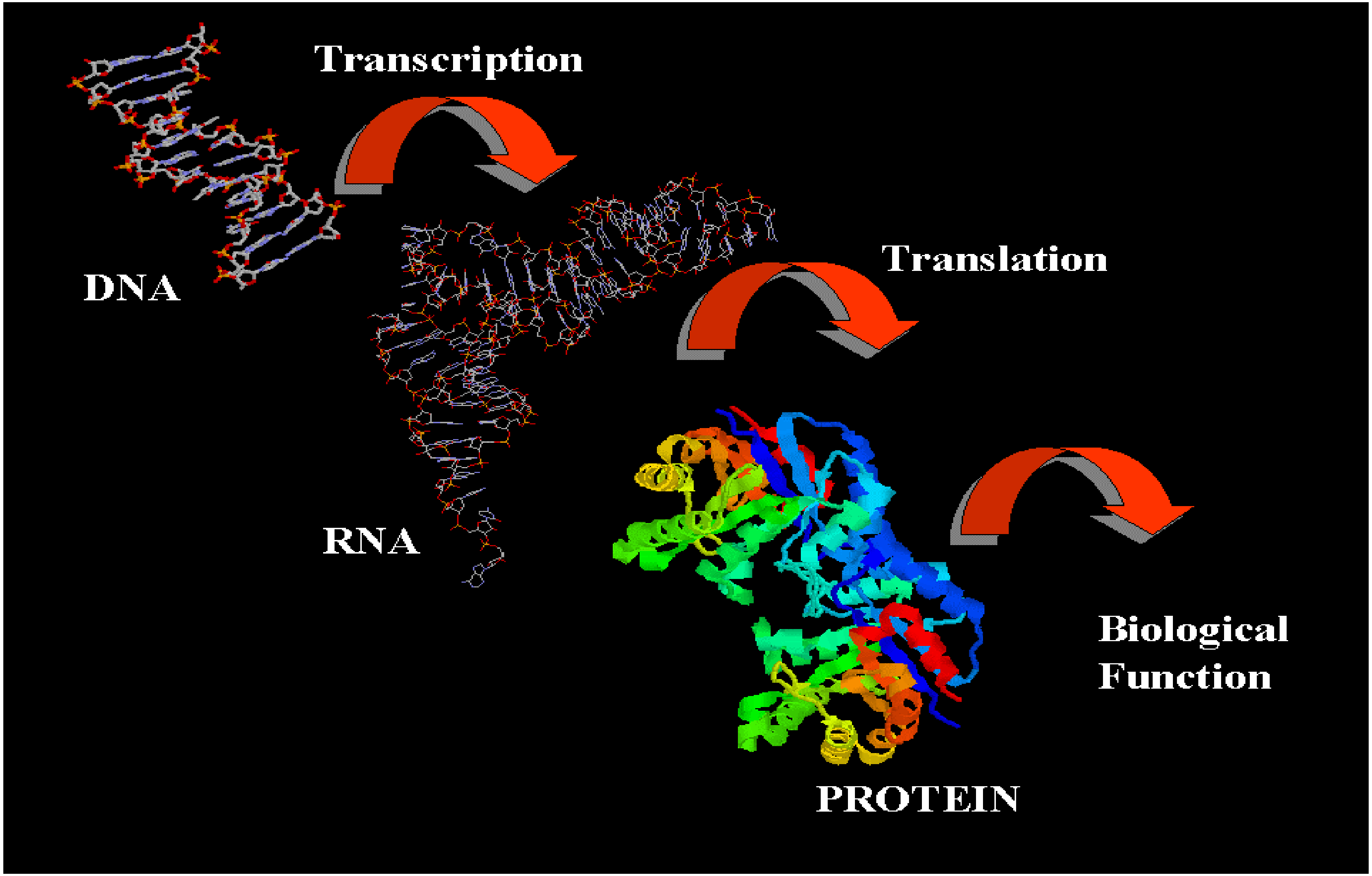
A CASE STUDY

- THE RKIP PATHWAY -

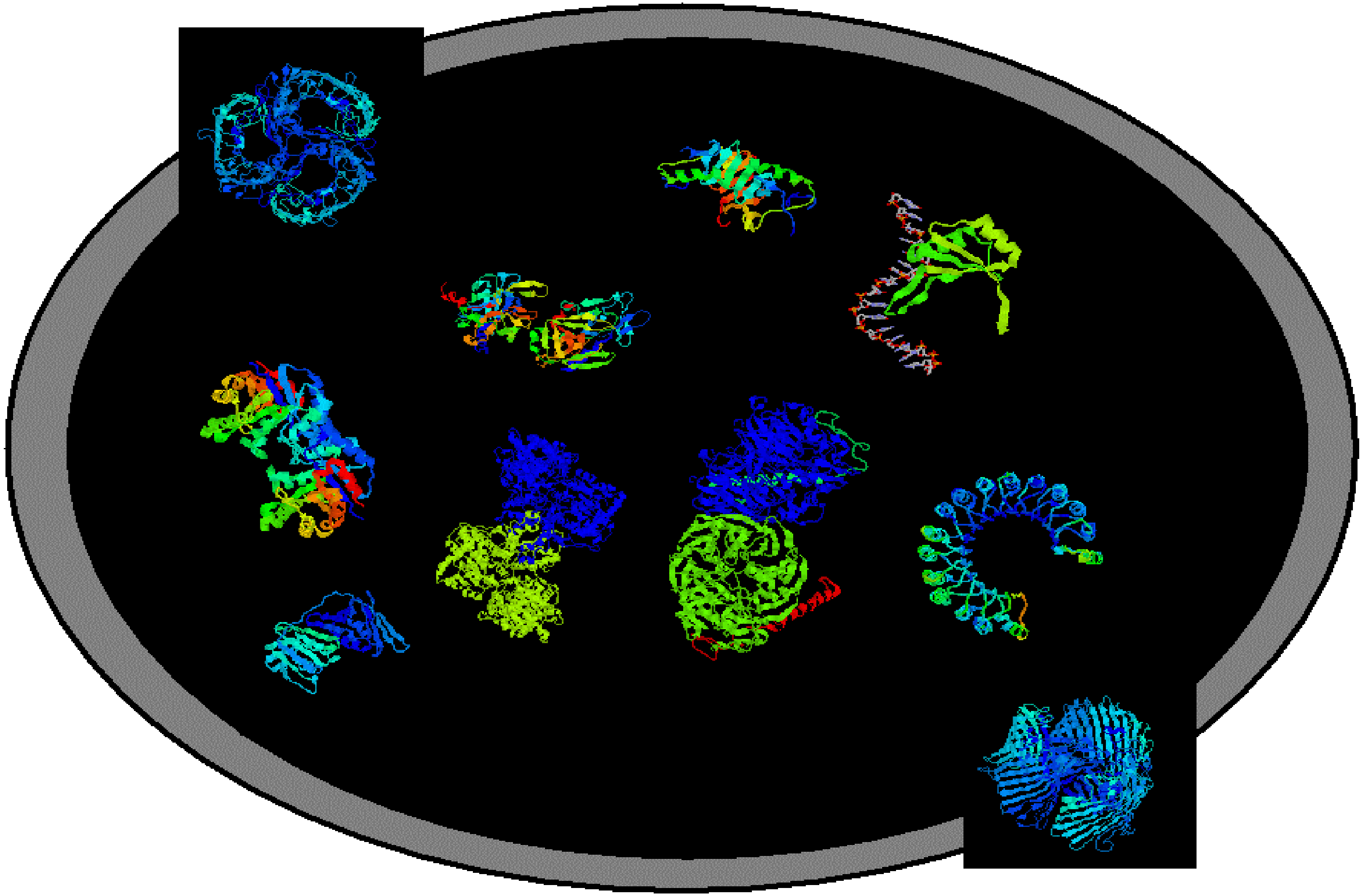
MONIKA HEINER

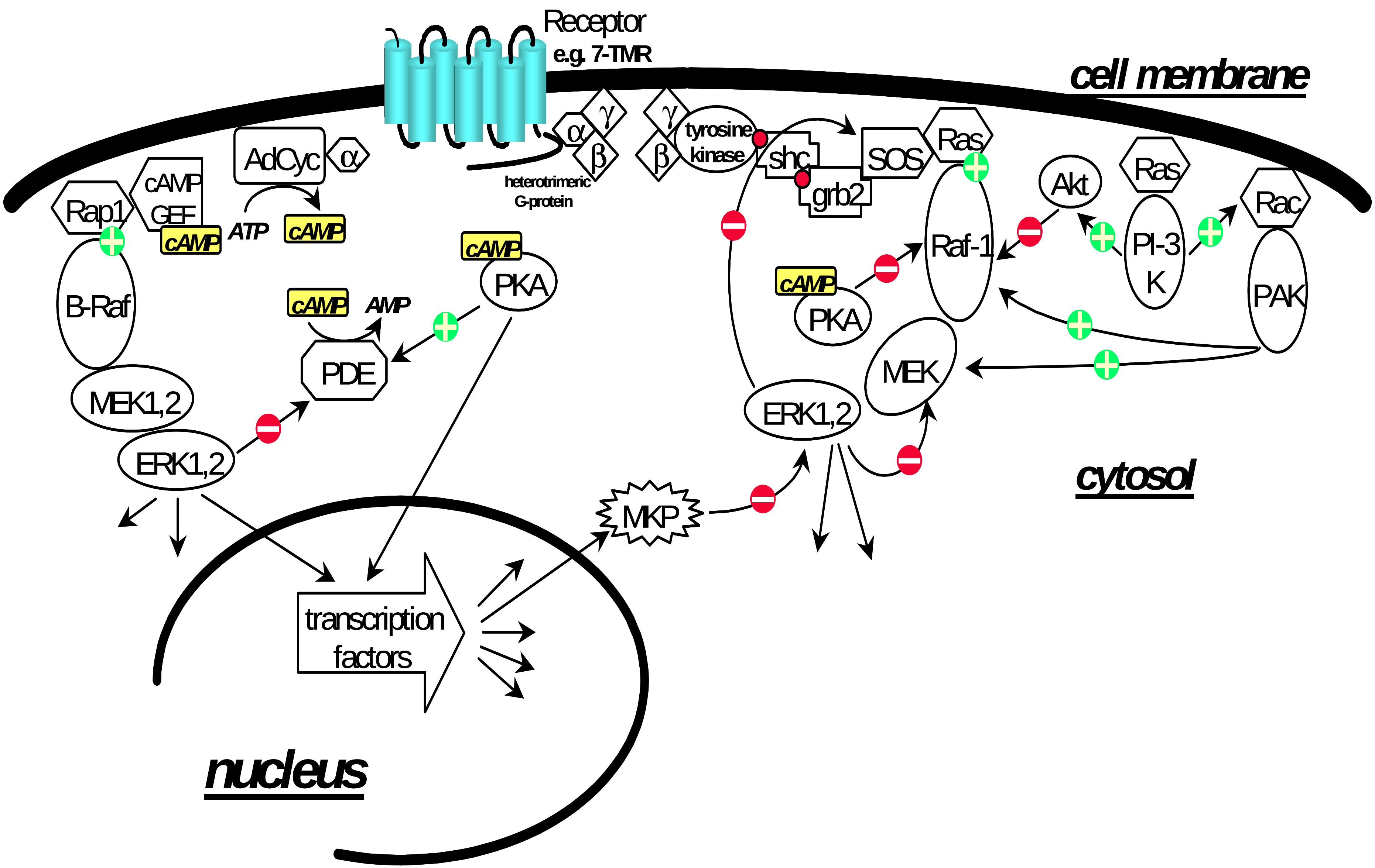
BRANDENBURG TECHNICAL UNIVERSITY COTTBUS-SENFTENBERG
COMPUTER SCIENCE INSTITUT

BIOLOGICAL FUNCTION ?

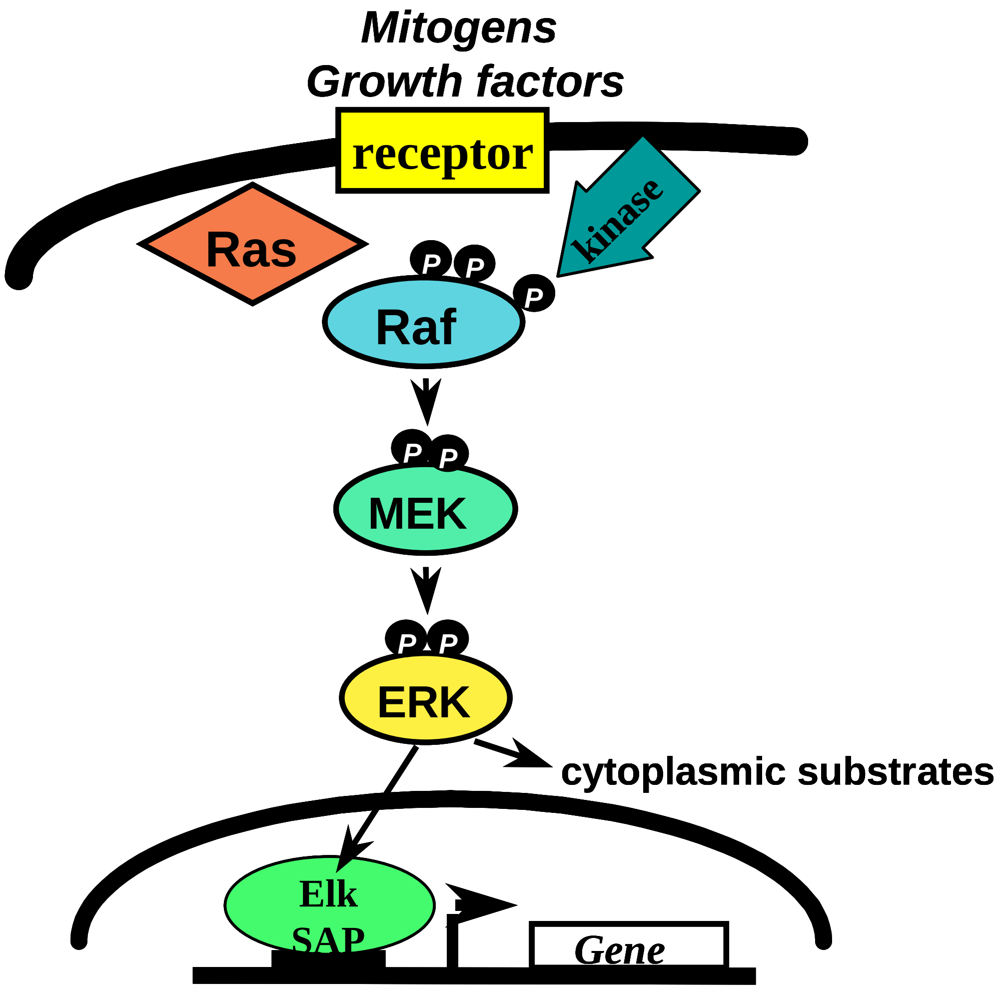


. . . BY INTERACTION IN NETWORKS

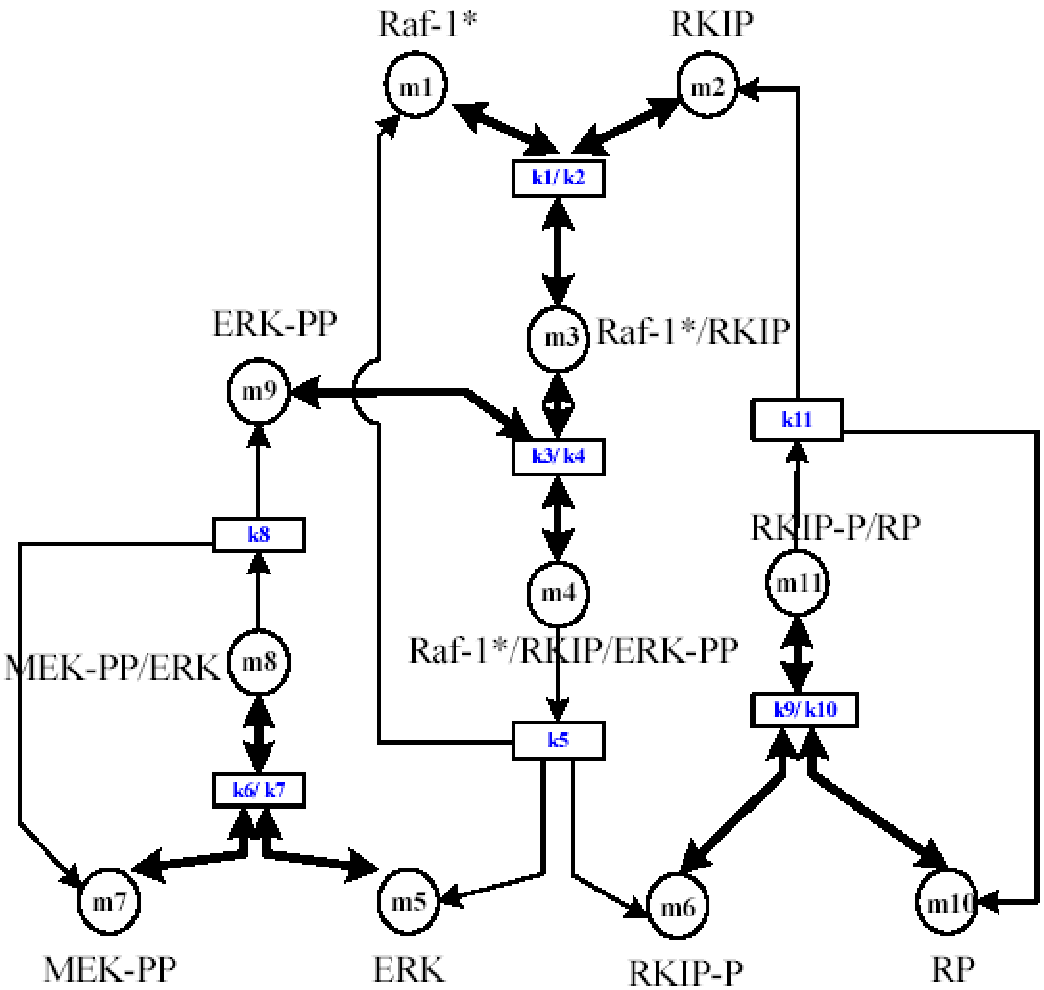



Receptor
e.g. 7-TMR
cell membrane
α
γ
β
heterotrimeric
G-protein
AdCyc
α
cAMP
GEF
Rap1
cAMP
ATP
cAMP
B-Raf
cAMP
PKA
cAMP
AMP
PDE
MEK1,2
ERK1,2
tyrosine
kinase
γ
β
shc
grb2
SOS
Ras
Raf-1
Akt
Ras
Rac
PI-3
K
PAK
cAMP
PKA
MEK
ERK1,2
cytosol
MKP
transcription
factors
nucleus

…one pathway…

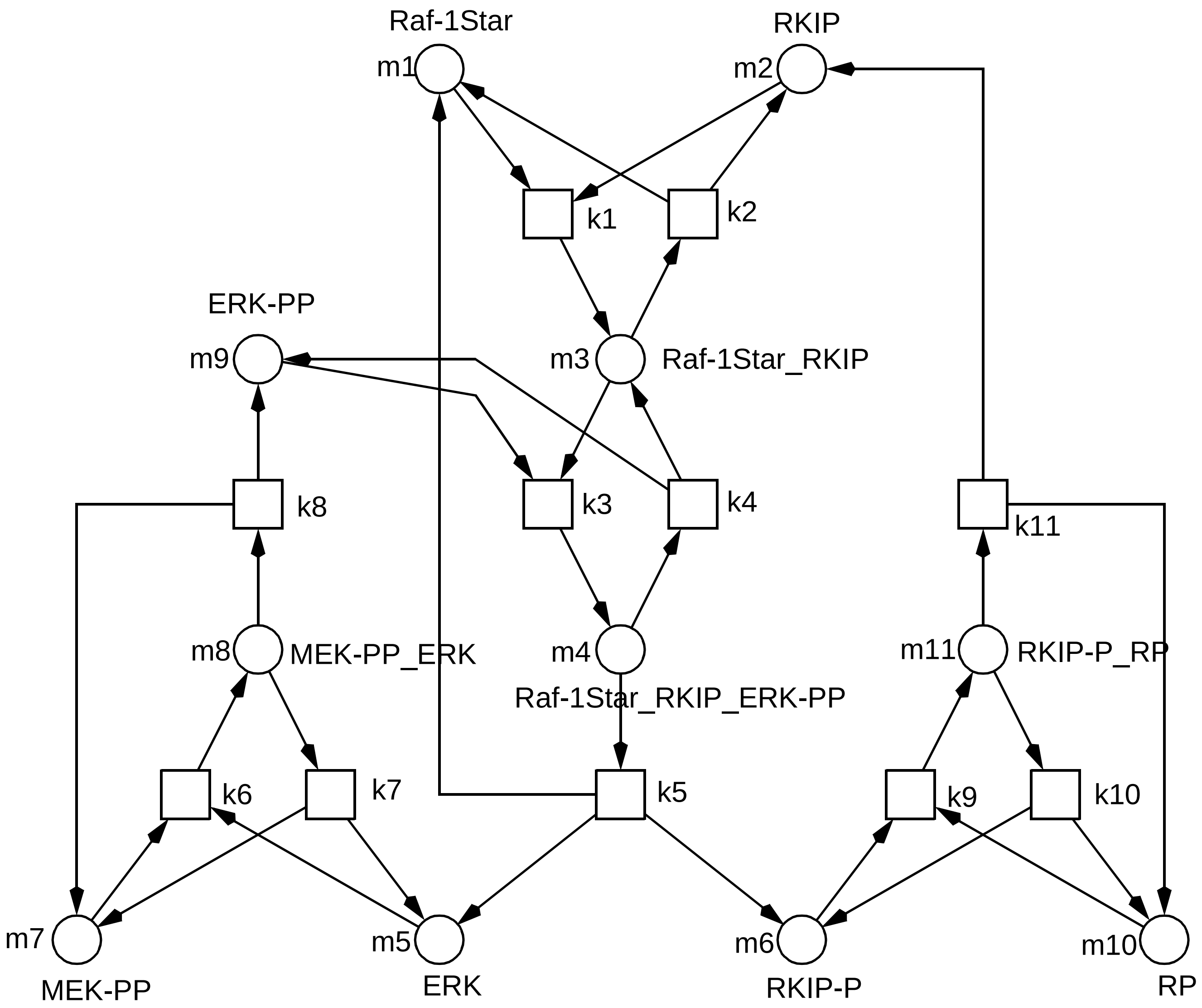

Mitogens
Growth factors
receptor
kinase
Ras
P
P
P
Raf
P
P
MEK
P
P
ERK
cytoplasmic substrates
Elk
SAP
Gene

The RKIP Pathway

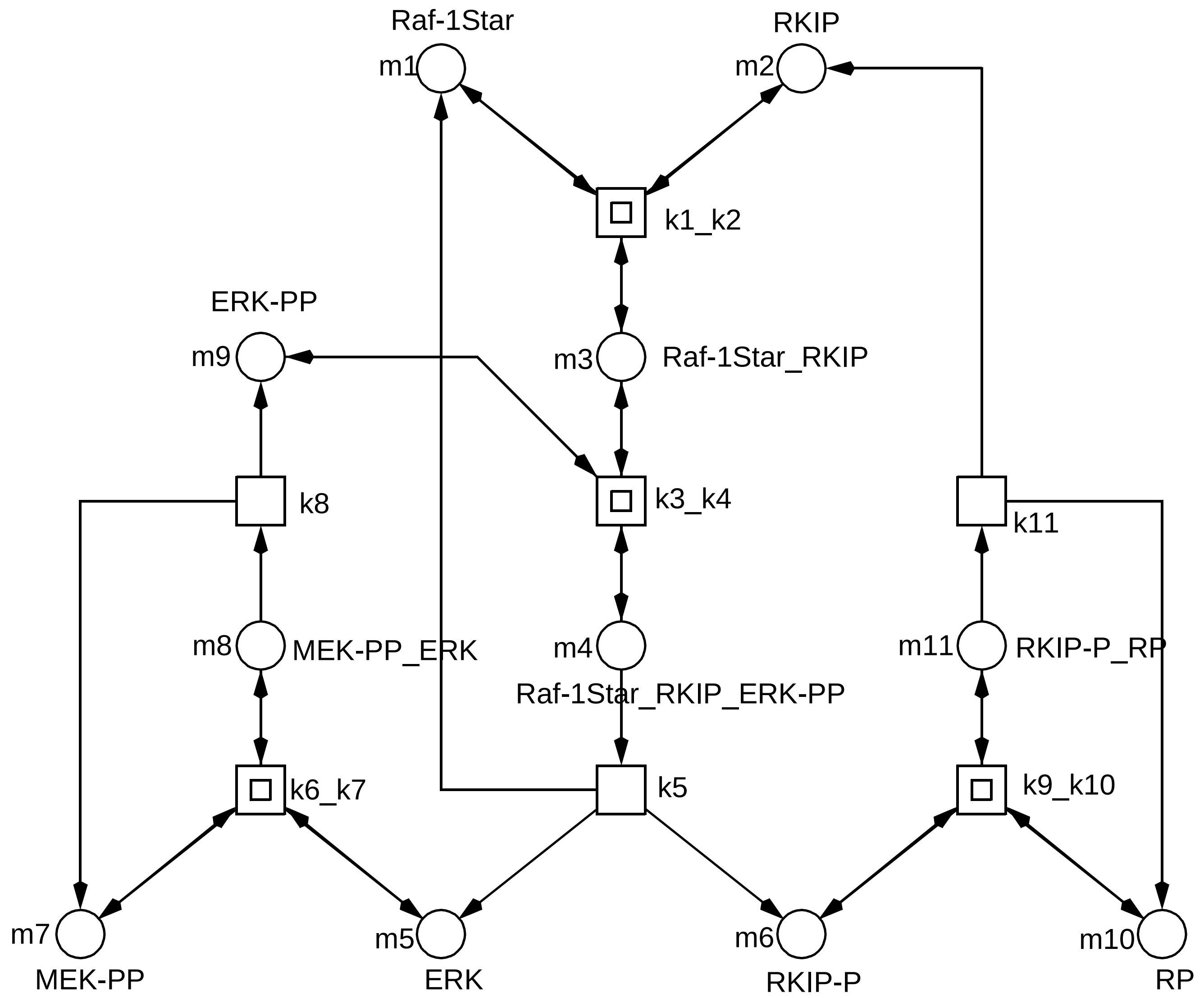


[Cho et al., CMSB 2003]

The RKIP Pathway, Petri Net



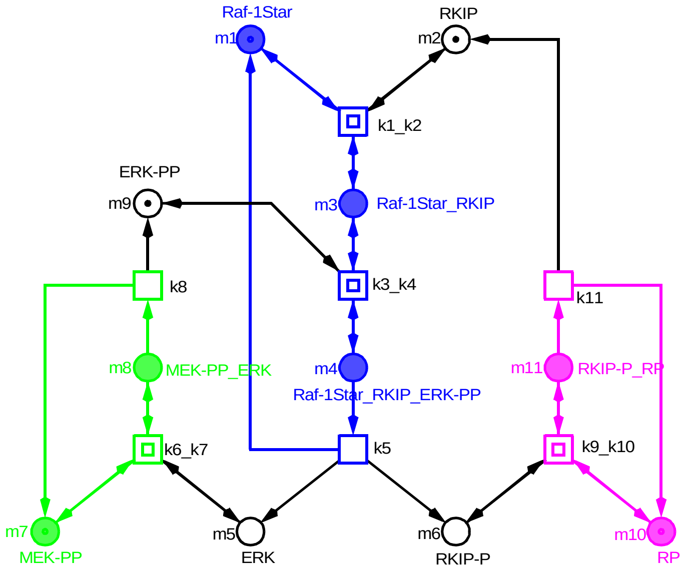
THE RKIP PATHWAY, HIERARCHICAL PETRI NET

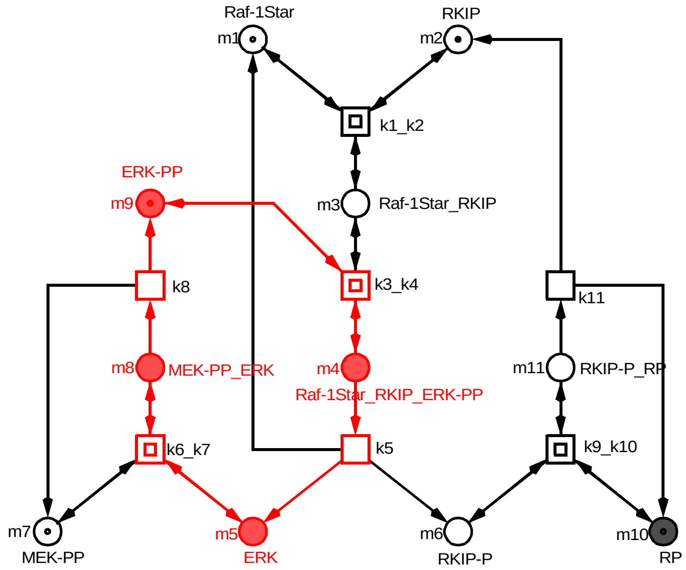


QUALITATIVE ANALYSES

STATIC ANALYSES

The RKIP Pathway, P-Invariants





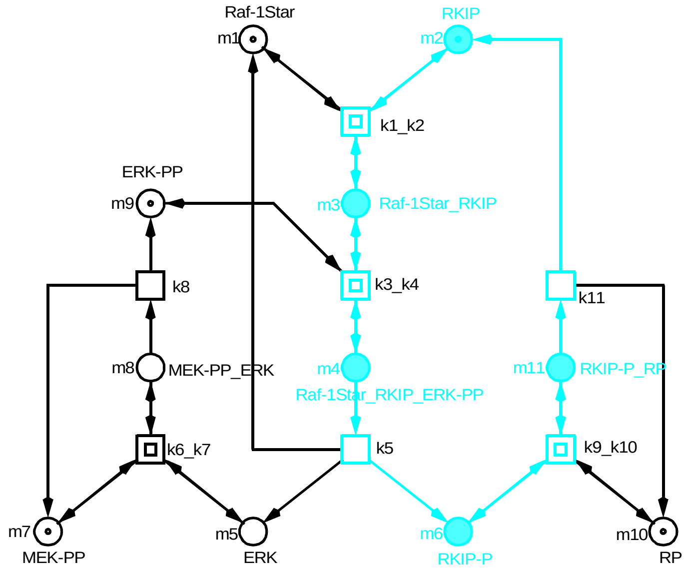
P-INV1: MEK

P-INV2: RAF-1STAR

P-INV3: RP

P-INV4: ERK

P-INV5: RKIP



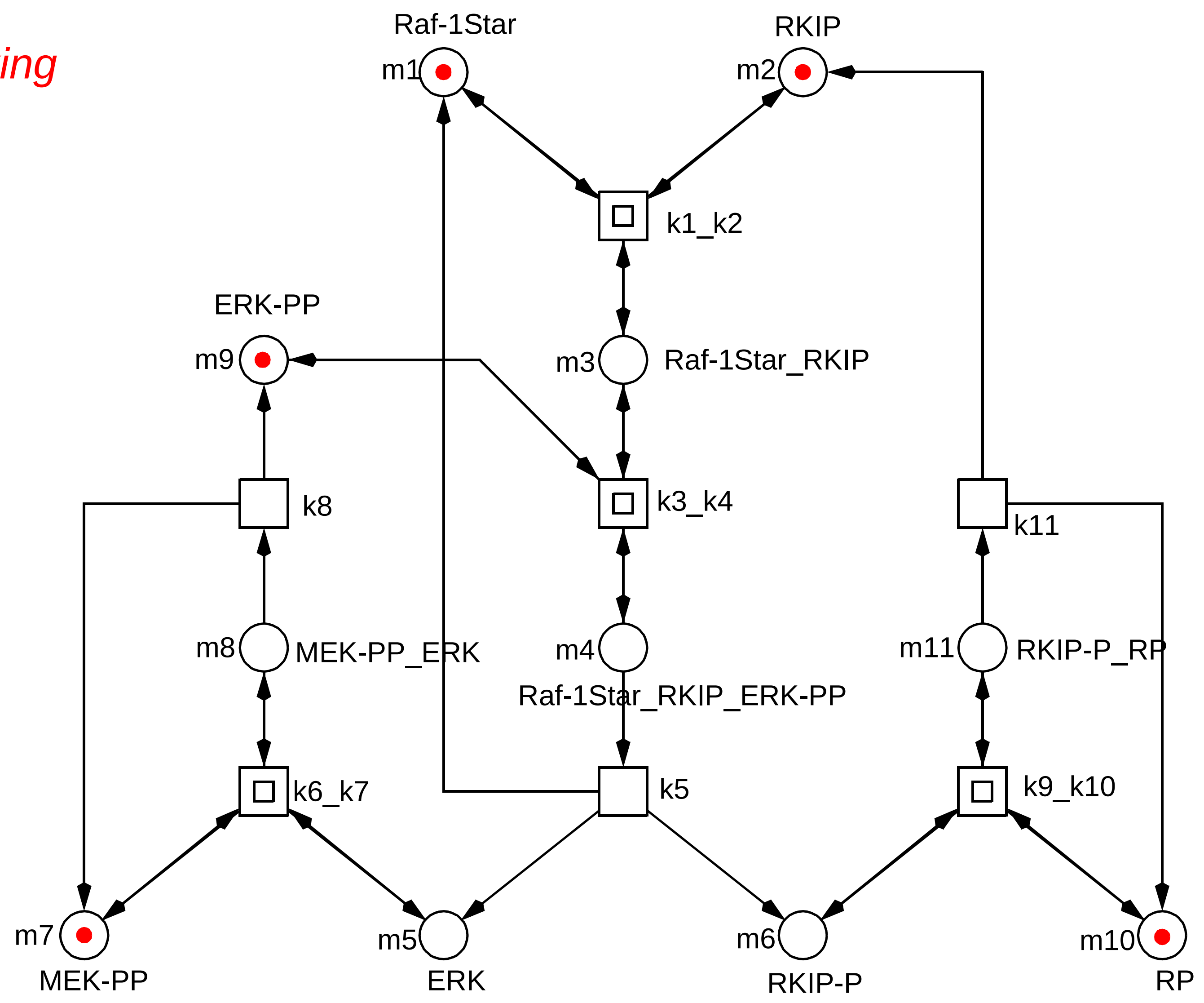
CCONSTRUCTION OF THE INITIAL MARKING

- **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 realizable**
  -> *to make the net live*
- **minimal marking**
  -> *minimization of the state space*

-> UNIQUE INITIAL MARKING <-

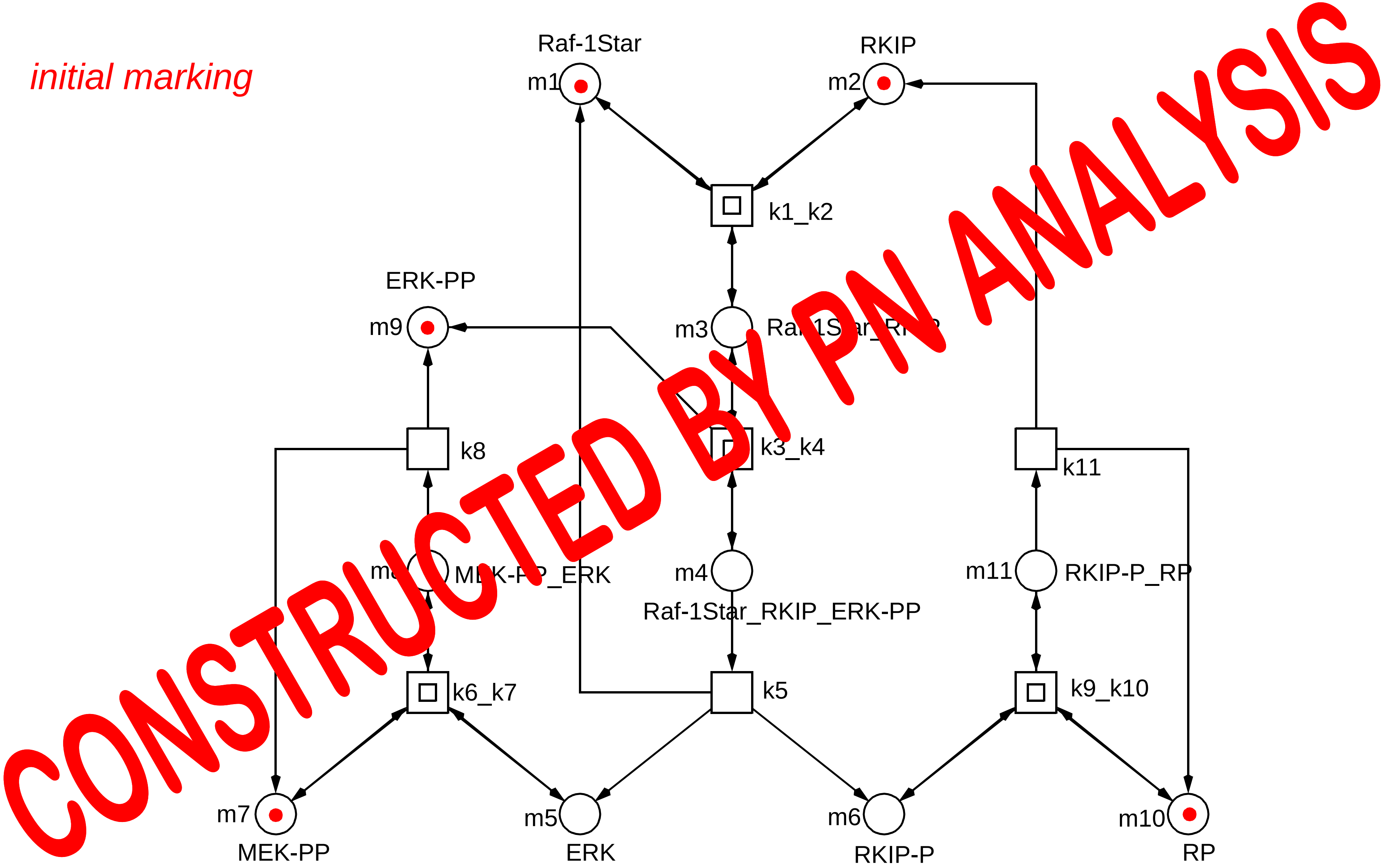
The RKIP Pathway, Hierarchical Petri Net

initial marking



THE RKIP PATHWAY, HIERARCHICAL PETRI NET

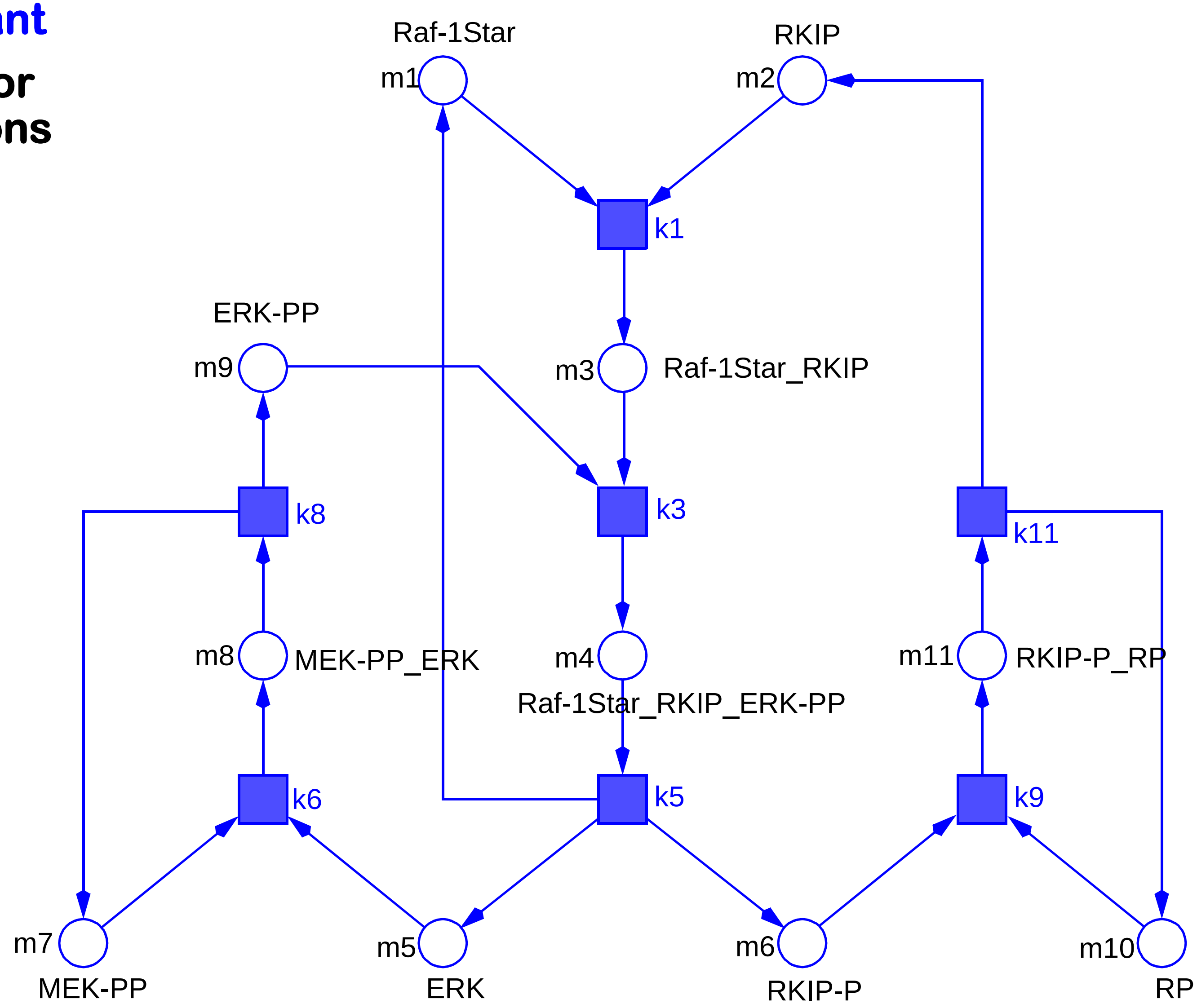
initial marking



The RKIP Pathway, Non-trivial T-invariant

-> non-trivial T-invariant

+ four trivial ones for reversible reactions



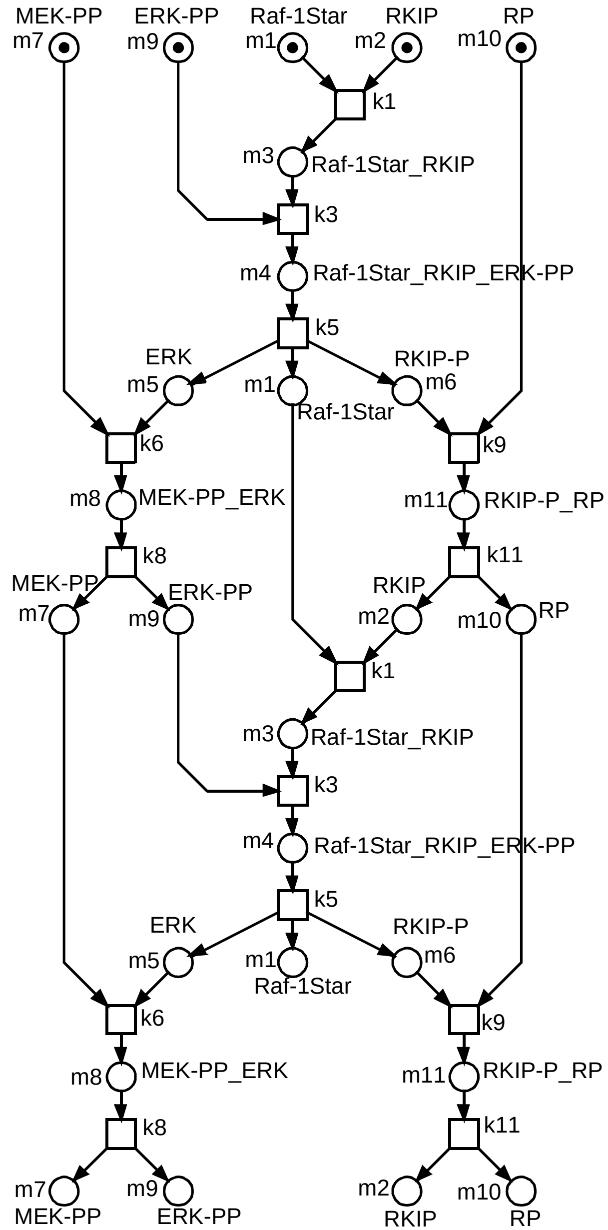
Non-trivial T-invariant, Run

- **realizability check under the constructed marking**

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

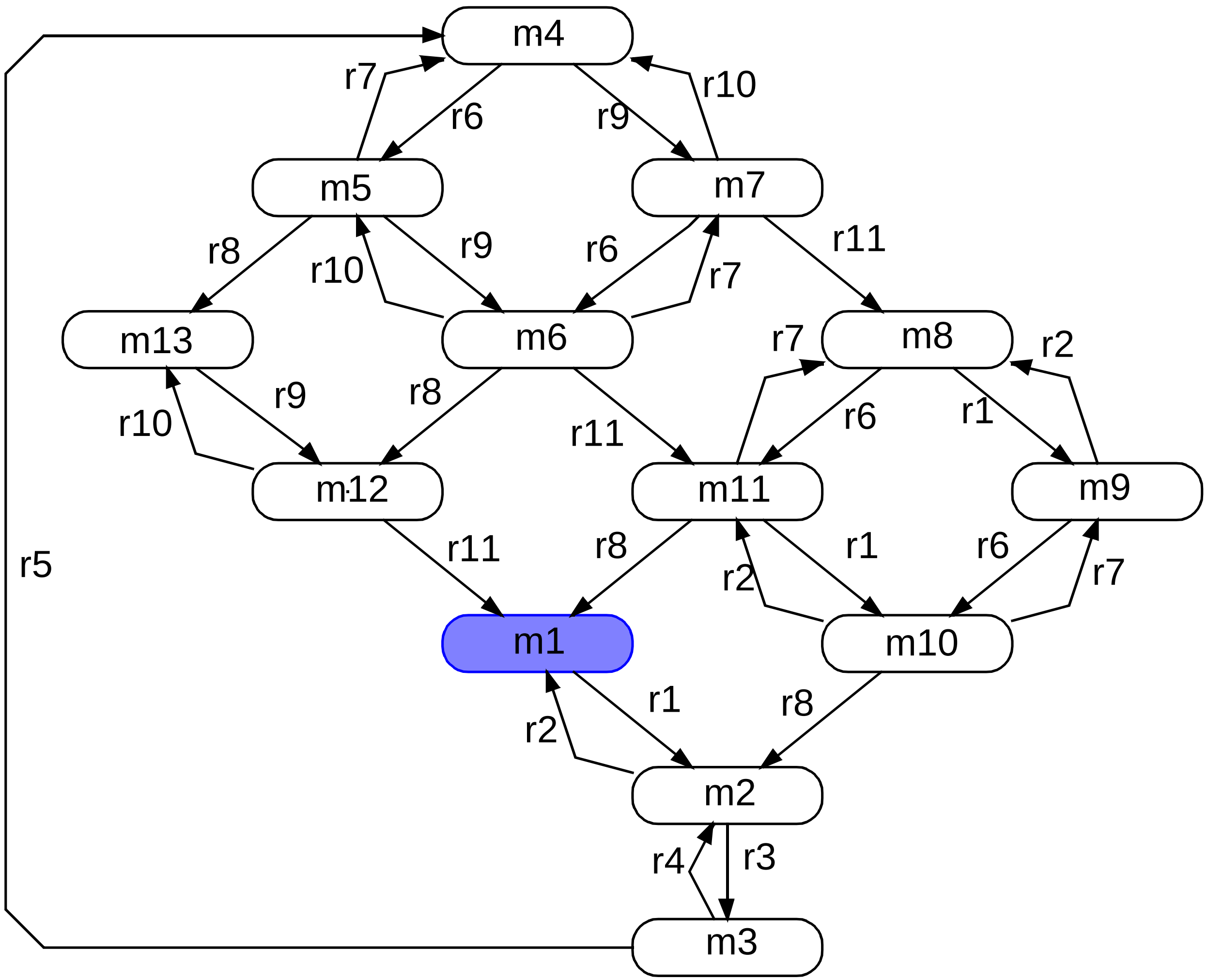
- **labelled condition / event net**
  - -> *events (boxes)*
    - transition occurences
  - -> *conditions (circles)*
    - involved compounds

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



DYNAMIC ANALYSES

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



- **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*

-> 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 !

- **quantitative model = qualitative model + quantitative parameters**

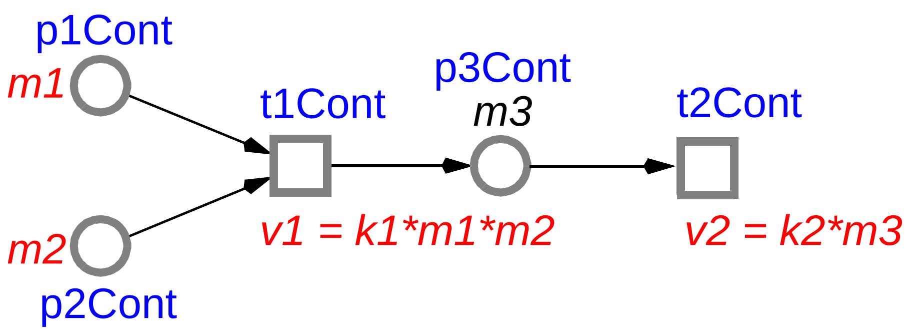
  -> known or estimated quantitative parameters

- **typical quantitative parameters of bionetworks**

  -> compound concentrations -> real numbers

  -> reaction rates / fluxes -> concentration-dependent

- **continuous Petri nets**



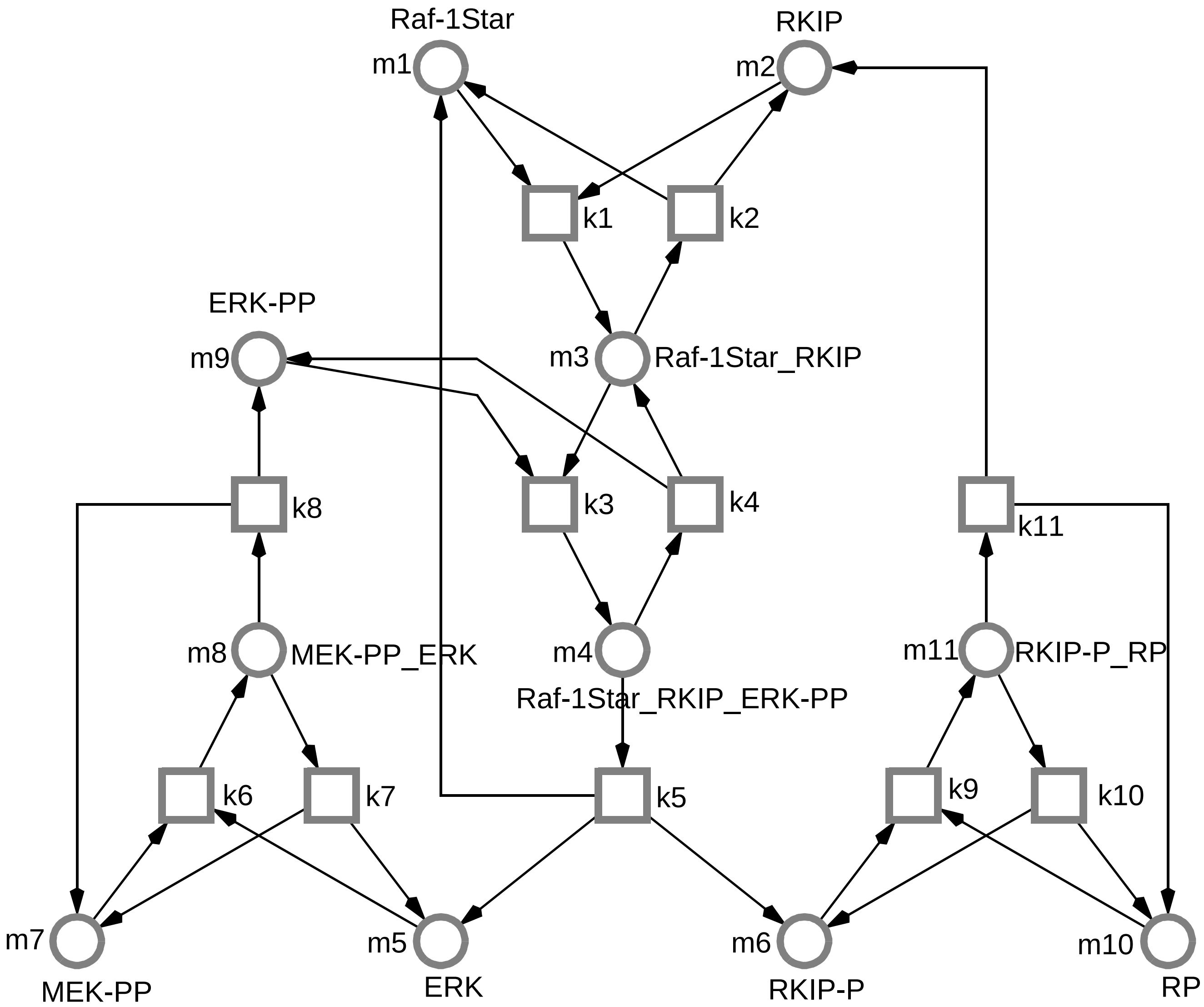
continuous nodes !

dm1 / dt = dm2 / dt = - v1

dm3 / dt = v1 - v2

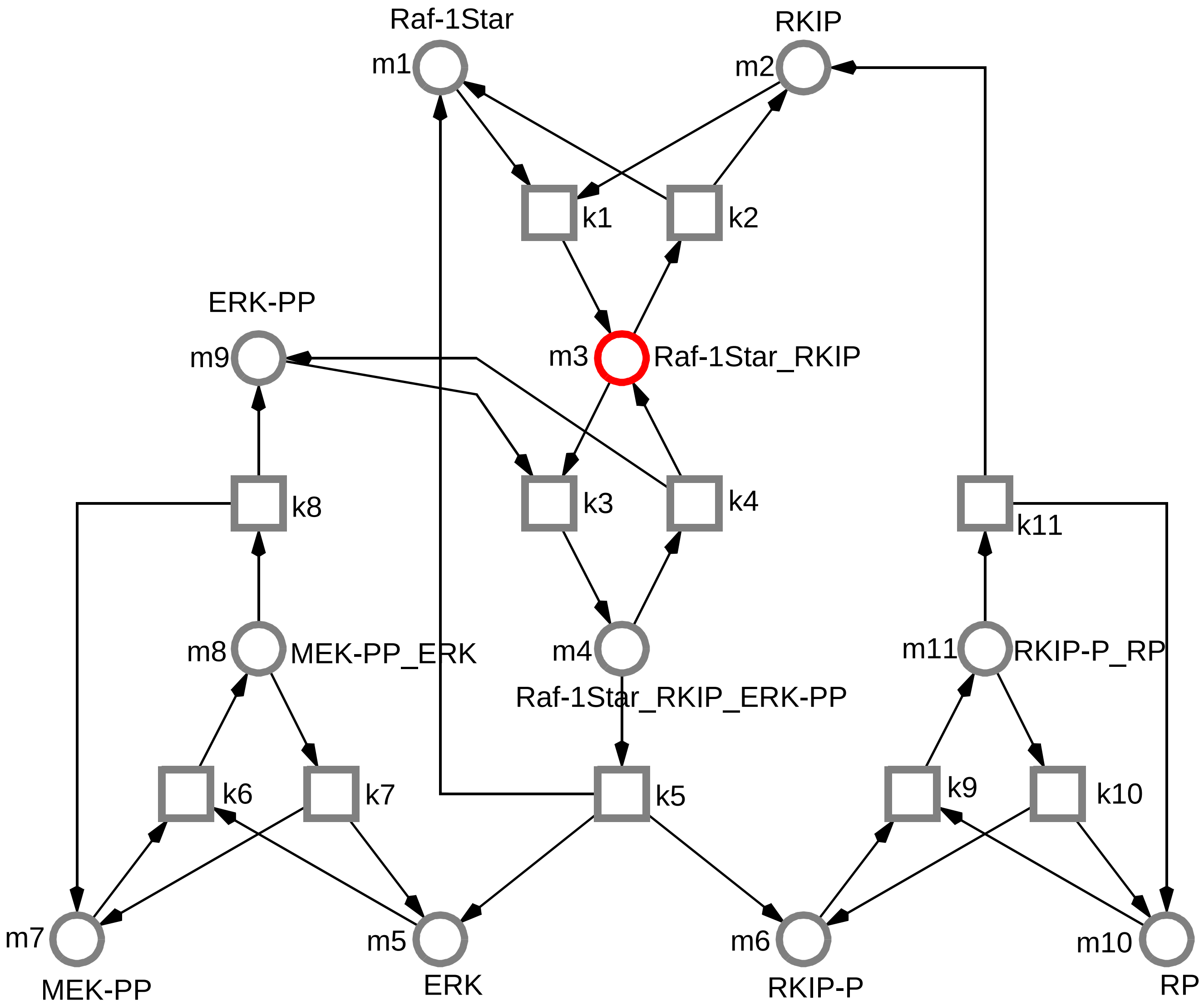
} **ODEs**

THE RKIP PATHWAY, CONTINUOUS PETRI NET



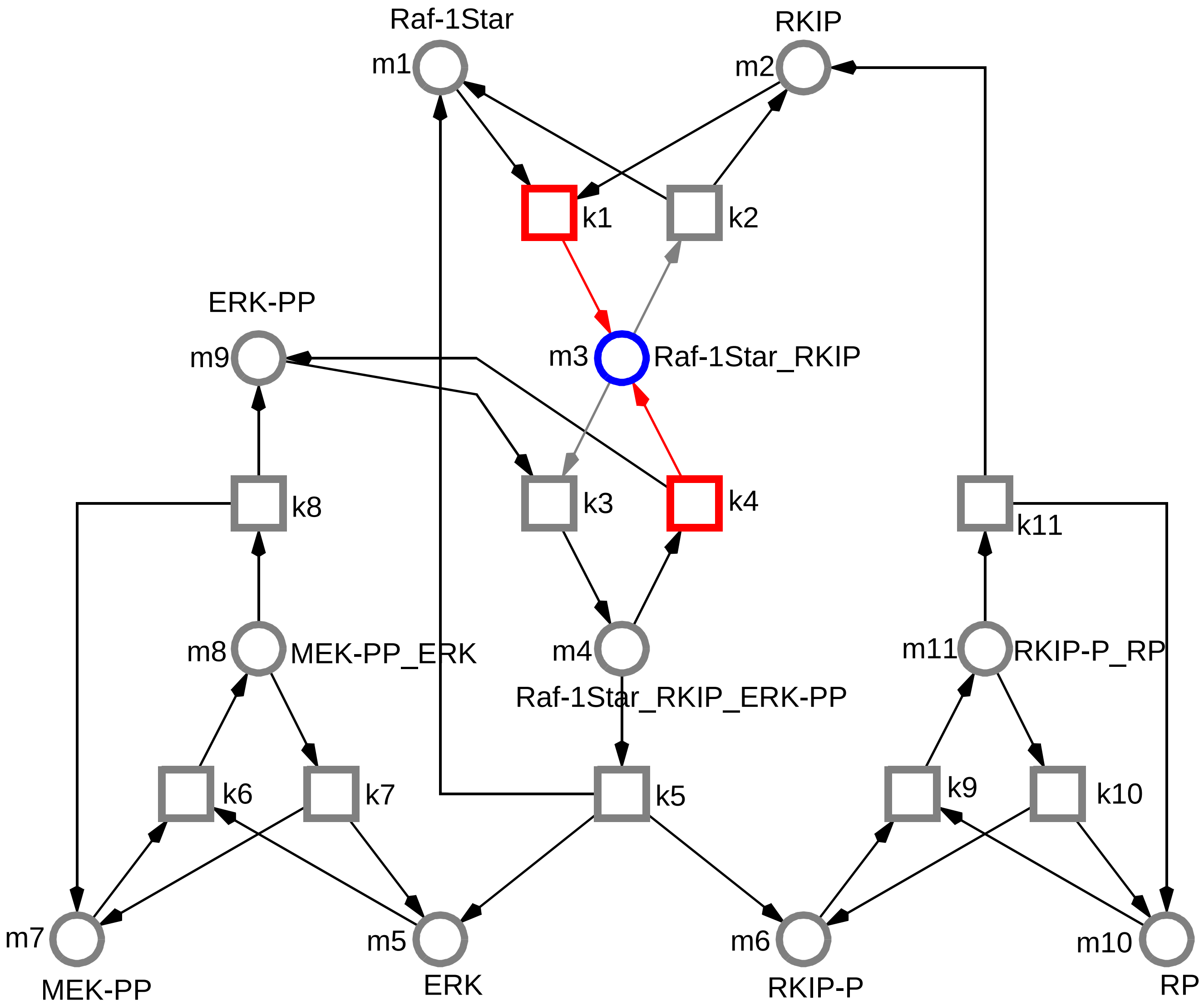
THE RKIP PATHWAY, CONTINUOUS PETRI NET

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



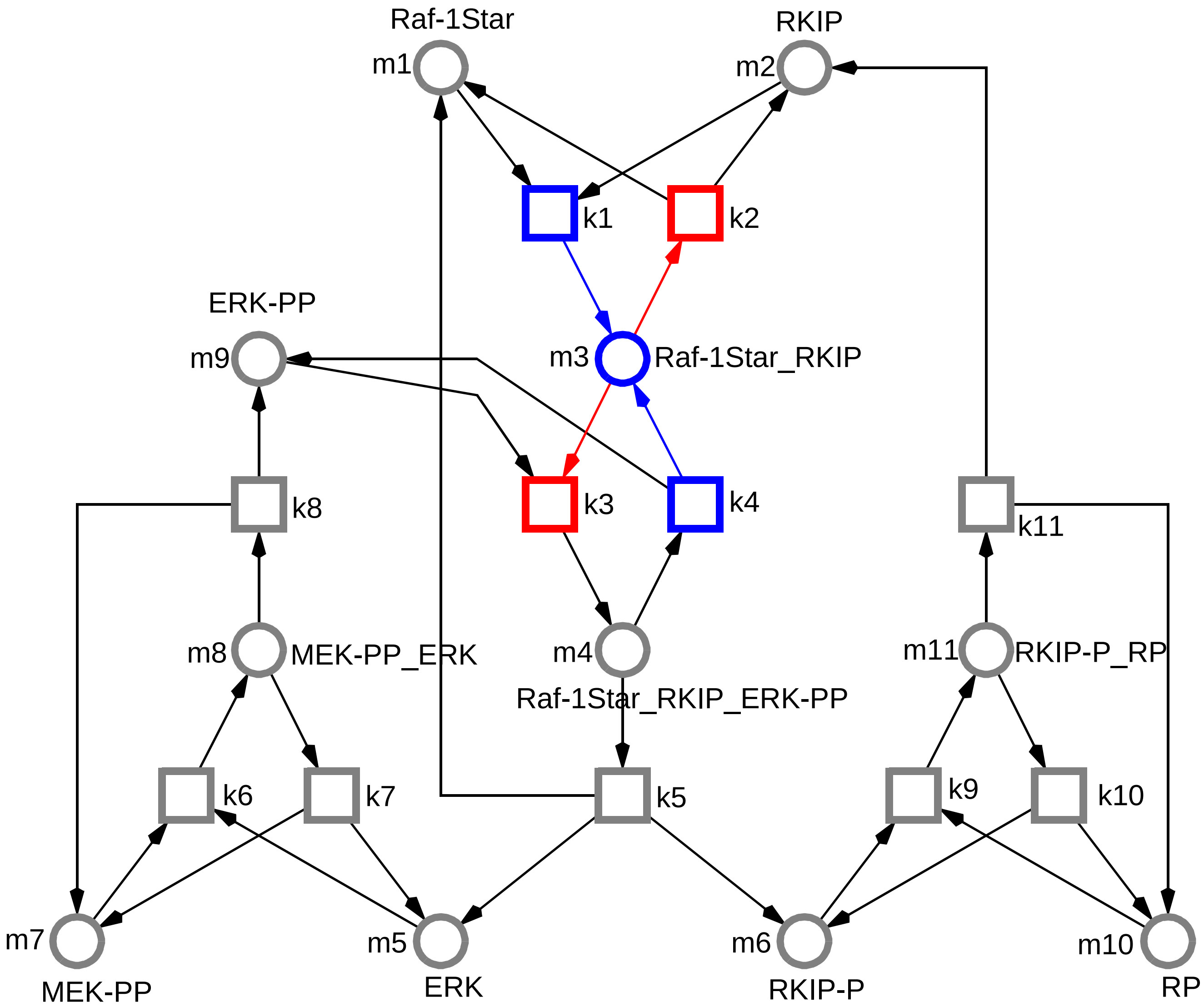
The RKIP Pathway, Continuous Petri Net

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

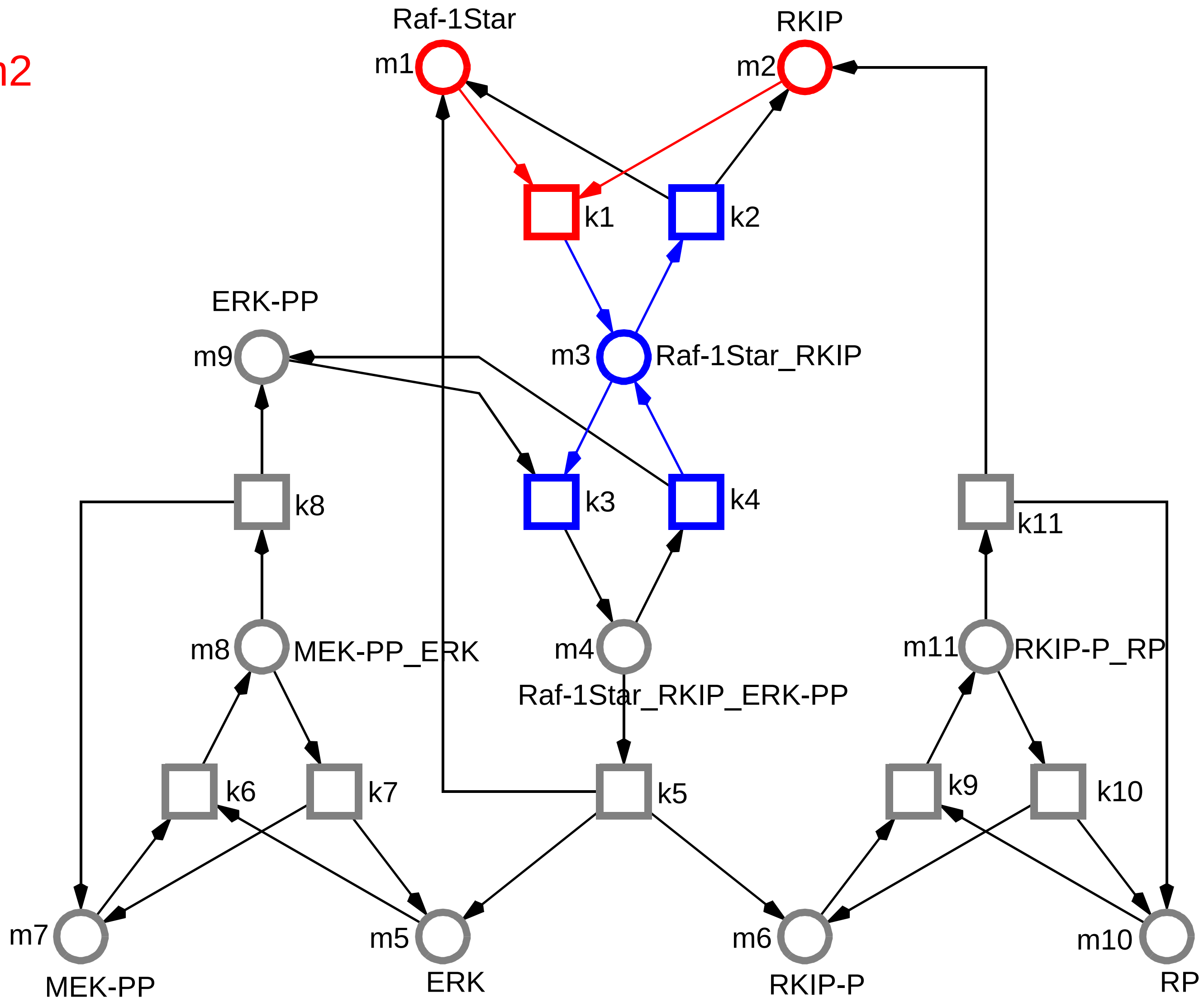


The RKIP Pathway, Continuous Petri Net

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

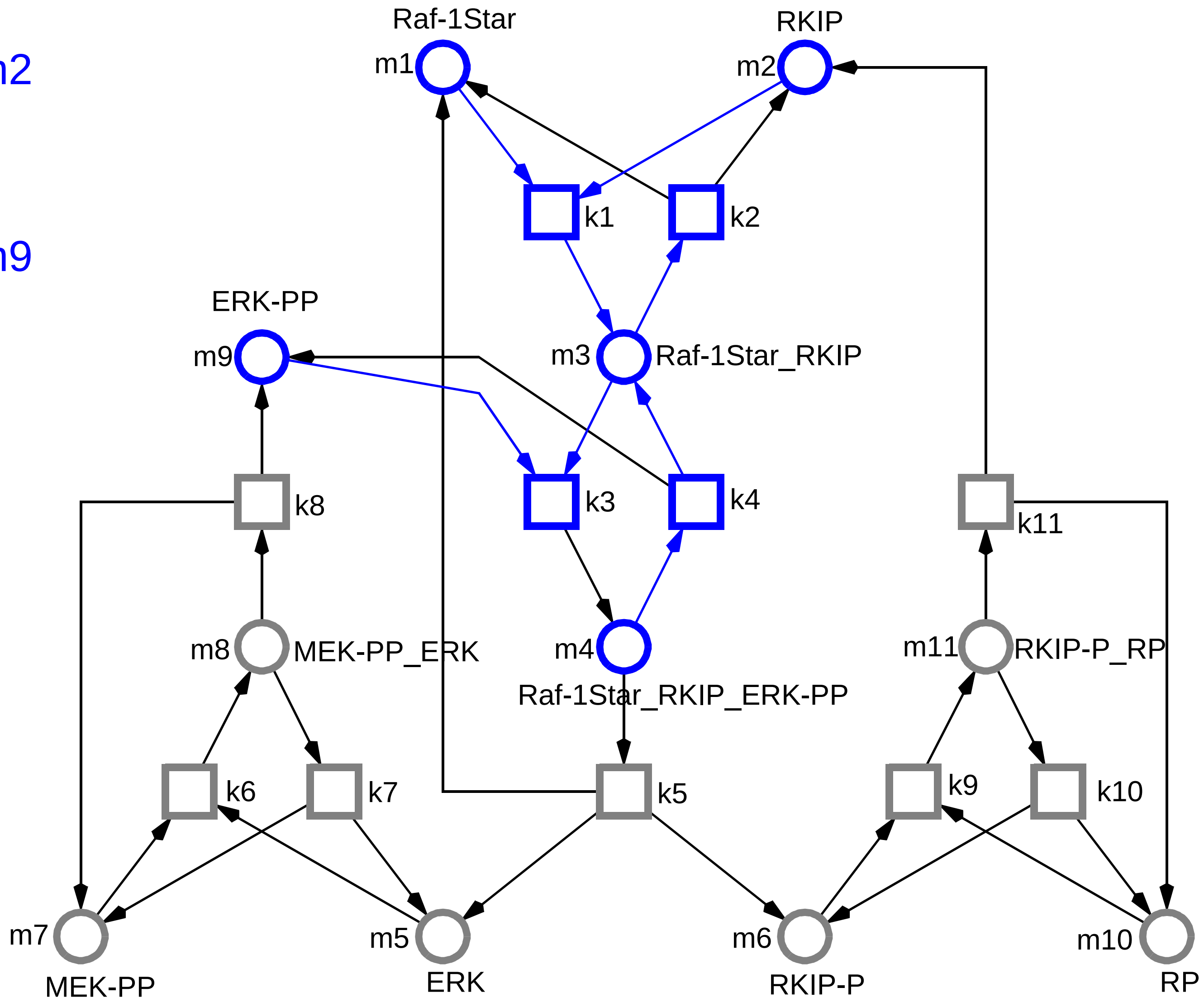


$$\frac{dm3}{dt} = + k1 * m1 * m2 + r4 - r2 - r3$$



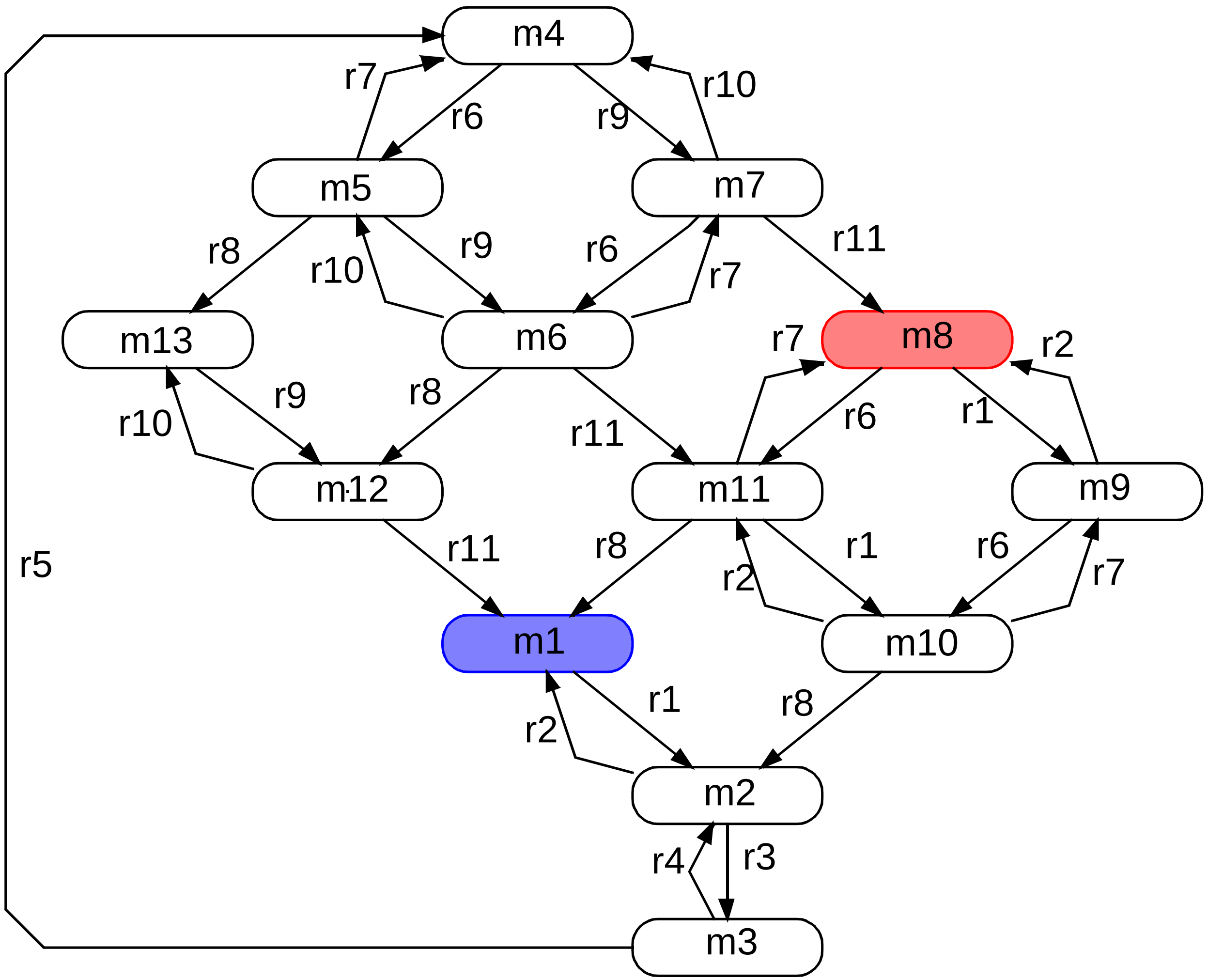
THE RKIP PATHWAY, CONTINUOUS PETRI NET

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



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

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

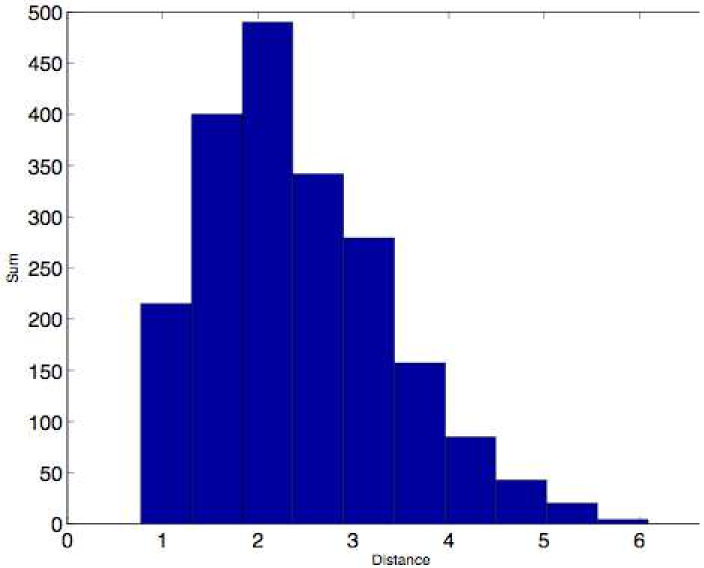


QUANTITATIVE ANALYSIS

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

Cho et al (S1)

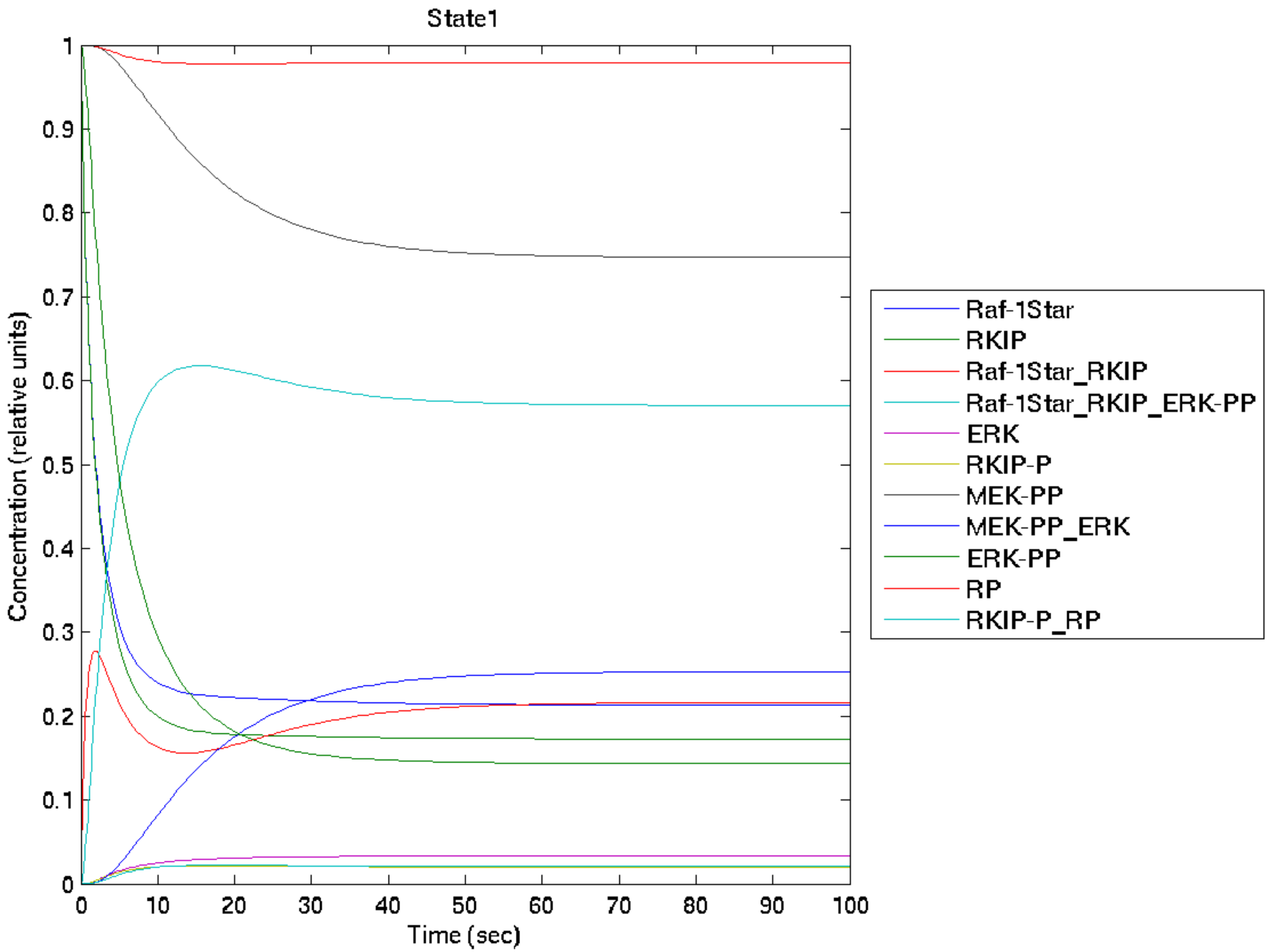
Biochemist (S8)

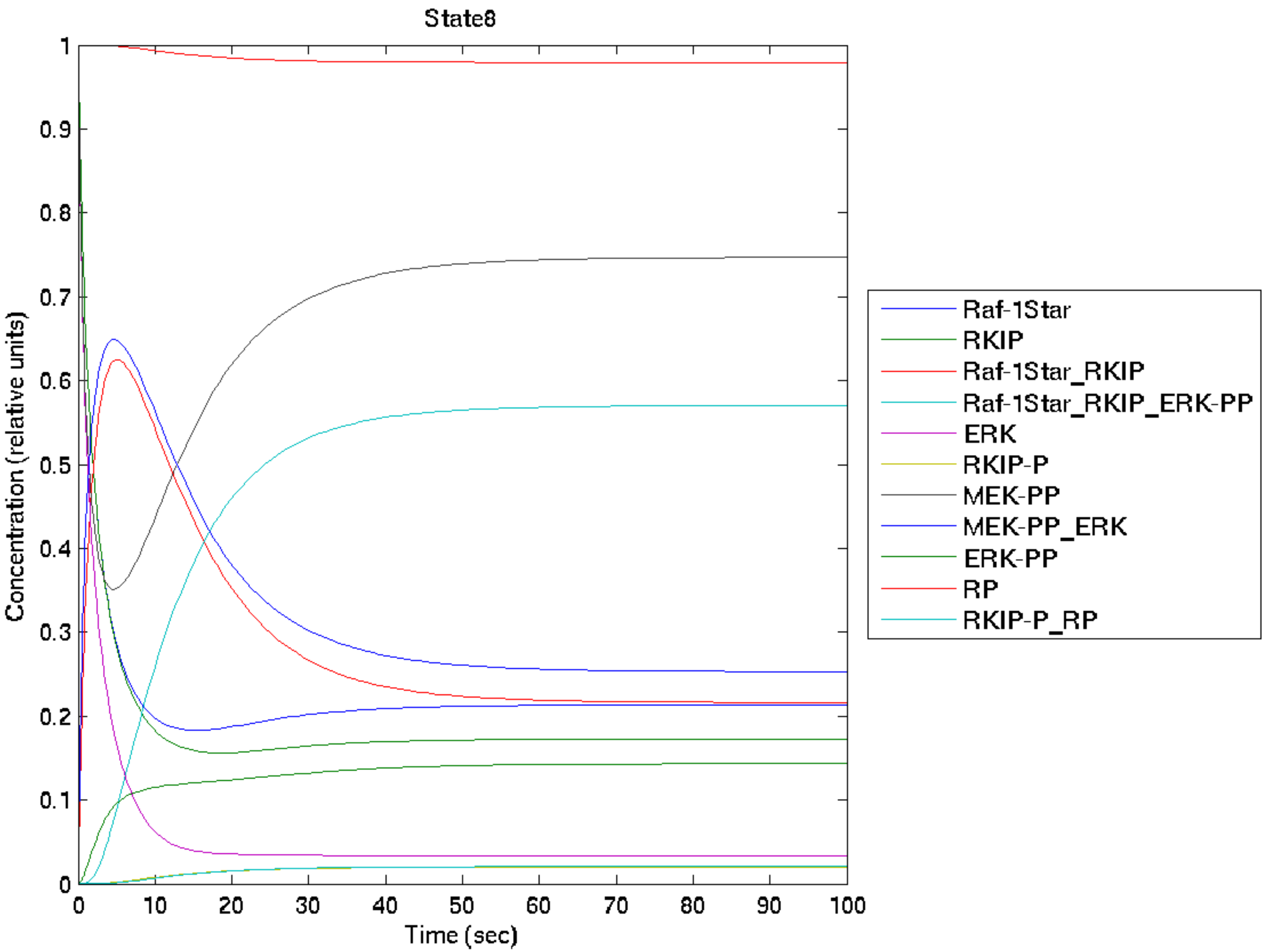


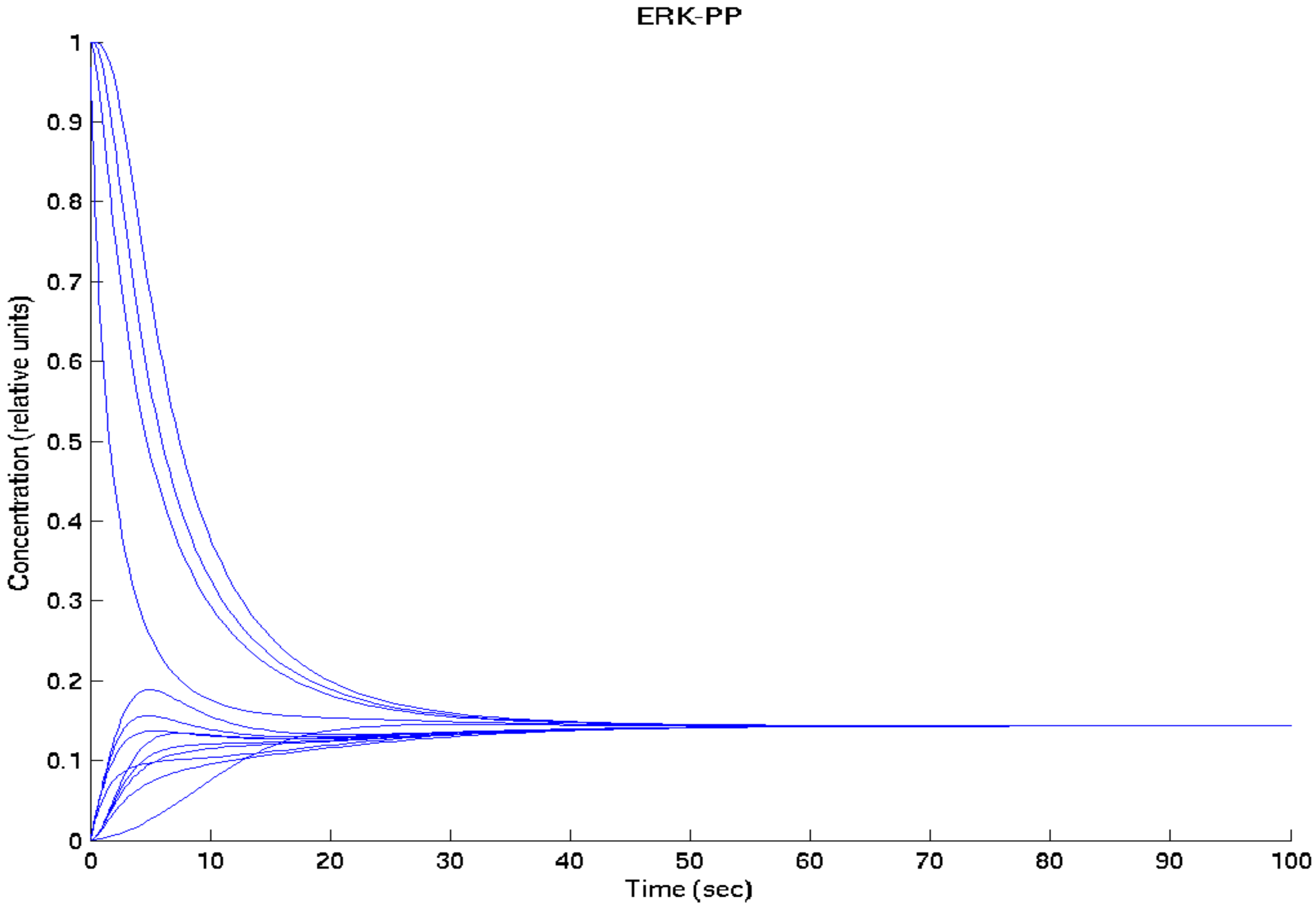
Distribution of `bad' steady states as euclidean distances from the `good' final steady state

13 “good” state configurations

the “bad” ones


State1
Concentration (relative units)
Time (sec)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60
70
80
90
100
Raf-1Star
RKIP
Raf-1Star_RKIP
Raf-1Star_RKIP_ERK-PP
ERK
RKIP-P
MEK-PP
MEK-PP_ERK
ERK-PP
RP
RKIP-P_RP


State8
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
Raf-1Star
RKIP
Raf-1Star_RKIP
Raf-1Star_RKIP_ERK-PP
ERK
RKIP-P
MEK-PP
MEK-PP_ERK
ERK-PP
RP
RKIP-P_RP



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

Case Study, Summary

- **representation of bionetworks by Petri nets**
  - -> *partial order representation* -> *better comprehension*
  - -> *formal semantics* -> *sound analysis techniques*
  - -> *unifying view*
- **purposes**
  - -> *animation* -> *to experience the model*
  - -> *model validation against consistency criteria* -> *to increase confidence*
  - -> *qualitative / quantitative behaviour prediction* -> *new insights*
- **two-step model development**
  - -> *qualitative model* -> *discrete Petri nets*
  - -> *quantitative model* -> *continuous Petri nets = ODEs*