Concepts for functional analysis of signaling pathways in complex networks based on Manatee invariants

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ECCB

CEF
Macromolecular Complexes
Cluster of Excellence Frankfurt

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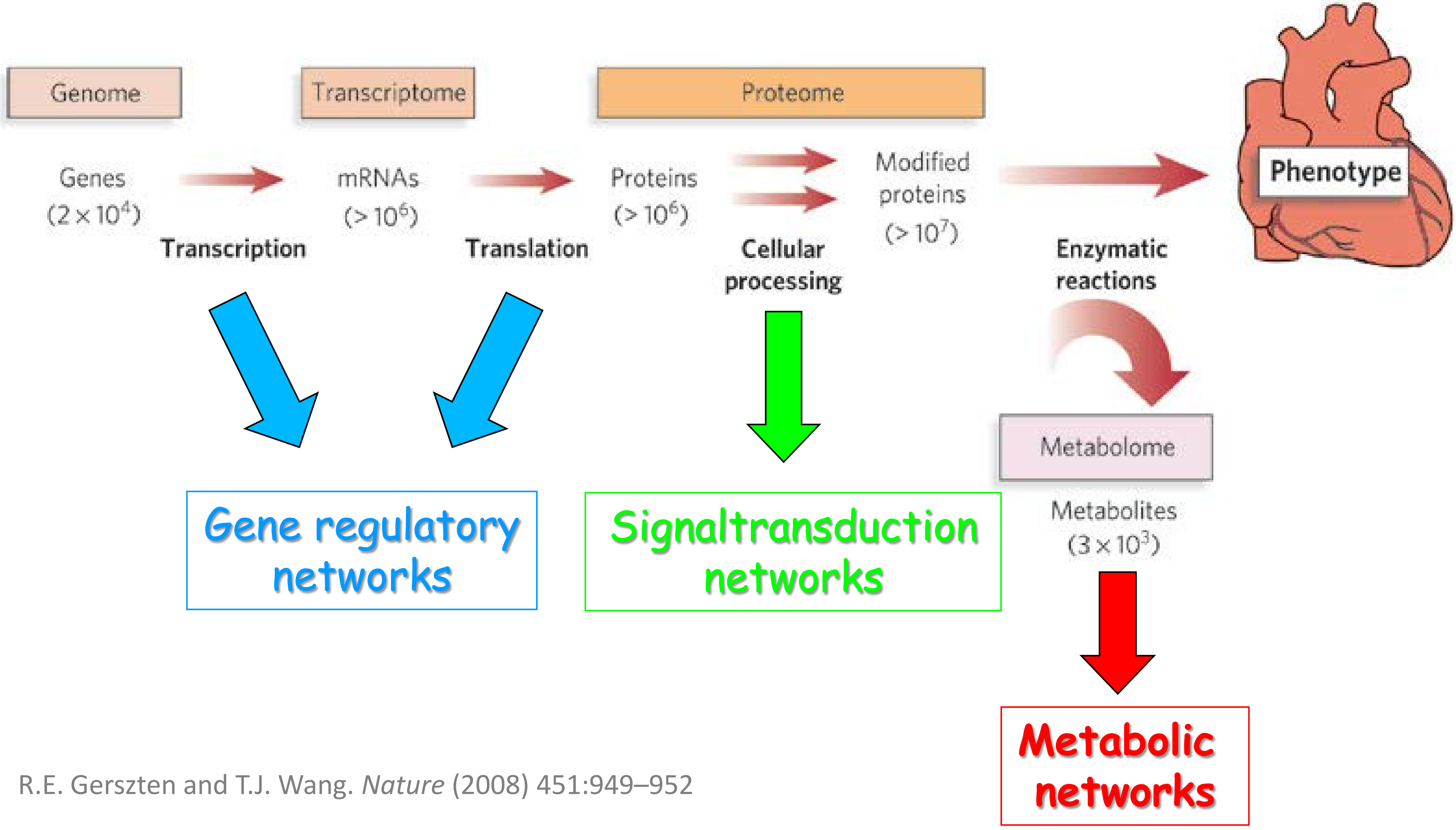
Functionality at different levels of abstraction



Genome

Transcriptome

Proteome

Genes (2×10^4)

Transcription

mRNAs $(> 10^6)$

Translation

Proteins $(> 10^6)$

Cellular processing

Modified proteins $(> 10^7)$

Enzymatic reactions

Phenotype

Metabolome

Metabolites (3×10^3)

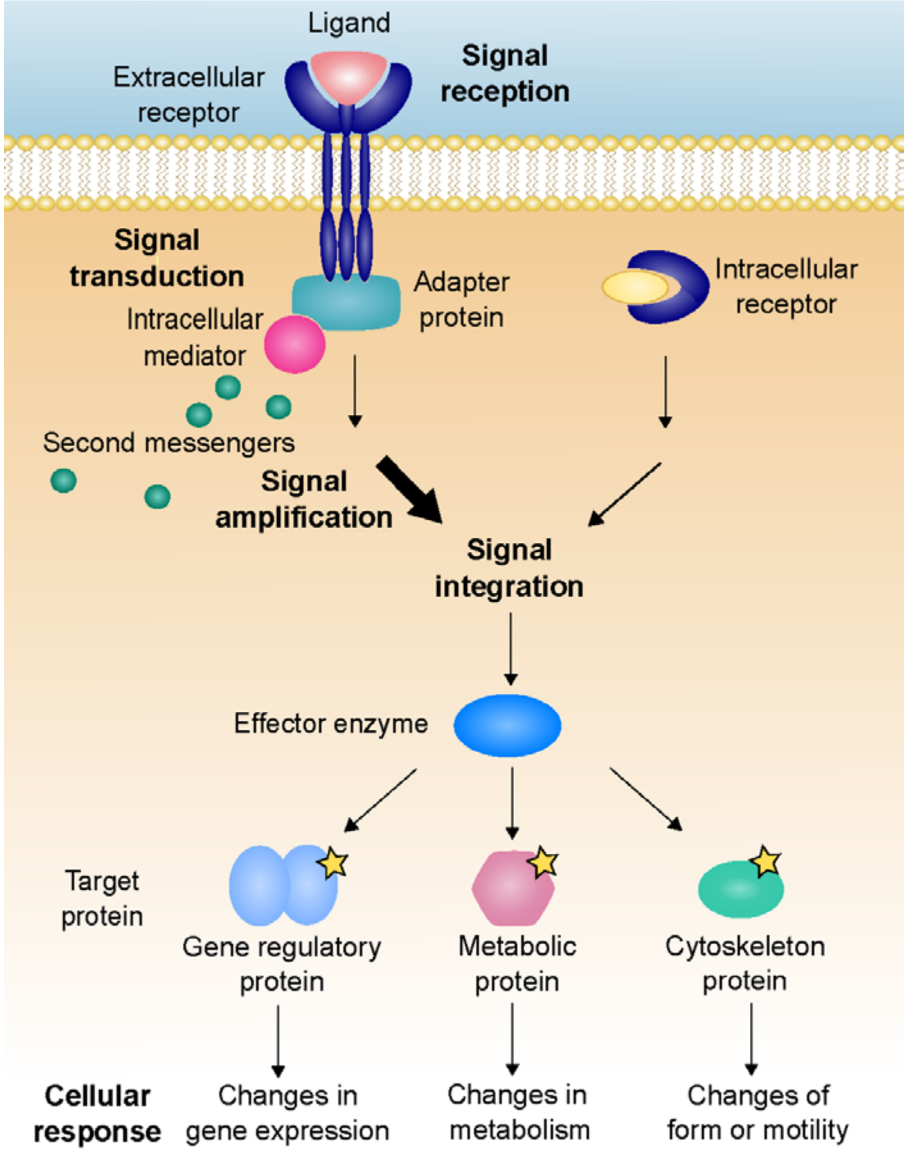
Gene regulatory networks

Signaltransduction networks

Metabolic networks

R.E. Gerszten and T.J. Wang. *Nature* (2008) 451:949–952

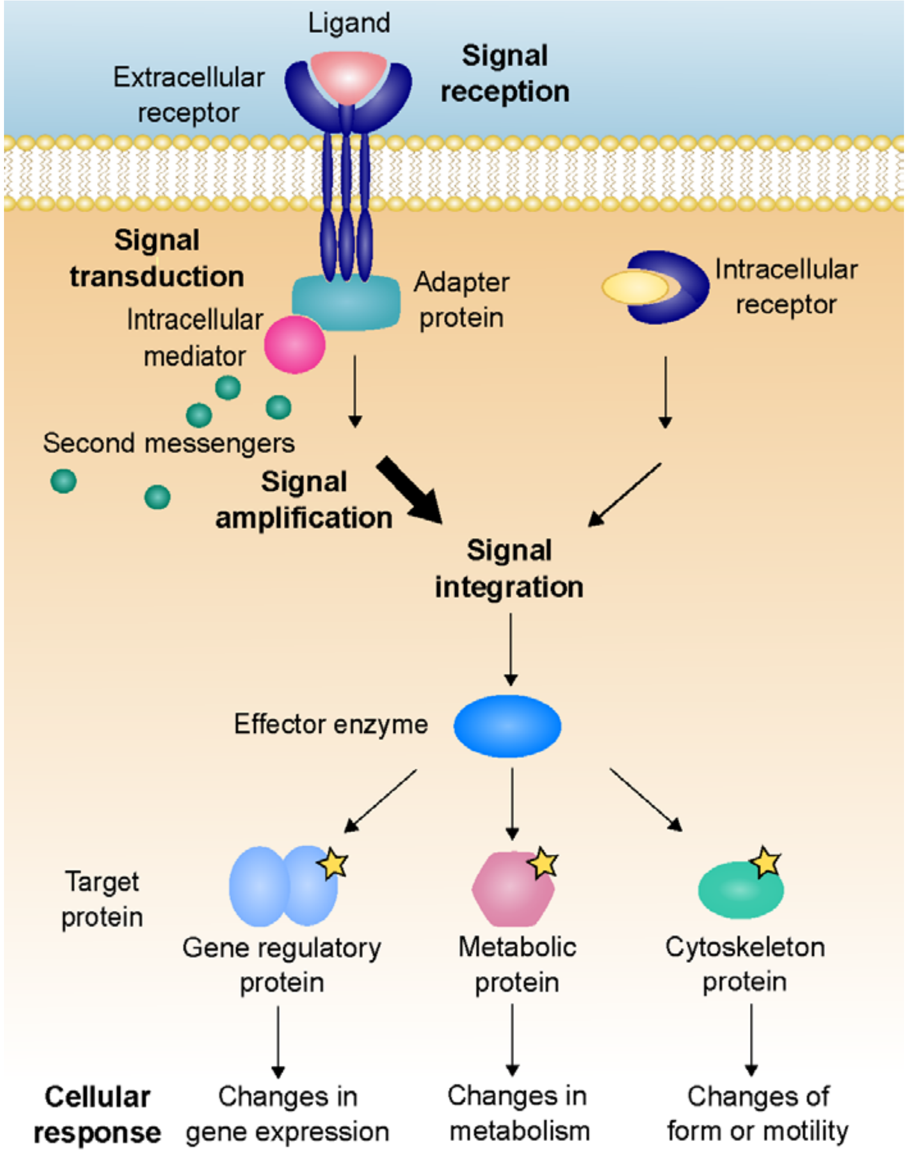
Functionality in signal transduction pathways



Leonie Amstein

Amstein *et al*. (2017)
BMC Systems Biology, 11:72

Functionality in signal transduction pathways



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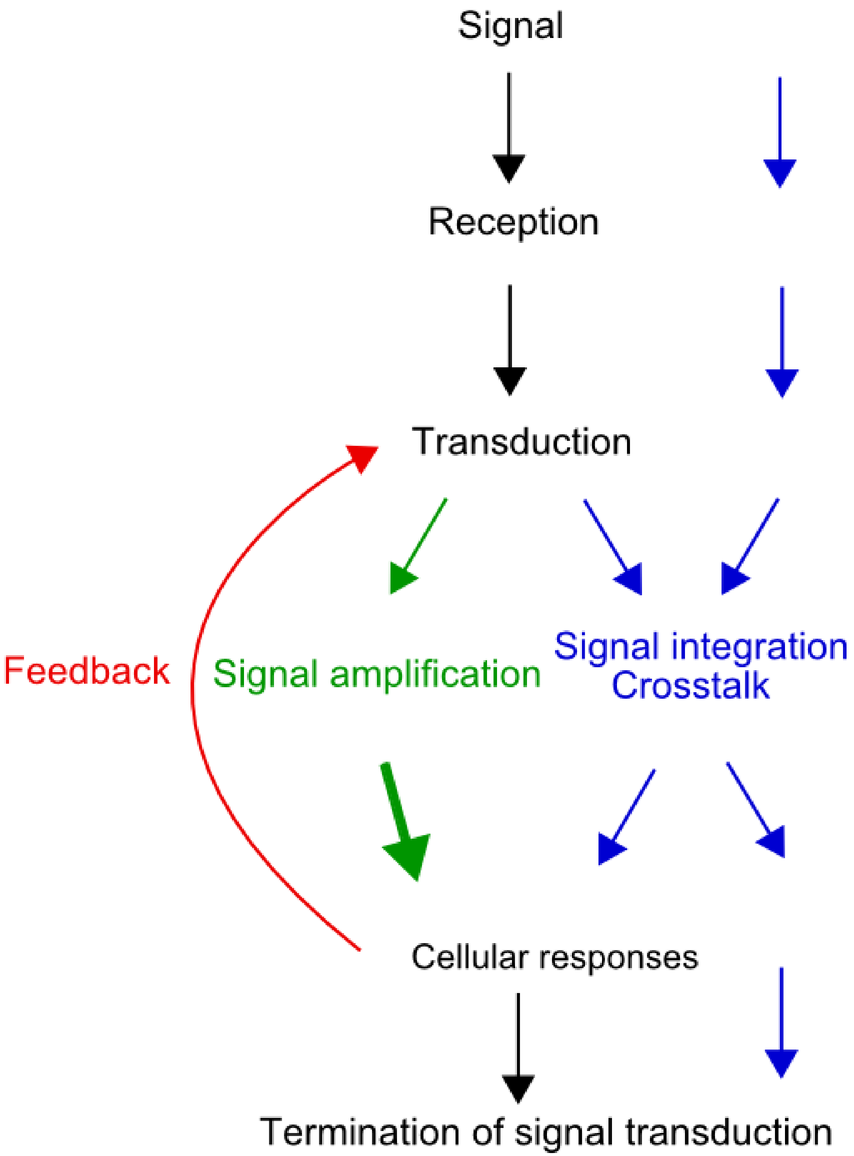


Signal

Reception

Transduction

Feedback

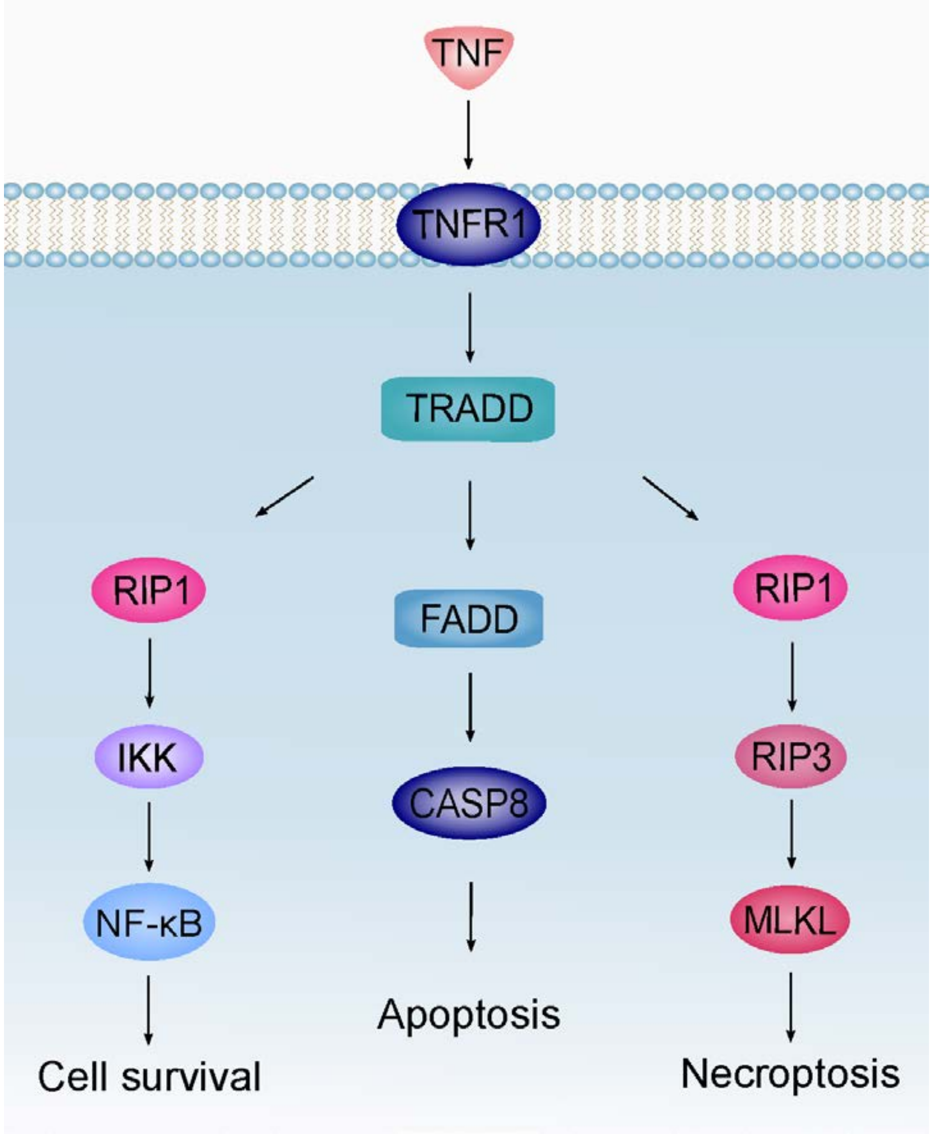
Signal amplification

Signal integration
Crosstalk

Cellular responses

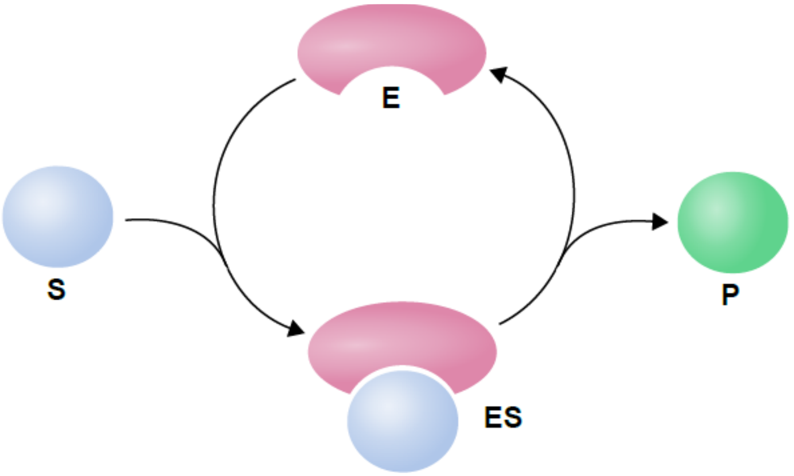
Termination of signal transduction

Overview of the TNFR1 signaling pathway

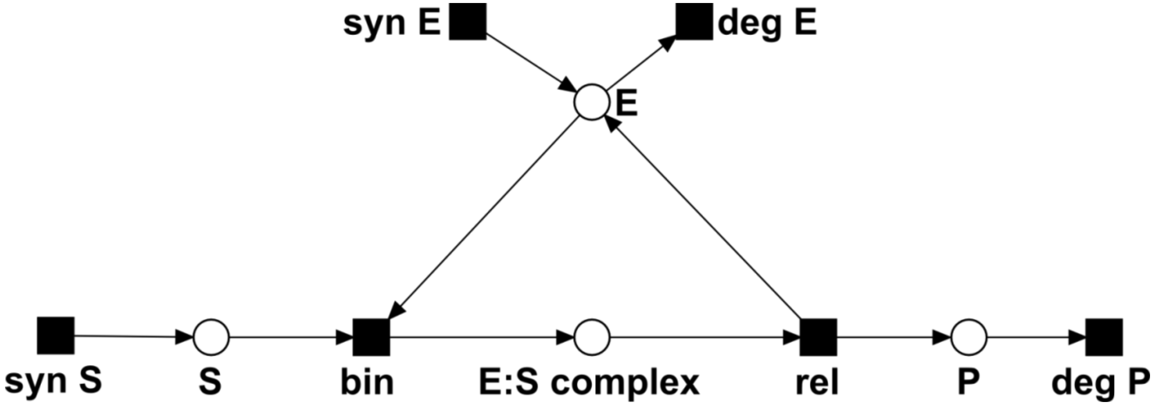


Petri net formalism

Biological process



Petri net



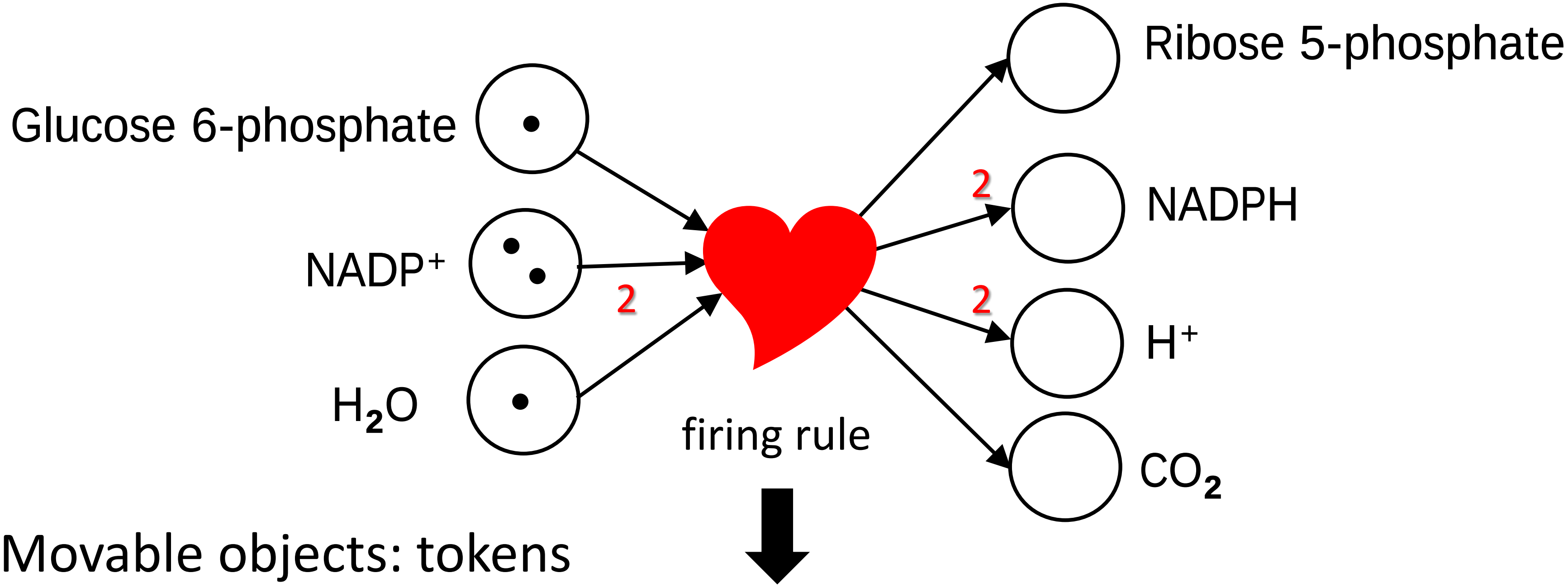
■ Transitions

○ Places

Koch, Reisig, Schreiber (2011) Modeling in Systems Biology – The Petri Net Approach, Springer

Molecular Petri nets

$\mathrm{G6P} + 2\ \mathrm{NADP}^+ + \mathrm{H_2O} \rightarrow \mathrm{R5P} + 2\ \mathrm{NADPH} + 2\ \mathrm{H}^+ + \mathrm{CO_2}$



Movable objects: tokens

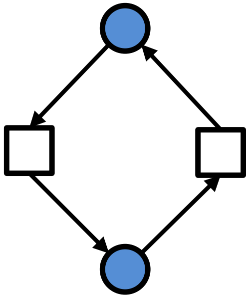
Untimed: classical P/T net
timed-discrete
stochastic (random variables, distribution function, Gillespie’s algorithm)
continuous (ODEs, kinetic modeling)

Koch, Reisig, Schreiber (2011) Modeling in Systems Biology – The Petri Net Approach, Springer

Petri net analysis

- **Minimal place invariants (PI)**

  substance conservations at steady state



- **Minimal transition invariants (TI)**

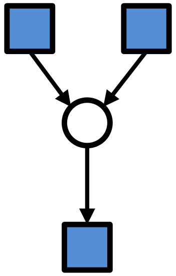
  basic functional processes at steady state

  cyclic firing sequences to the initial state

  Lautenbach, 1973, *GMD Report* No. 82

  correspond to elementary modes

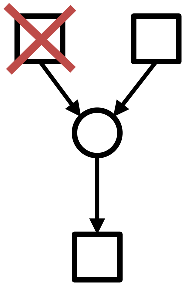
  Schuster *et al.*, 1993, *Second Gauss Symposium*



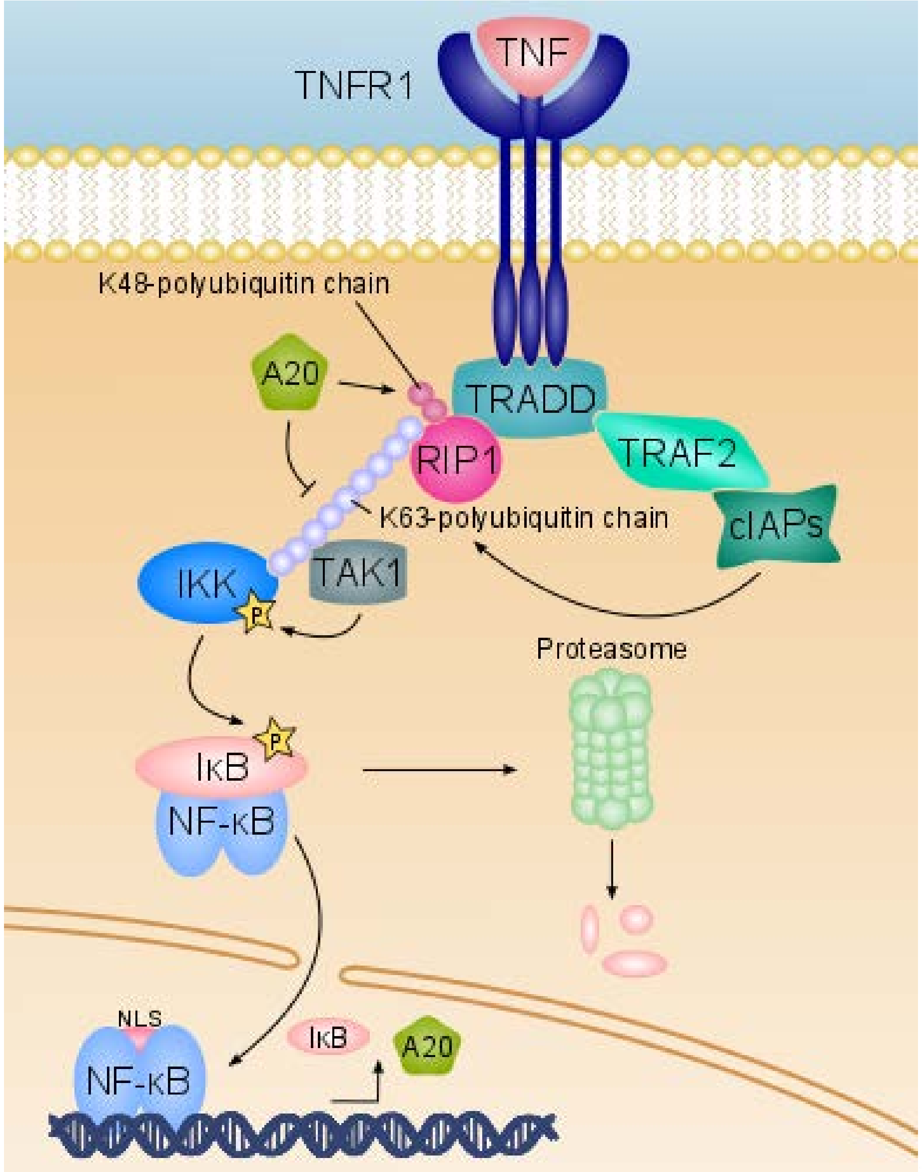
- ***In silico* knockout**

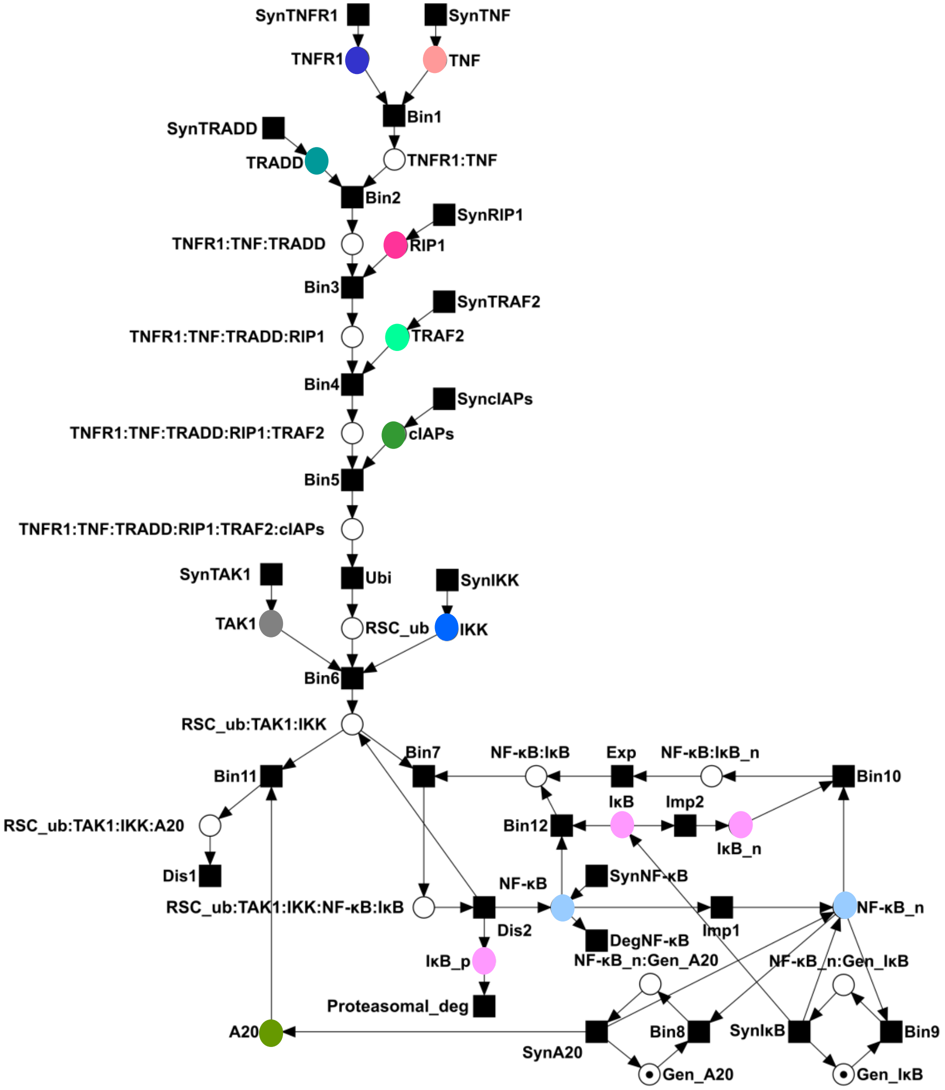
  knockout matrix at steady state

  Scheidel *et al.*, 2016, *PLoS Computational Biology,* 12(12):e1005200



Case study


TNFR1
TNF
K48-polyubiquitin chain
A20
TRADD
RIP1
TRAF2
K63-polyubiquitin chain
cIAPs
IKK
TAK1
P
Proteasome
P
IκB
NF-κB
NLS
NF-κB
IκB
A20


SynTNFR1
SynTNF
TNFR1
TNF
Bin1
SynTRADD
TNFR1:TNF
TRADD
Bin2
SynRIP1
TNFR1:TNF:TRADD
RIP1
Bin3
SynTRAF2
TNFR1:TNF:TRADD:RIP1
TRAF2
Bin4
SyncIAPs
TNFR1:TNF:TRADD:RIP1:TRAF2
cIAPs
Bin5
TNFR1:TNF:TRADD:RIP1:TRAF2:cIAPs
SynTAK1
Ubi
SynIKK
TAK1
RSC_ub
IKK
Bin6
RSC_ub:TAK1:IKK
Bin7
NF-κB:IκB
Exp
NF-κB:IκB_n
Bin11
Bin10
IκB
Imp2
RSC_ub:TAK1:IKK:A20
Bin12
IκB_n
Dis1
SynNF-κB
NF-κB
RSC_ub:TAK1:IKK:NF-κB:IκB
NF-κB_n
Dis2
DegNF-κB
Imp1
IκB_p
NF-κB_n:Gen_A20
NF-κB_n:Gen_IκB
Proteasomal_deg
A20
Bin8
SynIκB
Bin9
SynA20
Gen_A20
Gen_IκB

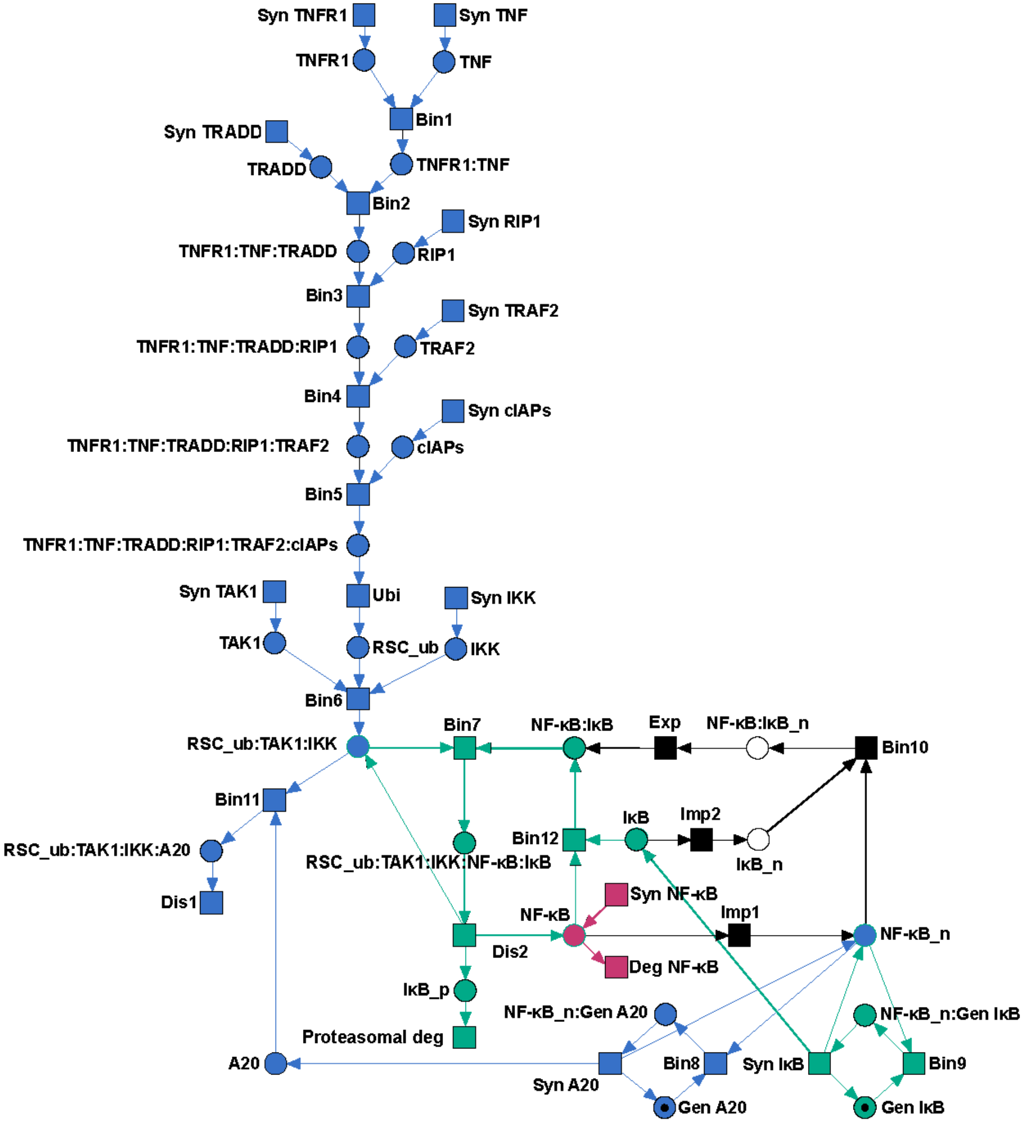
Motivation for Manatee invariants

- **Observation**: transition invariants (TI) are unable to represent signal flows from the receptor to the cell response in networks with cycles

- **Aim**: mathematical concept to compute all signal flows from the receptor to the cell response

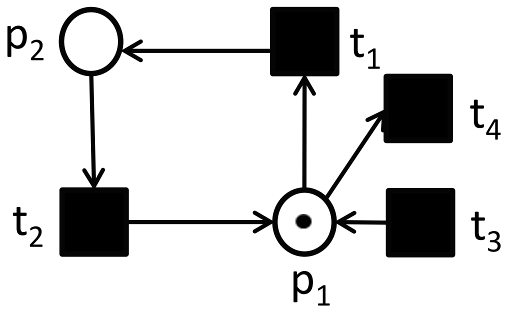
- **Method**: linear combination of TI to get Manatee invariants (MI) based on feasible TI

  Sackmann *et al.*, 2006, *BMC Bioinformatics* 7(1):482



Feasible transition invariants

A TI is feasible if all transitions of the TI can fire as firing sequence in the initial marking m_0.



$m_0 = (0, 0)$:
$TI_1 = (t_1, t_{2,})$ not feasible
$TI_2 = (t_3, t_4)$ feasible

$m_0 = (1, 0)$:
TI_1, TI_2 → feasible

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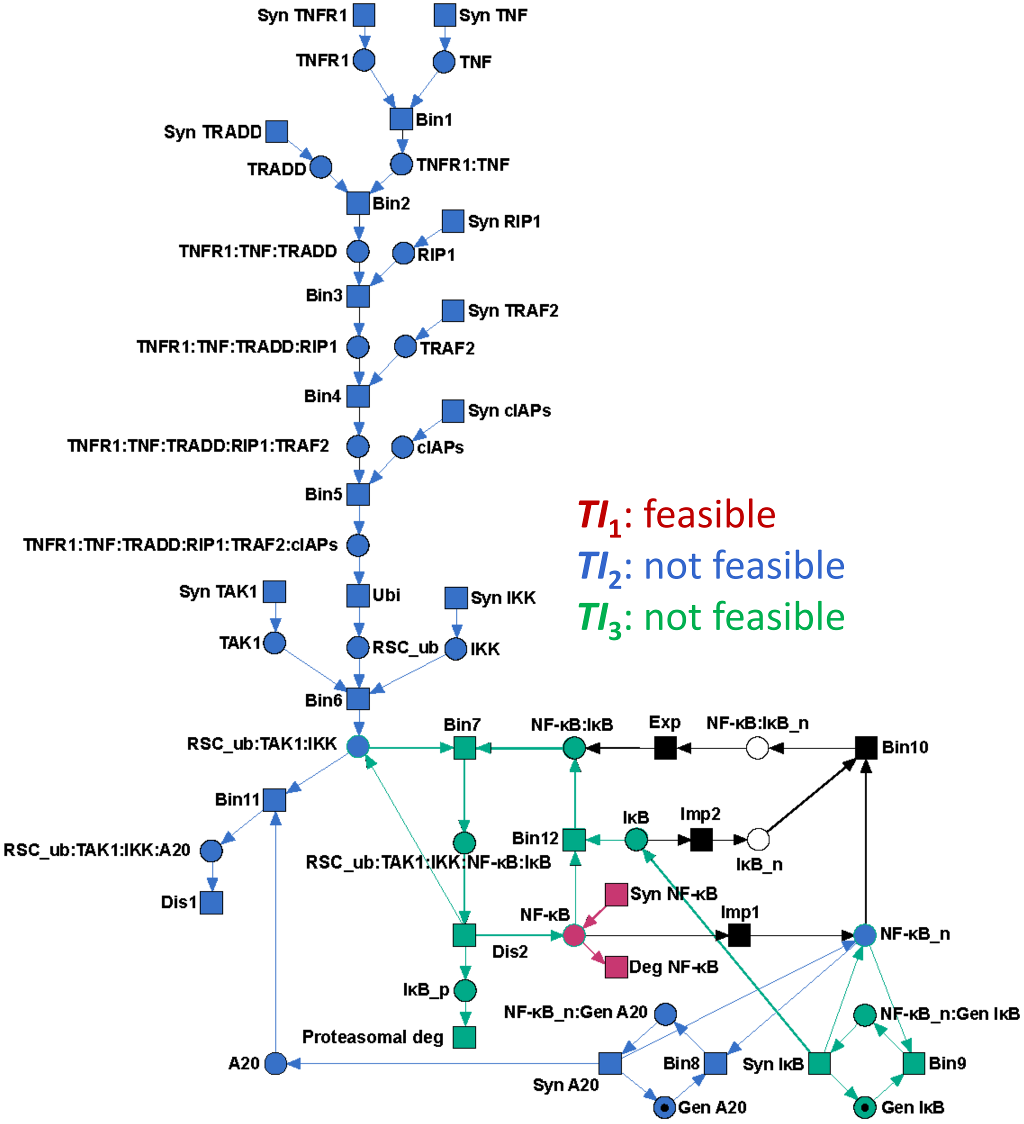

***TI*$_1$**: feasible

***TI*$_2$**: not feasible

***TI*$_3$**: not feasible

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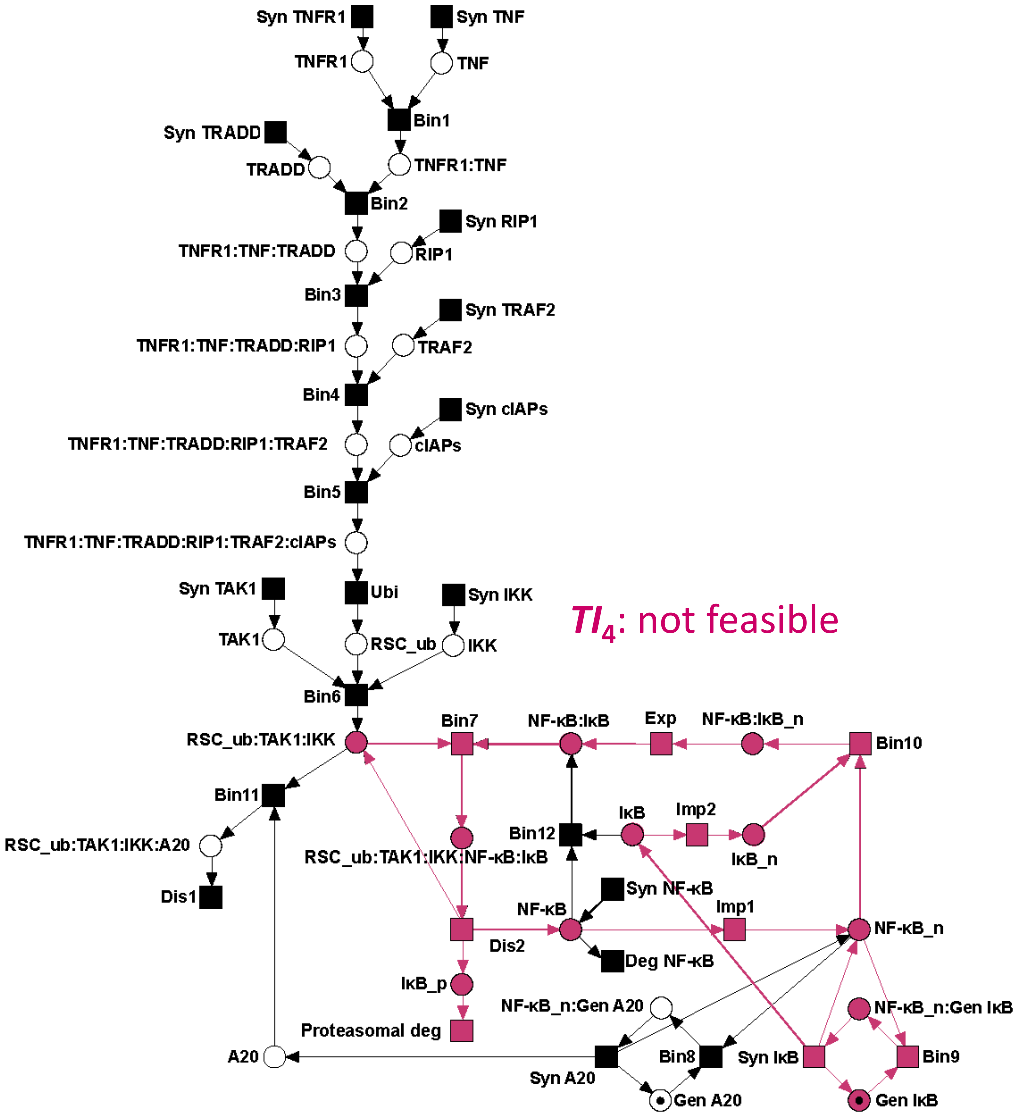
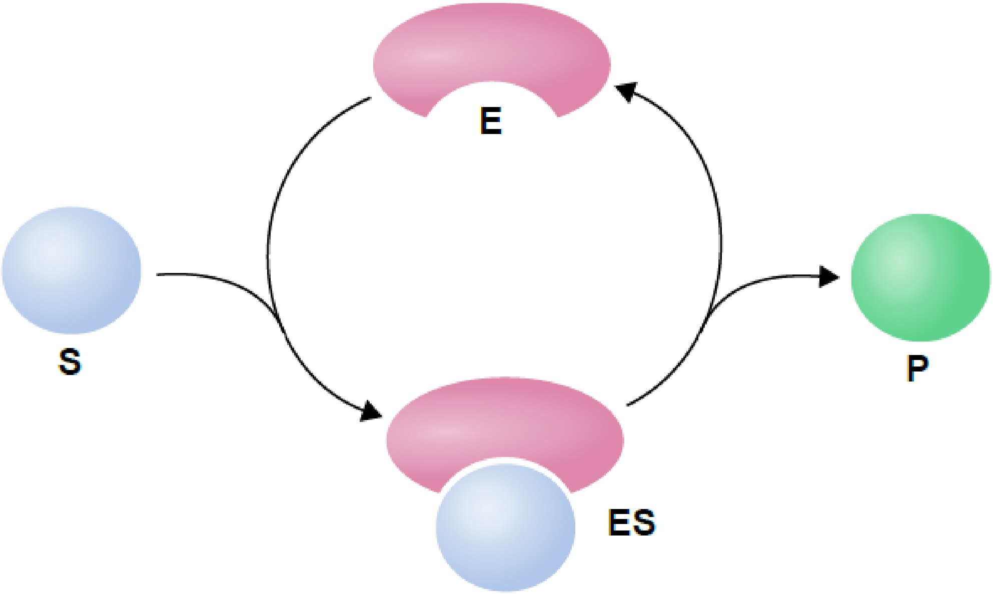
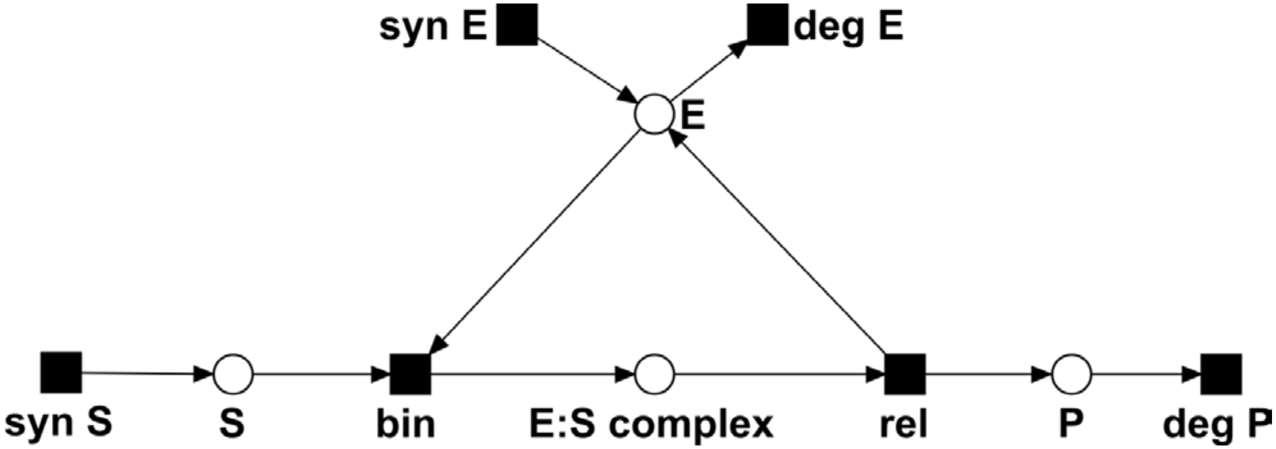


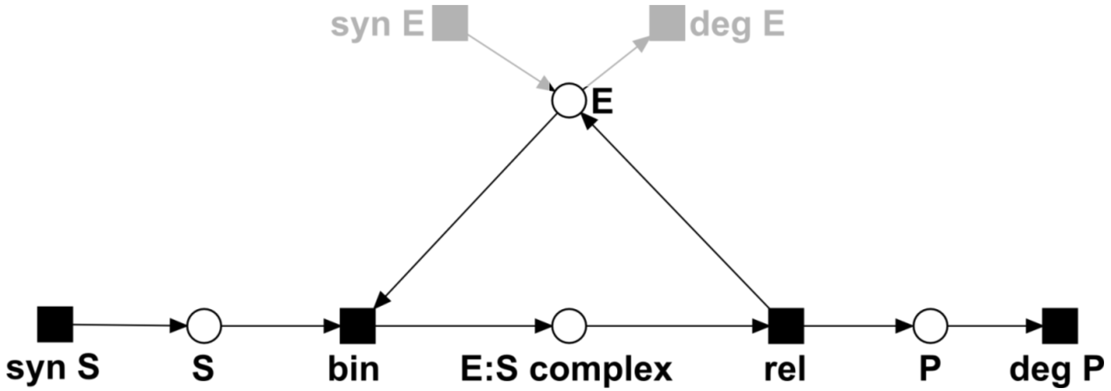
TI_4: not feasible

Enzyme-catalyzed reaction





Enzyme-catalyzed reaction

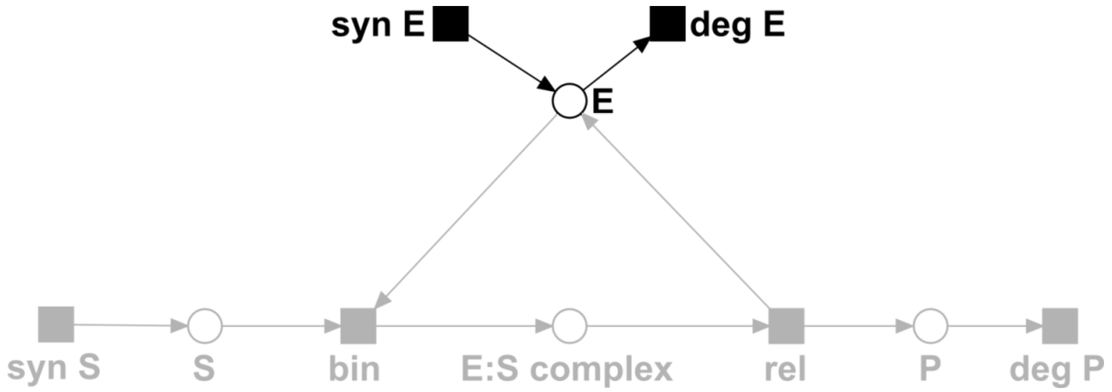


The TI_1-induced network

Transition invariants:

TI_1 = (syn S, bin, rel, deg P)

Enzyme-catalyzed reaction



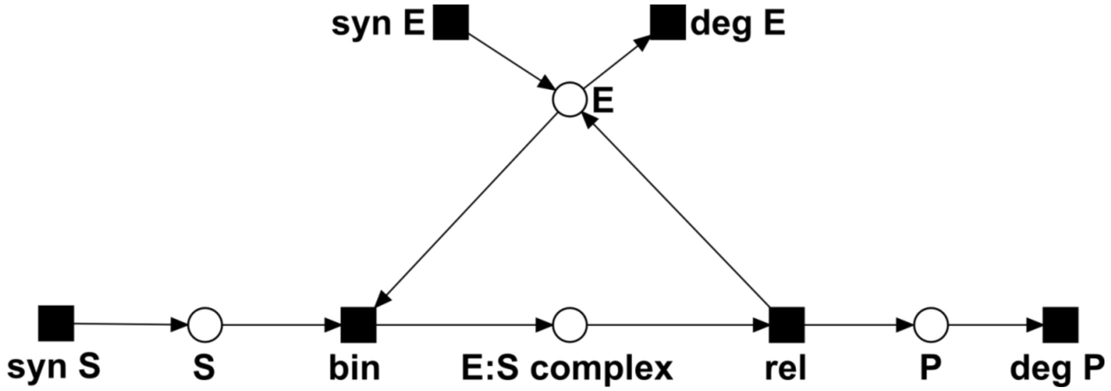
The TI_2-induced network

Transition invariants:

TI_1 = (syn S, bin, rel, deg P)

TI_2 = (syn E, deg E)

Enzyme-catalyzed reaction



Transition invariants:

TI_1 = (syn S, bin, rel, deg P)

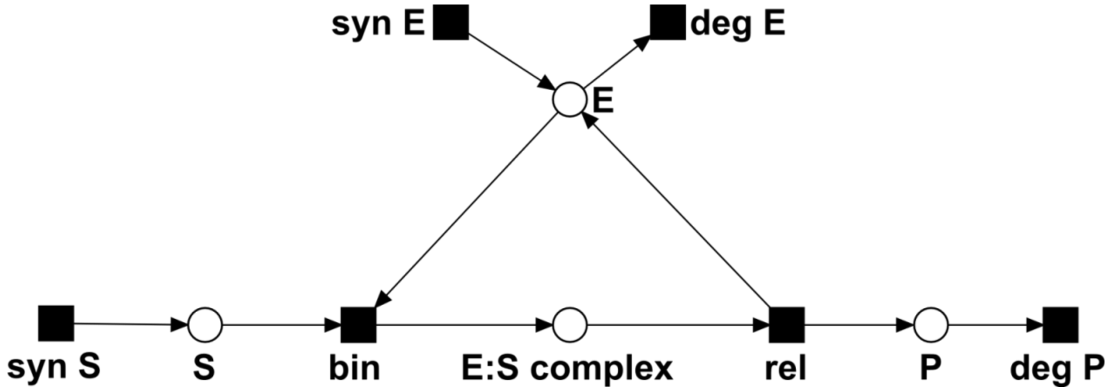
TI_2 = (syn E, deg E)

- Both processes are biologically interrelated
- TI_1 is dependent on TI_2

For m_0 = (0,0,0,0):

- TI_1 = not feasible
- TI_2 = feasible

Place invariant analysis

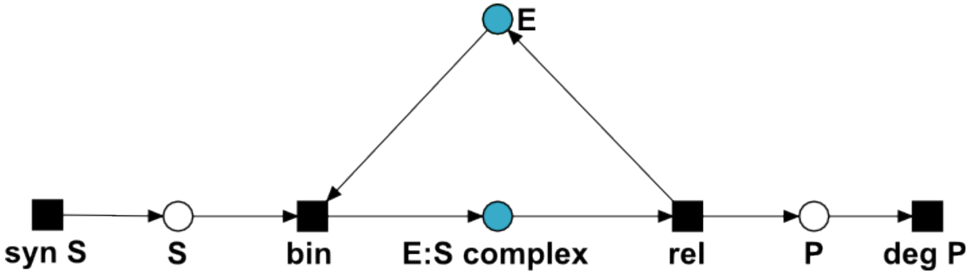


Transition invariants:

TI_1 = (syn S, bin, rel, deg P)

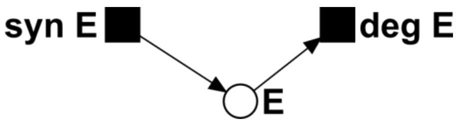
TI_2 = (syn E, deg E)

TI_1 – induced network



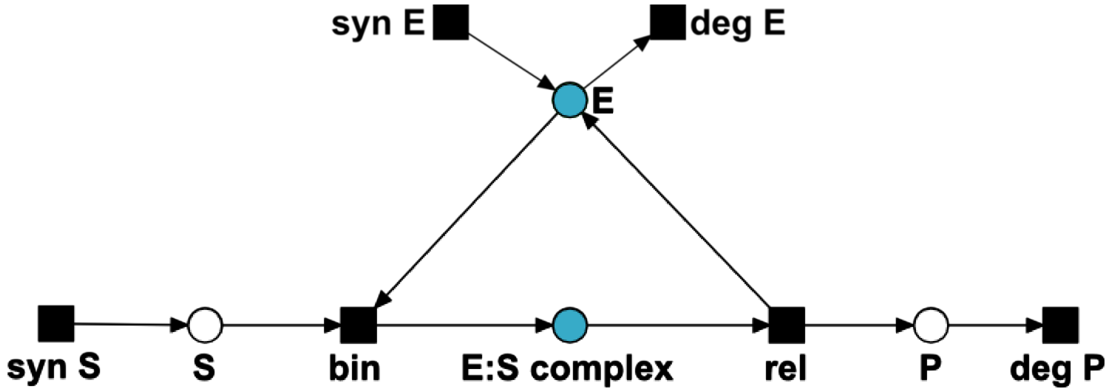
PI (TI_1) = (E, E:S complex) → **not feasible**

TI_2 – induced network



PI-free → **feasible**

Construction of Manatee invariants



Transition invariants:

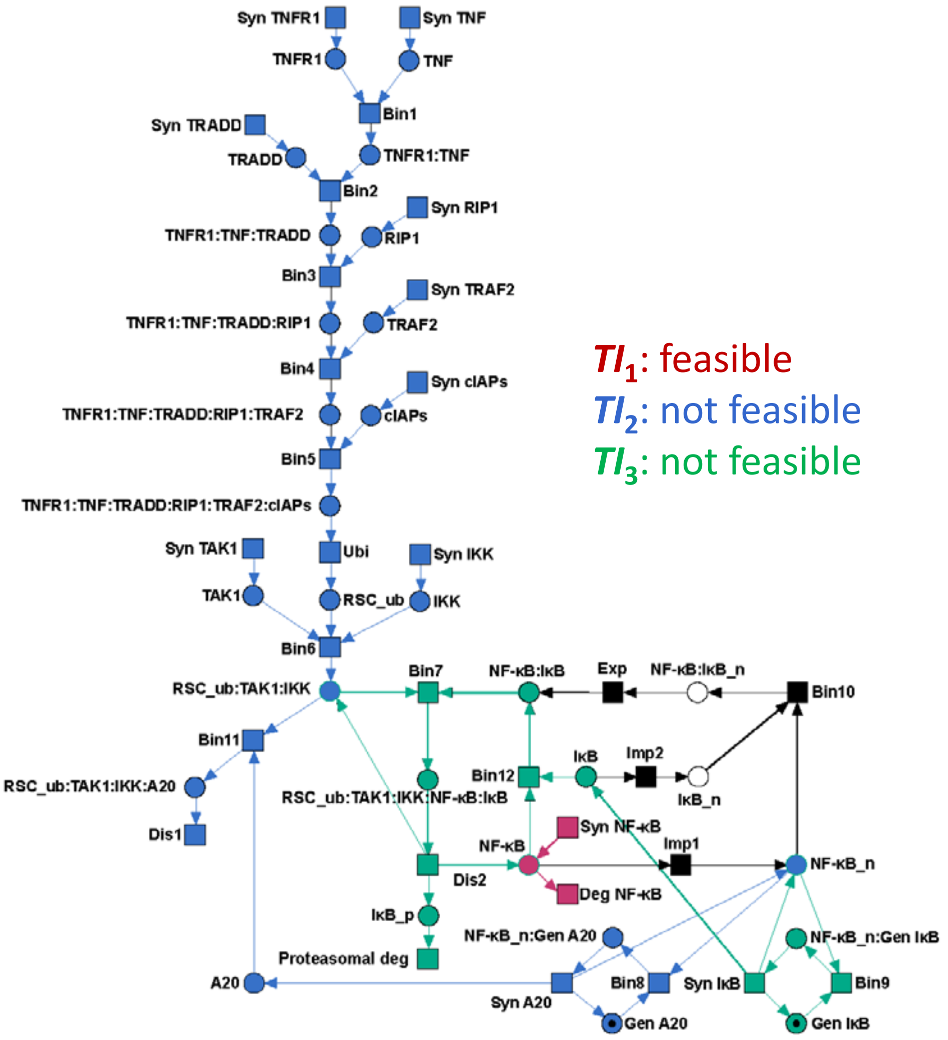
TI_1 = (syn S, bin, rel, deg P)

TI_2 = (syn E, deg E)

Manatee invariants	**PI-free MI-induced network**	**feasible**
$MI_1 = TI_1 + TI_2$	yes	yes
$MI_2 = TI_2$	yes	yes

Manatee invariants are linear combinations of TI that induce PI-free networks!

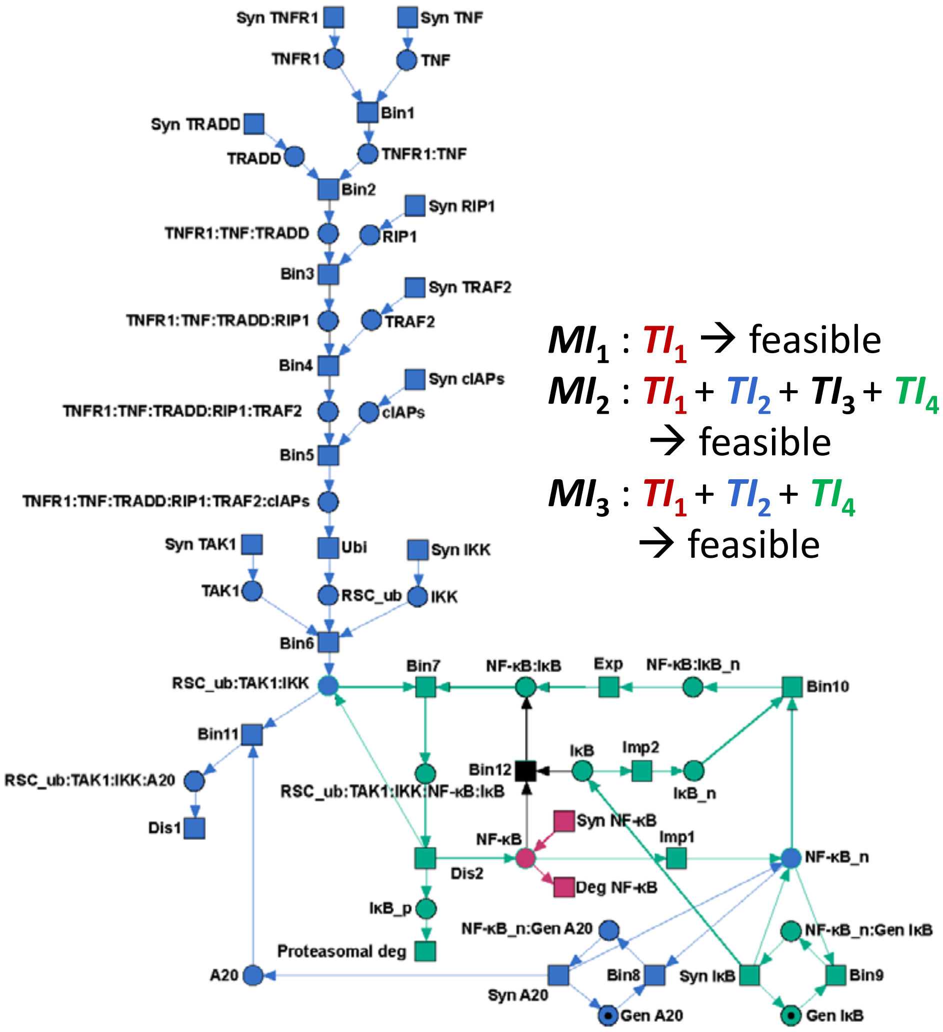
Signal flows described by Manatee invariants



TI_1: feasible

TI_2: not feasible

TI_3: not feasible



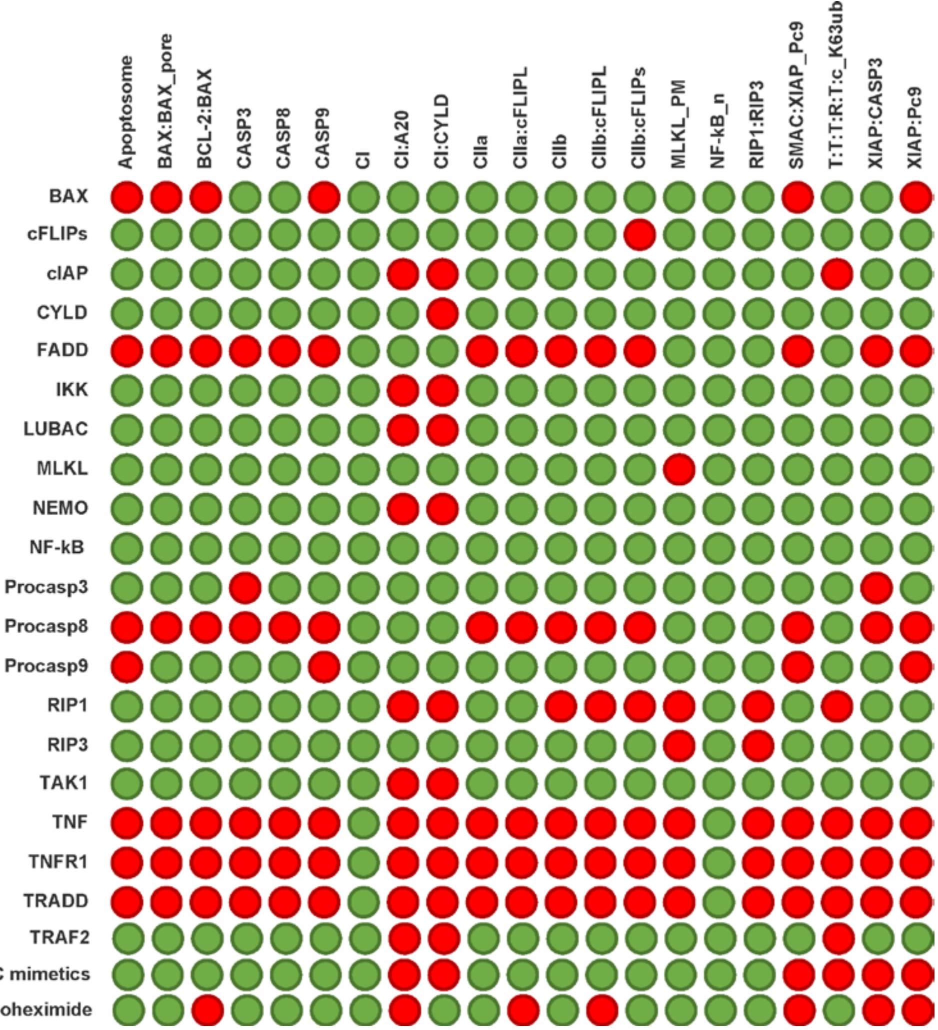
MI_1 : TI_1 → feasible

MI_2 : $TI_1 + TI_2 + TI_3 + TI_4$
→ feasible

MI_3 : $TI_1 + TI_2 + TI_4$
→ feasible

Knockout analysis of TNFR1 signaling

Based on transition invariants (48)



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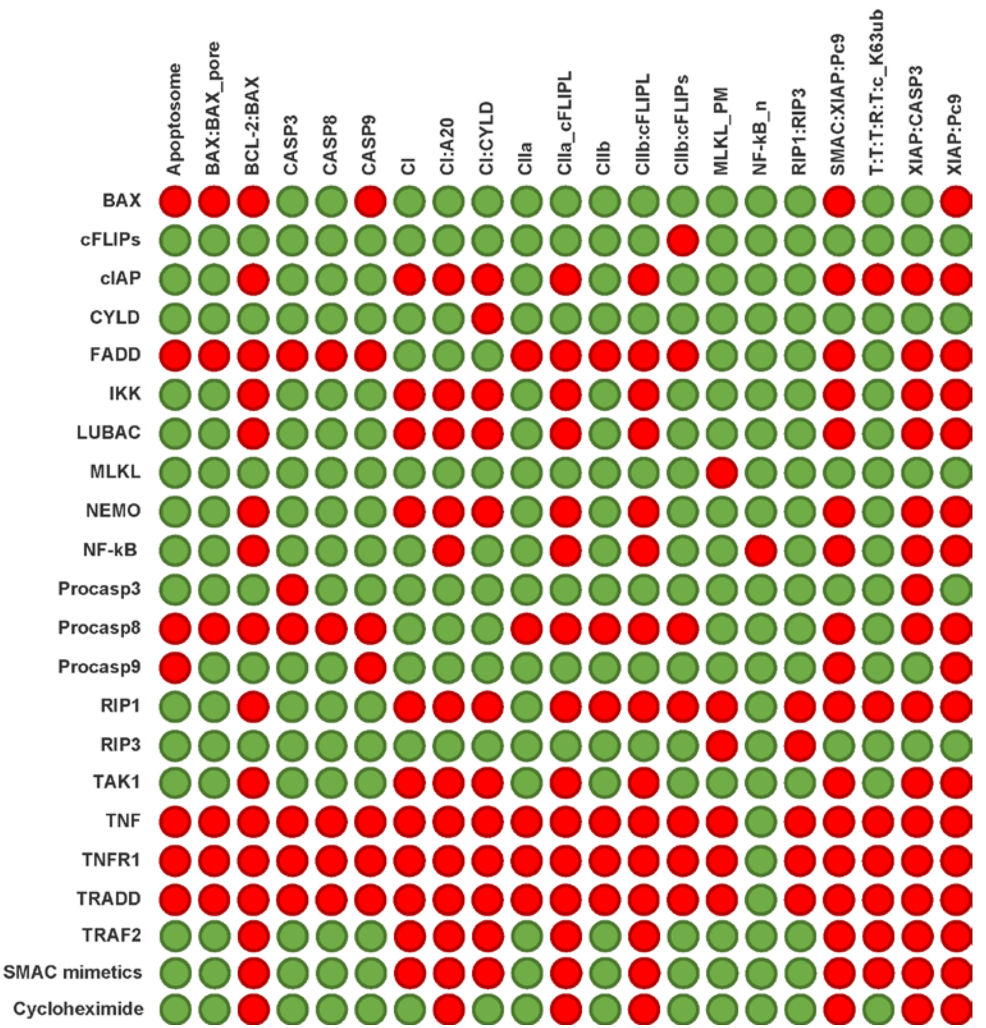
BAX	🔴	🔴	🔴	🟢	🟢	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🔴
cFLIPs	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢
cIAP	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🟢
CYLD	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
FADD	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🟢	🟢	🔴	🔴	🔴	🔴	🔴	🟢	🟢	🟢	🔴	🟢	🔴	🔴
IKK	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
LUBAC	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
MLKL	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🟢	🟢	🟢	🟢
NEMO	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
NF-kB	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
Procasp3	🟢	🟢	🟢	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢
Procasp8	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🟢	🟢	🔴	🔴	🔴	🔴	🔴	🟢	🟢	🟢	🔴	🟢	🔴	🔴
Procasp9	🔴	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🔴
RIP1	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🔴	🟢	🟢	🔴	🔴	🔴	🔴	🟢	🔴	🟢	🔴	🟢	🟢
RIP3	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🔴	🟢	🟢	🟢	🟢
TAK1	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
TNF	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🔴	🔴	🔴	🔴	🔴
TNFR1	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🔴	🔴	🔴	🔴	🔴
TRADD	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🔴	🔴	🔴	🔴	🔴
TRAF2	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🟢
mimetics	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🔴	🔴	🔴
oheximide	🟢	🟢	🔴	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🔴	🟢	🔴	🟢	🟢	🟢	🟢	🔴	🟢	🔴	🔴

Based on Manatee invariants (279)



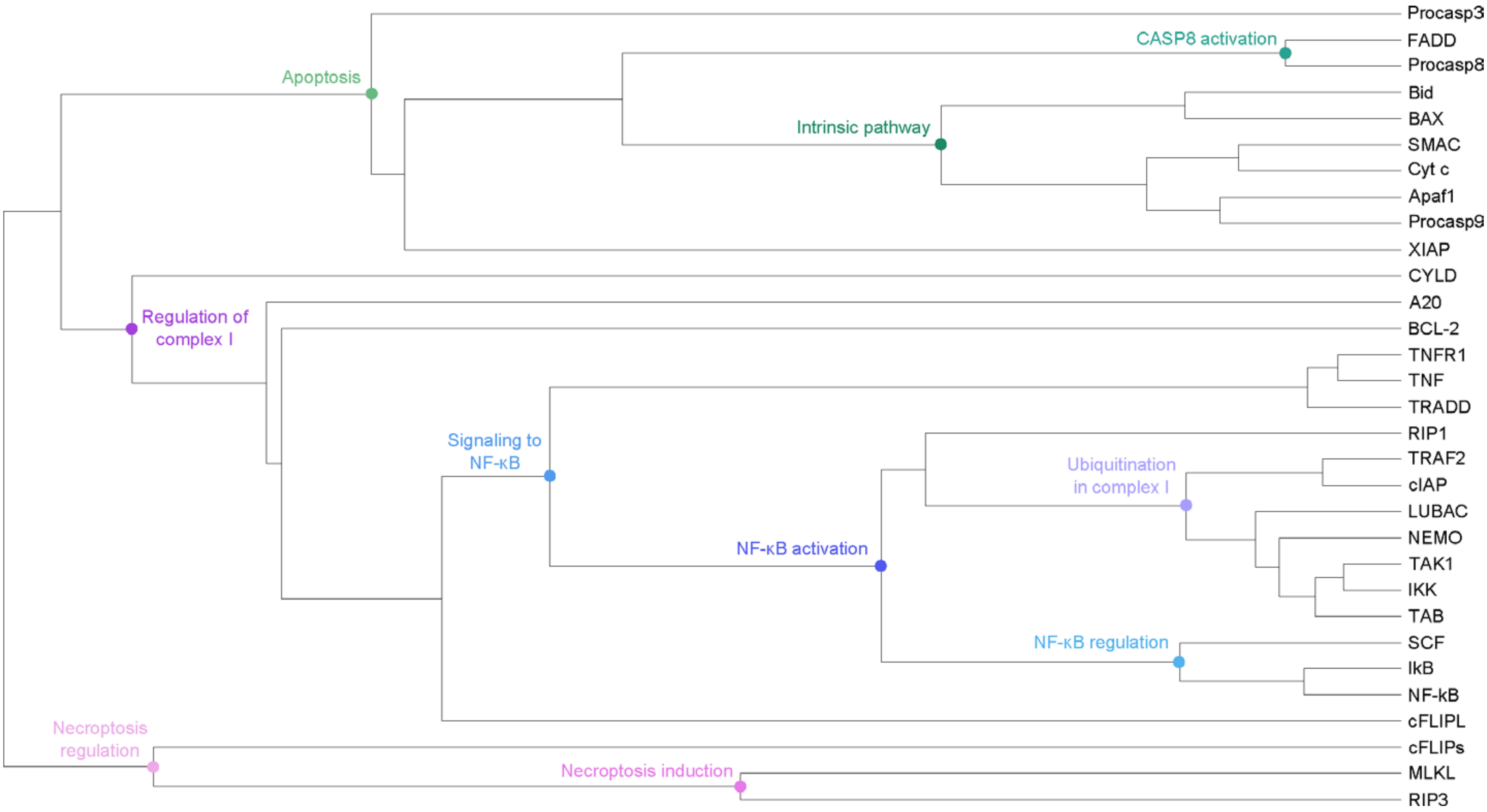
	Apoptosome	BAX:BAX_pore	BCL-2:BAX	CASP3	CASP8	CASP9	CI	CI:A20	CI:CYLD	CIIa	CIIa_cFLIPL	CIIb	CIIb:cFLIPL	CIIb:cFLIPs	MLKL_PM	NF-kB_n	RIP1:RIP3	SMAC:XIAP:Pc9	T:T:T:R:T:c_K63ub	XIAP:CASP3	XIAP:Pc9
BAX	🔴	🔴	🔴	🟢	🟢	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🔴
cFLIPs	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢
cIAP	🟢	🟢	🔴	🟢	🟢	🟢	🔴	🔴	🔴	🟢	🔴	🟢	🔴	🟢	🟢	🟢	🟢	🔴	🔴	🔴	🔴
CYLD	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
FADD	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🟢	🟢	🔴	🔴	🔴	🔴	🔴	🟢	🟢	🟢	🔴	🟢	🔴	🔴
IKK	🟢	🟢	🔴	🟢	🟢	🟢	🔴	🔴	🔴	🟢	🔴	🟢	🔴	🟢	🟢	🟢	🟢	🔴	🟢	🔴	🔴
LUBAC	🟢	🟢	🔴	🟢	🟢	🟢	🔴	🔴	🔴	🟢	🔴	🟢	🔴	🟢	🟢	🟢	🟢	🔴	🟢	🔴	🔴
MLKL	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🟢	🟢	🟢	🟢
NEMO	🟢	🟢	🔴	🟢	🟢	🟢	🔴	🔴	🔴	🟢	🔴	🟢	🔴	🟢	🟢	🟢	🟢	🔴	🟢	🔴	🔴
NF-kB	🟢	🟢	🔴	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🔴	🟢	🔴	🟢	🟢	🔴	🟢	🔴	🟢	🔴	🔴
Procasp3	🟢	🟢	🟢	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢
Procasp8	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🟢	🟢	🔴	🔴	🔴	🔴	🔴	🟢	🟢	🟢	🔴	🟢	🔴	🔴
Procasp9	🔴	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🔴
RIP1	🟢	🟢	🔴	🟢	🟢	🟢	🔴	🔴	🔴	🟢	🔴	🔴	🔴	🔴	🔴	🟢	🔴	🔴	🔴	🔴	🔴
RIP3	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🔴	🟢	🟢	🟢	🟢
TAK1	🟢	🟢	🔴	🟢	🟢	🟢	🔴	🔴	🔴	🟢	🔴	🟢	🔴	🟢	🟢	🟢	🟢	🔴	🟢	🔴	🔴
TNF	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🔴	🔴	🔴	🔴	🔴
TNFR1	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🔴	🔴	🔴	🔴	🔴
TRADD	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🔴	🔴	🔴	🔴	🔴
TRAF2	🟢	🟢	🔴	🟢	🟢	🟢	🔴	🔴	🔴	🟢	🔴	🟢	🔴	🟢	🟢	🟢	🟢	🔴	🔴	🔴	🔴
SMAC mimetics	🟢	🟢	🔴	🟢	🟢	🟢	🔴	🔴	🔴	🟢	🔴	🟢	🔴	🟢	🟢	🟢	🟢	🔴	🔴	🔴	🔴
Cycloheximide	🟢	🟢	🔴	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🔴	🟢	🔴	🟢	🟢	🟢	🟢	🔴	🟢	🔴	🔴

🟢 unaffected

🔴 affected

unpublished

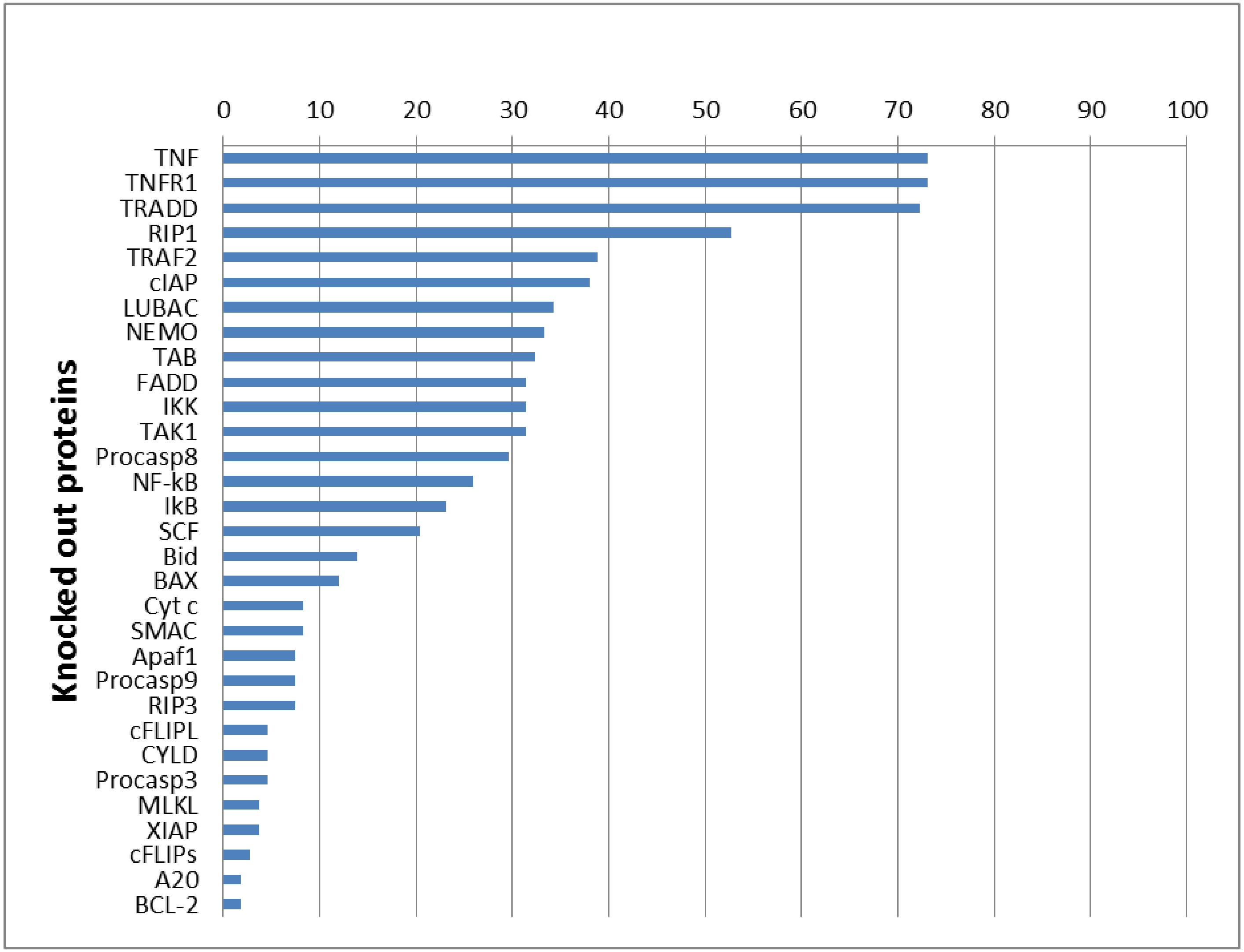
Cluster tree based on the knockout matrix



Giese *et al.*, 2015, *Bioinformatics,* 31(3):440-441

unpublished

Percentage of affected pathway entities

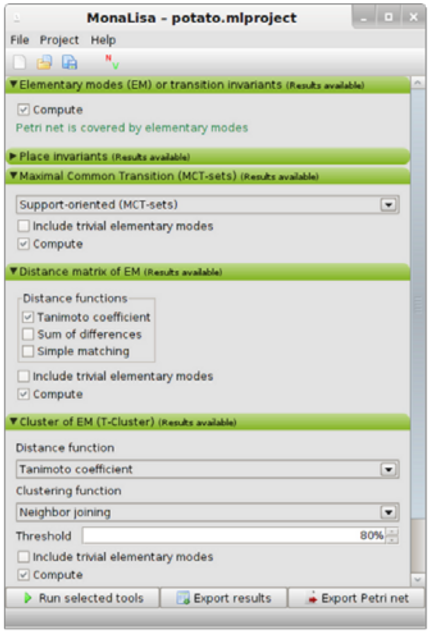


unpublished

MonaLisa

Jens Einloft, Jörg Ackermann, Joachim Nöthen, Pavel Balazki, Lilya Mirzoyan, Daniel Noll, Leonie Amstein, Aneken Laß

- ❖ Editor for biochemical Petri nets
- ❖ Network decomposition
- ❖ Knockout analysis, topological features
- ❖ Simulator for P/T nets and stochastic Petri nets



▼Elementary modes (EM) or transition invariants (Results available)

☑ Compute

Petri net is covered by elementary modes

▶ Place invariants (Results available)

▼Maximal Common Transition (MCT-sets) (Results available)

Support-oriented (MCT-sets)

☐ Include trivial elementary modes

☑ Compute

▼Distance matrix of EM (Results available)

Distance functions

☑ Tanimoto coefficient

☐ Sum of differences

☐ Simple matching

☐ Include trivial elementary modes

☑ Compute

▼Cluster of EM (T-Cluster) (Results available)

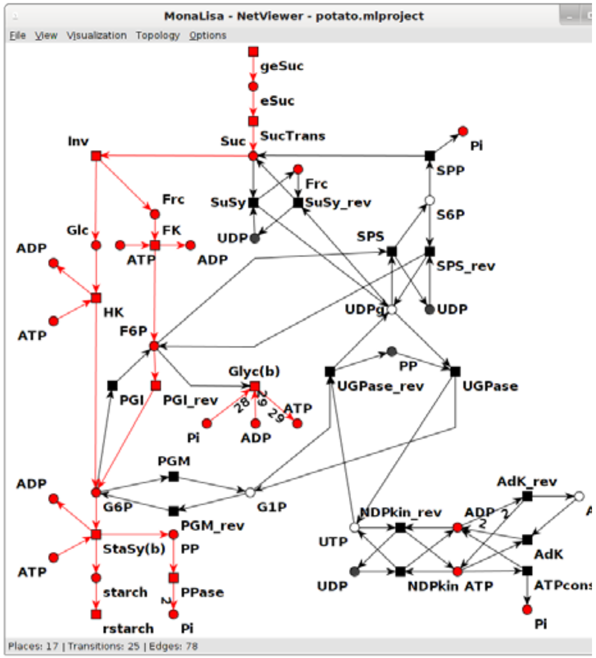
Distance function: Tanimoto coefficient

Clustering function: Neighbor joining

Threshold: 80%

☐ Include trivial elementary modes

☑ Compute



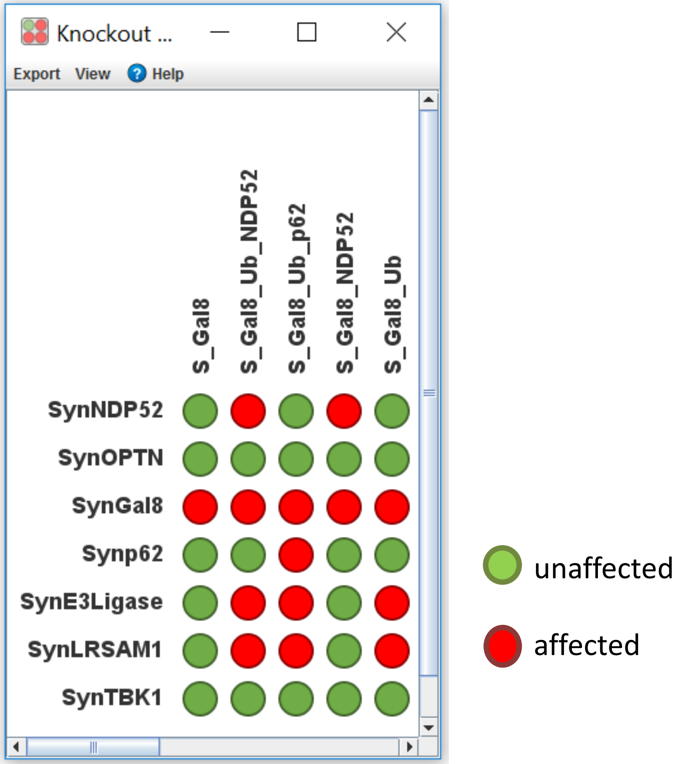
Places: 17 | Transitions: 25 | Edges: 78

Einloft *et al.* (2013) *Bioinformatics* **29**:1469-1470
Balazki *et al.* (2015) *BMC Bioinformatics* **16**:371

isiKnock

Jennifer Hannig, Heiko Giese, Börje Schweizer, Jörg Ackermann

- ❖ Tool for single and multiple *in silico* knockouts
- ❖ Graphical representation of the knockout matrix



	S_Gal8	S_Gal8_Ub_NDP52	S_Gal8_Ub_p62	S_Gal8_NDP52	S_Gal8_Ub
SynNDP52	🟢	🔴	🟢	🔴	🟢
SynOPTN	🟢	🟢	🟢	🟢	🟢
SynGal8	🔴	🔴	🔴	🔴	🔴
Synp62	🟢	🟢	🔴	🟢	🟢
SynE3Ligase	🟢	🔴	🔴	🟢	🔴
SynLRSAM1	🟢	🔴	🔴	🟢	🔴
SynTBK1	🟢	🟢	🟢	🟢	🟢

🟢 unaffected

🔴 affected

Hannig *et al.* (2018) *Bioinformatics, in press*

Conclusions

- Aim: computation of all signaling pathways from the reception to the cell response at steady state
- Use of the Petri net formalism as mathematical language
- Feasibility of transition invariants
- Manatee invariants as linear combinations of TI to attain feasibility
- Networks induced by Manatee invariants are PI-free
- Transition invariants can reveal cyclic regulations like feedback loops, whereas Manatee invariants detect complete signal flows
- The application of Manatee invariants is beneficial for models with cyclic structures, especially for signal transduction pathways, and necessary for follow-up analyses: *in silico* knockouts, cross-talks, …

Amstein *et al.*, *BMC System Biology*, 2017, 11:72

Challenges

- Integration of molecular data
  - sequence data
  - proteome data
  - protein protein interaction data
  - protein structure data
  - image data
- Hybrid modeling techniques
- Modeling signaling pathways and the dependencies on metabolism and on gene regulation
- Topological network analysis
- Visualization

Acknowledgments

Mol BI