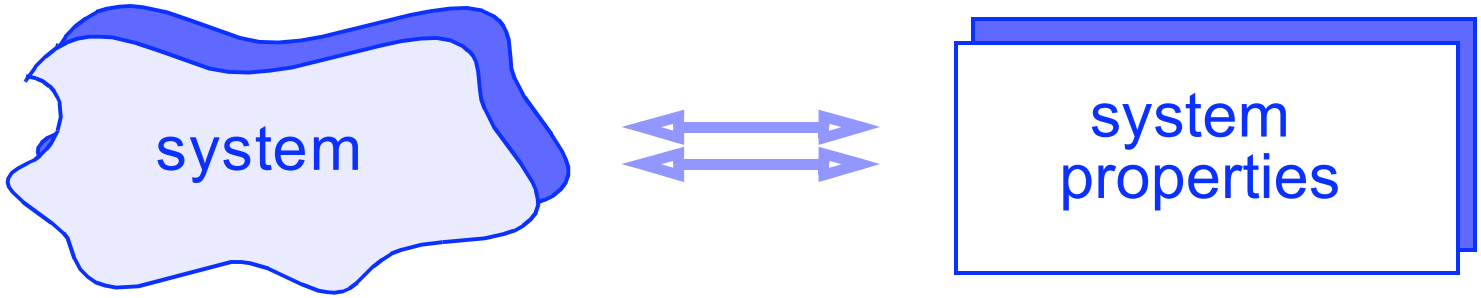
BIOCHEMICAL NETWORKS
- A PETRI NET PERSPECTIVE -

Monika Heiner

Brandenburg University of Technology Cottbus

Dept. of CS

MODEL- BASED SYSTEM ANALYSIS



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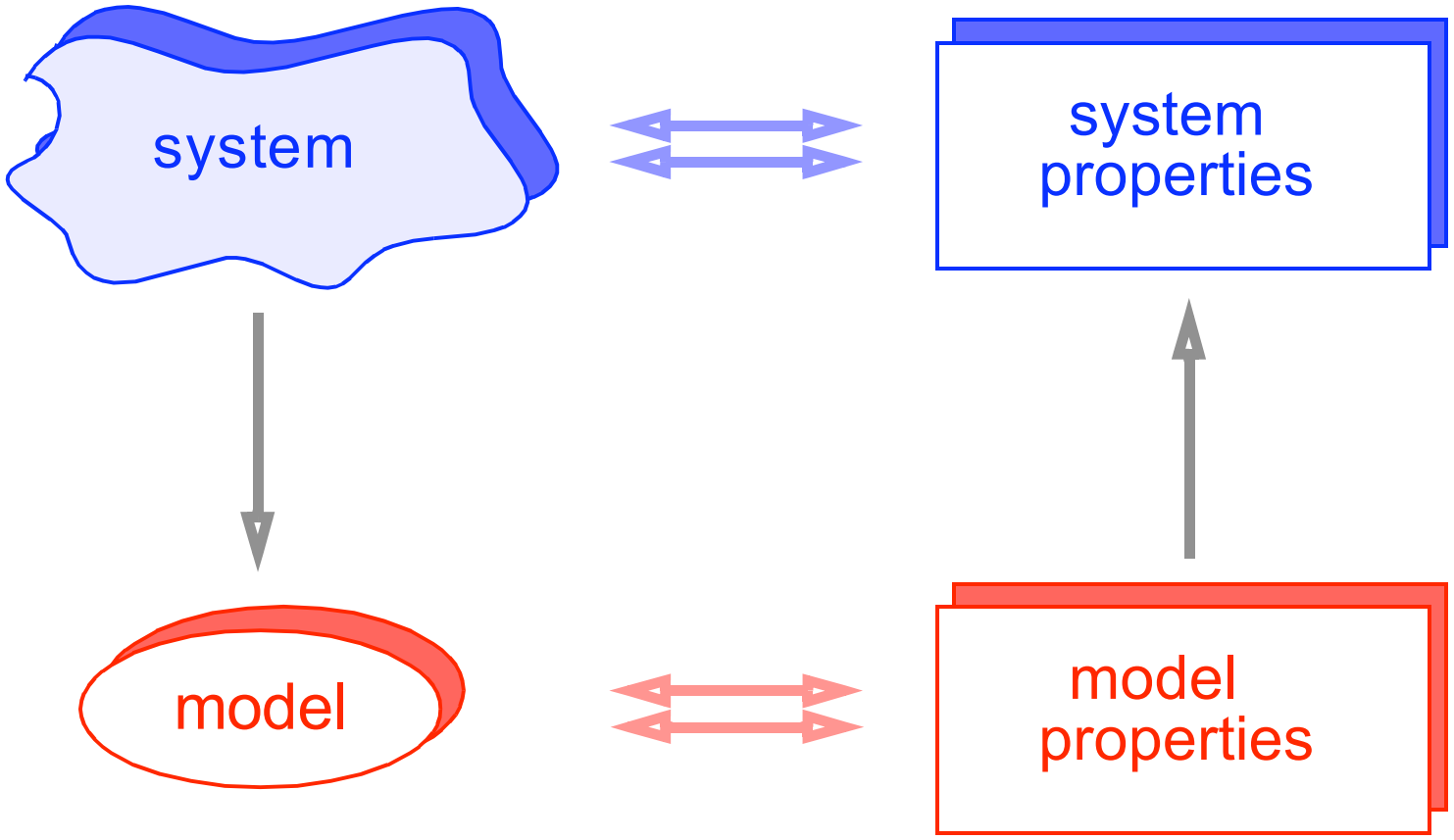

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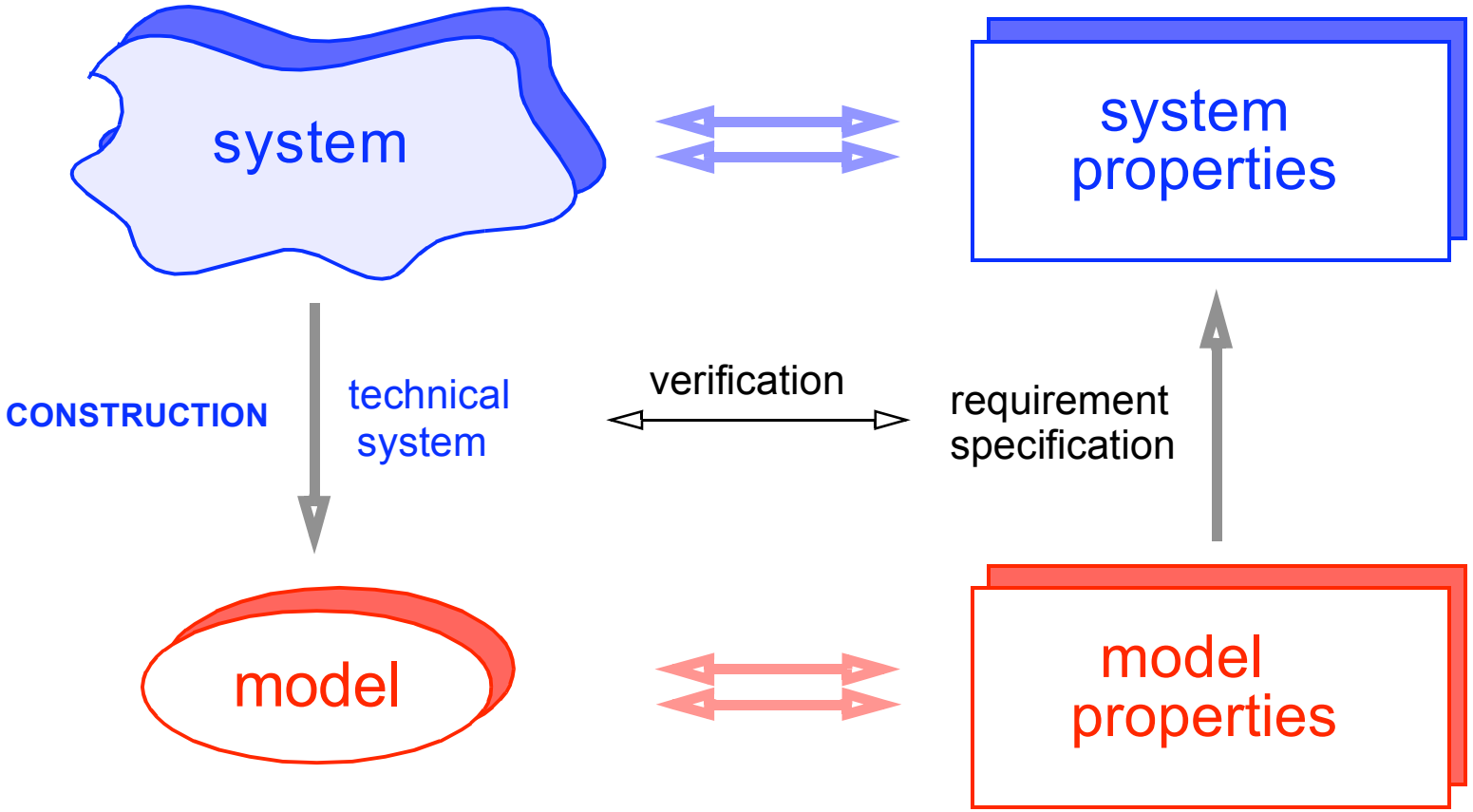

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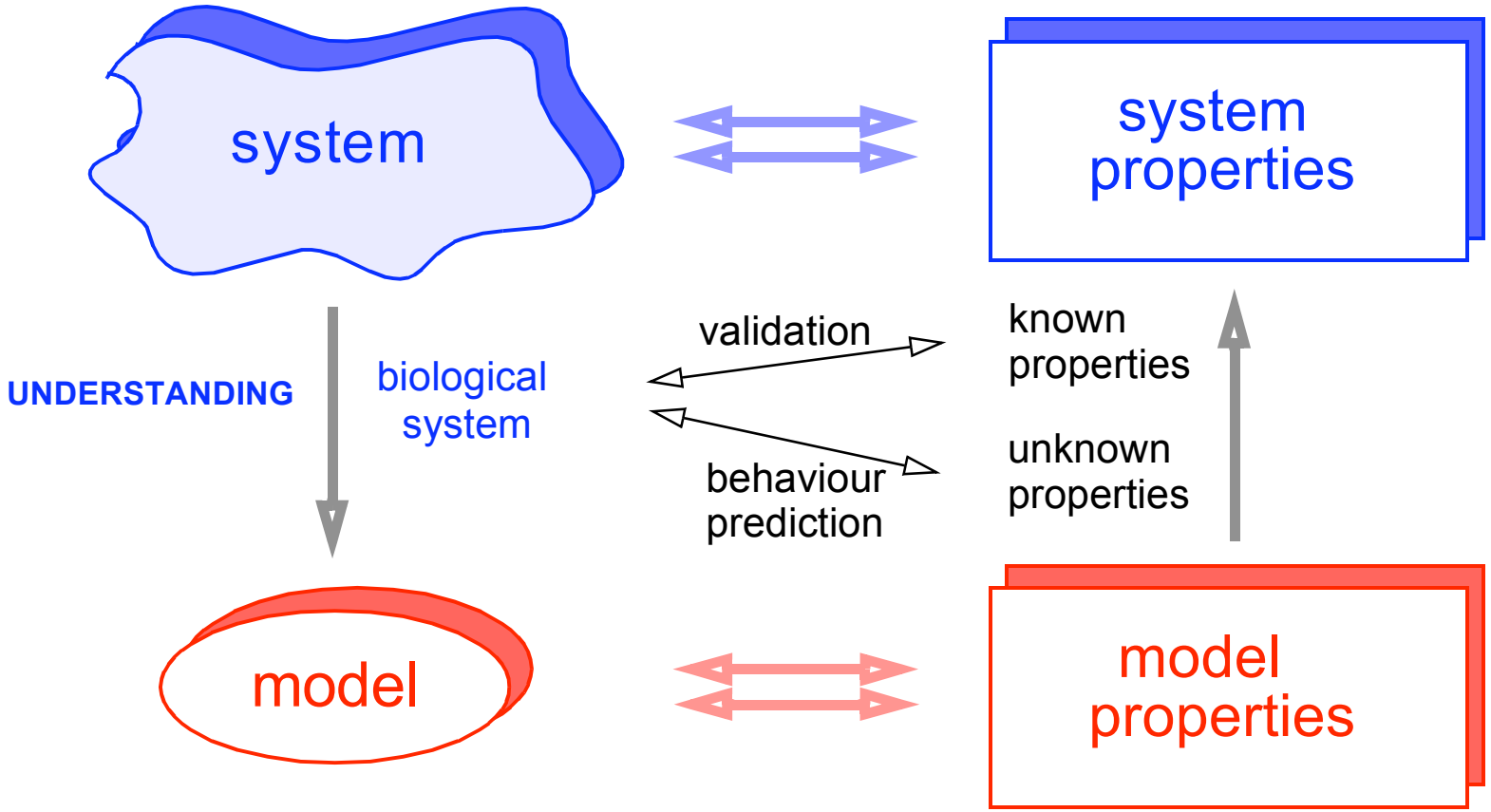
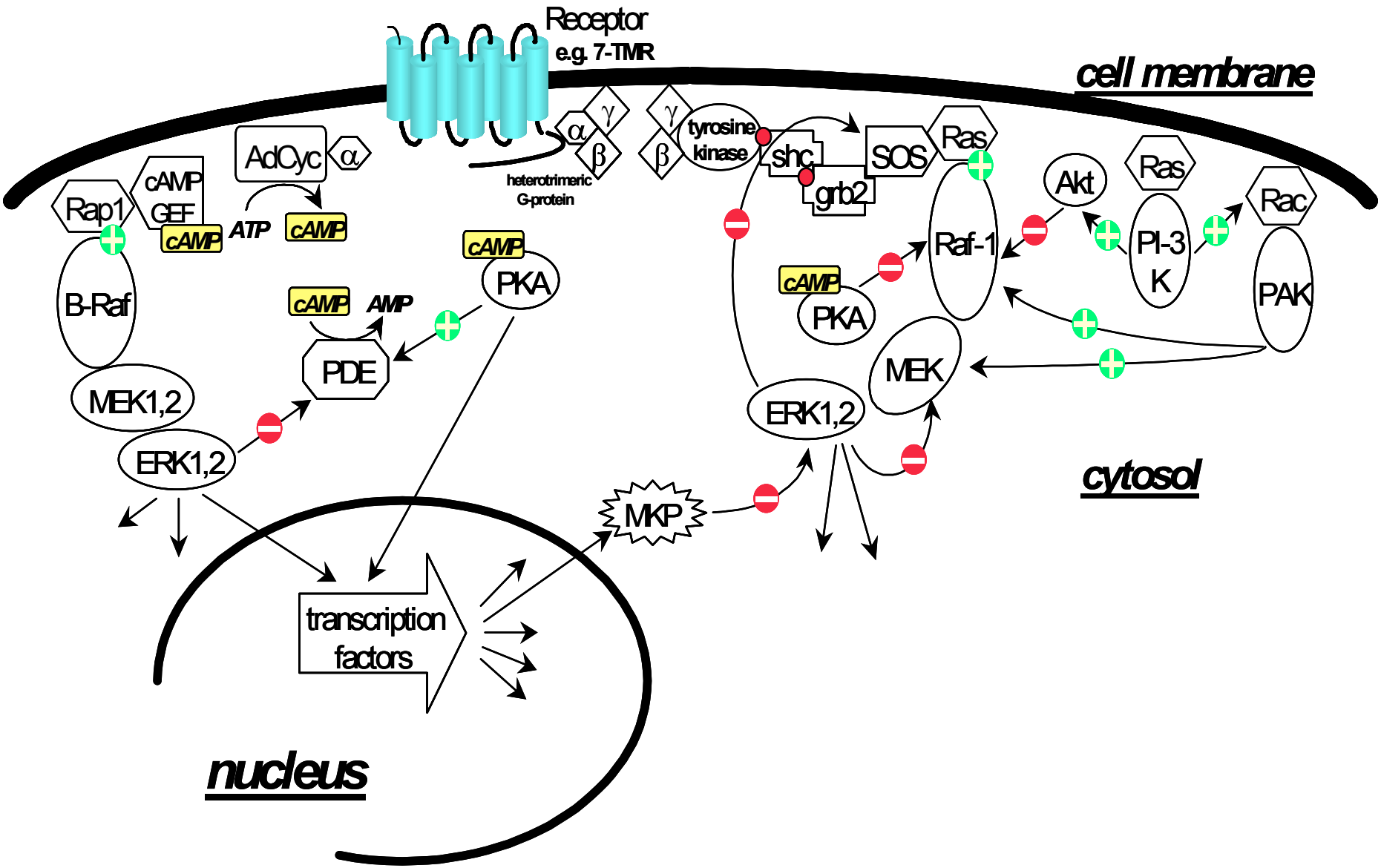


WHAT KIND OF MODEL SHOULD BE USED?

NETWORK REPRESENTATIONS, EX1



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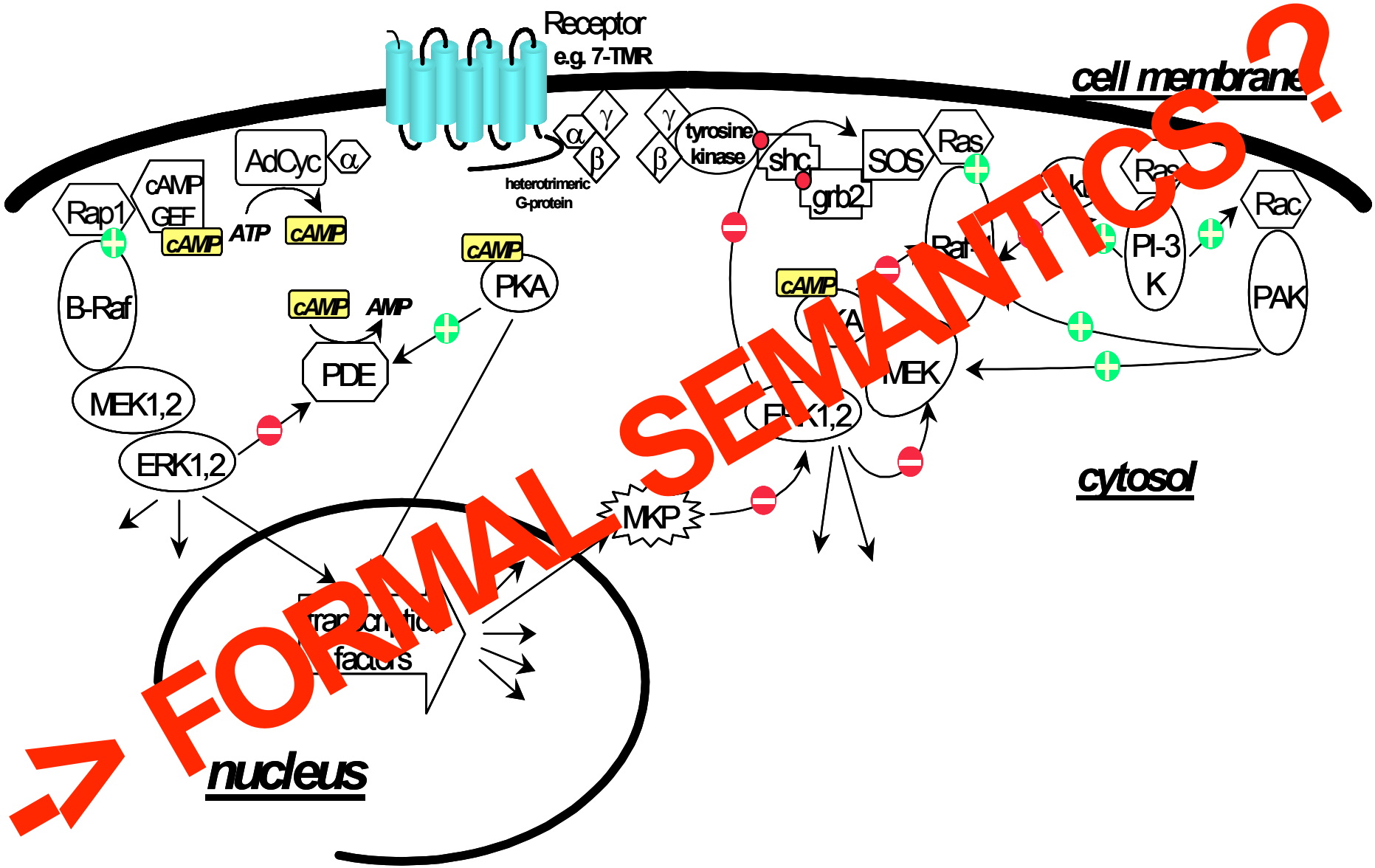

NETWORK REPRESENTATIONS, EX2

$$\frac{d\alpha}{dt} = -v_1$$
$$\frac{dSte2}{dt} = -v_2 + v_3 - v_5$$
$$\frac{dSte2_{active}}{dt} = v_2 - v_3 - v_4$$
$$\frac{dSst2_{active}}{dt} = v_{46} - v_{47}$$
$$\frac{dG\alpha\beta\gamma}{dt} = -v_6 + v_9$$
$$\frac{dG\alpha GTP}{dt} = v_6 - v_7 - v_8$$
$$\frac{dG\alpha GDP}{dt} = v_7 + v_8 - v_9$$
$$\frac{dG\beta\gamma}{dt} = v_6 - v_9 - v_{10} + v_{11} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32} - v_{42} + v_{43}$$
$$\frac{dSte5}{dt} = -v_{12} + v_{13} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32}$$
$$\frac{dSte11}{dt} = -v_{12} + v_{13} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32}$$
$$\frac{dSte7}{dt} = -v_{14} + v_{15} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32}$$
$$\frac{dFus3}{dt} = -v_{14} + v_{15} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} - v_{29} + v_{30} + v_{33}$$
$$\frac{dSte20}{dt} = -v_{18} + v_{19} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32}$$

$$v_1 = \alpha[t] \cdot Bar1_{active}[t] \cdot k_1$$
$$v_2 = Ste2[t] \cdot \alpha[t] \cdot k_2$$
$$v_3 = Ste2_{active}[t] \cdot k_3$$
$$v_4 = Ste2_{active}[t] \cdot k_4$$
$$v_5 = Ste2[t] \cdot k_5$$
$$v_6 = Ste2_{active}[t] \cdot G\alpha\beta\gamma[t] \cdot k_6$$
$$v_7 = G\alpha GTP[t] \cdot k_7$$
$$v_8 = G\alpha GTP[t] \cdot Sst2_{active}[t] \cdot k_8$$
$$v_9 = G\alpha GDP[t] \cdot G\beta\gamma[t] \cdot k_9$$
$$v_{10} = G\beta\gamma[t] \cdot C[t] \cdot k_{10}$$
$$v_{11} = D[t] \cdot k_{11}$$
$$v_{12} = Ste5[t] \cdot Ste11[t] \cdot k_{12}$$
$$v_{13} = A[t] \cdot k_{13}$$
$$v_{14} = Ste7[t] \cdot Fus3[t] \cdot k_{14}$$
$$v_{15} = B[t] \cdot k_{15}$$
$$v_{16} = A[t] \cdot B[t] \cdot k_{16}$$
$$v_{17} = C[t] \cdot k_{17}$$
$$v_{18} = D[t] \cdot Ste20[t] \cdot k_{18}$$

NETWORK REPRESENTATIONS, EX2

$$\frac{d\alpha}{dt} = -\nu_1$$
$$\frac{dSte2}{dt} = -\nu_2 + \nu_3 - \nu_5$$
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$$\frac{dSte20}{dt} = -\nu_{18} + \nu_{19} + \nu_{21} + \nu_{23} + \nu_{25} + \nu_{27} + \nu_{32}$$

$$\nu_1 = \alpha[t] \cdot Bar1_{active}[t] \cdot k_1$$
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$$\nu_{17} = C[t] \cdot k_{17}$$
$$\nu_{18} = D[t] \cdot Ste20[t] \cdot k_{18}$$

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 structure** -> ***PROBLEM 3***
  - -> *tends to grow fast*
  - -> *dense, apparently unstructured*
  - -> *hard to read*

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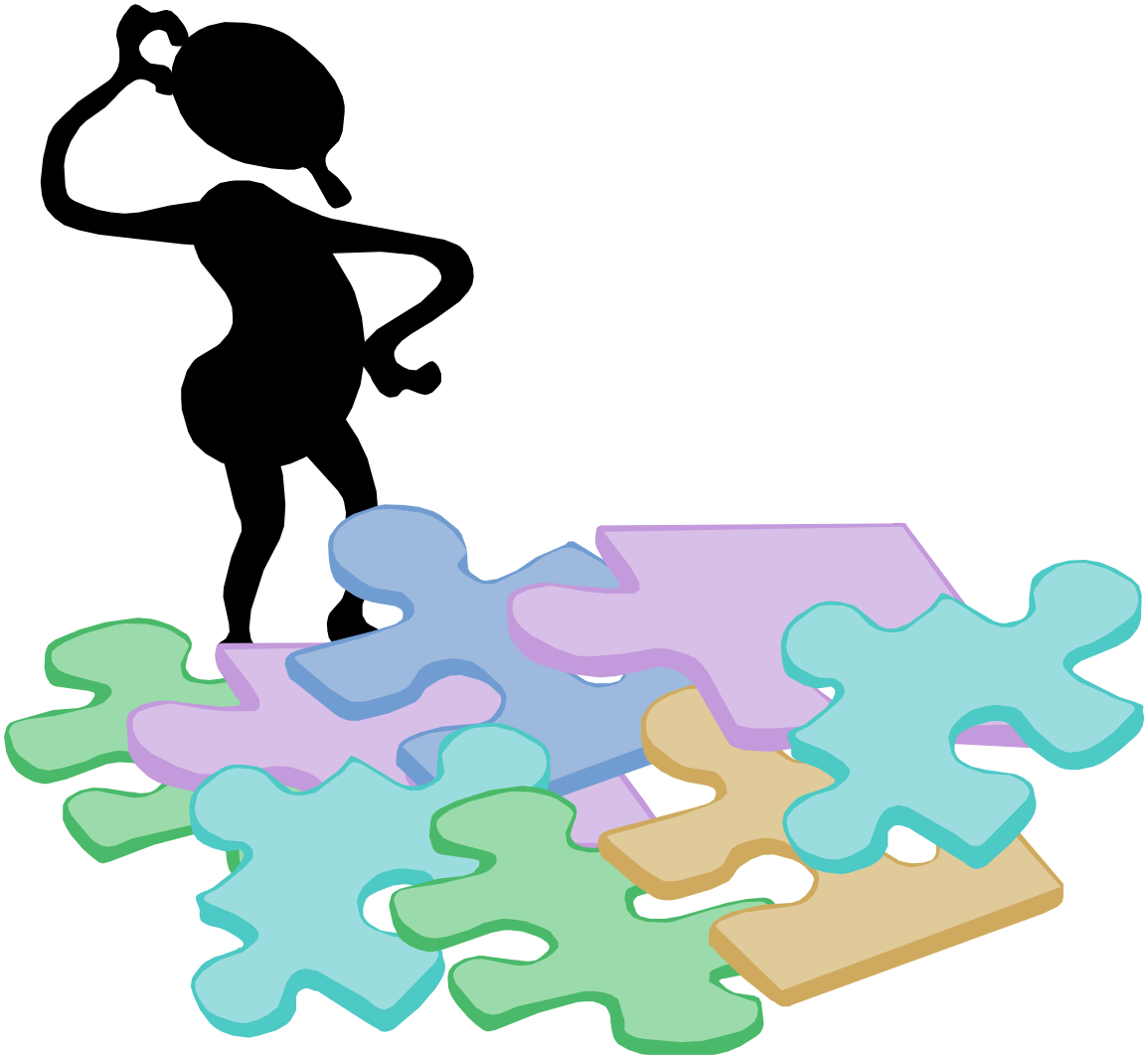
-> MODELS ARE FULL OF ASSUMPTIONS <-

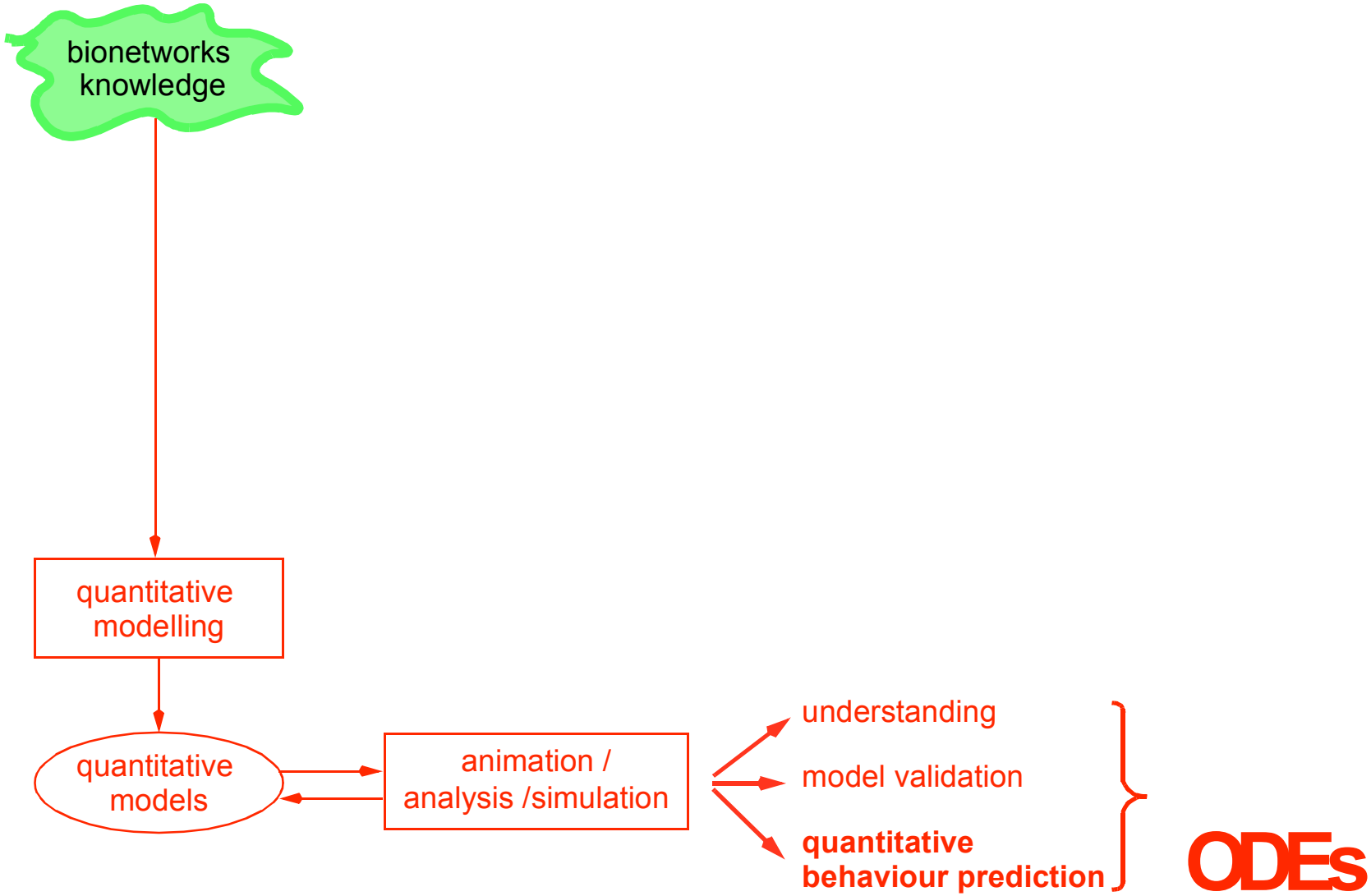
BIO NETWORKS, SOME PROBLEMS



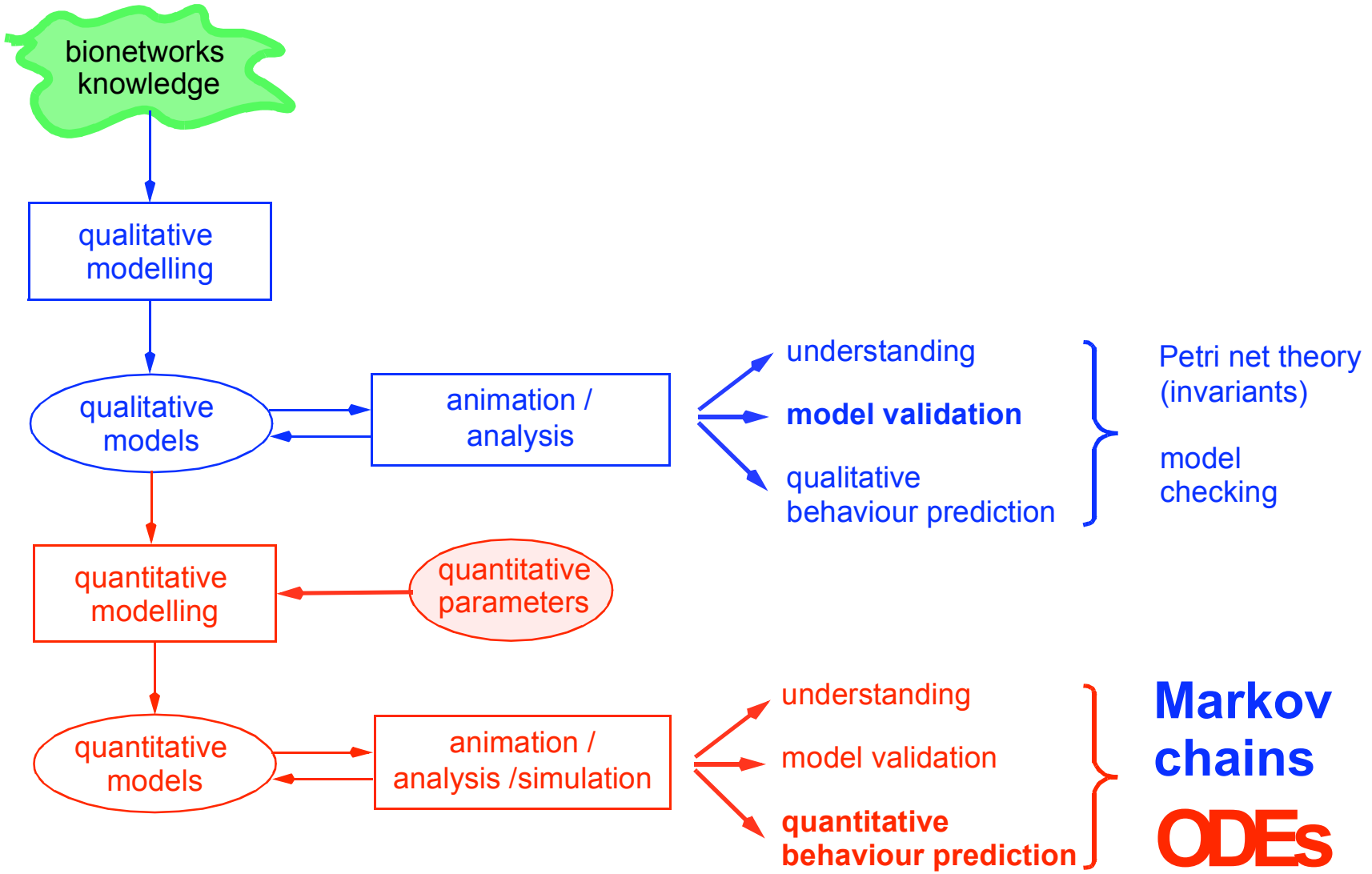
BIO NETWORK REPRESENTATIONS SHOULD BE

- **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*

FRAMEWORK


bionetworks knowledge
quantitative modelling
quantitative models
animation / analysis /simulation
understanding
model validation
quantitative behaviour prediction
ODEs

FRAMEWORK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Petri net theory (invariants)
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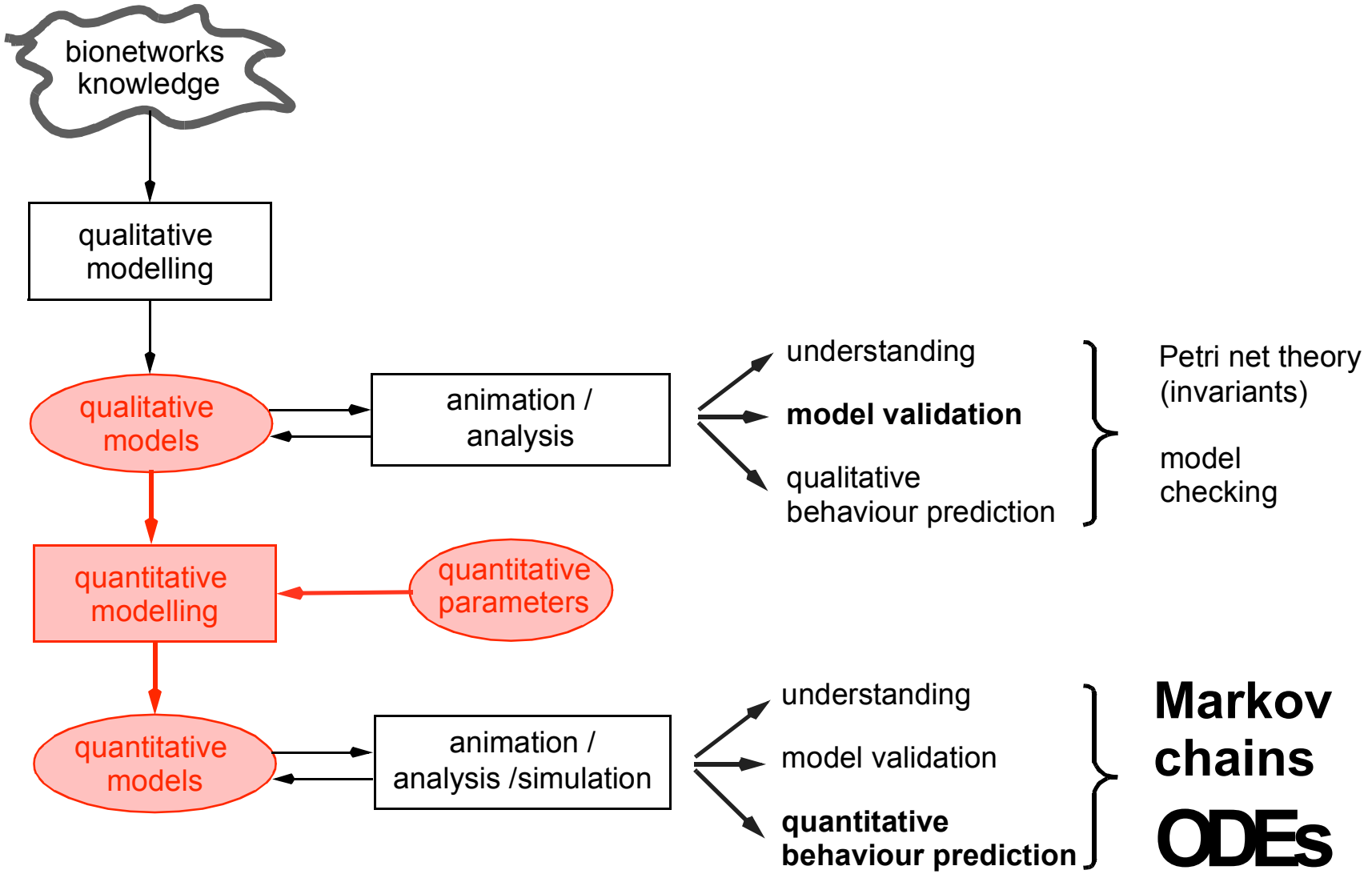
FRAMEWORK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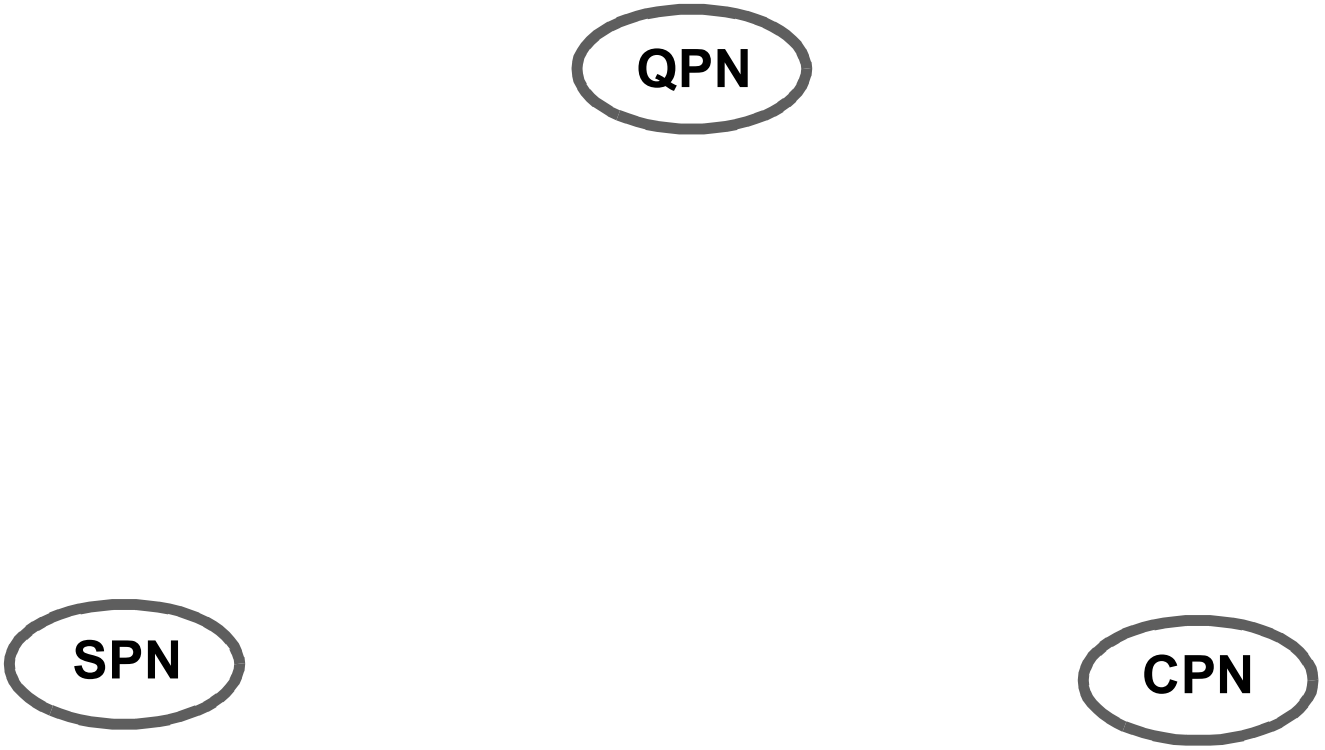
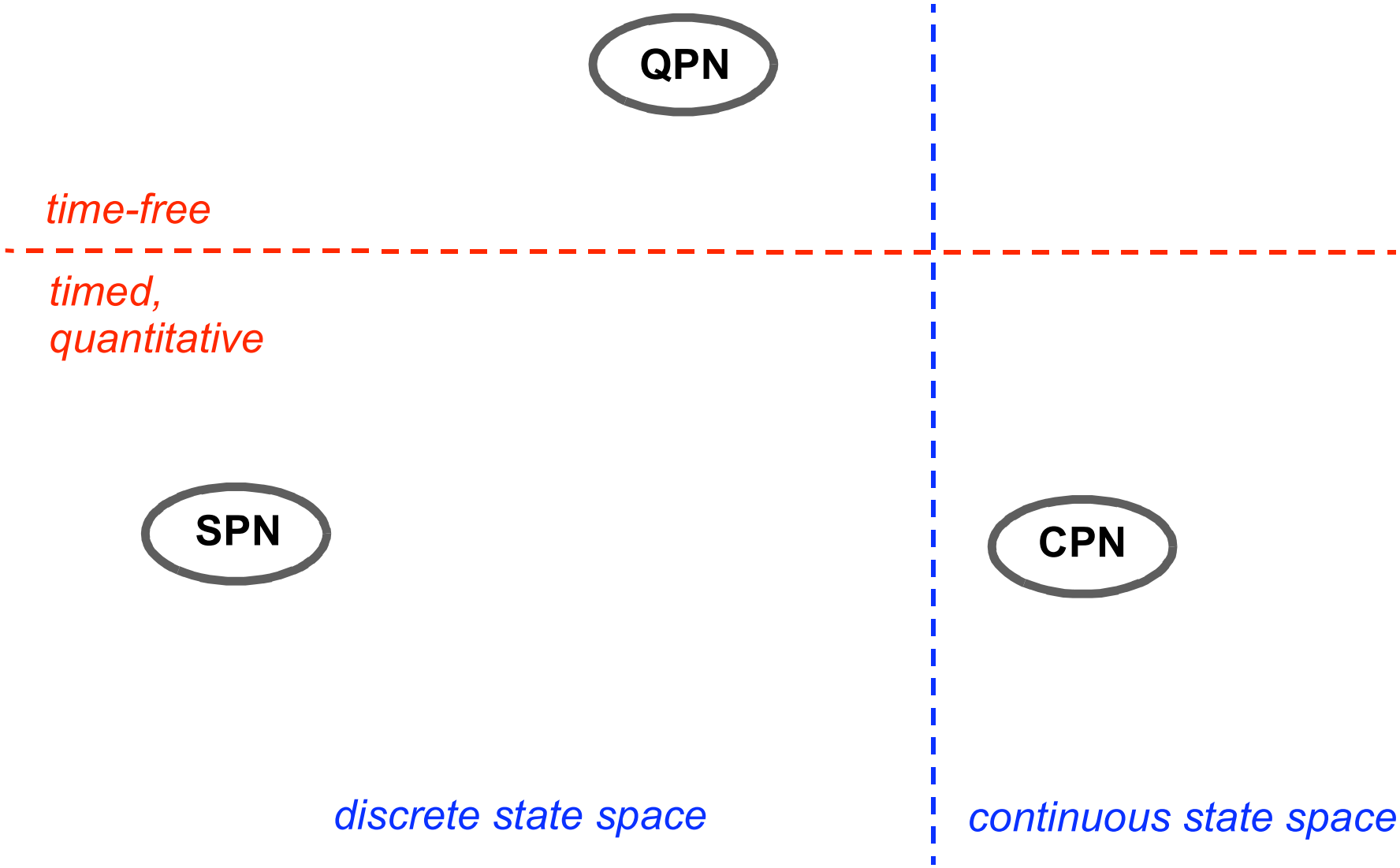
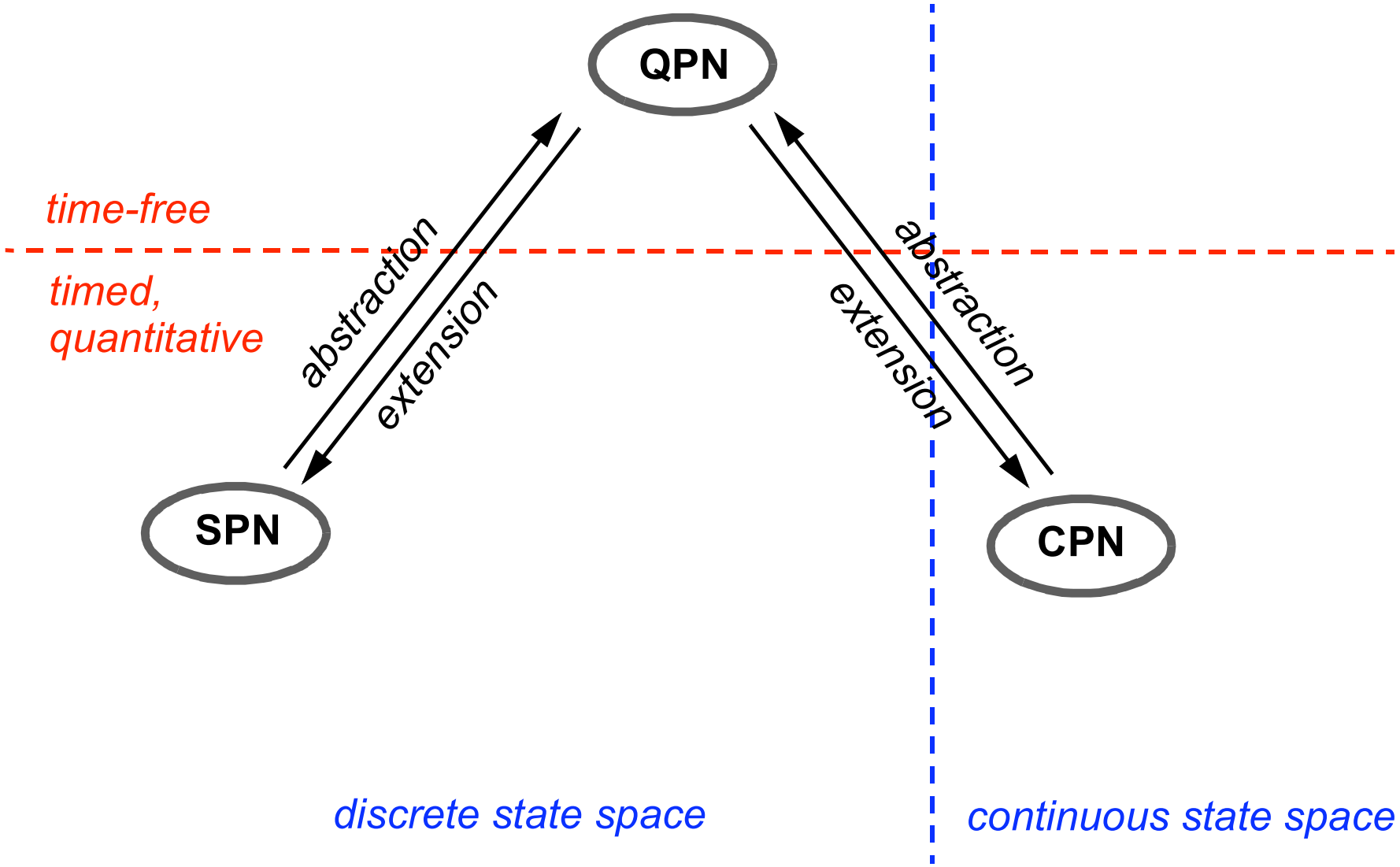


FRAMEWORK


QPN
time-free
timed,
quantitative
SPN
CPN
discrete state space
continuous state space

FRAMEWORK


QPN
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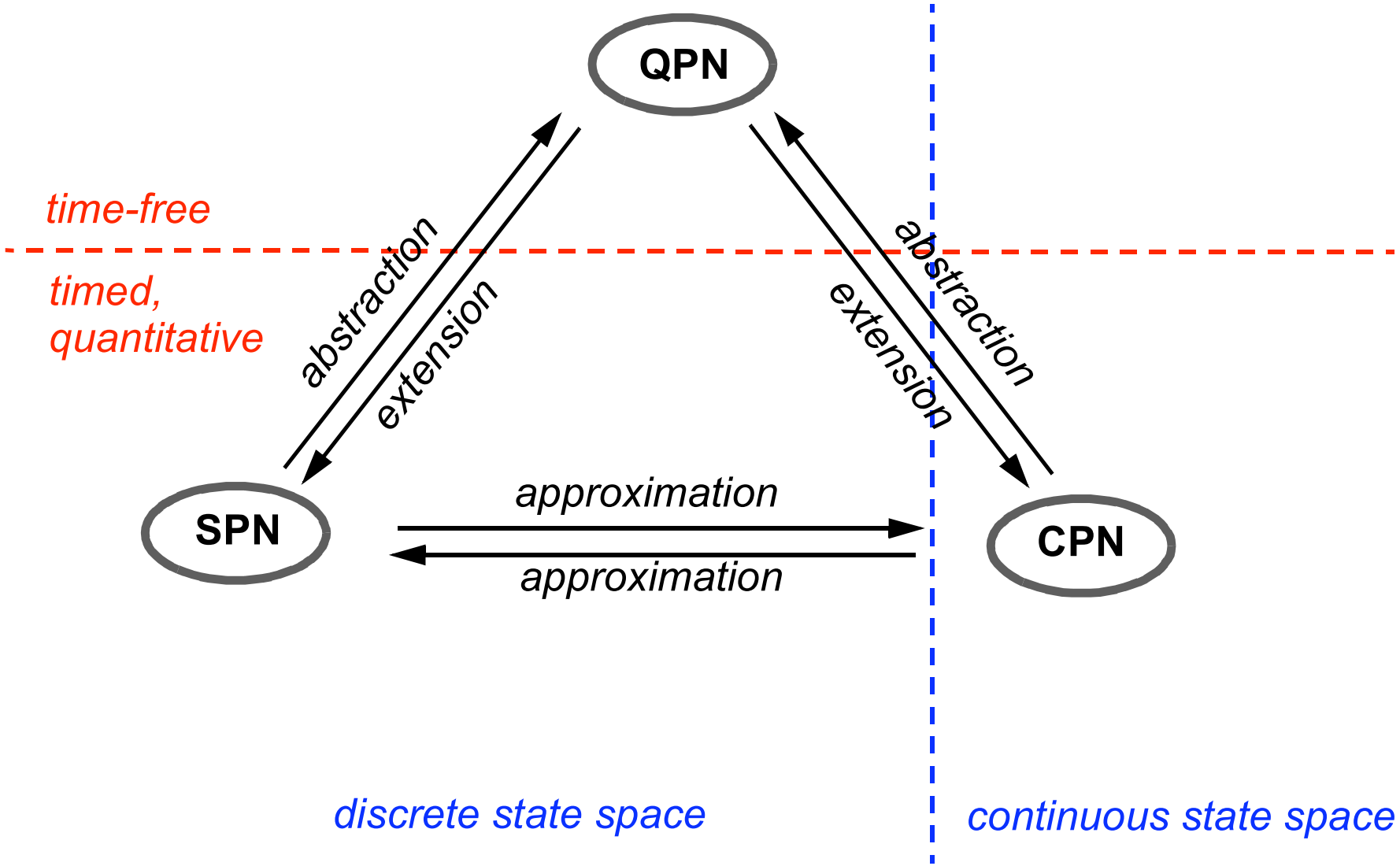
QPN
time-free
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extension
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extension
SPN
approximation
approximation
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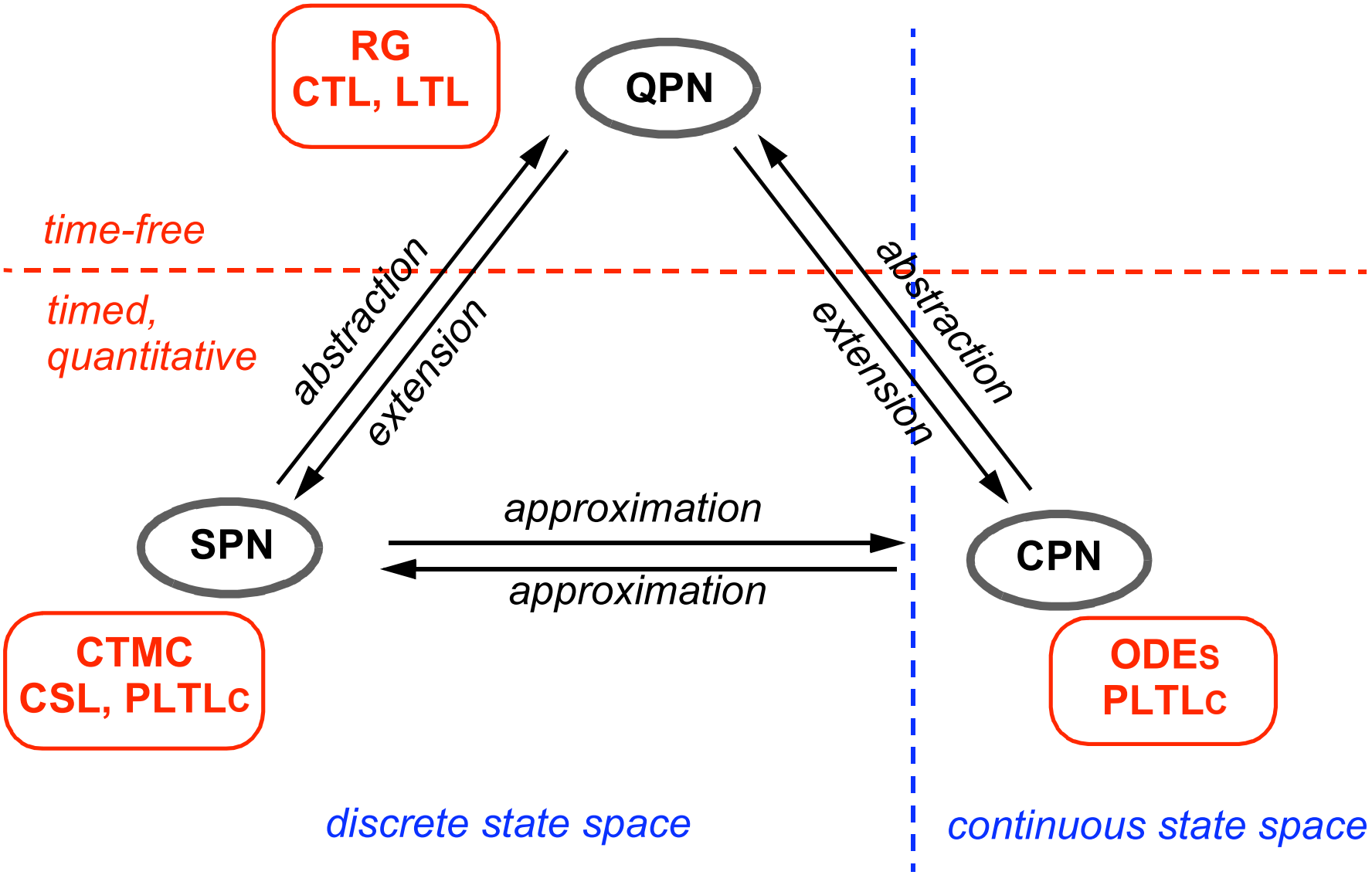
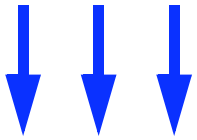
discrete state space
continuous state space

FRAMEWORK


RG
CTL, LTL
QPN
time-free
timed,
quantitative
abstraction
extension
abstraction
extension
approximation
approximation
SPN
CPN
CTMC
CSL, PLTLc
ODEs
PLTLc
discrete state space
continuous state space

THREE MODELS SHARING STRUCTURE



QUANTITATIVE MODEL = QUALITATIVE MODEL

+

QUANTITATIVE PARAMETERS (KINETICS)

QUALITATIVE PETRI NETS - QPN -

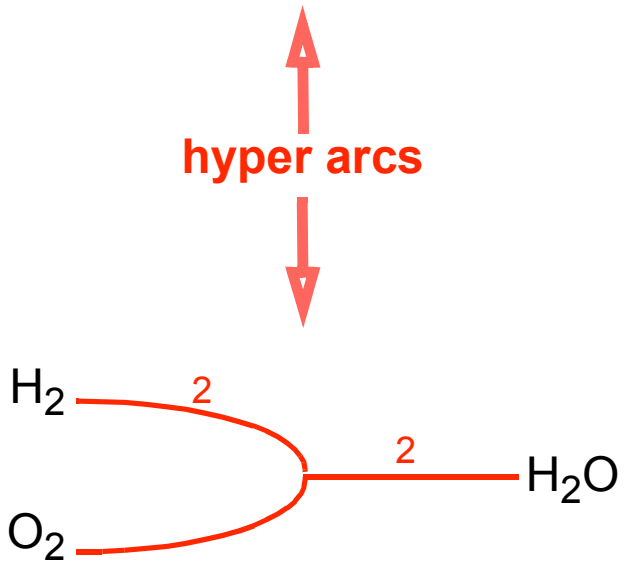
. . . ARE
NETWORKS OF
(BIO-) CHEMICAL REACTIONS

-

$$2\ H_2 + O_2 \rightarrow 2\ H_2O$$

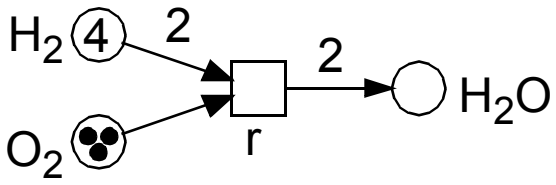
❑

$$2\ H_2 + O_2 \rightarrow 2\ H_2O$$

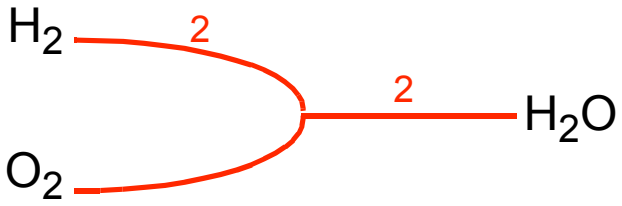


❑

$2\ H_2 + O_2 \rightarrow 2\ H_2O$

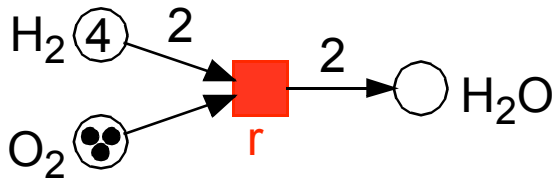


hyper arcs



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

$$2\ H_2 + O_2 \rightarrow 2\ H_2O$$



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

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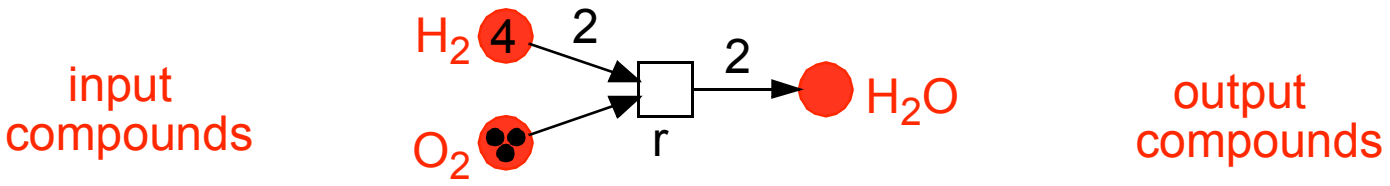

- **local conditions -> Petri net places -> chemical compounds**

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

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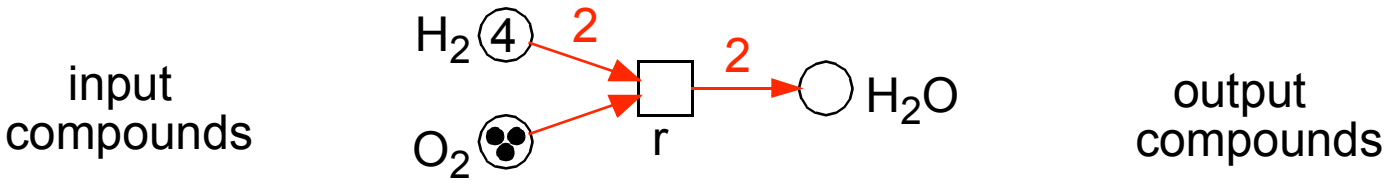

- **local conditions -> Petri net places -> chemical compounds**
- **multiplicities -> Petri net arc weights -> stoichiometric relations**

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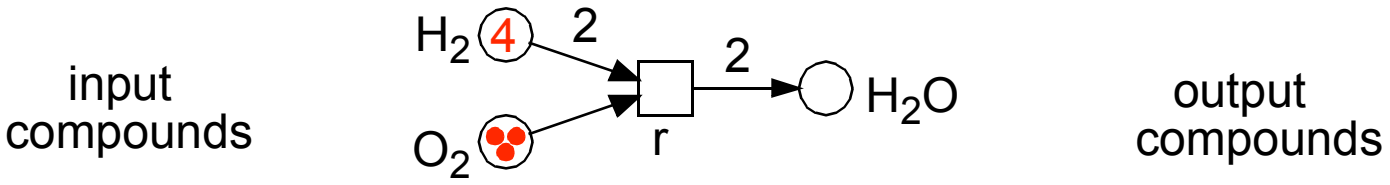
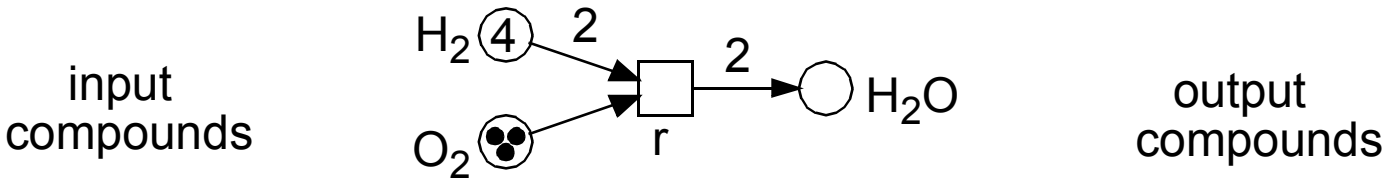


- local conditions -> Petri net places -> chemical compounds
- multiplicities -> Petri net arc weights -> stoichiometric relations
- condition's state -> token(s) in its place -> available amount (e.g. mol)
- system state -> marking -> compounds distribution

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

$$2\ H_2 + O_2 \rightarrow 2\ H_2O$$



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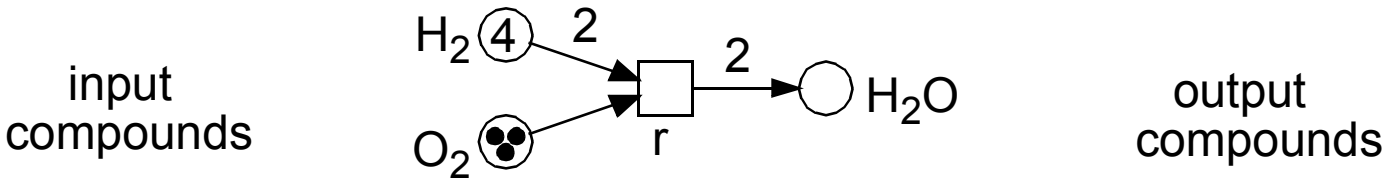
- **multiplicities -> Petri net arc weights -> stoichiometric relations**
- **condition's state -> token(s) in its place -> available amount (e.g. mol)**
- **system state -> marking -> compounds distribution**
- **PN = (P, T, F, m_0), F: (P x T) U (T x P) -> N_0, m_0: P -> N_0**

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*
  - -> *time-less*

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

$$2\ H_2 + O_2 \rightarrow 2\ H_2O$$



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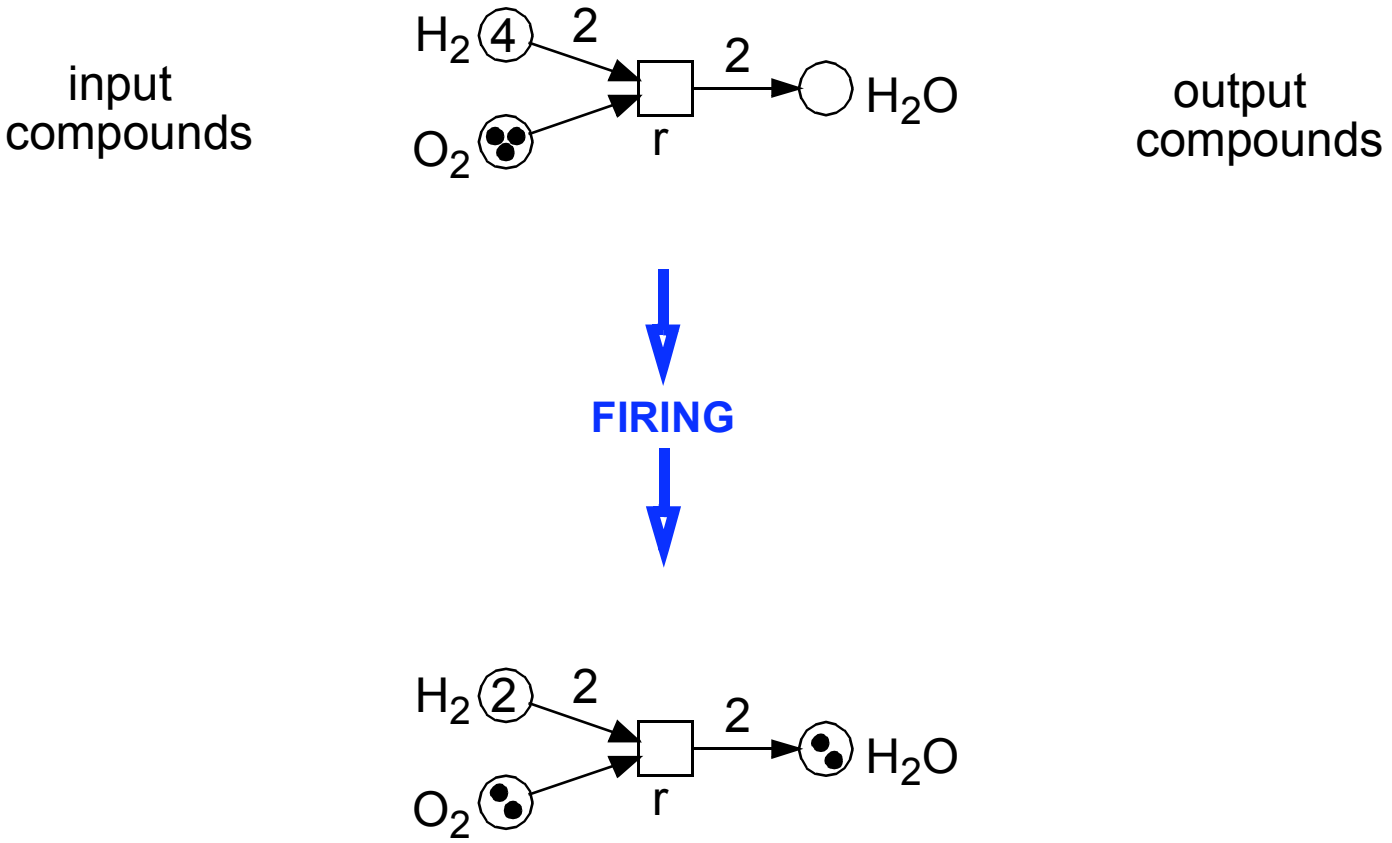

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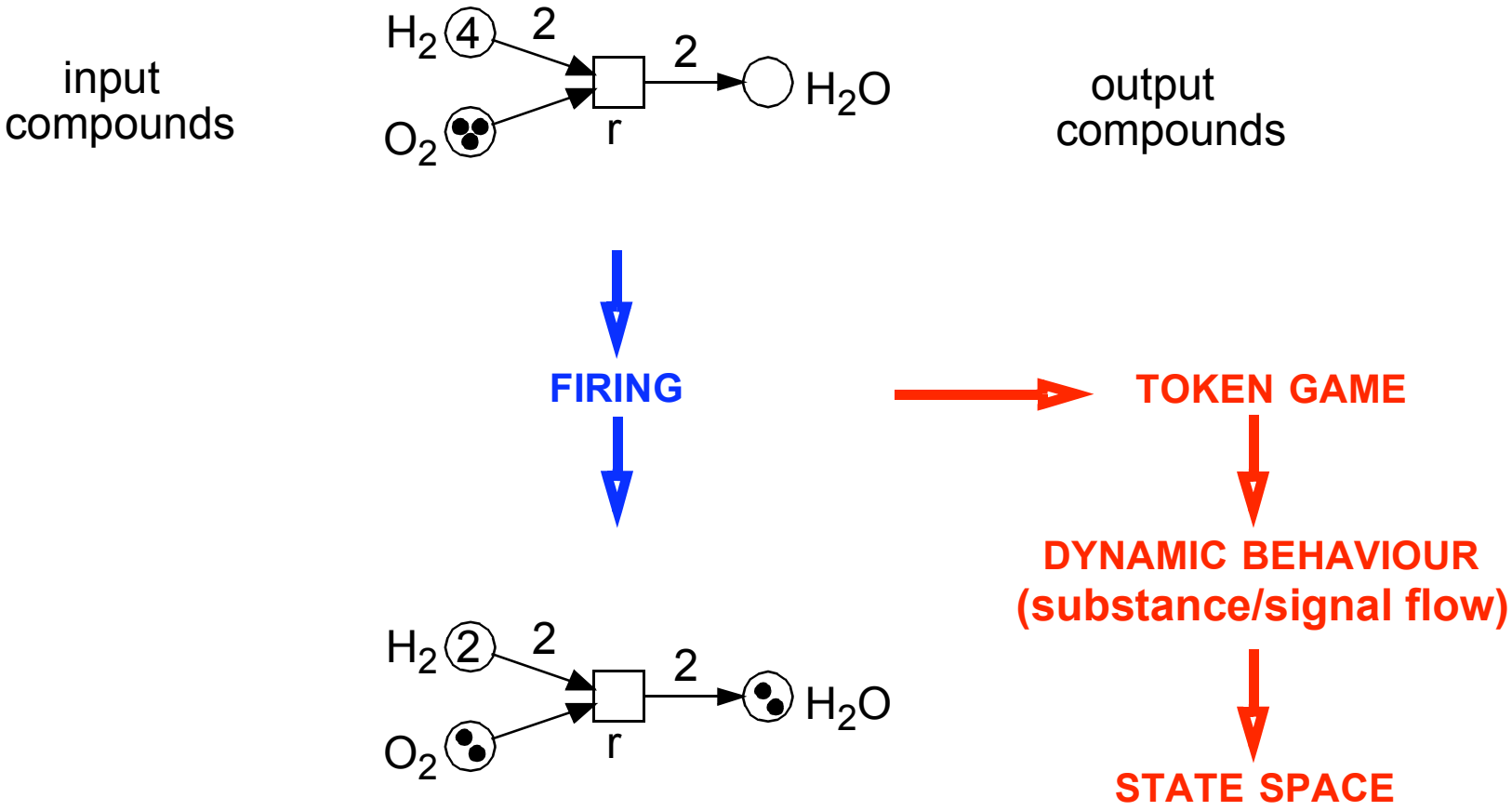
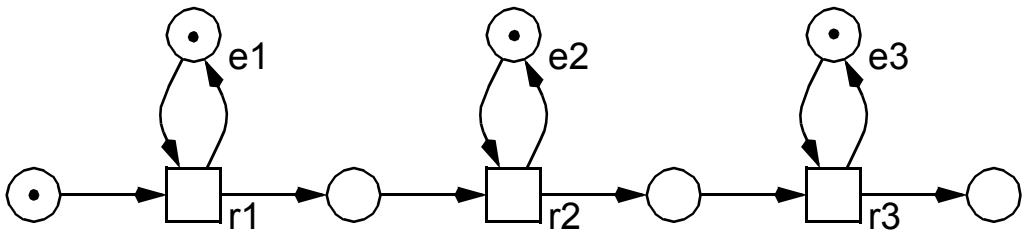
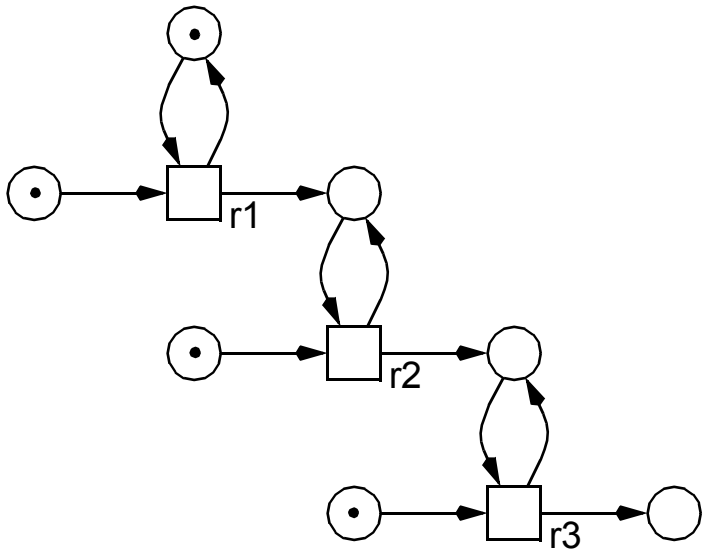


TYPICAL BASIC STRUCTURES

- **metabolic networks**
  -> *substance flows*



- **signal transduction networks**
  -> *signal flows*



PETRI NET ELEMENTS, INTERPRETATIONS

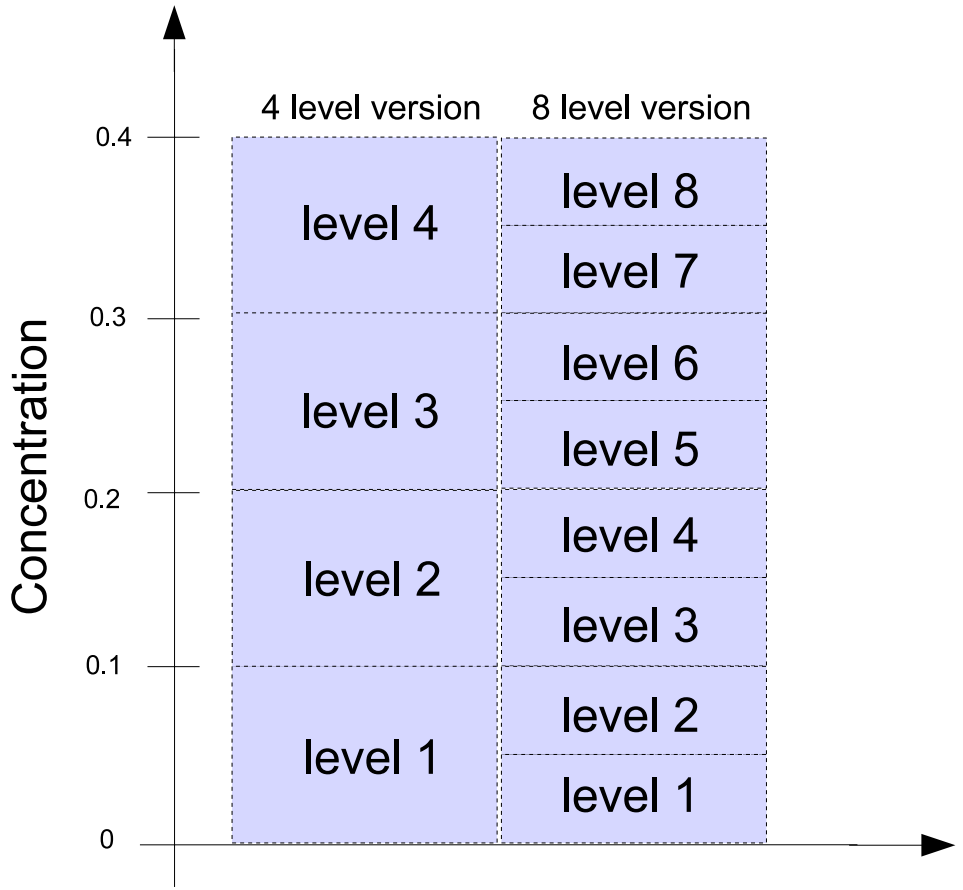
- **metabolic networks**
  signal transduction networks
  gene regulatory networks

- **transitions**
  - -> *(reversible, stoichiometric, enzyme-catalyzed) chemical reactions,*
  - -> *conversions/transport 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 integer number

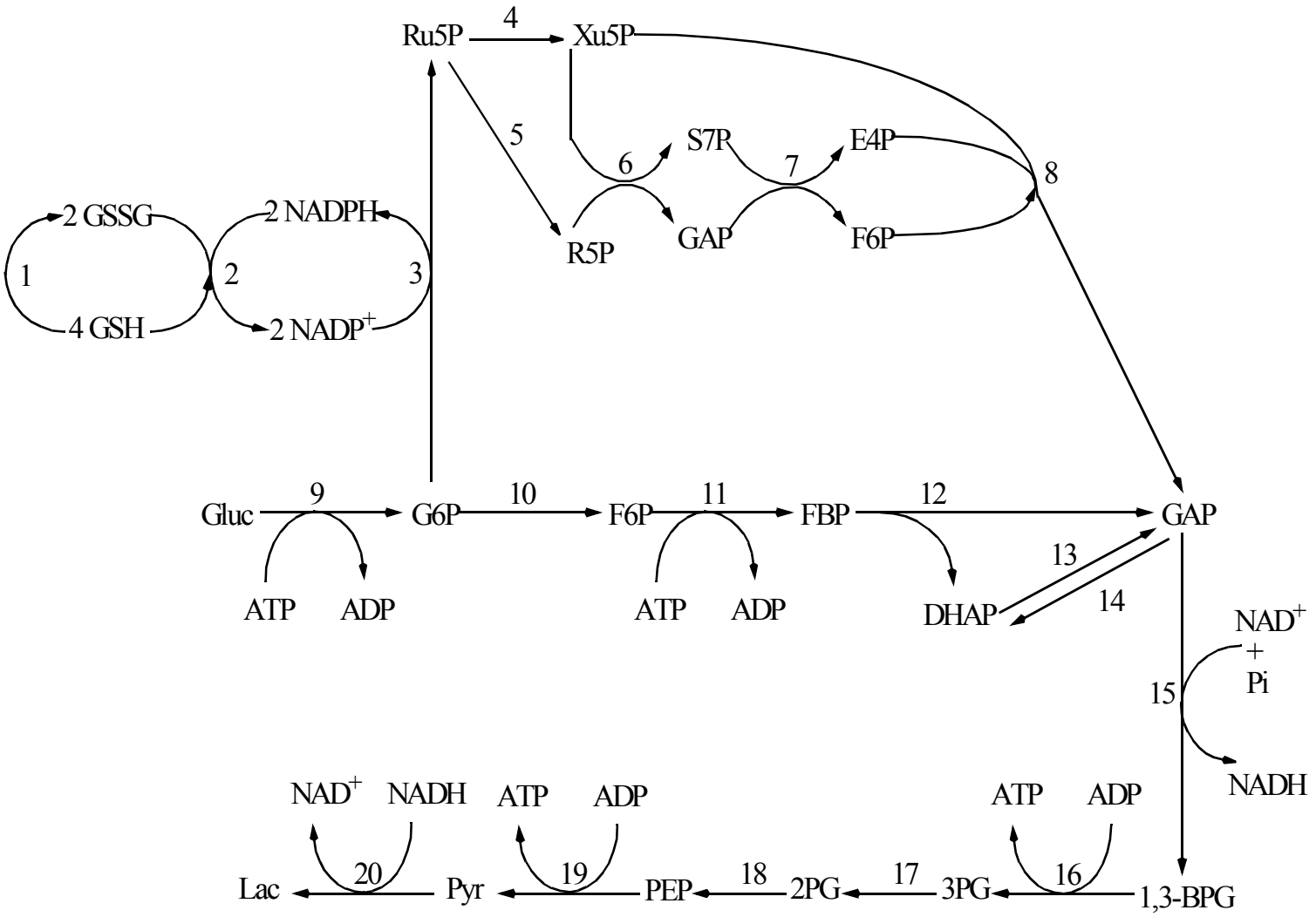
LEVEL CONCEPT



BIO PETRI NETS - SOME EXAMPLES

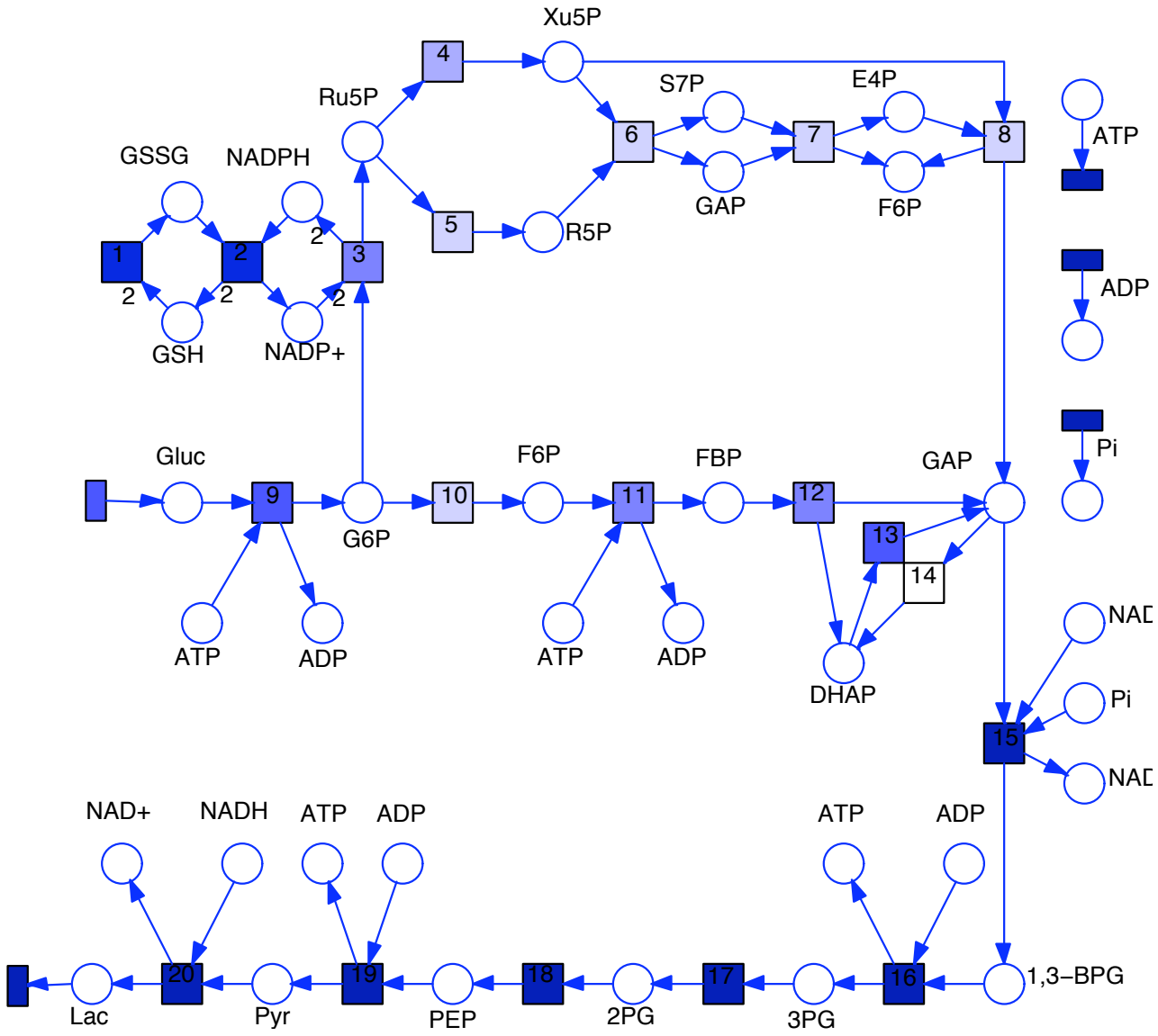
Ex1 - Glycolysis and Pentose Phosphate Pathway

[Reddy 1993]

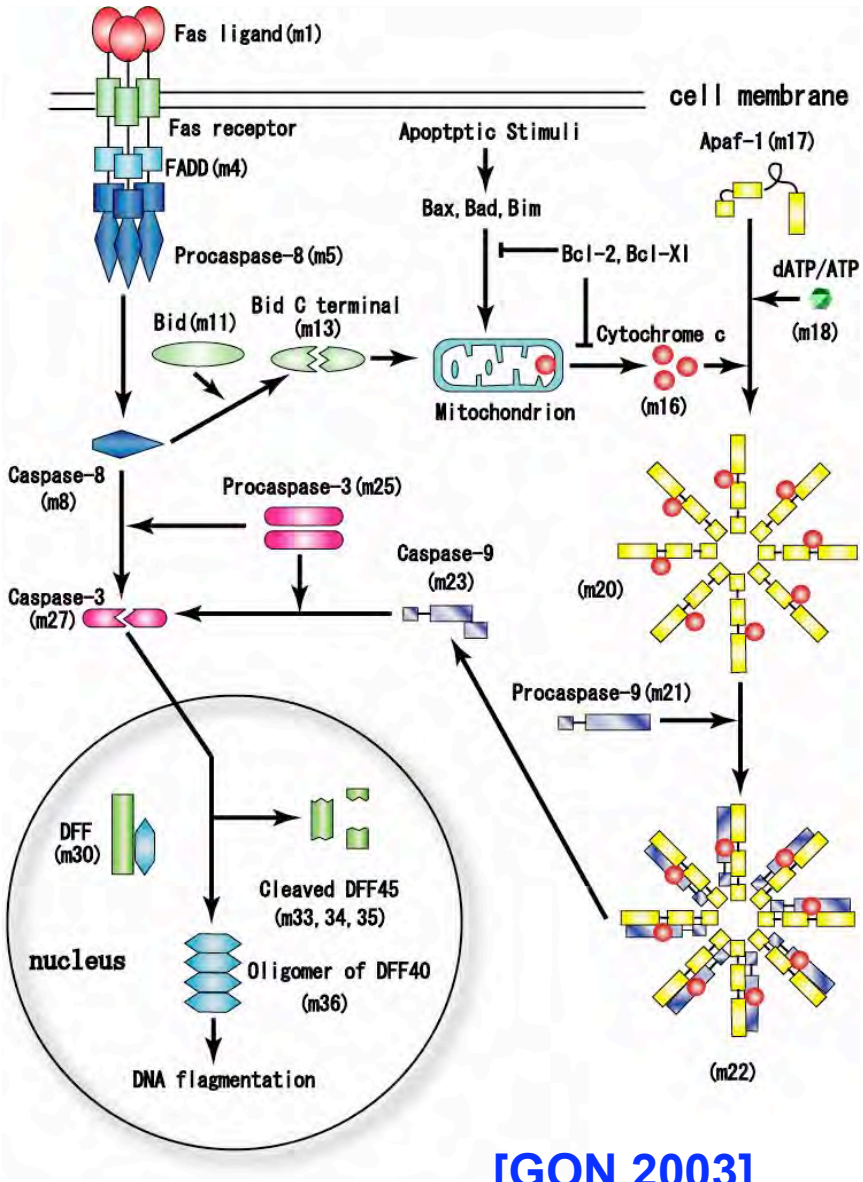


Ex1 - Glycolysis and Pentose Phosphate Pathway

[Reddy 1993]
[Heiner 1998]



EX2: APOPTOSIS IN MAMMALIAN CELLS



[GON 2003]

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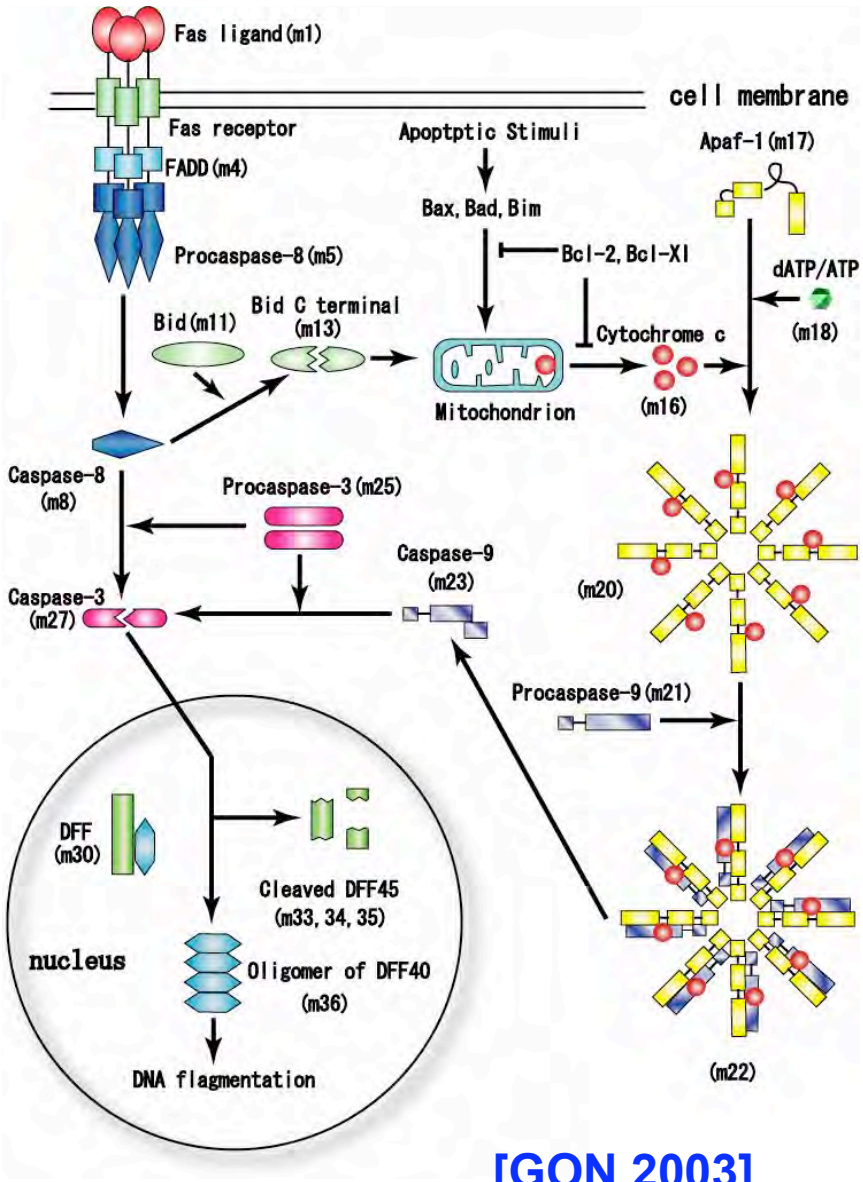

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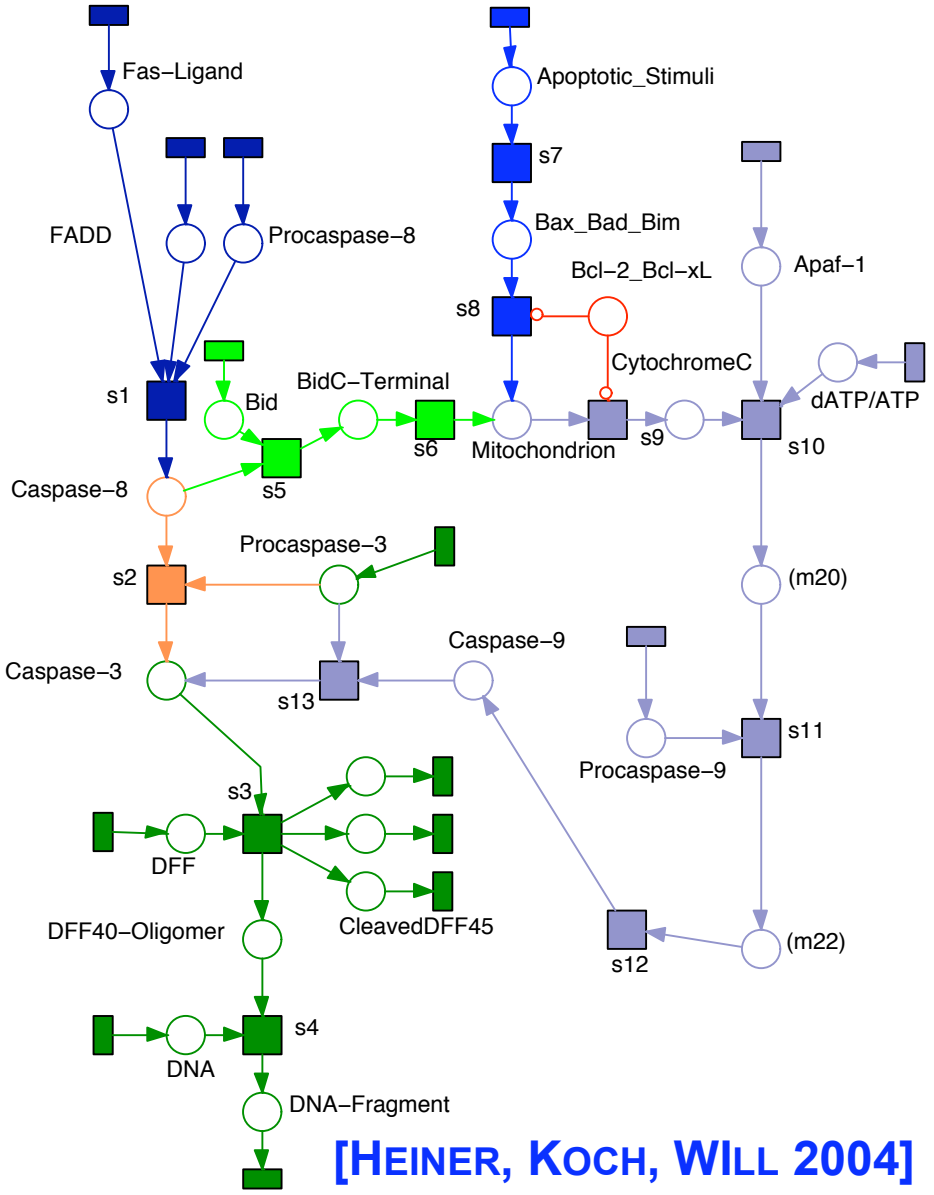


[HEINER, KOCH, WILL 2004]

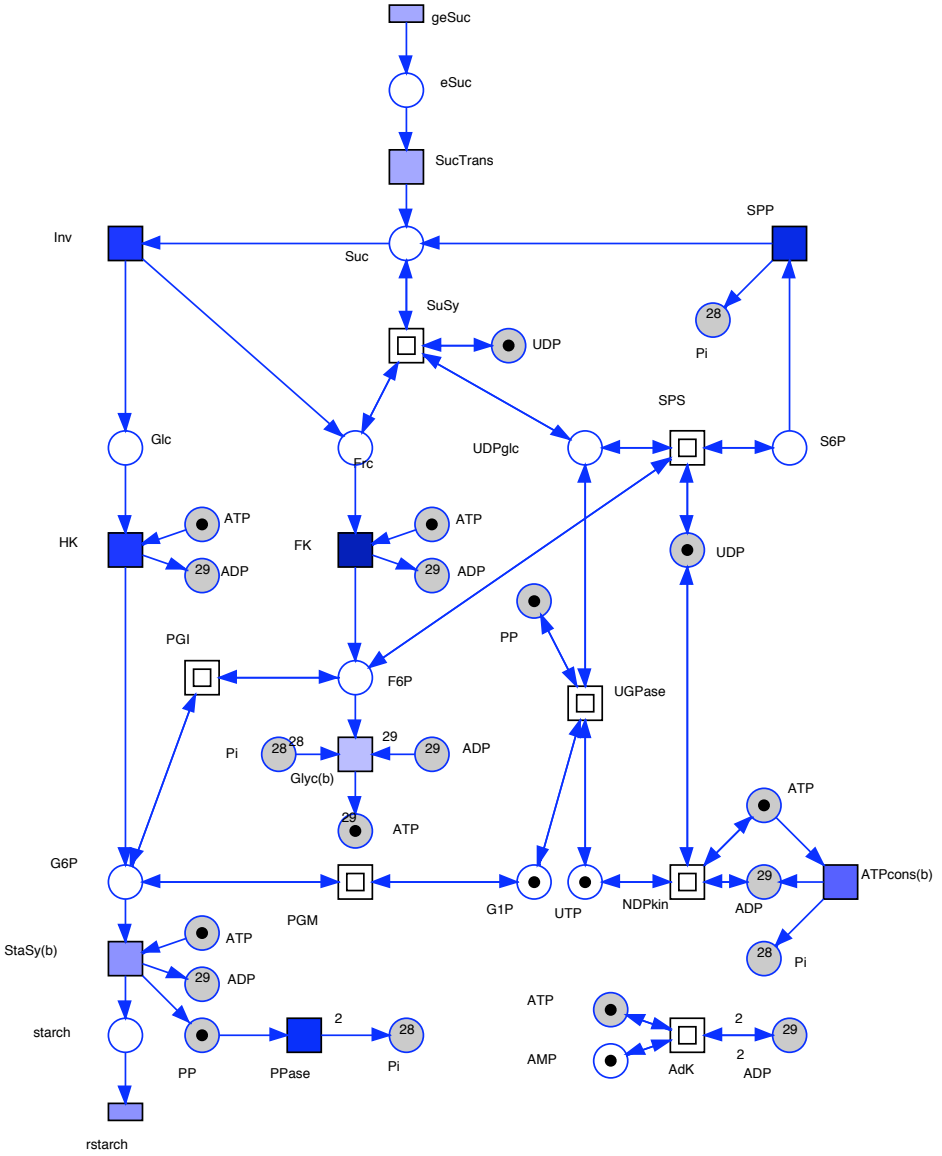
Ex3 - Carbon Metabolism in Potato Tuber

[KOCH, JUNKER, HEINER 2005]

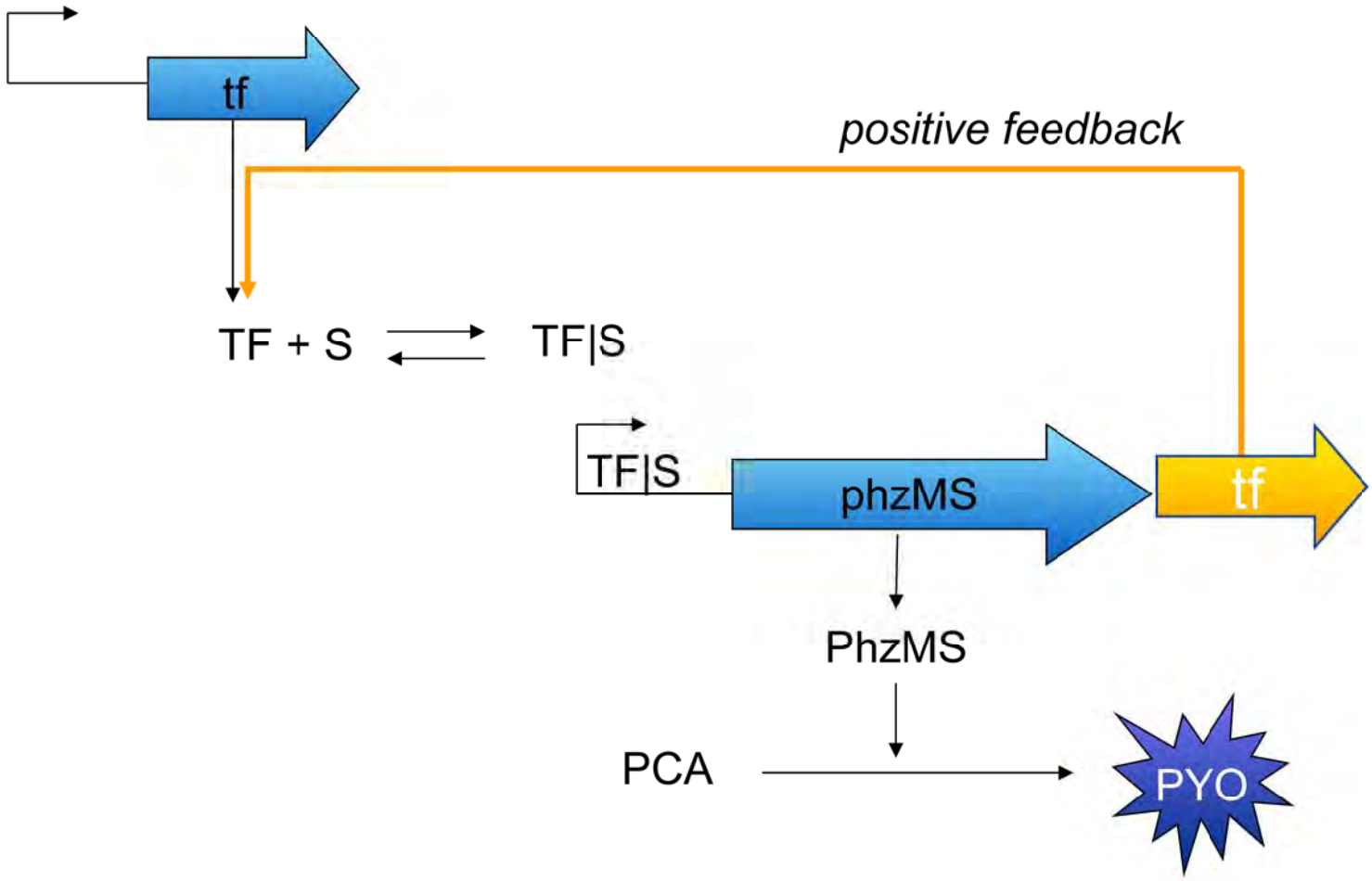
monika.heiner@informatik.tu-cottbus.de

Ex3 - Carbon Metabolism in Potato Tuber

[KOCH, JUNKER, HEINER 2005]



EX4 - BIOSENSOR



[GILBERT, HEINER, ROSSER, FULTON, GU, TRYBILO 2008]

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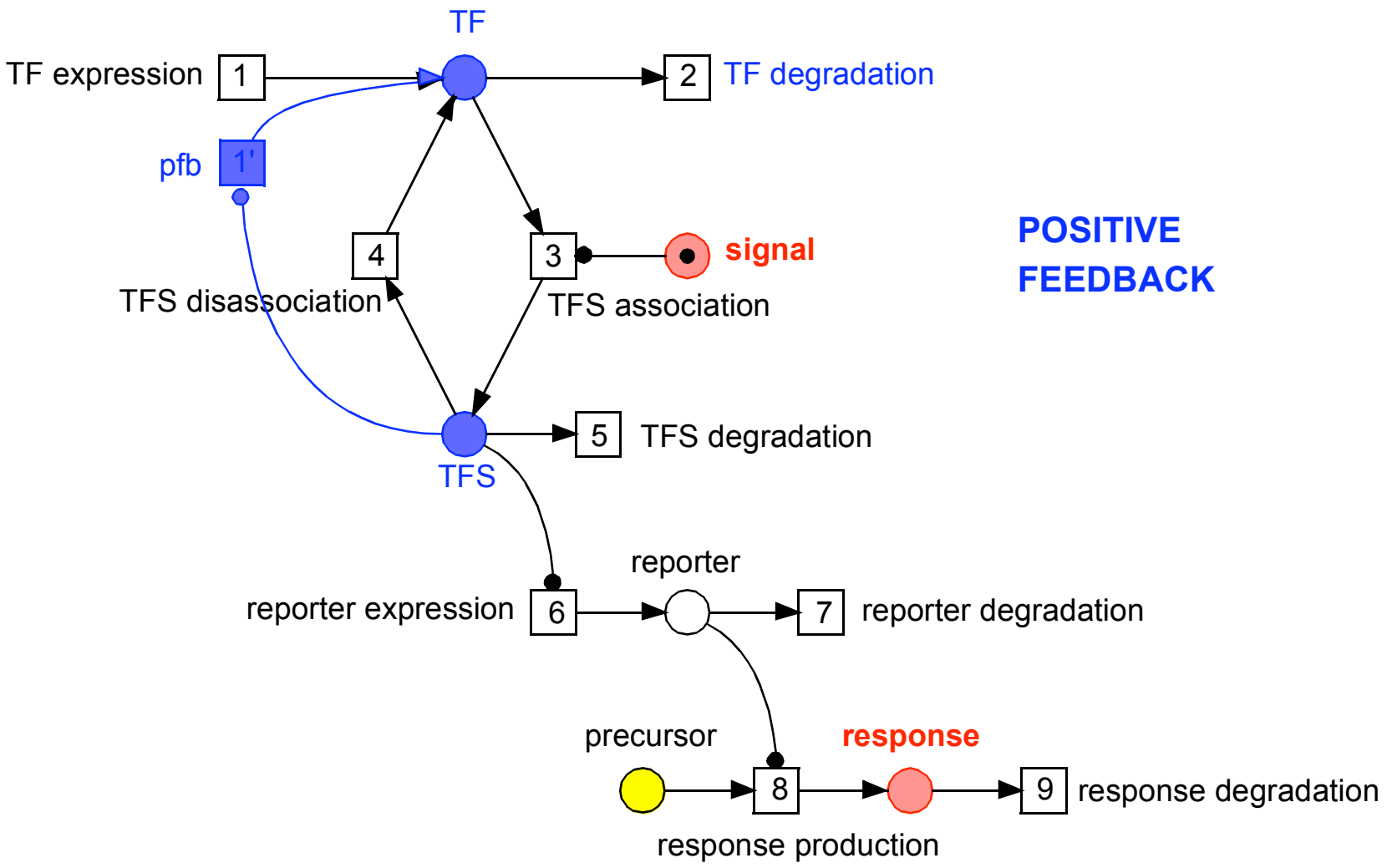
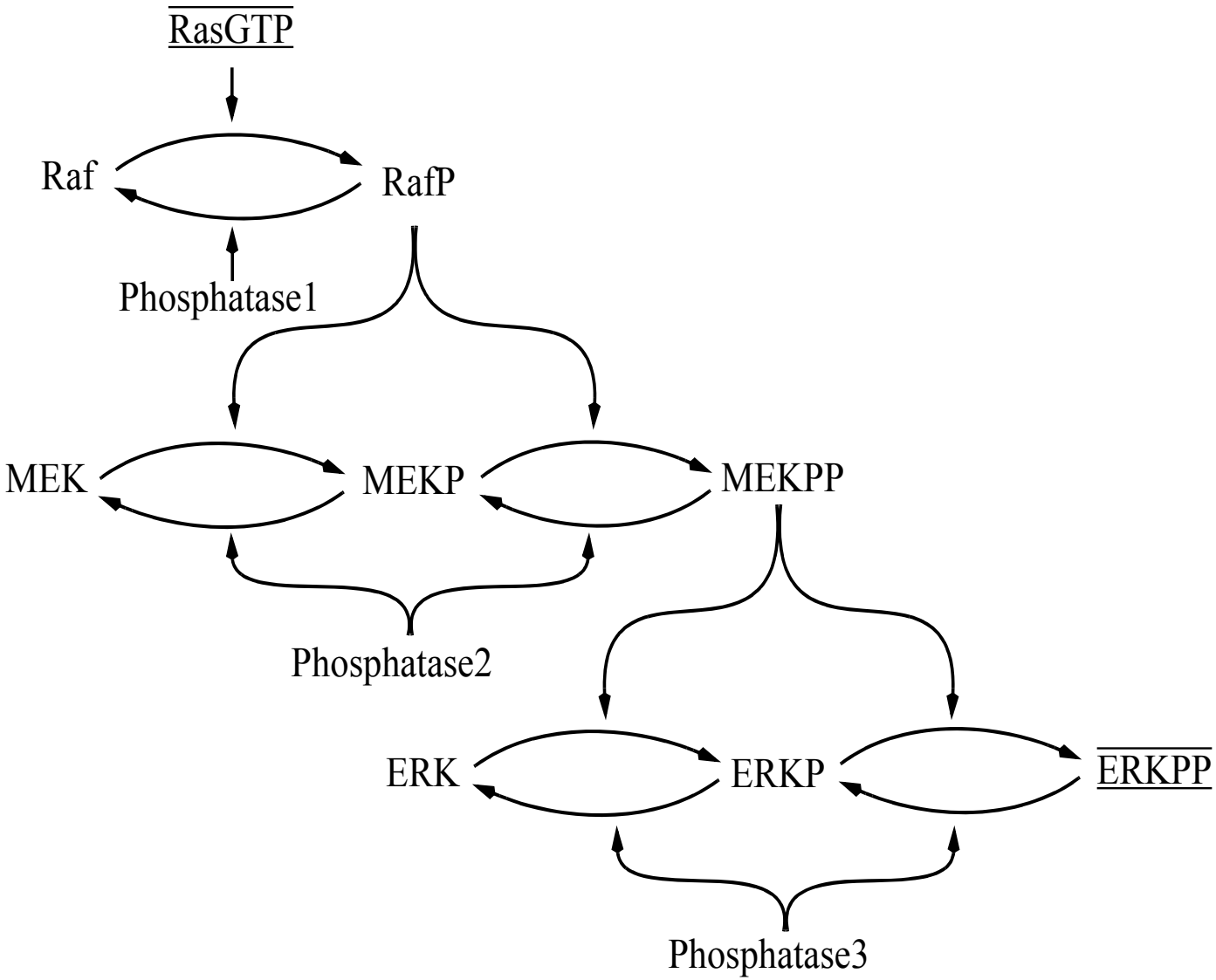


[GILBERT, HEINER, ROSSER, FULTON, GU, TRYBILO 2008]

EX5 - SIGNALLING CASCADE



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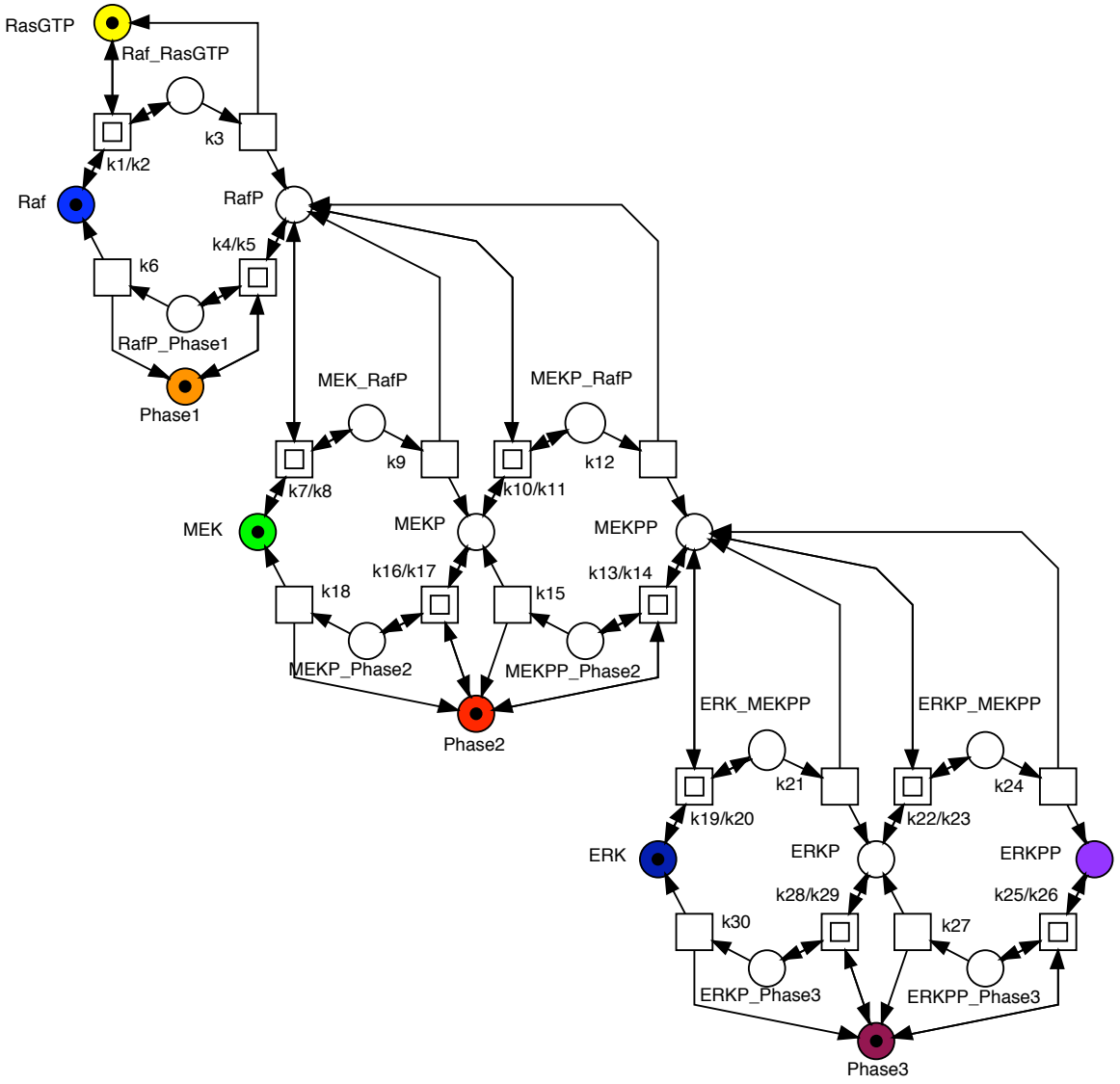
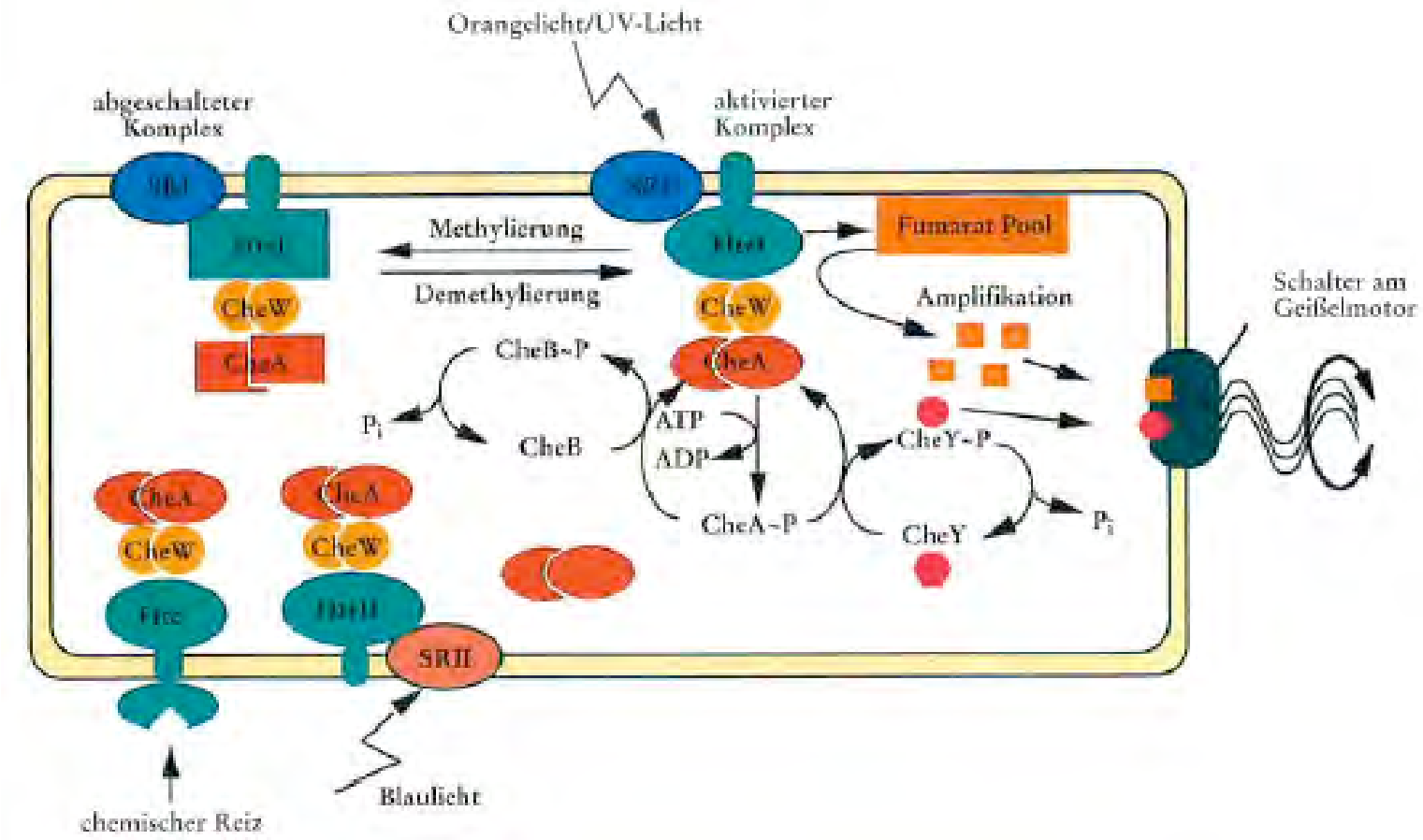


[GILBERT, HEINER, LEHRACK 2007]

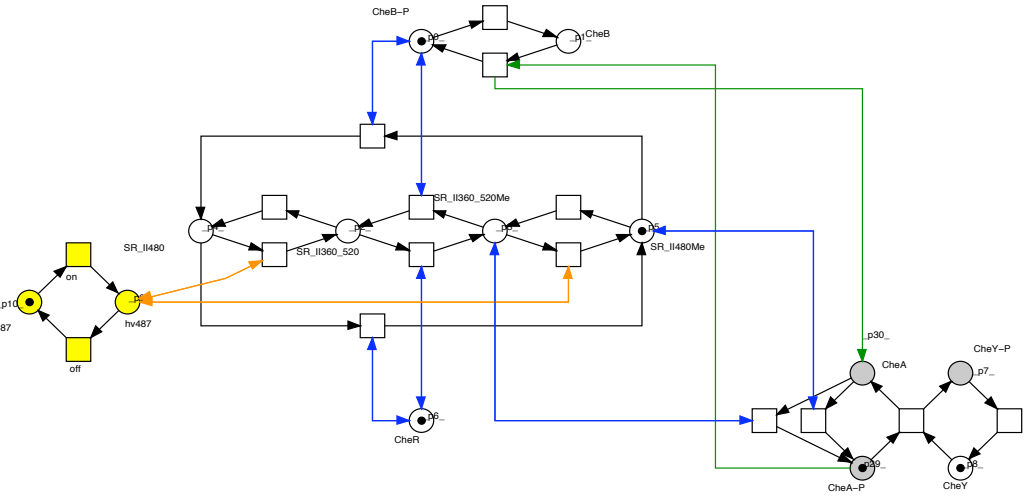
[HEINER, GILBERT, DONALDSON 2008]

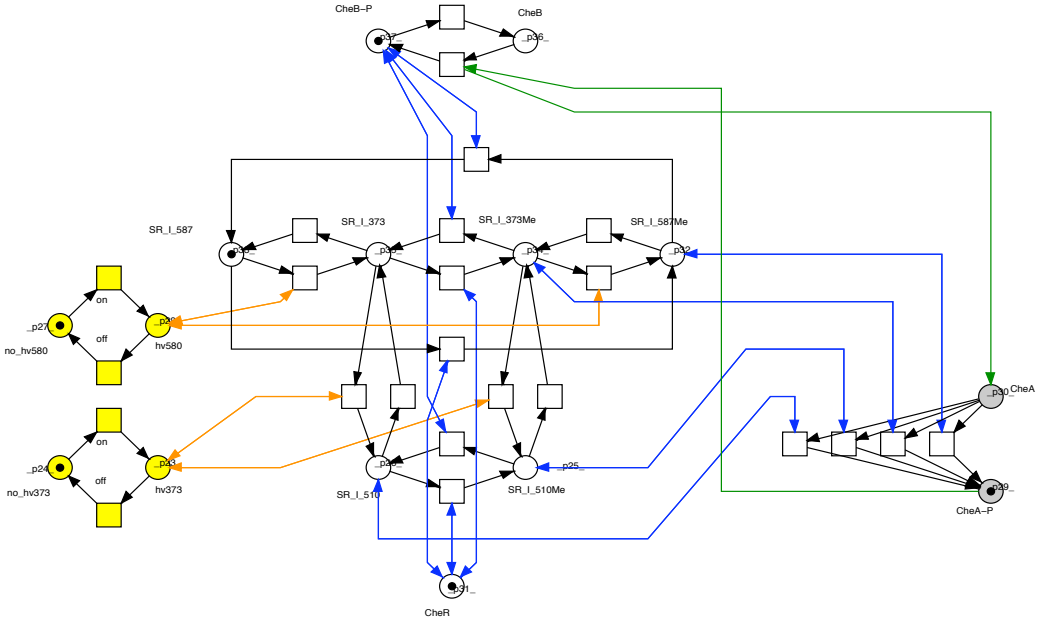
EX6 - SWITCH CYCLE HALOBACTERIUM SALINARUM

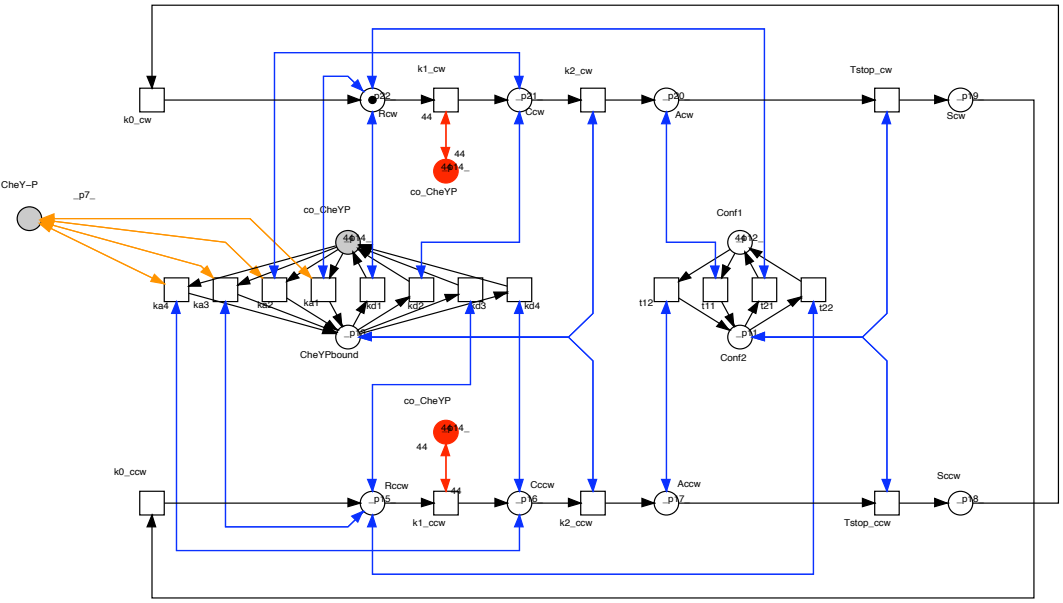


[MARWAN, OESTERHELT 1999]

Ex6 - Switch Cycle Halobacterium Salinarum







QUALITATIVE ANALYSES

TYPICAL PETRI NET QUESTIONS

- **How many tokens can reside at most in a given place ?**

  -> *(0, 1, k, oo)* -> *BOUNDEDNESS*

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GENERAL BEHAVIOURAL PROPERTIES

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SPECIAL BEHAVIOURAL PROPERTIES

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ANALYSIS TECHNIQUES

- **static analyses** -> **no state space construction**
- **dynamic analyses** -> **total/ partial state space construction**

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  - -> *structural properties (graph theory)*
  - -> *P / T - invariants (linear algebra)*
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  - -> *P / T - invariants (linear algebra)*
- **dynamic analyses** **-> total/ partial state space construction**

-> *analysis of general behavioural system properties,*

e.g. boundedness, liveness, reversibility, . . .

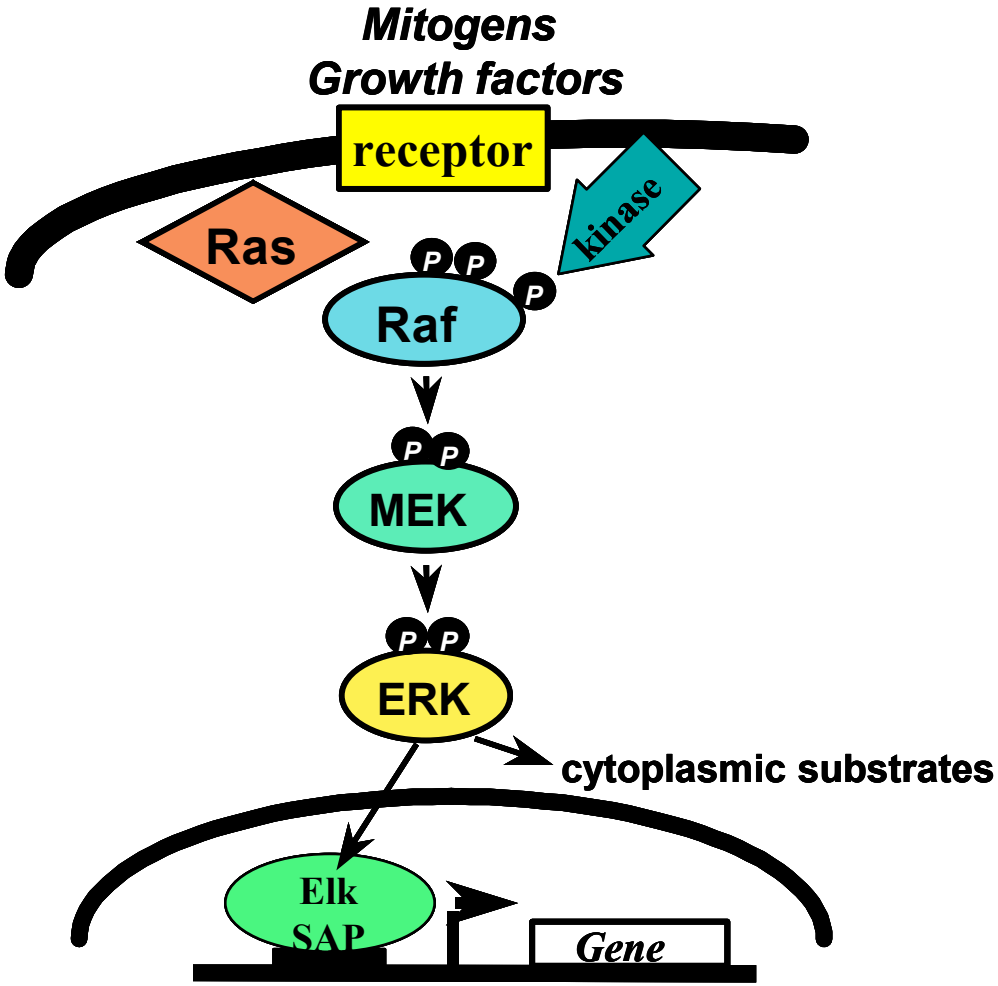
-> *model checking of special behavioural system properties,*

e.g. reachability of a given (sub-) system state (with constaints),
reproducability of a given (sub-) system state (with constraints)

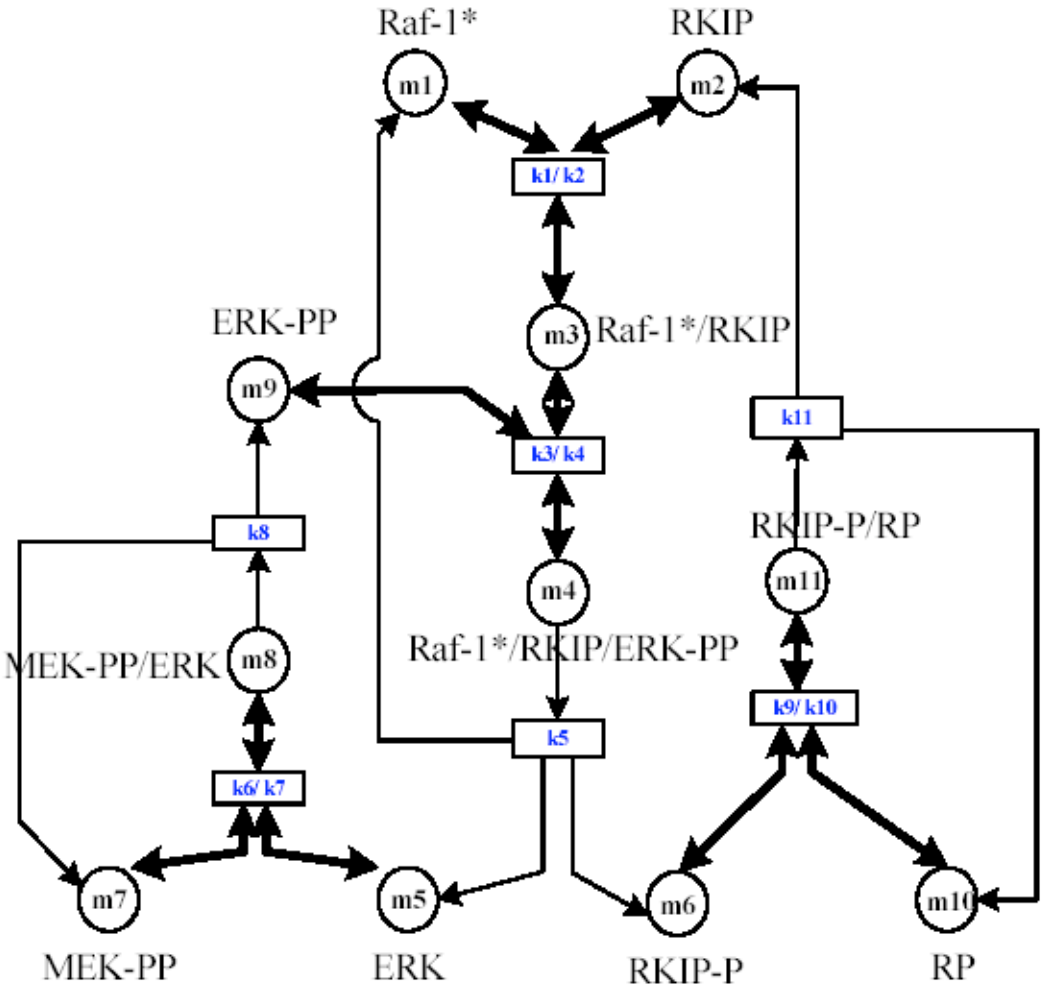
expressed in temporal logics (CTL / LTL),
-> *very flexible, powerful querry language*

A CASE STUDY

…one pathway…


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

THE RKIP PATHWAY



[Cho et al., CMSB 2003]

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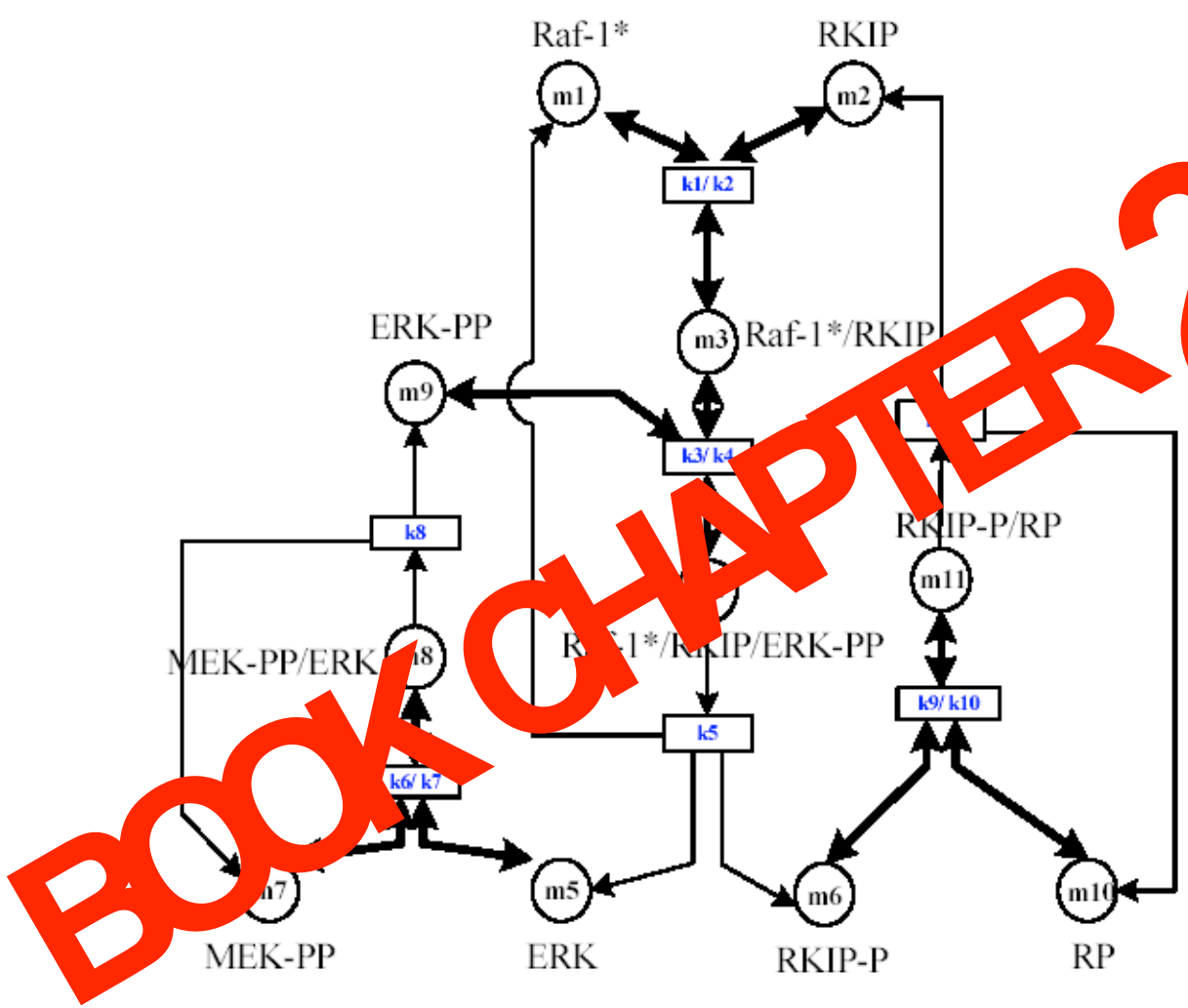

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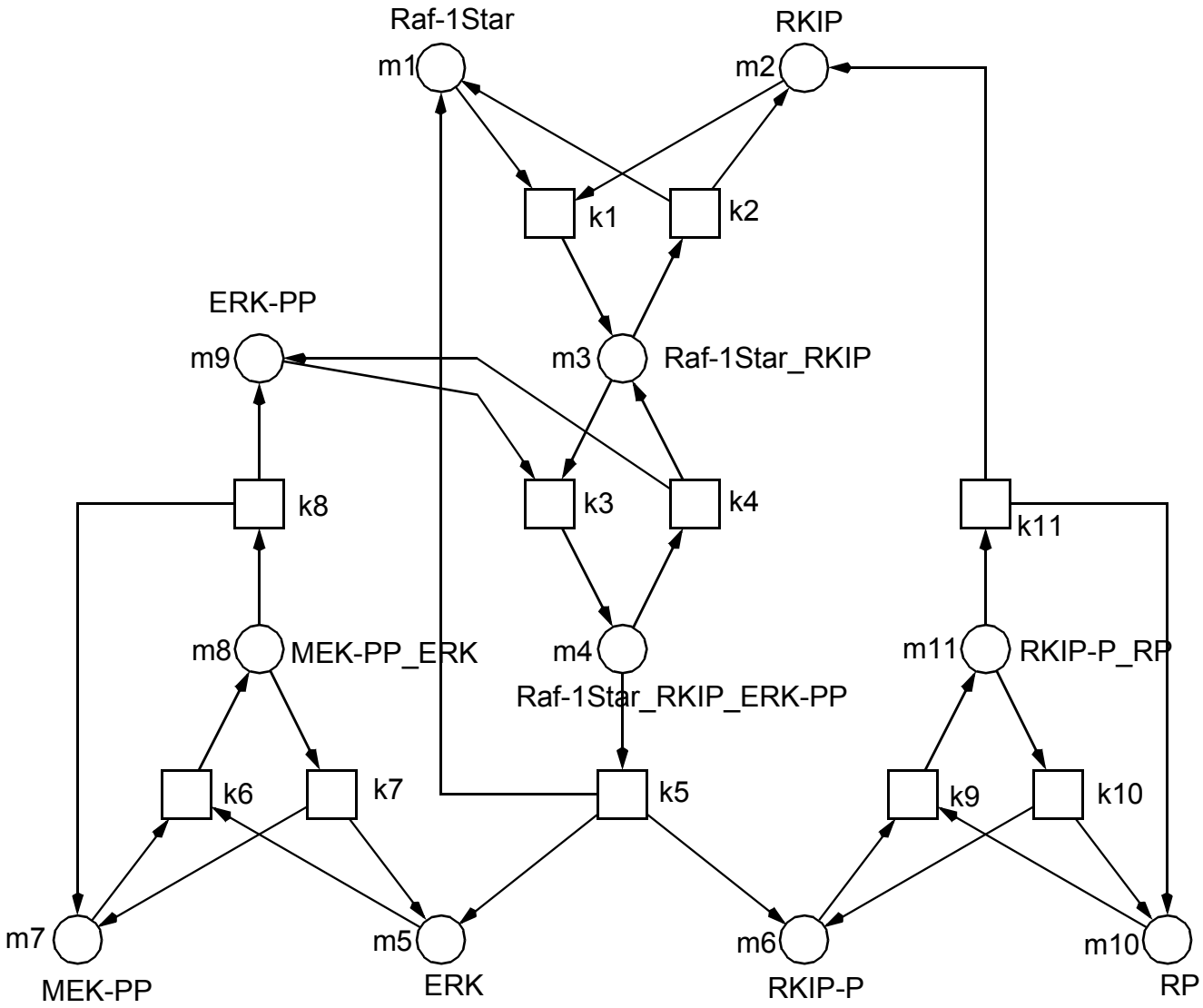
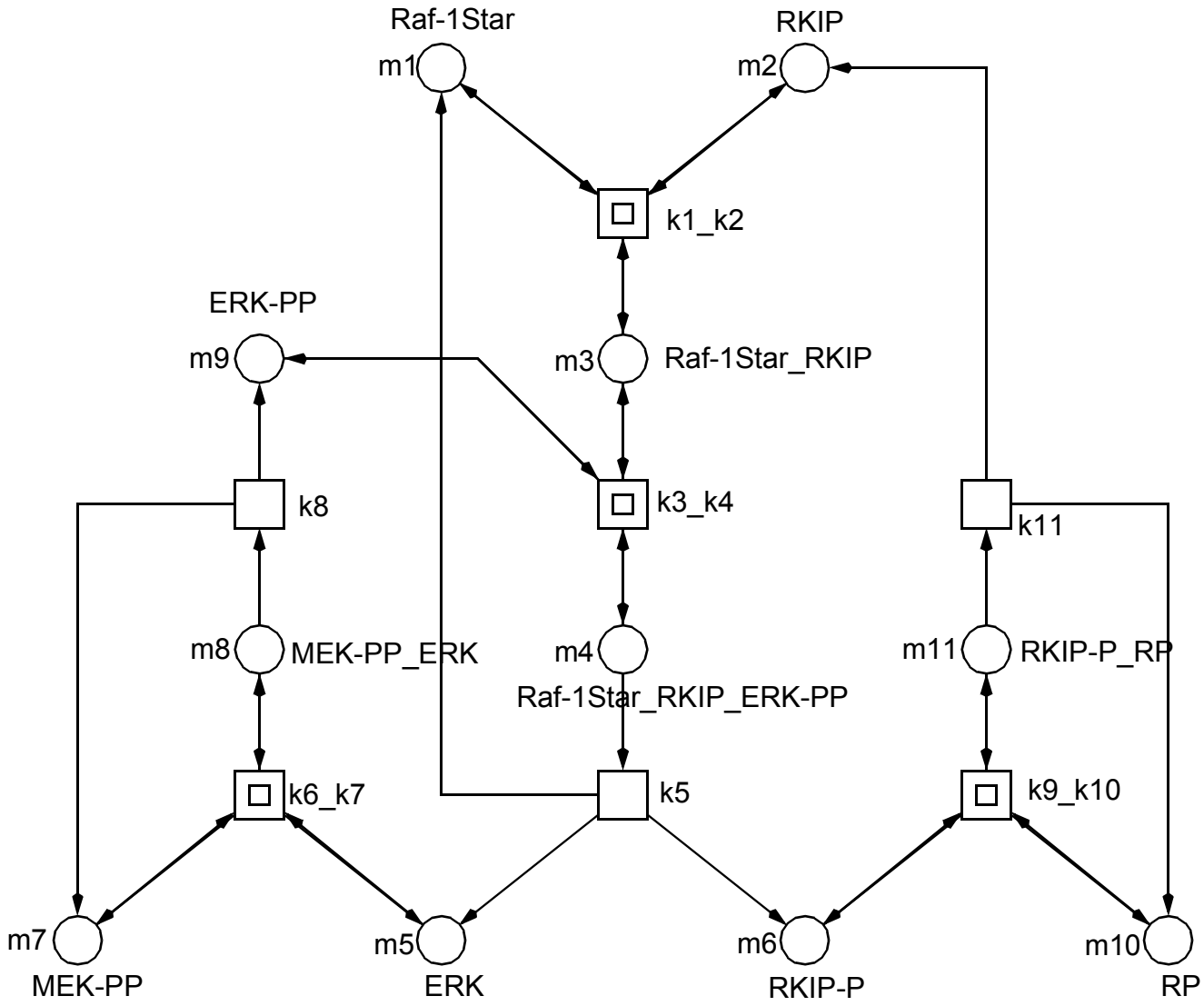
THE RKIP PATHWAY, PETRI NET

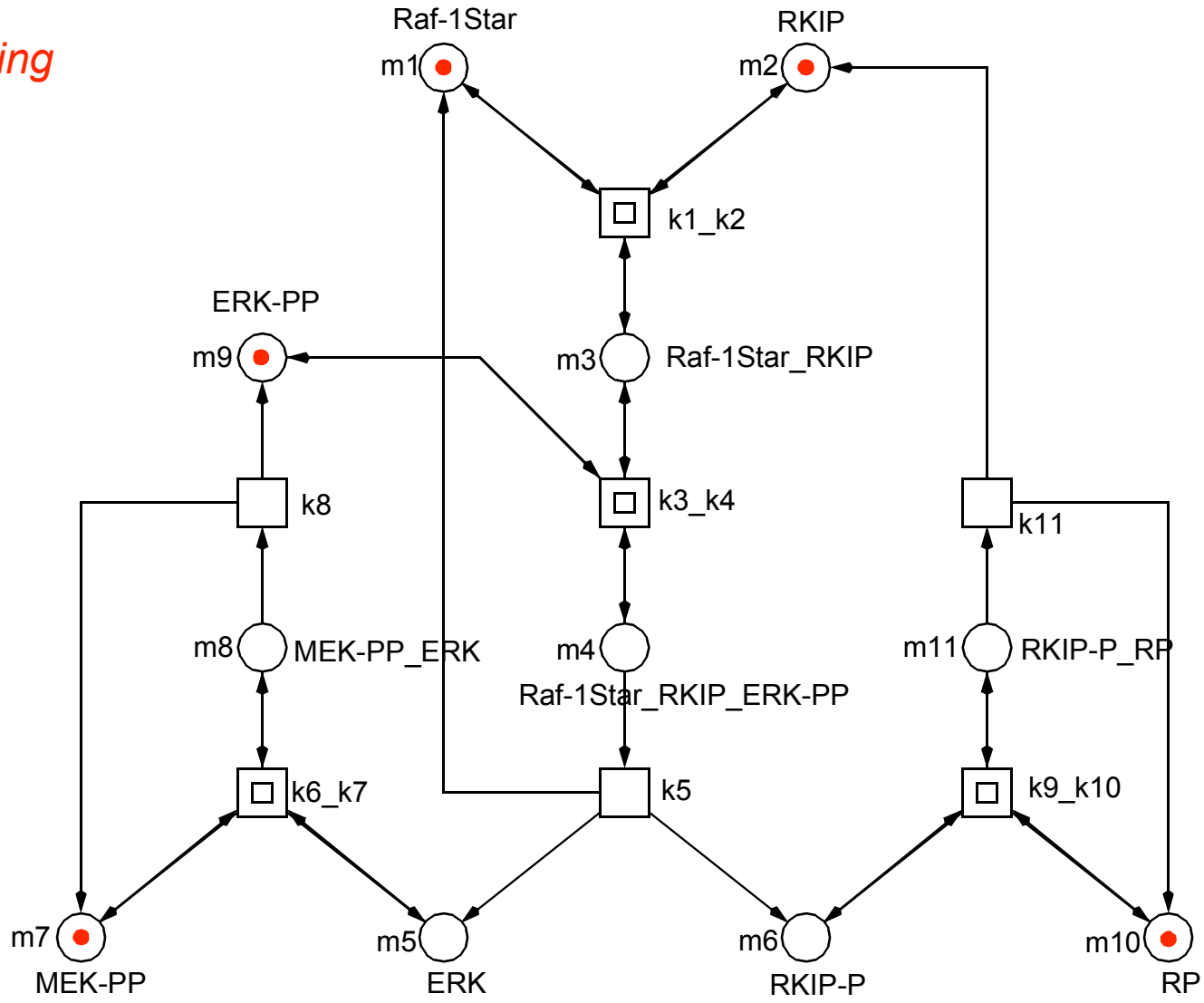


THE RKIP PATHWAY, HIERARCHICAL PETRI NET



THE RKIP PATHWAY, HIERARCHICAL PETRI NET

initial marking



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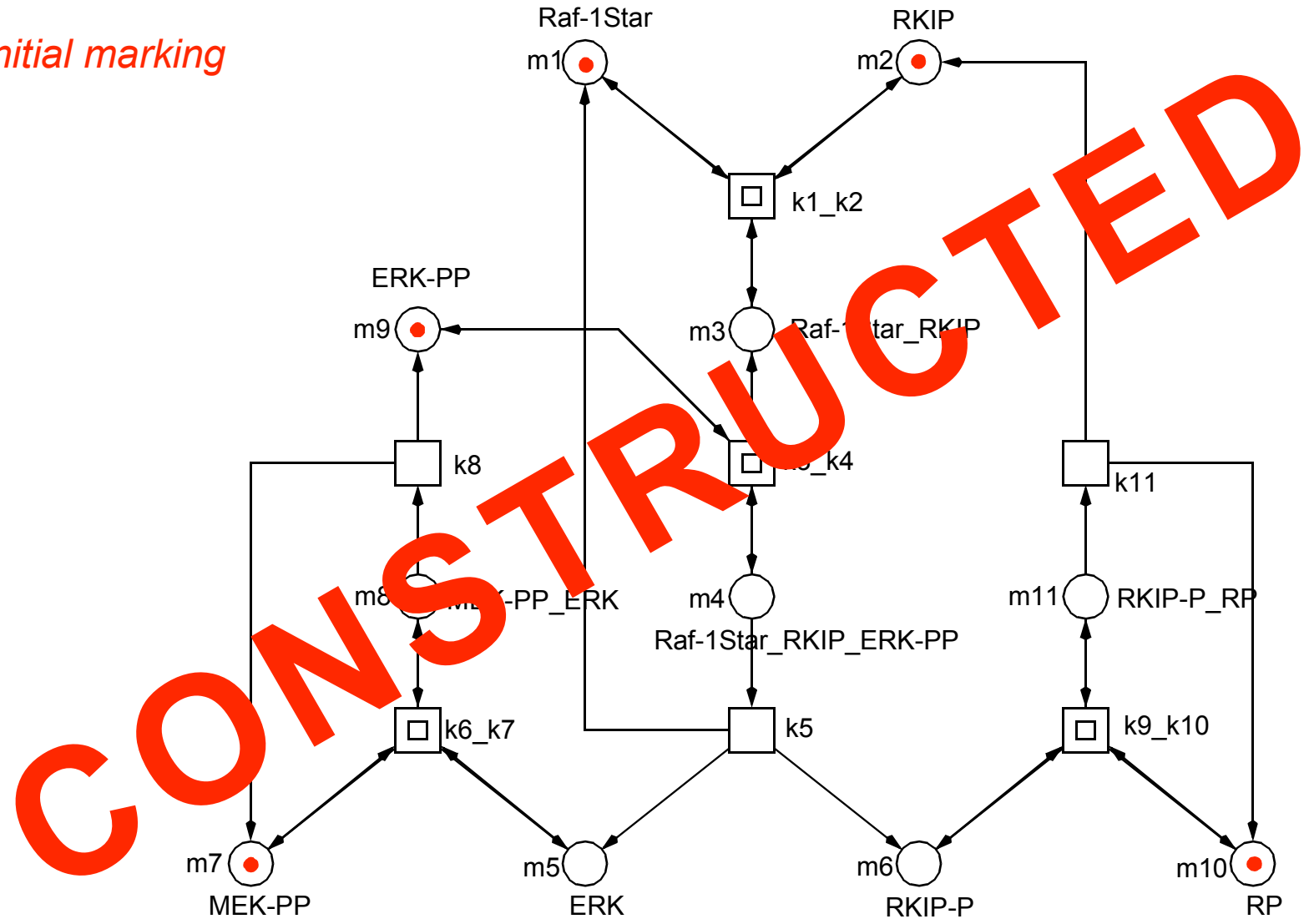
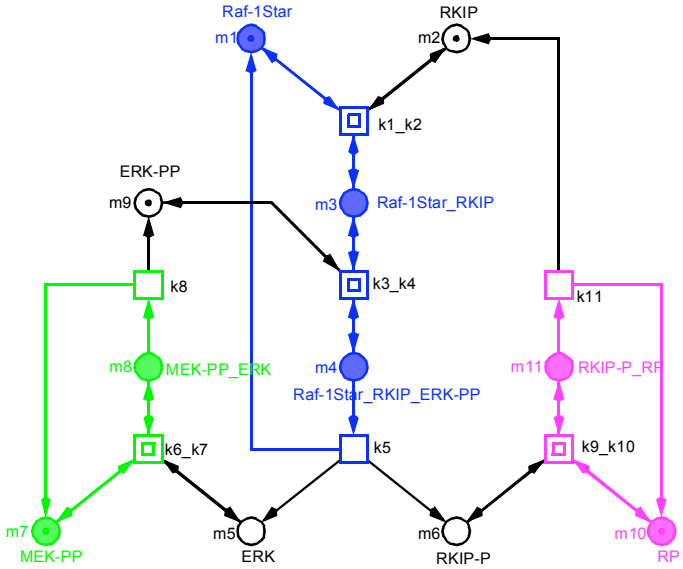
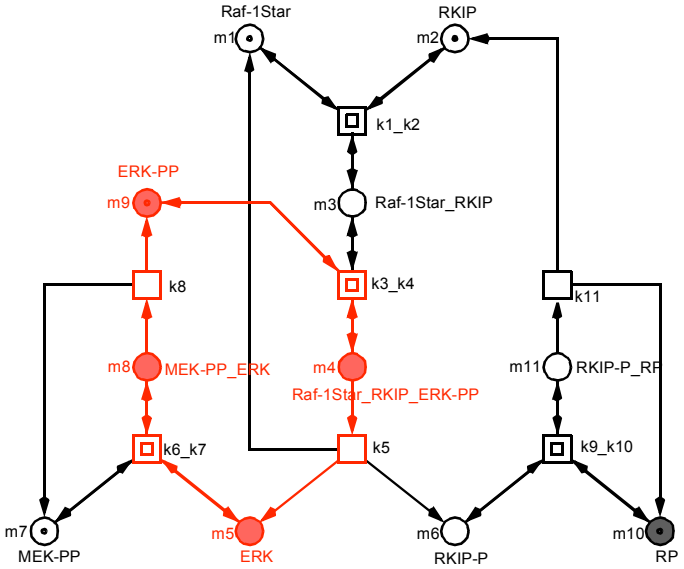


THE RKIP PATHWAY, P-INVARIANTS





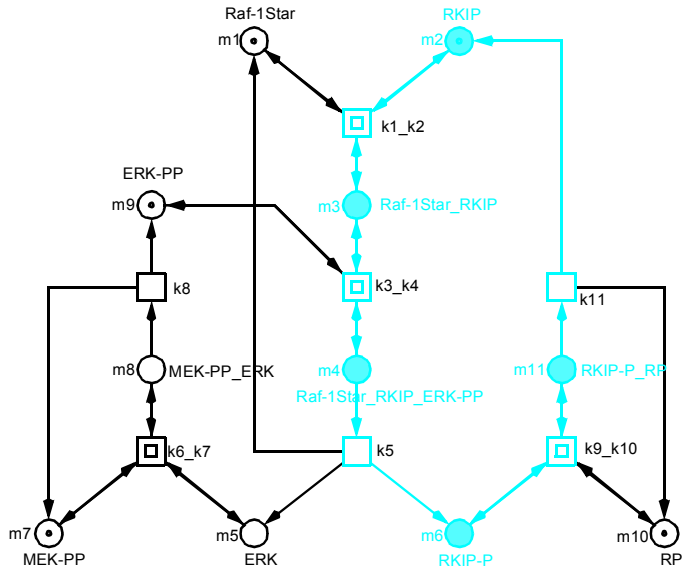
P-INV1: MEK

P-INV2: RAF-1STAR

P-INV3: RP

P-INV4: ERK

P-INV5: RKIP



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*

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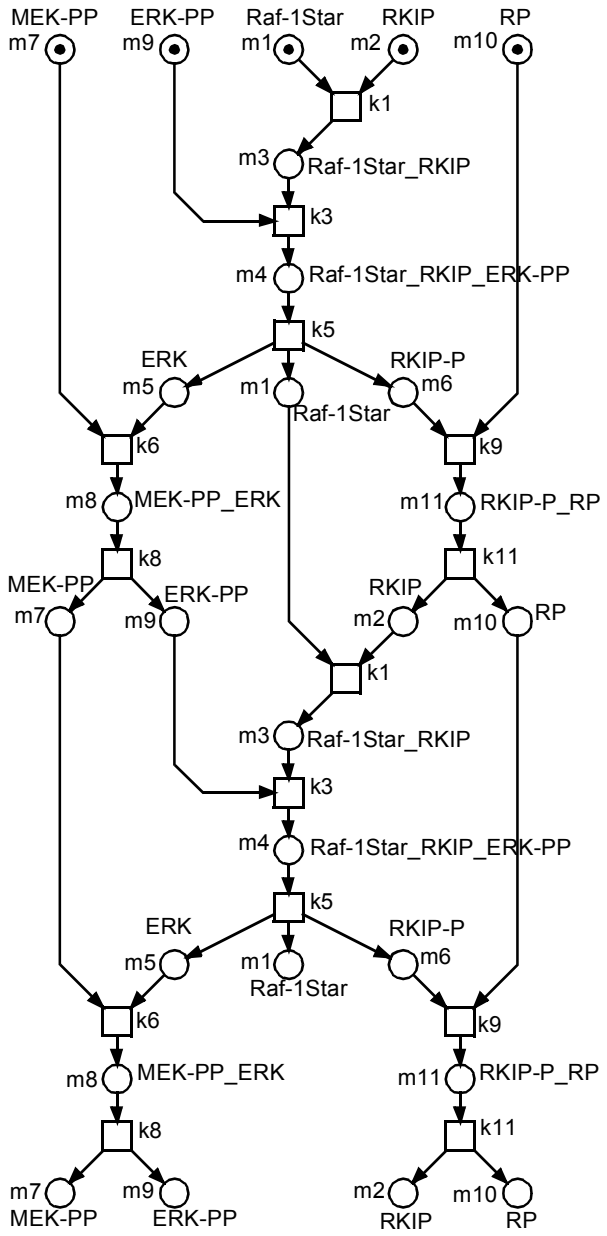
-> UNIQUE INITIAL MARKING <-

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*



MODEL CHECKING, EXAMPLES

- **property 1**

  Is a given (sub-) marking (system state) reachable ?

  *EF (ERK * RP);*

- **property 2**

  Liveness of transition k8 ?

  AG EF (MEK-PP_ERK);

- **property 3**

  Is it possible to produce ERK-PP neither creating nor using MEK-PP ?

  E (! MEK-PP U ERK-PP);

- **property 4**

  Is there cyclic behaviour w.r.t. the presence / absence of RKIP ?

  *EG ((RKIP -> EF (! RKIP)) * (! RKIP -> EF (RKIP)));*

QUALITATIVE ANALYSIS RESULTS, SUMMARY

- **structural decisions of behavioural properties** **-> static analysis**
  - -> *CPI* -> *BND*
  - -> *ES & DTP* -> *LIVE*
- **CPI & CTI**
  - -> *all minimal T-invariant / P-invariants enjoy biological interpretation*
  - -> *non-trivial T-invariant -> partial order description of the essential behaviour*
- **reachability graph** **-> dynamic analysis**
  - -> *finite* -> *BND*
  - -> *the only SCC contains all transitions* -> *LIVE*
  - -> *one Strongly Connected Component (SCC)* -> *REV*
- **model checking** **-> requires professional understanding**
  - -> *all expected properties are valid*

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-> VALIDATED QUALITATIVE MODEL

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

NOW WE ARE READY FOR SOPHISTICATED QUANTITATIVE ANALYSES !

STOCHASTIC PETRI NETS
- SPN *(xSPN)* -

STOCHASTIC PETRI NETS, BASICS

- **transitions get a stochastic waiting time**
  -> *exponential distribution with parameter lambda*

- **state-dependent lambda defined by rate function**
  -> *any arithmetic function including*
  the transition's pre-places as integer variables and
  user-defined real-valued parameters
  -> *modifier arcs*
  -> *popular kinetics:*
  mass-action semantics,
  level semantics

- **semantics: Continuous Time Markov Chain (CTMC)**
  -> *reachability graph + state transition rates*

- **stochastic simulation algorithms (SSA)**
  -> *e.g. Gillespie's SSA*

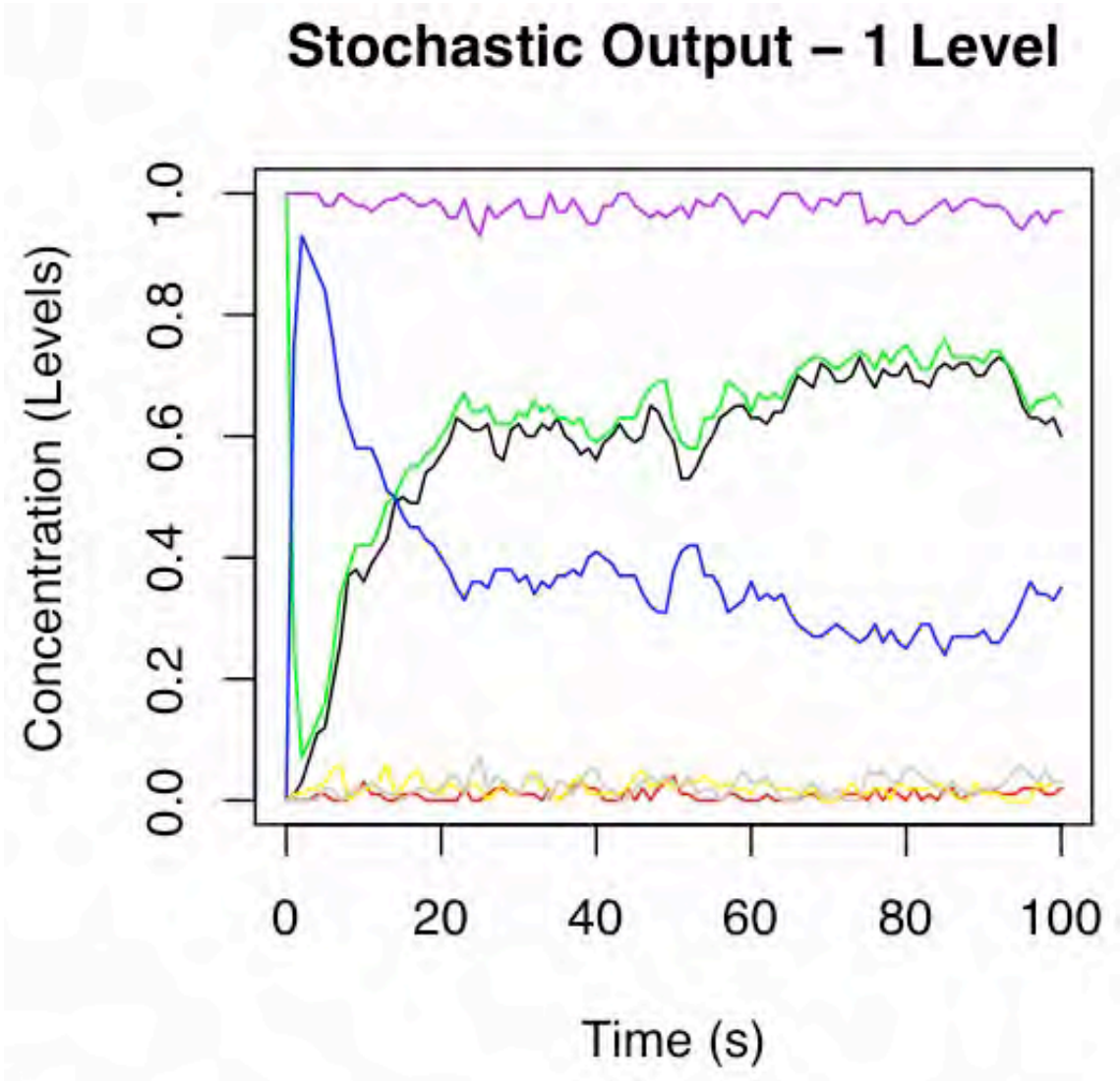
RATE FUNCTIONS

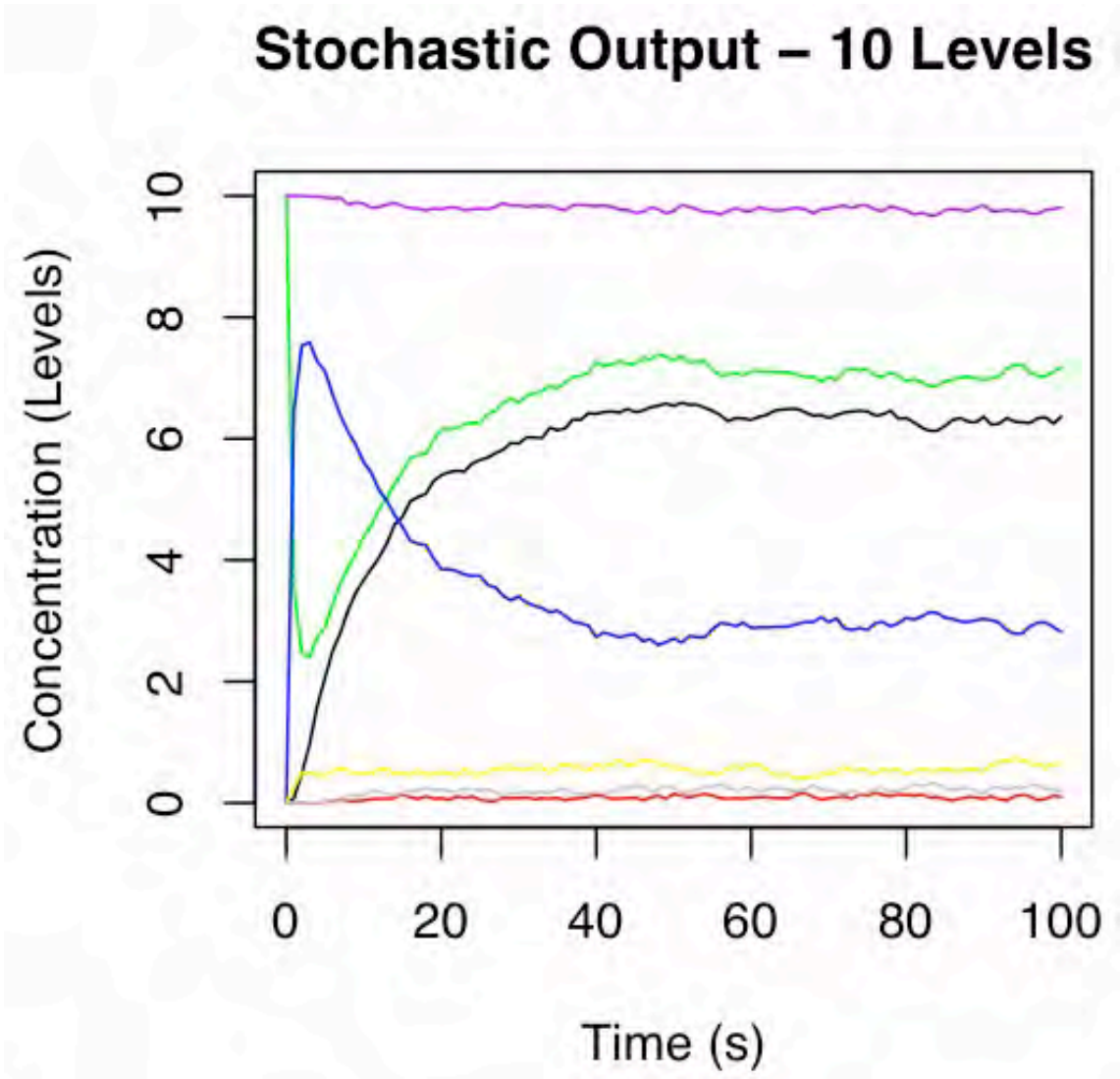
- *molecules semantics*

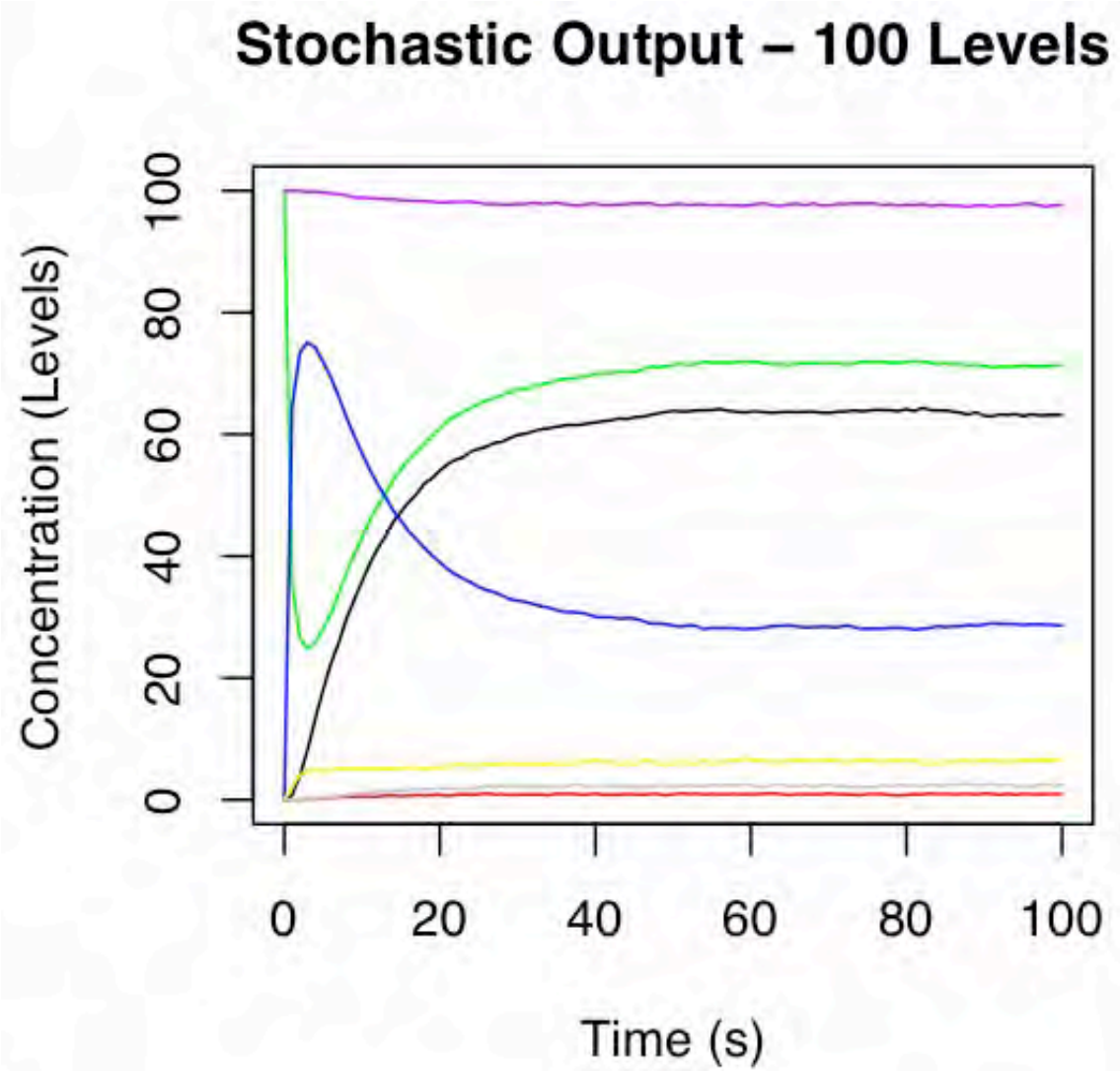
$$h_t := c_t \cdot \prod_{p \in {}^\bullet t} \binom{m(p)}{f(p,t)}$$

- *concentration levels semantics*

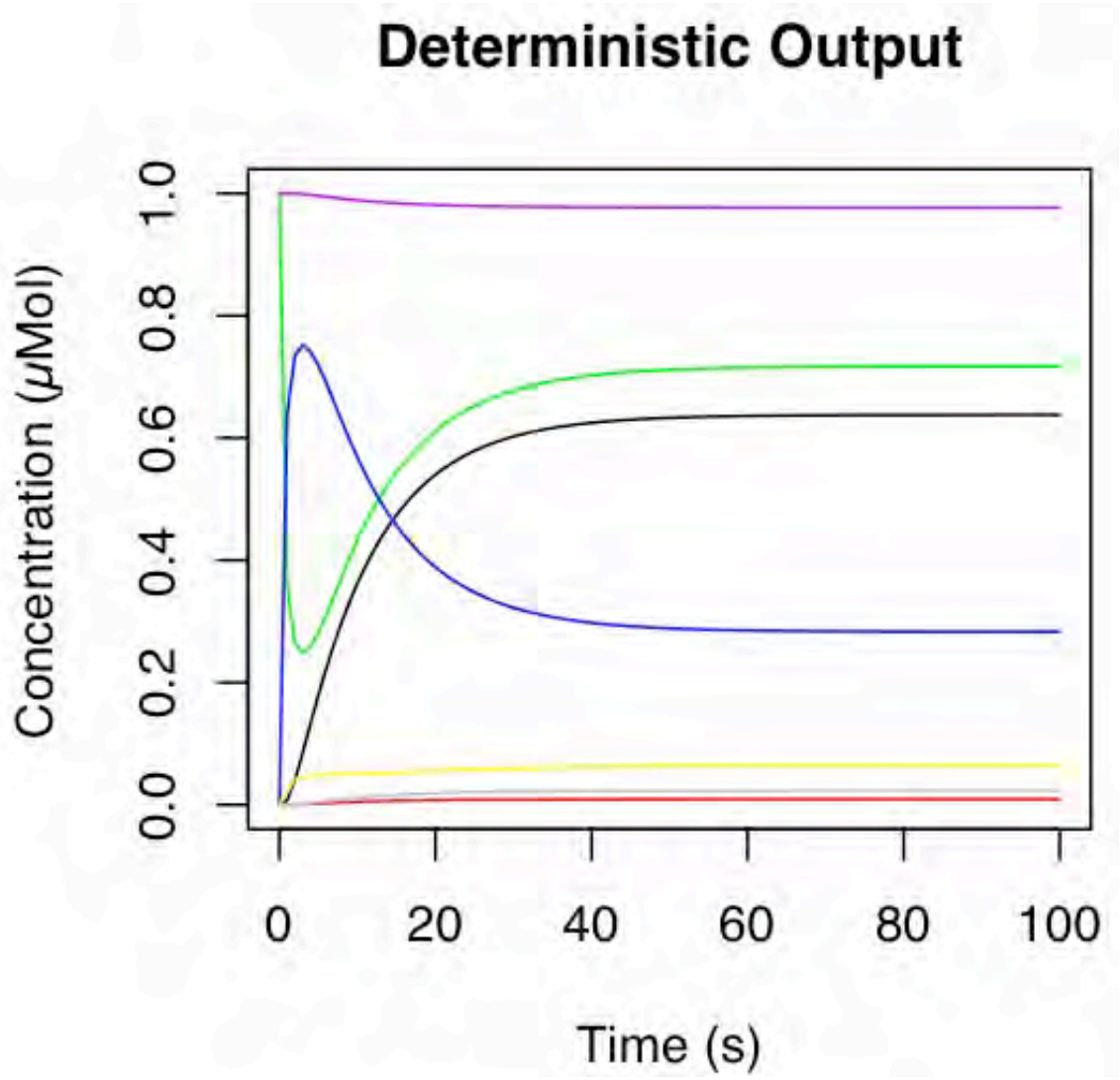
$$h_t := k_t \cdot N \cdot \prod_{p \in {}^\bullet t} \left(\frac{m(p)}{N}\right)$$


Stochastic Output – 1 Level
Concentration (Levels)
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
Time (s)


Stochastic Output – 10 Levels
Concentration (Levels)
0
2
4
6
8
10
0
20
40
60
80
100
Time (s)


Stochastic Output – 100 Levels
Concentration (Levels)
0
20
40
60
80
100
0
20
40
60
80
100
Time (s)

DETERMINISTIC SIMULATION

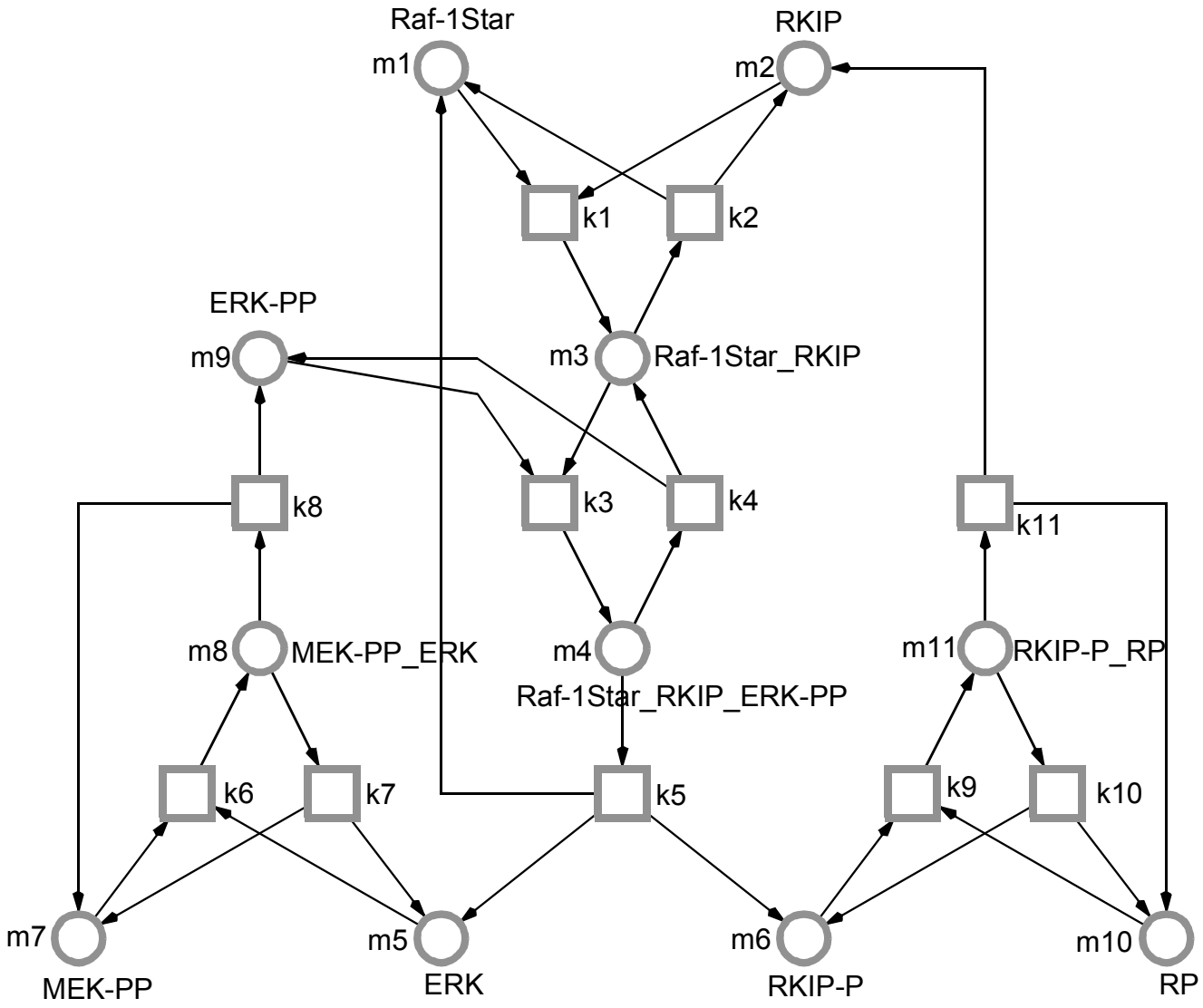


CONTINUOUS PETRI NETS - CPN -

CONTINUOUS PETRI NETS, BASICS

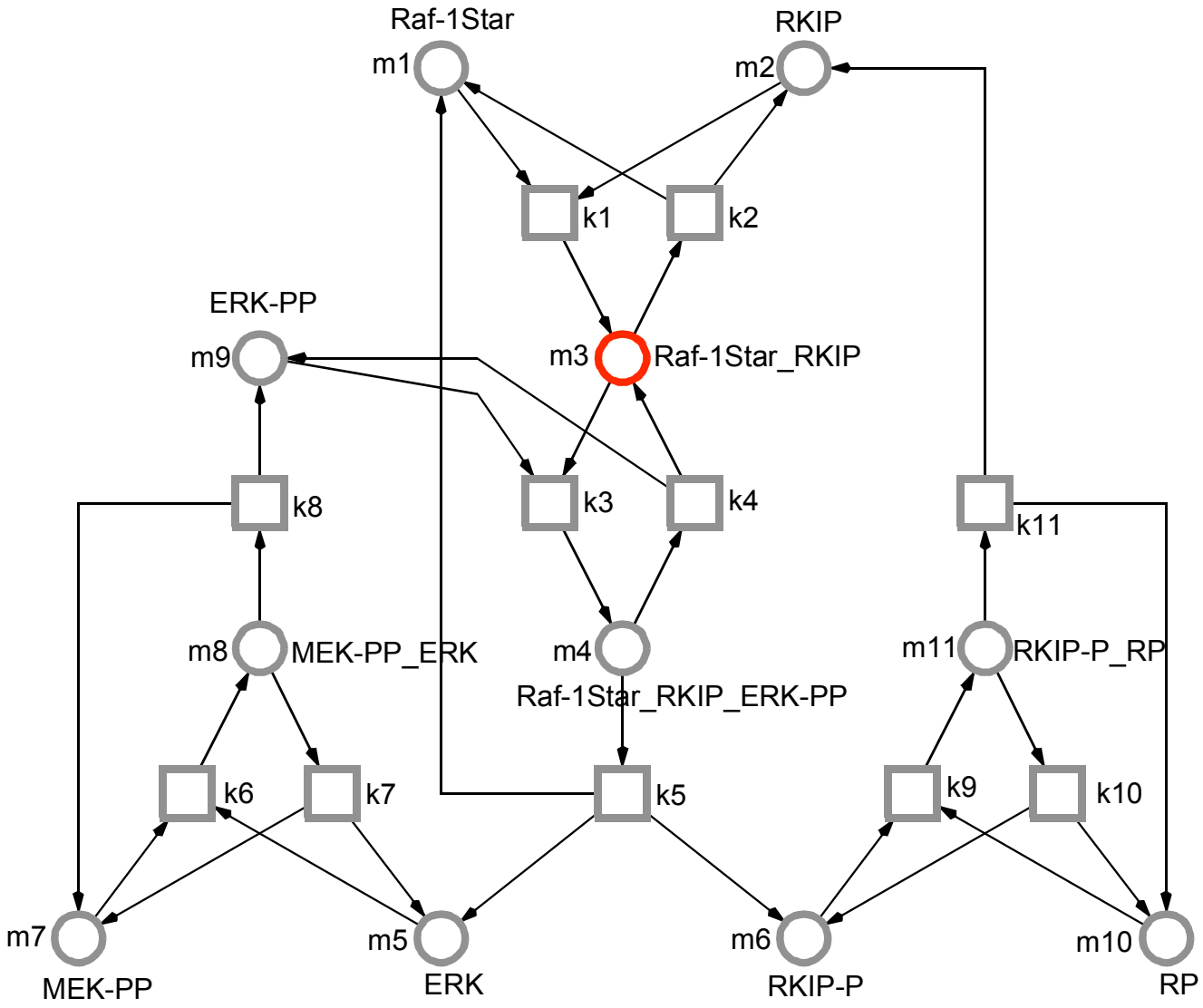
- **transitions fire continuously**
- **rate functions**
  - -> *any arithmetic function including the transition's pre-places as real-valued variables and user-defined real-valued parameters*
- **real-valued tokens**
  - -> *concentrations*
- **semantics: set of Ordinary Differential Equations (ODEs)**
  - -> *uniquely defined, but not vice versa*
  - -> *typically non-linear*
- **simulation (numerical integration)**
  - -> *stiff/unstiff solvers*

CONTINUOUS PETRI NET DEFINES ODES



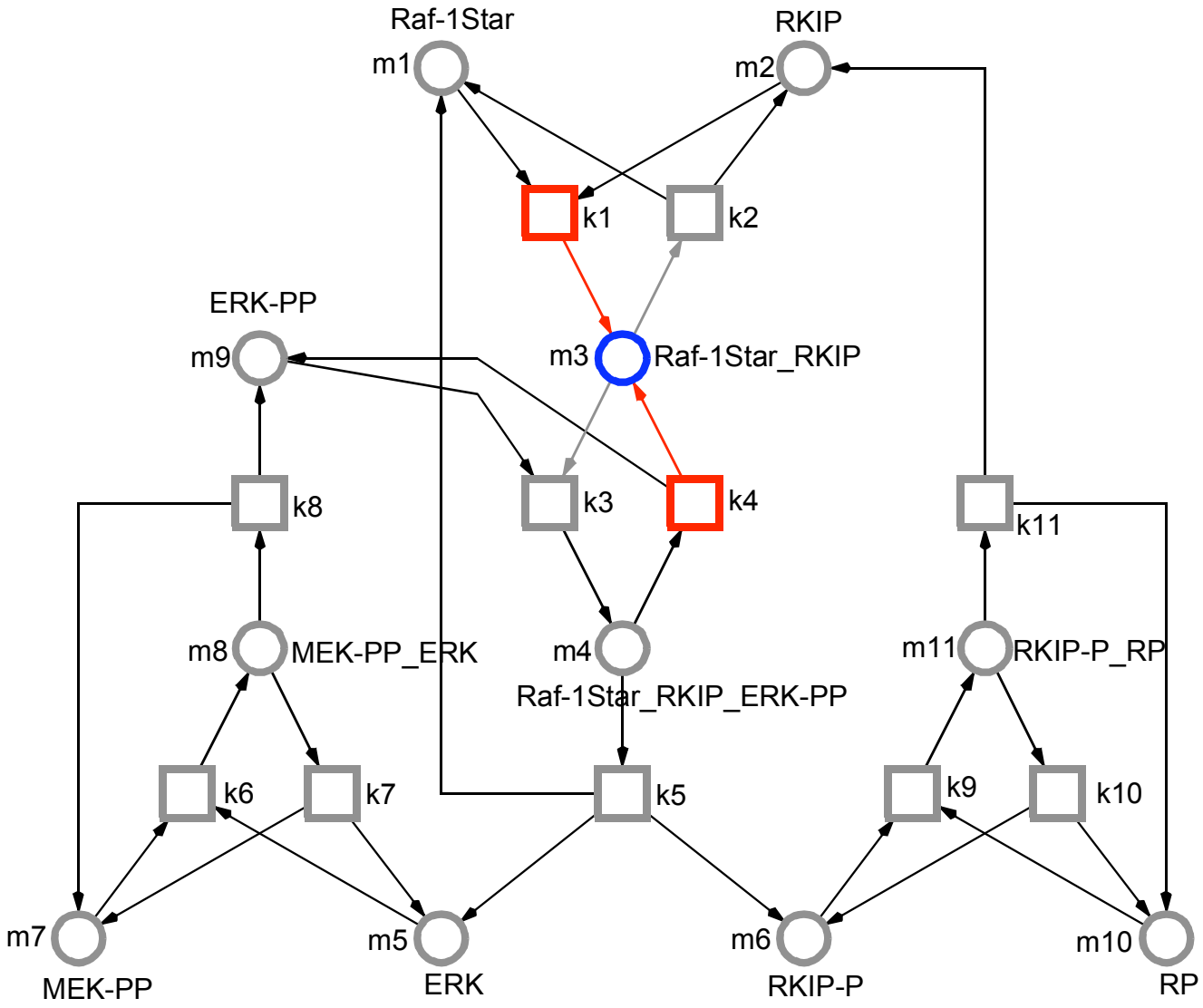
CONTINUOUS PETRI NET DEFINES ODES

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



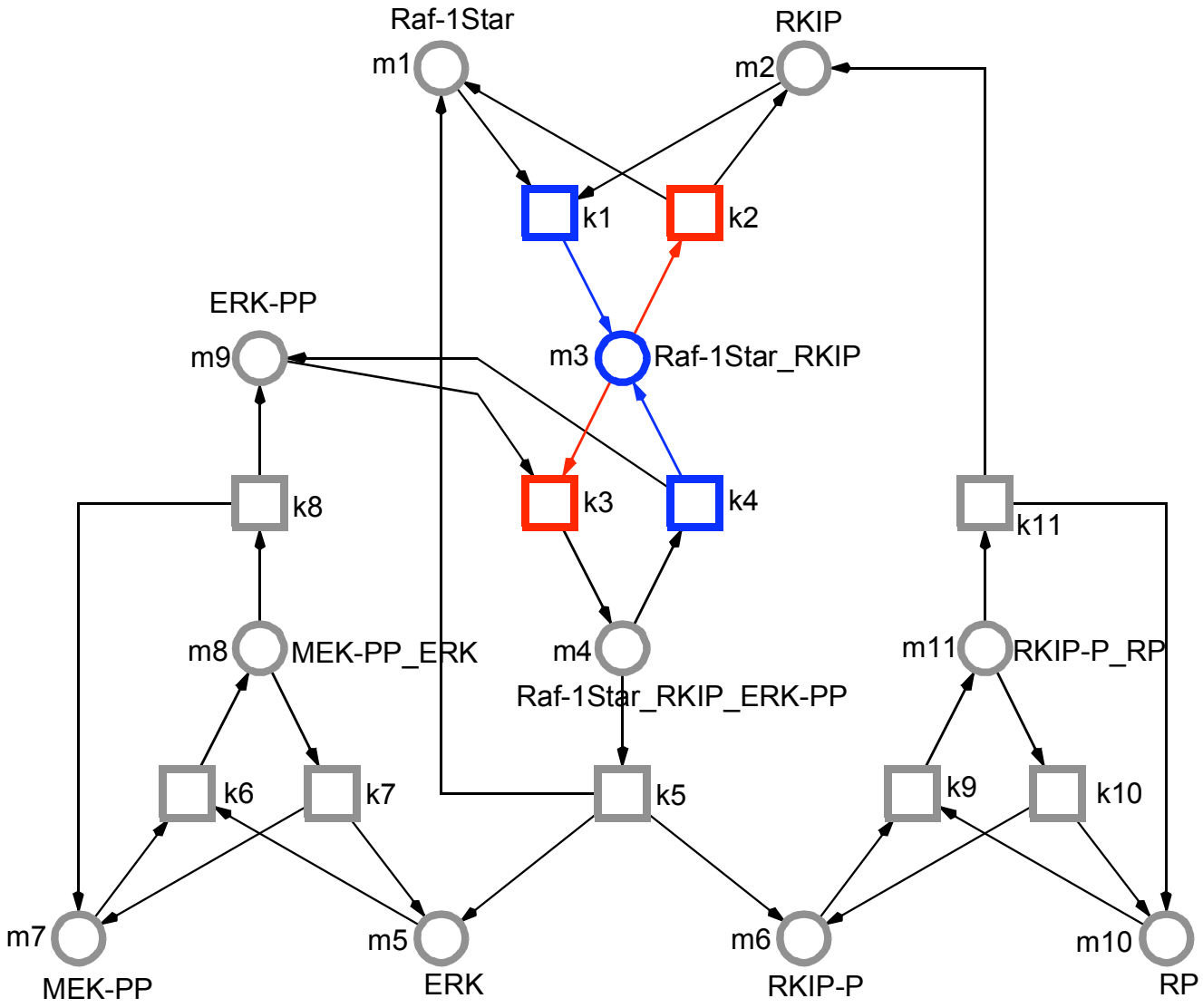
CONTINUOUS PETRI NET DEFINES ODES

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



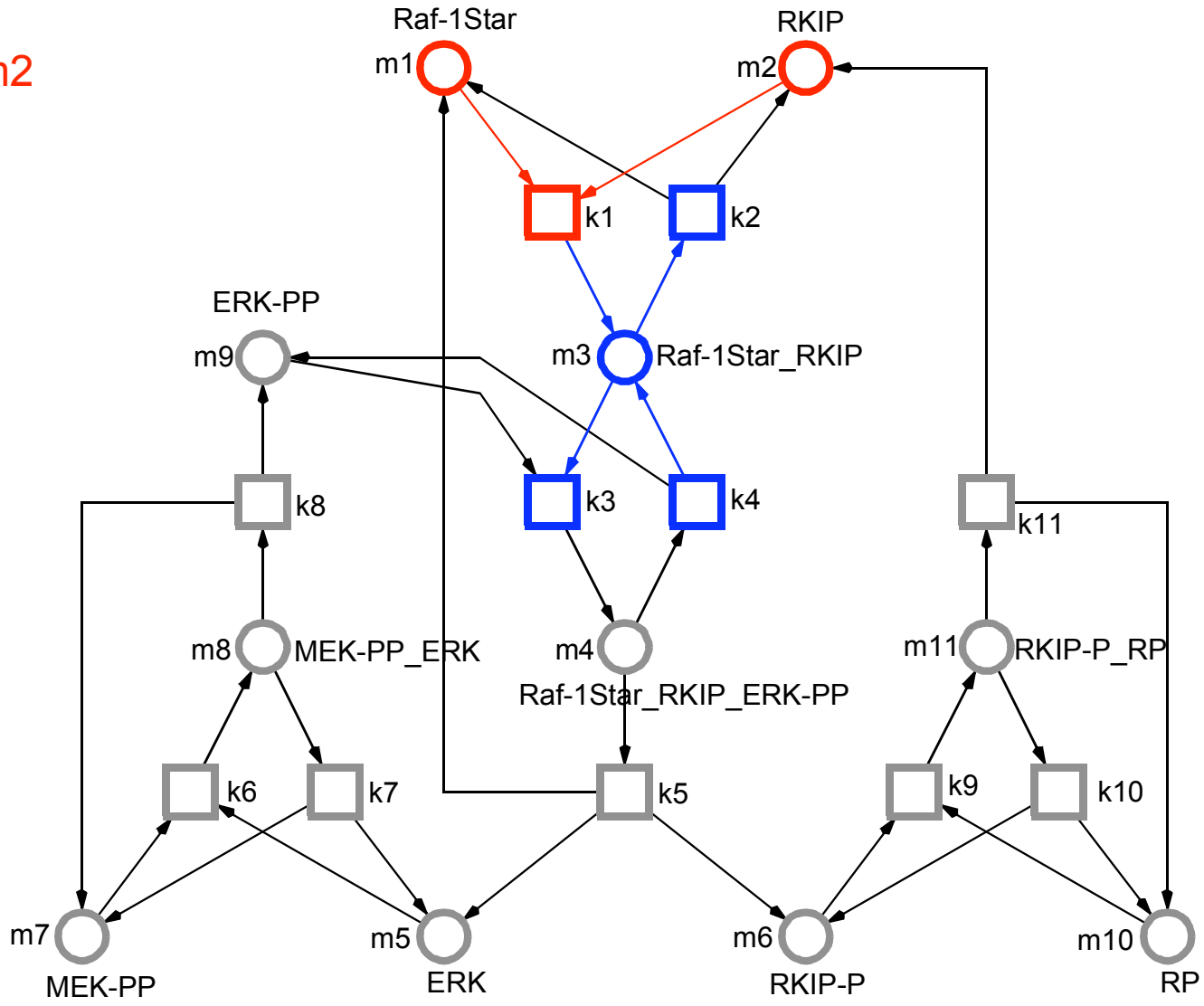
CONTINUOUS PETRI NET DEFINES ODES

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



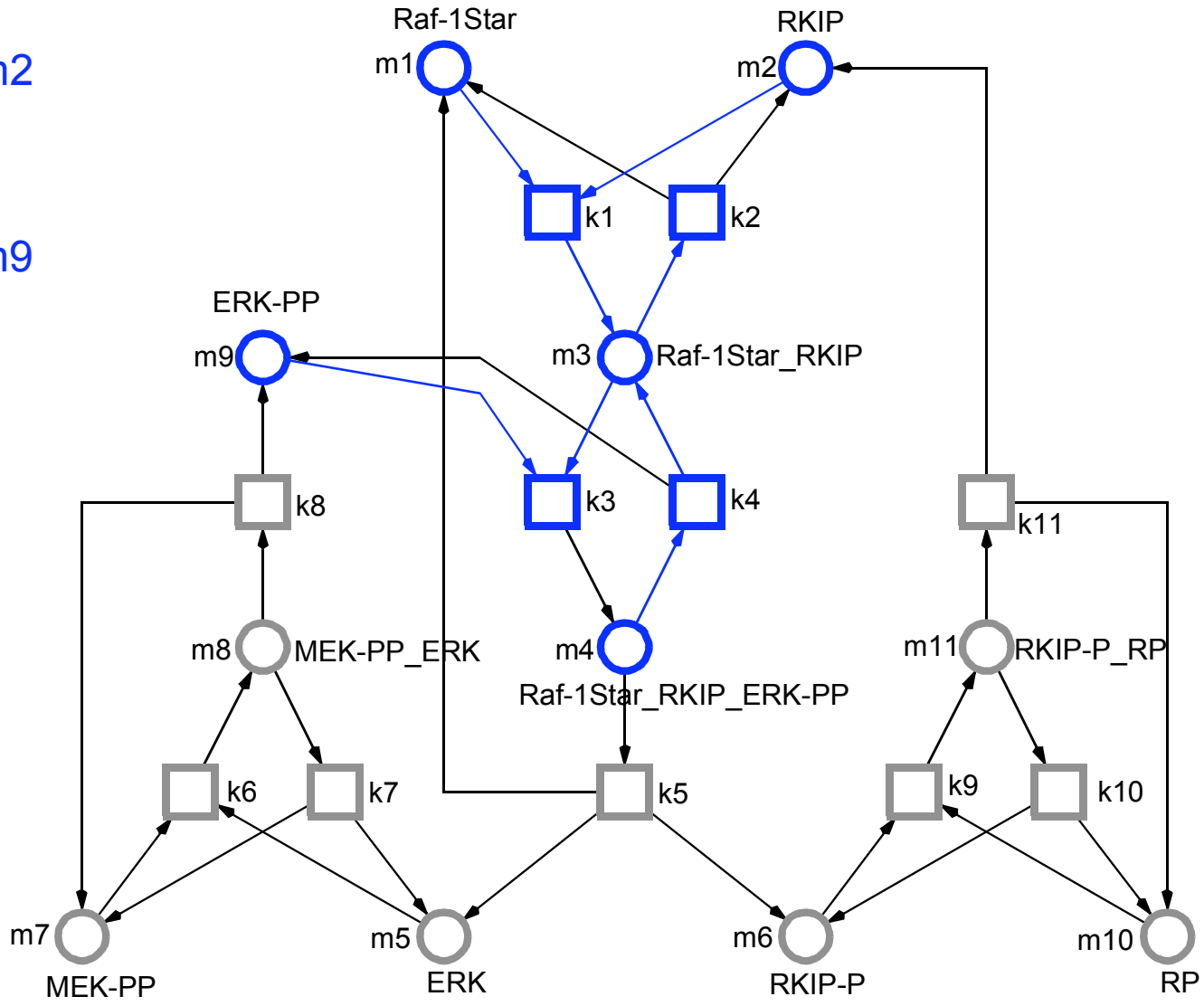
CONTINUOUS PETRI NET DEFINES ODES

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



CONTINUOUS PETRI NET DEFINES ODES

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



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

QUANTITATIVE ANALYSIS

Species	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
Raf-1*	1	0	0	1	1	1	1	1	0	0	1	1	1
RKIP	1	0	0	0	0	0	0	1	0	0	1	0	0
Raf-1*_RKIP	0	1	0	0	0	0	0	0	1	1	0	0	0
Raf-1*_RKIP_ERK-PP	0	0	1	0	0	0	0	0	0	0	0	0	0
ERK	0	0	0	1	0	0	1	1	1	0	0	0	0
RKIP-P	0	0	0	1	1	0	0	0	0	0	0	0	1
MEK-PP	1	1	1	1	0	0	1	1	1	0	0	1	1
MEK-PP_ERK	0	0	0	0	1	1	0	0	0	1	1	0	0
ERK-PP	1	1	0	0	0	0	0	0	0	0	0	1	1
RP	1	1	1	1	1	0	0	1	1	1	1	0	1
RKIP-P_RP	0	0	0	0	0	1	1	0	0	0	0	1	0

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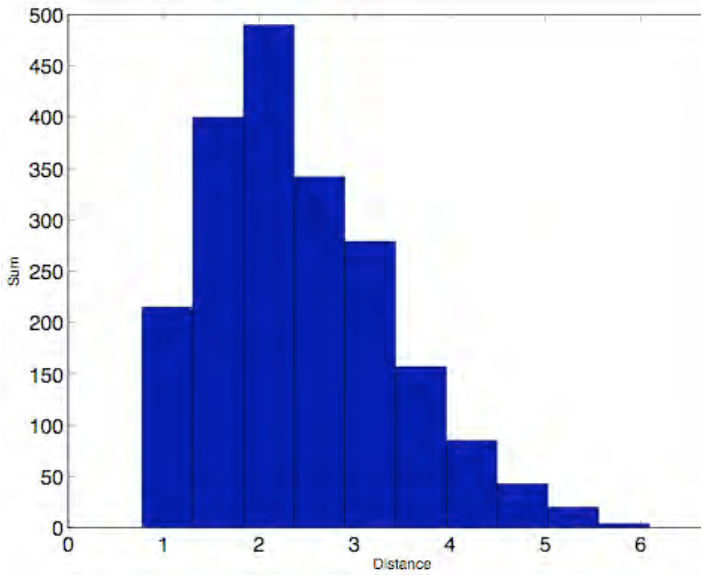
Biochemist

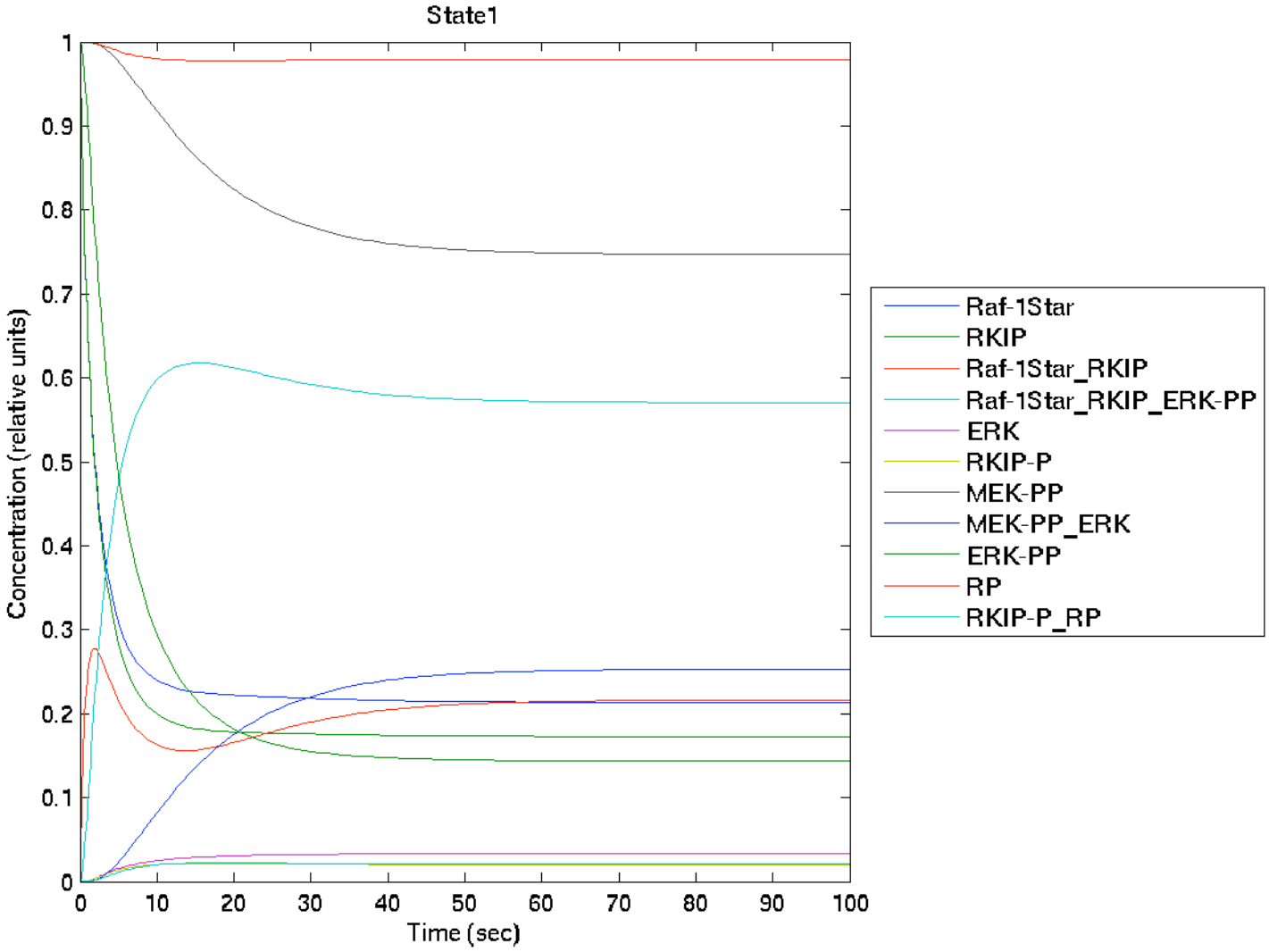


Distribution of `bad' steady states as euclidean distances from the `good' final steady state

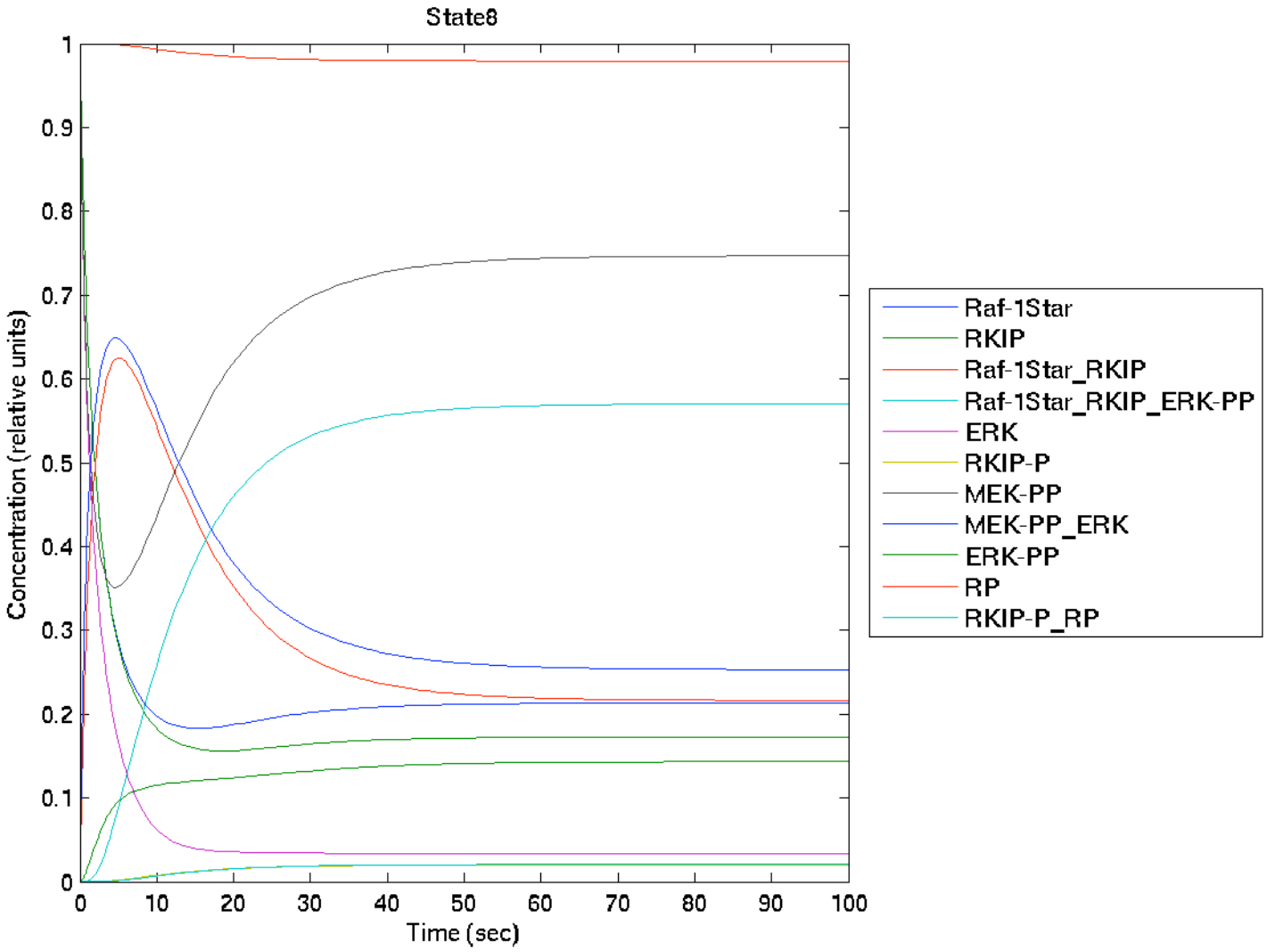
13 “good” state configurations

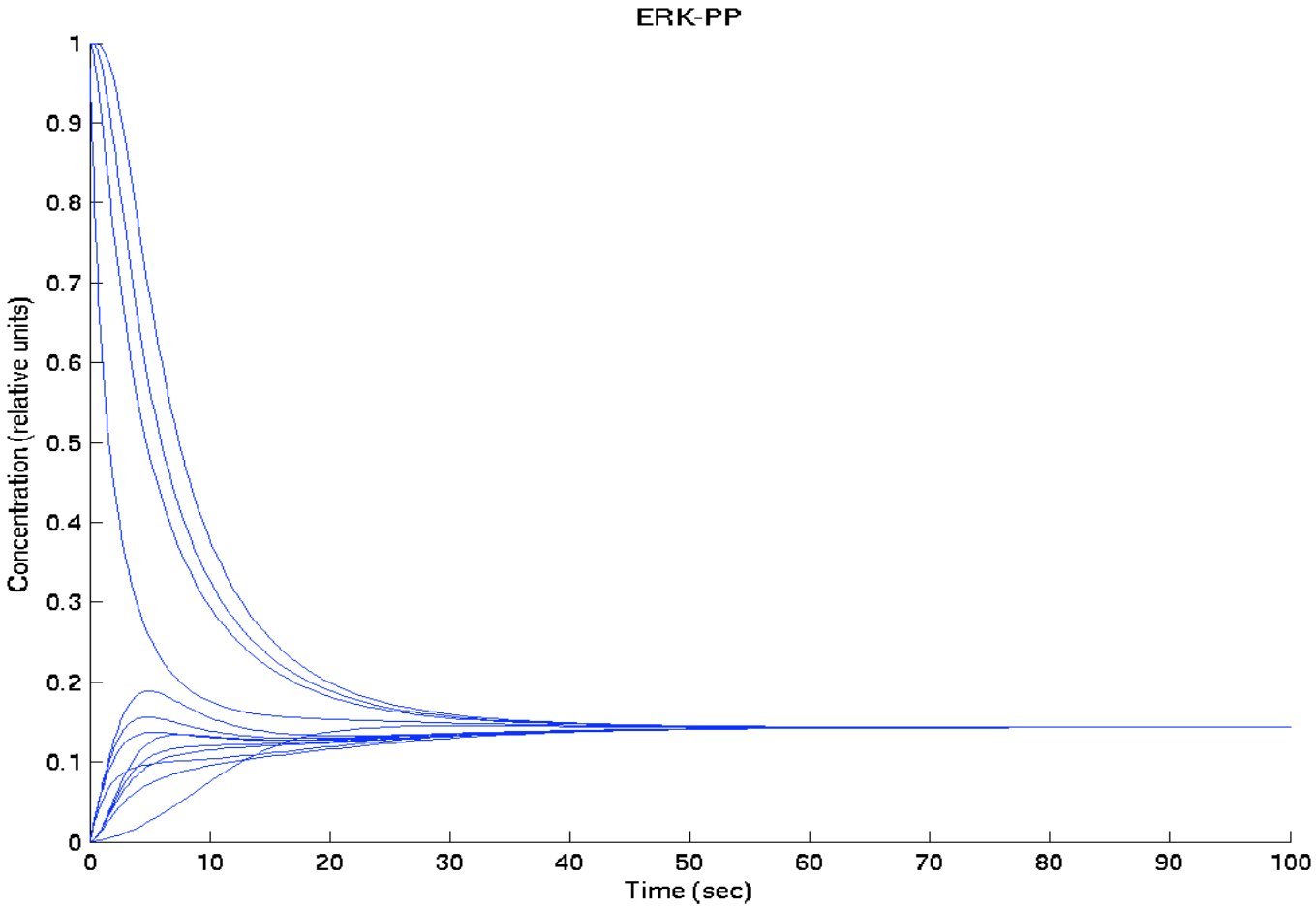
the “bad” ones

QUANTITATIVE ANALYSIS



QUANTITATIVE ANALYSIS




ERK-PP
Concentration (relative units)
Time (sec)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
10
20
30
40
50
60
70
80
90
100

SUMMARY

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- **representation of bionetworks by Petri nets**
  - -> *partial order representation* -> *better comprehension*
  - -> *formal semantics* -> *sound analysis techniques*
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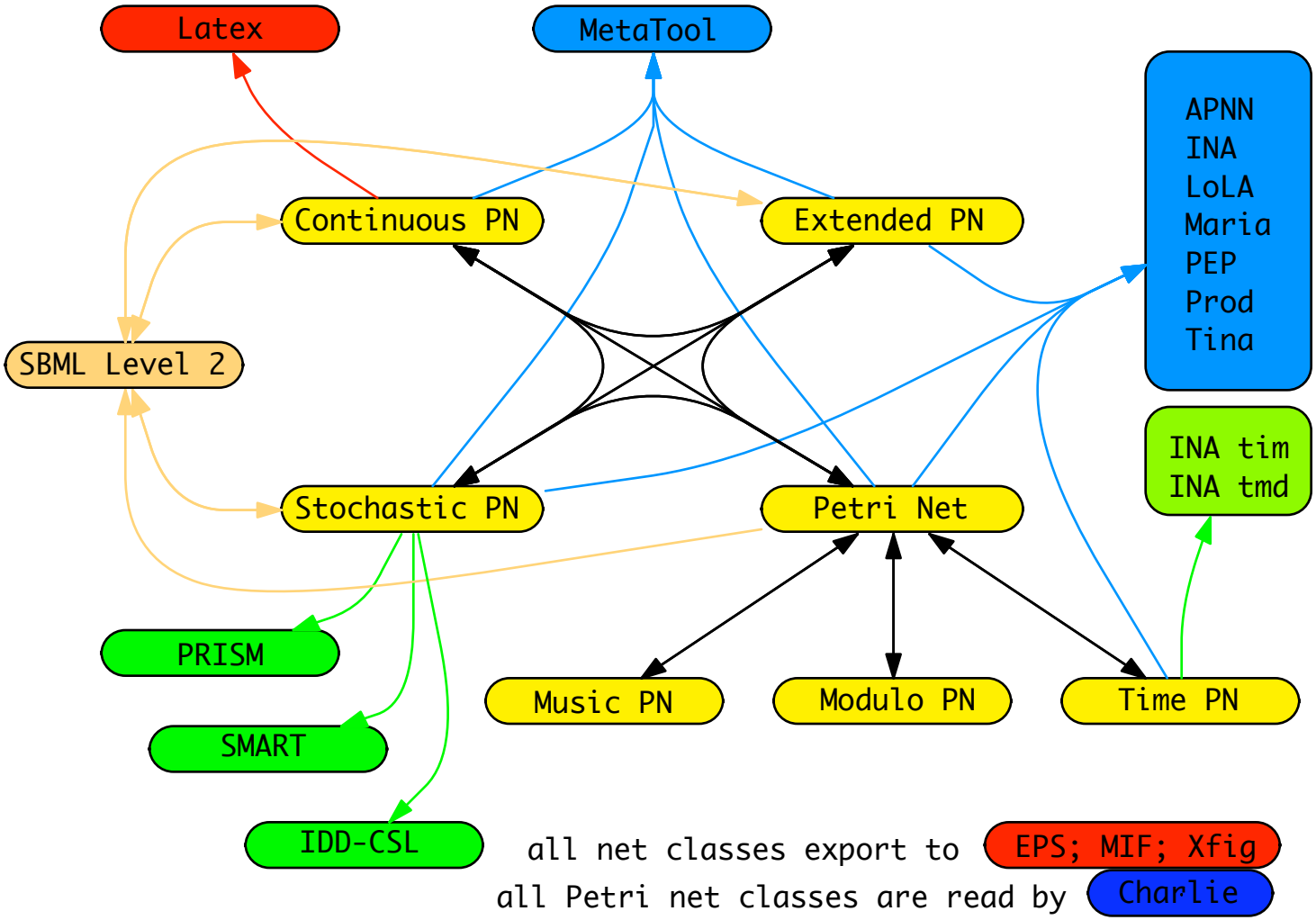
- **step-wise model development**
  - -> *qualitative model* -> *discrete Petri nets*
  - -> *discrete quantitative model* -> *stochastic Petri nets*
  - -> *continuous quantitative model* -> *continuous Petri nets = ODEs*

TOOLKIT - SNOOPY'S EXPORT FEATURES



PRINCIPAL COLLABORATORS

- **Rainer Breitling**

  University Glasgow, Integrative and Systems Biology &
  University of Groningen, Groningen Bioinformatics Centre

- **David Gilbert**

  Brunel University London/Uxbridge,
  School of Information Systems, Computing and Mathematics

- **Wolfgang Marwan**

  Otto von Guericke University & Magdeburg Centre for Systems Biology &
  Max Planck Institute for Dynamics of Complex Technical Systems

- **Louchka Popova-Zeugmann**

  Humboldt University Berlin, Computer Science Institute

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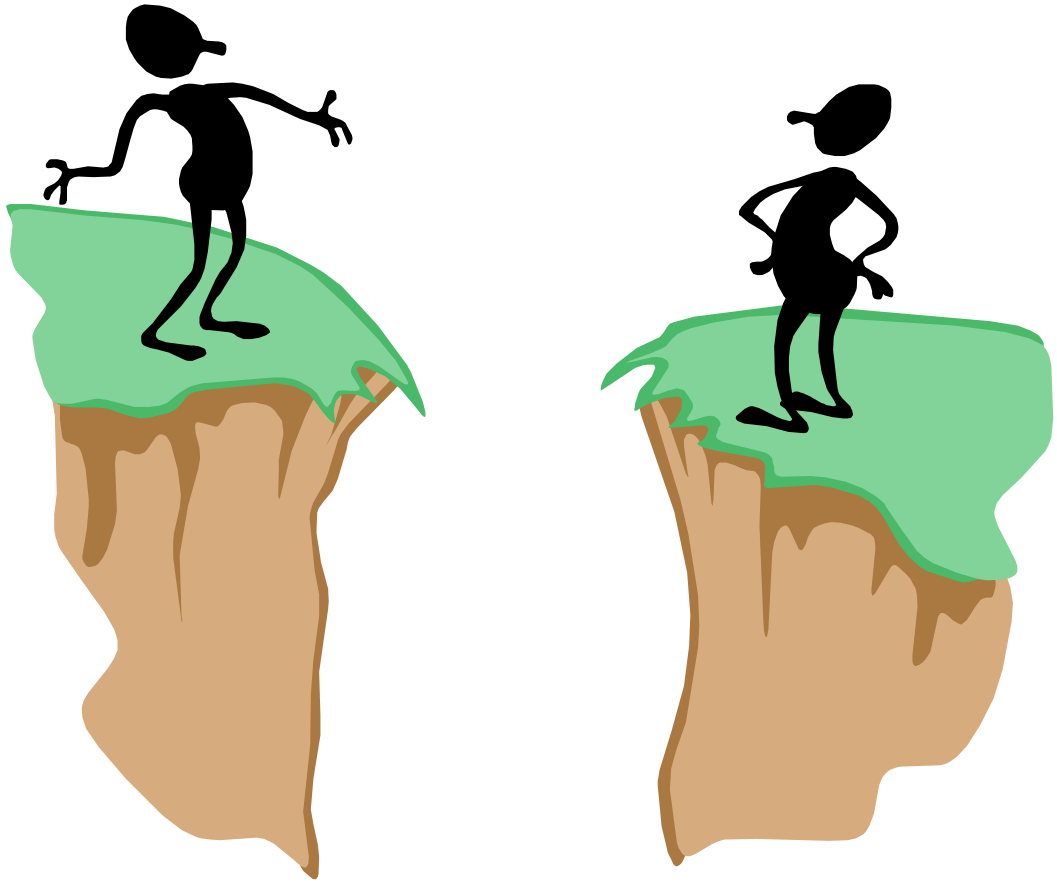
OUTLOOK



THANKS !

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