HOW MIGHT PETRI NETS ENHANCE YOUR SYSTEMS BIOLOGY TOOLKIT !

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HOW MIGHT PETRI NETS ENHANCE MY SYSTEMS BIOLOGY TOOLKIT ?

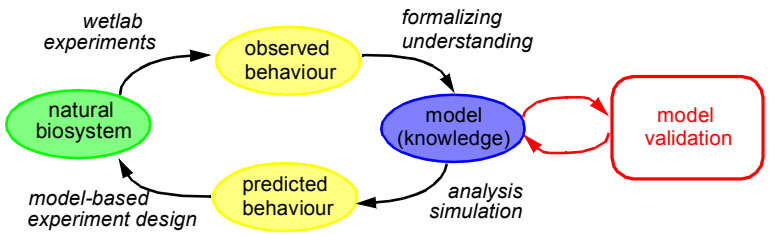
- **BACKGROUND**
  - -> *modelling, what for*
  - -> *some case studies*
- **BIOMODEL ENGINEERING**
  - -> *a unifying framework: QPN - SPN - CPN*
  - -> *relation SPN - CPN ?*
- **OUR TOOL BOX**
  - -> *Snoopy*
  - -> *Charlie*
  - -> *Marcie*
- **LATEST NEWS**
  - -> *Generalized Hybrid Petri Nets*
  - -> *colored framework*

-> MANY BUZZWORDS <-

BACKGROUND

MODELS IN SYSTEMS BIOLOGY

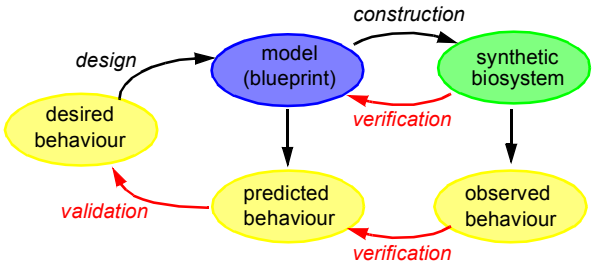
MODELLING = FORMAL KNOWLEDGE REPRESENTATION



MODEL VALIDATION = CONFIDENCE INCREASE

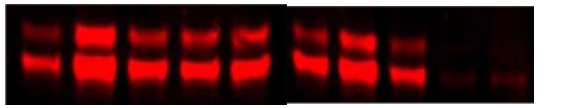
MODELS IN SYNTHETIC BIOLOGY

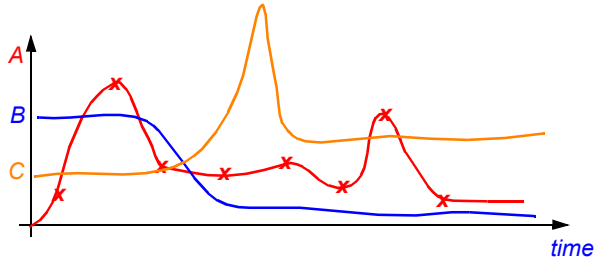
MODELLING = BLUEPRINT FOR SYSTEM CONSTRUCTION



RELIABLE AND ROBUST ENGINEERING REQUIRES VERIFIED MODELS

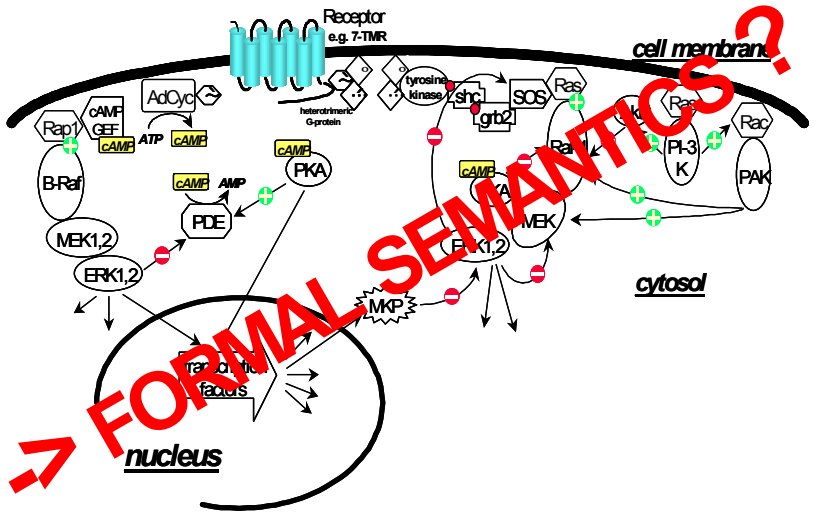
MODELS IN SYSTEMS BIOLOGY - OBSERVED BEHAVIOUR





WHAT KIND OF MODEL SHOULD BE USED?

NETWORK REPRESENTATIONS, EX1



-> FORMAL SEMANTICS?

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NETWORK REPRESENTATIONS, EX2

$$\frac{d\alpha}{dt} = -\nu_1$$
$$\frac{dSte2}{dt} = -\nu_2 + \nu_3 - \nu_5$$
$$\frac{dSte2_{active}}{dt} = \nu_2 - \nu_3 - \nu_4$$
$$\frac{dSst2_{active}}{dt} = \nu_{46} - \nu_{47}$$
$$\frac{dG\alpha\beta\gamma}{dt} = -\nu_6 + \nu_9$$
$$\frac{dG\alpha GTP}{dt} = \nu_6 - \nu_7 - \nu_8$$
$$\frac{dG\alpha GDP}{dt} = \nu_7 + \nu_8 - \nu_9$$
$$\frac{dG\beta\gamma}{dt} = \nu_6 - \nu_9 - \nu_{10} + \nu_{11} + \nu_{21} + \nu_{23} + \nu_{25} + \nu_{27} + \nu_{32} - \nu_{42} + \nu_{43}$$
$$\frac{dSte5}{dt} = -\nu_{12} + \nu_{13} + \nu_{17} + \nu_{23} + \nu_{25} + \nu_{27} + \nu_{32}$$
$$\frac{dSte11}{dt} = -\nu_{12} + \nu_{13} + \nu_{17} + \nu_{23} + \nu_{25} + \nu_{27} + \nu_{32}$$
$$\frac{dSte7}{dt} = -\nu_{14} + \nu_{15} + \nu_{21} + \nu_{23} + \nu_{25} + \nu_{27} + \nu_{32}$$
$$\frac{dFus3}{dt} = -\nu_{14} + \nu_{17} + \nu_{21} + \nu_{23} + \nu_{25} + \nu_{27} - \nu_{29} + \nu_{30} + \nu_{33}$$
$$\frac{dSte20}{dt} = -\nu_{18} + \nu_{19} + \nu_{21} + \nu_{23} + \nu_{25} + \nu_{27} + \nu_{32}$$

$$\nu_1 = \alpha[t] \cdot Bar1_{active}[t] \cdot k_1$$
$$\nu_2 = Ste2[t] \cdot \alpha[t] \cdot k_2$$
$$\nu_3 = Ste2_{active}[t] \cdot k_3$$
$$\nu_4 = Ste2_{active}[t] \cdot k_4$$
$$\nu_5 = Ste2[t] \cdot k_5$$
$$\nu_6 = Ste2_{active}[t] \cdot G\alpha\beta\gamma[t] \cdot k_6$$
$$\nu_7 = G\alpha GTP[t] \cdot k_7$$
$$\nu_8 = G\alpha GTP[t] \cdot Sst2_{active}[t] \cdot k_8$$
$$\nu_9 = G\alpha GDP[t] \cdot G\beta\gamma[t] \cdot k_9$$
$$\nu_{10} = G\beta\gamma[t] \cdot C[t] \cdot k_{10}$$
$$\nu_{11} = D[t] \cdot k_{11}$$
$$\nu_{12} = Ste5[t] \cdot Ste11[t] \cdot k_{12}$$
$$\nu_{13} = A[t] \cdot k_{13}$$
$$\nu_{14} = Ste7[t] \cdot Fus3[t] \cdot k_{14}$$
$$\nu_{15} = B[t] \cdot k_{15}$$
$$\nu_{16} = A[t] \cdot B[t] \cdot k_{16}$$
$$\nu_{17} = C[t] \cdot k_{17}$$
$$\nu_{18} = D[t] \cdot Ste20[t] \cdot k_{18}$$

-> READABILITY?

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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*
  - -> *tend to grow fast*
  - -> *dense, apparently unstructured*
  - -> *hard to read*

-> MODELS ARE PATHWORKS FULL OF ASSUMPTIONS

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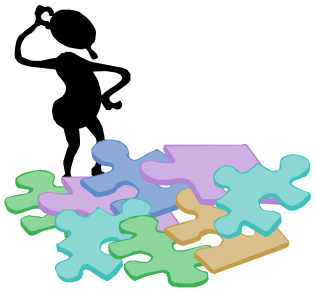
BIO NETWORK REPRESENTATIONS SHOULD BE



- **readable**
  - *-> fault avoidance*
  - *-> informal = cartoon-like representations ?*
- **executable**
  - *-> to experience the model, spec. causality*
- **analysable**
  - *-> formal = mathematical representations*
- **unifying power**
  - *-> high-level description for various analysis approaches*

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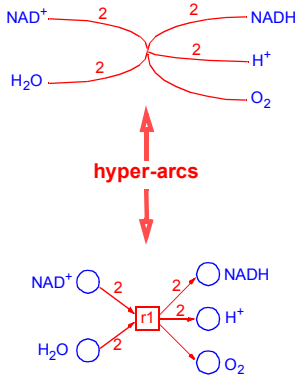
. . .

ARE NETWORKS OF BIOCHEMICAL REACTIONS

. . .

NATURALLY EXPRESSIBLE AS PETRI NETS

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



C. A. PETRI
INTERPRETATIONS OF NET THEORY
GMD, INTERNAL REPORT 75-07, 2ND IMPROVED EDITION 1976

places	transitions
state elements	transitional elements
conditions	events/facts
statements	dependencies
model domains	specifications
chemical compounds	chemical reactions
open one-point sets	closed one-point sets
channels	offices
languages	translators
products	poduction activities

[Peterson 1981]

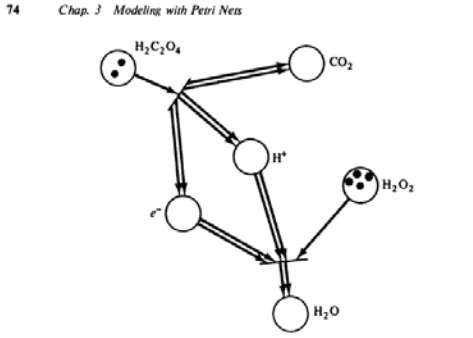


Figure 3.38 A Petri net representing the oxidation-reduction of oxalic acid and hydrogen peroxide into carbon dioxide and water.

$$H_2C_2O_4 \longrightarrow 2CO_2 + 2H^+ + 2e^-$$
$$2\,e^- + 2H^+ + H_2O_2 \longrightarrow 2H_2O$$

[Murata 1989]

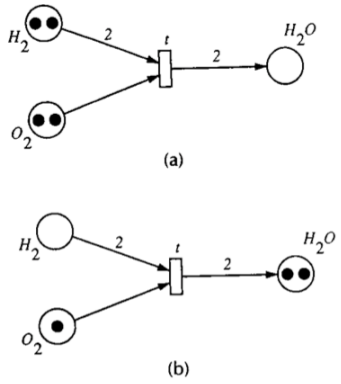
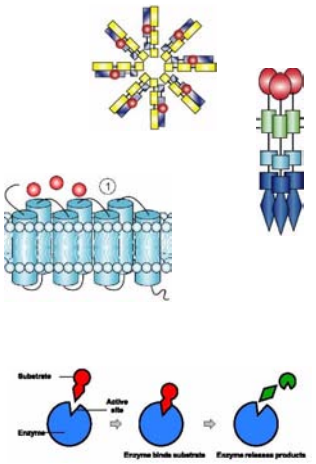


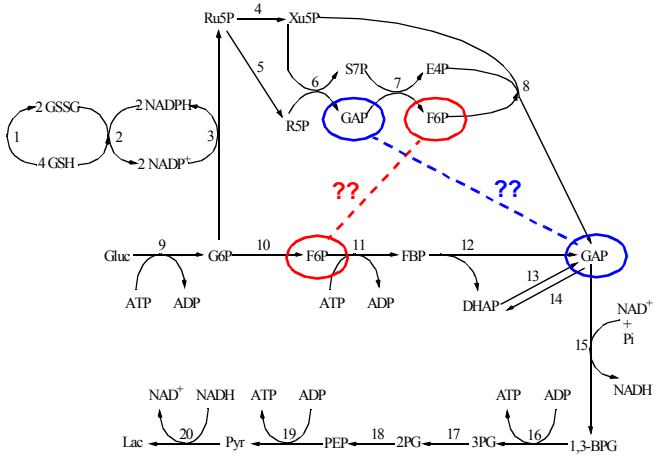
Fig. 1. Example 1: An illustration of a transition (firing) rule: (a) The marking before firing the enabled transition t. (b) The marking after firing t, where t is disabled.

- **places -> atomic species**
  - -> *(bio-) chemical compounds*
  - -> *proteins*
  - -> *protein conformations*
  - -> *complexes*
  - -> *genes, . . . etc.*
  - *. . . in different locations*
- **transitions -> atomic reactions**
  - -> *(stoichiometric) chemical reaction*
  - -> *complexation / decomplexation*
  - -> *phosphorylation / dephosphorylation*
  - -> *conformational change*
  - -> *transport step, . . . etc.*
  - *. . . in different locations*

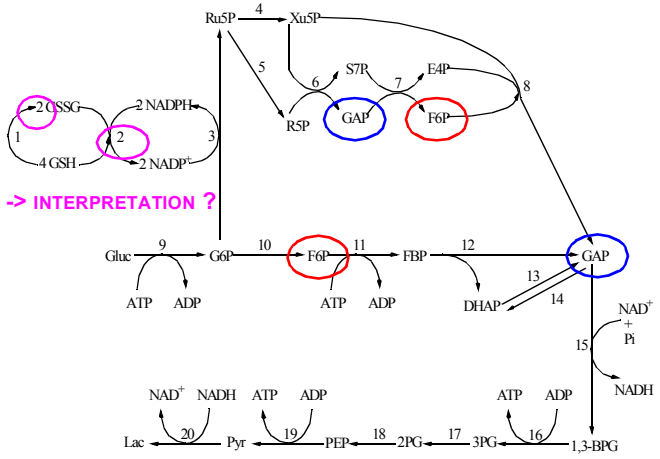


BIO PETRI NETS - SOME EXAMPLES

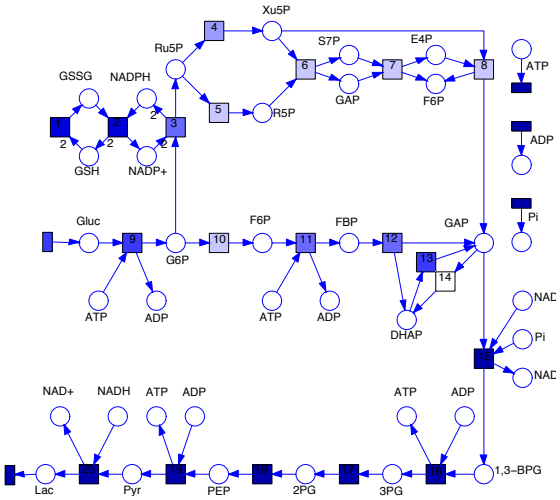
[Reddy 1993]



[Reddy 1993]

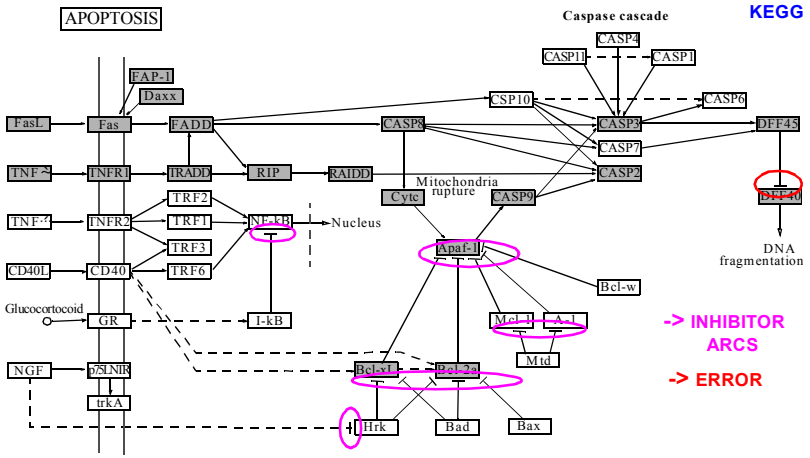


Ex1 - Glycolysis and Pentose Phosphate Pathway

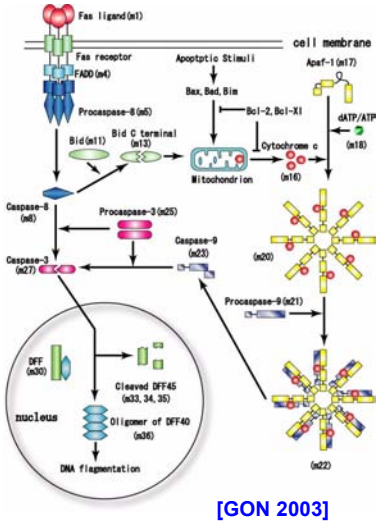


[Reddy 1993]
[Heiner 1998]
. . .
[Koch, Heiner 2010]

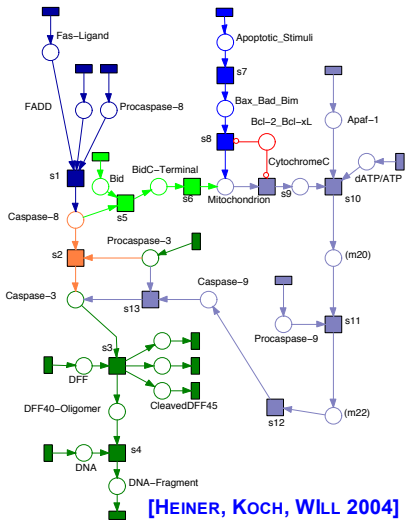
Ex2 - Apoptosis in Mammalian Cells



Ex2 - Apoptosis in Mammalian Cells



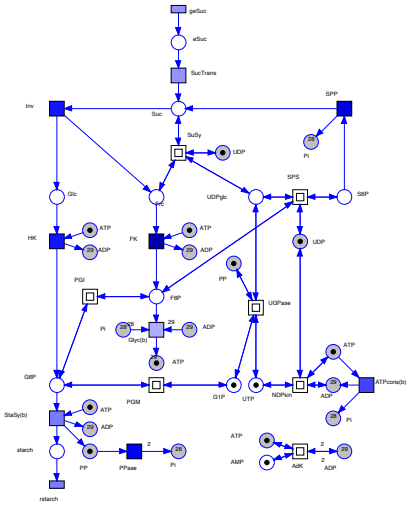
[GON 2003]

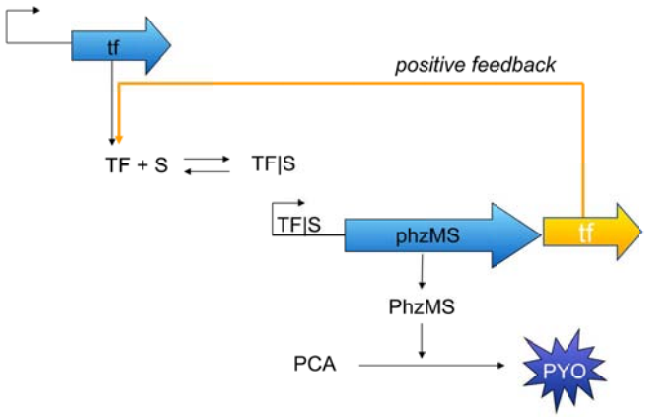


[Heiner, Koch, Will 2004]

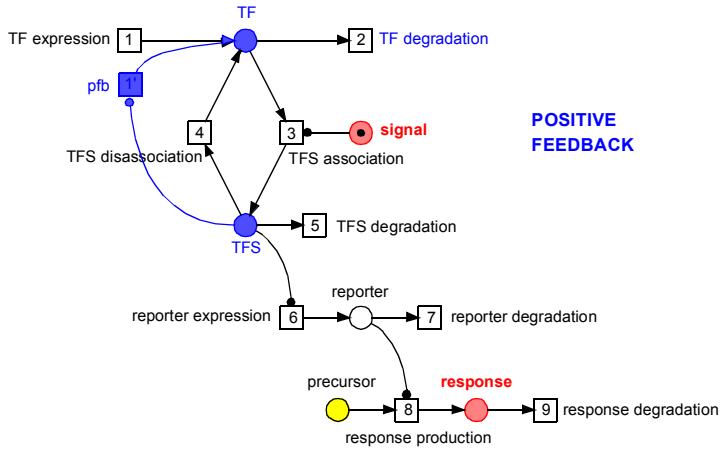
Ex3 - Carbon Metabolism in Potato Tuber

[Koch, Junker, Heiner 2005]

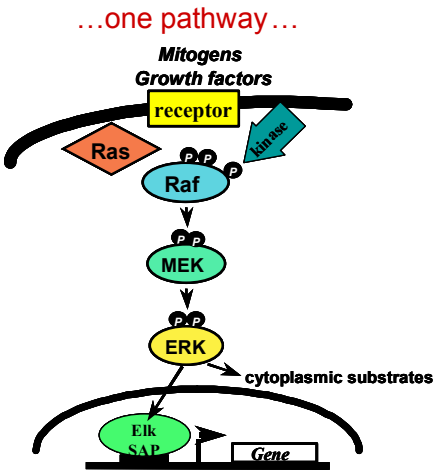


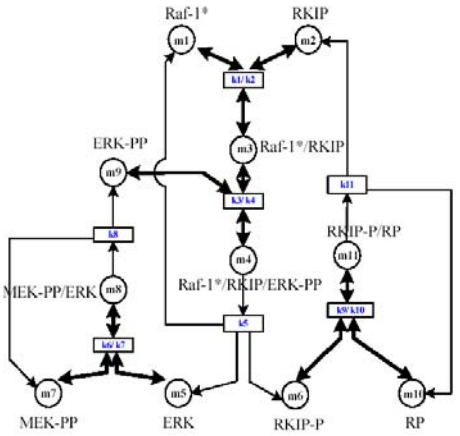


[GILBERT, HEINER, ROSSER, FULTON, GU, TRYBILO 2008]



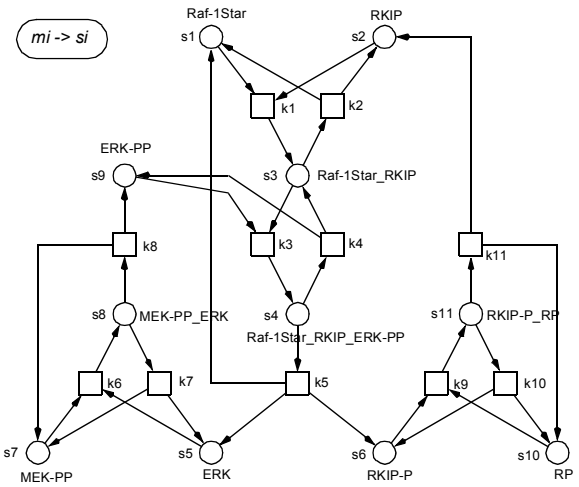
[GILBERT, HEINER, ROSSER, FULTON, GU, TRYBILO 2008]





[Cho et al. 2003]

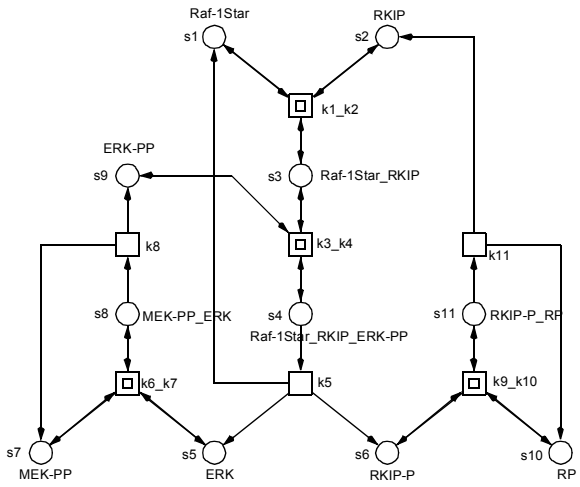
EX5 - RKIP SIGNALLING PATHWAY, PETRI NET



[HEINER, GILBERT 2006]

[HEINER, DONALDSON, GILBERT 2010]

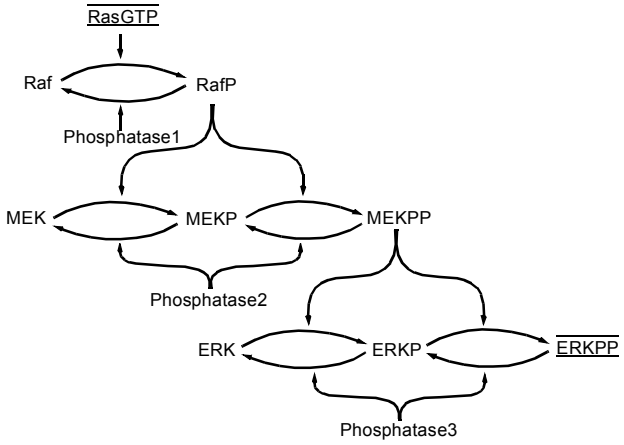
EX5 - RKIP SIGNALLING PATHWAY, HIERARCHICAL PETRI NET



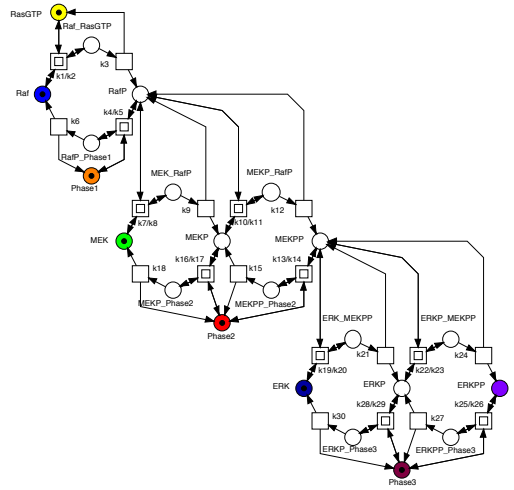
[HEINER, GILBERT 2006]

[HEINER, DONALDSON, GILBERT 2010]

EX6 - SIGNALLING CASCADE



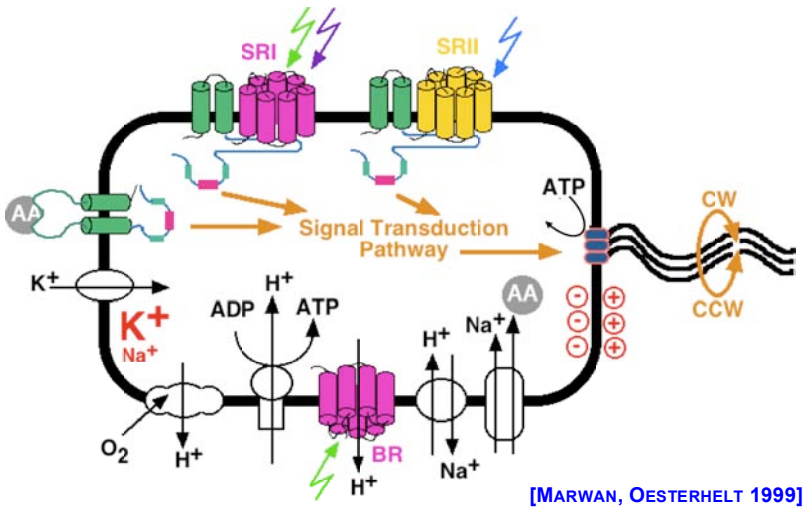
EX6 - SIGNALLING CASCADE



[GILBERT, HEINER, LEHRACK 2007]

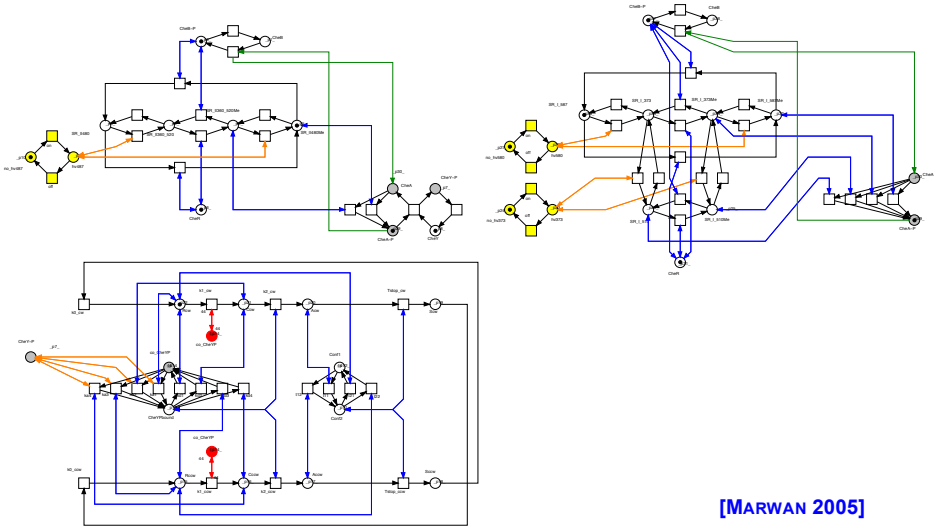
[HEINER, GILBERT, DONALDSON 2008]

EX7 - HALOBACTERIUM SALINARUM



[MARWAN, OESTERHELT 1999]

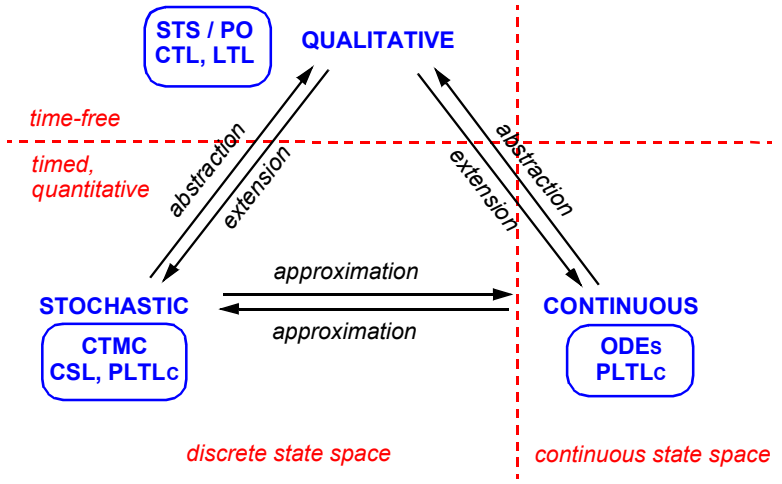
EX6 - HALOBACTERIUM SALINARUM



[MARWAN 2005]

THE FRAMEWORK

FRAMEWORK



3 MODELS SHARING STRUCTURE

QUANTITATIVE MODEL = QUALITATIVE MODEL
+
RATE FUNCTIONS
(KINETICS)

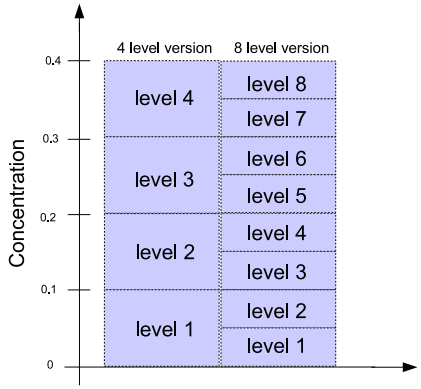
- **transitions r_i get a stochastic waiting time**
  - -> *exponential distribution with parameter lambda*
- **state-dependent lambda defined by rate function $v_i(r_i)$**
  - -> *any arithmetic function including the transition's pre-places as integer variables and user-defined real-valued parameters*
  - -> *modifier arcs*
  - -> *popular kinetics: mass-action semantics, level semantics*
- **semantics: Continuous Time Markov Chain (CTMC)**
  - -> *reachability graph + state transition rates*
- **analysis**
  - -> *standard Markov analysis techniques: transient, steady state*
  - -> *stochastic simulation algorithms (SSA), e.g. Gillespie's SSA*

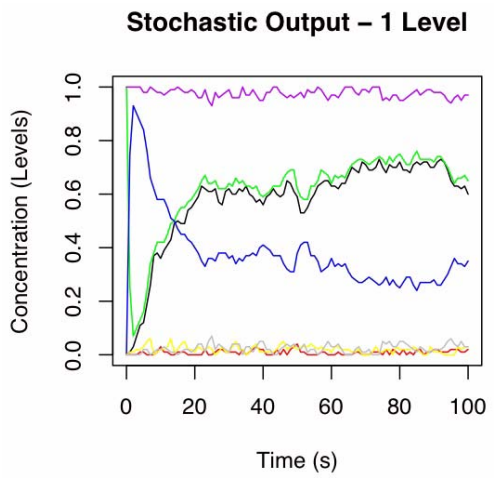
- **mass-action semantics**

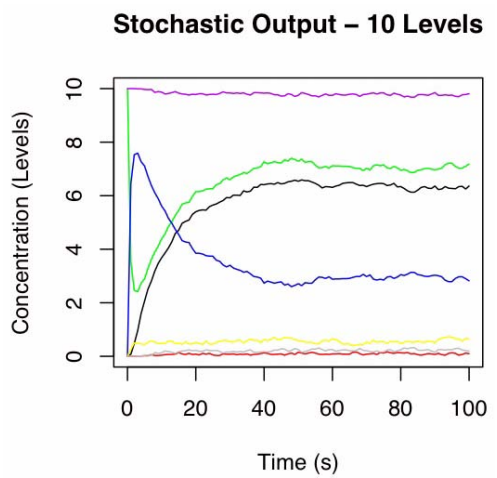
$$h_t := c_t \cdot \prod_{p \in {}^\bullet t} \binom{m(p)}{f(p,t)}$$

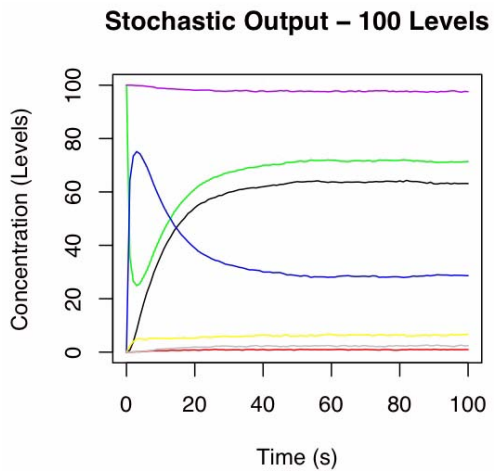
- **level semantics**

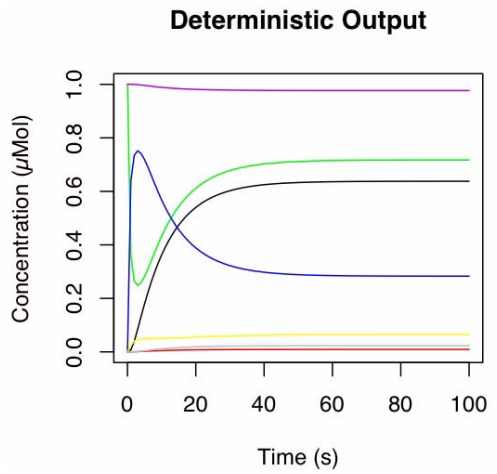
$$h_t := k_t \cdot N \cdot \prod_{p \in {}^\bullet t} \left(\frac{m(p)}{N}\right)$$




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)

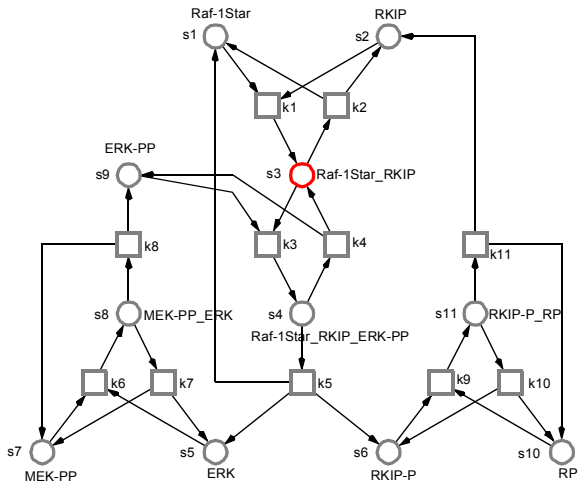

Deterministic Output
Concentration (μMol)
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
Time (s)

CONTINUOUS PETRI NETS, BASICS

- **transitions r_i fire continuously**
- **rate functions $v_i(r_i)$**
  - -> *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* -> [SOLIMAN, HEINER 2010]
  - -> *typically non-linear*
- **simulation (numerical integration)**
  - -> *stiff / unstiff solvers*

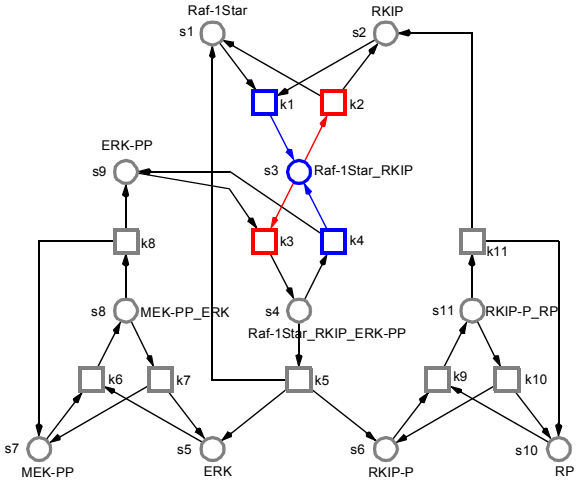
CONTINUOUS PETRI NET DEFINES ODES

$\frac{ds3}{dt} =$



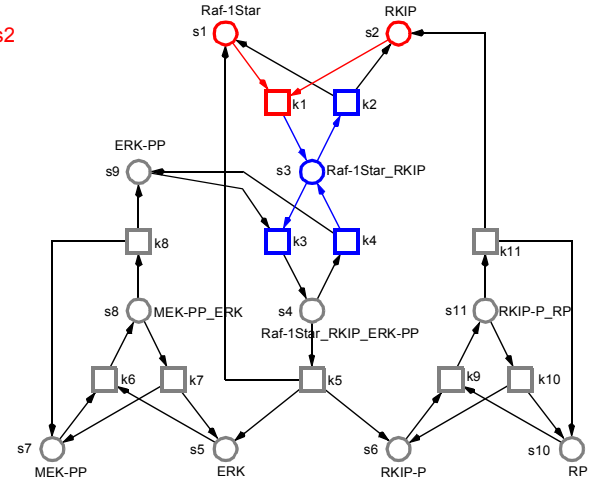
CONTINUOUS PETRI NET DEFINES ODES

$\frac{ds3}{dt} =$ + v1
+ v4
- v2
- v3



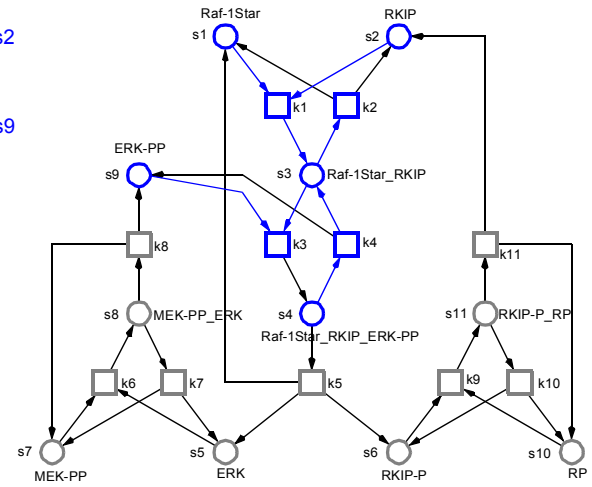
CONTINUOUS PETRI NET DEFINES ODES

$\frac{ds3}{dt} =$ + k1 * s1 * s2
+ v4
- v2
- v3



Continuous Petri Net Defines ODEs

$$\frac{ds3}{dt} = + k1 * s1 * s2 + k4 * s4 - k2 * s3 - k3 * s3 * s9$$



Crucial Point

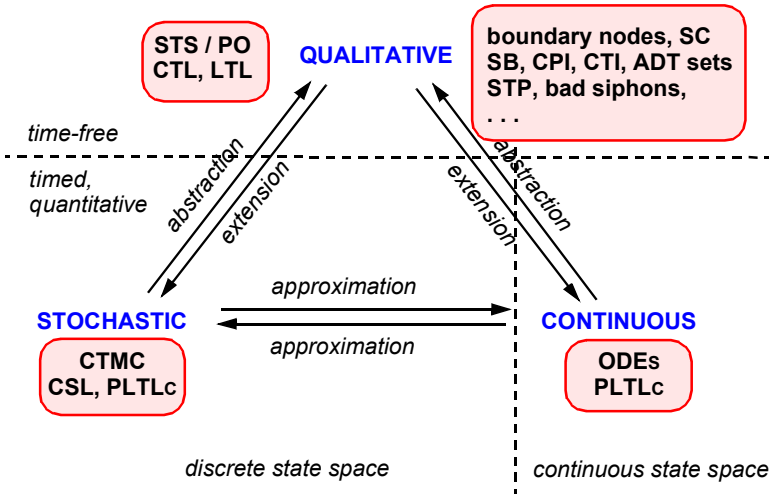
STATE-DEPENDENT RATE FUNCTION

LAMBDA OF EXPONENTIAL WAITING TIME → **CTMC**

STRENGTH OF CONTINUOUS FLOW → **ODEs**

-> supported by, e.g., COPASI, Dizzy, ..., Snoopy

Framework



Framework and Model Checking

- **Qualitative**

  Protein rises then falls:

  $$\mathbf{P}_{=?}\,[\,(\,d(\text{Protein}) > 0\,)\,U\,(\,G(\,d(\text{Protein}) < 0\,)\,)\,].$$

- **Semi-qualitative**

  Protein rises then falls to less than 50% of its peak concentration:

  $$\mathbf{P}_{=?}\,[\,(\,d(\text{Protein}) > 0\,)\,U\,(\,G(\,d(\text{Protein}) < 0\,)\,\wedge\, F(\,[\text{Protein}] < 0.5 * max[\text{Protein}]\,)\,)\,].$$

- **Semi-quantitative**

  Protein rises then falls to less than 50% of its peak concentration at 60 minutes:

  $$\mathbf{P}_{=?}\,[\,(\,d(\text{Protein}) > 0\,)\,U\,(\,G(\,d(\text{Protein}) < 0\,)\,\wedge\, F(\,\text{time} = 60 \wedge \text{Protein} < 0.5 * max(\text{Protein})\,)\,)\,].$$

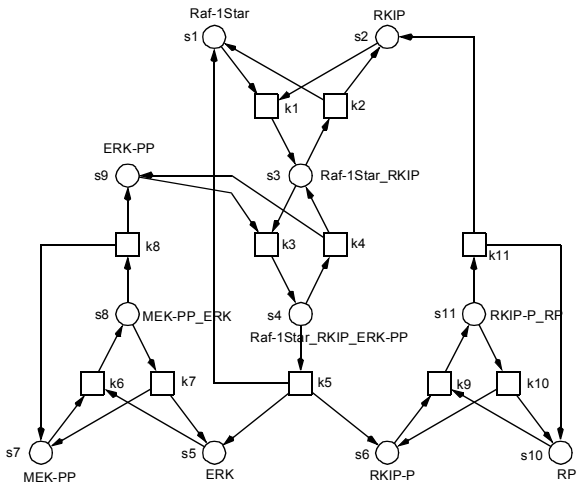
- **Quantitative**

  Protein rises then falls to less than 100 microMol at 60 minutes::

  $$\mathbf{P}_{=?}\,[\,(\,d(\text{Protein}) > 0\,)\,U\,(\,G(\,d(\text{Protein}) < 0\,)\,\wedge\, F(\,\text{time} = 60 \wedge \text{Protein} < 100\,)\,)\,].$$

About the Relation SPN - CPN

Ex1 - RKIP Signalling Pathway, Petri Net

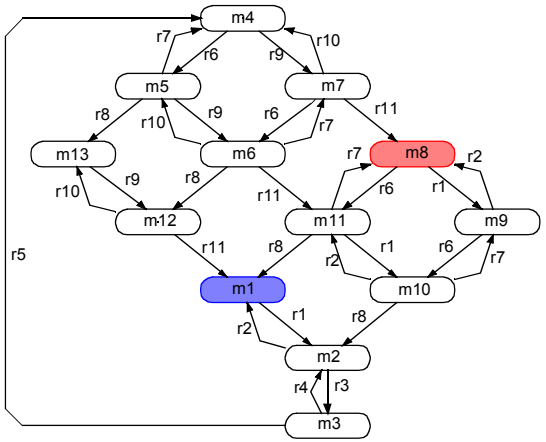


[Heiner, Gilbert 2006]

[Heiner, Donaldson, Gilbert 2010]

Ex1 - RKIP, Reachability Graph (STS)

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



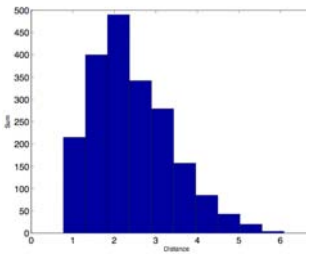
Ex1 - RKIP, Quantitative Analysis

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

Biochemist

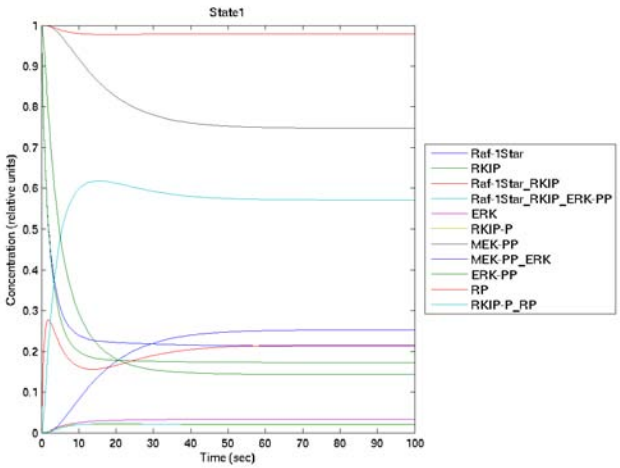
13 "good" state configurations



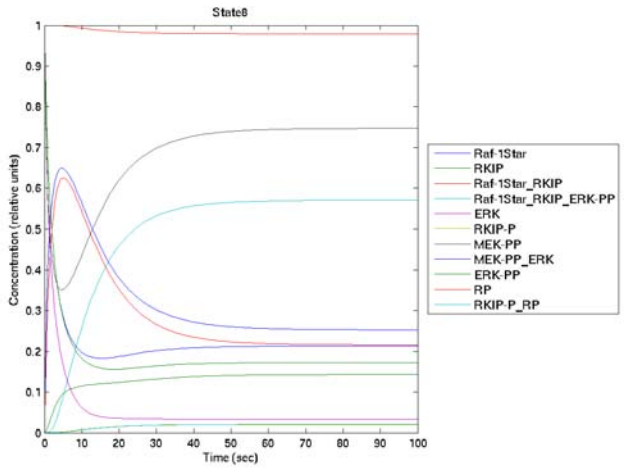
Distribution of `bad' steady states as euclidean distances from the `good' final steady state

the "bad" ones

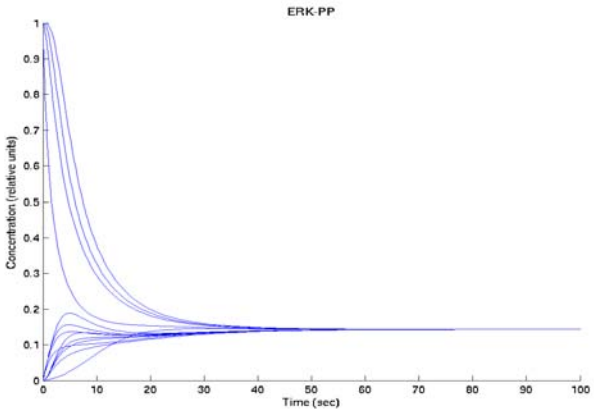
Ex1 - RKIP, Quantitative Analysis



Ex1 - RKIP, Quantitative Analysis

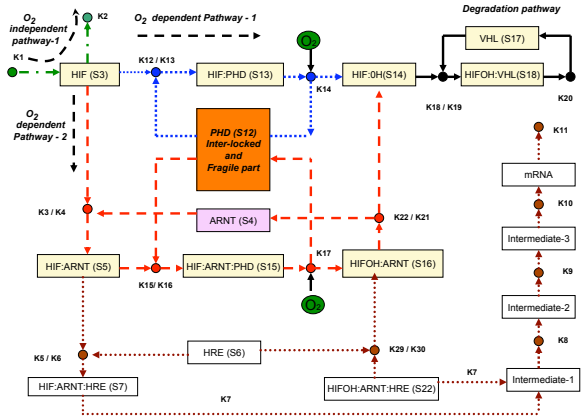


Ex1 - RKIP, Quantitative Analysis

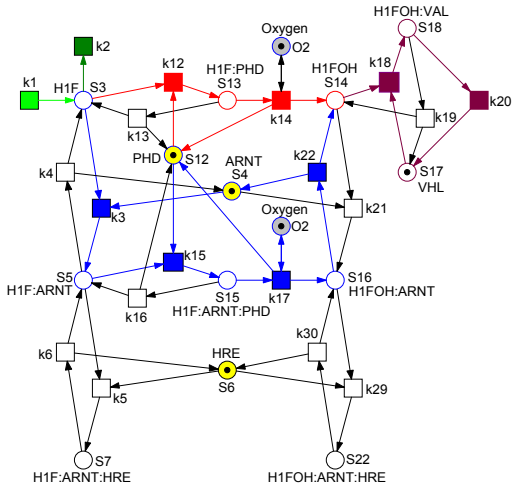


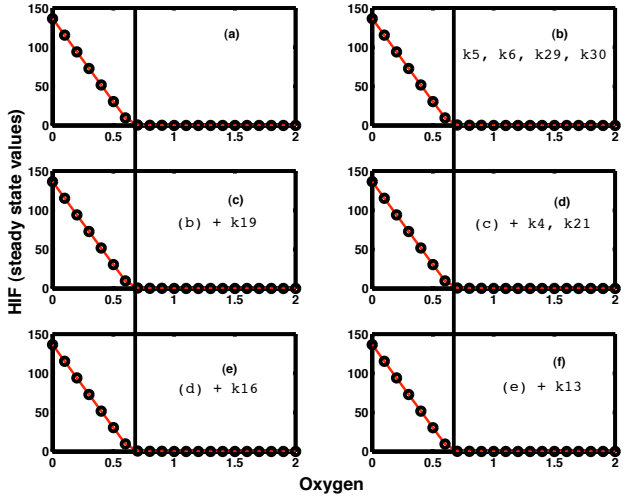
Ex2 - Hypoxia

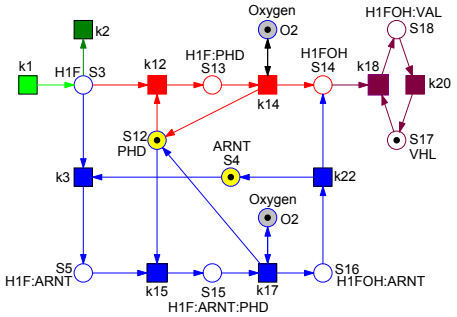
[Yu et al. 2007]

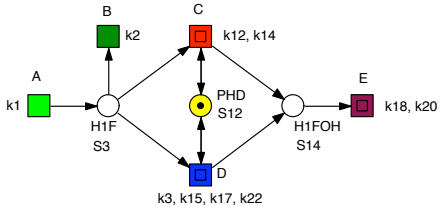


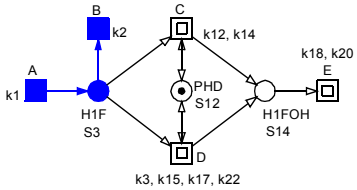
[HEINER, SRIRAM 2010]

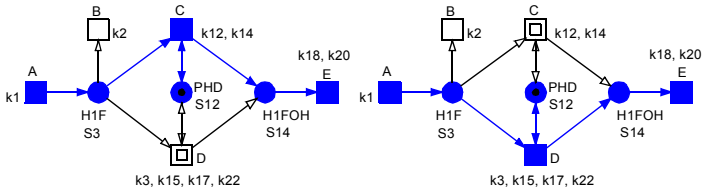










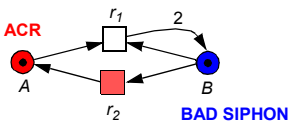


BUT,

TRANSITION SPN -> CPN
MAY COME WITH
COUNTERINTUITIVE EFFECTS.

- **ACR: steady state value of variable (place) does not depend on total mass, only on kinetic constants** *-> [SHINAR, FEINBERG 2010]*

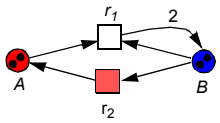
- **simple example** **mass-action kinetics**
  r_1: $A + B \rightarrow 2B$ $v_1(r_1) = k_1AB$
  r_2: $B \rightarrow A$ $v_2(r_2) = k_2B$



- **ODEs**
  $dA/dt = v_2 - v_1 = k_2B - k_1AB$
  $dB/dt = v_1 - v_2 = k_1AB - k_2B$

CPI: $m_0(A) + m_0(B) = total$

- **steady state**
  $dA/dt = k_2B - k_1AB = 0$
  $dB/dt = k_1AB - k_2B = 0$



-> *steady_state(A) = k_2/k_1*
steady_state(B) = total - k_2/k_1

OUR TOOL BOX

Our Tool Box

- **SNOOPY**
  - -> *modelling and animation/simulation of hierarchical graphs,*
    e.g. (extended) fault trees,
    various Petri net classes, e.g. QPN, XQPN, SPN, XSPN, CPN, TPN,
    . . . ,
    free style graphs
- **CHARLIE**
  - -> *QPN, XQPN, Time/Timed Petri nets (TPN)*
  - -> *mostly standard analysis techniques of Petri net theory*
- **MARCIE**
  - -> *XQPN, SPN, XSPN*
  - -> *symbolic and simulative model checking*
- **many complementary foreign tools**
  - -> *e.g. INA, LoLa, PEP, Tina, . . . , PRISM, SMART, . . . , Adam, Anastasia*

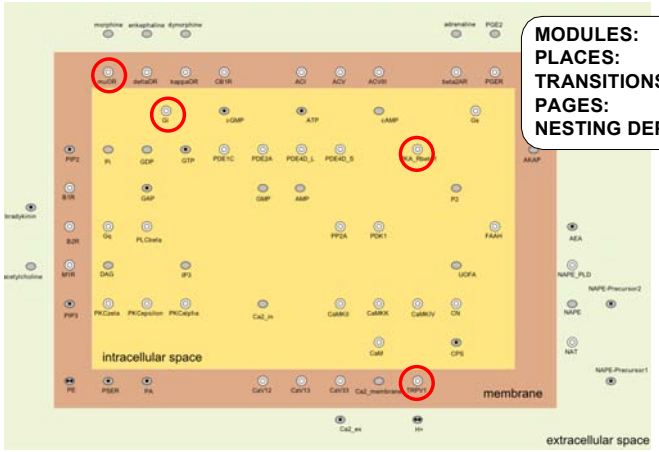
Snoopy

-> *some features inspired by Design/CPN (hierarchies, fusion nodes)*
-> *many features triggered by bio collaborators, spec. Wolfgang Marwan*

- **history**
  - -> *predecessor: Petri Net Editor PED, 1992 - 2004*
  - -> *initial implementation concepts 1997*
  - -> *core implementation 2004 + many Master Theses + students' projects*
- **key features**
  - -> *hierarchies: marco transitions, macro places*
  - -> *fusion nodes: logical transitions, logical places*
  - -> *several initial markings, rate function sets, parameter sets*
  - -> *built-in stochastic/continuous simulation*
- **supported operating systems**
  - -> *MAC OS X, Windows, Linux (selected distributions)*
  - -> *C++, wxWidgets, CVODE / PVODE, . . .*

Ex - Pain Signalling

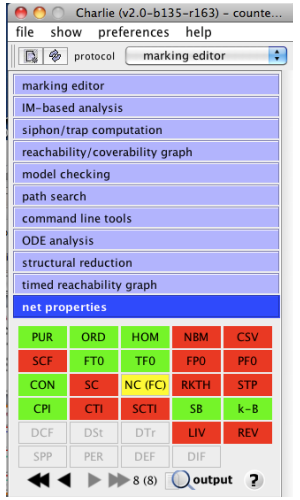
[Blätke, Meyer, Marwan 2011]
-> a protein-oriented modular modelling concept



Charlie

-> *inspired by Integrated Net Analyser (INA)*

- **structural analysis**
  - -> *net classes: SM, SG, FC, EFS, ES*
  - -> *siphon/trap property, rank theorem*
- **analysis based on incidence matrix**
  - -> *structural boundedness test*
  - -> *place/transition invariants, CPI, CTI*
  - -> *Abstract Dependent Transition sets (ADT sets, connected ADT sets)*
- **reachability/coverability graph**
  - -> *explicite model checking ->RG of XQPN*
- **structural reduction**
- **Java thread programming with GUI**



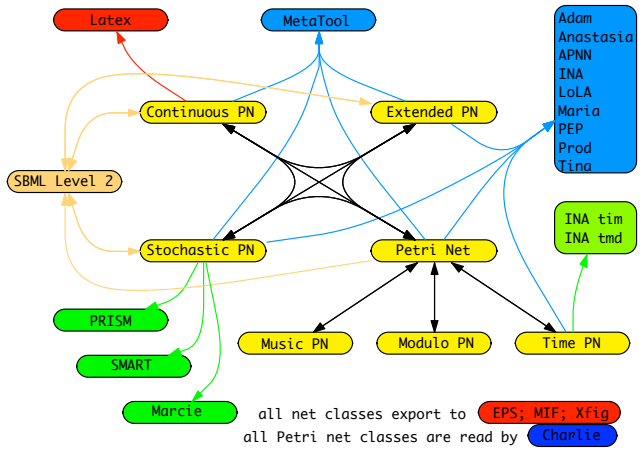
MARCIE

-> *builds on lessons learnt by . . . , SMART, PRISM*

- **Model checking And Reachability analysis done effiCIEntly**
  - -> *Interval Decision Diagrams*
  - -> *model checking of XQPN, SPN, XSPN*
- **XQPN: symbolic CTL model checking**
- **SPN: symbolic CSL model checking**
  - -> *"matrix free" transient and steady state analysis*
  - -> *parallelized*
  - -> *full CSL model checking + rewards*
- **XSPN: simulative PLTL model checking**
  - -> *distributed*
  - -> *in-line/ off-line traces*
- **command line tool, written in C++**

-> PETRI NET 2009
CMSB 2009
CMSB 2010
QEST 2011

SNOOPY'S EXPORT FEATURES

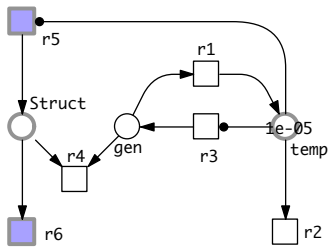


LATEST NEWS

-> MULTI-SCALE ISSUES
- TIME / SPACE -

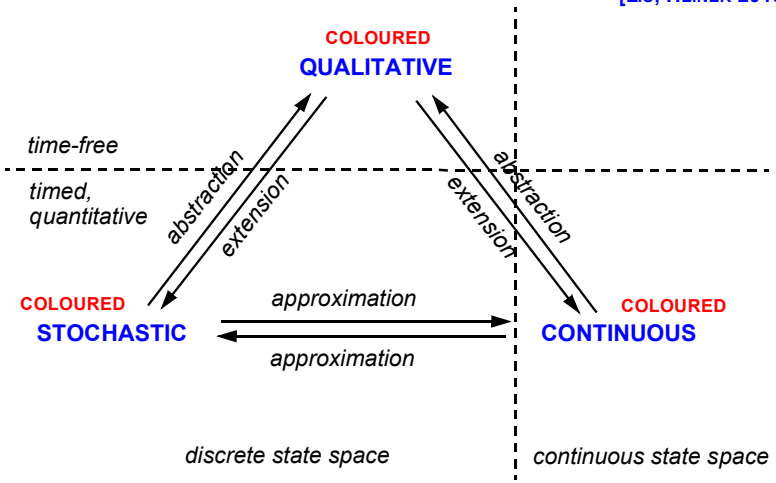
GENERALIZED HYBRID PETRI NETS (GHPN)

- **GHPN = XSPN + CPN** [HERAJY, HEINER 2010]
- **XSPN - Extended Generalized Stochastic Petri Nets**
  - -> *discrete places*
  - -> *discrete transitions: stochastic, immediate, deterministically delayed, scheduled*
  - -> *special arcs: read, inhibitor, equal, reset*
- **CPN - Continuous Petri Nets**
  - -> *continuous places*
  - -> *continuous transitions*
  - -> *special arcs: read, inhibitor*
- **hybrid simulation engine**
  - -> *static partitioning*
  - -> *dynamic partitioning*



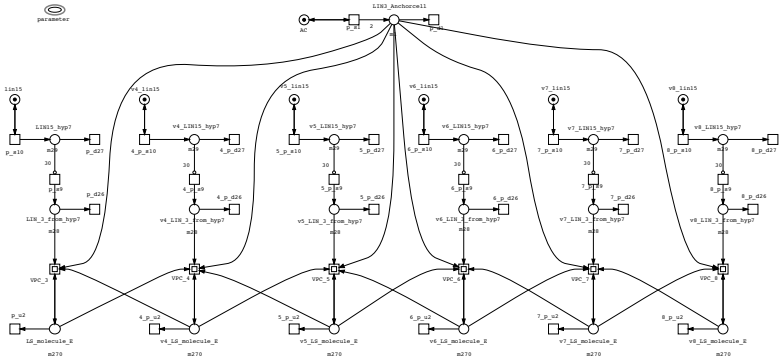
COLOURED FRAMEWORK

[LIU, HEINER 2010]

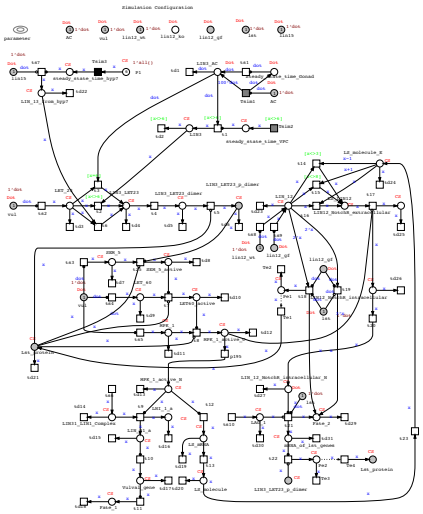


EX1 - C. ELEGANS

[LI ET AL. 2009]
[BONZANNI ET AL. 2009]

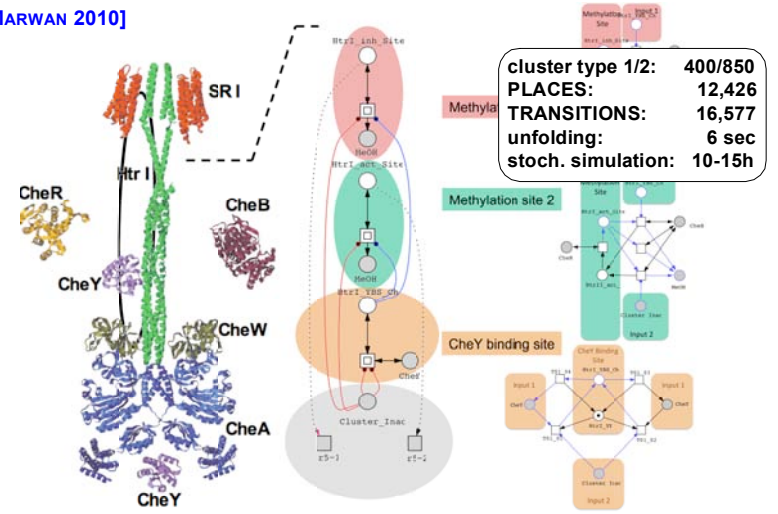


EX1 - C. ELEGANS

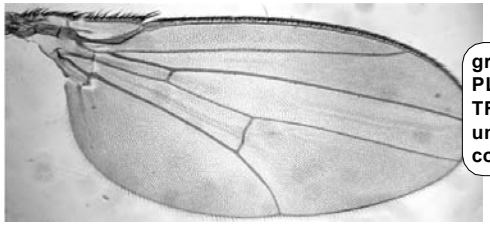


EX2 - HALOBACTERIUM SALINARUM

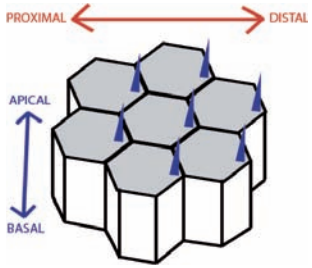
[MARWAN 2010]

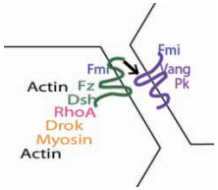


EX3 - PLANAR CELL POLARITY



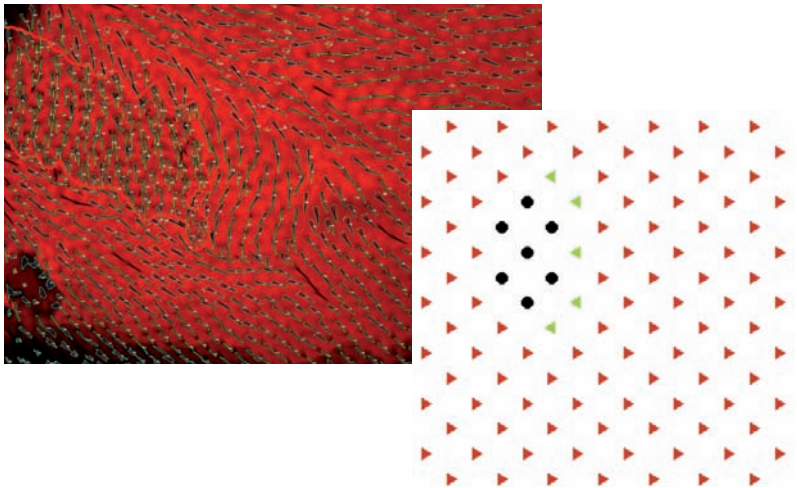
grid size:	100 x 100
PLACES:	385,000
TRANSITIONS:	410,000
unfolding:	3.8 h
cont. simulation:	18 min





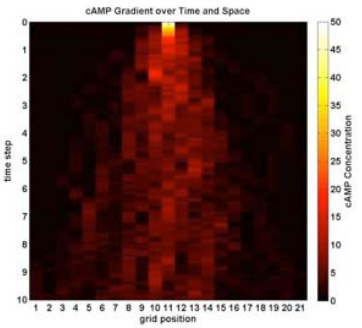
[BIOPPN 2011]
[CMSB 2011]

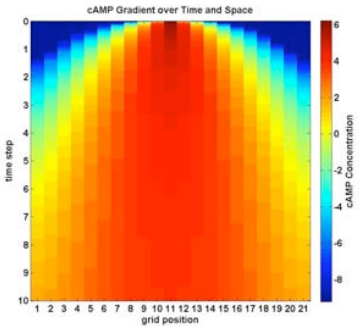
EX3 - PLANAR CELL POLARITY



EX4 - MOBILITY / MOTILITY

-> GRADIENTS





[DAGSTUHL 2011]

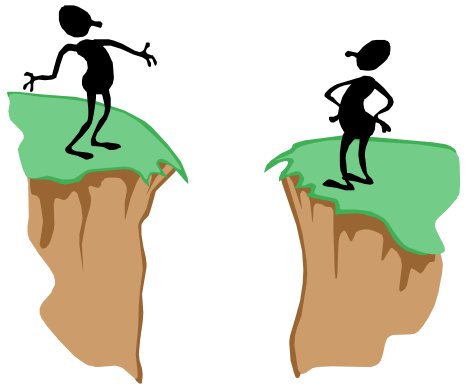
COLOURED PETRI NETS, APPLICATIONS

- **get multiple copies of patterns**
  - -> *Halo model, new order of net sizes*
- **encode locality**
  - -> *Ca channel models*
  - -> *cell tissue + communication between cells*
  - -> *motility, gradients, . . .*
- **dynamic membrane systems**
- **differentiate between submodels within a master net**
  - -> *T-invariants*
  - -> *generated models in conformance with wet-lab data*
  - -> *mutants*
  - -> *algorithmic folding*
- . . .

- **modelling patterns**
  - -> *library*
  - -> *predefined functions to address neighbours of cells*
- **model reduction**
  - -> *equal server semantics*
- **analysis / simulation on the coloured level**
  - -> *well-formed colored stochastic Petri nets (GSPN)*
- **sophisticated result visualisation**
  - -> *2D / 3D*

SUMMARY

- **representation of bio networks 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* -> *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*



THANKS !

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