From Petri Nets to Differential Equations
An Integrative Approach for Biochemical Network Analysis

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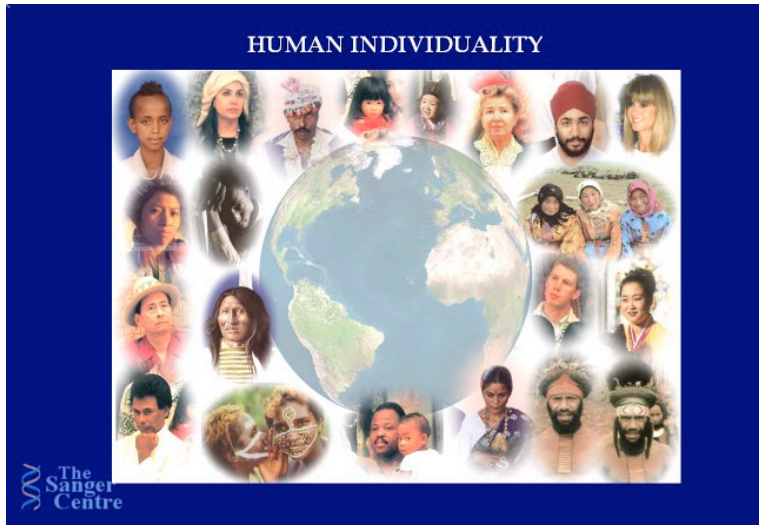
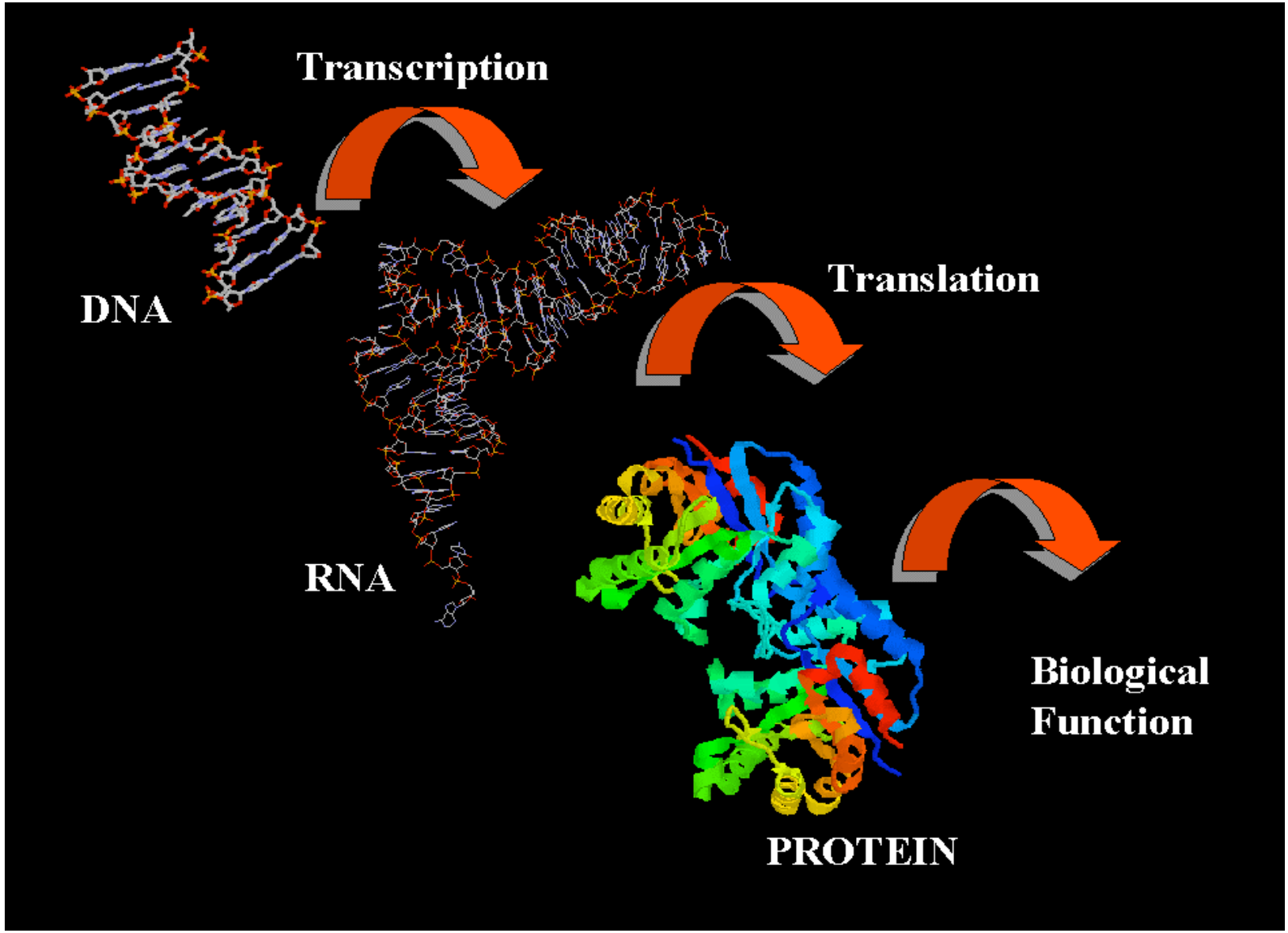
Long-term vision

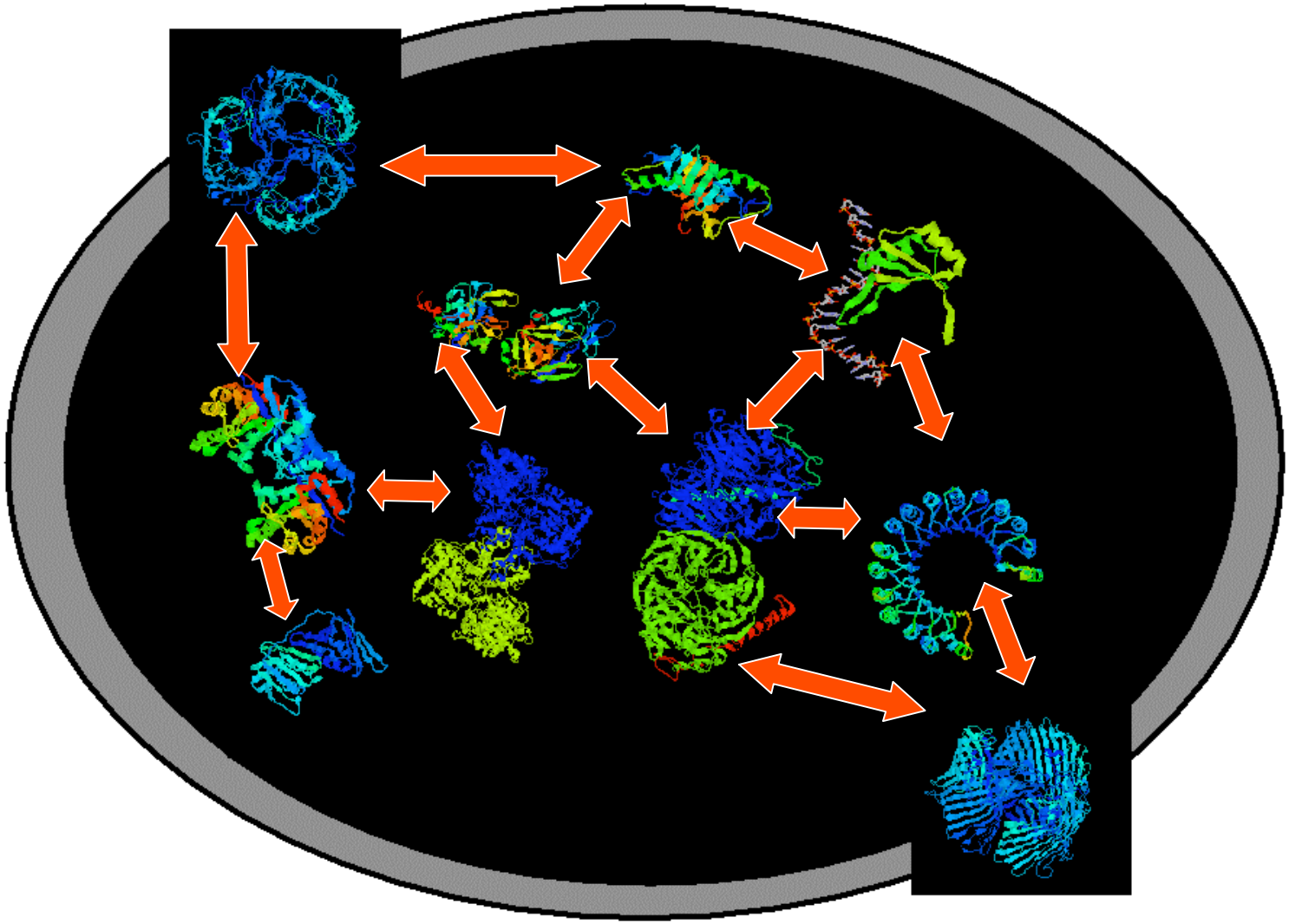


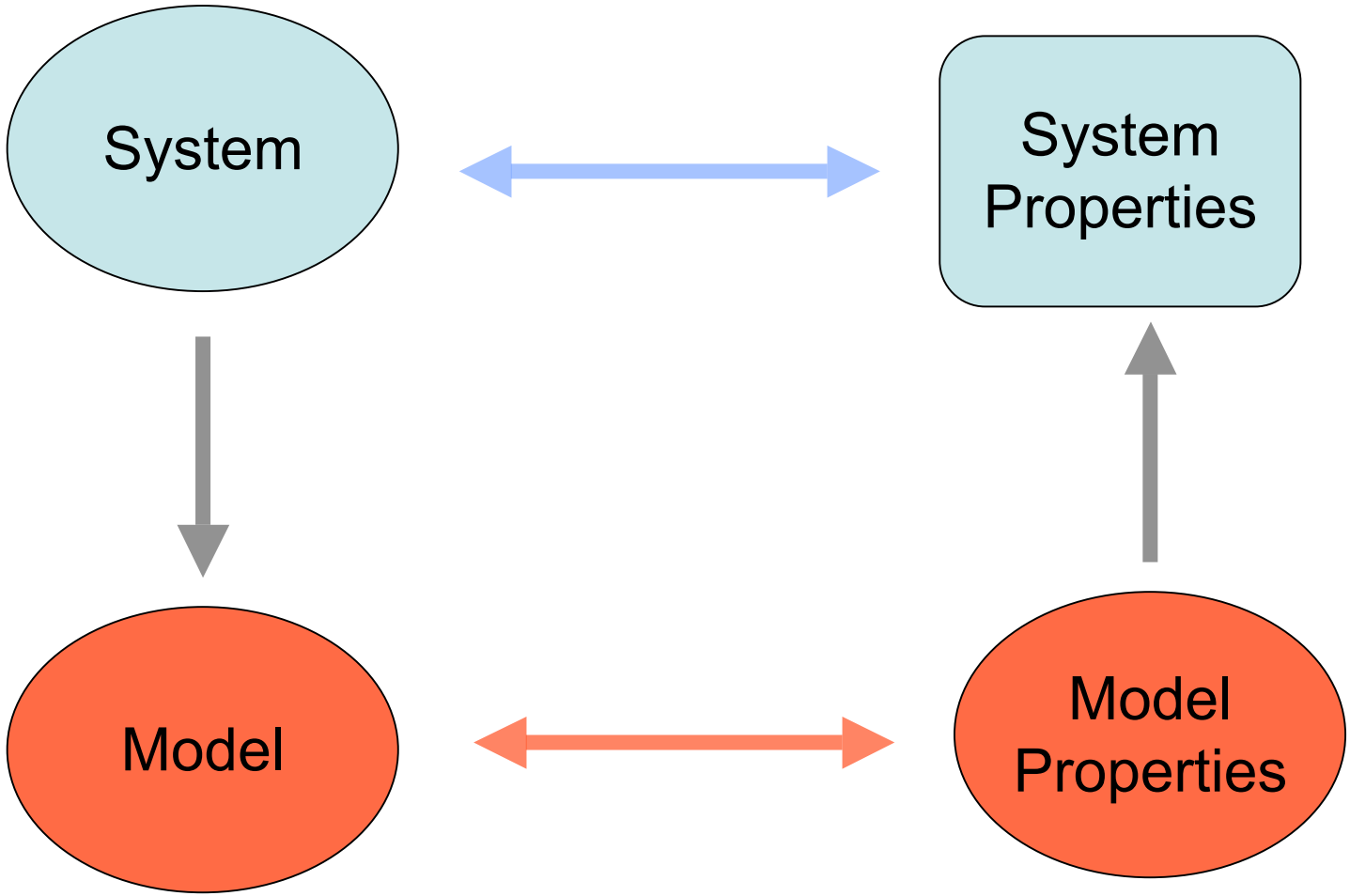
- Current trial-and-error drug prescription procedure
  - "adjust" an individual to a given drug by testing reaction over several weeks
- Vision: model-based drug prescription as part of medical patient treatment by a physician:
  - what and how much is necessary to substitute a given underfunction/malfunction
- Drug discovery process:
  - *in-silico* ***quantitative*** modelling before major development of drug

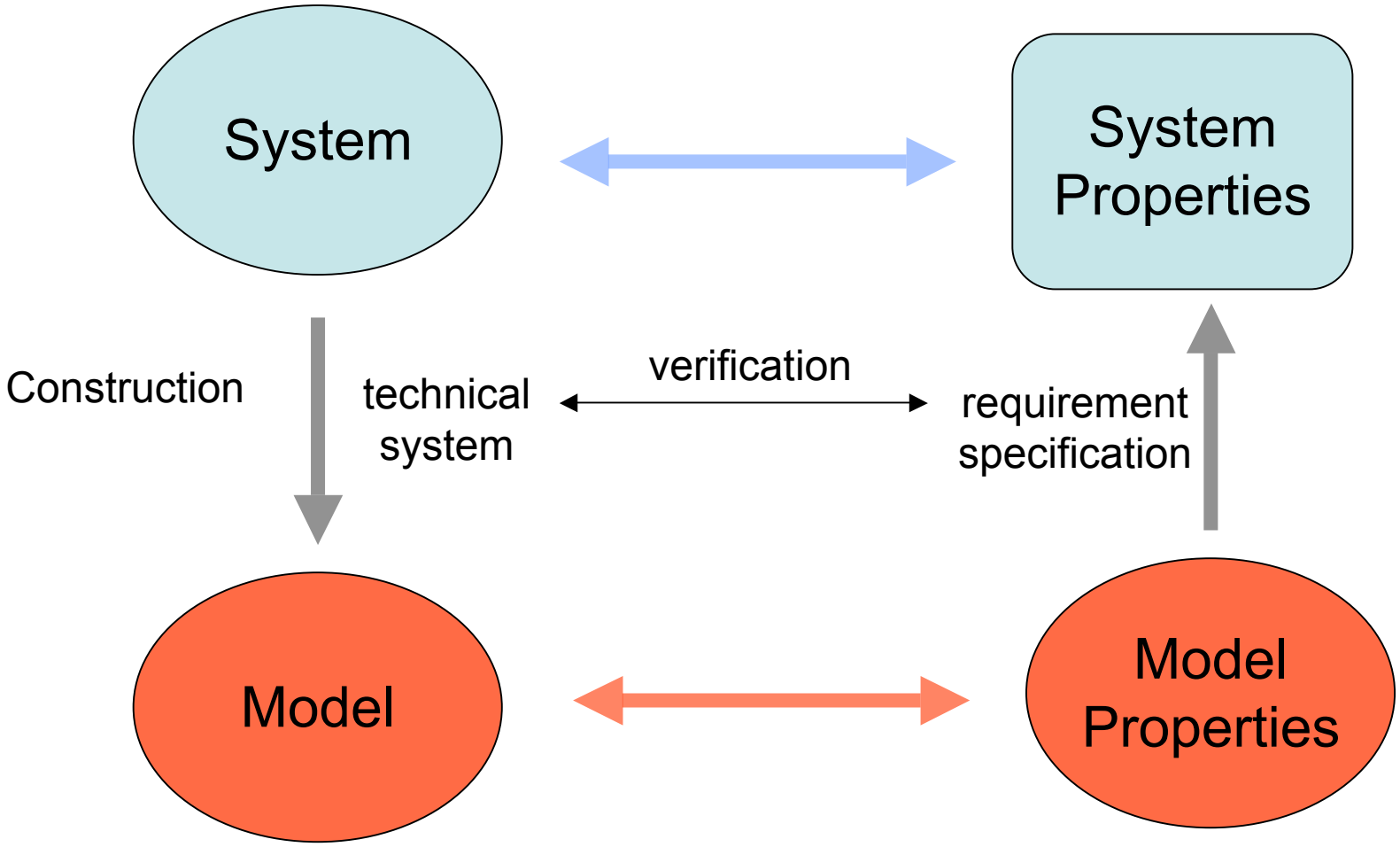
Biological function?

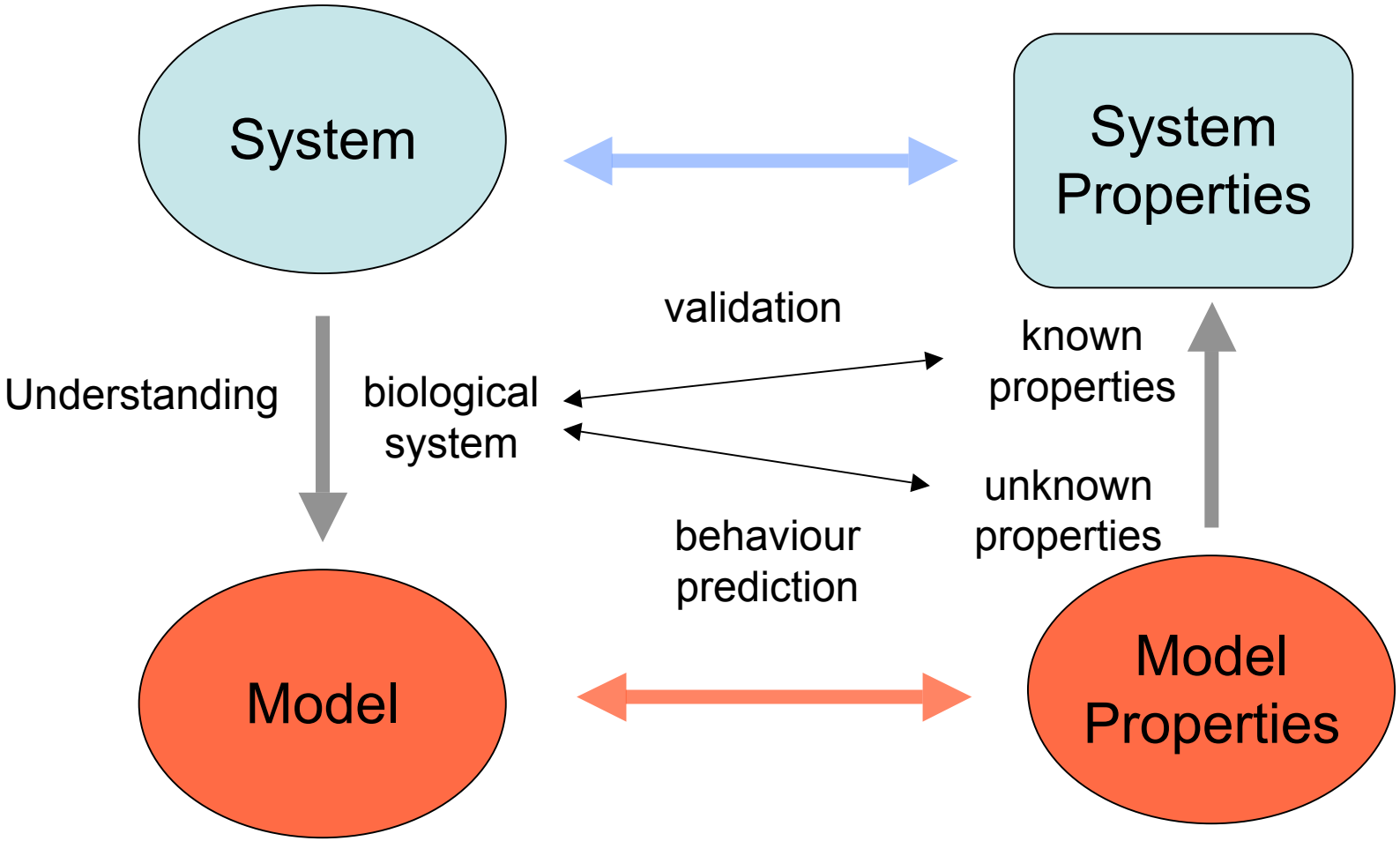


… by interaction in *networks*



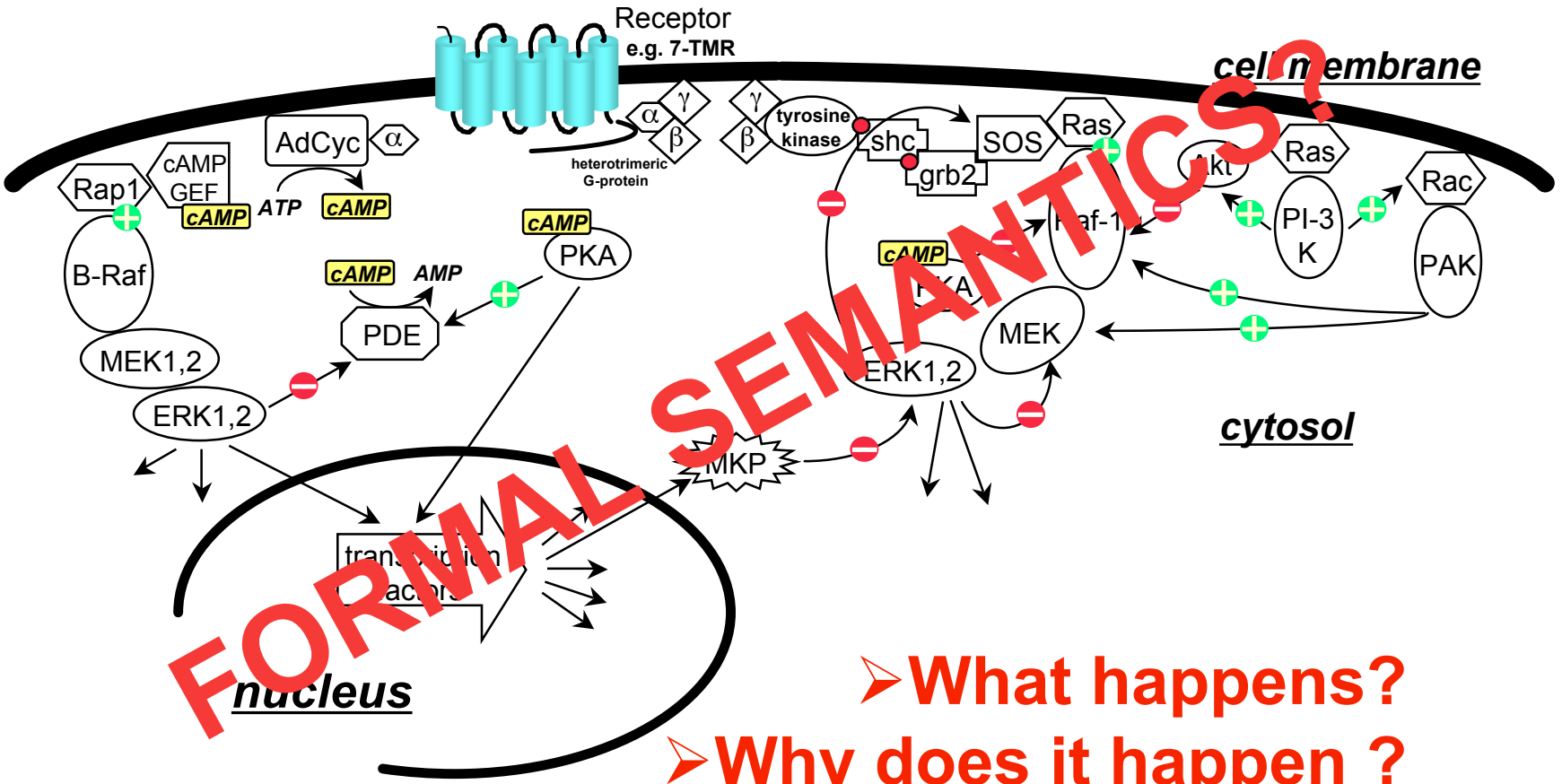

System
System Properties
Model
Model Properties


System
System Properties
Construction
technical system
verification
requirement specification
Model
Model Properties


System
System Properties
validation
Understanding
biological system
known properties
unknown properties
behaviour prediction
Model
Model Properties

Biochemical networks

We can describe the general topology and single biochemical steps.
However, we do not understand the network function as a whole.



- What happens?
- Why does it happen ?
- How is specificity achieved?

$$\frac{d\alpha}{dt} = -v_1$$

$$\frac{dSte2}{dt} = -v_2 + v_3 - v_5$$

$$\frac{dSte2_{active}}{dt} = v_2 - v_3 - v_4$$

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

$$\frac{dG\alpha\beta\gamma}{dt} = -v_6 + v_9$$

$$\frac{dG\alpha GTP}{dt} = v_6 - v_7 - v_8$$

$$\frac{dG\alpha GDP}{dt} = v_7 + v_8 - v_9$$

$$\frac{dG\beta\gamma}{dt} = v_6 - v_9 - v_{10} + v_{11} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32} - v_{42} + v_{43}$$

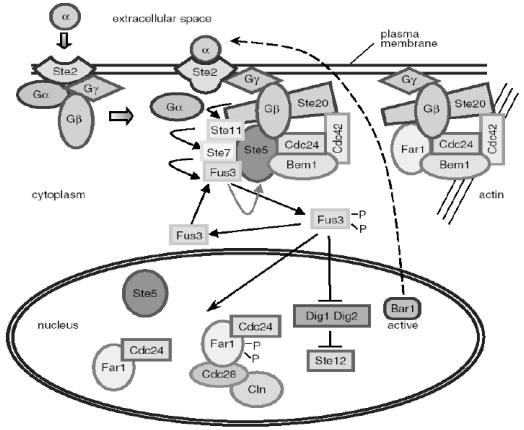
$$\frac{dSte5}{dt} = -v_{12} + v_{13} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32}$$

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

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

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

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



$$v_1 = \alpha[t] \cdot Bar1_{active}[t] \cdot k_1$$

$$v_2 = Ste2[t] \cdot \alpha[t] \cdot k_2$$

$$v_3 = Ste2_{active}[t] \cdot k_3$$

$$v_4 = Ste2_{active}[t] \cdot k_4$$

$$v_5 = Ste2[t] \cdot k_5$$

$$v_6 = Ste2_{active}[t] \cdot G\alpha\beta\gamma[t] \cdot k_6$$

$$v_7 = G\alpha GTP[t] \cdot k_7$$

$$v_8 = G\alpha GTP[t] \cdot Sst2_{active}[t] \cdot k_8$$

$$v_9 = G\alpha GDP[t] \cdot G\beta\gamma[t] \cdot k_9$$

$$v_{10} = G\beta\gamma[t] \cdot C[t] \cdot k_{10}$$

$$v_{11} = D[t] \cdot k_{11}$$

$$v_{12} = Ste5[t] \cdot Ste11[t] \cdot k_{12}$$

$$v_{13} = A[t] \cdot k_{13}$$

$$v_{14} = Ste7[t] \cdot Fus3[t] \cdot k_{14}$$

$$v_{15} = B[t] \cdot k_{15}$$

$$v_{16} = A[t] \cdot B[t] \cdot k_{16}$$

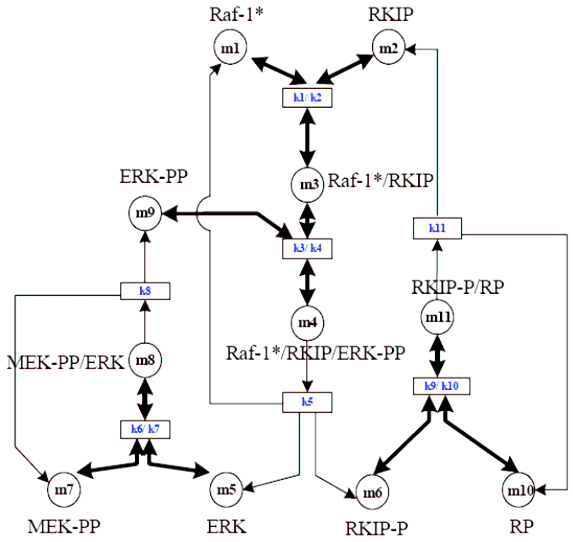
$$v_{17} = C[t] \cdot k_{17}$$

$$v_{18} = D[t] \cdot Ste20[t] \cdot k_{18}$$

READABILITY?

What is a biochemical network model?

1. Structure



graph
QUALITATIVE

2. Kinetics (if you can)

$d[Raf1^*]/dt = k1*m1*m2 + k2*m3 + k5*m4$

$k1 = 0.53;\ k2 = 0.0072;\ k5 = 0.0315$

reaction rates
QUANTITATIVE

3. Initial conditions

$[Raf1^*]_{t=0} = 2\ \mu Molar$

marking , concentrations

Bionetworks: some problems

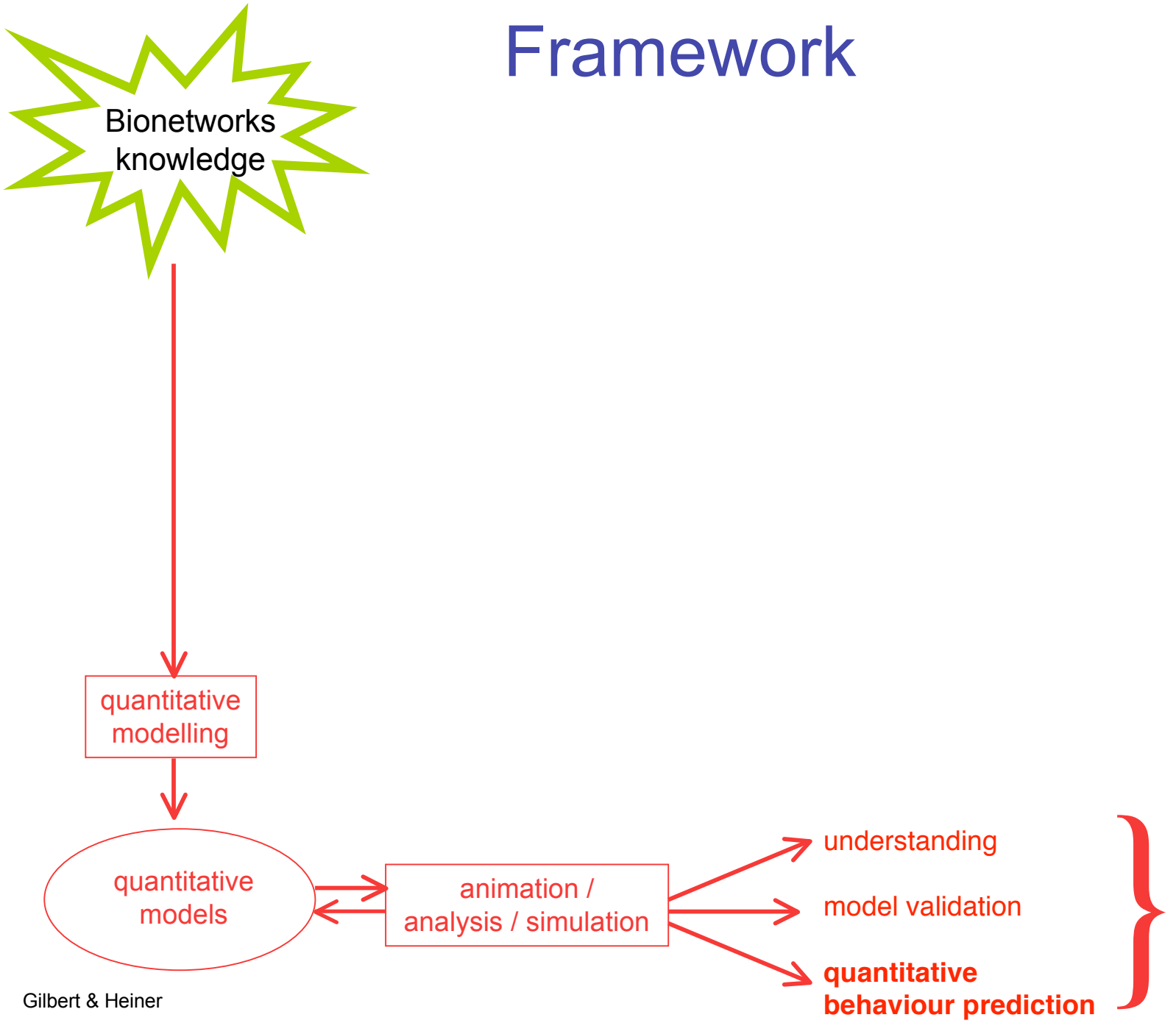
- knowledge → **PROBLEM 1**
  - ⇒ uncertain
  - ⇒ growing, changing
  - ⇒ time-consuming wet-lab experiments
  - ⇒ some data estimated
  - ⇒ results distributed over independent data bases, papers, journals, . . .

- various, mostly ambiguous representations → **PROBLEM 2**
  - ⇒ verbose descriptions
  - ⇒ diverse graphical representations
  - ⇒ contradictory and / or fuzzy statements

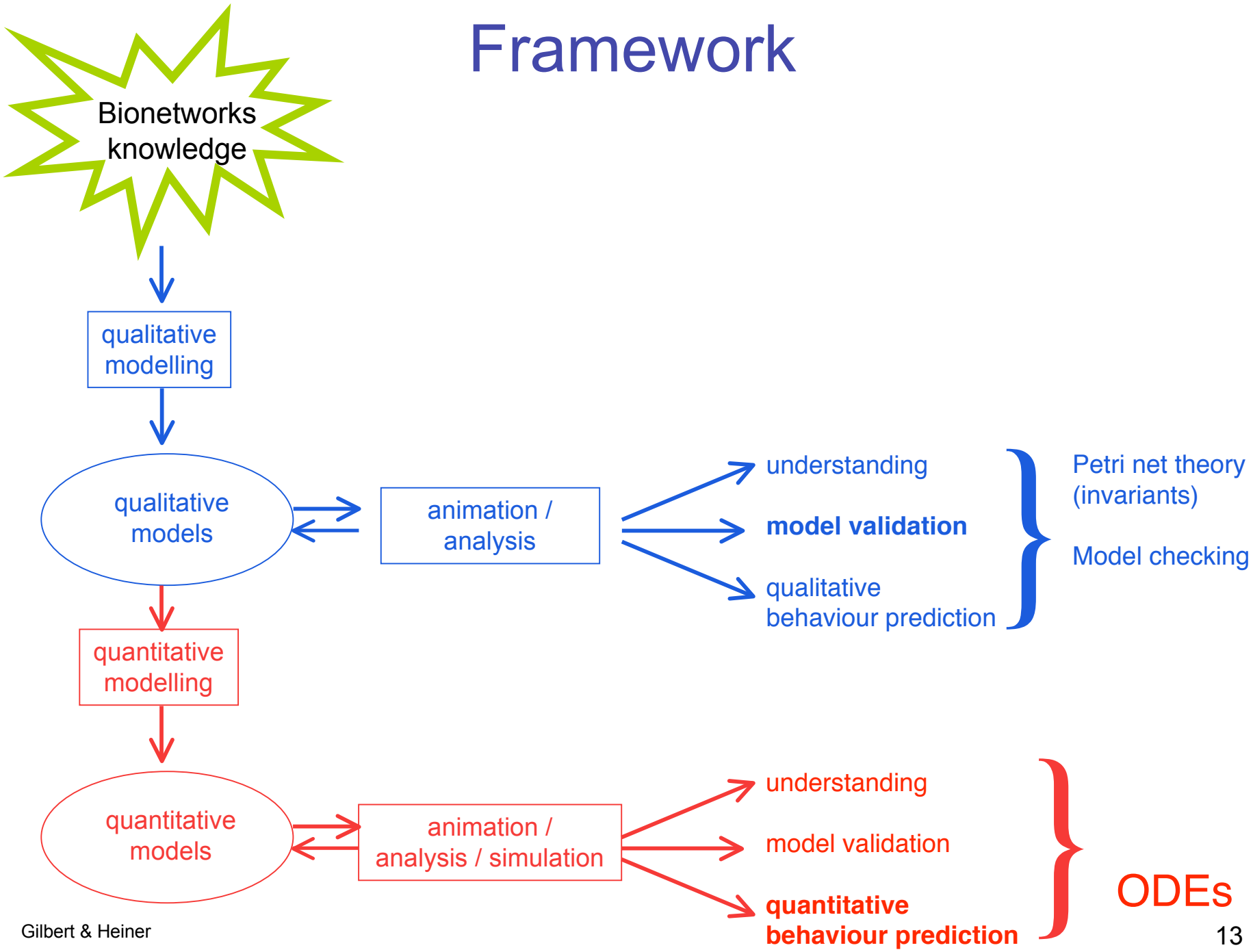
- network structure → **PROBLEM 3**
  - ⇒ tend to grow fast
  - ⇒ dense, apparently unstructured
  - ⇒ hard to read

⇒ models are full of ASSUMPTIONS ⇐

Framework


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

Framework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qualitative behaviour prediction
Petri net theory (invariants)
Model checking
quantitative modelling
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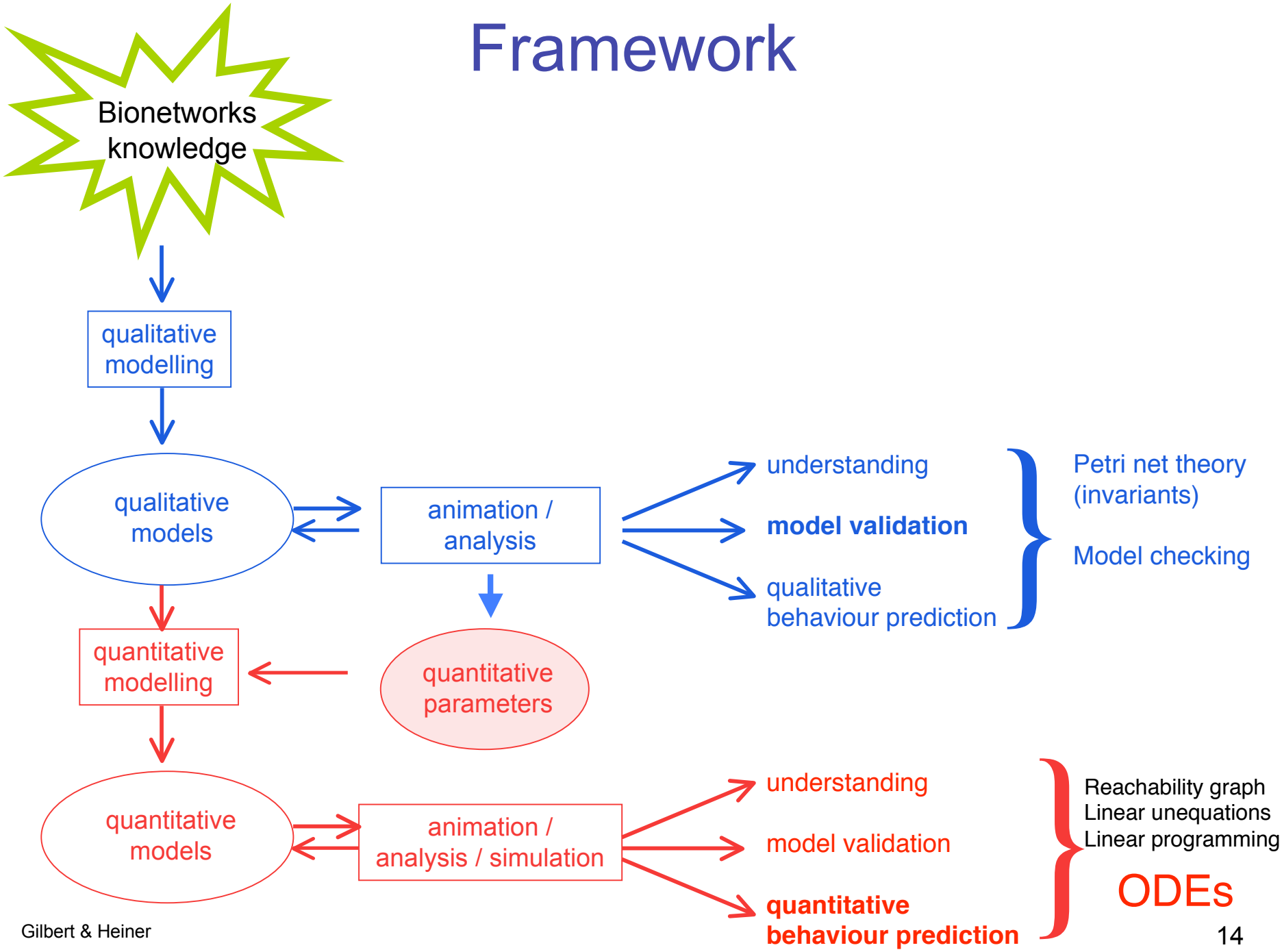
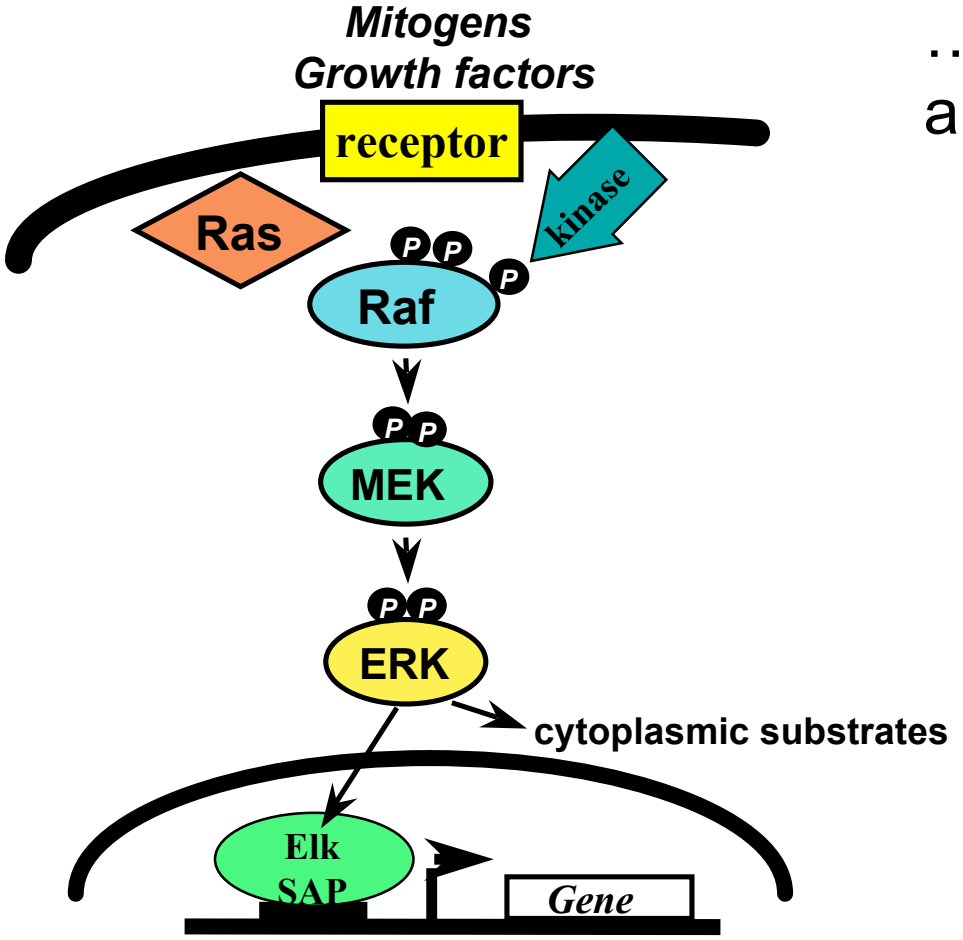
ODEs

Framework


Bionetworks knowledge
qualitative modelling
qualitative models
animation / analysis
understanding
model validation
qualitative behaviour prediction
Petri net theory (invariants)
Model checking
quantitative modelling
quantitative parameters
quantitative models
animation / analysis / simulation
understanding
model validation
quantitative behaviour prediction
Reachability graph
Linear unequations
Linear programming
ODEs

Ras-Raf-MEK-ERK signalling pathway

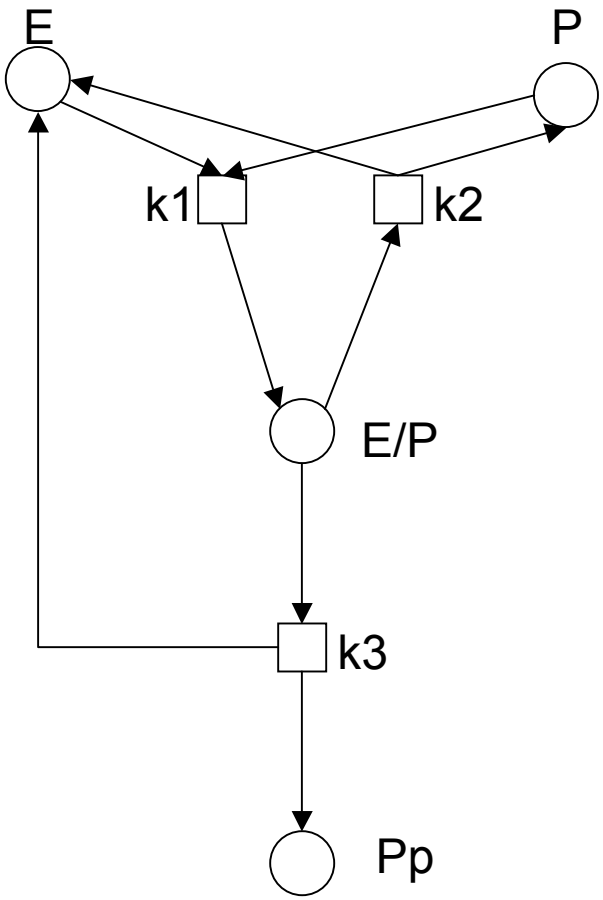
…one pathway…

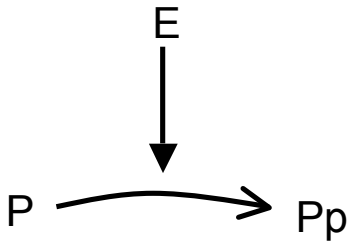


…mediates many functions, and hence diseases…

- Proliferation
- Cell cycle
- Differentiation
- Transformation
- Survival
- Cytoskeleton
- Adhesion
- Motility
- etc.

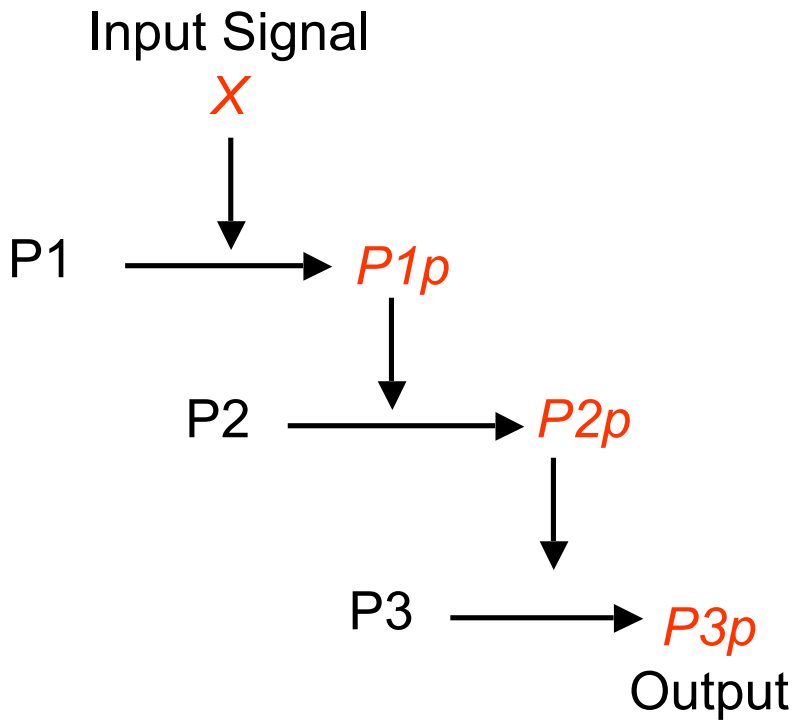
Network building block - enzymatic reaction





$$E + P \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E \mid P \xrightarrow{k_3} E + Pp$$

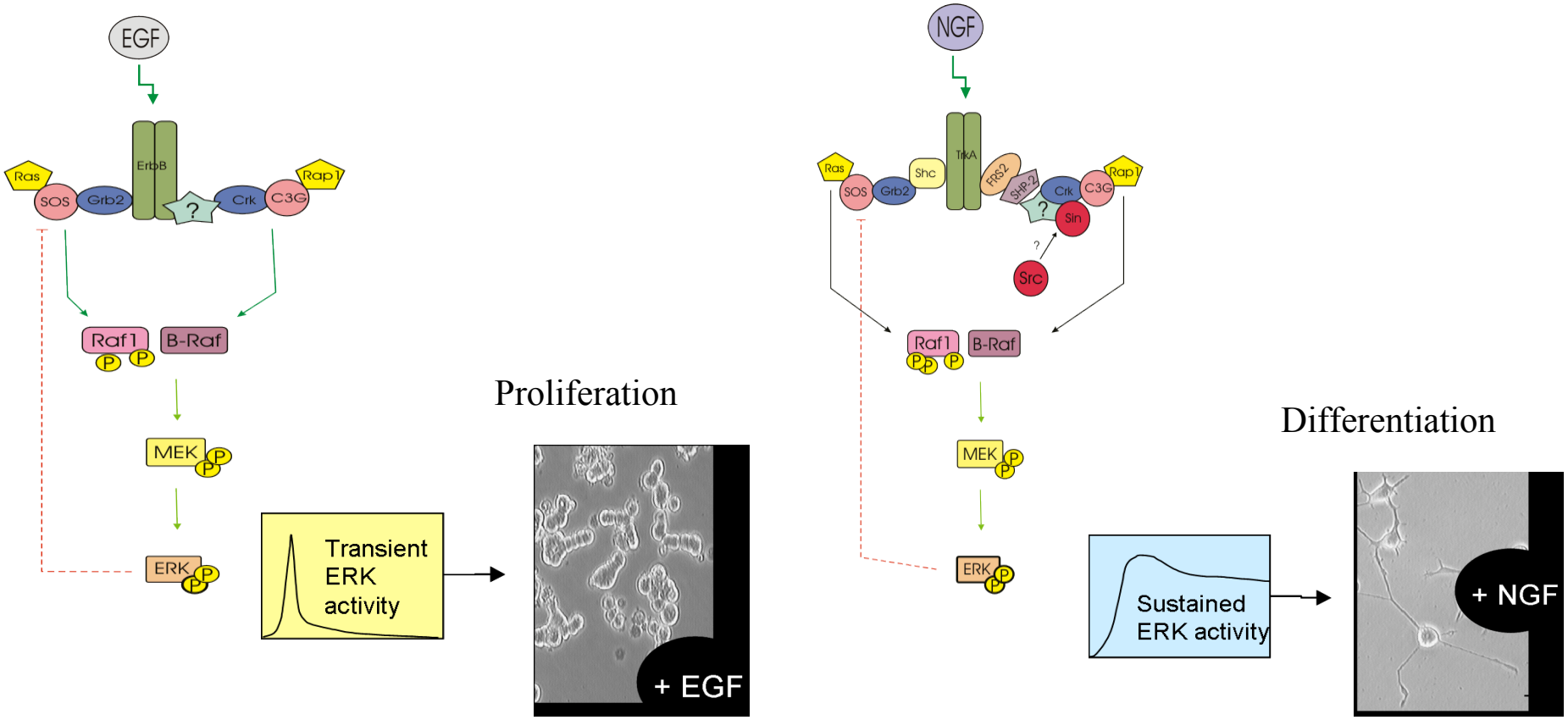
Signalling cascade of enzymatic reactions



Product become enzyme at next stage

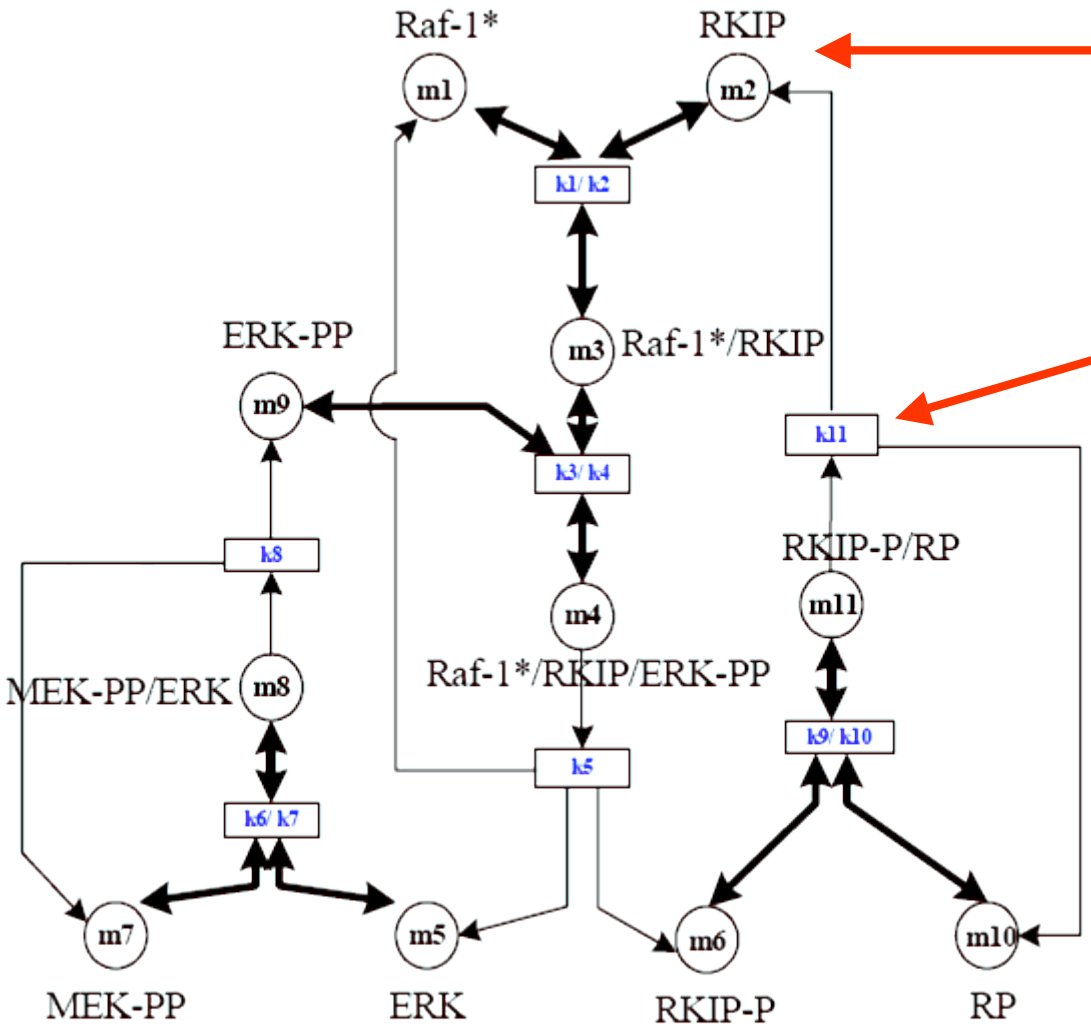
Continuous system & model

Effect of addition of EGF vs NGF



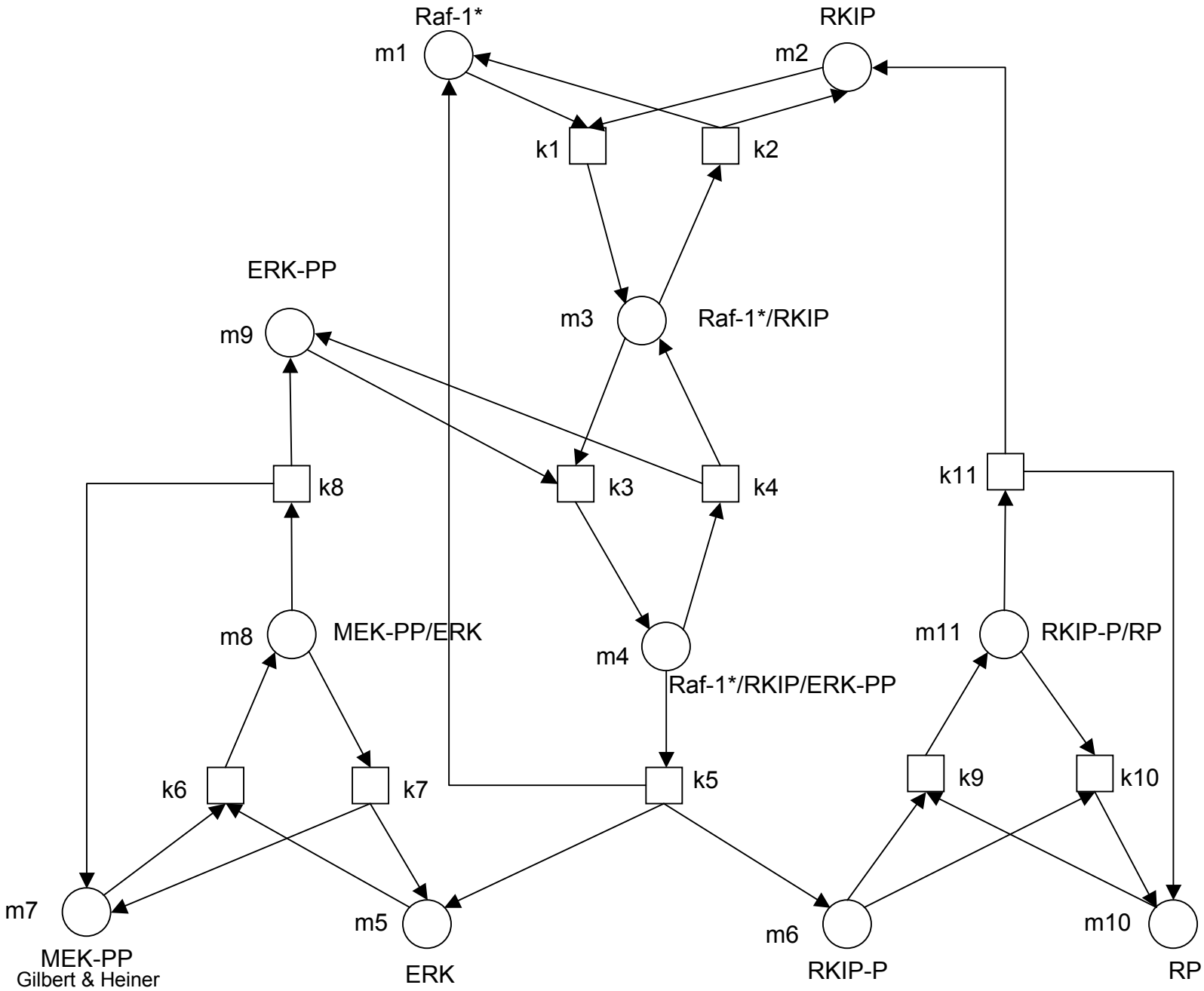
Case study: small model network
RKIP inhibited ERK pathway

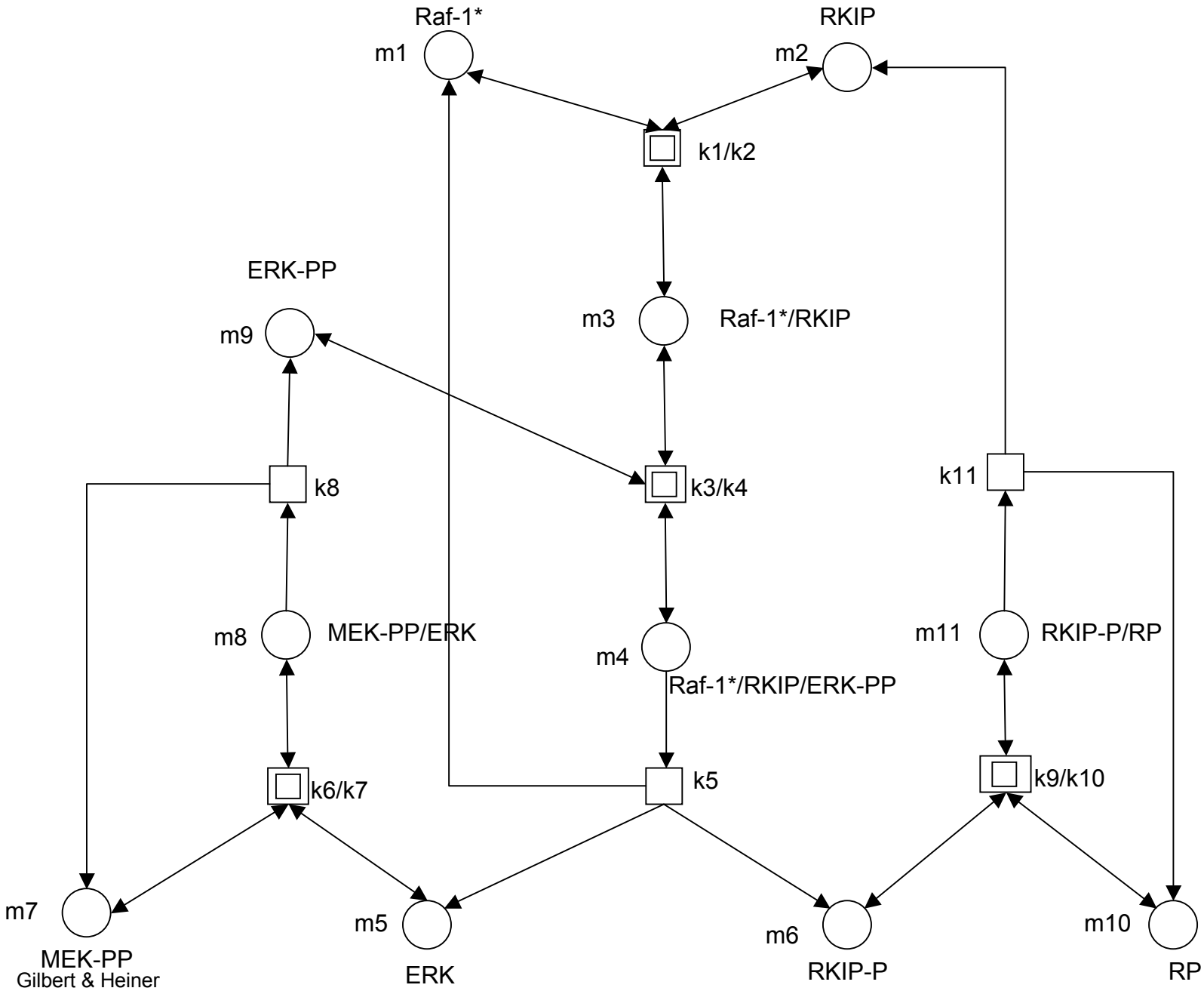
Cho et al, CMSB03



Protein
(concentration)

Reaction
+ rate


Raf-1*
m1
RKIP
m2
k1
k2
ERK-PP
m9
m3
Raf-1*/RKIP
k8
k3
k4
k11
m8
MEK-PP/ERK
m4
Raf-1*/RKIP/ERK-PP
m11
RKIP-P/RP
k6
k7
k5
k9
k10
m7
MEK-PP
Gilbert & Heiner
m5
ERK
m6
RKIP-P
m10
RP


Raf-1*
m1
RKIP
m2
k1/k2
ERK-PP
m9
m3
Raf-1*/RKIP
k8
k3/k4
k11
m8
MEK-PP/ERK
m4
Raf-1*/RKIP/ERK-PP
m11
RKIP-P/RP
k6/k7
k5
k9/k10
m7
MEK-PP
Gilbert & Heiner
m5
ERK
m6
RKIP-P
m10
RP

Step 1 - Qualitative analysis

- Structural properties

Tool supported

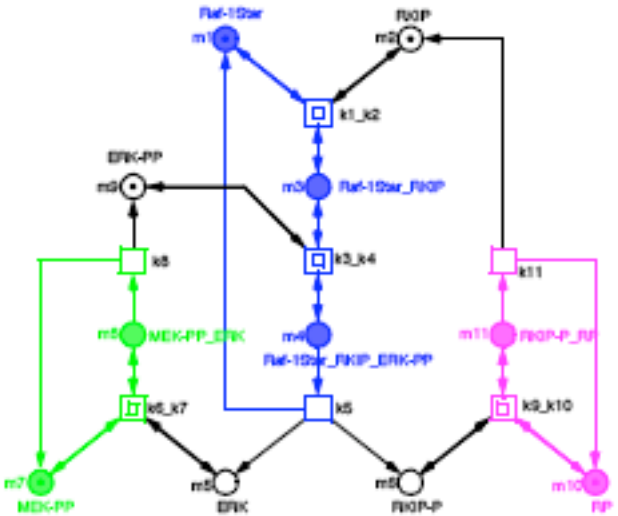
- General behavioural properties:
  - Boundedness
  - Liveness
  - Reversability
- Place & transition invariants:
  - CTI
  - CPI
- Model checking of temporal-logic properties:
  - special behavioural properties

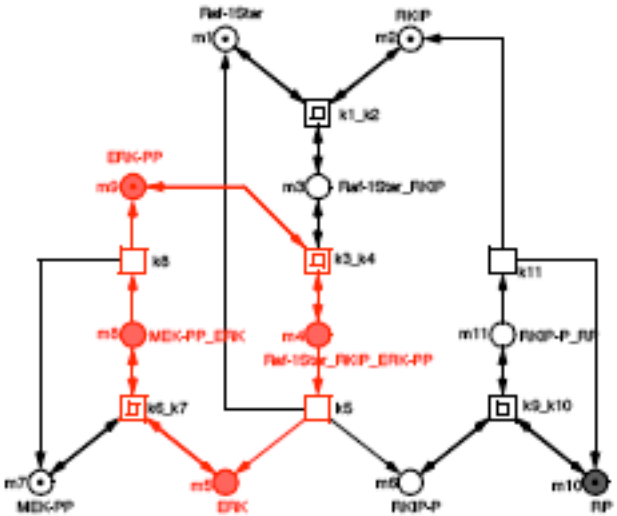
Systematic construction of the minimal marking

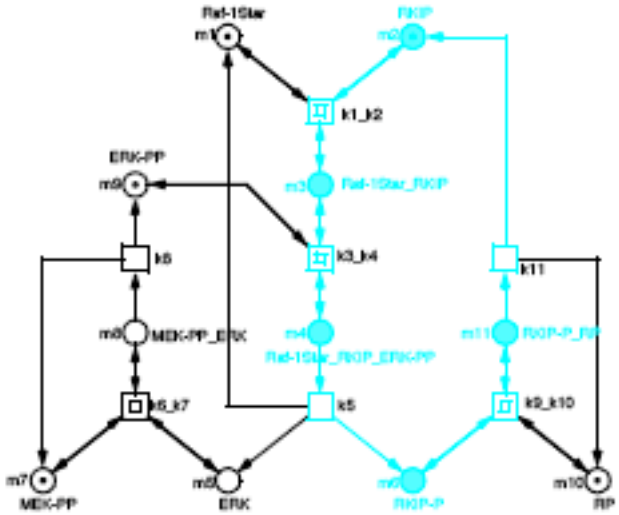
- Each P-invariant gets at least one token
- In signal transduction:
  - exactly 1 token, corresponding to species conservation
  - token in least active state
- All (non-trivial) T-invariants become realizable
  - to make the net live
- Minimal marking
  - minimization of the state space

$\Rightarrow$ UNIQUE INITIAL MARKING $\Leftarrow$

P-invariants







P-INV1: MEK

P-INV2: RAF-1STAR

P-INV3: RP

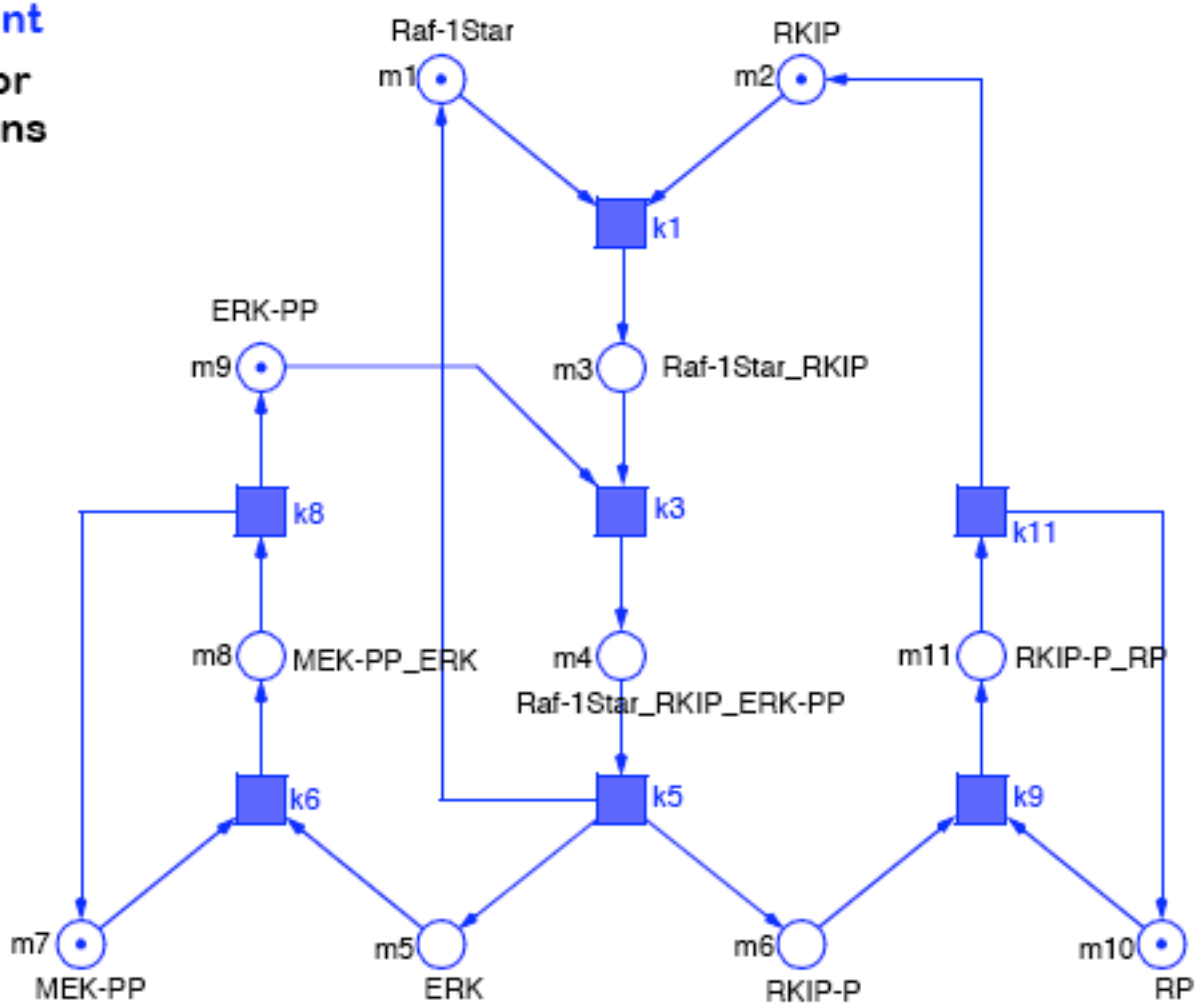
P-INV4: ERK

P-INV5: RKIP

T-invariants

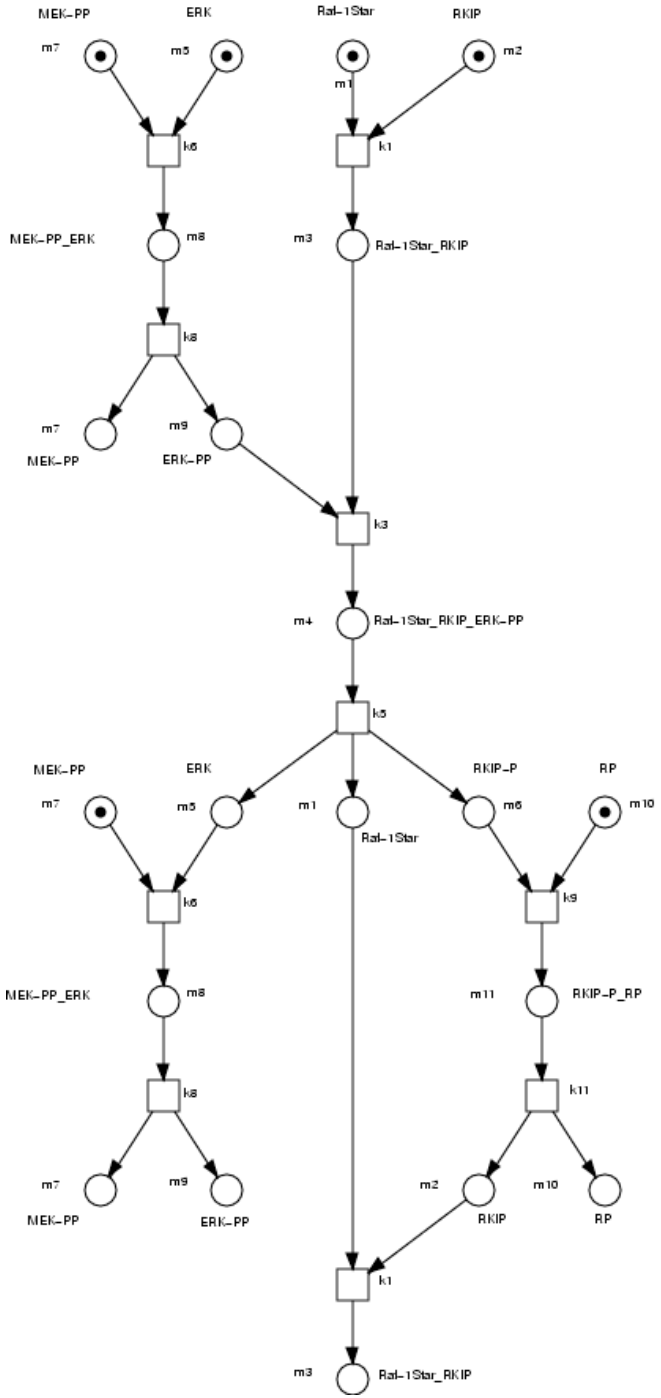
-> non-trivial T-invariant

+ four trivial ones for reversible reactions



Partial order run of the non-trivial T-invariant

- Partial order structure
- Illustrates *causal* & *concurrent* behaviour
- Labelled condition/event net
  - Events - transition occurrences
  - Conditions: input/output compounds



CTL queries

1. There are reachable states where ERK is phosphorylated and RKIP is not phosphorylated.

   EF [(ERK-PP ∨ RAF1*/RKIP/ERK-PP) ∧ RKIP]

2. The phosphorylation of ERK does not depend on the phosphorylation of RKIP.

   EG [ERK → **E** (¬RKIP-P ∨ RKIP-P/RP) U ERK-PP]

3. A cyclic behaviour w.r.t. RKIP is possible forever.

   AG [(RKIP → **EF** (¬RKIP)) ∧ (¬RKIP → **EF** (RKIP))]

Qualitative analysis - summary

- Validation criterion 0
  - all expected structural properties hold
  - all expected general behavioural properties hold
- Validation criterion 1
  - CTI
  - no minimal T-invariant without biological interpretation
  - no known biological behaviour without corresponding T-invariant
- Validation criterion 2
  - CPI
  - no minimal P-invariant without biological interpretation
- Validation criterion 3
  - all expected special behavioural properties expressed by temporal logic formulae hold

Step 2: quantitative analysis

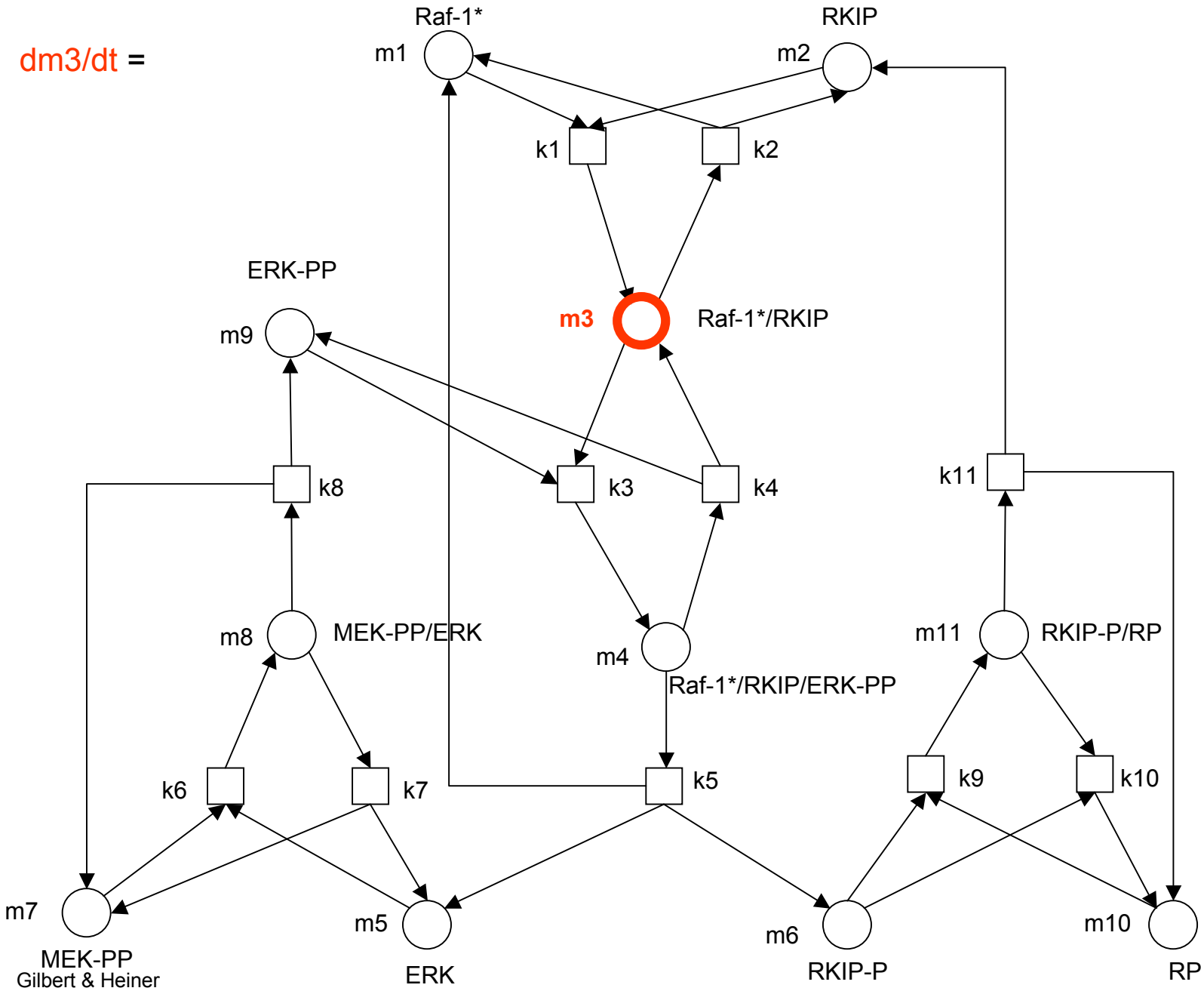
- Derive differential equations using graph structure & rate constants
- Add (13 alternative) initial conditions derived via qualitative analysis
- Compute steady-states to show “equivalence” of alternatives

From (time-less) discrete to (timed) continuous Petri nets

- Keep the structure
- Add rate function to each transition
  - Pre-places appear as parameters → state dependent
  - Function defines the *continuous* flow
- Each place gets 1 token (real number)
  - Represents current *continuous* concentration
- Continuous state space

⇒ Continuous Pn defines a system of ODEs ⇐

dm3/dt =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RKIP
m1
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m11
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k6
k7
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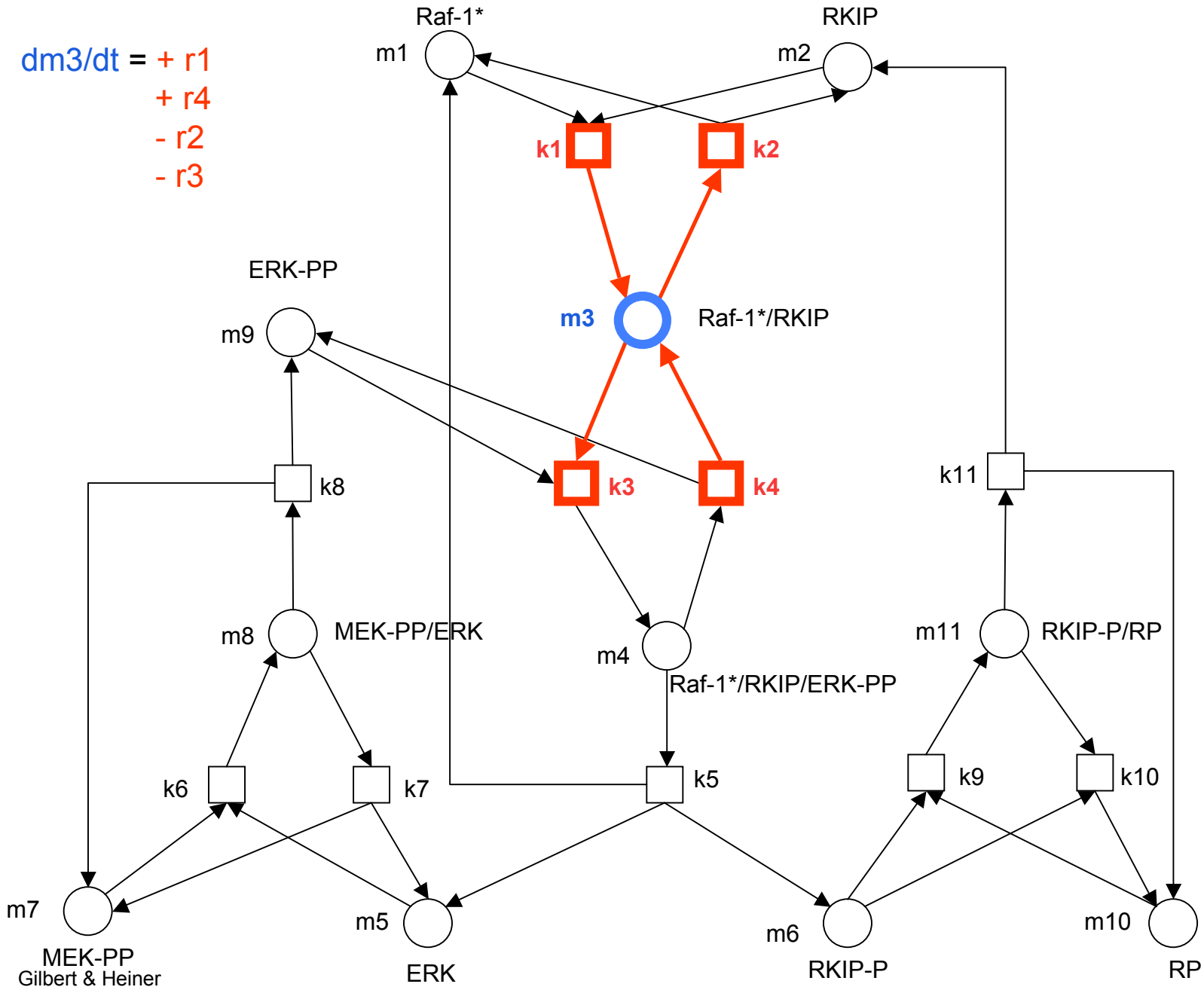
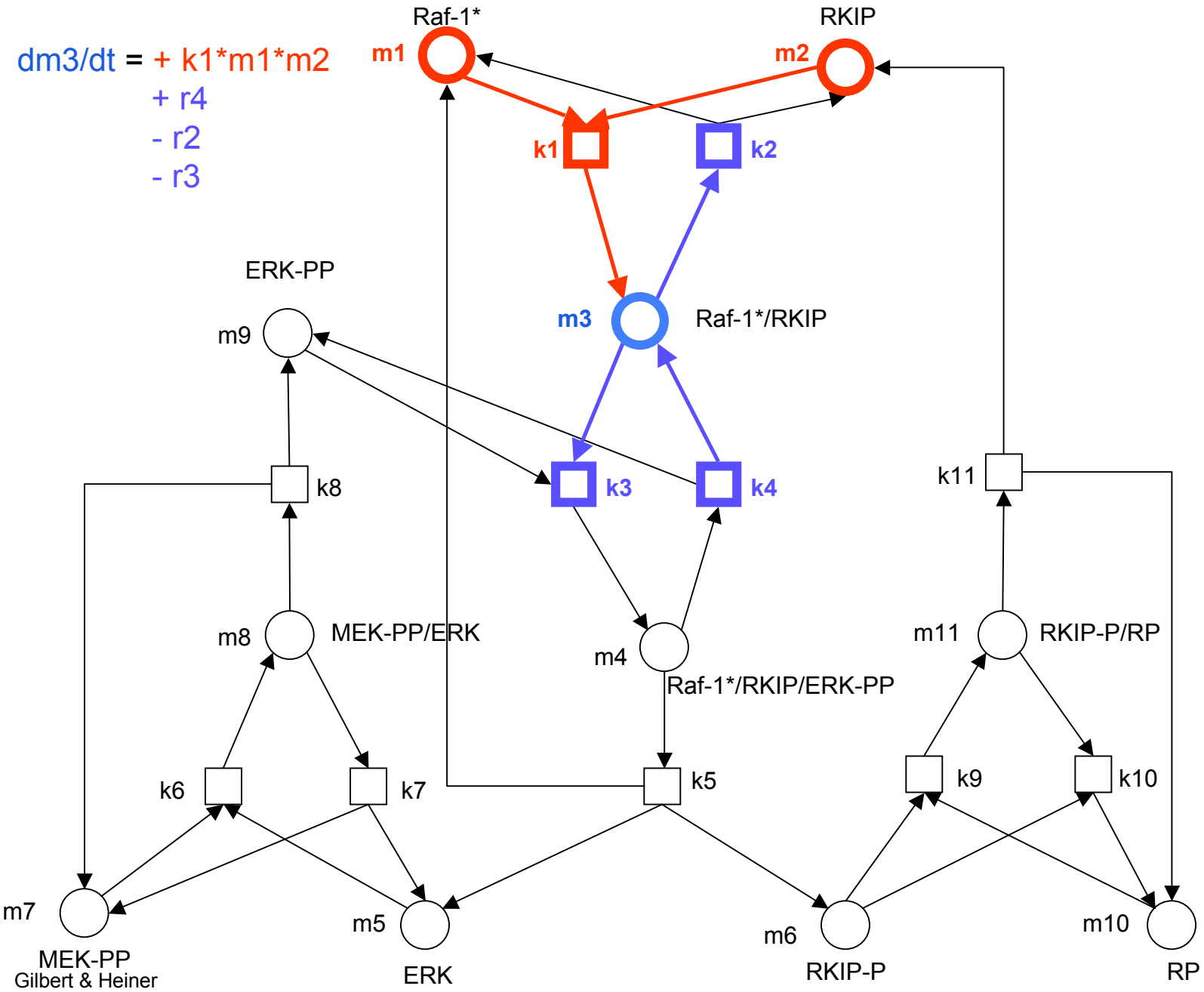

dm3/dt = + r1
+ r4
- r2
- r3
Raf-1*
m1
RKIP
m2
k1
k2
ERK-PP
m9
m3
Raf-1*/RKIP
k3
k4
k11
k8
m8
MEK-PP/ERK
m4
Raf-1*/RKIP/ERK-PP
m11
RKIP-P/RP
k6
k7
k5
k9
k10
m7
MEK-PP
Gilbert & Heiner
m5
ERK
m6
RKIP-P
m10
RP


dm3/dt = + k1*m1*m2
+ r4
- r2
- r3
Raf-1*
m1
RKIP
m2
k1
k2
ERK-PP
m9
m3
Raf-1*/RKIP
k3
k4
k8
k11
m8
MEK-PP/ERK
m4
Raf-1*/RKIP/ERK-PP
m11
RKIP-P/RP
k6
k7
k5
k9
k10
m7
MEK-PP
Gilbert & Heiner
m5
ERK
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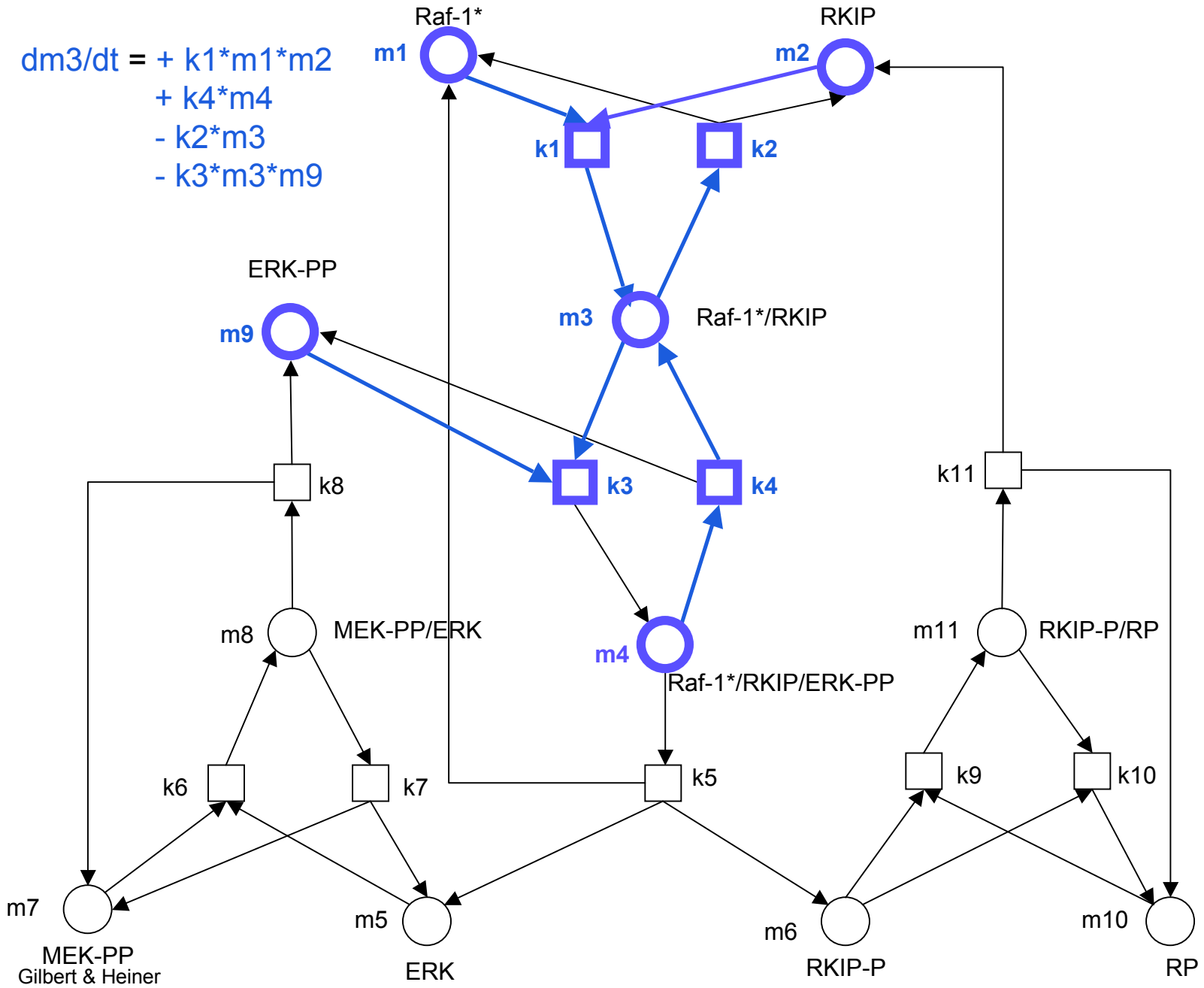
RKIP-P
m10
RP


dm3/dt = + k1*m1*m2
+ k4*m4
- k2*m3
- k3*m3*m9
Raf-1*
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RKIP
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k6
k7
k5
k9
k10
m7
MEK-PP
Gilbert & Heiner
m5
ERK
m6
RKIP-P
m10
RP

Validation via ODE simulation

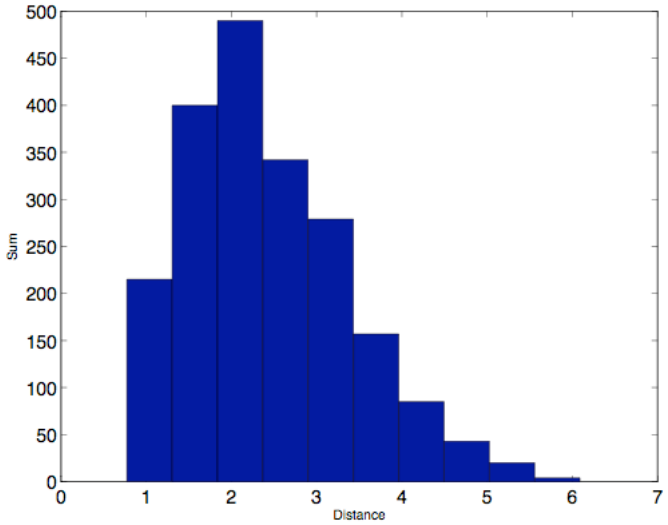
Species	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
Raf-1*	1	0	0	1	1	1	1	1	0	0	1	1	1
RKIP	1	0	0	0	0	0	0	1	0	0	1	0	0
Raf-1*_RKIP	0	1	0	0	0	0	0	0	1	1	0	0	0
Raf-1*_RKIP_ERK-PP	0	0	1	0	0	0	0	0	0	0	0	0	0
ERK	0	0	0	1	0	0	1	1	1	0	0	0	0
RKIP-P	0	0	0	1	1	0	0	0	0	0	0	0	1
MEK-PP	1	1	1	1	0	0	1	1	1	0	0	1	1
MEK-PP_ERK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

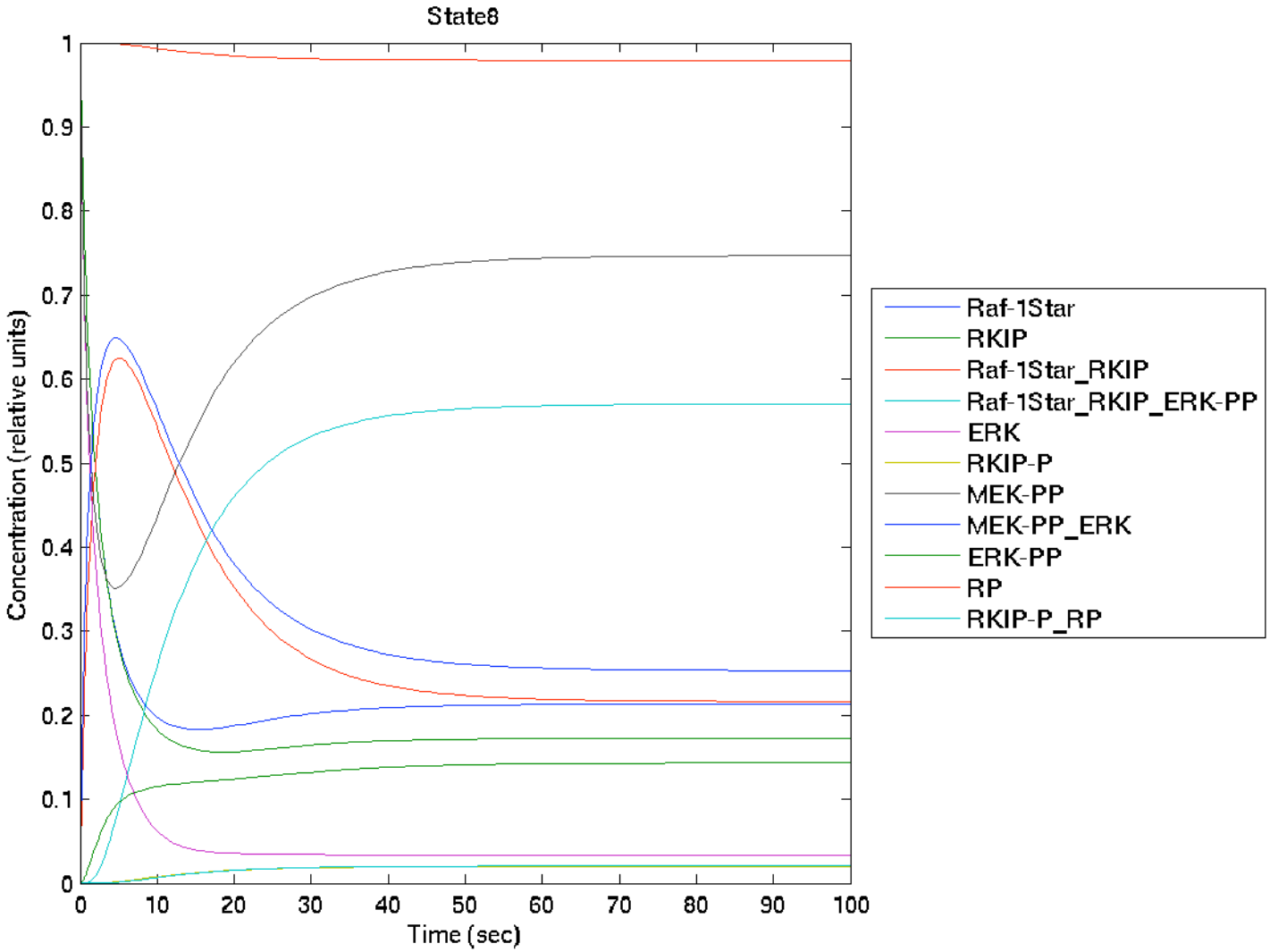
Biochemist

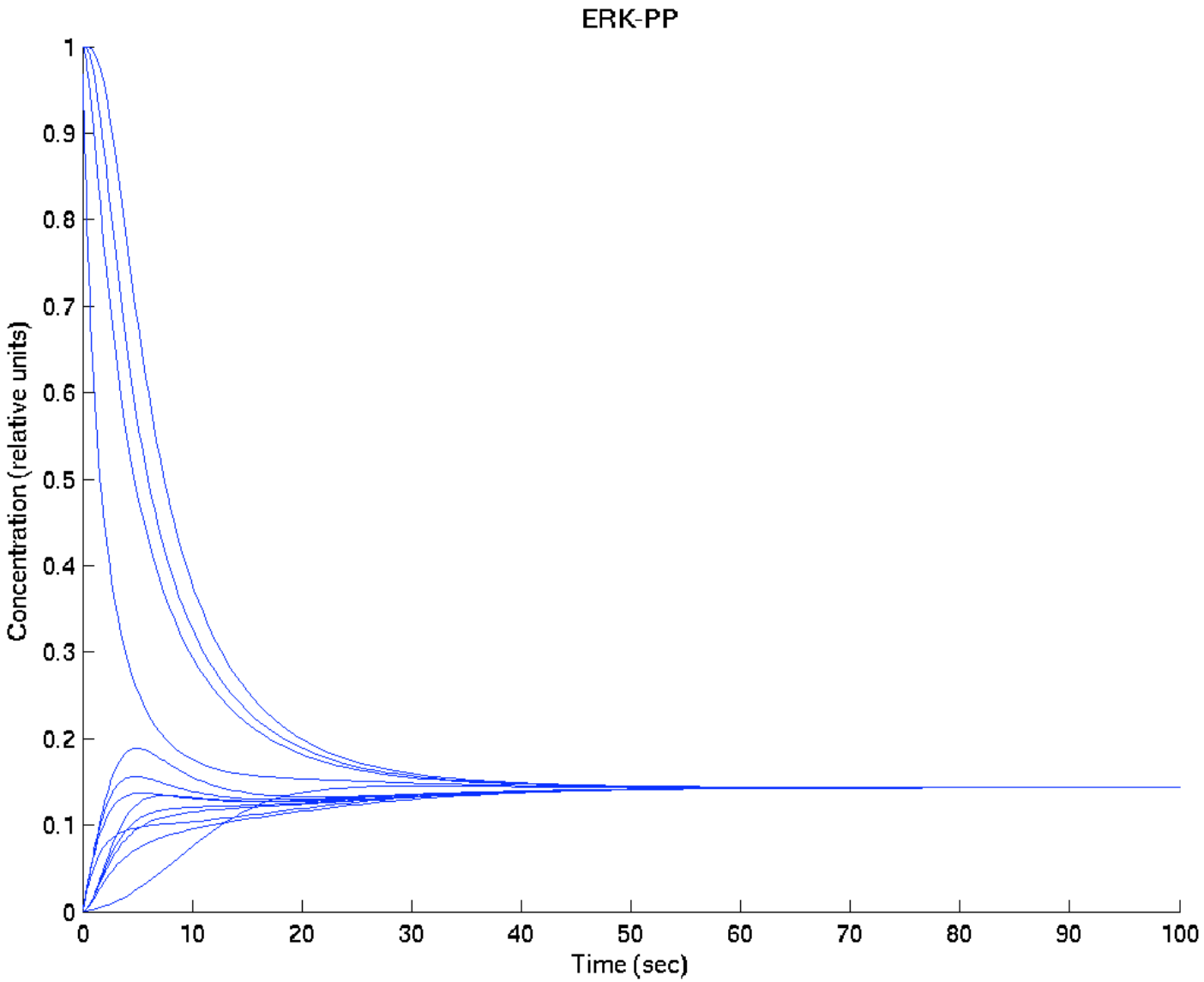
13 ‘Good’ state configurations

Species	Mean steady state concentration	Standard Deviation
Raf-1*	0.2133	0.1225 * 1.0e-04
RKIP	0.1727	0.0854 * 1.0e-04
Raf-1*_RKIP	0.2163	0.5546 * 1.0e-04
Raf-1*_RKIP_ERK-PP	0.5704	0.4346 * 1.0e-04
ERK	0.0332	0.0135 * 1.0e-04
RKIP-P	0.0200	0.0169 * 1.0e-04
MEK-PP	0.7469	0.6020 * 1.0e-04
MEK-PP_ERK	0.2531	0.6020 * 1.0e-04
ERK-PP	0.1433	0.1846 * 1.0e-04
RP	0.9793	0.0471 * 1.0e-04
RKIP-P_RP	0.0207	0.0473 * 1.0e-04



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


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

Quantitative analysis - summary

- Reasonable initial states of the quantitative model can be constructed by help of the qualitative model
- All “live” discrete states result in the same steady state
- No other (theoretically possible) initial states are “close” to this steady state
- Hold for this self-contained case study
  - Other case studies confirm these findings

Overall summary

- We use a 2-step approach;
  - qualitative model to validate the structure;
  - qualitative & quantitative model share the same structure

- Continuous pn are structured notations for ODEs

- Many open questions…

…Outlook

- Deeper insights into the relation between the discrete and the continuous world

- The whole truth about biomolecular systems

  $\Rightarrow$ inherently stochastic behaviour!!!

- Continuous models are just an approximation of reality

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