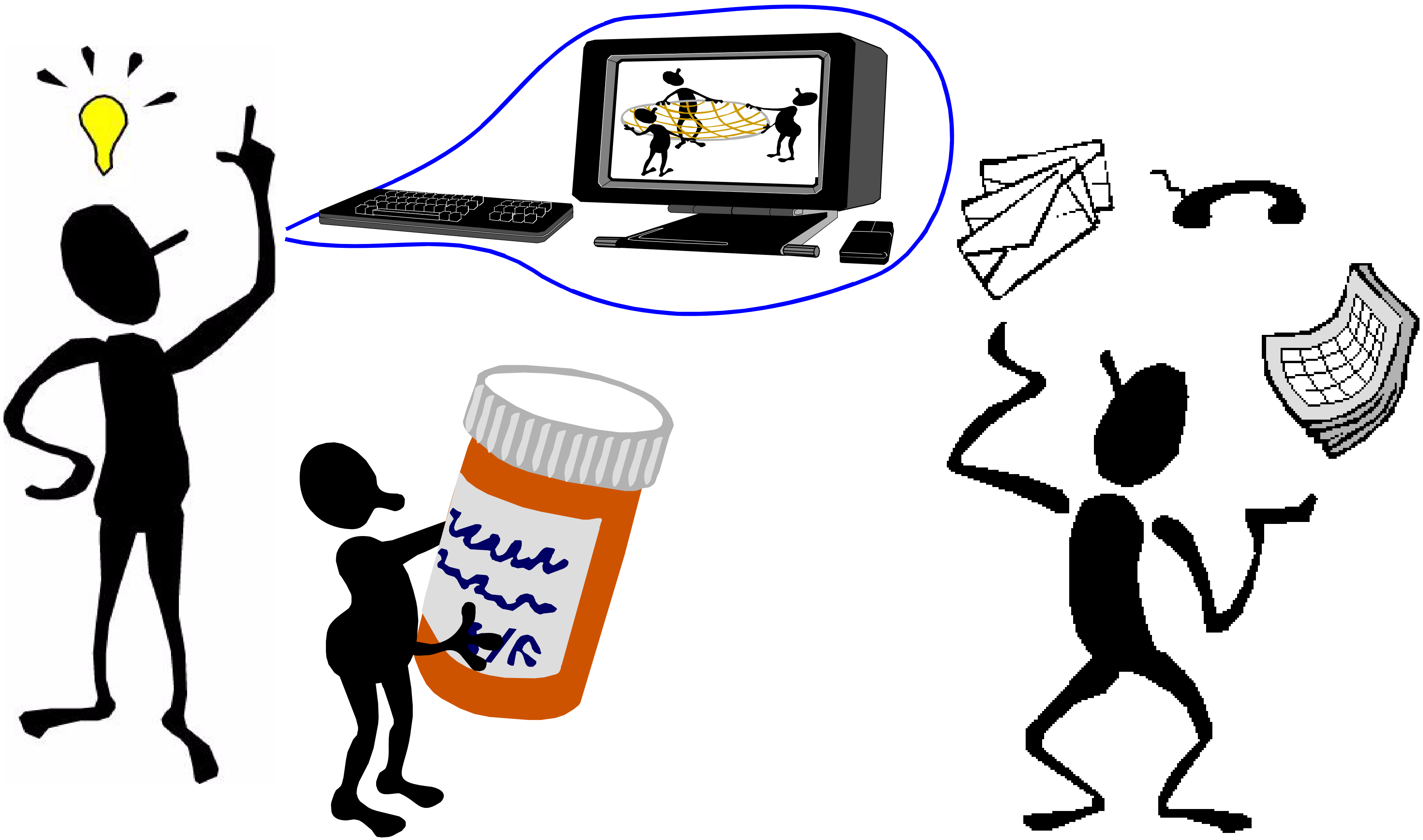
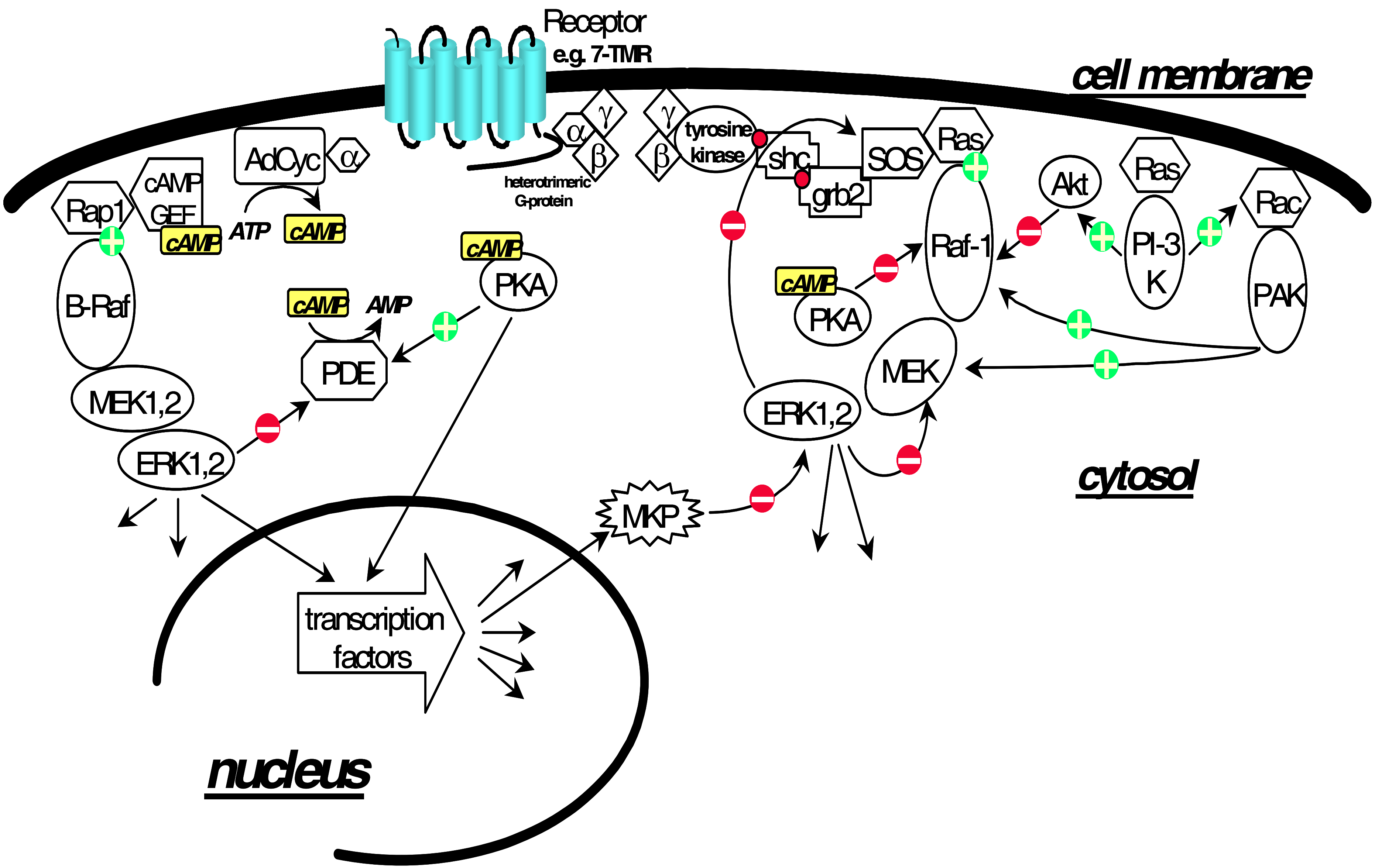
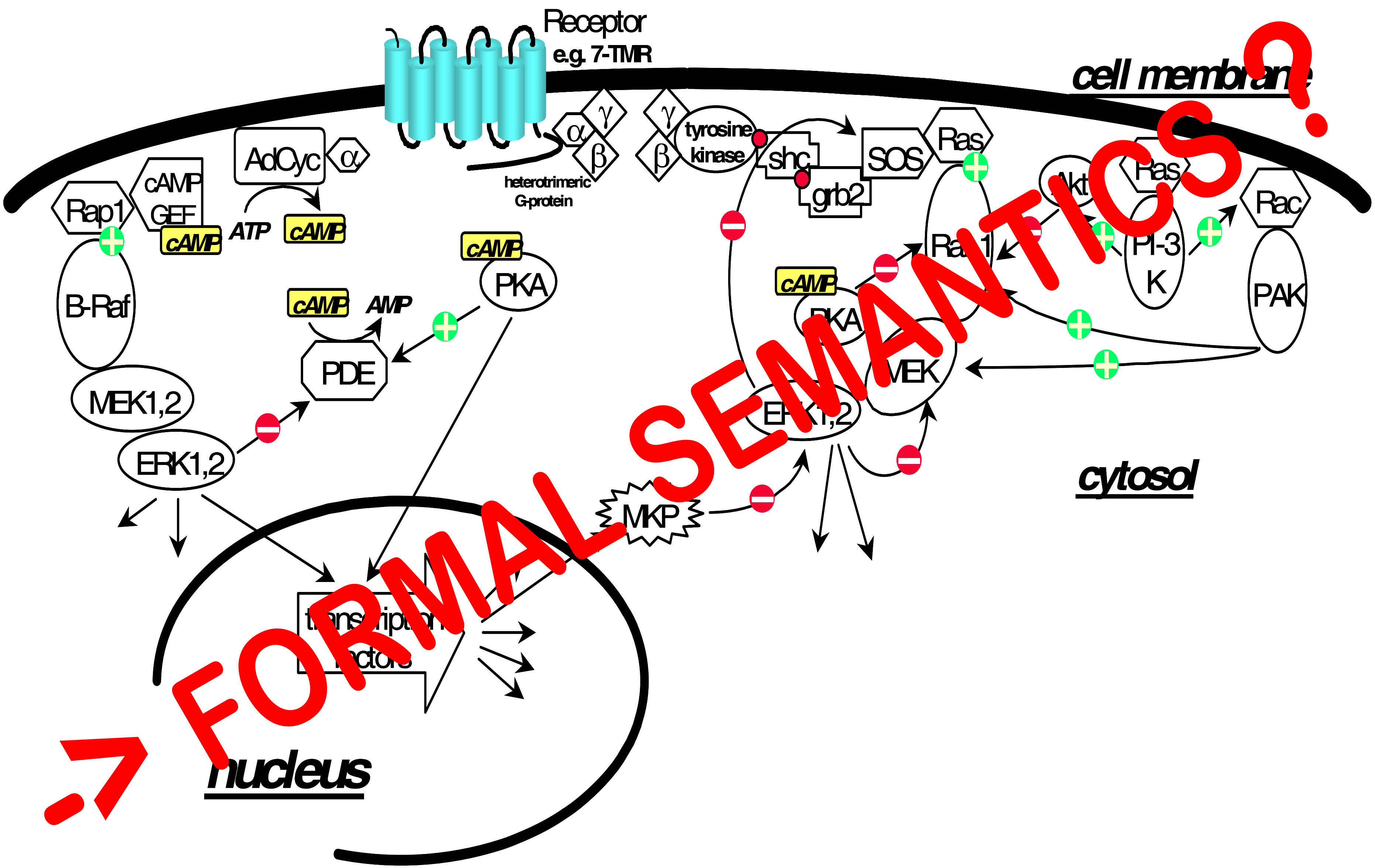


WHAT KIND OF MODEL SHOULD BE USED?



Receptor
e.g. 7-TMR
cell membrane
heterotrimeric
G-protein
α
β
γ
tyrosine
kinase
shc
grb2
SOS
Ras
Rap1
cAMP
GEF
AdCyc
ATP
cAMP
B-Raf
MEK1,2
ERK1,2
PKA
PDE
AMP
Raf-1
Akt
PI-3
K
Rac
PAK
MEK
ERK1,2
MKP
transcription
factors
cytosol
nucleus

Bio Network Representations, Ex1



$$\frac{d\alpha}{dt} = -\nu_1$$

$$\frac{dSte2}{dt} = -\nu_2 + \nu_3 - \nu_5$$

$$\frac{dSte2_{active}}{dt} = \nu_2 - \nu_3 - \nu_4$$

$$\frac{dSst2_{active}}{dt} = \nu_{46} - \nu_{47}$$

$$\frac{dG\alpha\beta\gamma}{dt} = -\nu_6 + \nu_9$$

$$\frac{dG\alpha GTP}{dt} = \nu_6 - \nu_7 - \nu_8$$

$$\frac{dG\alpha GDP}{dt} = \nu_7 + \nu_8 - \nu_9$$

$$\frac{dG\beta\gamma}{dt} = \nu_6 - \nu_9 - \nu_{10} + \nu_{11} + \nu_{21} + \nu_{23} + \nu_{25} + \nu_{27} + \nu_{32} - \nu_{42} + \nu_{43}$$

$$\frac{dSte5}{dt} = -\nu_{12} + \nu_{13} + \nu_{17} + \nu_{21} + \nu_{23} + \nu_{25} + \nu_{27} + \nu_{32}$$

$$\frac{dSte11}{dt} = -\nu_{12} + \nu_{13} + \nu_{17} + \nu_{21} + \nu_{23} + \nu_{25} + \nu_{27} + \nu_{32}$$

$$\frac{dSte7}{dt} = -\nu_{14} + \nu_{15} + \nu_{17} + \nu_{21} + \nu_{23} + \nu_{25} + \nu_{27} + \nu_{32}$$

$$\frac{dFus3}{dt} = -\nu_{14} + \nu_{15} + \nu_{17} + \nu_{21} + \nu_{23} + \nu_{25} + \nu_{27} - \nu_{29} + \nu_{30} + \nu_{33}$$

$$\frac{dSte20}{dt} = -\nu_{18} + \nu_{19} + \nu_{21} + \nu_{23} + \nu_{25} + \nu_{27} + \nu_{32}$$

$$\nu_1 = \alpha[t] \cdot Bar1_{active}[t] \cdot k_1$$
$$\nu_2 = Ste2[t] \cdot \alpha[t] \cdot k_2$$
$$\nu_3 = Ste2_{active}[t] \cdot k_3$$
$$\nu_4 = Ste2_{active}[t] \cdot k_4$$
$$\nu_5 = Ste2[t] \cdot k_5$$
$$\nu_6 = Ste2_{active}[t] \cdot G\alpha\beta\gamma[t] \cdot k_6$$
$$\nu_7 = G\alpha GTP[t] \cdot k_7$$
$$\nu_8 = G\alpha GTP[t] \cdot Sst2_{active}[t] \cdot k_8$$
$$\nu_9 = G\alpha GDP[t] \cdot G\beta\gamma[t] \cdot k_9$$
$$\nu_{10} = G\beta\gamma[t] \cdot C[t] \cdot k_{10}$$
$$\nu_{11} = D[t] \cdot k_{11}$$
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$$\nu_{13} = A[t] \cdot k_{13}$$
$$\nu_{14} = Ste7[t] \cdot Fus3[t] \cdot k_{14}$$
$$\nu_{15} = B[t] \cdot k_{15}$$
$$\nu_{16} = A[t] \cdot B[t] \cdot k_{16}$$
$$\nu_{17} = C[t] \cdot k_{17}$$
$$\nu_{18} = D[t] \cdot Ste20[t] \cdot k_{18}$$

Bio Network Representations, Ex2

$$\frac{d\alpha}{dt} = -\nu_1$$
$$\frac{dSte2}{dt} = -\nu_2 + \nu_3 - \nu_5$$
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$$\frac{dSst2_{active}}{dt} = \nu_{46} - \nu_{47}$$
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$$\nu_{10} = G\beta\gamma[t] \cdot C[t] \cdot k_{10}$$
$$\nu_{11} = D[t] \cdot k_{11}$$
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$$\nu_{13} = A[t] \cdot k_{13}$$
$$\nu_{14} = Ste7[t] \cdot Fus3[t] \cdot k_{14}$$
$$\nu_{15} = B[t] \cdot k_{15}$$
$$\nu_{16} = A[t] \cdot B[t] \cdot k_{16}$$
$$\nu_{17} = C[t] \cdot k_{17}$$
$$\nu_{18} = D[t] \cdot Ste20[t] \cdot k_{18}$$

-> READABILITY ?

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

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- models are full of assumptions -

BIO NETWORK REPRESENTATIONS SHOULD BE

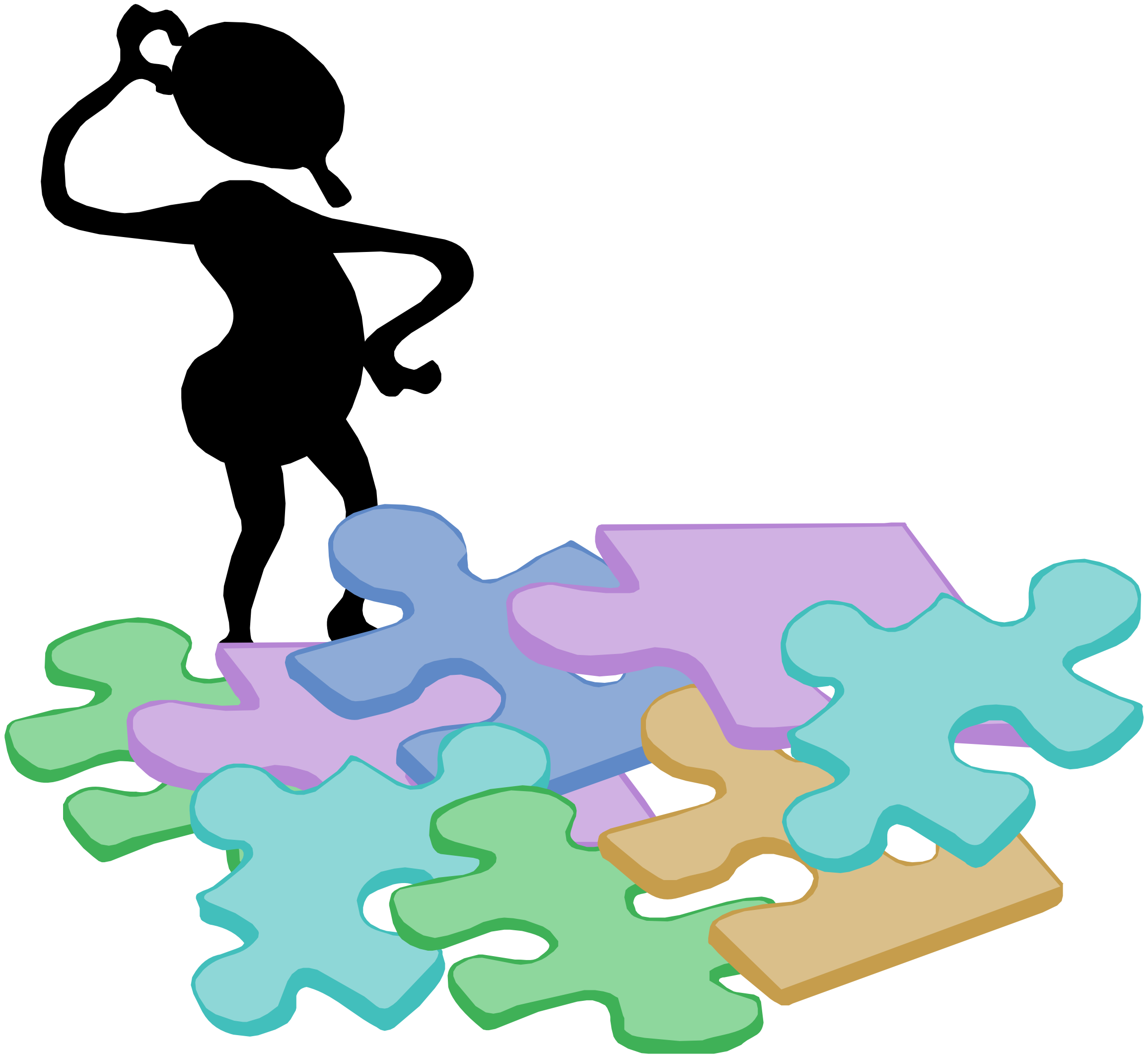
- **readable**
  - -> *fault avoidance*
  - -> *informal = cartoon-like representations ?*

- **analysable**
  - -> *formal = mathematical representations*

- **executable**
  - -> *to experience the model*

- **unifying power**
  - -> *high-level description for various analysis approaches*

WHAT KIND OF MODEL TO CHOSE?

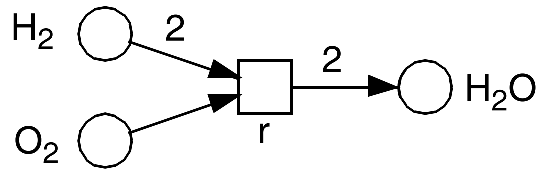


. . . ARE
NETWORKS OF
(BIO-) CHEMICAL REACTIONS

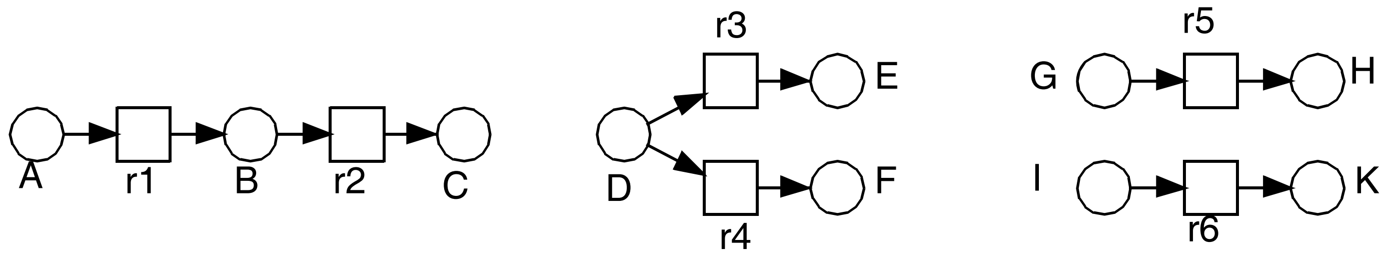
Bio Networks, Three Basic Properties

- **bipartite - species & reactions**

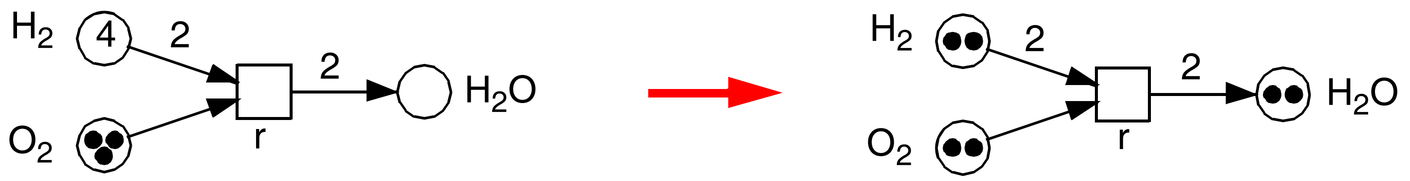
r: $2\ H_2 + O_2 \dashrightarrow 2\ H_2O$

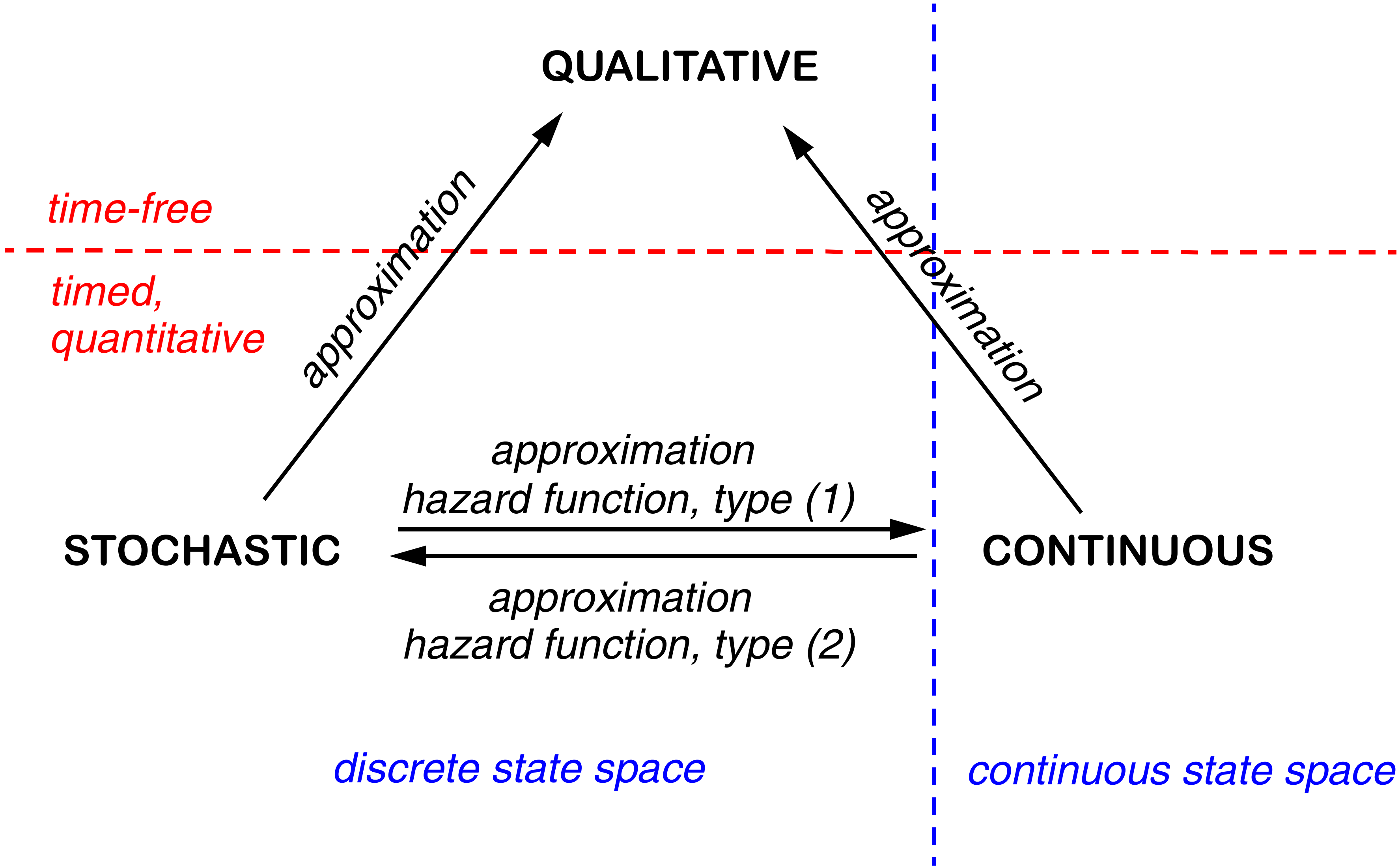


- **reactions - sequential, alternative, concurrent**



- **behaviour - stochastic**




QUALITATIVE
time-free
timed,
quantitative
approximation
approximation
approximation
hazard function, type (1)
STOCHASTIC
CONTINUOUS
approximation
hazard function, type (2)
discrete state space
continuous state space

PETRI NETS -
AN INFORMAL CRASH COURSE

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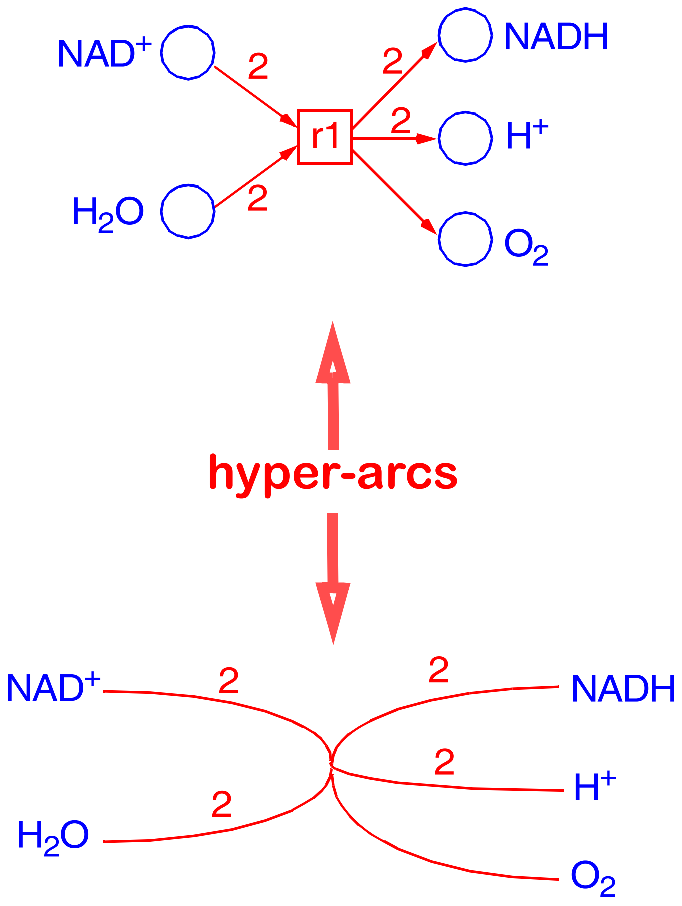
❑

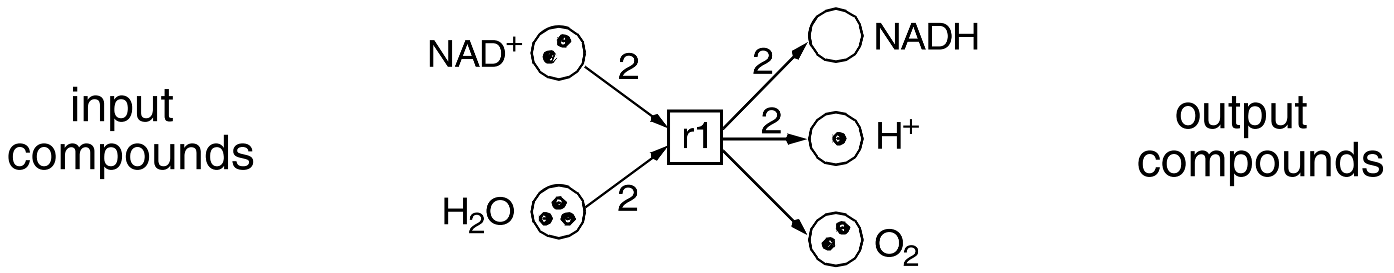
$$2\ NAD^{+} + 2\ H_2O \rightarrow 2\ NADH + 2\ H^{+} + O_2$$



Petri Nets, Basics - the Structure

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

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



- **local conditions -> places -> chemical compounds**
- **multiplicities -> arc weights -> stoichiometric relations**
- **condition's state -> token(s) -> available amount (e.g. mol)**
- **system state -> marking -> compounds distribution**
- **PN = (P, T, F, m_0), F: ((P x T) U (T x P)) -> N_0, m_0: P -> N_0**

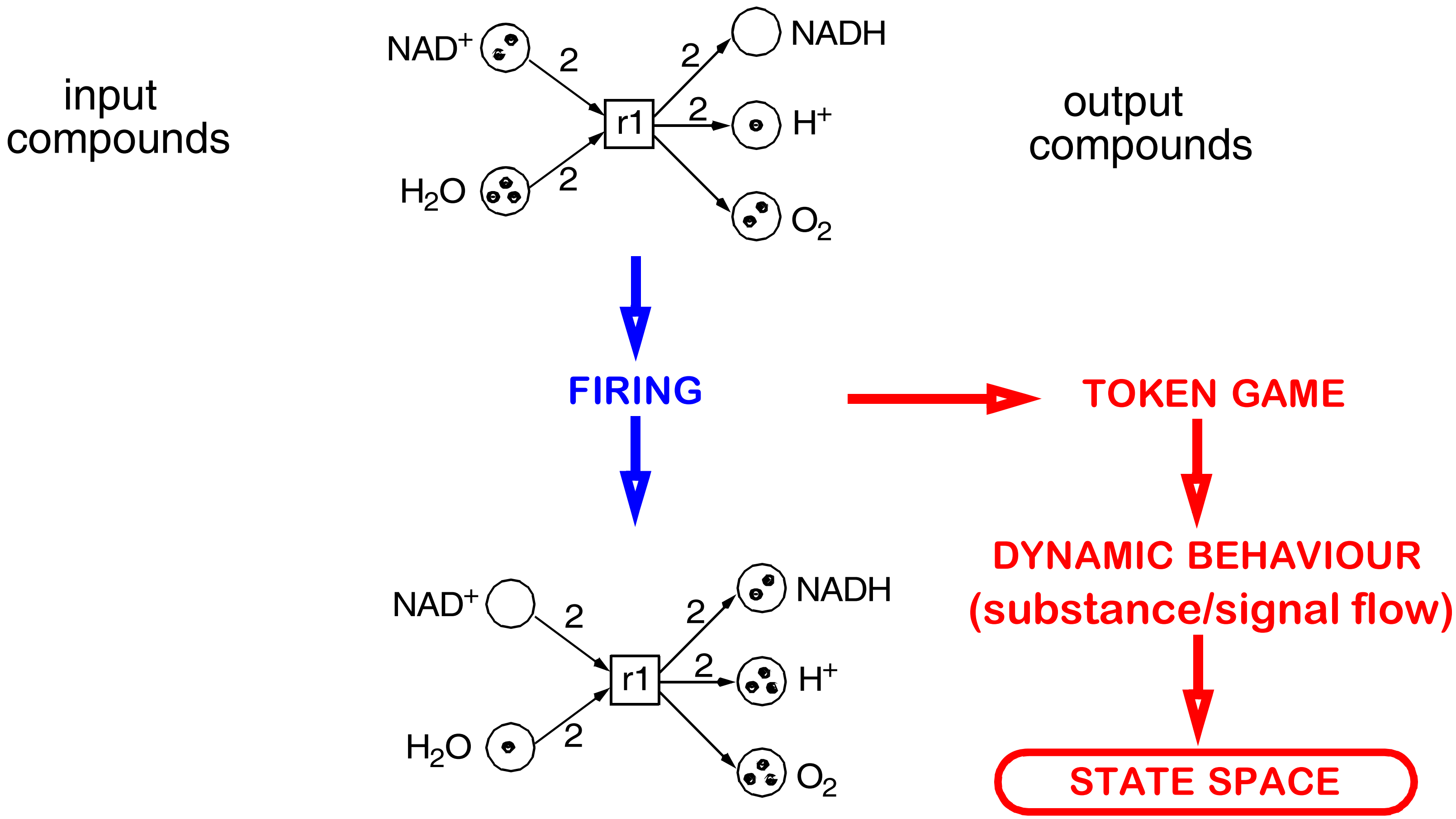
PETRI NETS, BASICS - THE FIRING RULE

- **an action may happen, if** -> **prerequisite**
  - -> *all preconditions are fulfilled (corresponding to the arc weights)*
- **if an action happens, then** -> **firing behaviour**
  - -> *tokens are removed from all preconditions (corresponding to the arc weights), and*
  - -> *tokens are added to all postconditions (corresponding to the arc weights)*
- **action happens (firing of a transition)** -> **model assumptions**
  - -> *atomic*
  - -> *no time consumption*

-> TIME-FREE MODEL, WHICH CONSIDERS ALL POSSIBLE TIMING BEHAVIOUR

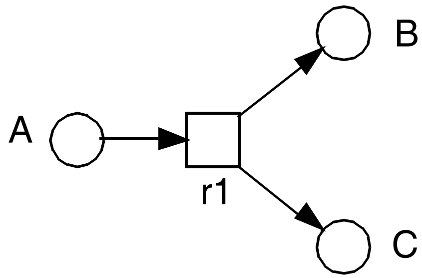
- **atomic actions -> transitions -> chemical reactions**

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

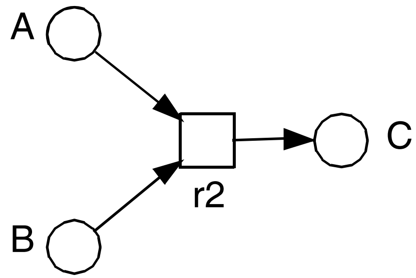


TYPICAL BASIC STRUCTURES I

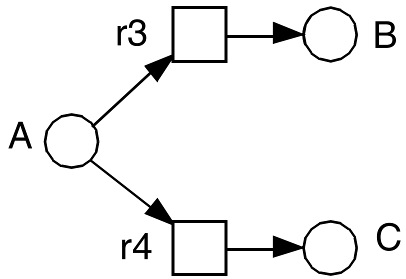
A --> B + C



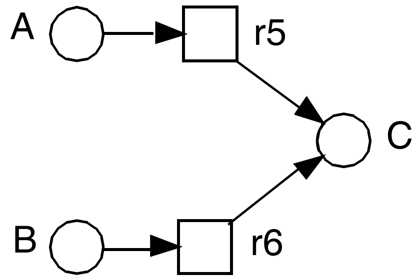
A + B --> C



A --> B,
A --> C

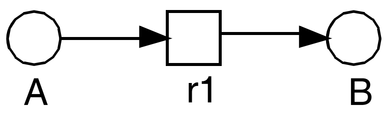


A --> C,
B --> C

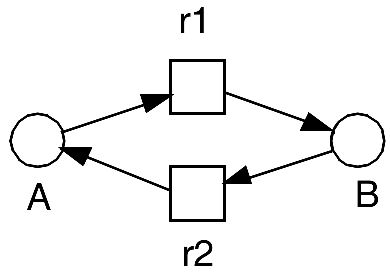


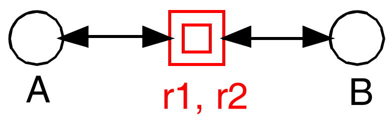
Typical Basic Structures II

A --> B

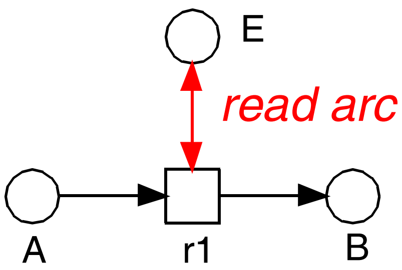


A <--> B

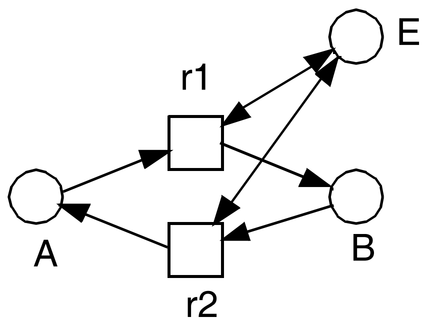


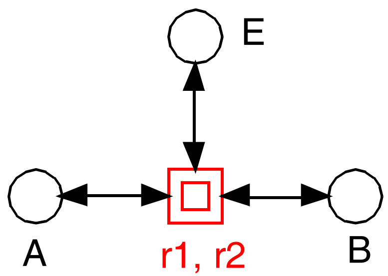


E
A --> B



E
A <--> B



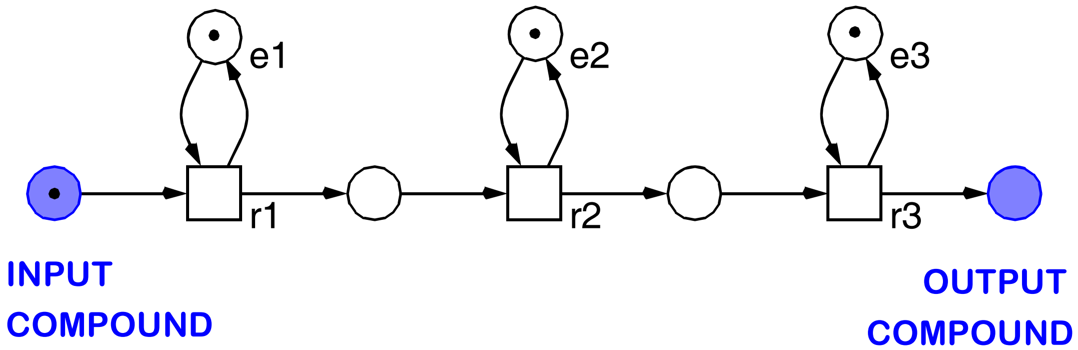


macro transition

Typical Basic Structures III

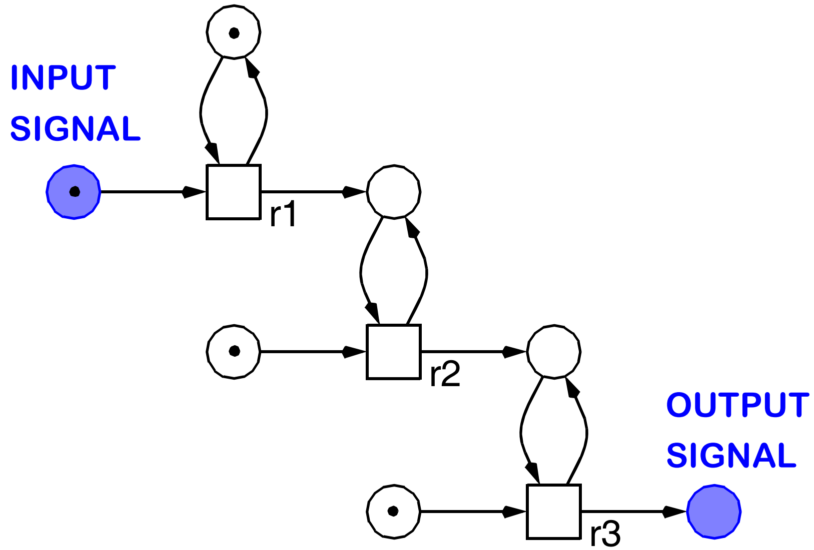
- **metabolic networks**

  -> *substance flows*



- **signal transduction networks**

  -> *signal flows*



Typical Basic Structures III

- **metabolic networks**

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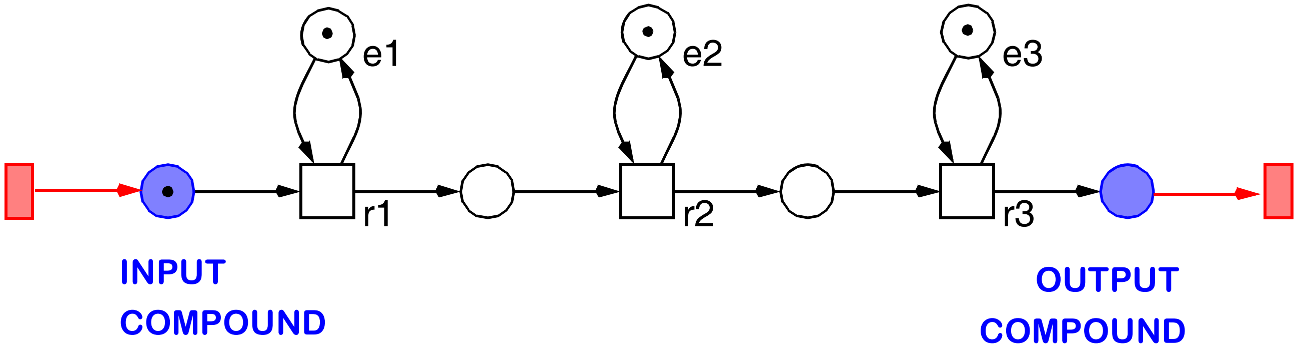

- **signal transduction networks**

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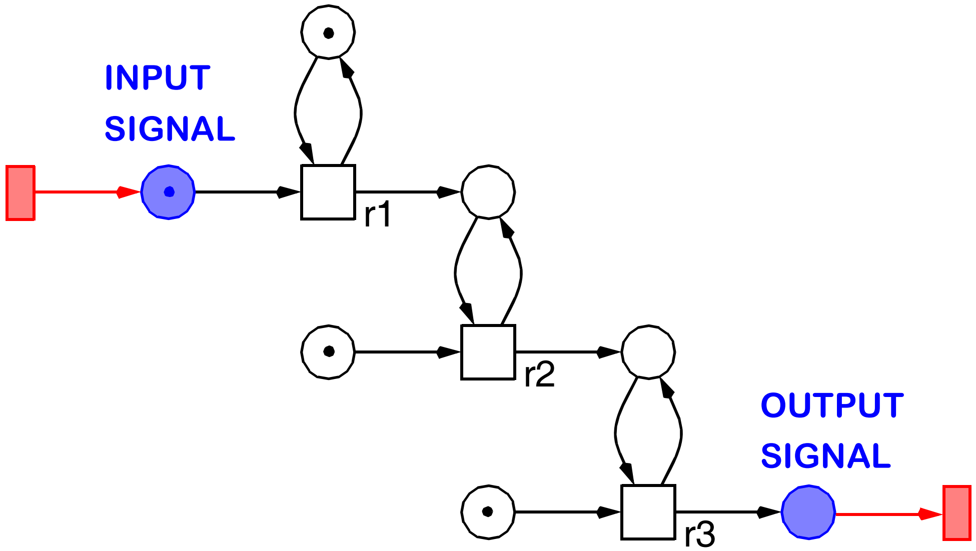
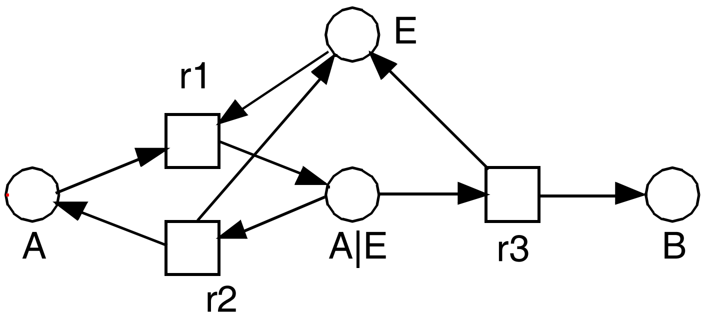


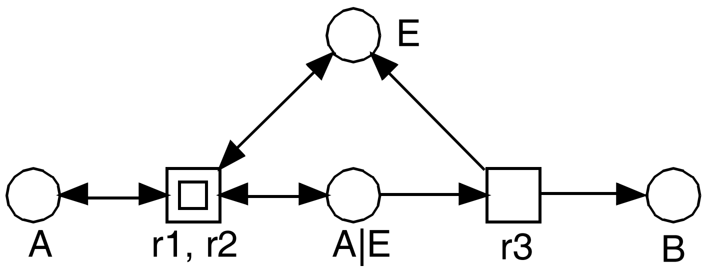
-> OPEN / CLOSED SYSTEMS

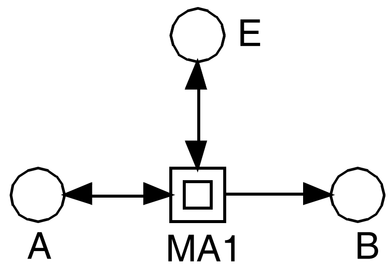
TYPICAL BASIC STRUCTURES IV

E
A <--> A|E --> B

enzymatic reaction,
mass-action approach 1



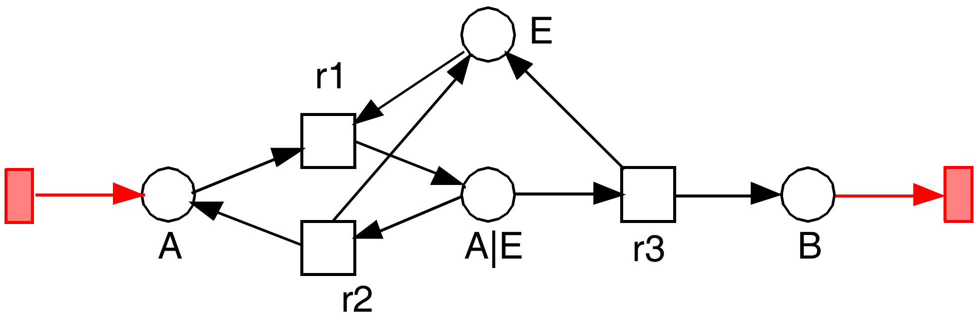


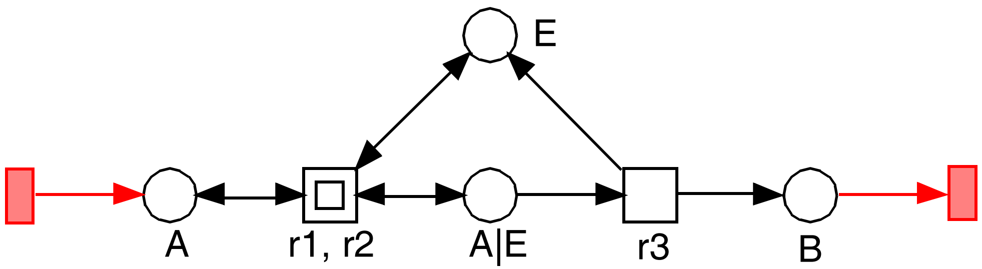


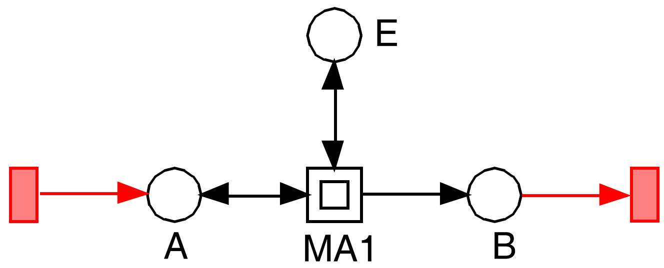
TYPICAL BASIC STRUCTURES IV

$$A \overset{E}{\longleftrightarrow} A|E \longrightarrow B$$

enzymatic reaction,
mass-action approach 1

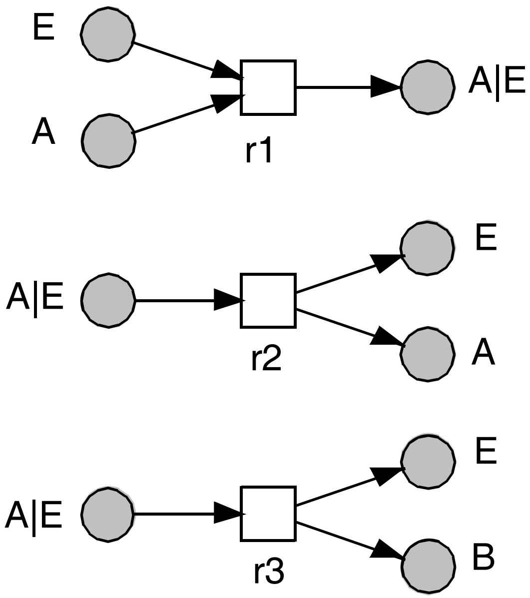






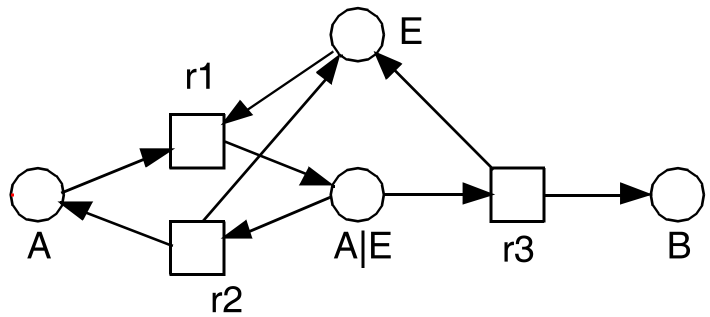
Layout Options

reaction-centred view

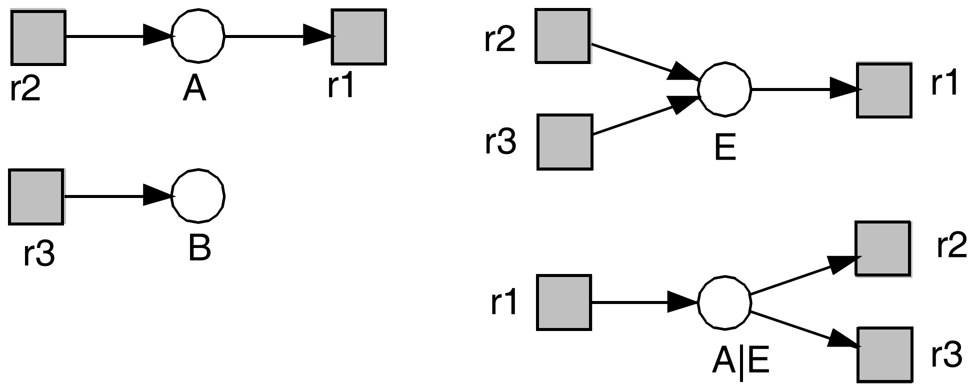


logical nodes
(fusion nodes)

process-oriented view

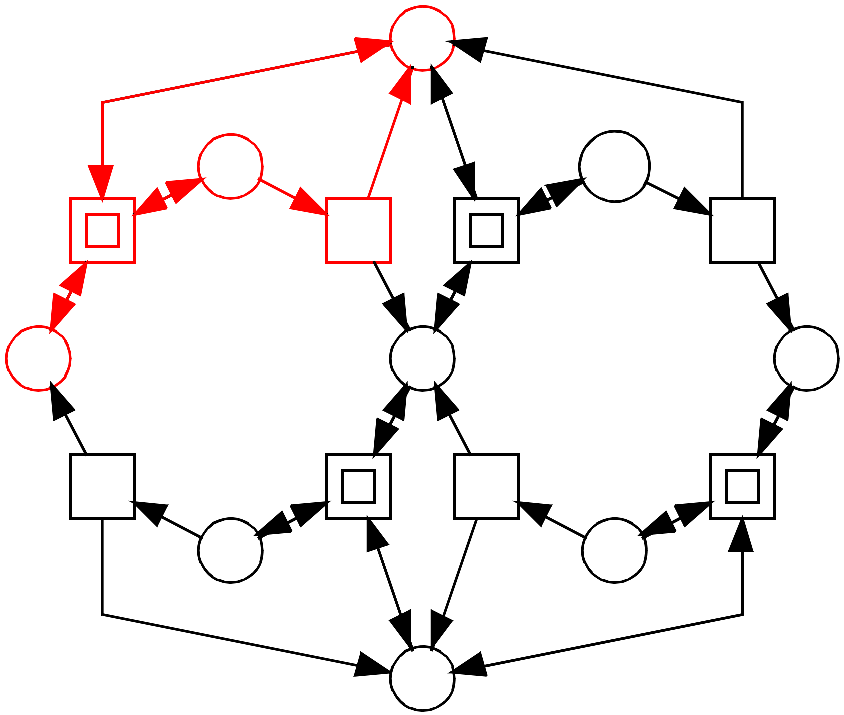


species-centred view

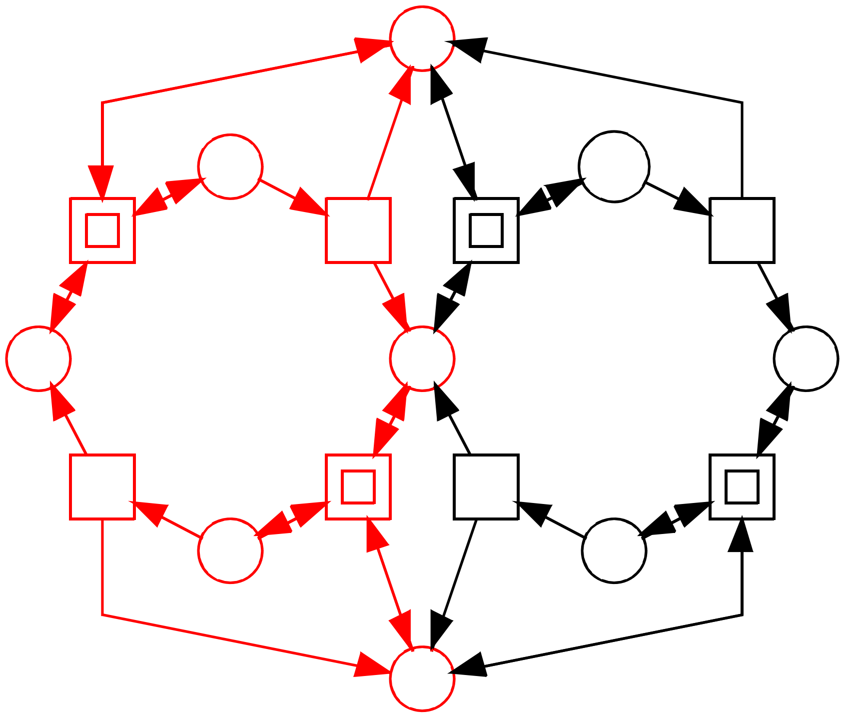


DOUBLE PHOSPHOYLATION/DEPHOSPHORYLATION

SINGLE
MASS-ACTION STEP

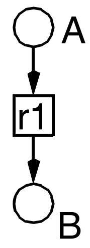


DOUBLE PHOSPHOYLATION / DEPHOSPHORYLATION



SINGLE
PHOSPHOYLATION / DEPHOSPHORYLATION

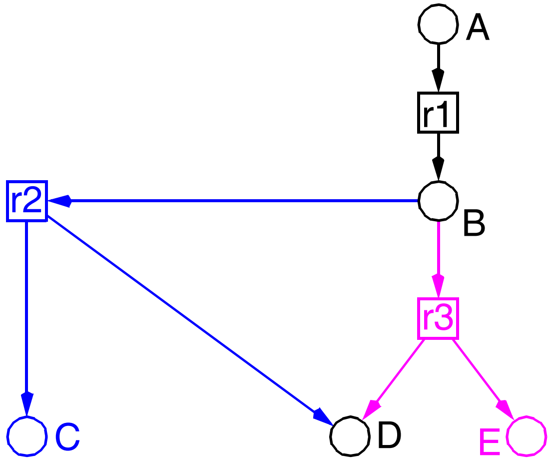
r1: A -> B



r1: A -> B

r2: B -> C + D

r3: B -> D + E



-> alternative reactions

r1: A -> B

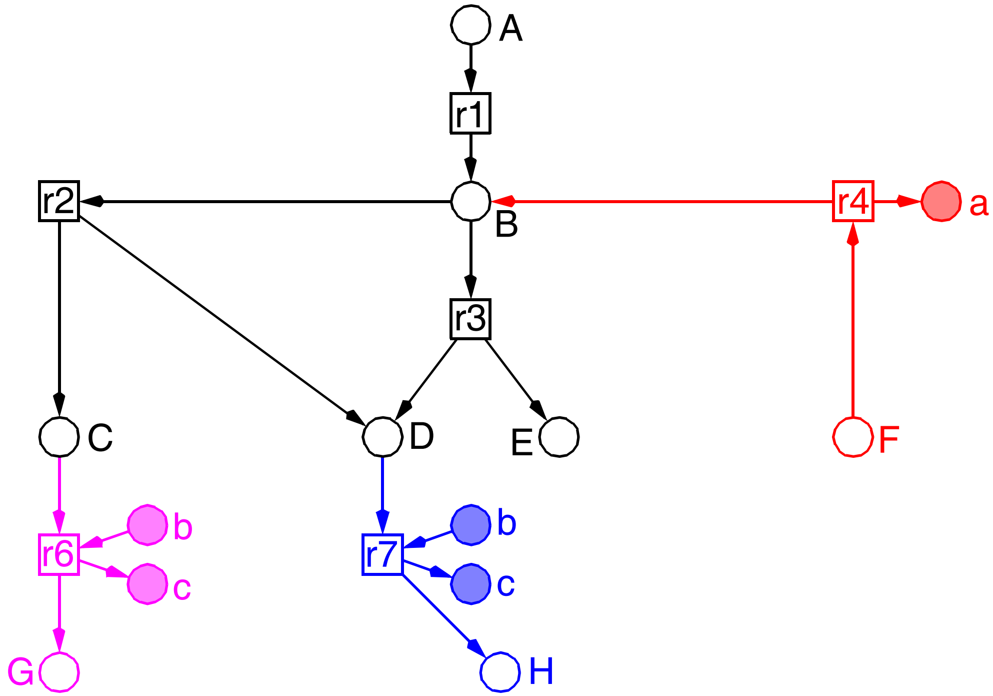
r2: B -> C + D

r3: B -> D + E

r4: F -> B + a

r6: C + b -> G + c

r7: D + b -> H + c



-> concurrent reactions

r1: A -> B

r2: B -> C + D

r3: B -> D + E

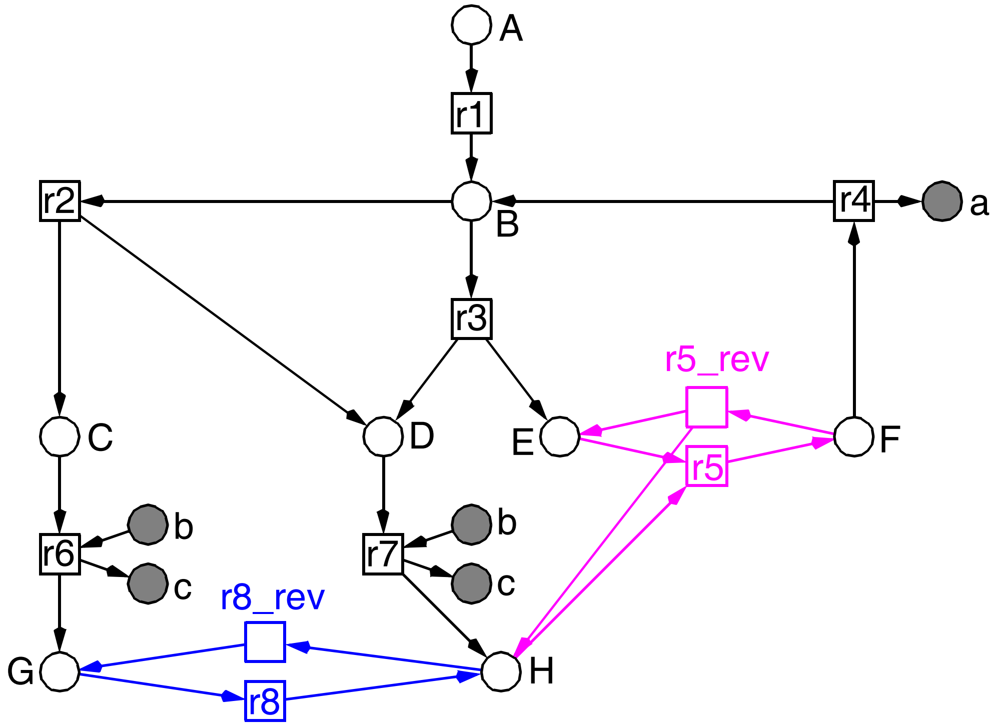
r4: F -> B + a

r5: E + H <-> F

r6: C + b -> G + c

r7: D + b -> H + c

r8: H <-> G



-> reversible reactions

r1: A -> B

r2: B -> C + D

r3: B -> D + E

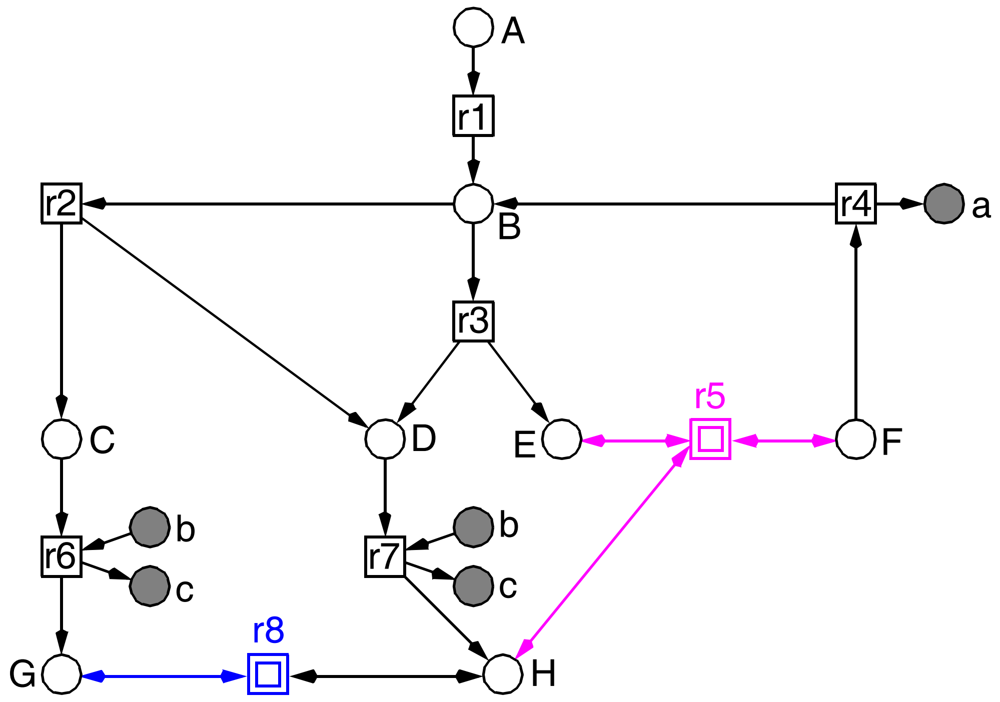
r4: F -> B + a

r5: E + H <-> F

r6: C + b -> G + c

r7: D + b -> H + c

r8: H <-> G



-> reversible reactions
- hierarchical nodes

r1: A -> B

r2: B -> C + D

r3: B -> D + E

r4: F -> B + a

r5: E + H <-> F

r6: C + b -> G + c

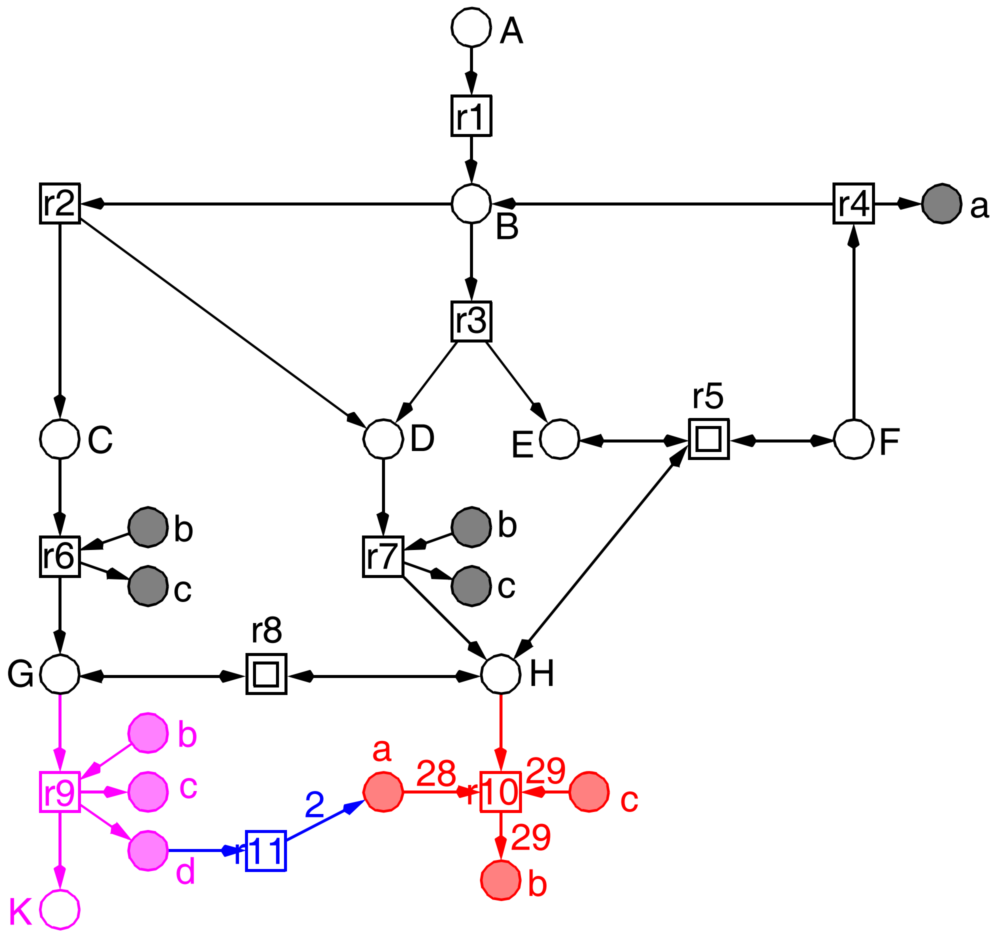
r7: D + b -> H + c

r8: H <-> G

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

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

r11: d -> 2a



r1: A -> B

r2: B -> C + D

r3: B -> D + E

r4: F -> B + a

r5: E + H <-> F

r6: C + b -> G + c

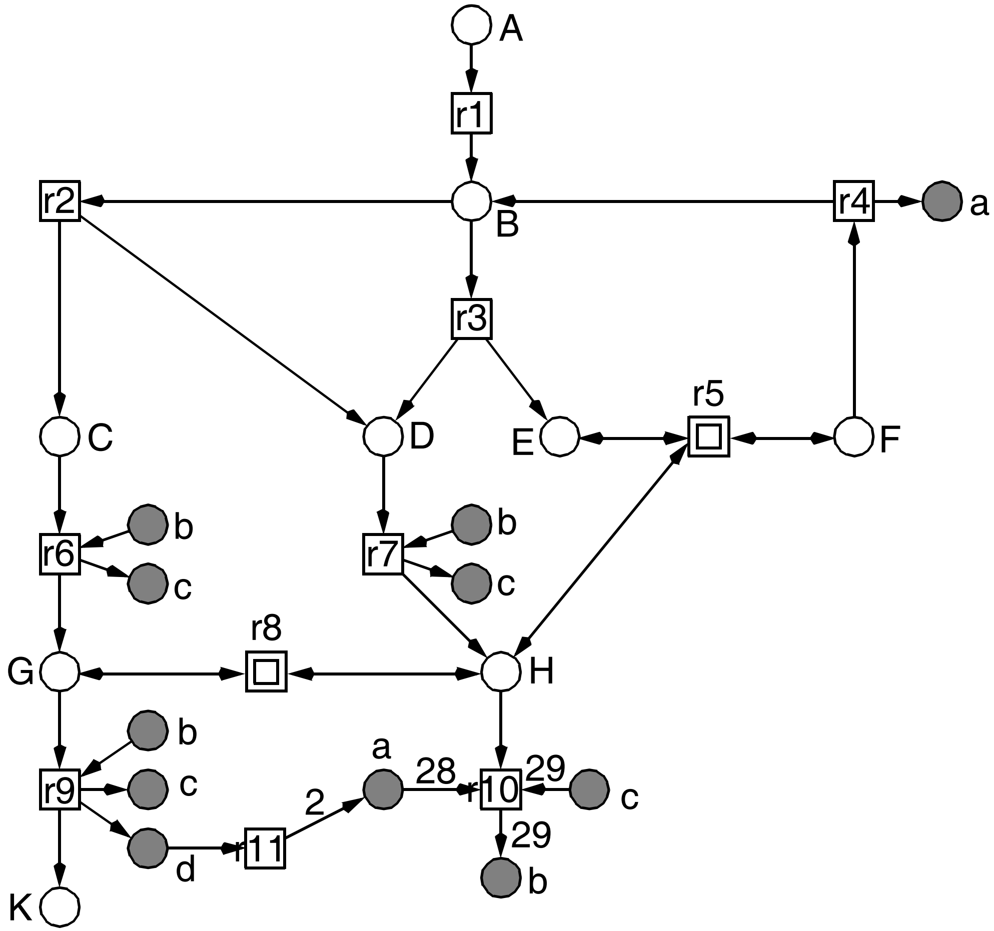
r7: D + b -> H + c

r8: H <-> G

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

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

r11: d -> 2a



r1: A -> B

r2: B -> C + D

r3: B -> D + E

r4: F -> B + a

r5: E + H <-> F

r6: C + b -> G + c

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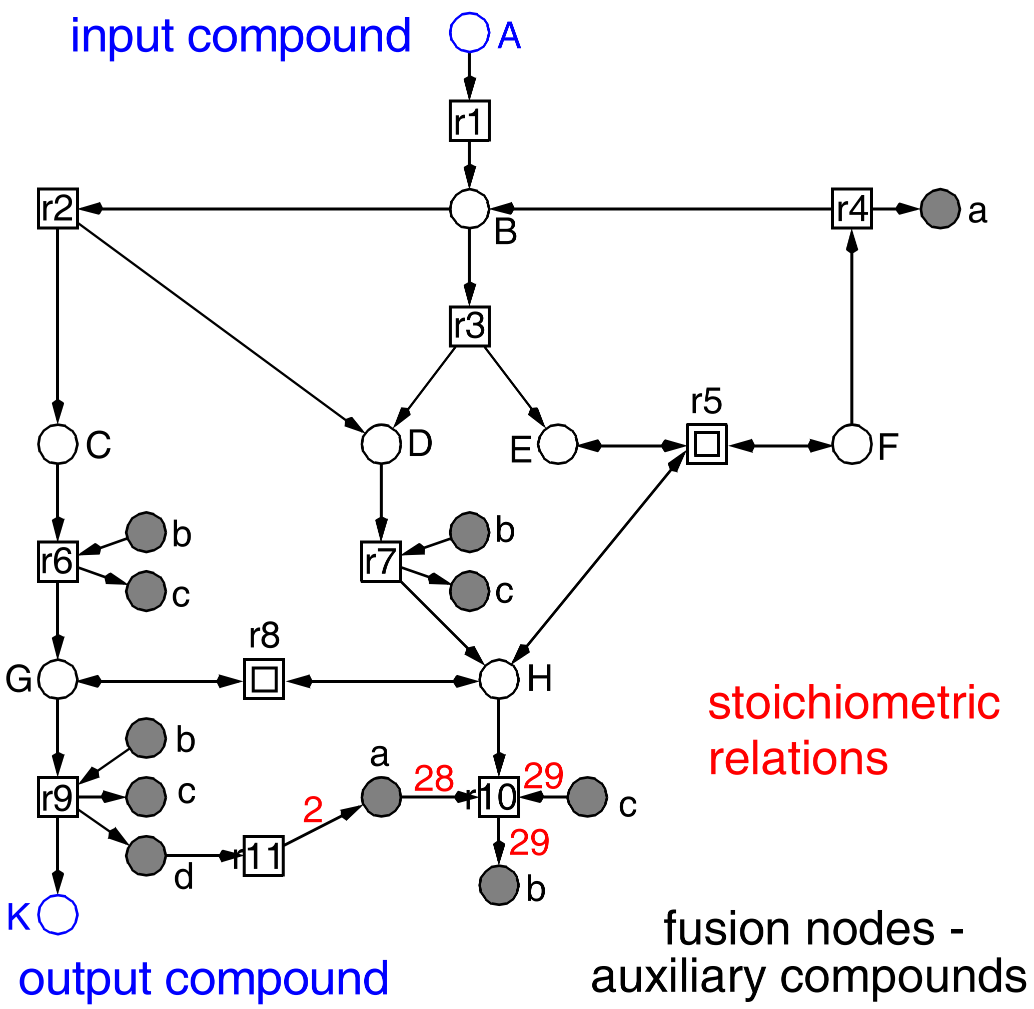
r10: H + 28a + 29c -> 29b

r11: d -> 2a



r1: A -> B

r2: B -> C + D

r3: B -> D + E

r4: F -> B + a

r5: E + H <-> F

r6: C + b -> G + c

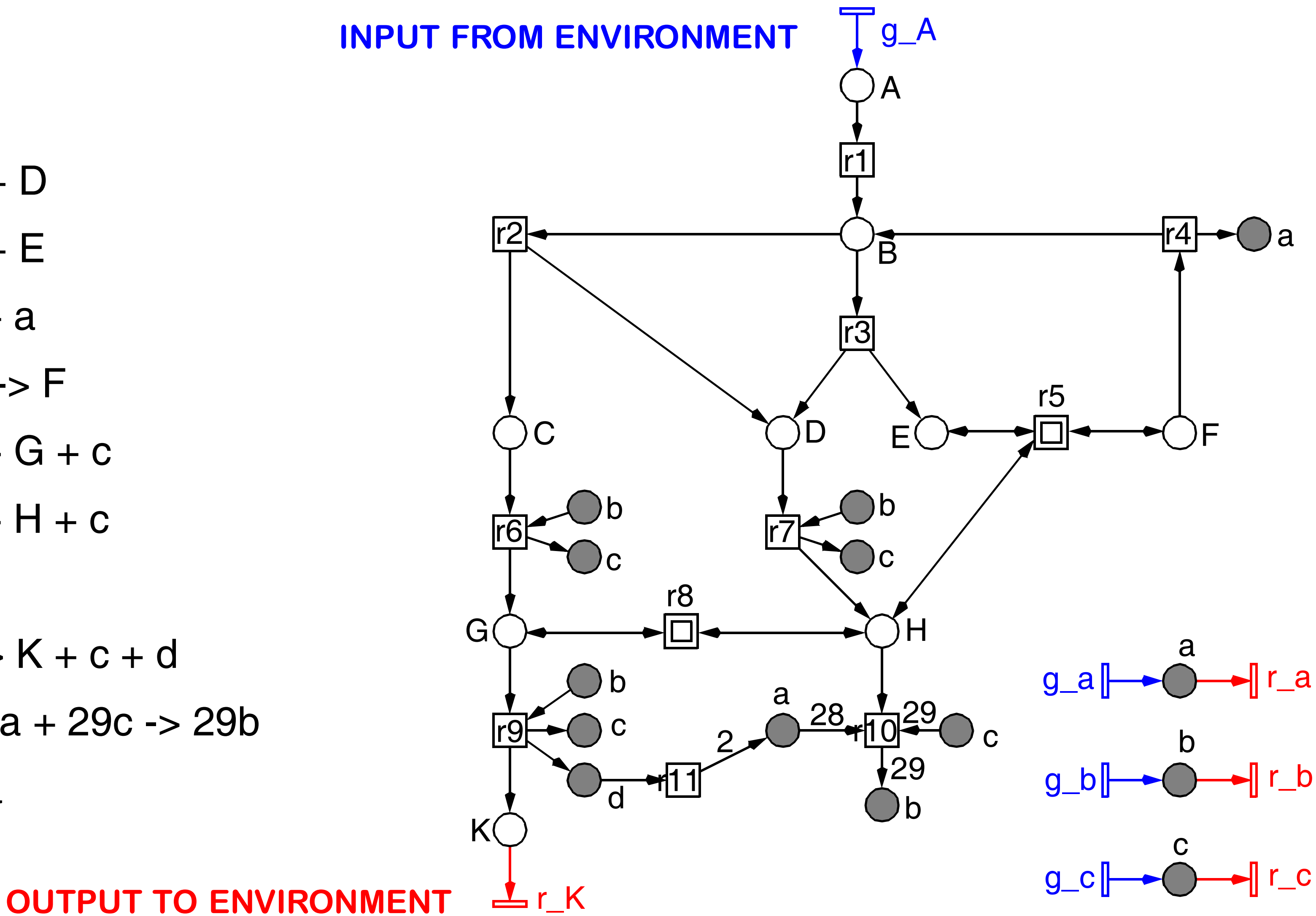
r7: D + b -> H + c

r8: H <-> G

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

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

r11: d -> 2a



PETRI NET ELEMENTS, INTERPRETATIONS

- **METABOLIC NETWORKS**
  . . . **SIGNAL TRANSDUCTION NETWORKS**
  . . . **GENE REGULATORY NETWORKS**

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

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

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

BIOCHEMICAL PETRI NETS, SUMMARY

- **biochemical networks**

  -> *networks of (abstract) chemical reactions*

- **biochemically interpreted Petri net**

  -> *partial order sequences of chemical reactions (= elementary actions)*
  transforming input into output compounds / signals

  [respecting the given stoichiometric relations, if any]

  -> *set of all pathways*
  from the input to the output compounds / signals

  [respecting the stoichiometric relations, if any]

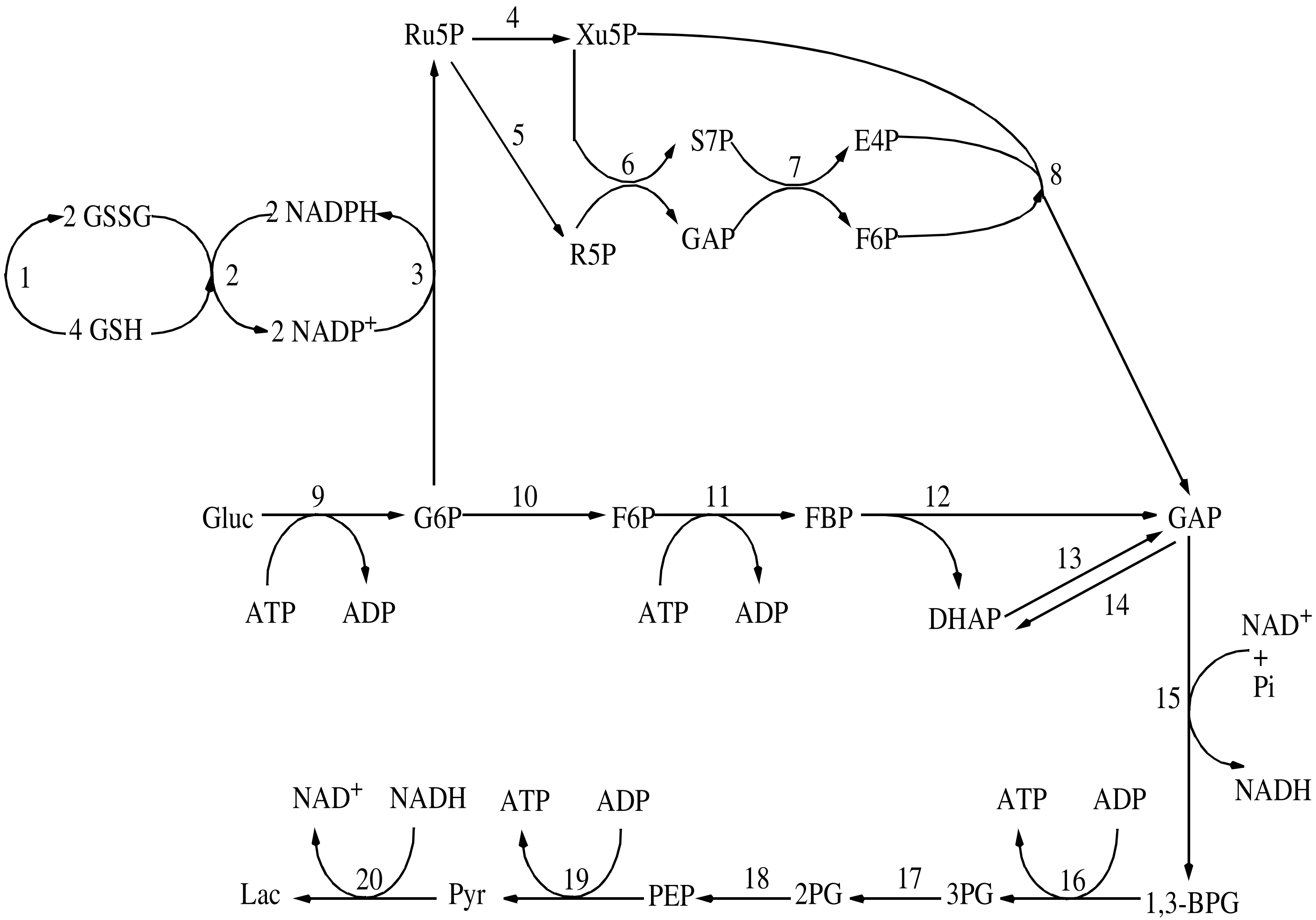
- **pathway**

  -> *self-contained partial order sequence of elementary (re-) actions*

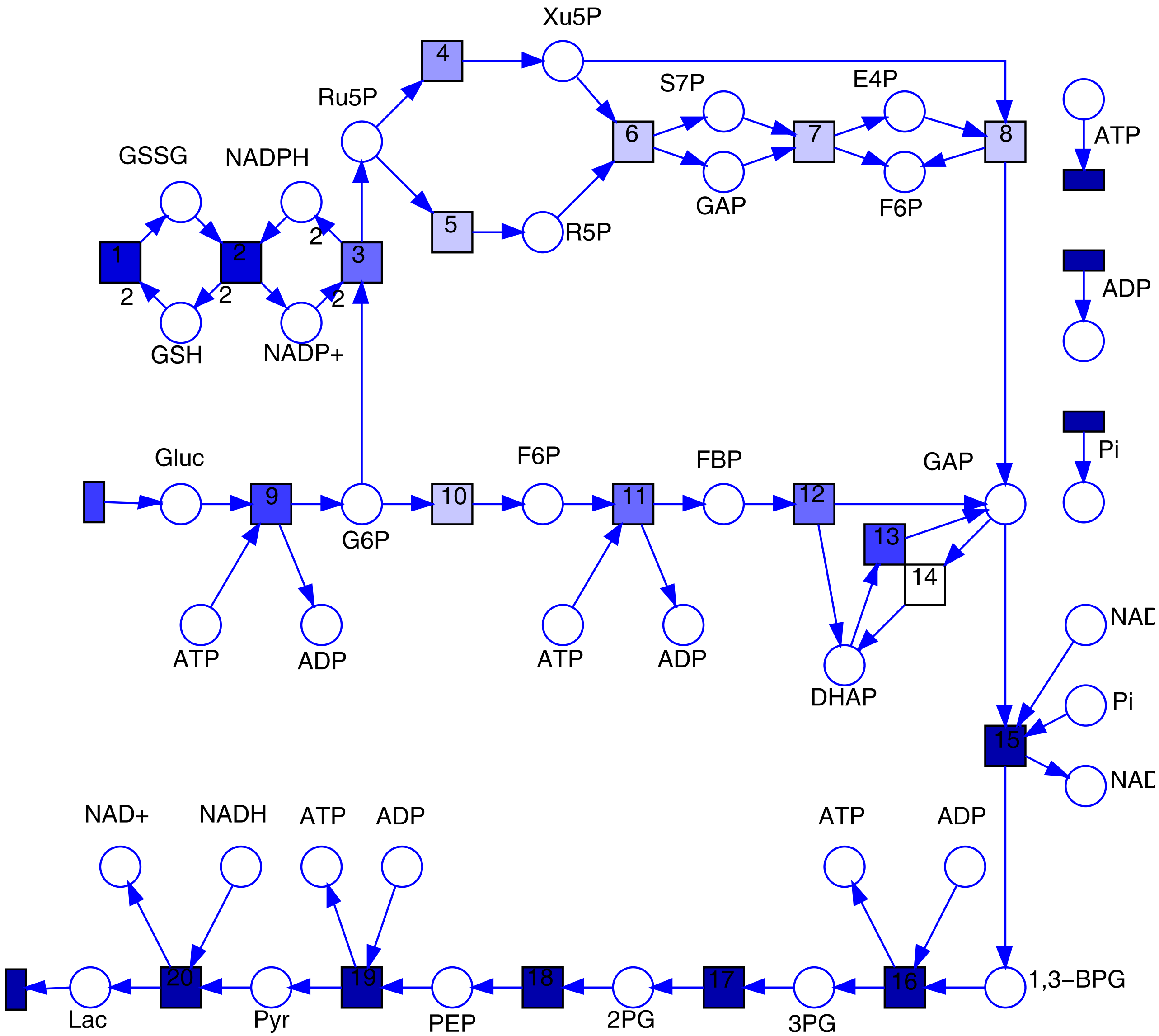
BIO PETRI NETS - SOME EXAMPLES

Ex1 - Glycolysis and Pentose Phosphate Pathway

[Reddy 1993]



Ex1 - Glycolysis and Pentose Phosphate Pathway

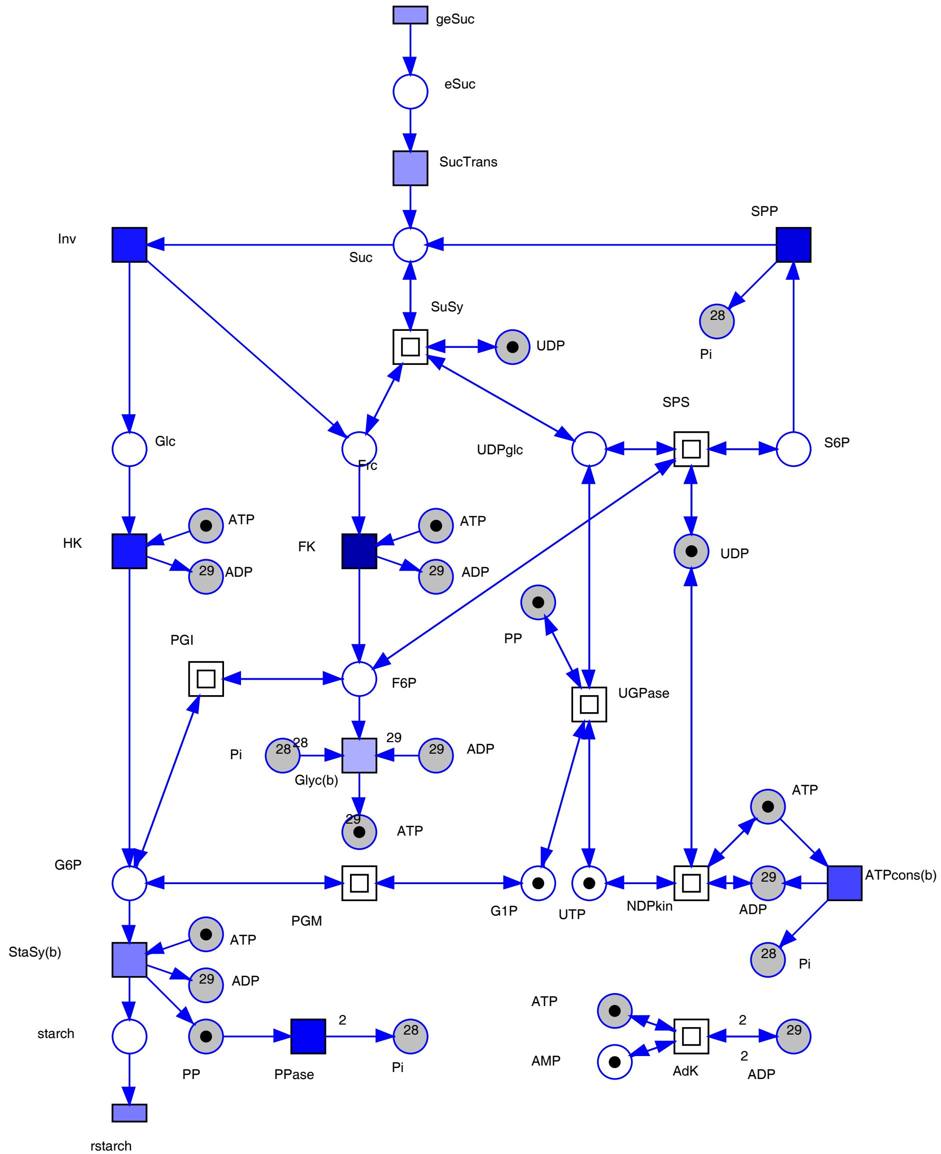


[Reddy 1993]

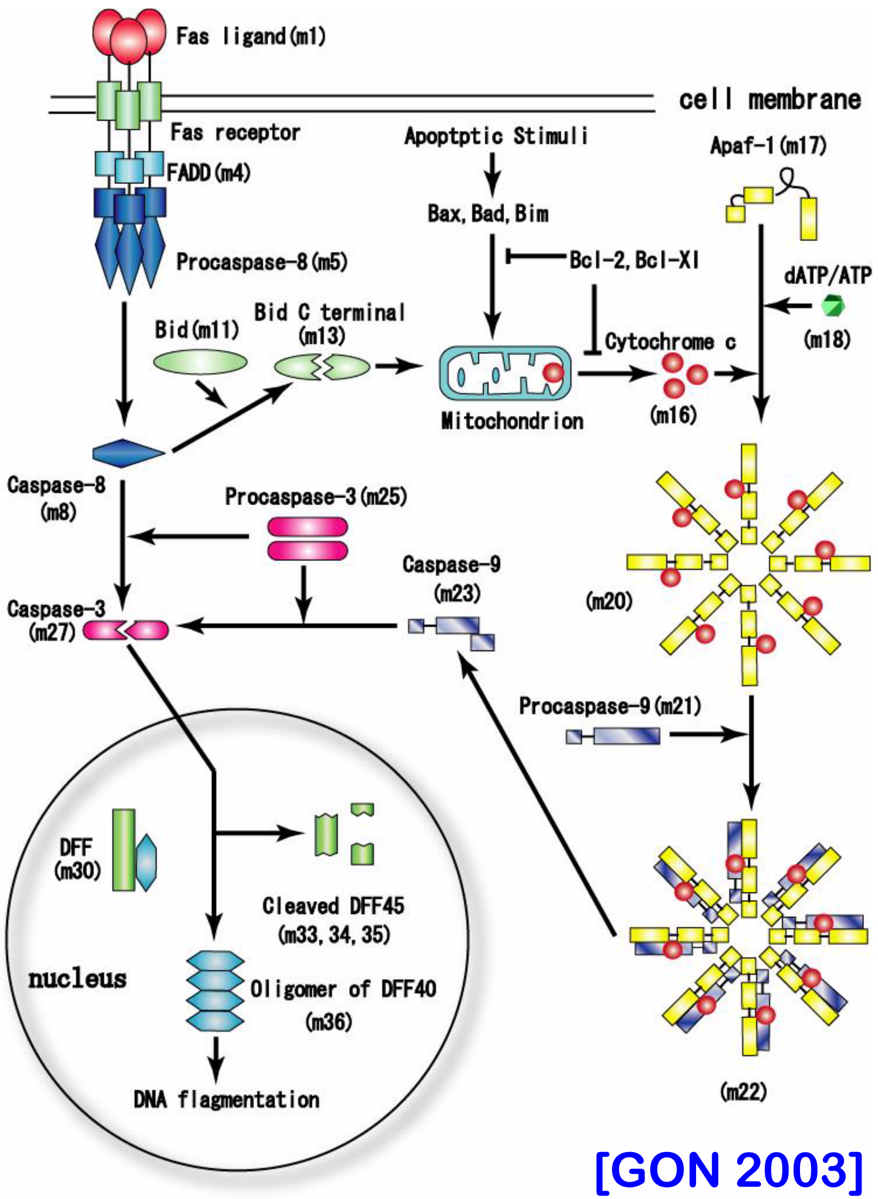
[Koch, Heiner 2008]

Ex2 - Carbon Metabolism in Potato Tuber

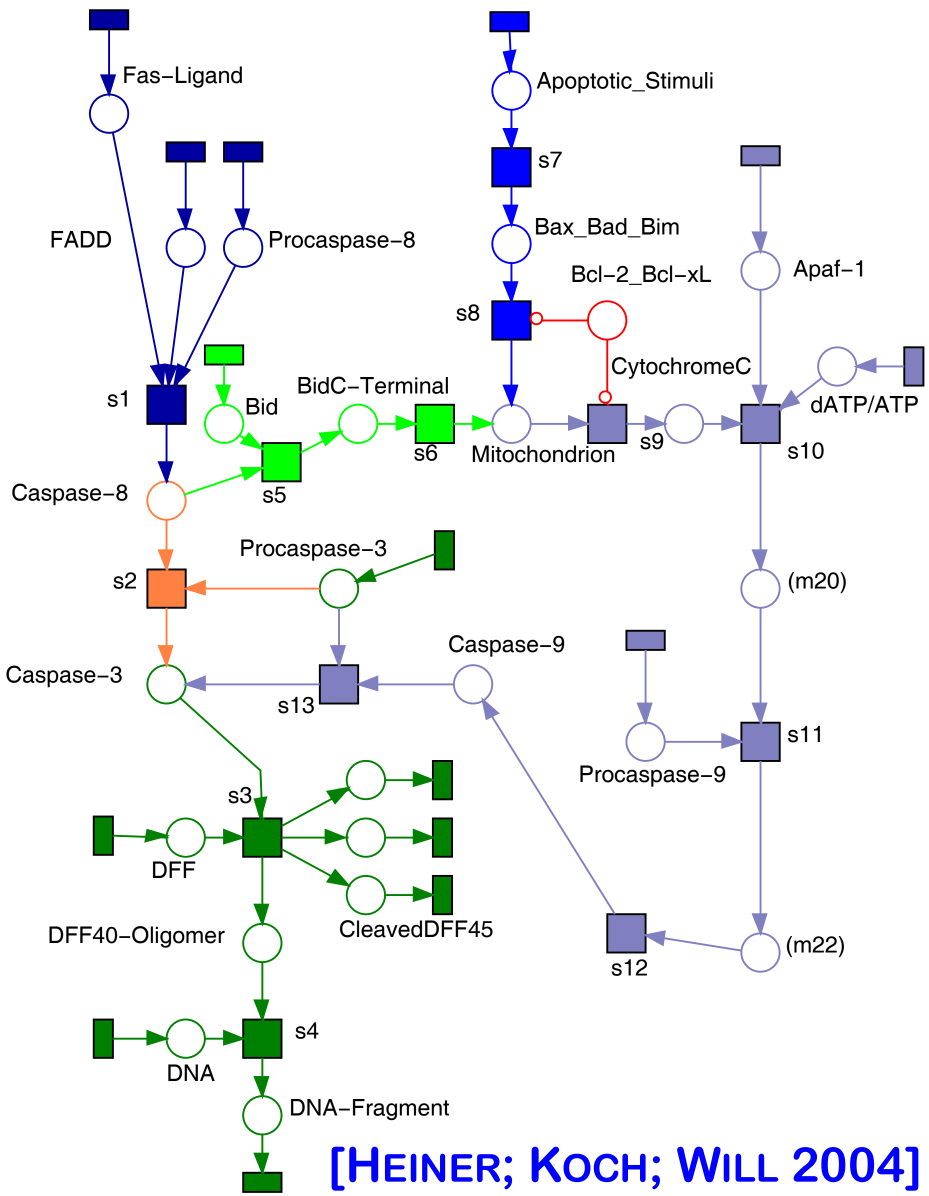
[KOCH; JUNKER; HEINER 2005]



Ex3: Apoptosis in Mammalian Cells

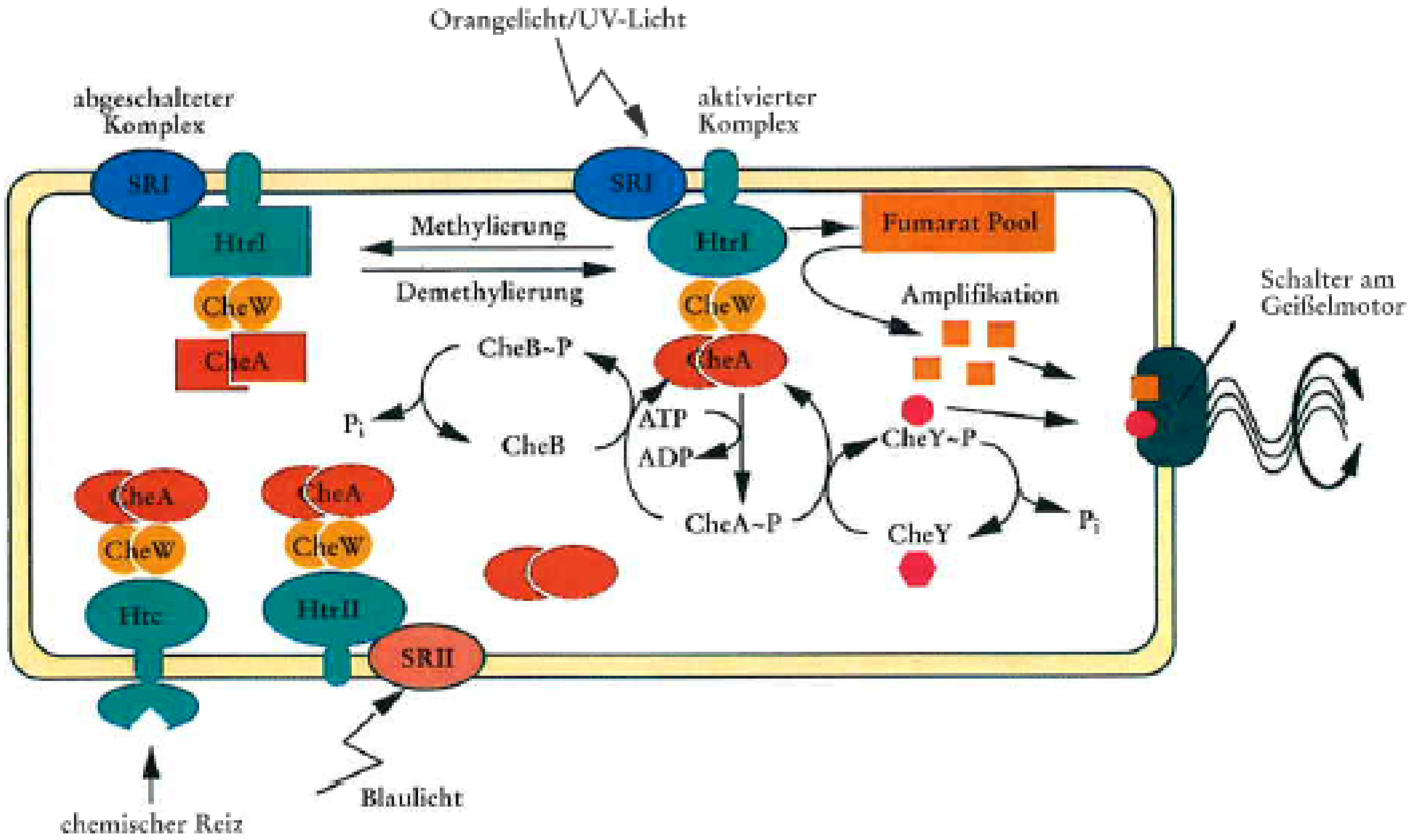


[GON 2003]



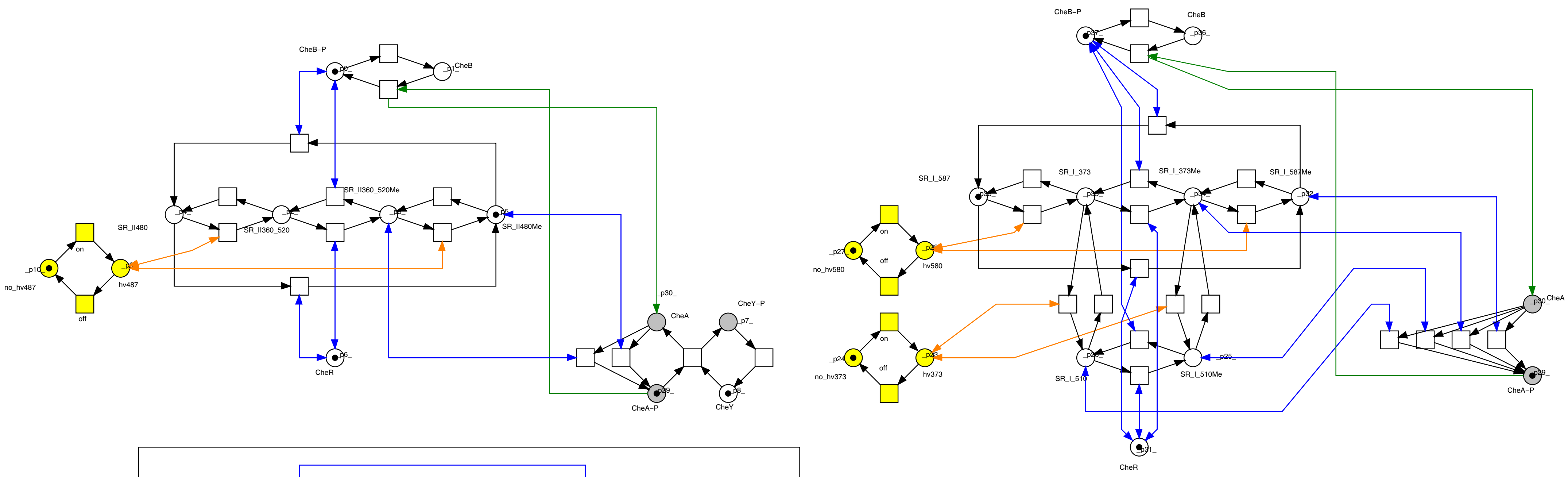
[Heiner; Koch; Will 2004]

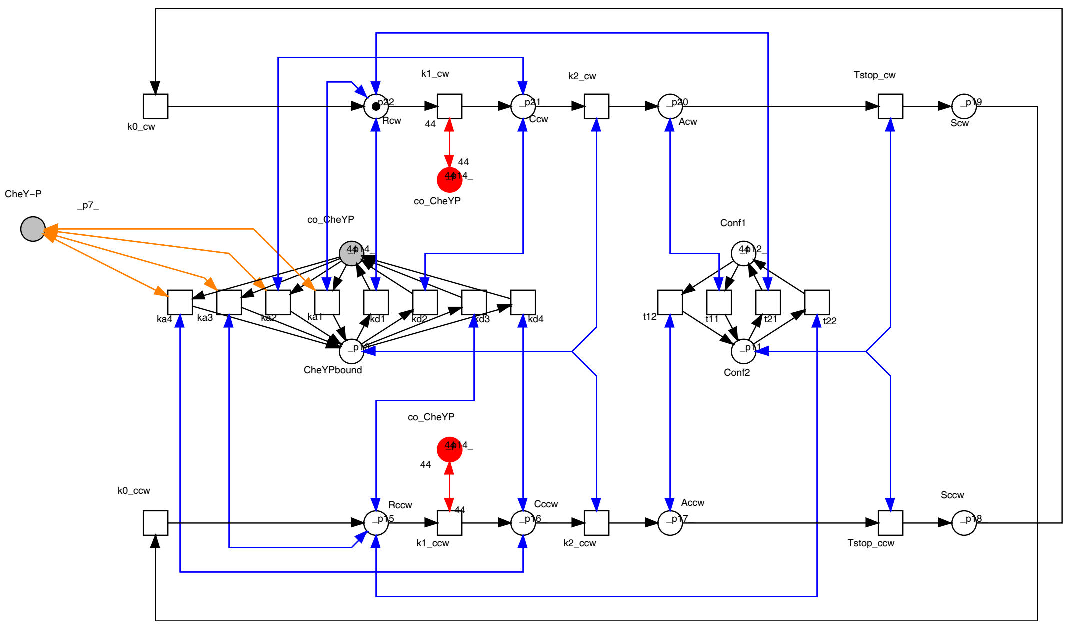
Ex4 - Switch Cycle Halobacterium Salinarum



[Marwan; Oesterhelt 1999]

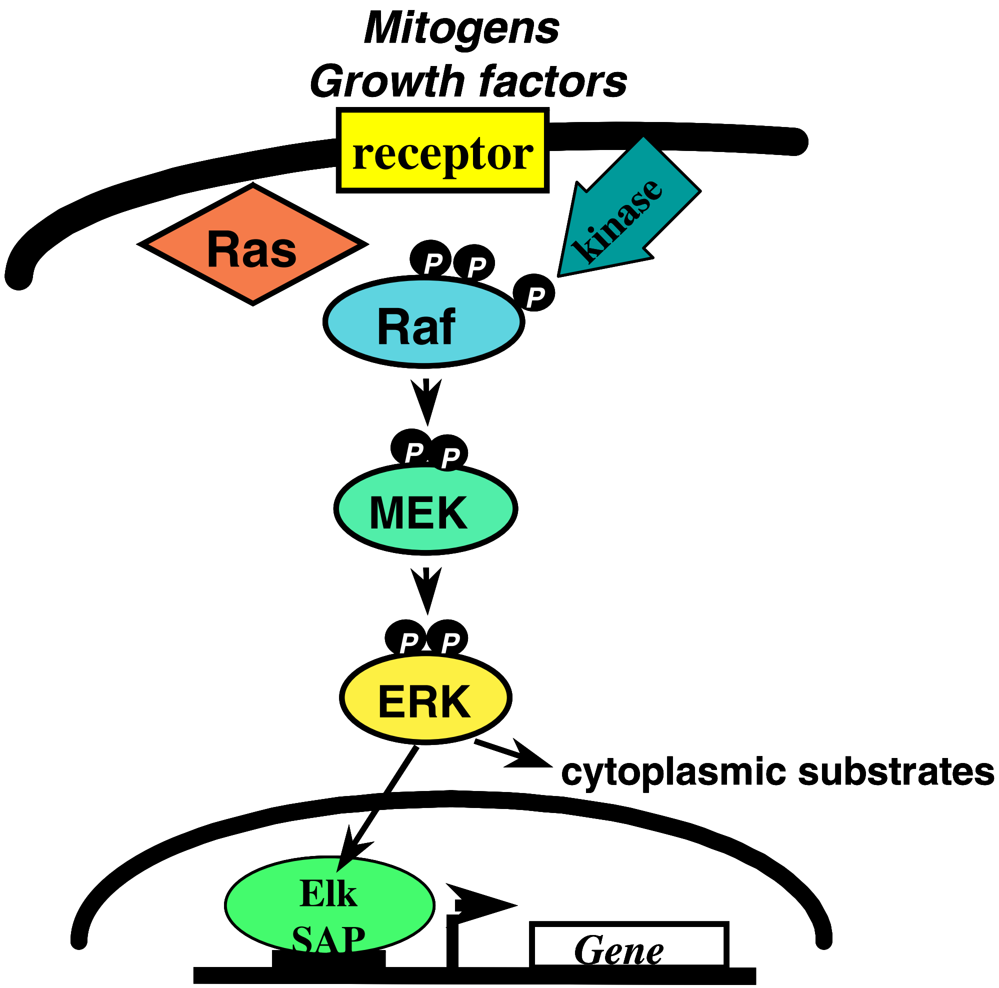
Ex4 - Switch Cycle Halobacterium Salinarum





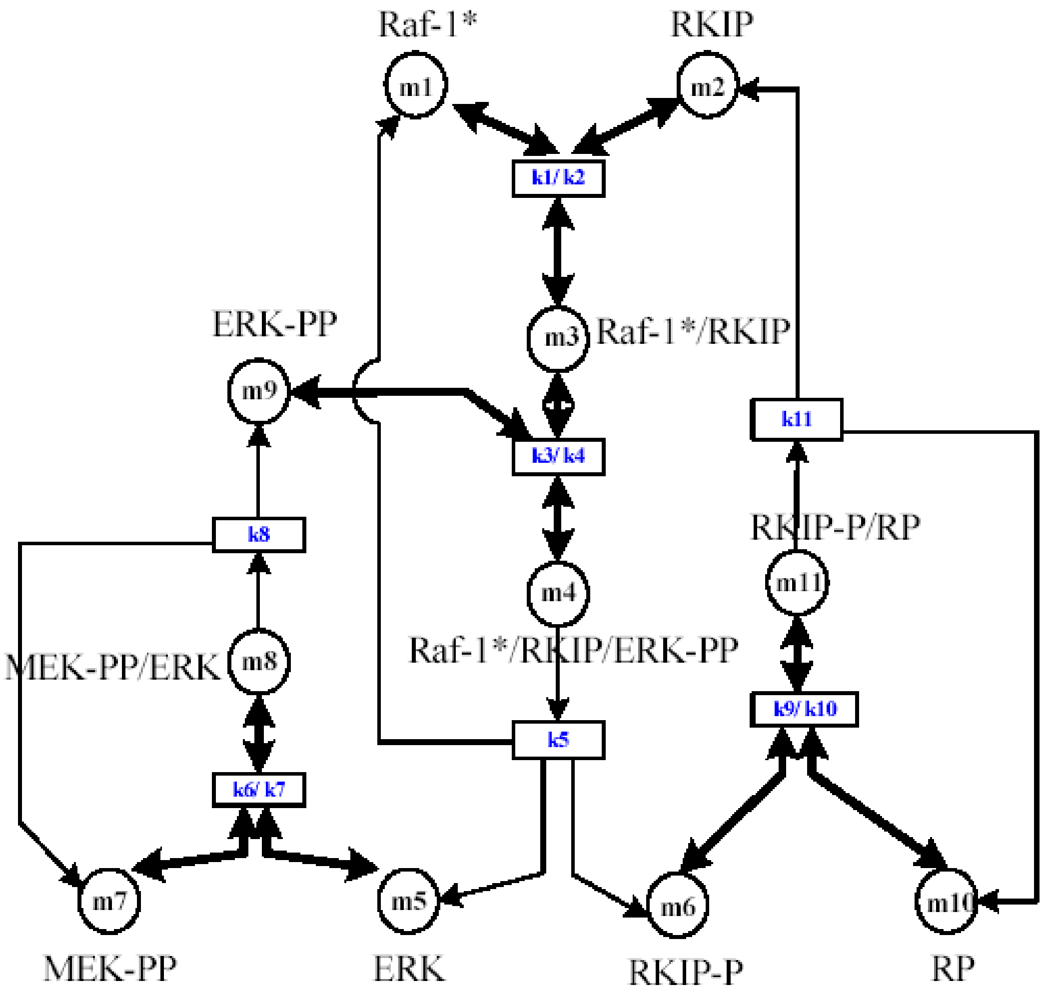
[UNPUBLISHED]

…one pathway…


Mitogens
Growth factors
receptor
Ras
kinase
P
P
P
Raf
P
P
MEK
P
P
ERK
cytoplasmic substrates
Elk
SAP
Gene

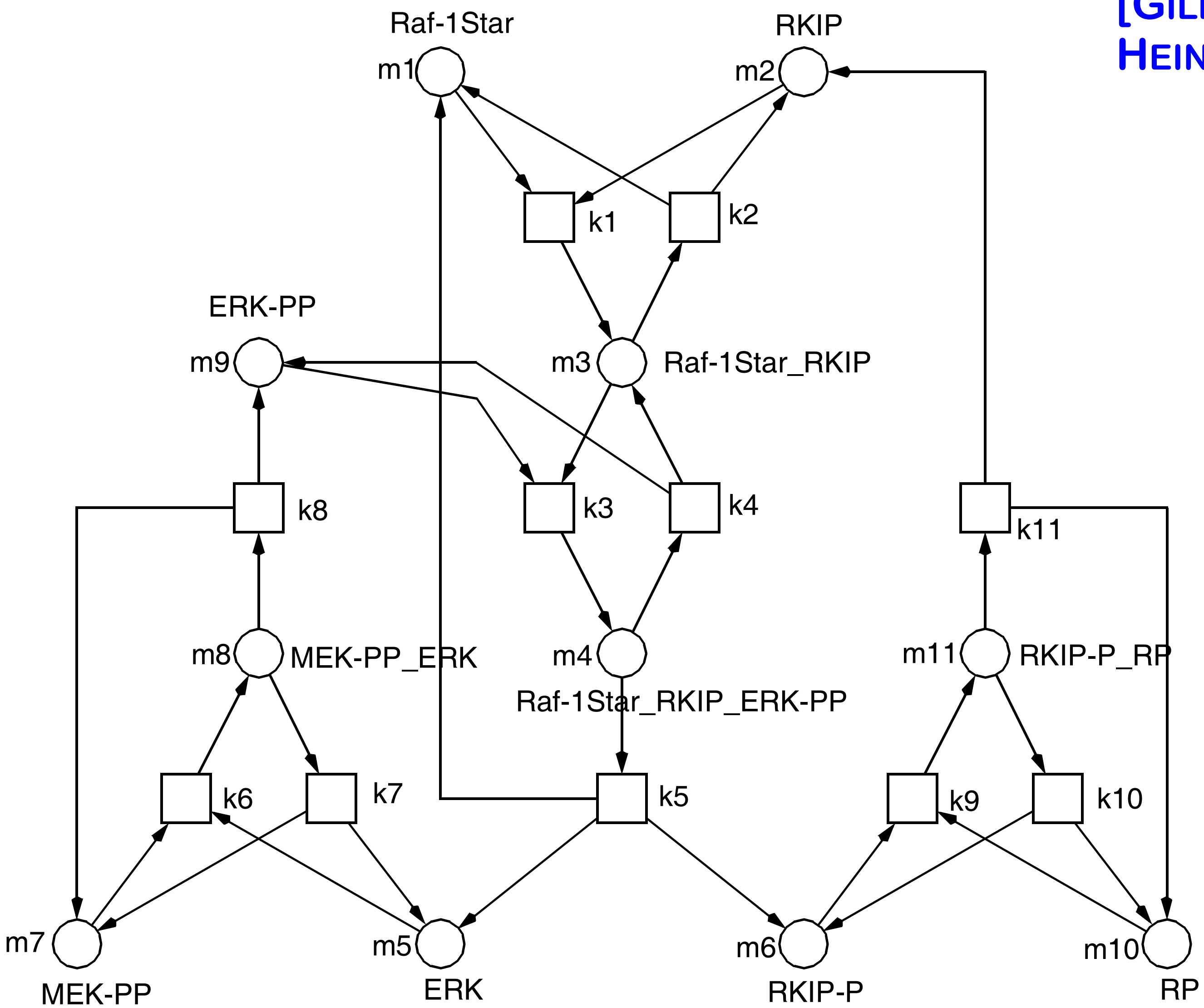
EX5 - THE RKIP PATHWAY

[Cho et al., CMSB 2003]



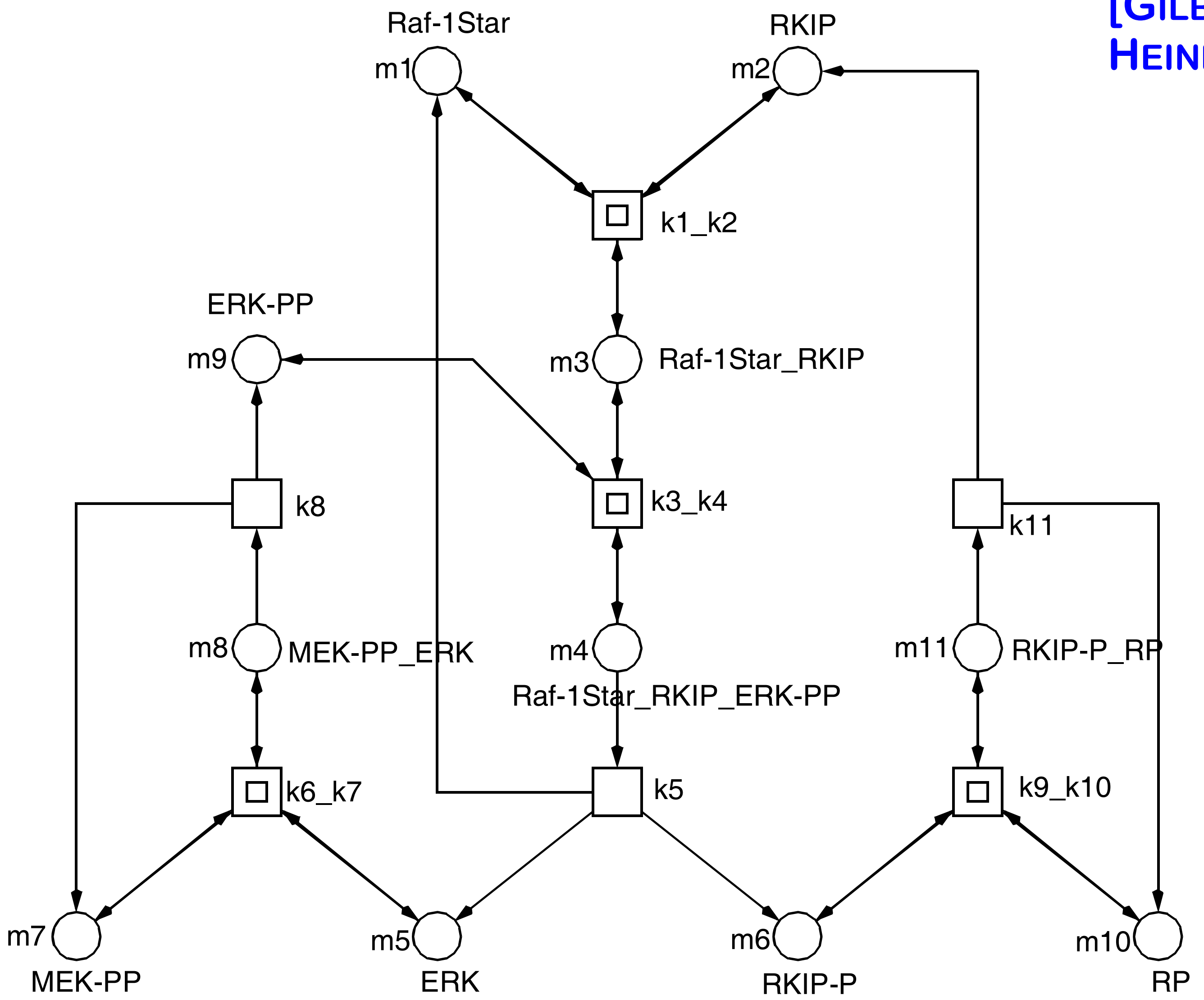
Ex5 - The RKIP Pathway, Petri Net

[Gilbert, Heiner 2006]



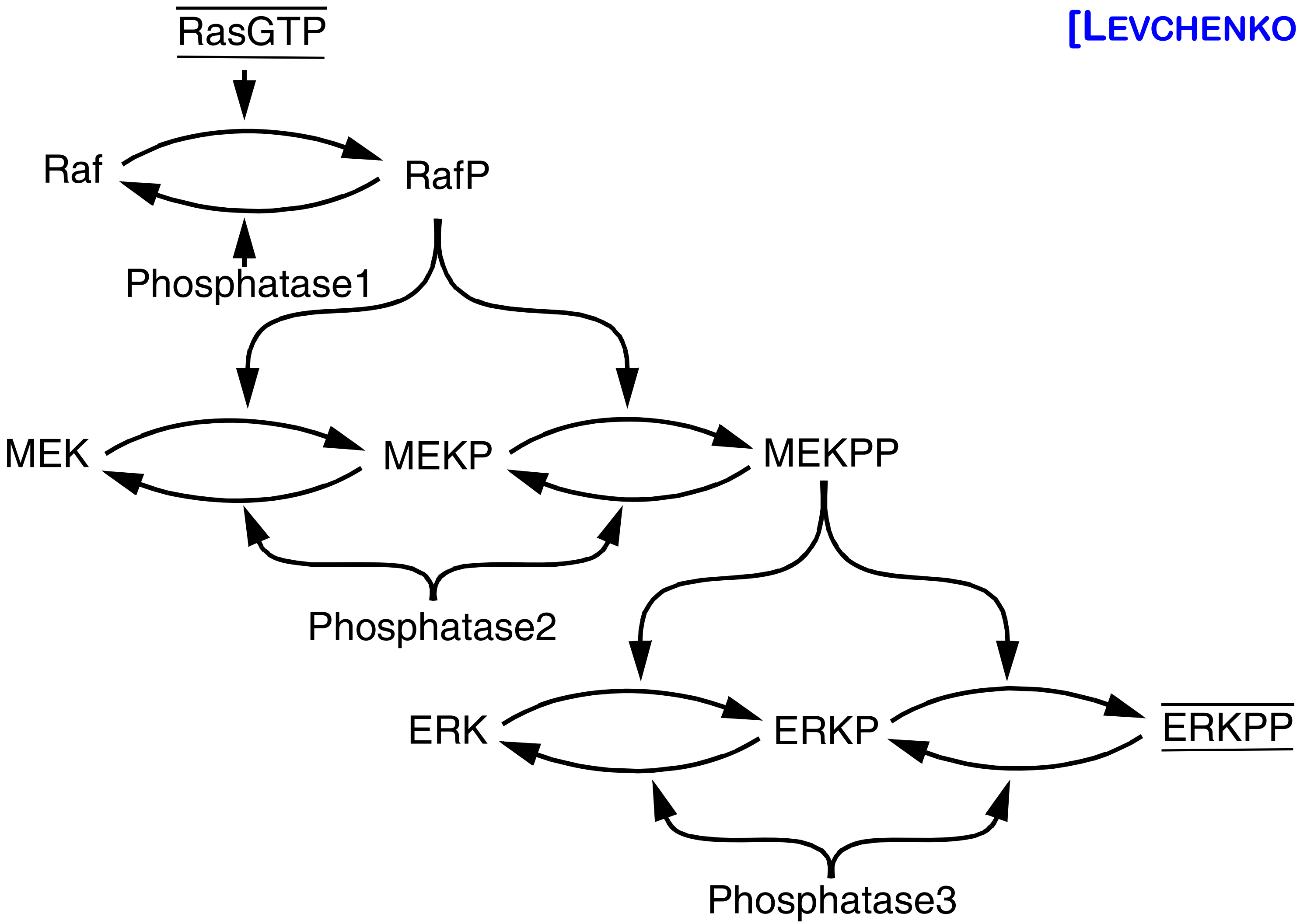
Ex5 - The RKIP Pathway, Hierarchical Petri Net

[Gilbert, Heiner 2006]

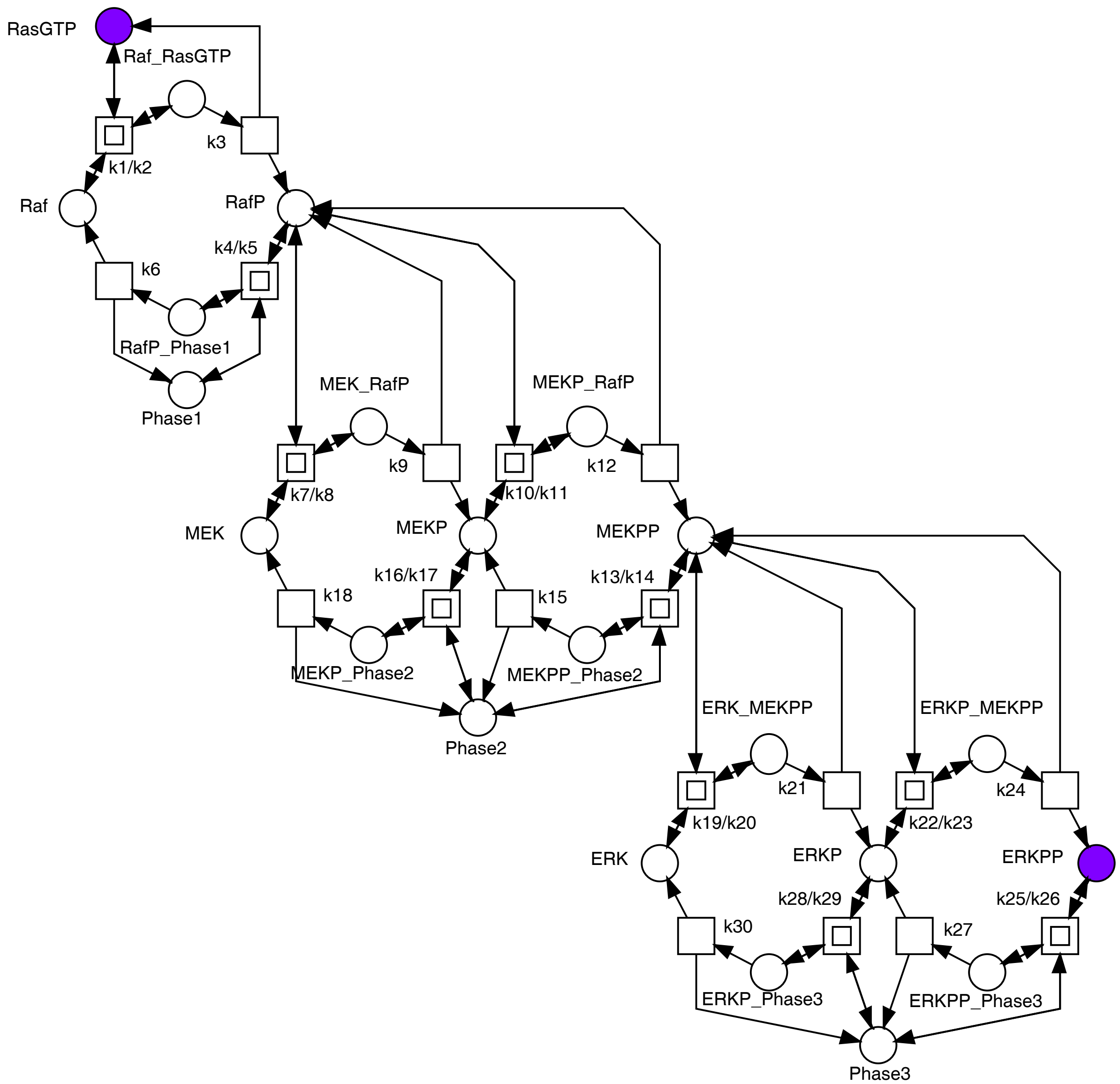


EX6 - SIGNALLING CASCADE

[LEVCHENKO 2000]



Ex6 - Signalling cascade



[Gilbert, Heiner, Lehrack 2007]

[Heiner, Gilbert, Donaldson 2008]

END OF

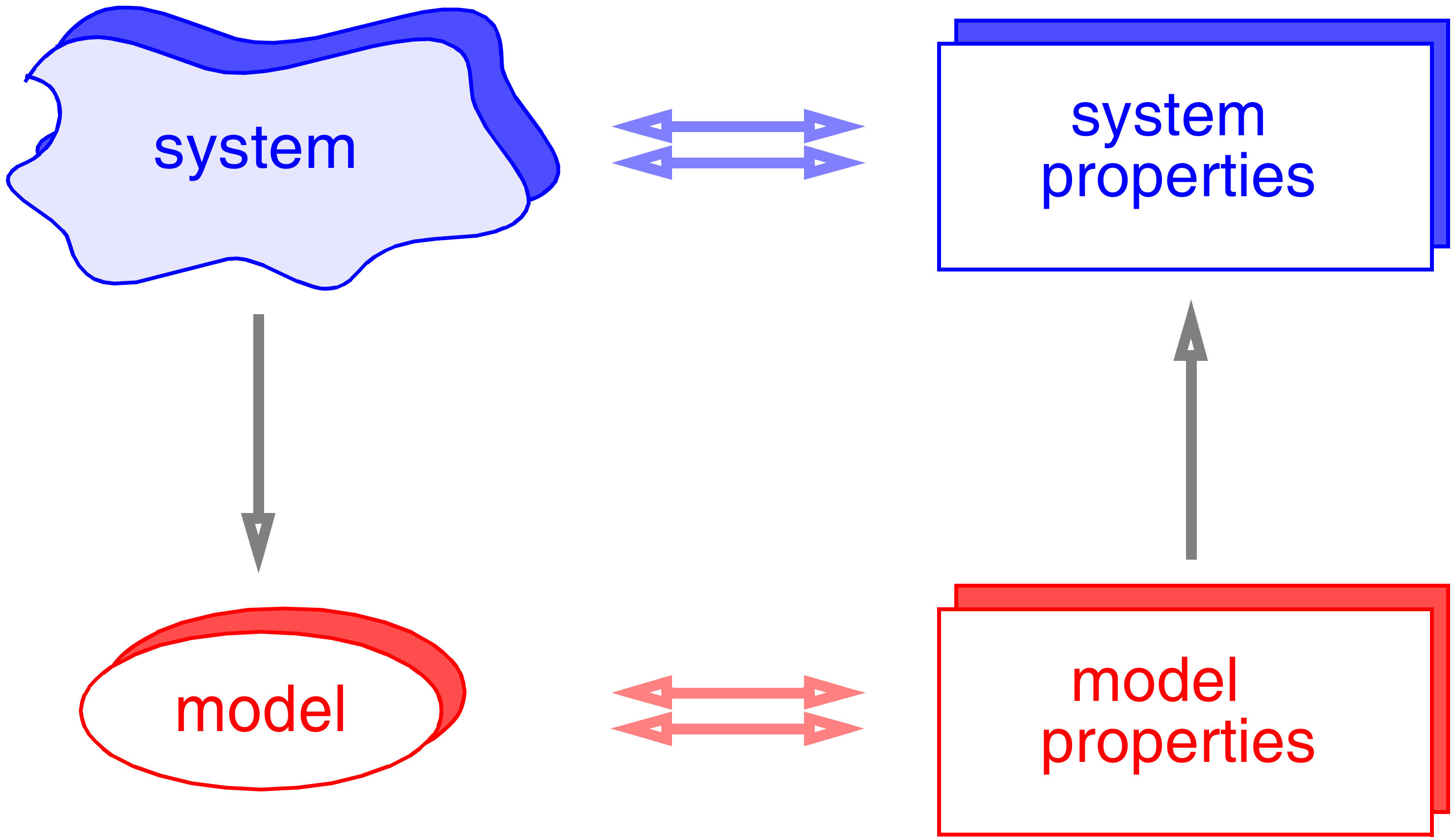
PART I

Bio Petri Nets, Part II

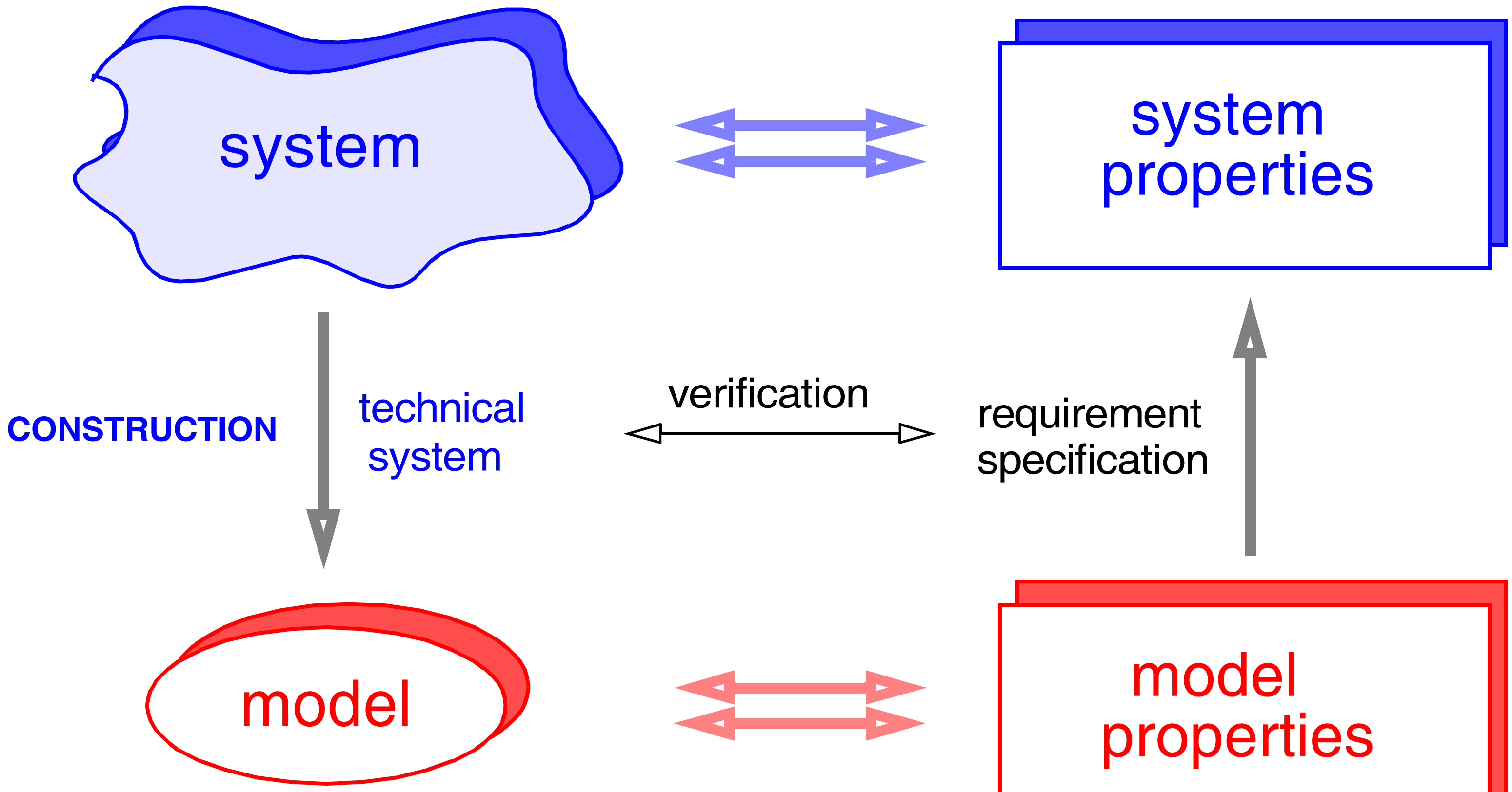
Model-based System Analysis

monika.heiner@tu-cottbus.de

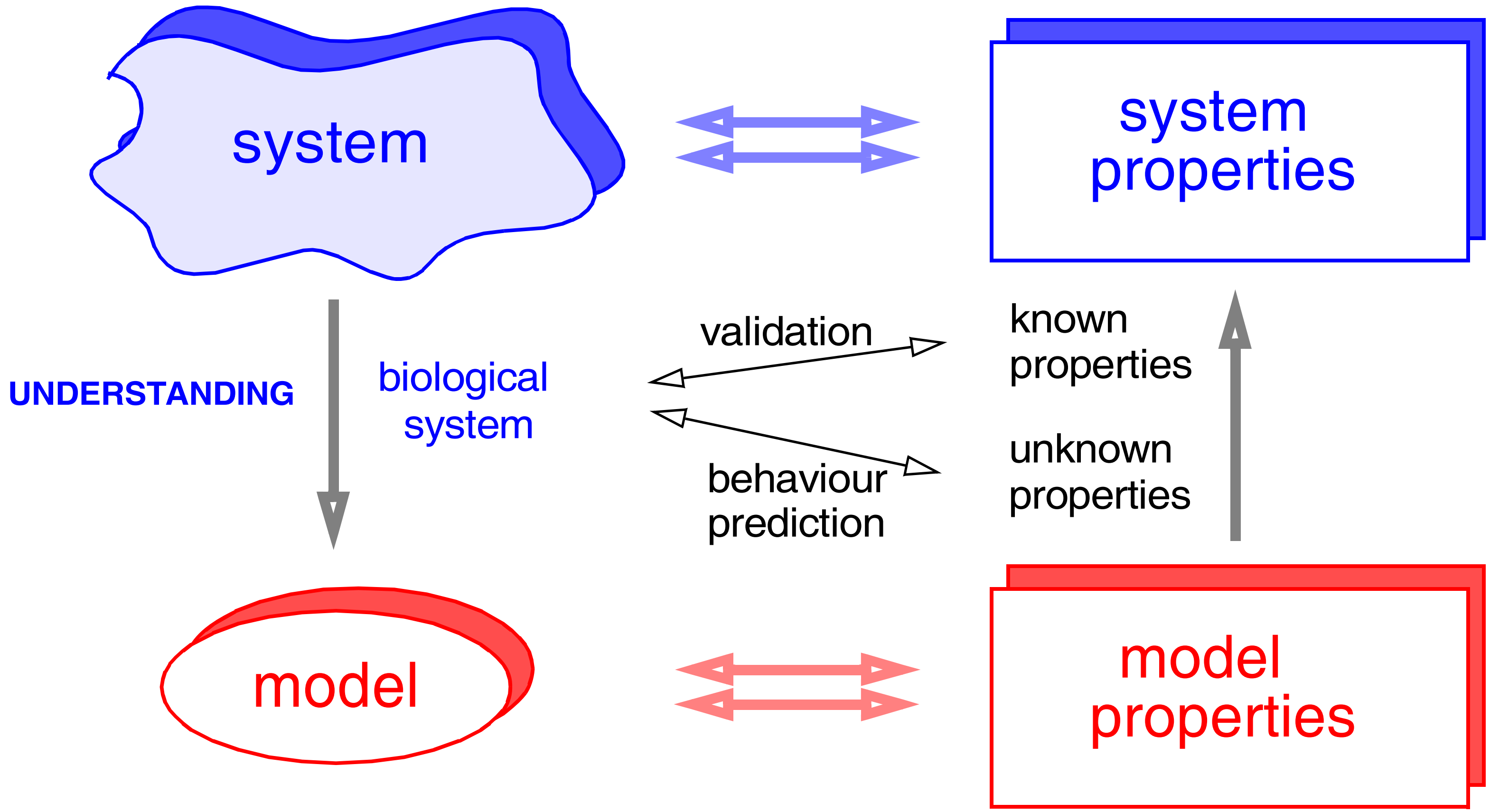
MODEL- BASED SYSTEM ANALYSIS



MODEL- BASED SYSTEM ANALYSIS



MODEL- BASED SYSTEM ANALYSIS



QUALITATIVE ANALYSES

TYPICAL PETRI NET QUESTIONS

- **How many tokens can reside at most in a given place ?**
  - -> *(0, 1, k, oo)* -> ***BOUNDEDNESS***
- **How often can a transition fire ?**
  - -> *(0-times, n-times, oo-times)* -> ***LIVENESS***
- **How often can a system state be reached ?**
  - -> *never* -> *UNREACHABLE -> SAFETY PROPERTIES*
  - -> *n-times* -> *REPRODUCIBLE*
  - -> *always reachable again* -> *REVERSIBLE (HOME STATE)*
  - -> *reversible initial state* -> ***REVERSIBILITY***

GENERAL BEHAVIOURAL PROPERTIES

-> *orthogonal*

-> *general decidable*

MODEL ANIMATION (?)

DYNAMIC ANALYSES

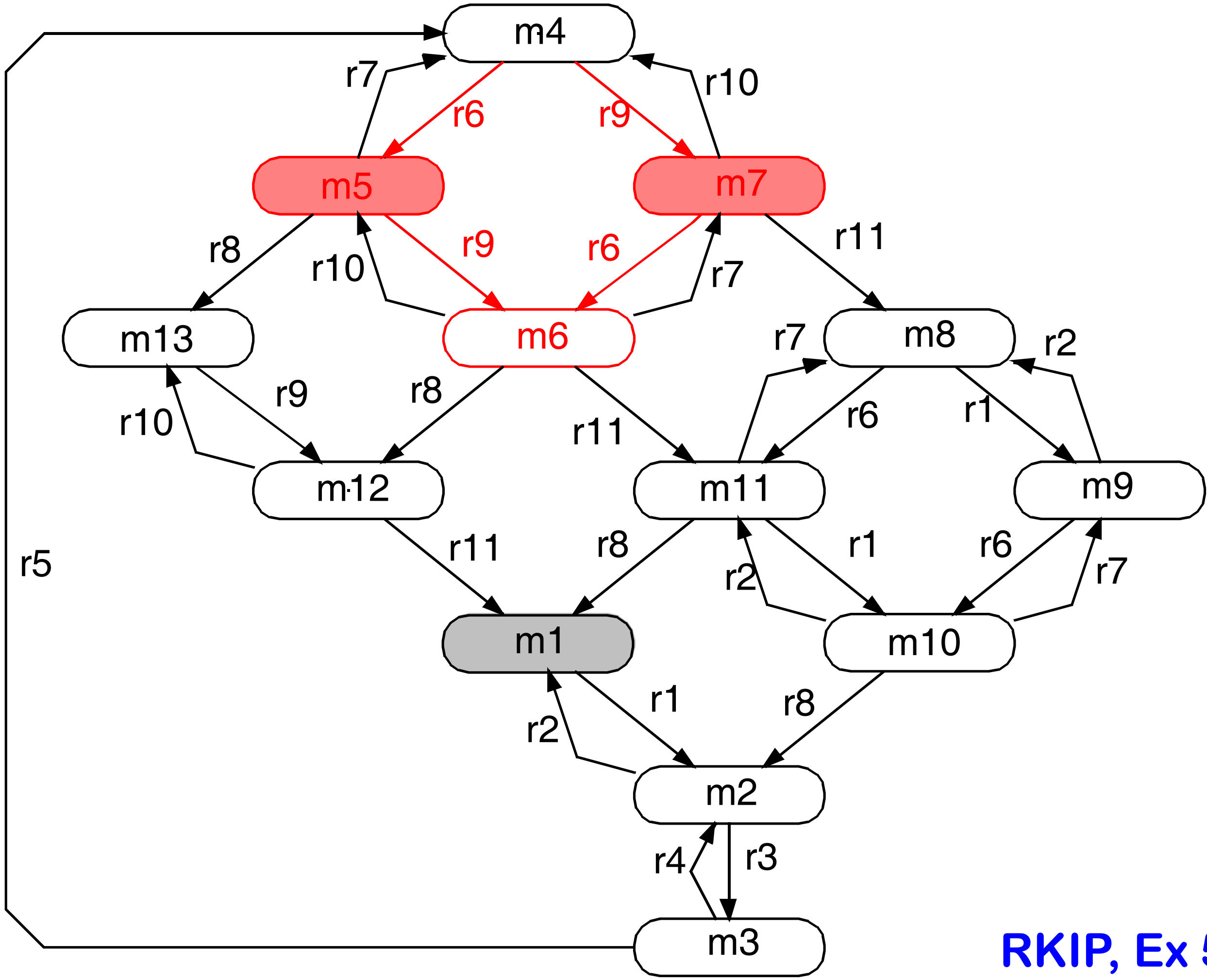
DYNAMIC ANALYSES

reachability / occurrence graph,

(labelled) state transition graph

CTMC, Kripke structure

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



RKIP, Ex 5

REACHABILITY GRAPH EVALUATION

- **interleaving semantics**

  -> *(sequential) finite automaton*

  -> *concurrency == enumerating all interleaving sequences*

- **boundedness**

  -> *finite graph*

- **reversibility**

  -> *one Strongly Connected Component (SCC)*

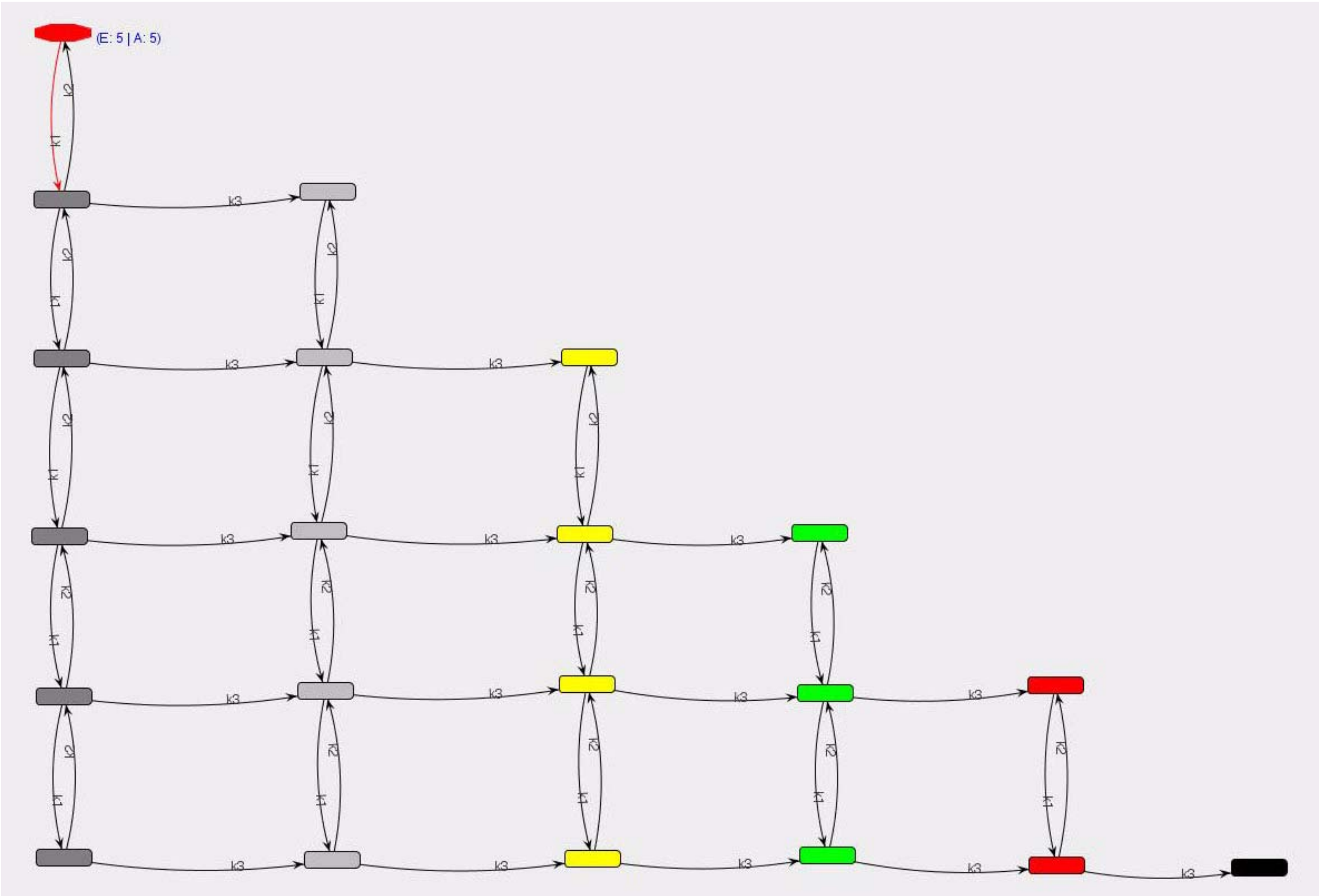
- **liveness**

  -> *every transition contained in all terminal SCC*

- **dead states**

  -> *terminal nodes*

REACHABILITY GRAPH, EX: MA1, 5 TOKENS

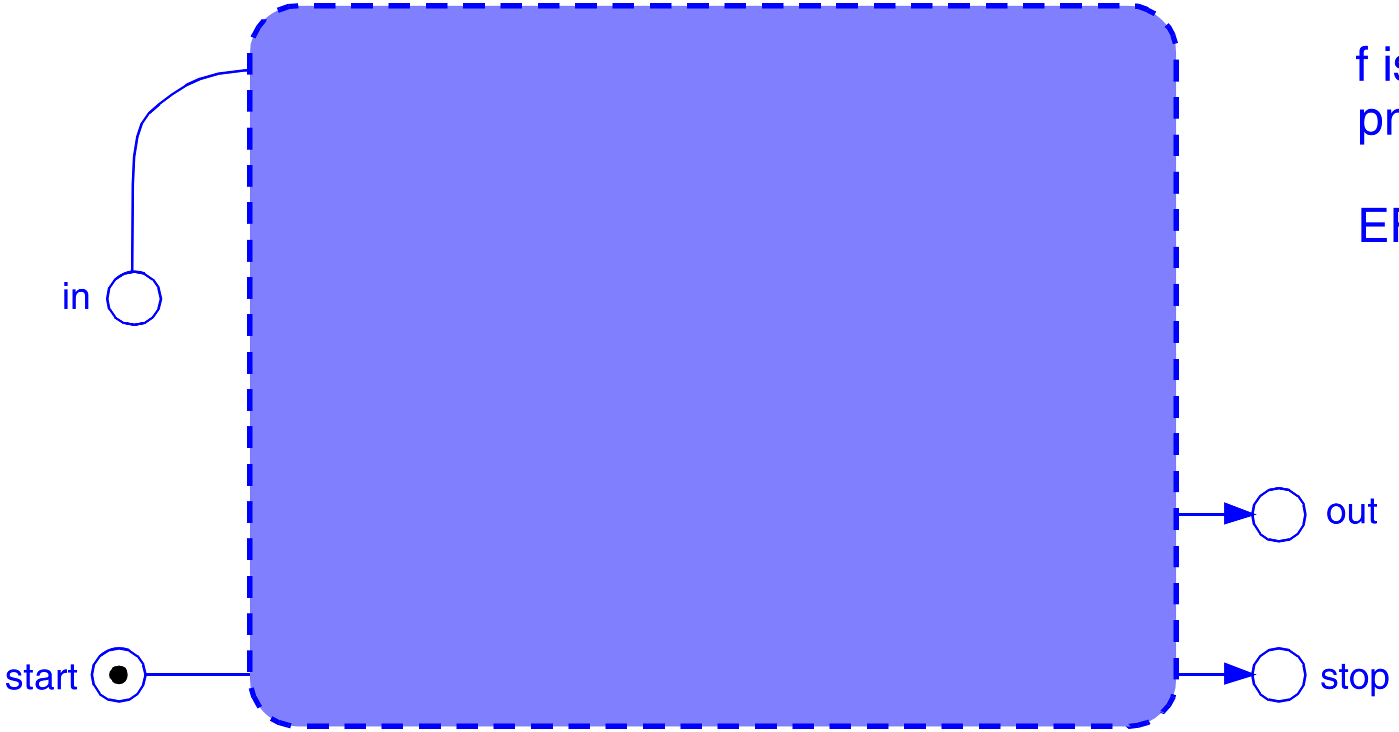


- infinite for unbounded nets

- worst-case for finite state spaces [Priese, Wimmel 2003]

  ... cannot be bounded by a primitive recursive function ...

- **proof** -> Petri net computer for a function **f:** $\mathbf{N_0^m \rightarrow N_0}$



f is weakly pn-computable:

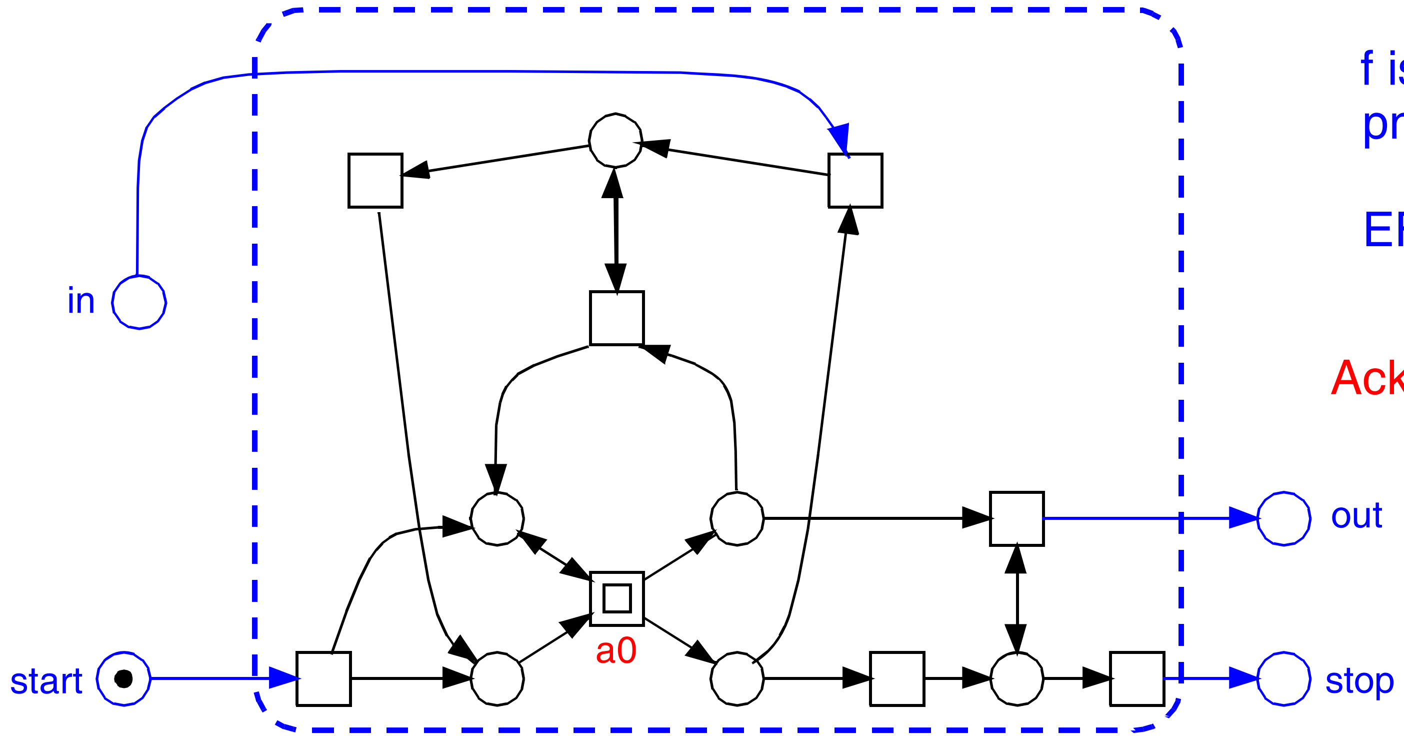
EF(out = f(in) &
stop = 1)

- **infinite for unbounded nets**

- **worst-case for finite state spaces** **[Priese, Wimmel 2003]**

  ... cannot be bounded by a primitive recursive function ...

- **proof** -> Petri net computer for a function **f: N_0^m -> N_0**



f is weakly pn-computable:

EF(out = f(in) &
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Ackermann function a1

- infinite for unbounded nets

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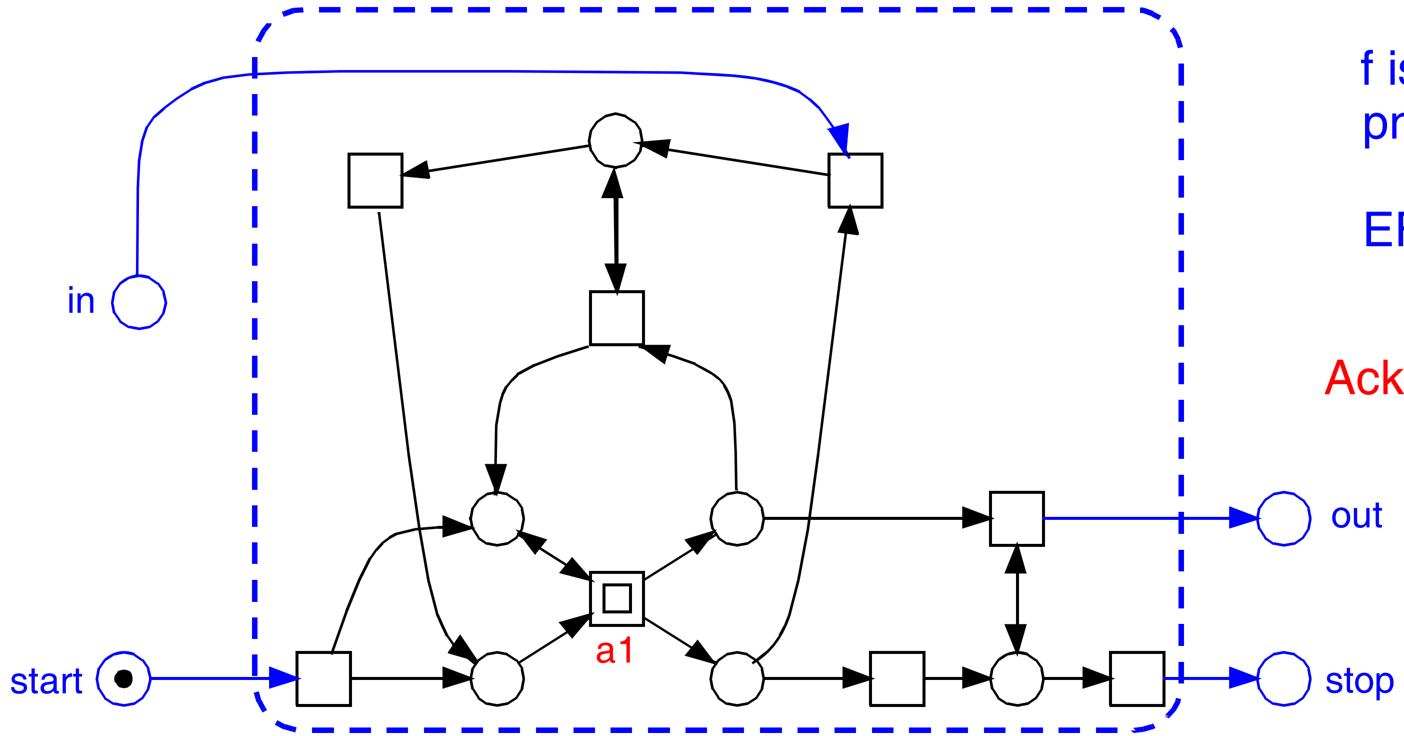
  ... cannot be bounded by a primitive recursive function ...

- **proof** -> Petri net computer for a function $\mathbf{f: N_0^m \to N_0}$

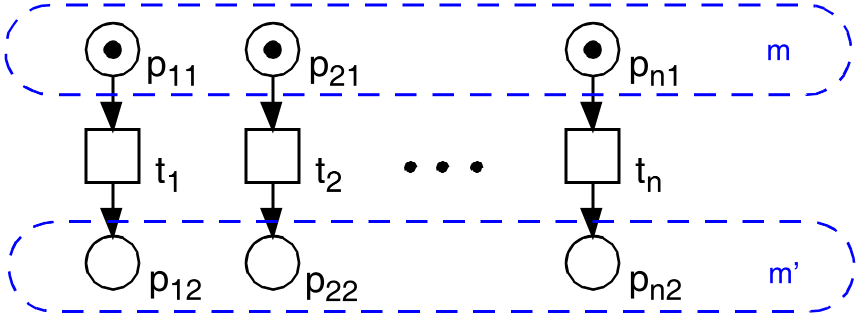


f is weakly pn-computable:

EF(out = f(in) &
stop = 1)

Ackermann function a2

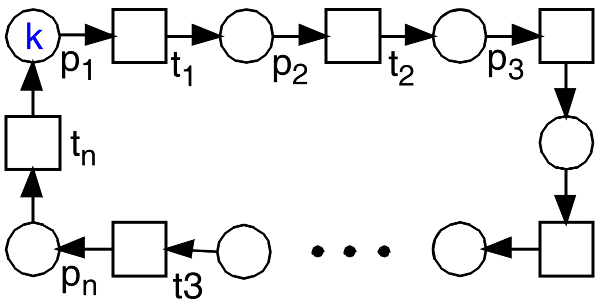
State Space Complexity, Causes



n! interleaving sequences

m -> m'

$2^n - 2$ intermediate states



$$\frac{(n + k - 1)!}{(n - 1)!\, k!}$$ states

(combination with repetition)

ANALYSIS TECHNIQUES

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

- **dynamic analyses** **-> total / partial state space construction**

  -> analysis of general behavioural system properties,
  i.e. boundedness, liveness, reversibility

  -> model checking of special behavioural system properties,
  e.g. reachability of a given (sub-) system state (with constraints),
  reproducability of a given (sub-) system state (with constraints)

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

TYPICAL PETRI NET QUESTIONS

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  - -> *n-times* -> *REPRODUCIBLE*
  - -> *always reachable again* -> *REVERSIBLE (HOME STATE)*
  - -> *reversible initial state* -> *REVERSIBILITY*
- **Are there behaviourally invariant subnet structures ?**
  - -> *token conservation* -> *P - INVARIANTS*
  - -> *token distribution reproduction* -> *T - INVARIANTS*
- **. . . and many more -> temporal logics (CTL, LTL)**

GENERAL BEHAVIOURAL PROPERTIES

SPECIAL BEHAVIOURAL PROPERTIES

Petri net theory

- -> *INA (HU Berlin)*
- -> *TINA (LAAS/CNRS)*
- -> *Charlie*

model checking

		CTL	LTL
-> *reachability graph*	->	*INA, Charlie*	*Charlie*
		PROD, MARIA	*PROD, MARIA*
-> *lazy state spaces*			
- stubborn set reduction	->	*LoLA*	*PROD (LTL\X)*
- symmetry reduction	->	*LoLA*	
-> *compressed state spaces (BDD, NDD, ... , IDD)*	->	*bdd-CTL, SMART* *idd-CTL*	*bdd-LTL* *idd-LTL*
-> *Kronecker algebra*	->	*[Kemper]*	
-> *prefix*	->	*PEP* (CTL_0)	*QQ (LTL\X)*
-> *process automata*	->	*[pd]*	

Petri net theory

- -> *INA (HU Berlin)*
- -> *TINA (LAAS/CNRS)*
- -> *Charlie*

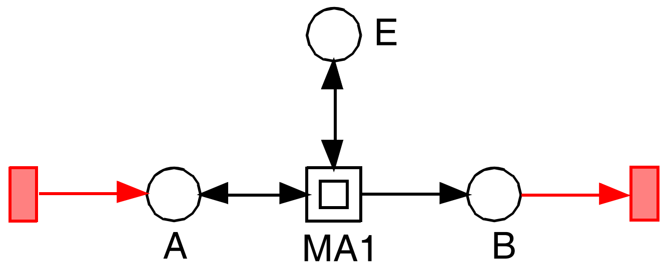
model checking

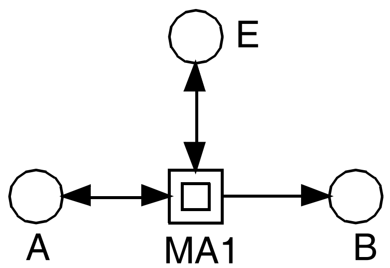
		CTL	LTL
-> *reachability graph*	->	*INA, Charlie*	*Charlie*
		PROD, MARIA	*PROD, MARIA*
-> *lazy state spaces*			
- stubborn set reduction	->	*LoLA*	*PROD (LTL\X)*
- symmetry reduction	->	*LoLA*	
-> *compressed state spaces (BDD, NDD, ... , IDD)*	->	*bdd-CTL, SMART*	*bdd-LTL*
		idd-CTL	*idd-LTL*
-> *Kronecker algebra*	->	*[Kemper]*	
-> *prefix*	->	*PEP (CTL_0)*	*QQ (LTL\X)*
-> *process automata*	->	*[pd]*	

-> TOOL BOX <-

STATIC ANALYSES

- **boundary nodes**
  - -> *input transitions -> not BND*
  - -> *input places -> not LIVE*
  - -> *LIVE & BND -> no boundary nodes*
- **conservative -> BND**
- **Deadlock-Trap Property (DTP)**
  - -> *no structural deadlock -> live*
  - -> *ORD & DTP -> no dead states (Ex6)*
  - -> *ORD & ES & DTP -> LIVE (Ex5)*
  - -> *ORD & EFC & DTP <-> LIVE*





INCIDENCE MATRIX C

- a representation of the net structure **=> stoichiometric matrix**

C =

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

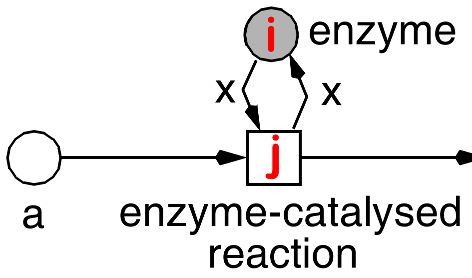
$cij = (pi, tj) = F(tj,pi) - F(pi, tj) = \Delta\ tj(pi)$

$\Delta tj = \Delta\ tj(*)$

- **matrix entry cij:**
  token change in place pi by firing of transition tj

- **matrix column Δtj:**
  vector describing the change of the whole marking by firing of tj

- **side-conditions are neglected**



cij = 0

P-INVARIANTS, BASICS

- Lautenbach, 1973

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

- minimal P-invariants
  - -> *there is no P-invariant with a smaller support* -> *sets of places*
  - -> *gcD of all entries is 1*

- any P-invariant is a non-negative linear combination of minimal ones
  - -> *multiplication with a positive integer*
  - -> *addition*
  - -> *Division by gcD*

$$ky = \sum_i a_i y_i$$

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

- **the firing of any transition has no influence on the weighted sum of tokens on the P-invariant's places**

  -> *for all t: the effect of the arcs, removing tokens from a P-invariant's places is equal to the effect of the arcs, adding tokens to a P-invariant's places*

- **set of places with**

  -> *a constant weighted sum of tokens for all markings m reachable from* m_0

  $$ym = ym_0$$

  -> *token / compound preservation,*

  -> *moieties*

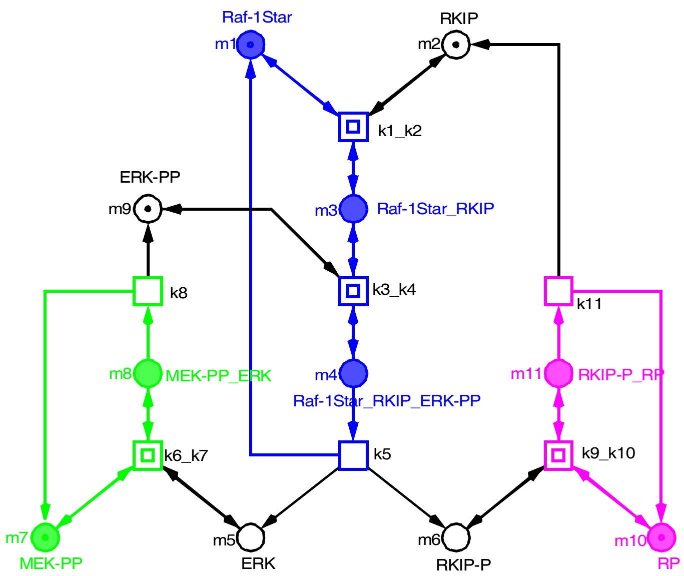
  -> *a place belonging to a P-invariant is bounded*

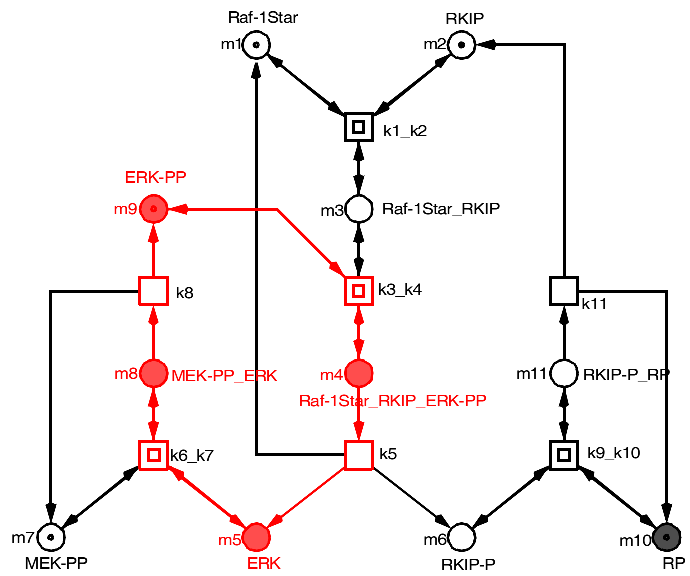
- **a P-invariant defines a subnet**

  -> *the P-invariant's places (the support),*
  + all their pre- and post-transitions
  + the arcs in between

  -> *pre-sets of supports = post-sets of supports* -> **self-contained**

THE RKIP PATHWAY, P-INVARIANTS





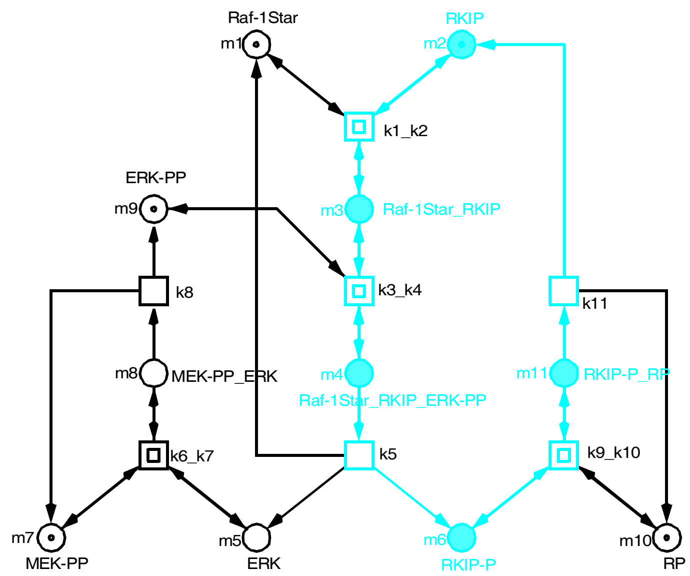
P-INV1: MEK

P-INV2: RAF-1STAR

P-INV3: RP

P-INV4: ERK

P-INV5: RKIP



T-INVARIANTS, BASICS

- **Lautenbach, 1973** -> **Schuster, 1993**
- **T-invariants** -> *multisets of transitions*
  - -> *integer solutions x of* $Cx = 0, x \neq 0, x \geq 0$ -> *Parikh vector*
- **minimal T-invariants**
  - -> *there is no T-invariant with a smaller support* -> *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*

$$kx = \sum_i a_i x_i$$

- **Covered by T-Invariants (CTI)**
  - -> *each transition belongs to a T-invariant*
  - -> *BND & LIVE => CTI (necessary condition)*

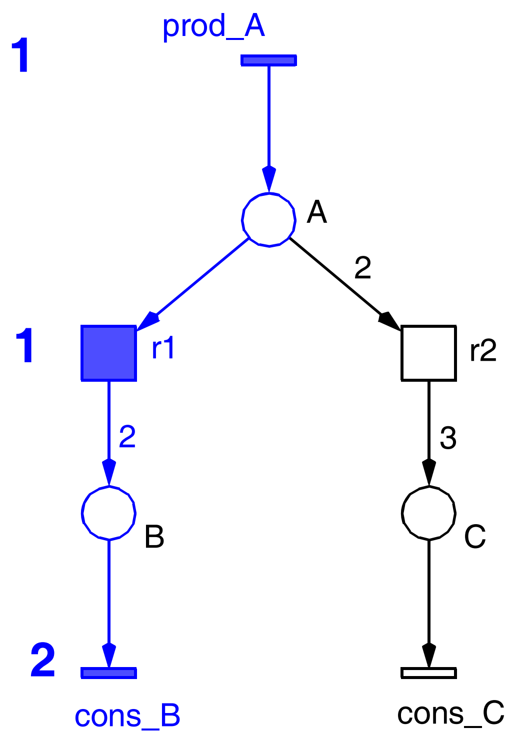
T-INVARIANTS, INTERPRETATIONS

- **T-invariants = (multi-) sets of transitions = Parikh vector**
  - -> *zero effect on marking*
  - -> *reproducing a marking / system state*

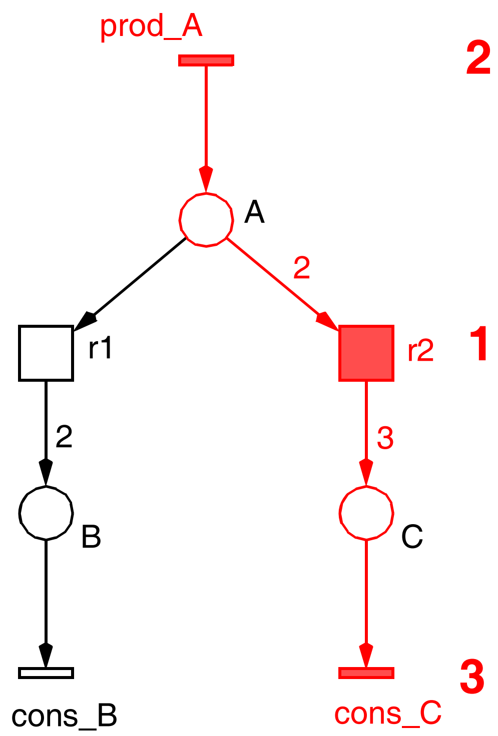
- **two interpretations**
  1. *partially ordered transition sequence* **-> behaviour understanding**
     of transitions occuring one after the other
     - -> *substance / signal flow*
  2. *relative transition firing rates* **-> steady state behaviour**
     of transitions occuring permanently & concurrently
     - -> *steady state behaviour*

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

r1: A -> 2 B
r2: 2 A -> 3 C



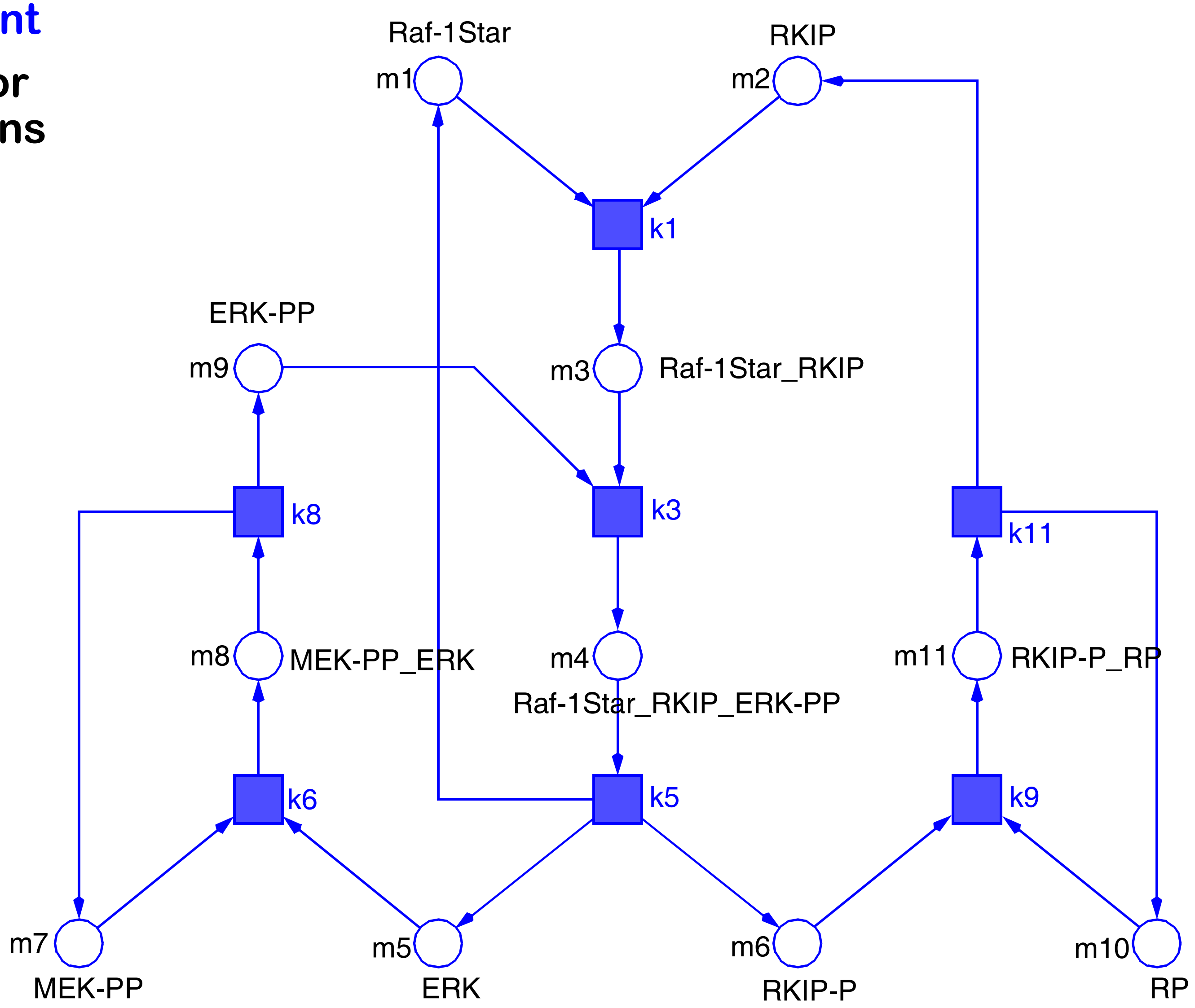
T-INVARIANT 1



T-INVARIANT 2

-> non-trivial T-invariant

+ four trivial ones for reversible reactions



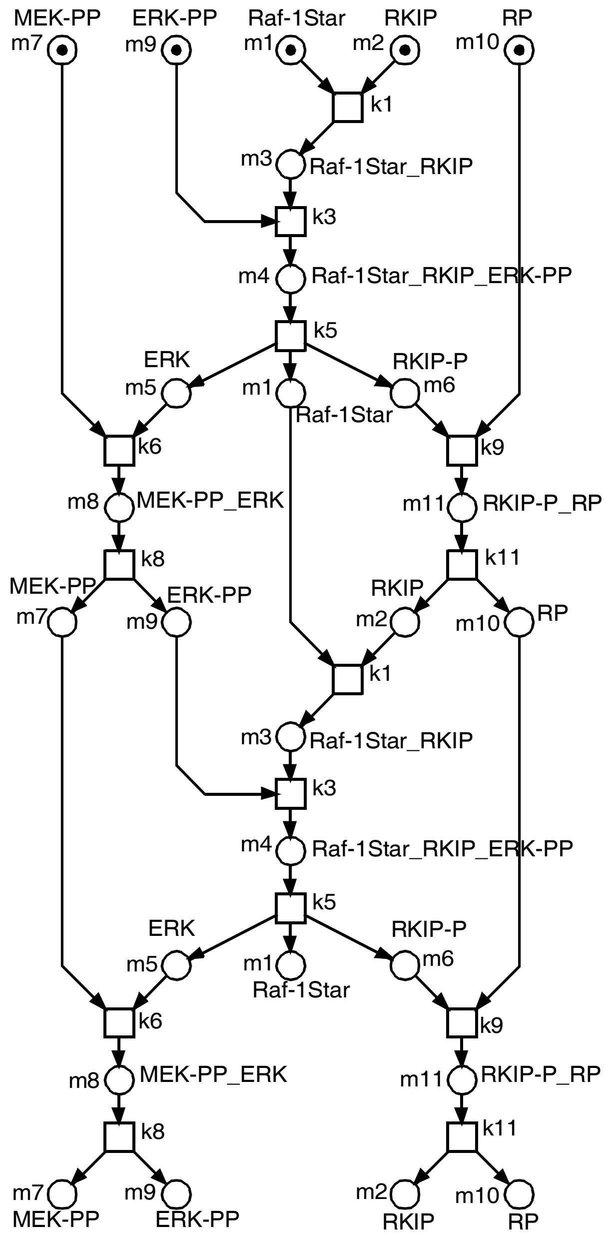
NON-TRIVIAL T-INVARIANT, RUN

- **realizability check under the constructed marking**

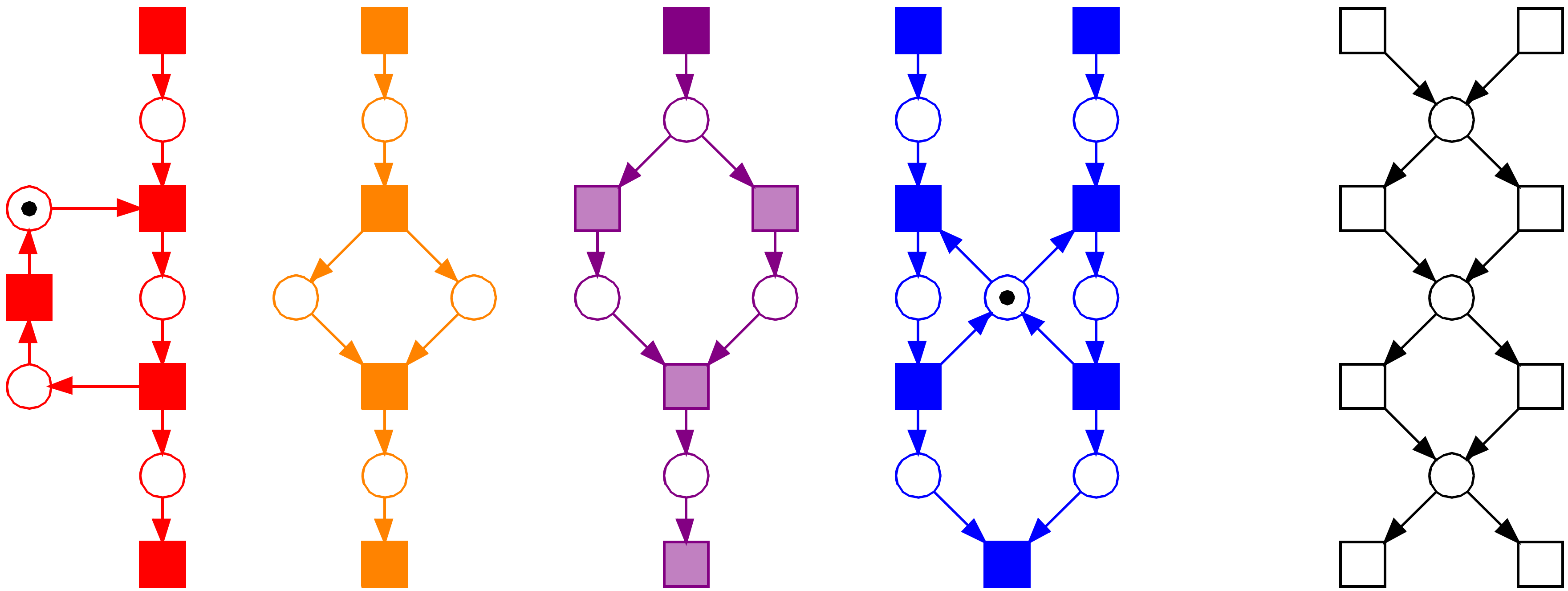
- **T-invariant's unfolding to describe its behaviour**
  - -> **partial order structure**

- **labelled condition / event net**
  - -> *events (boxes)*
    - transition occurences
  - -> *conditions (circles)*
    - involved compounds

- **occurrence net**
  - -> *acyclic*
  - -> *no backward branching conditions*
  - -> *infinite*



- T-invariants may contain any structure



- T-invariants generally overlap

  -> combinatorial effect brings explosion in the number of min. T-invariants (2^4)

CONSTRUCTION OF THE INITIAL MARKING

- **each P-invariant gets at least one token**
  - -> *P-invariants are structural deadlocks and traps*
- **in signal transduction**
  - -> *exactly 1 token, corresponding to species conservation*
  - -> *token in least active state*
- **all (non-trivial) T-invariants get feasible**
  - -> *to make the net live*
- **minimal marking**
  - -> *minimization of the state space*

-> UNIQUE INITIAL MARKING <-

- **validation criterion 1**
  - -> *all expected structural properties hold*
  - -> *all expected general behavioural properties hold*
- **validation criterion 2**
  - -> *CPI (if closed model)*
  - -> *no minimal P-invariant without biological interpretation*
- **validation criterion 3**
  - -> *CTI*
  - -> *no minimal T-invariant without biological interpretation*
  - -> *no known biological behaviour without corresponding T-invariant*
- **validation criterion 4**
  - -> *all expected special behavioural properties hold*
  - -> *temporal-logic properties -> TRUE*

QUALITATIVE ANALYSIS, SUMMARY

- **construction of initial marking**
- **subnetwork identification**
  - -> *P-invariants: token preserving modules (mass conservation)*
  - -> *T-invariants: state repeating modules (elementary modes)*
- **network validation**
  - -> *structure (topology)*
  - -> *initial conditions*
- **choice of stochastic analysis techniques**

NOW WE ARE READY FOR SOPHISTICATED QUANTITATIVE ANALYSES !

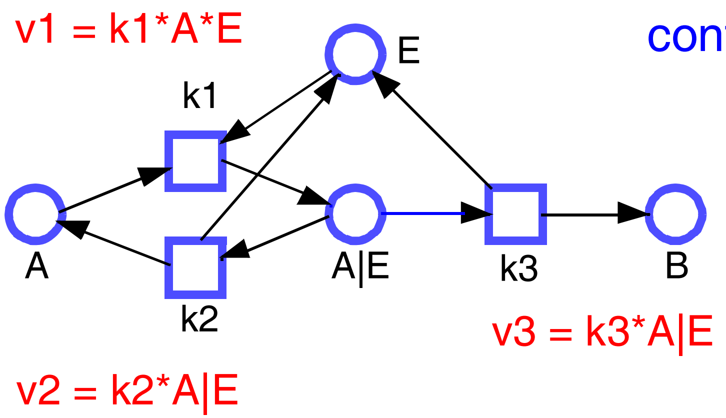
STOCHASTIC PETRI NETS

- **transitions**
  - -> *exponentially distributed waiting time*
  - -> *state-dependent propensity (hazard) function*
- **semantics**
  - -> *Continuous Time Markov Chain (CTMC)*
- **CTMC ~ reachability graph + transition rates**
  - -> *all qualitative properties are preserved*
  - -> *reversibility -> ergodicity*
- **(sufficiently) finite CTMC**
  - -> *analytical = exact CSL model checking*
- **(practically) infinite CTMC**
  - -> *simulative = approximative PLTLc model checking*
  - -> *approximation: finite number of finite simulation traces*

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

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

- **continuous Petri nets = ODEs**



continuous nodes !

dA / dt = -v1 + v2

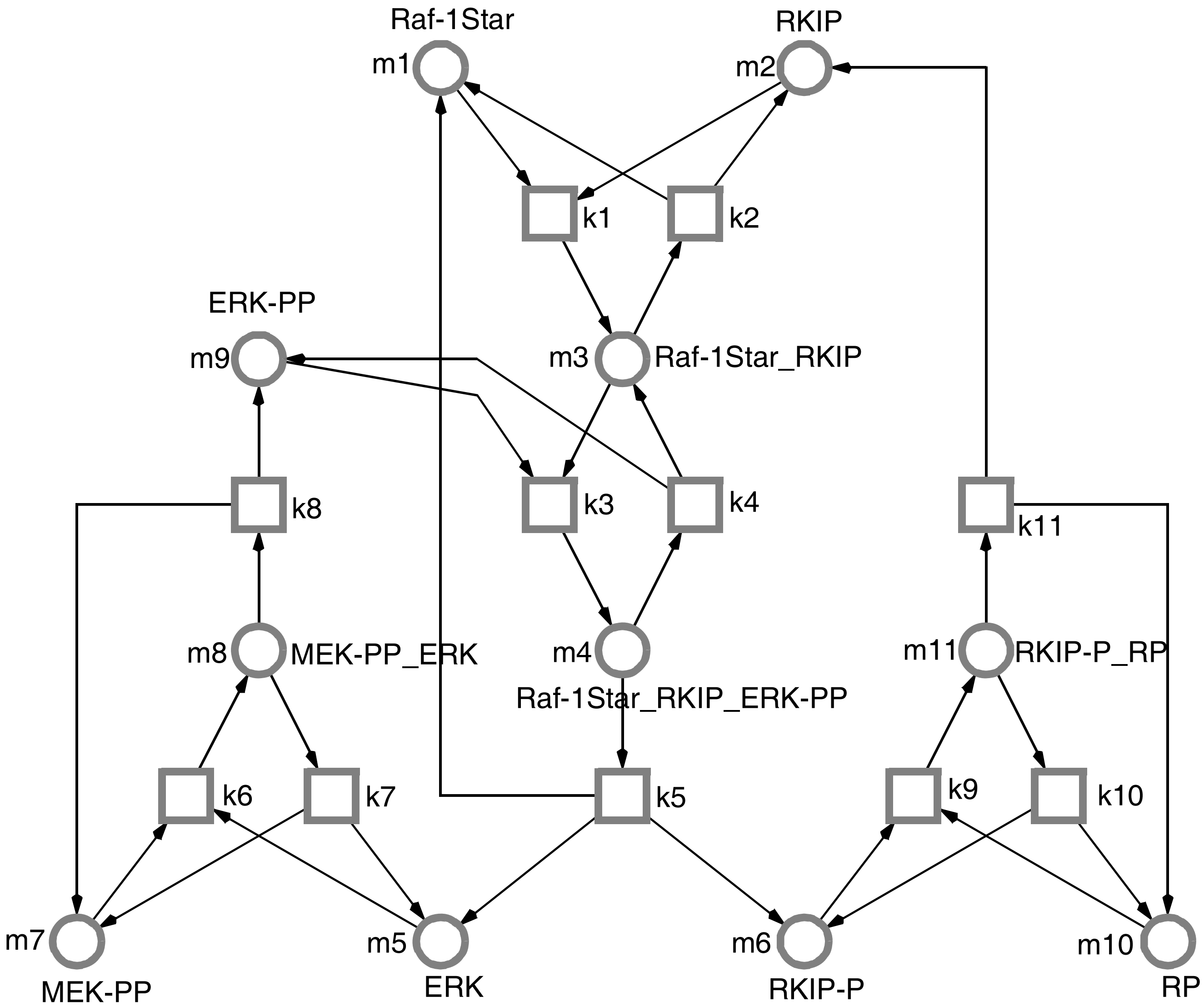
dA|E / dt = v1 - v2 - v3

dB / dt = v3

dE / dt = -v1 + v2 + v3

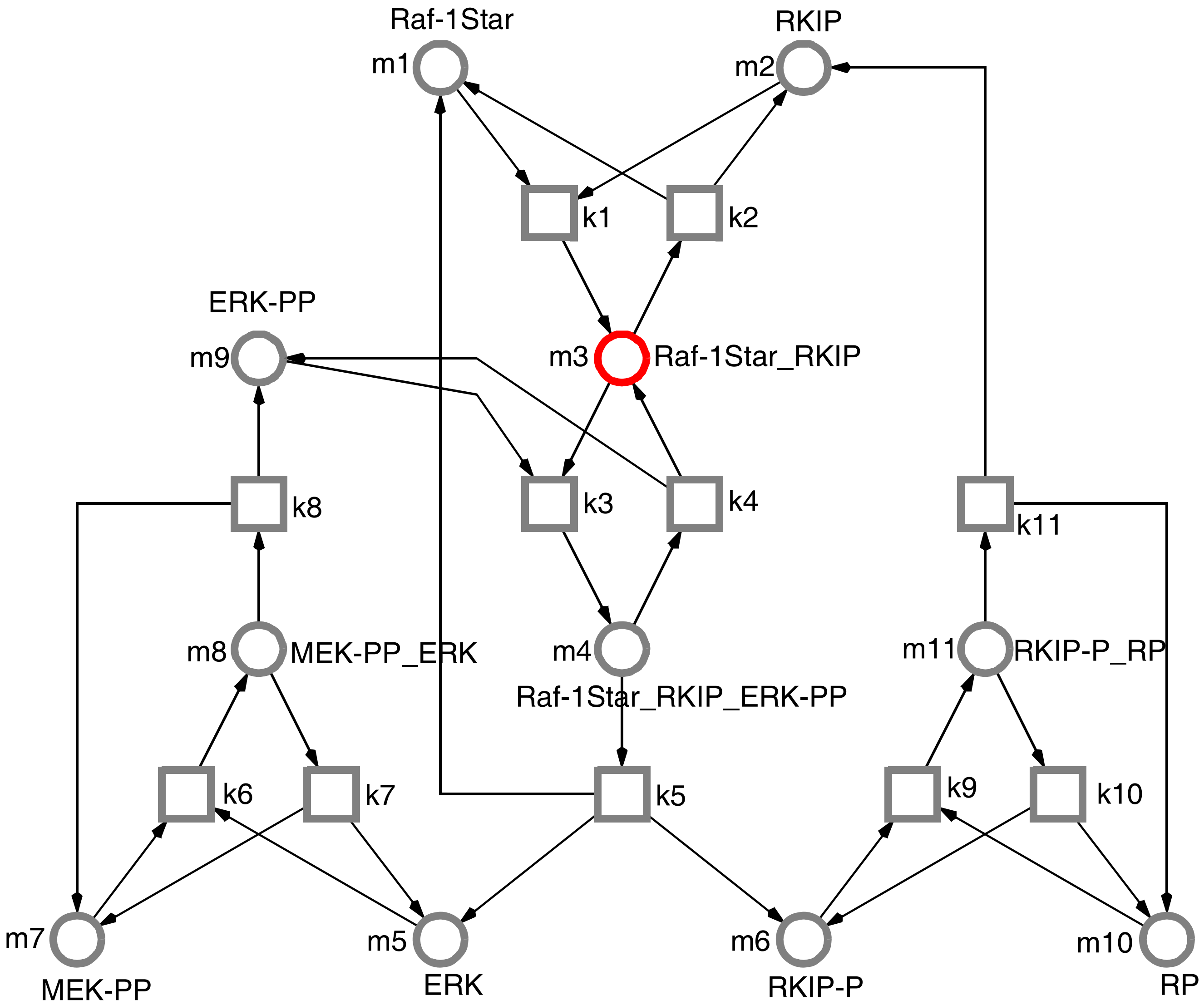
- **analysis**
  -> *all standard ODEs techniques + LTLc model checking*

THE RKIP PATHWAY, CONTINUOUS PETRI NET



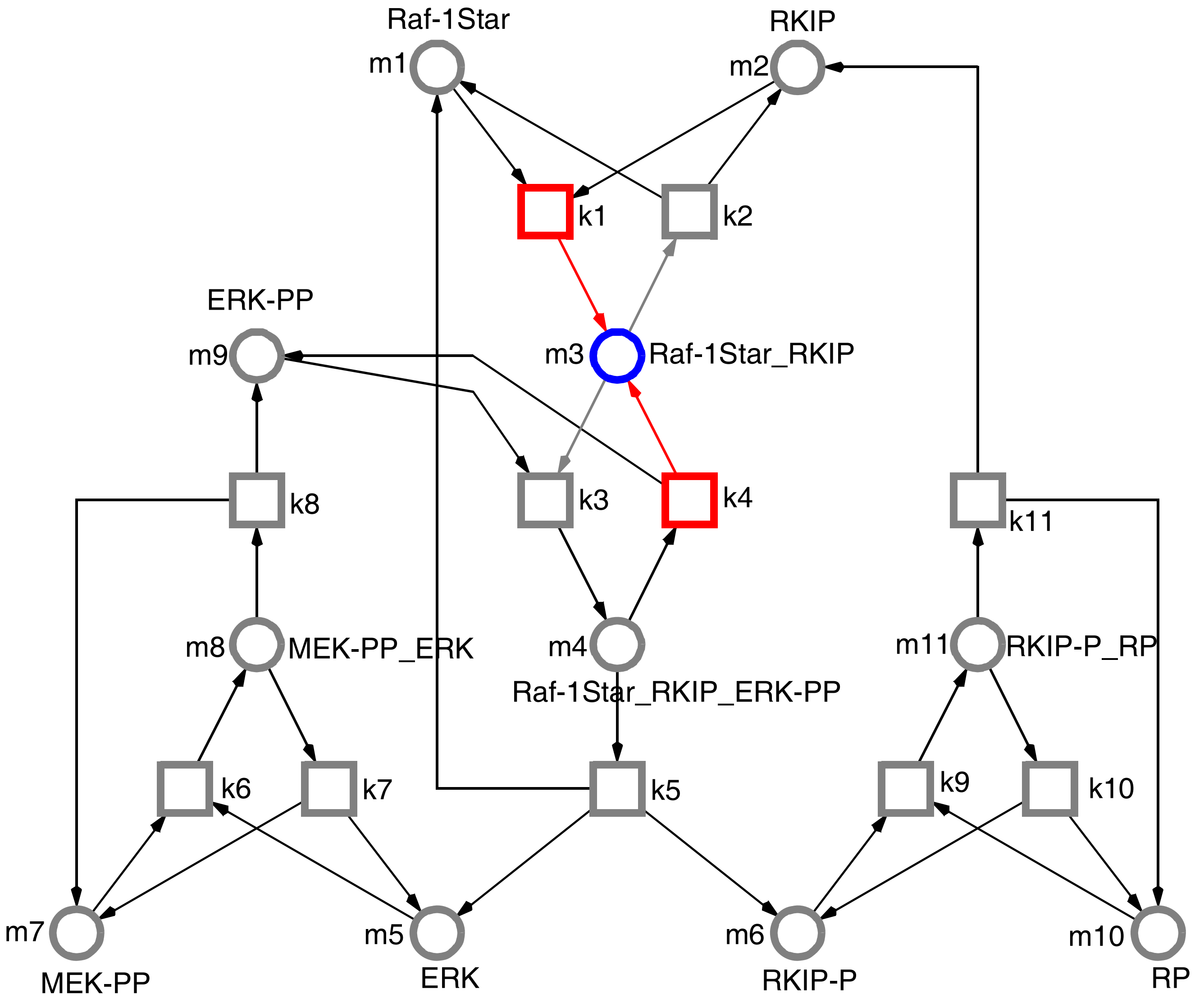
The RKIP Pathway, Continuous Petri Net

$$\frac{dm3}{dt} =$$



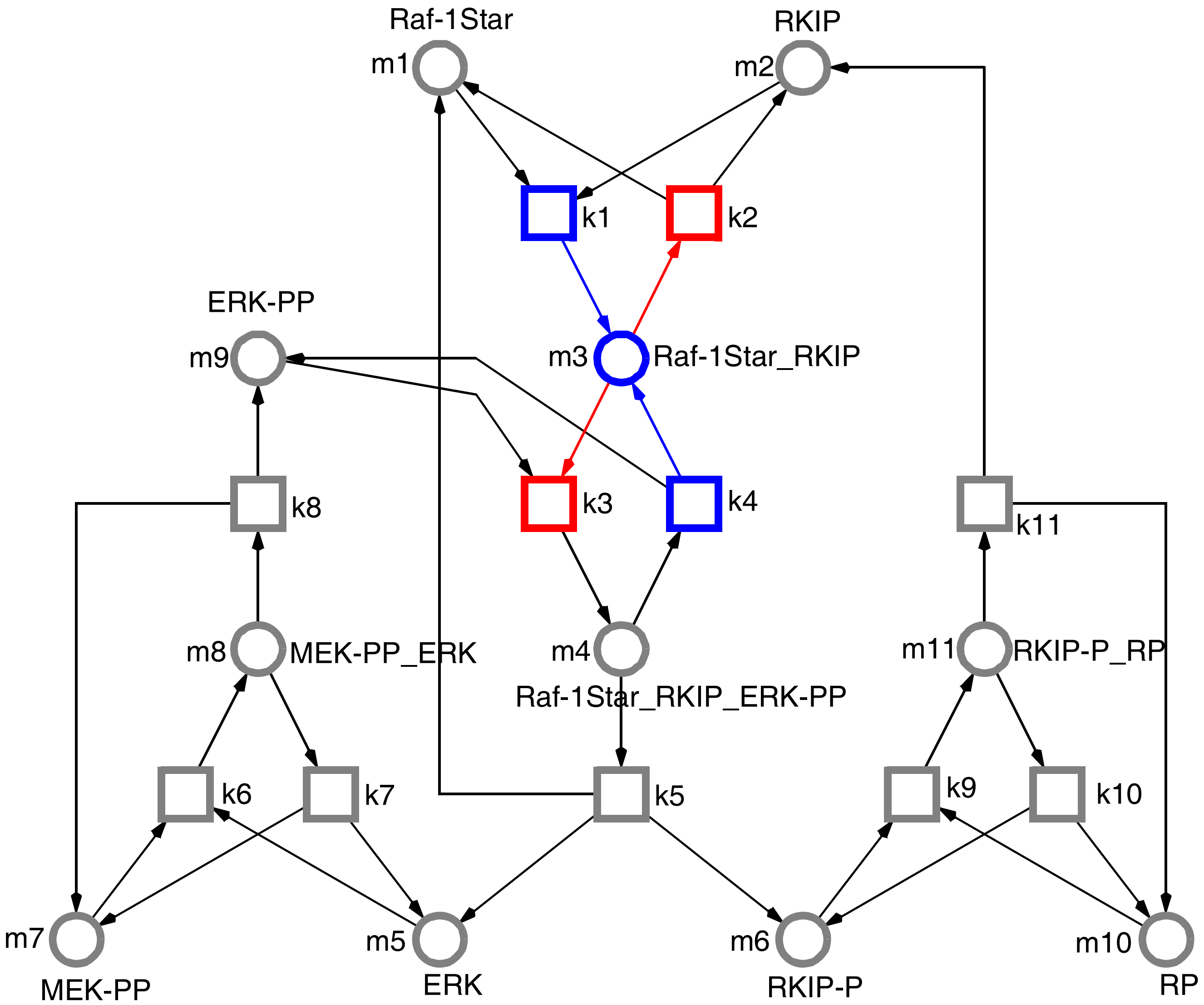
THE RKIP PATHWAY, CONTINUOUS PETRI NET

$$\frac{dm3}{dt} = + r1 + r4$$



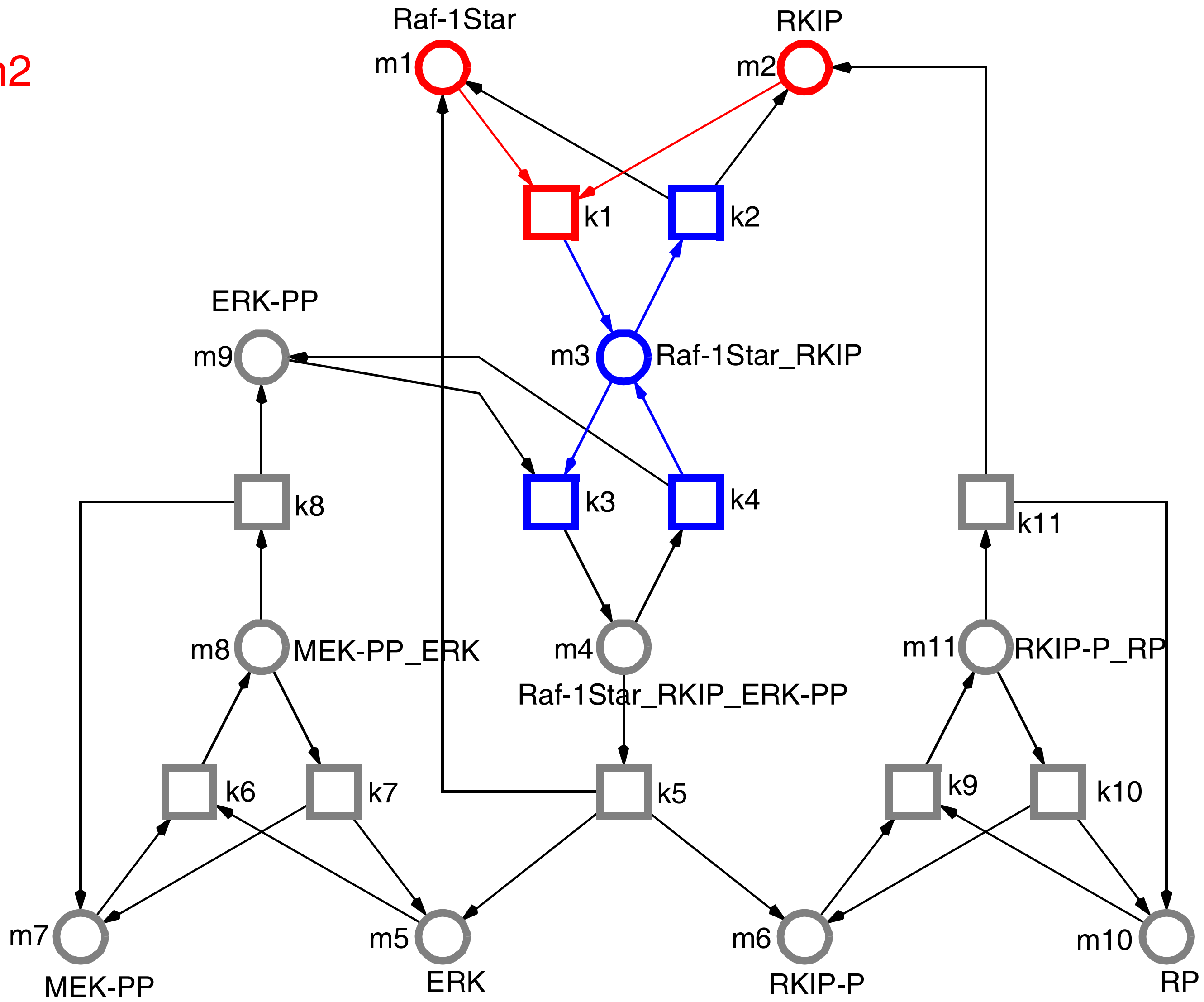
THE RKIP PATHWAY, CONTINUOUS PETRI NET

$$\frac{dm3}{dt} = + r1 + r4 - r2 - r3$$



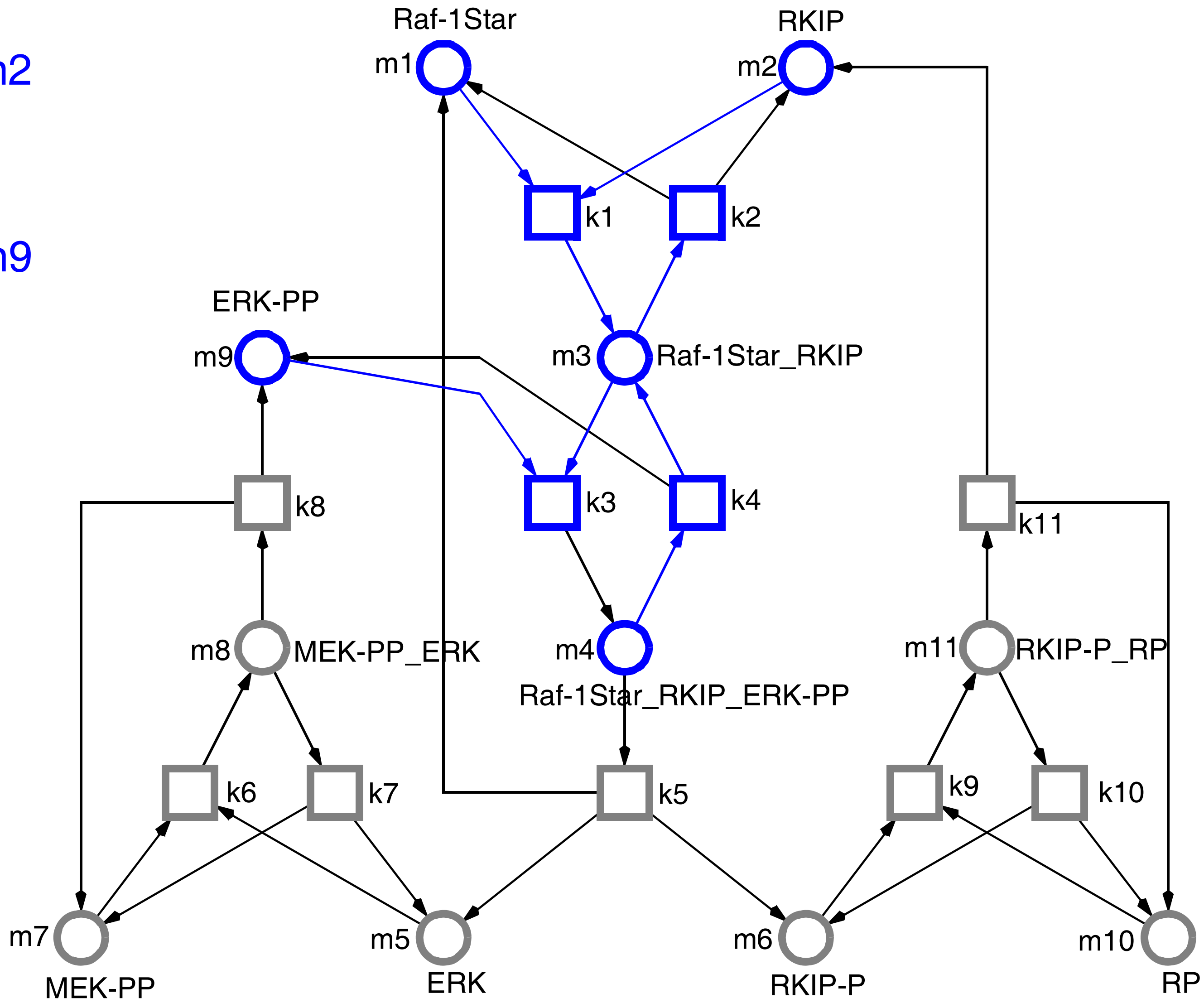
THE RKIP PATHWAY, CONTINUOUS PETRI NET

$$\frac{dm3}{dt} = + k1 * m1 * m2 + r4 - r2 - r3$$



THE RKIP PATHWAY, CONTINUOUS PETRI NET

$$\frac{dm3}{dt} = + k1 * m1 * m2 + k4 * m4 - k2 * m3 - k3 * m3 * m9$$



THE QUALITATIVE MODEL
BECOMES
THE STRUCTURED DESCRIPTION
OF THE QUANTITATIVE MODELS !

Bio Petri Nets, Part III A Case Study

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

Bertinoro, June, 2008

Definition :

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

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

Interleaving Semantics : reachability graph / CTL, LTL

Definition :

A **biochemically interpreted stochastic Petri net** is a quintuple $\mathcal{SPN}_{Bio} = (P, T, f, v, m_0)$, where

- P, T - finite, non empty, disjoint sets *(***places, transitions***)*
- $f : ((P \times T) \cup (T \times P)) \rightarrow \mathbb{N}_0$ *(***weighted directed arcs***)*
- $m_0 : P \rightarrow \mathbb{N}_0$ *(***initial marking***)*
- $v : T \rightarrow H$ *(***stochastic firing rate functions***)* with
  - $H := \bigcup_{t \in T} \left\{ h_t \mid h_t : \mathbb{N}_0^{|^\bullet t|} \rightarrow \mathbb{R}^+ \right\}$
  - $v(t) = h_t$ for all transitions $t \in T$

Semantics : Continuous Time Markov Chain / CSL, PLTLc

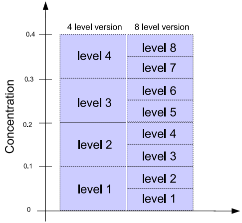
Definition :
A **biochemically interpreted continuous Petri net** is a quintuple $\mathcal{CPN}_{Bio} = (P, T, f, v, m_0)$, where

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

Semantics : ODEs / LTLc

Interpretation of tokens :

- *tokens = molecules, moles*
- *tokens = concentration levels*



Specialised stochastic firing rate function, two examples :

- *molecules semantics*

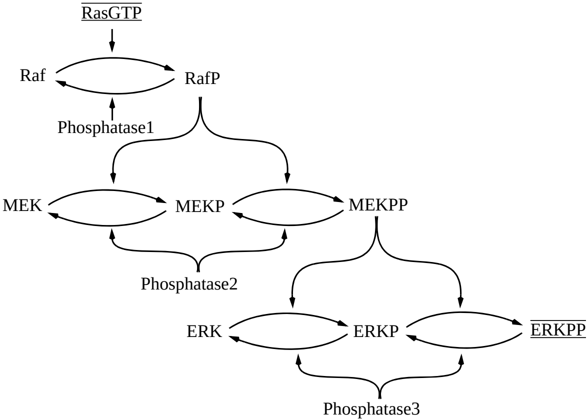
$$h_t := c_t \cdot \prod_{p \in {}^\bullet t} \binom{m(p)}{f(p,t)} \qquad (1)$$

- *concentration levels semantics*

$$h_t := k_t \cdot N \cdot \prod_{p \in {}^\bullet t} (\frac{m(p)}{N}) \qquad (2)$$

Running Case Study

- . . . *a typical signalling cascade*



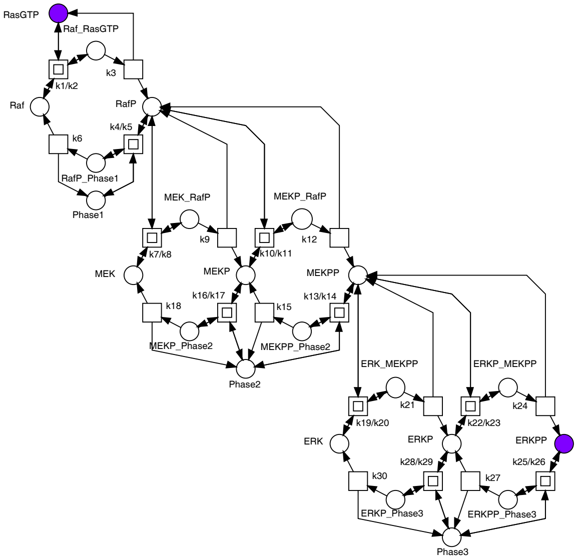
modelled in [Levchenko et al. 2000] like this . . .

Running Case Study - Origin

[Levchenko et al. 2000], *Supplemental Material : ODEs*

$$
\begin{aligned}
d\mathrm{Raf}/dt &= k_2 * \mathrm{Raf\_RasGTP} + k_6 * \mathrm{RafP\_Phase1} - k_1 * \mathrm{Raf} * \mathrm{RasGTP} \\
d\mathrm{RasGTP}/dt &= k_2 * \mathrm{Raf\_RasGTP} + k_3 * \mathrm{Raf\_RasGTP} - k_1 * \mathrm{Raf} * \mathrm{RasGTP} \\
d\mathrm{Raf\_RasGTP}/dt &= k_1 * \mathrm{Raf} * \mathrm{RasGTP} - k_2 * \mathrm{Raf\_RasGTP} - k_3 * \mathrm{Raf\_RasGTP} \\
d\mathrm{RafP}/dt &= k_3 * \mathrm{Raf\_RasGTP} + k_{12} * \mathrm{MEKP\_RafP} + k_9 * \mathrm{MEK\_RafP} + \\
&\quad k_5 * \mathrm{RafP\_Phase1} + k_8 * \mathrm{MEK\_RafP} + k_{11} * \mathrm{MEKP\_RafP} - \\
&\quad k_7 * \mathrm{RafP} * \mathrm{MEK} - k_{10} * \mathrm{MEKP} * \mathrm{RafP} - k_4 * \mathrm{Phase1} * \mathrm{RafP} \\
d\mathrm{RafP\_Phase1}/dt &= k_4 * \mathrm{Phase1} * \mathrm{RafP} - k_5 * \mathrm{RafP\_Phase1} - k_6 * \mathrm{RafP\_Phase1} \\
d\mathrm{MEK\_RafP}/dt &= k_7 * \mathrm{RafP} * \mathrm{MEK} - k_8 * \mathrm{MEK\_RafP} - k_9 * \mathrm{MEK\_RafP} \\
d\mathrm{MEKP\_RafP}/dt &= k_{10} * \mathrm{MEKP} * \mathrm{RafP} - k_{11} * \mathrm{MEKP\_RafP} - k_{12} * \mathrm{MEKP\_RafP} \\
d\mathrm{MEKP\_Phase2}/dt &= k_{16} * \mathrm{Phase2} * \mathrm{MEKP} - k_{18} * \mathrm{MEKP\_Phase2} - k_{17} * \mathrm{MEKP\_Phase2} \\
d\mathrm{MEKPP\_Phase2}/dt &= k_{13} * \mathrm{MEKPP} * \mathrm{Phase2} - k_{15} * \mathrm{MEKPP\_Phase2} - k_{14} * \mathrm{MEKPP\_Phase} \\
d\mathrm{ERK}/dt &= k_{20} * \mathrm{ERK\_MEKPP} + k_{30} * \mathrm{ERKP\_Phase3} - k_{19} * \mathrm{MEKPP} * \mathrm{ERK} \\
d\mathrm{ERK\_MEKPP}/dt &= k_{19} * \mathrm{MEKPP} * \mathrm{ERK} - k_{20} * \mathrm{ERK\_MEKPP} - k_{21} * \mathrm{ERK\_MEKPP} \\
d\mathrm{ERKP\_MEKPP}/dt &= k_{22} * \mathrm{MEKPP} * \mathrm{ERKP} - k_{24} * \mathrm{ERKP\_MEKPP} - k_{23} * \mathrm{ERKP\_MEKPP} \\
etcetera &= \ldots
\end{aligned}
$$

Running Case Study



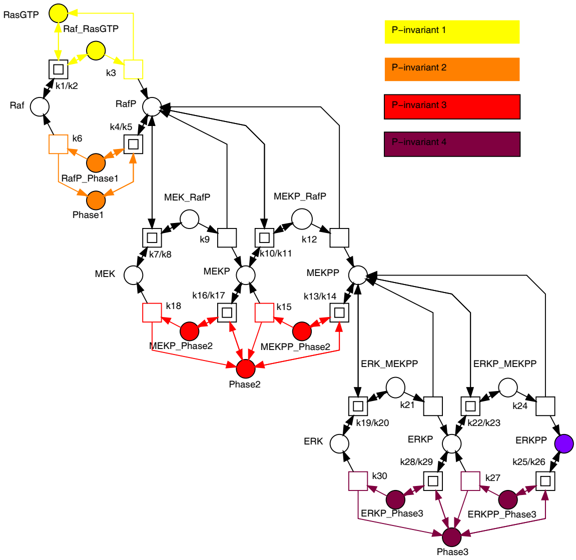
- **initial marking construction**
  P-invariants

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

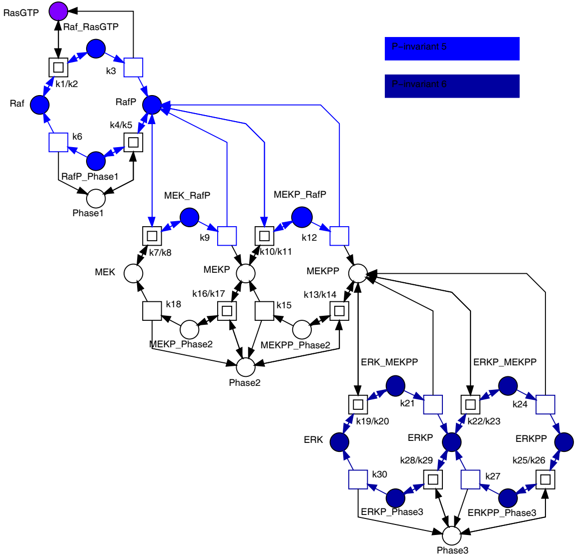
- **general behavioural properties**
  - *boundedness :* every place gets finite token number only
  - *liveness :* every transition may happen forever
  - *reversibility :* every state may be reached forever

- **special behavioural properties**
  CTL / LTL model checking

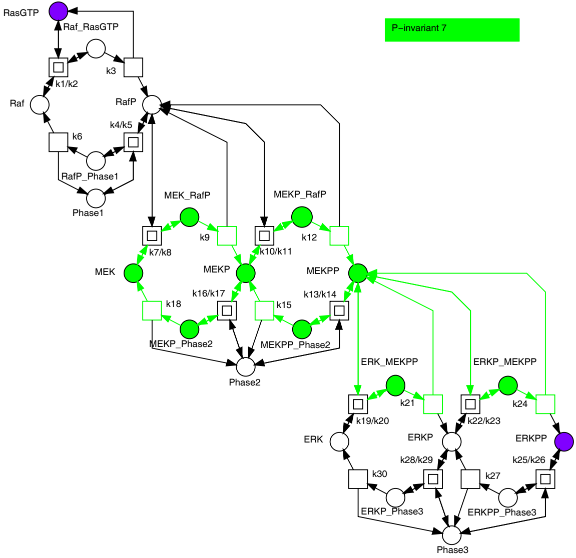
Running Case Study - P-invariants



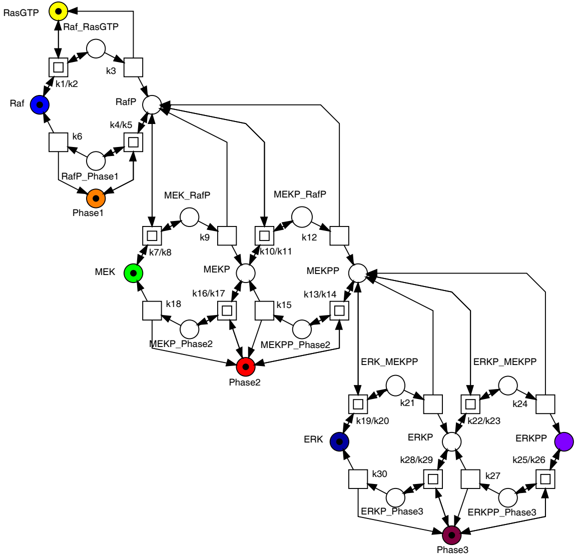
Running Case Study - P-invariants



Running Case Study - P-invariants



Running Case Study - initial marking



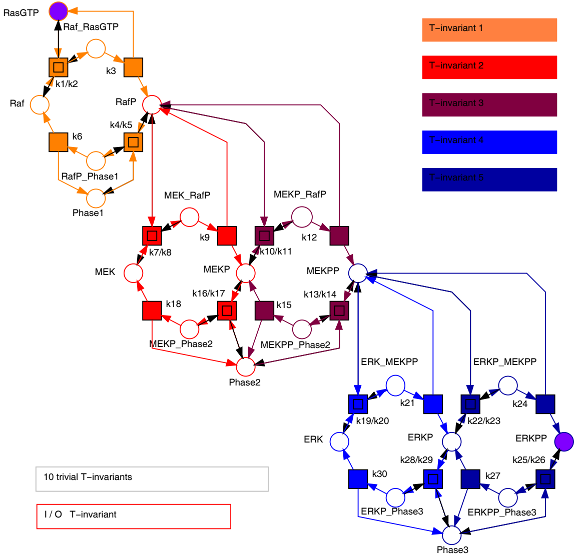
Running Case Study - general properties

- *state space*

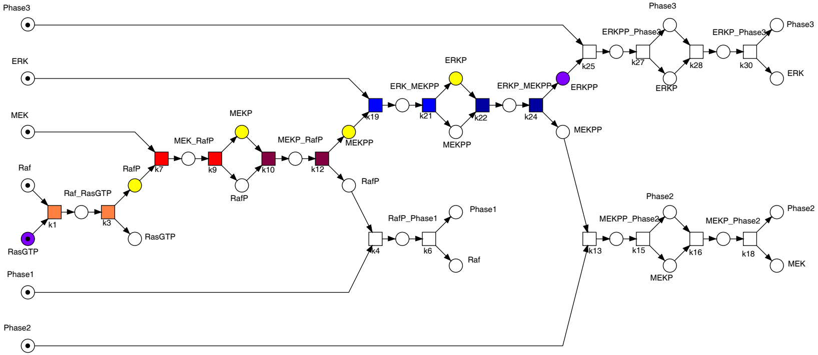
levels	reachability graph number of states	IDD data structure number of nodes
1	118	52
4	$2.4 \cdot 10^4$	115
8	$6.1 \cdot 10^6$	269
80	$5.6 \cdot 10^{18}$	13,472
120	$1.7 \cdot 10^{21}$	29,347

- Covered by P-invariants (CPI) $\Rightarrow$ **bounded**
- Deadlock-Trap Property (DTP) holds $\Rightarrow$ **no dead states**
- reachability graph
  - strongly connected $\Rightarrow$ **reversible**
  - contains every transition (reaction) $\Rightarrow$ **live**

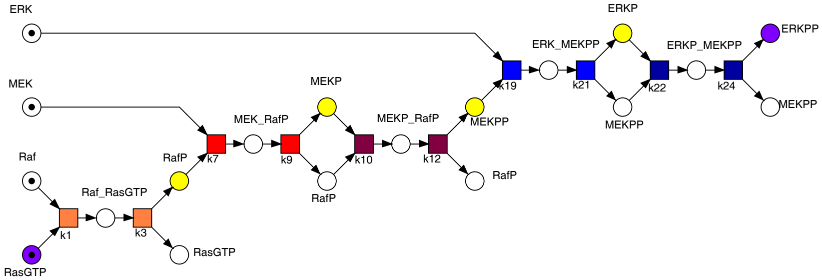
Running Case Study - T-invariants

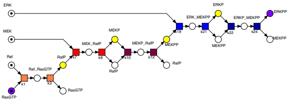


Running Case Study - partial order run of I/O T-invariant



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





property Q1 :

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

$$
\begin{aligned}
\neg\ [\ &\mathbf{E}\ (\ \neg\ \text{RafP}\ \mathbf{U}\ \text{MEKP}\ )\ \vee \\
&\mathbf{E}\ (\ \neg\ \text{MEKP}\ \mathbf{U}\ \text{MEKPP}\ )\ \vee \\
&\mathbf{E}\ (\ \neg\ \text{MEKPP}\ \mathbf{U}\ \text{ERKP}\ )\ \vee \\
&\mathbf{E}\ (\ \neg\ \text{ERKP}\ \mathbf{U}\ \text{ERKPP}\ )\ \ ]
\end{aligned}
$$

- *isomorphy of reachability graph and CTMC,*
  thus all qualitative properties still valid

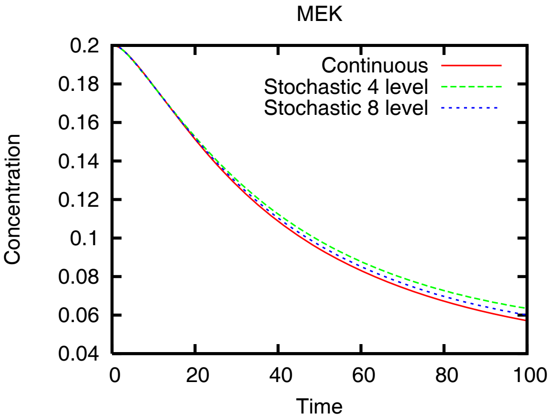
- *How many levels needed for quantitative evaluation ?*
  - state space(1 levels) = 118 (Boolean interpretation)
  - state space(4 levels) = 24,065
  - state space(8 levels) = 6,110,643

- *equivalence check*

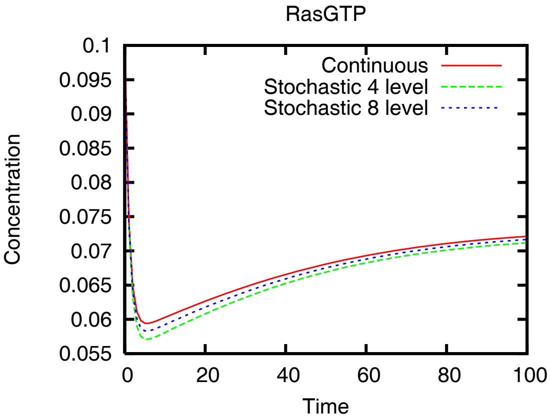
$$C_{RafP}(t) \;=\; \frac{0.1}{s} \cdot \underbrace{\sum_{i=1}^{4s} (i \cdot P(L_{RafP}(t) = i))}_{expected\ \ value\ \ of\ \ L_{RafP}(t)}$$

Stochastic Model Checking - Preparation

- *equivalence check, results, e.g. for MEK :*



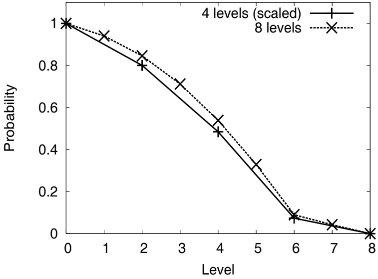
- *equivalence check, results, e.g. for RasGTP :*



property S1 :

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

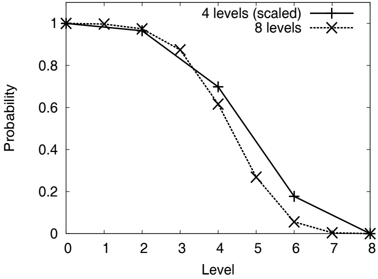
$$\mathbf{P}_{=?}\ [\ (\ \text{RafP}\ =\ \text{L}\ )\ \mathbf{U}^{<=100}\ (\ \text{RafP} > \text{L}\ )\ \{\ \text{RafP} = \text{L}\ \}\ ]$$



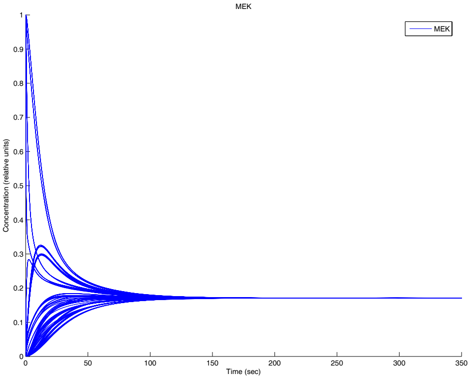
property S2 :

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

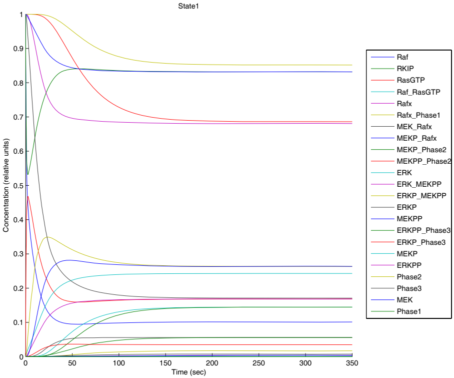
$$P_{=?}\,[\,(\,(\,MEKPP = 0\,) \wedge (\,ERKPP = 0\,)\,)\,\mathbf{U}^{<=100}\,(\,RafP > L\,)\; \{\,(\,MEKPP = 0\,) \wedge (\,ERKPP = 0\,) \wedge (\,RafP = 0\,)\,\}\,]$$



- *steady state analysis, results for all 118 'good' states, e.g. for MEK :*

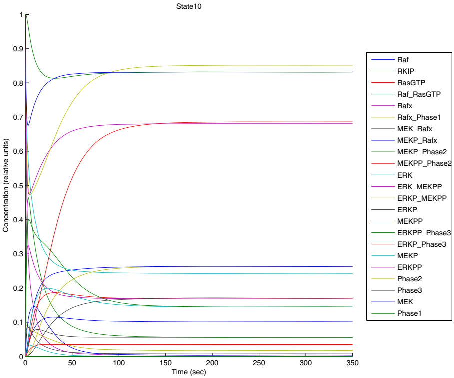


- *steady state analysis for state 1 :*



Continuous Model Checking - Preparation

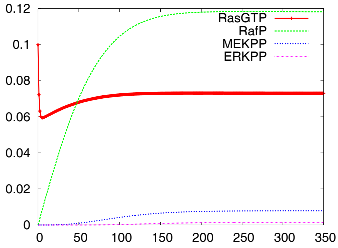
- *steady state analysis for state 10 :*

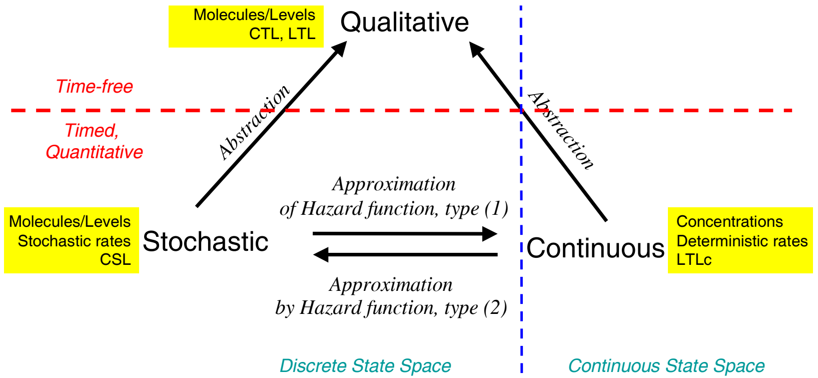


property C1 :

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

$$((MEKPP < 0.001) \wedge (ERKPP < 0.0002)) \; \mathbf{U} \; (RafP > 0.06)$$




Molecules/Levels
CTL, LTL
Qualitative
Time-free
Timed,
Quantitative
Abstraction
Abstraction
Molecules/Levels
Stochastic rates
CSL
Stochastic
Approximation
of Hazard function, type (1)
Approximation
by Hazard function, type (2)
Continuous
Concentrations
Deterministic rates
LTLc
Discrete State Space
Continuous State Space

Tools

- *model construction, animation, simulation*
  - Snoopy *(Cottbus)*
- *qualitative analysis*
  - Charlie *(Cottbus)*, INA
  - BDD-CTL model checker (Boolean semantics) *(Cottbus)*
  - IDD-CTL model checker (integer semantics) *(Cottbus)*
- *stochastic analysis*
  - analytical model checking : PRISM/CSL
  - simulative model checking : MC2(PLTLc) *(Glasgow)*
- *continuous analysis*
  - MATLAB
  - BioNessie *(Glasgow)*
  - LTLc model checking : MC2(PLTLc) *(Glasgow)*, BioCham

end of part III

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

BIO PETRI NETS, PART IV

SUMMARY

A Bit of History

- **Carl Adam Petri, 1962, PhD University of Technology Darmstadt**

  -> *basic ideas introduced*

- **early 1970's**

  -> *first papers contributing to Petri net theory*

- **Petri, 1976**

  -> *application to chemical networks mentioned*

- **early 1980's**

  -> *first monographs on Petri net theory*

- **Reddy, 1993**

  -> *first paper on bio application*

- **late 1990's**

  -> *increasing interest for modelling and analysis of bio networks*

C. A. PETRI, NOVEMBER 2006

A BIT OF HISTORY

SUMMARY

- **representation of bio networks by Petri nets**

-> *partial order representation*	-> *better comprehension*
-> *formal semantics*	-> *sound analysis techniques*
-> *unifying view*	-> *various abstraction levels*

- **purposes**

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

- **step-wise model development**

-> *qualitative model*	-> *discrete Petri nets*
-> *discrete quantitative model*	-> *stochastic Petri nets*
-> *continuous quantitative model*	-> *continuous Petri nets = ODEs*

CHALLENGES

- **increasing level number = increasing accuracy**
  BUT, *monotonous liveness holds for substructures only !*

- **unbounded qualitative model + time = bounded model**
  BUT, *that's not always the case !*
  -> (structural) criteria for time-dependent boundedness ?

- **continuous behaviour = averaged stochastic behaviour**
  BUT, *that's not always the case !*
  -> stochastic and continuous behaviour may differ; why ? when ?

- **sharing structure = sharing properties**
  BUT, *to which extend ?*
  -> relation: qualitative & continuous behaviour ?

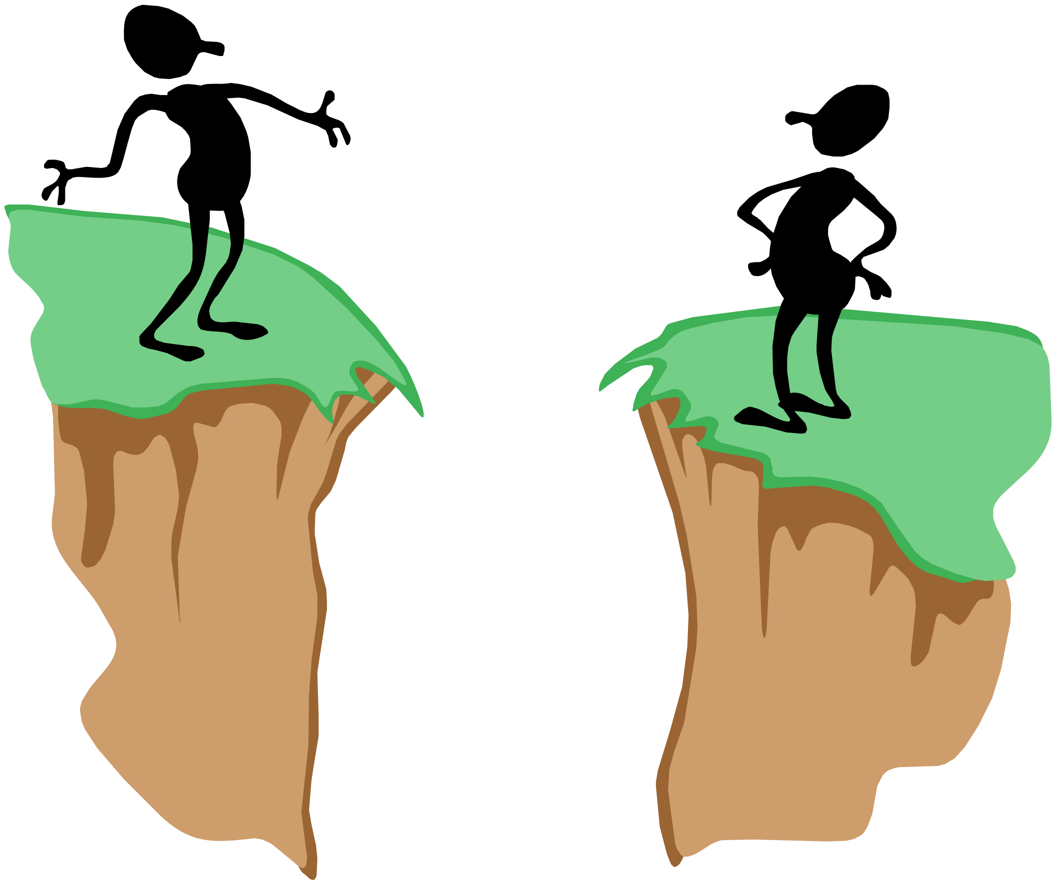
Further Reading

- M Heiner, D Gilbert, R Donaldson:
  Petri Nets for Systems and Synthetic Biology
  Springer LNCS 5016, pp. 215-264, 2008.

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

- D Gilbert, M Heiner, S Rosser, R Fulton, X Gu, M Trybilo:
  A Case Study in Model-driven Synthetic Biology;
  IFIP WCC 2008, 2nd IFIP Conference on Biologically Inspired Collaborative Computing (BICC 2008), Milano, Sept. 2008, to appear.

OUTLOOK



THANKS !

HHTP://WWW-DSSZ.INFORMATIK.TU-COTTBUS.DE