Petri nets for the analysis of disease maps: applied to the TNFR1 signaling pathway

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To model disease systems, we have to understand host-pathogen interactions and the healthy system, too.

Challenges for construction and analysis of disease maps

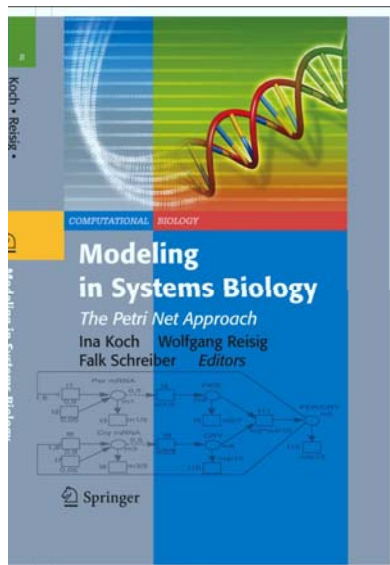**Data influence the choice of the modeling approach**
- ❖ Incomplete
- ❖ Different time points and different locations in the cell
- ❖ Different patients
- ❖ Different experiments with partly varying experimental conditions
- ❖ Different scales: genomics, transcriptomics, proteomics, metabolomics, interactomics, imaging, …

Need:
- ❖ Standards
- ❖ Scalability
- ❖ Systematic formal network verification methods
- ❖ Pathway comparison techniques
- ❖ System's state analysis

Petri nets for analysis at semi-quantitative level

Low *a priori* knowledge
Qualitative data

Logical modeling
→ Boolean modeling

Glass & Kauffmann (1973) *J. Theoretical Biology* 39:103-129
Akutsu & Miyano (1999) *Pacific Symposium on Biocomputing* 4:17
Wang *et al*. (2012) *Physical Biology* 9(5):055001
Abou-Jaoude *et al*. (2016) *Frontiers in Genetics* 7: 94

Stoichiometric modeling
→ Elementary modes, extreme pathways

Schuster & Hilgetag (1994) *Journal of Biological Systems* 2:165
Papin *et al*. (2003*) Trends in Biochemical Sciences* 28:250

Flux balance modeling
→Metabolic flux analysis

Review: Varma & Palsson (1994) *Nature Biotechnology* 12:994

Petri net modeling

Koch, Reisig, Schreiber (Eds.) (2011) Modeling in
    Systems Biology – The Petri Net Approach, Springer
→ Hybrid models

Matsuno *et al. Miyano* (2003) *In Silico Biology* 3:389

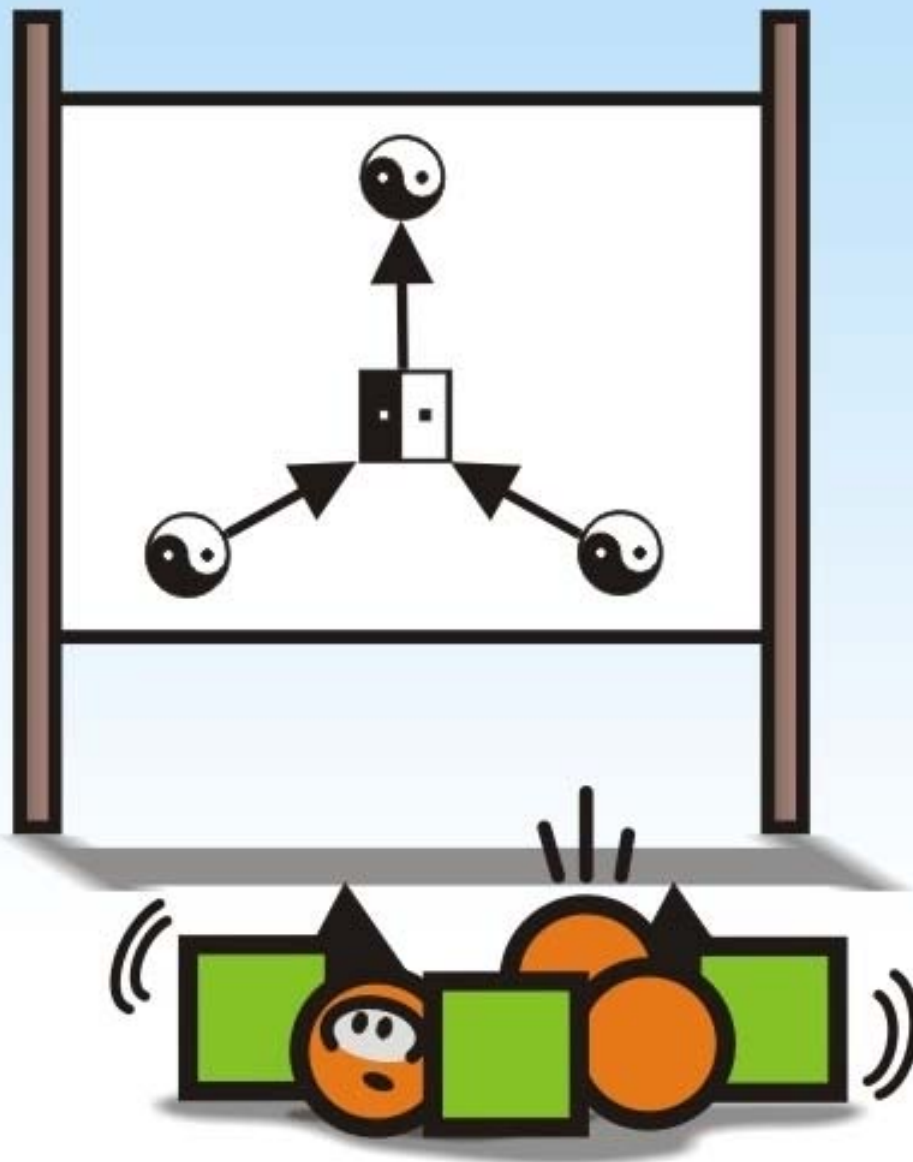**Kinetic modeling**
→ Metabolic control analysis

Kacser & Burns (1973) *Symposia of the Society Experiment Biol* 27:65
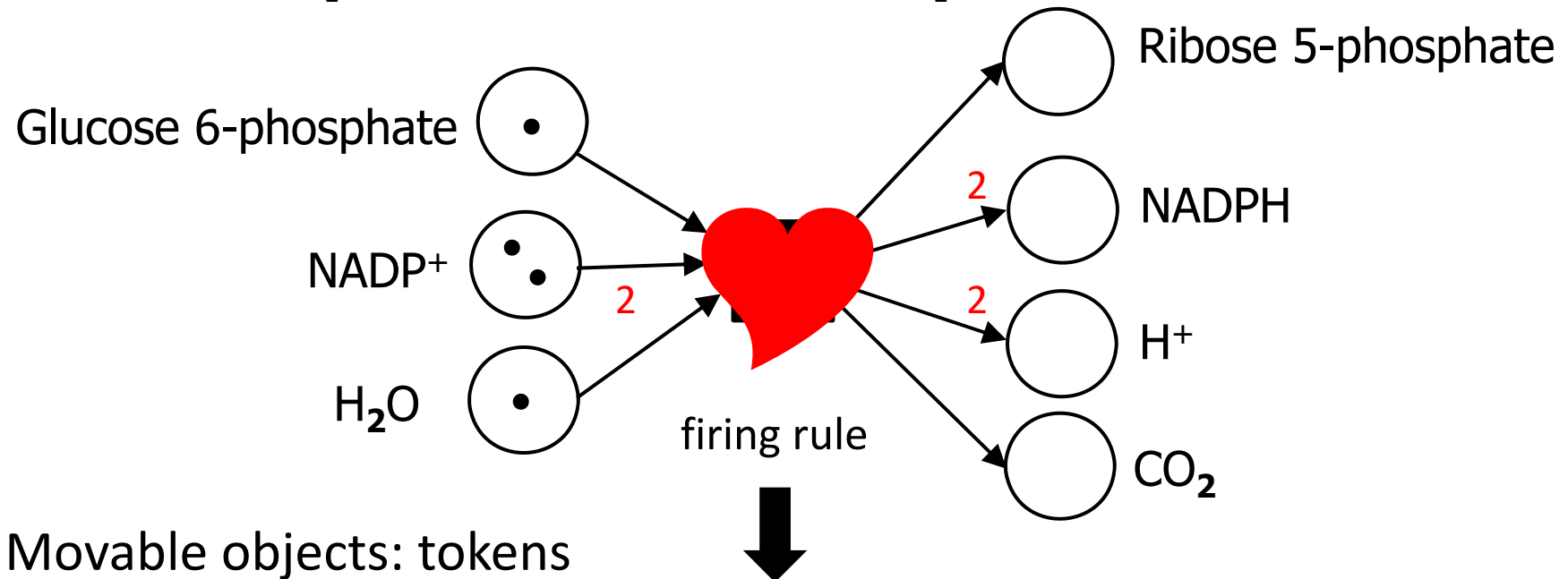Heinrich & Rapoport (1974) *European Journal Biochemistry* 42: 89, 97

High *a priori* knowledge
Kinetic data

Petri nets

❖ Mathematical theory that describes systems with **concurrent** processes

❖ Developed by **Carl Adam Petri** (1926 – 2010)

❖ Basic definitions in his **PhD thesis** at University of Technology Darmstadt in **1962**

❖ **Main applications** in computer communication, operating systems, manufacturing systems, business processes

❖ Carl Adam Petri mentioned application to **chemical networks** already in **1976**

❖ First paper on biochemical application by Venkatramana Reddy *et al.* in 1993

❖ Applications to **metabolic networks, signal transduction pathways, gene regulatory networks** using discrete, stochastic and continuous methods

Lesson I
"Petri Nets"

Molecular Petri nets

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

Glucose 6-phosphate

$NADP^+$

H_2O

2

firing rule

Ribose 5-phosphate

2 NADPH

2 H^+

CO_2

Movable objects: tokens

untimed
timed-discrete
stochastic (random variables, distribution function,
                 Gillespie's algorithm)
continuous (ODEs, kinetic modeling)

What are Petri nets good for?

Analysis: model verification, reduction, decomposition,
invariant analysis, reachability analysis, liveness

Simulation: token game, deadlocks, token accumulation

Strength:
❖ Different levels of abstraction (Boolean, discrete untimed & timed, stochastic, continuous) → **hybrid models**

❖ Flexibility in changing firing concepts (e.g., Fuzzy logics)

❖ Intuitive visualization and animation combined with analysis and simulation

❖ Easy knockout analysis

Petri net analysis at steady state

❖ **Minimal place invariants (PI)**
  Lautenbach (1973) *GMD Report* No. 82
  substance conservations

❖ **Minimal transition invariants (TI)**
  Lautenbach (1973) *GMD Report* No. 82
  basic functional processes, cyclic firing sequences
  to the initial state, correspond to elementary modes
  Schuster *et al.* (1993) *Second Gauss Symposium*
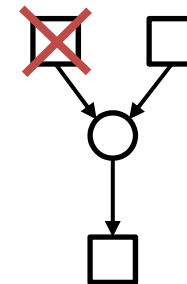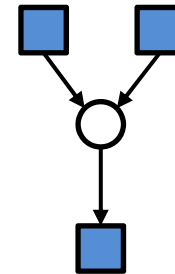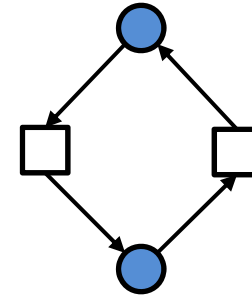
❖ **Manatee invariants (MI)**
  Amstein et al. (2017) BMC Systems Biology 11:72
  linear combinations of TI to represent a complete
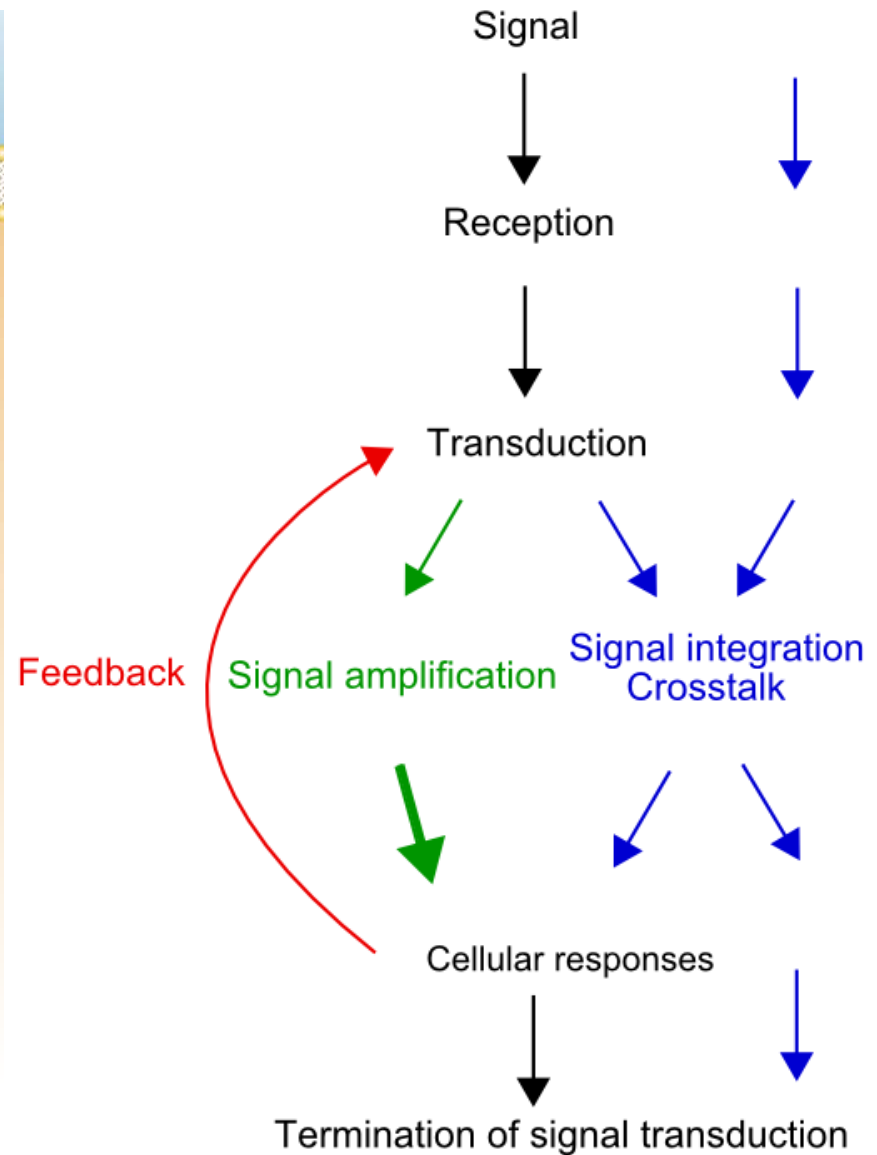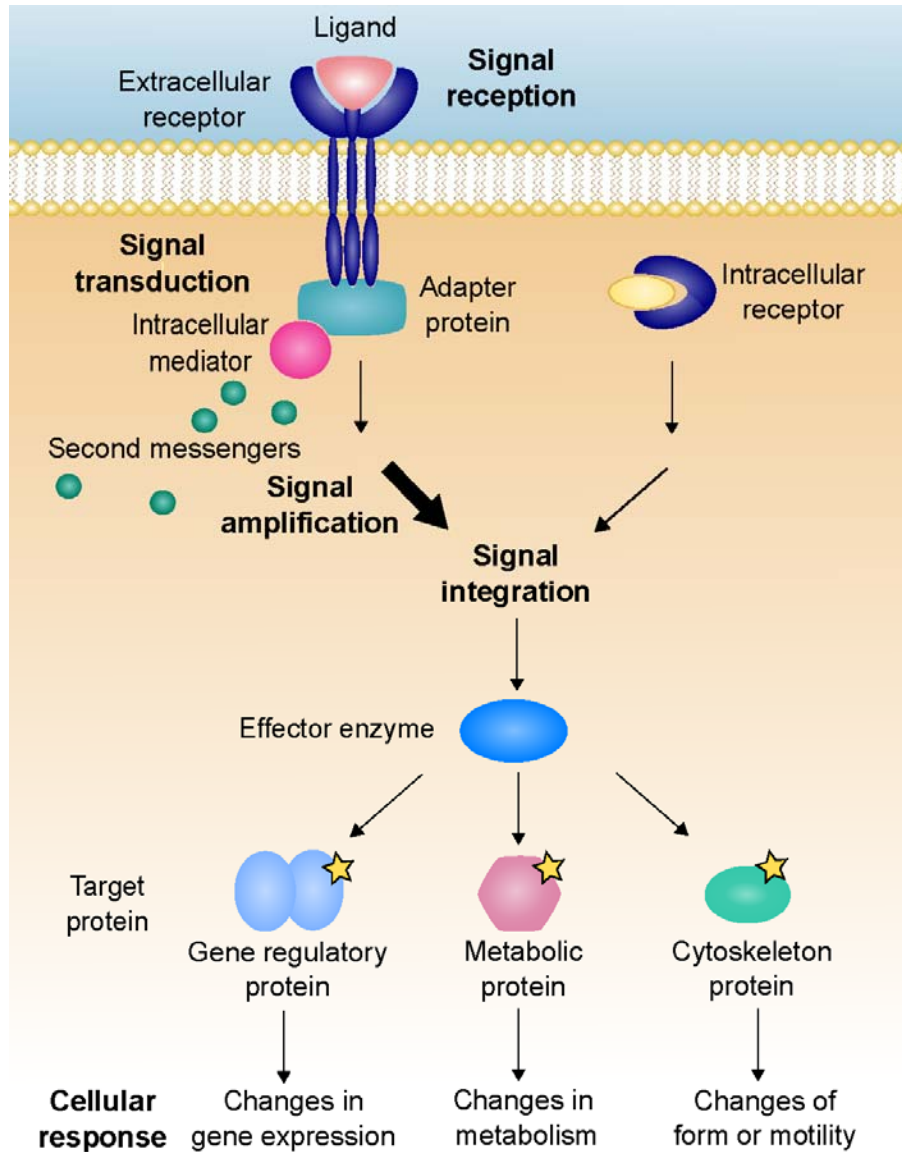 signal flow from the receptor to the cell response

❖ ***In silico* knockout**
  Scheidel *et al.* (2016) *PLoS Computational Biology* 12(12):e1005200
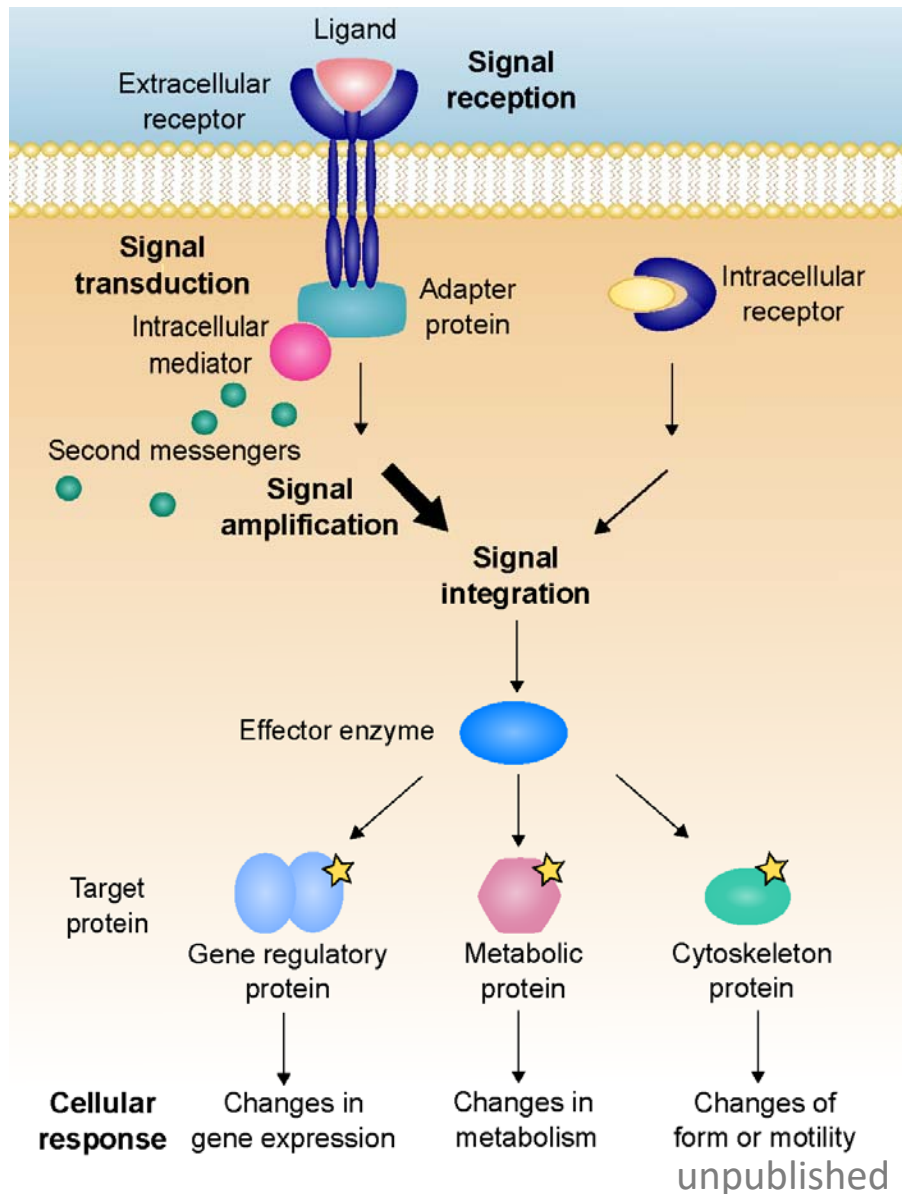 knockout matrix

The TNFR1 signaling pathway

Signaling pathways

Modeling of signaling pathways

Tasks

❖ Compilation of the molecular processes in computer models

❖ Development of a regulatory network

❖ Elucidation of the system's behavior

❖ Investigation of the robustness and vulnerability

Motivation and aim of the work

❖ Joint project with physicians from Goethe-University Frankfurt/Main

Simone Fulda   Ivan Dikic

❖ Knowledge compilation into a mathematical model

❖ Computational verification of the model

❖ Role of RIP1 and other proteins as molecular switch between apoptosis and necroptosis

Leonie Amstein

Nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB)
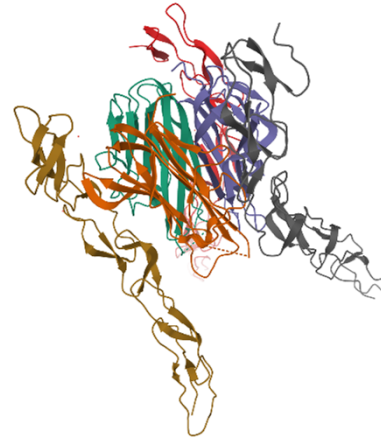

1NFK
NF-κB p50 homodimer bound to a κB site

Ghosh *et al.* (1995)
Nature **373**: 303-310

❖ Initiates gene expression of mainly pro-inflammatory and anti-apoptotic operating genes
❖ Is often referred to as the survival pathway triggered by TNFR1 stimulation

The tumor necrosis factor receptor 1 (TNFR1)



3 ALQ
TNF-TNFR2 complex

Mukai *et al.* (2010)
Sci Signal **3**: ra83-ra83

❖ Controls pivotal cellular reactions involved in immunity and developmental processes
❖ Mediates signaling pathways, which induce opposing cellular responses from initiation of gene expression to two forms of cell death, apoptosis and necroptosis

❖ Permanent activation of NF-κB can result in chronical inflammation and promote the formation of tumors
❖ In various diseases, the TNFR1 signaling pathway has been found to be disrupted
❖ Resistance against cell death induction is caused by imbalance of pathway regulation
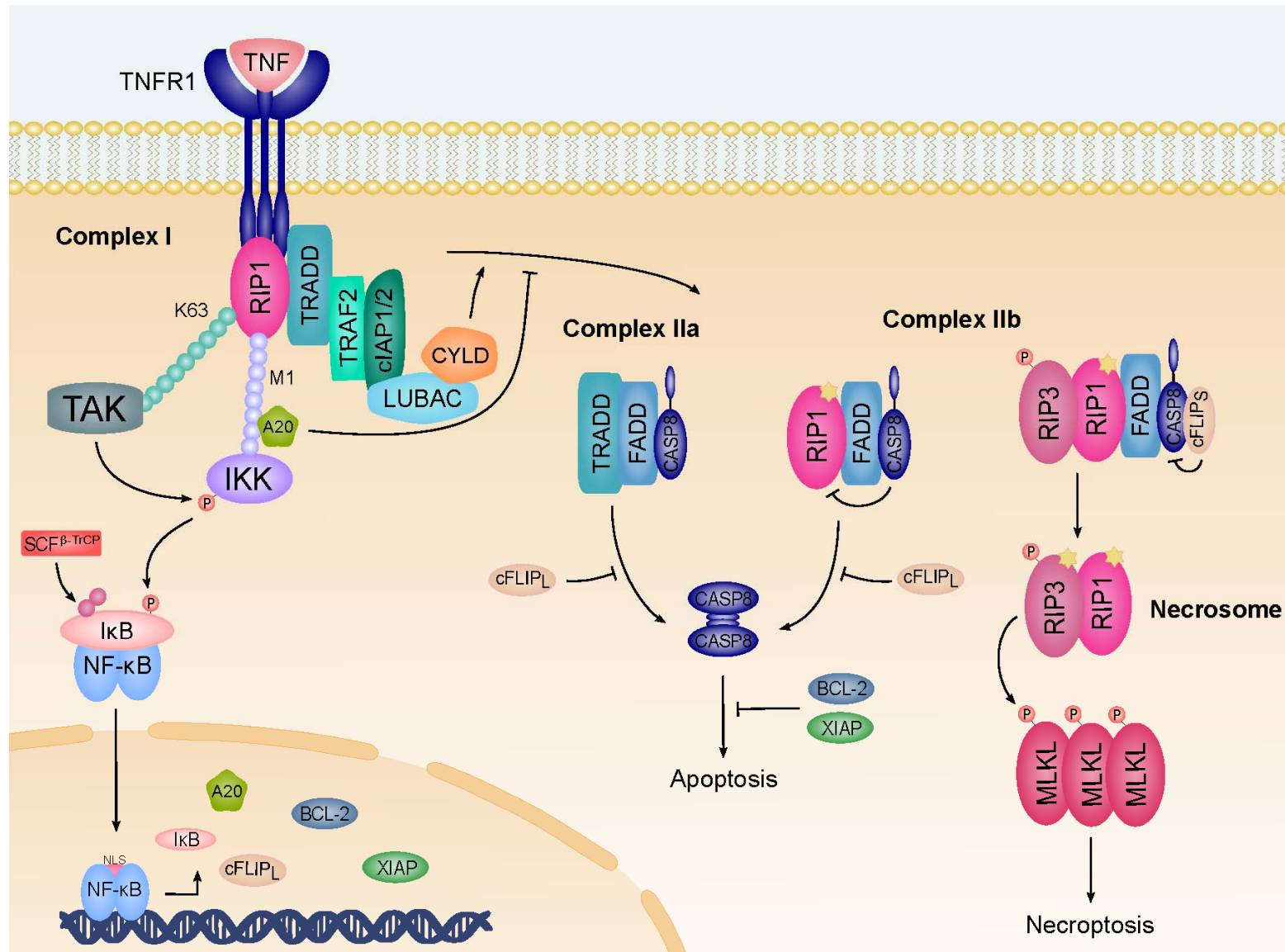
The TNFR1 signaling pathway
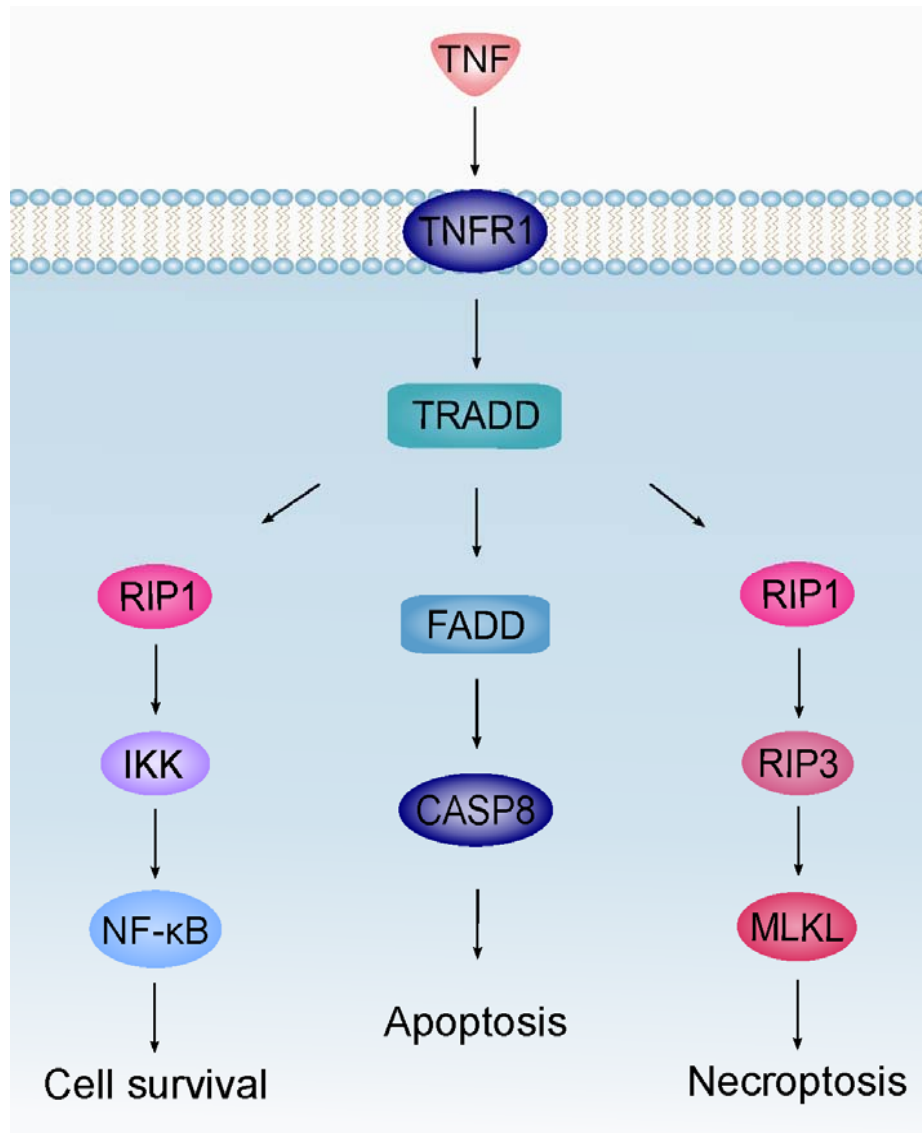
- ❖ Involved in essential cellular processes as
  - development
  - immune reactions

- ❖ Oppositional cell responses:
  - survival by NF-κB activation
  - apoptosis and necroptosis

- ❖ Regulation by
  - macromolecular complexes
  - posttranslational modifications
  → *molecular switch*

The TNFR1 signaling pathway
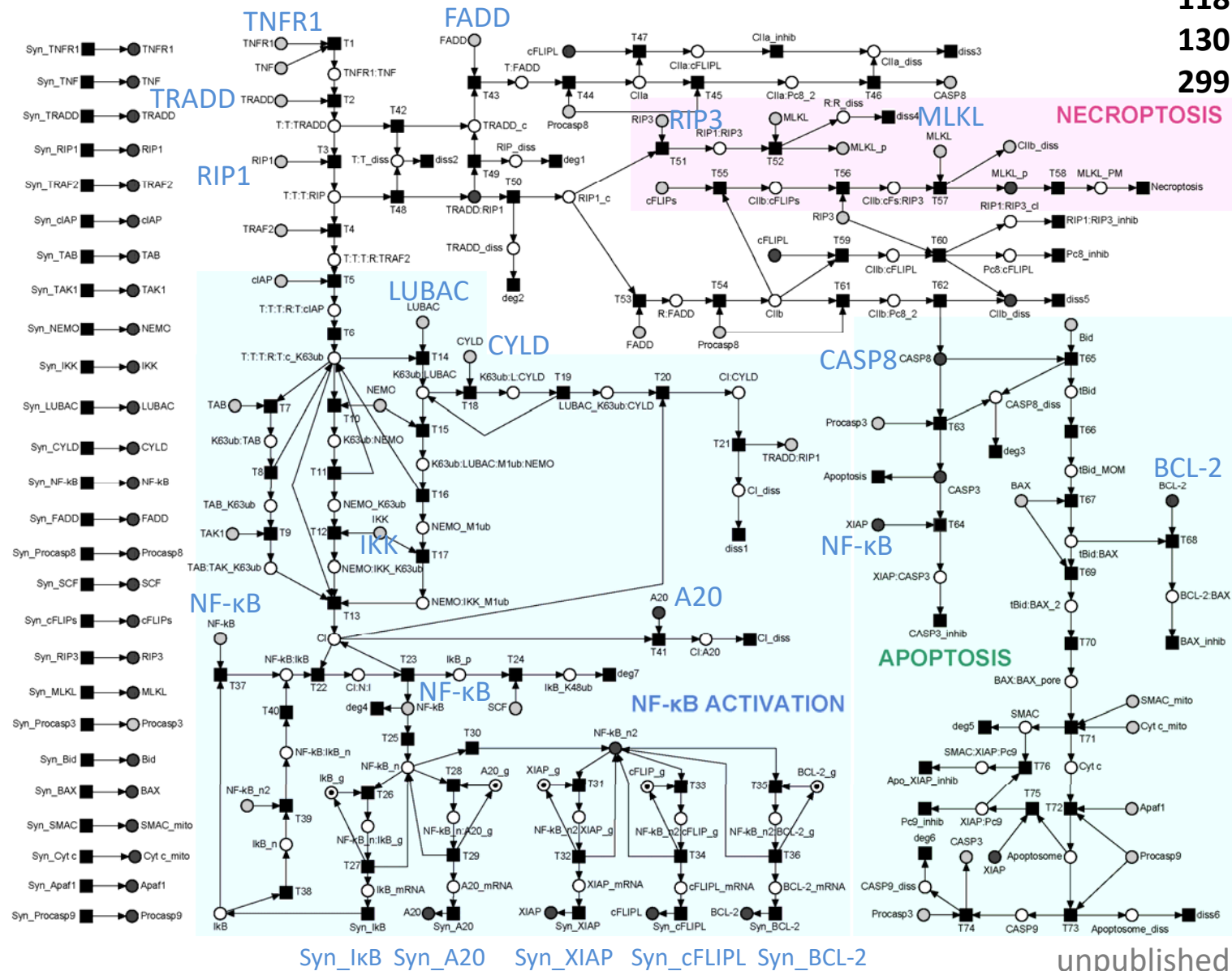
Petri net of the TNFR1 signaling pathway

118 places
130 transitions
299 edges

unpublished

Model verification

Model verification through invariant analysis

Incidence matrix

Transition Place	r_1	r_2	r_{3f}	r_{3b}
C	−2	−1	−1	+1
O_2	−1	−1	0	0
CO	+2	0	+2	−2
CO_2	0	+1	−1	+1
init	0	0	0	0

place (P-) invariant: $C^T x = 0$

transition (T-) invariant: $C y = 0$

0: steady-state constraint

Search for **minimal nonnegative, nontrivial integer** solutions

Minimal: $\nexists z$: supp $(z) \subseteq$ supp (u) and the largest common divisor of all non-zero entries of u is 1

❖ Correctness by biological interpretation
❖ Completeness
❖ Basic dynamic behavior

Model verification through invariant analysis

Incidence matrix

Transition / Place	r_1	r_2	r_{3f}	r_{3b}
C	-2	-1	-1	$+1$
O_2	-1	-1	0	0
CO	$+2$	0	$+2$	-2
CO_2	0	$+1$	-1	$+1$
init	0	0	0	0

place (P-) invariant: $C^T x = 0$

transition (T-) invariant: $C y = 0$

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Search for **minimal nonnegative, nontrivial integer** solutions

Minimal: $\nexists z$: supp $(z) \subseteq$ supp (u) and the largest common divisor of all non-zero entries of u is 1
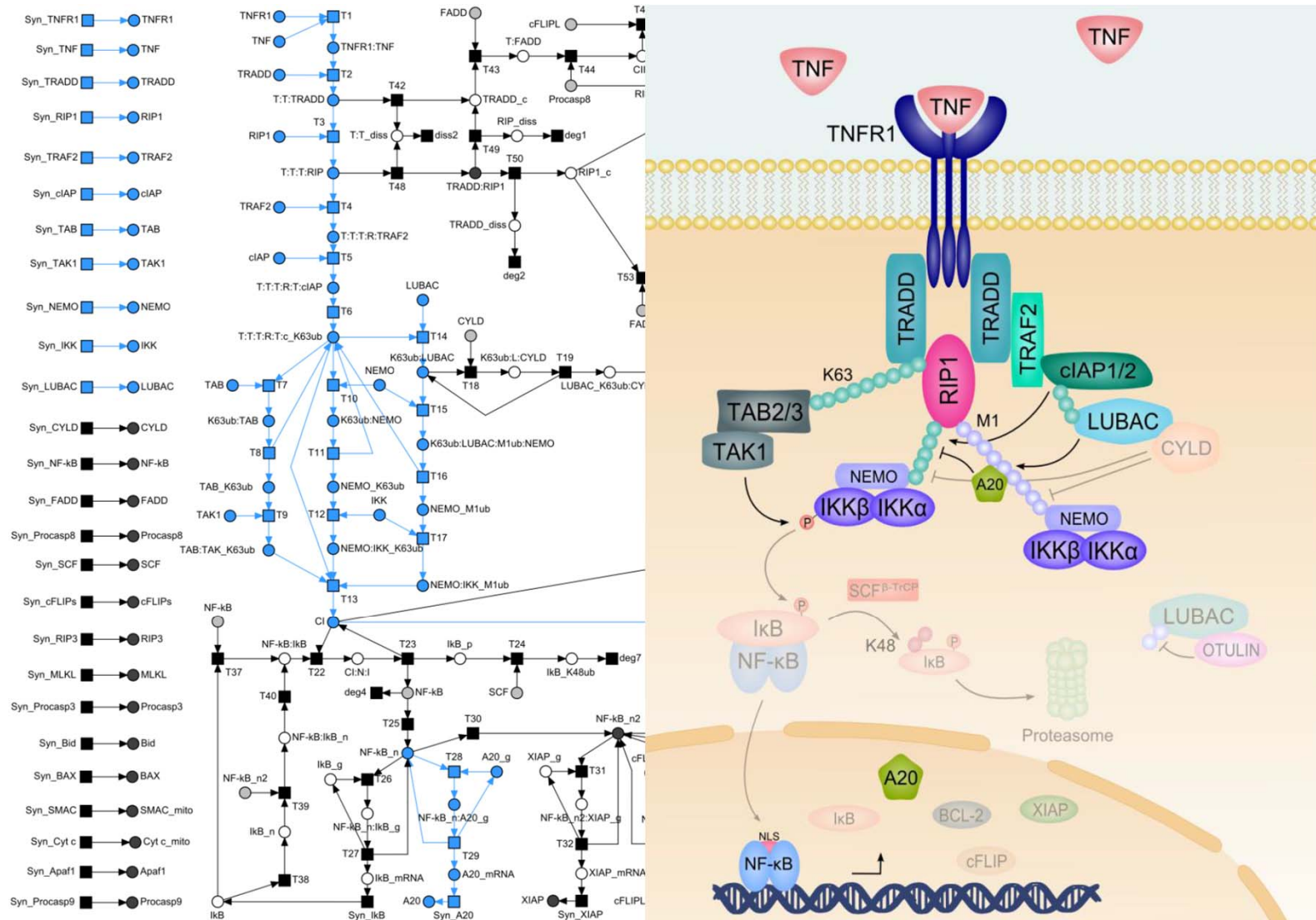
P-invariants

$$-2\,x_1\ -1\,x_2\ +2\,x_3 = 0$$
$$-1\,x_1\ -1\,x_2\ +1\,x_4 = 0$$
$$-1\,x_1\ +2\,x_3\ -1\,x_4 = 0$$
$$+1\,x_1\ -2\,x_3\ +1\,x_4 = 0$$
$$+3\,x_1\ +2\,x_2\ -1\,x_5 = 0$$
$$-2\,x_3\ -1\,x_4\ +1\,x_5 = 0$$

T-invariants

$$-2\,y_1\ -1\,y_2\ -1\,y_3\ +1\,y_4\ +3\,y_5 = 0$$
$$-1\,y_1\ -1\,y_2\ +2\,y_5 = 0$$
$$+2\,y_1\ +2\,y_3\ -2\,y_4\ -2\,y_6 = 0$$
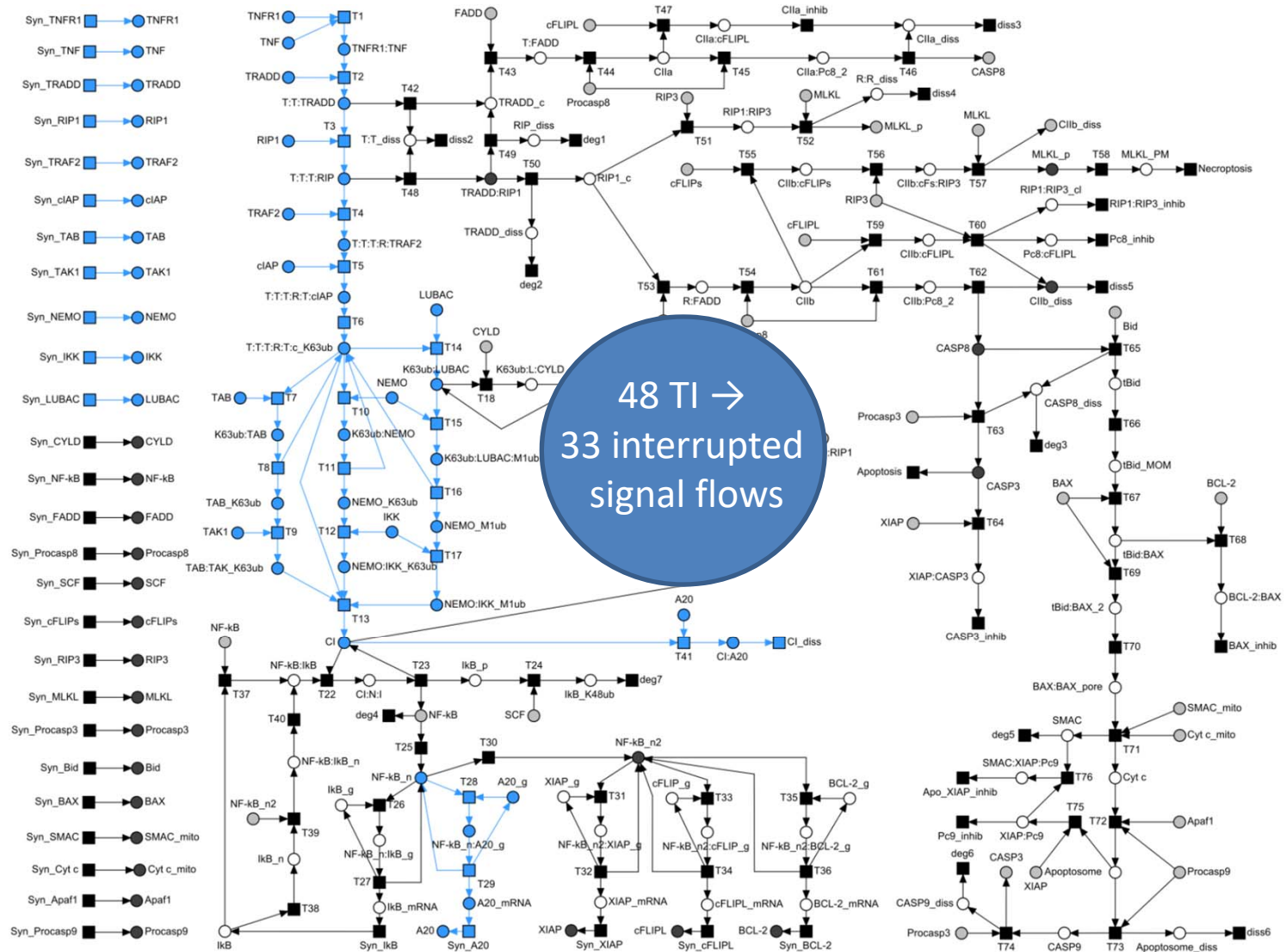$$+1\,y_2\ -1\,y_3\ +1\,y_4\ -1\,y_6 = 0$$
$$-1\,y_5\ +1\,y_6 = 0$$

Parikh vector: vector of firing frequencies

CTI property

Example for a transition invariant (TI)

Transition invariants

48 TI →
33 interrupted signal flows
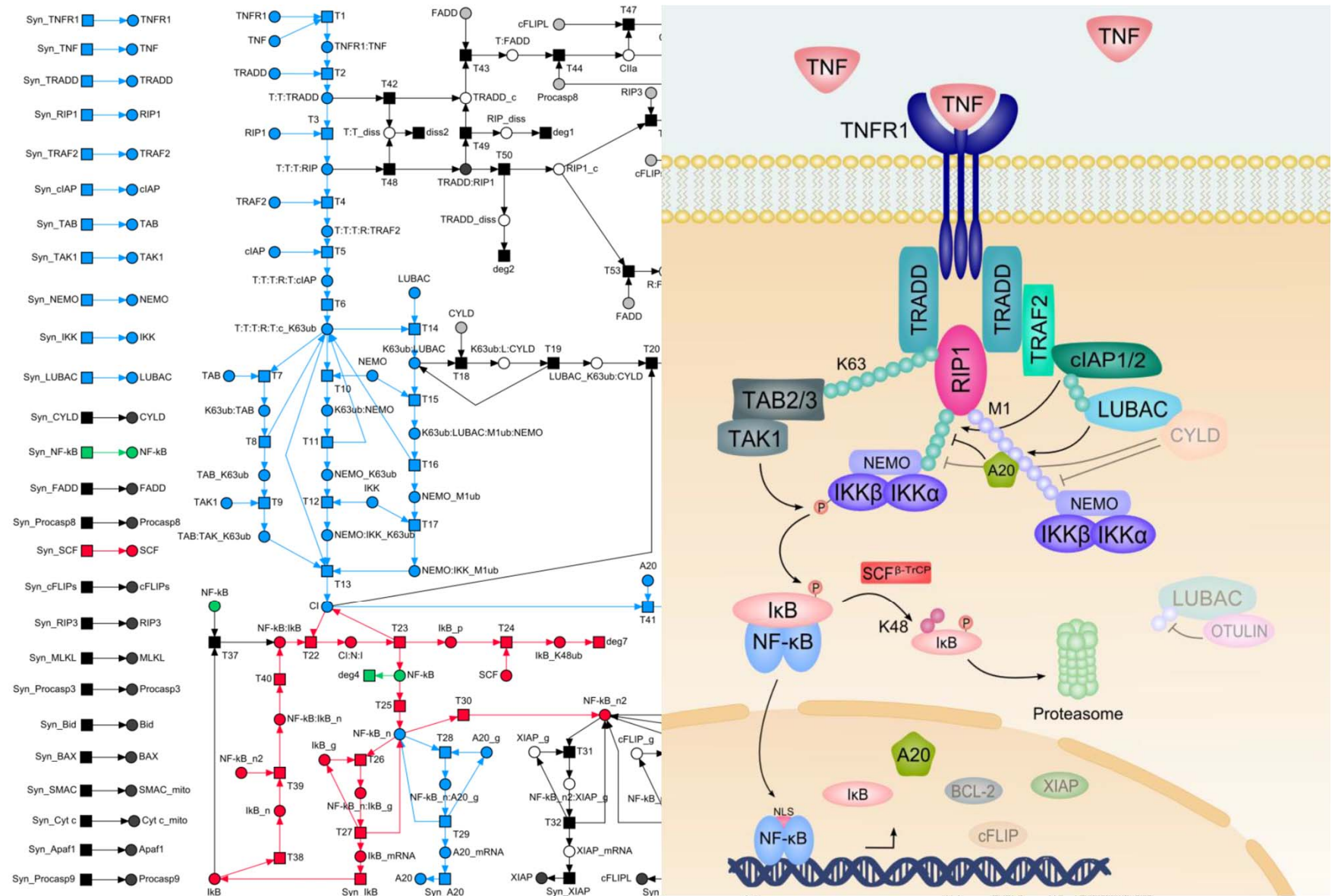
Manatee invariants (MI)

❖ Characterize **complete** signal flows
→ from the signal reception to the cell response

❖ Adapt the concept of *feasible* transitions invariants
- *feasible*: all transitions of a transition invariant can sequentially fire in the initial marking
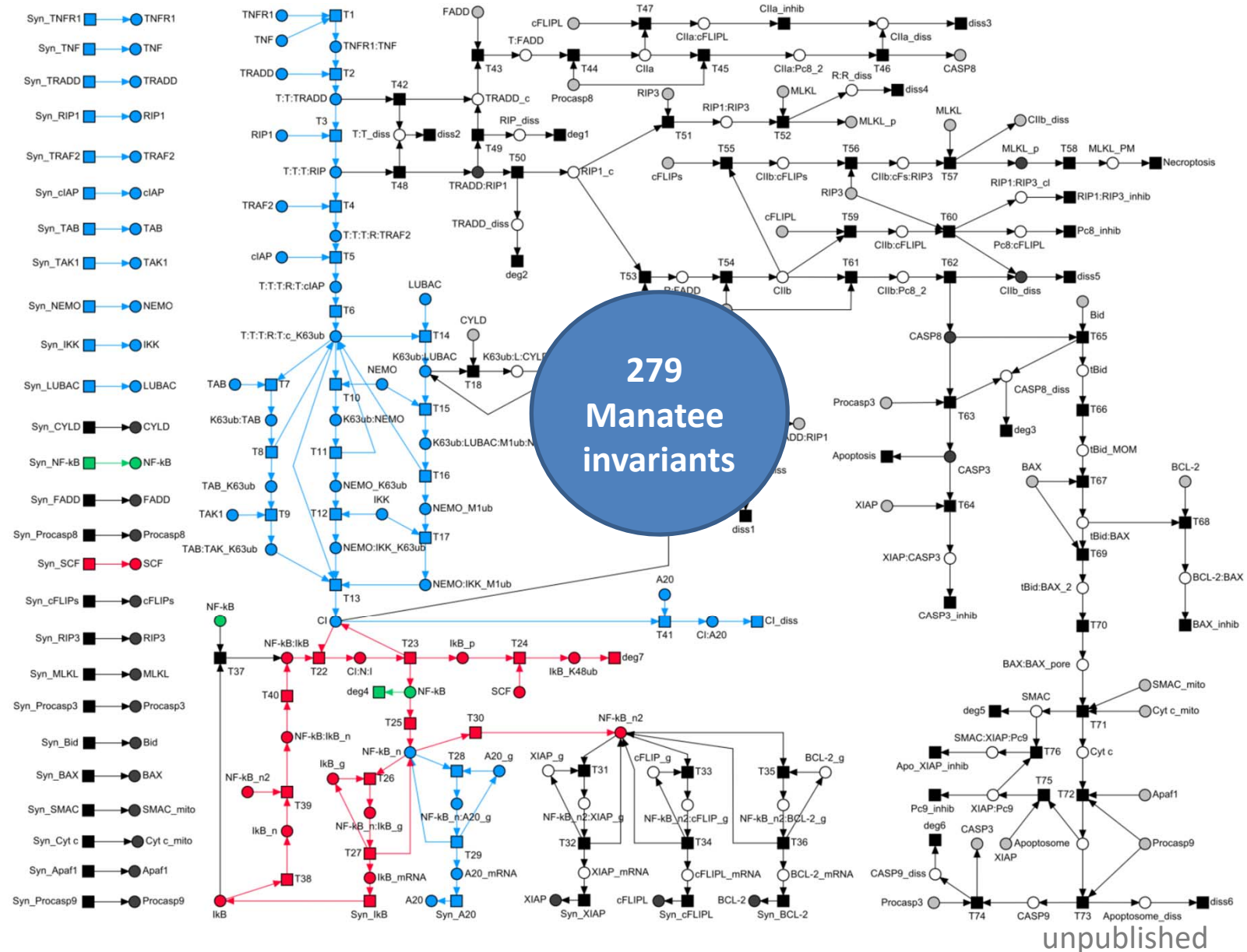- formation of specific **linear combinations** of transition invariants

Amstein *et al.* (2017) *BMC Systems Biology* 11:72
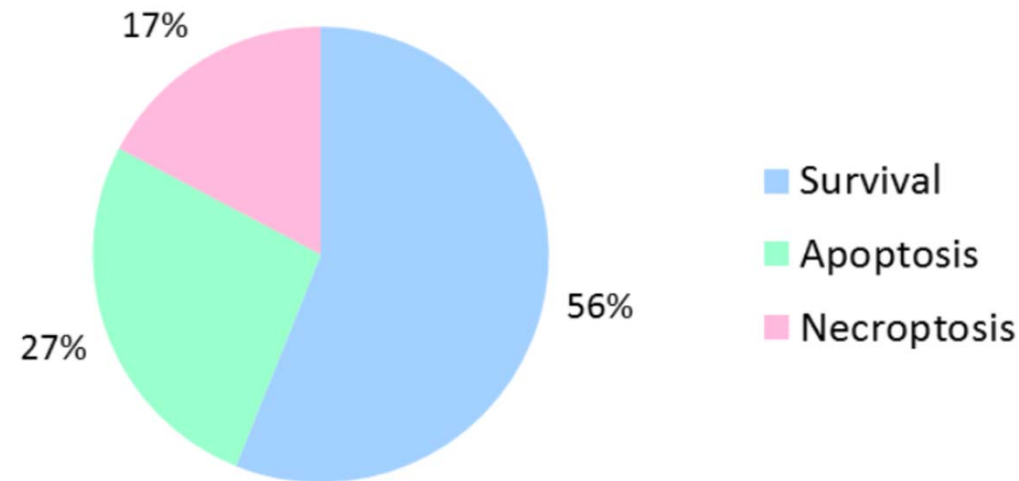
Example for a Manatee invariant

unpublished

Manatee invariants

279 Manatee invariants

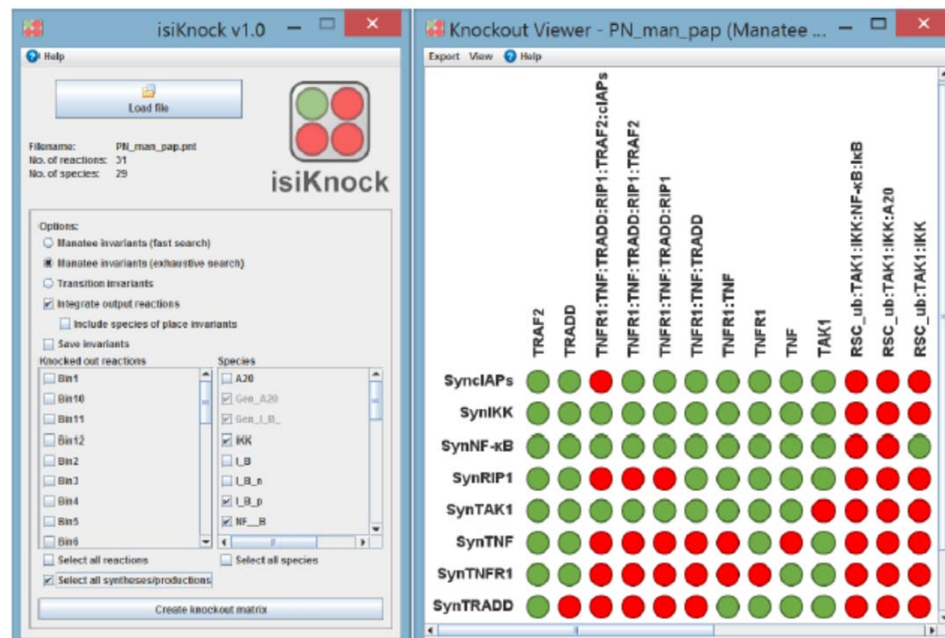unpublished

Pathway classification of Manatee invariants

❖ Classification of 214 Manatee invariants according to their cellular outcome
❖ Robust *survival* response: in agreement with experimental observations

Ting & Bertrand (2016) *Trends in Immunology* 37(8):535

unpublished

In silico knockout analysis

In silico knockout analysis

❖ Allows for perturbation analyses based on transition and Manatee invariants

❖ Gives the effects of the knockouts of each network component, thus revealing vulnerable parts of the network

❖ Provides a matrix representation

❖ The software **isiKnock** combines the concept of *in silico* knockouts with the computation of Manatee invariants

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

Amstein *et al.* (2017) *BMC Systems Biology*, 11(1)72
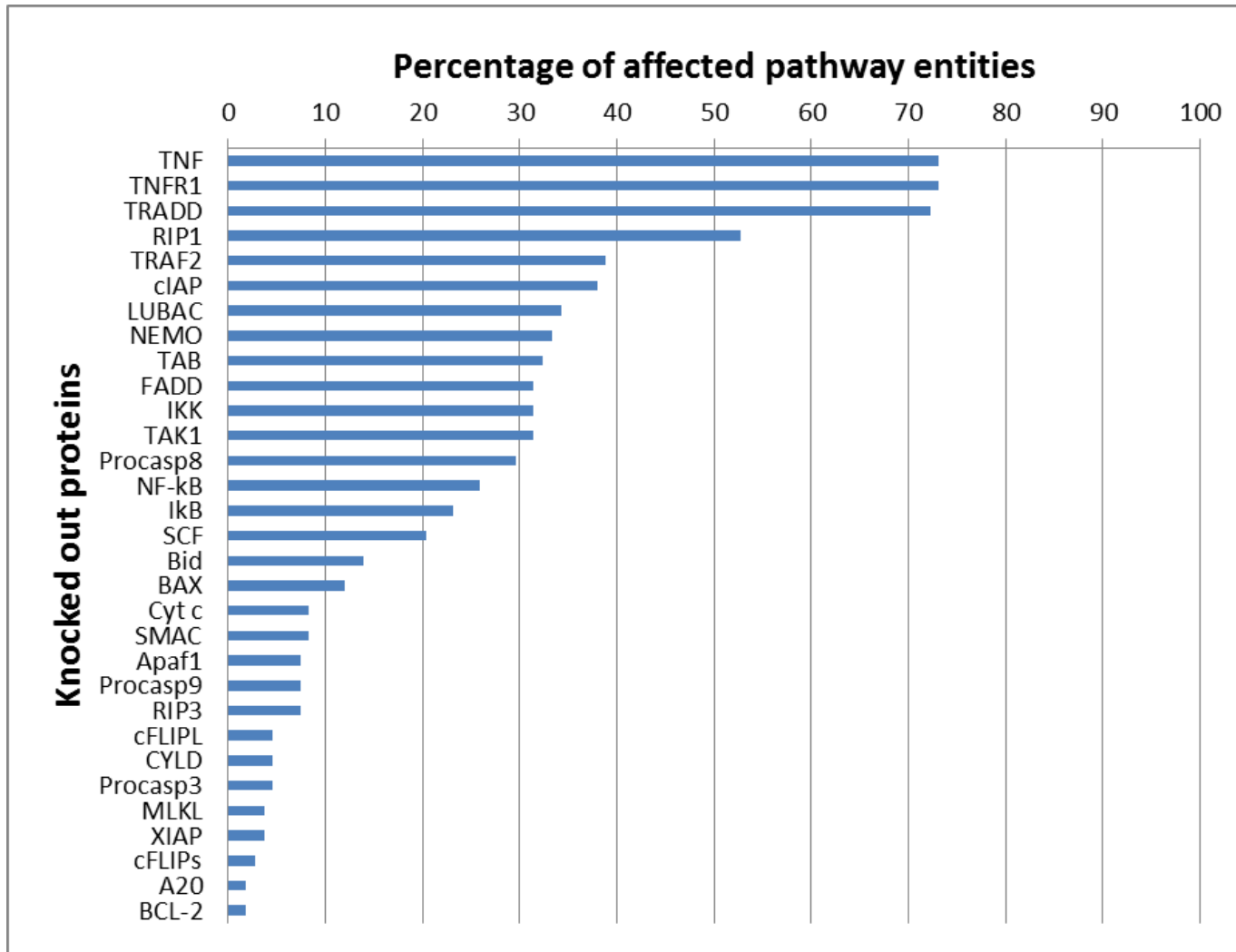
Hannig *et al.* (2018)*Bioinformatics,* 5:892

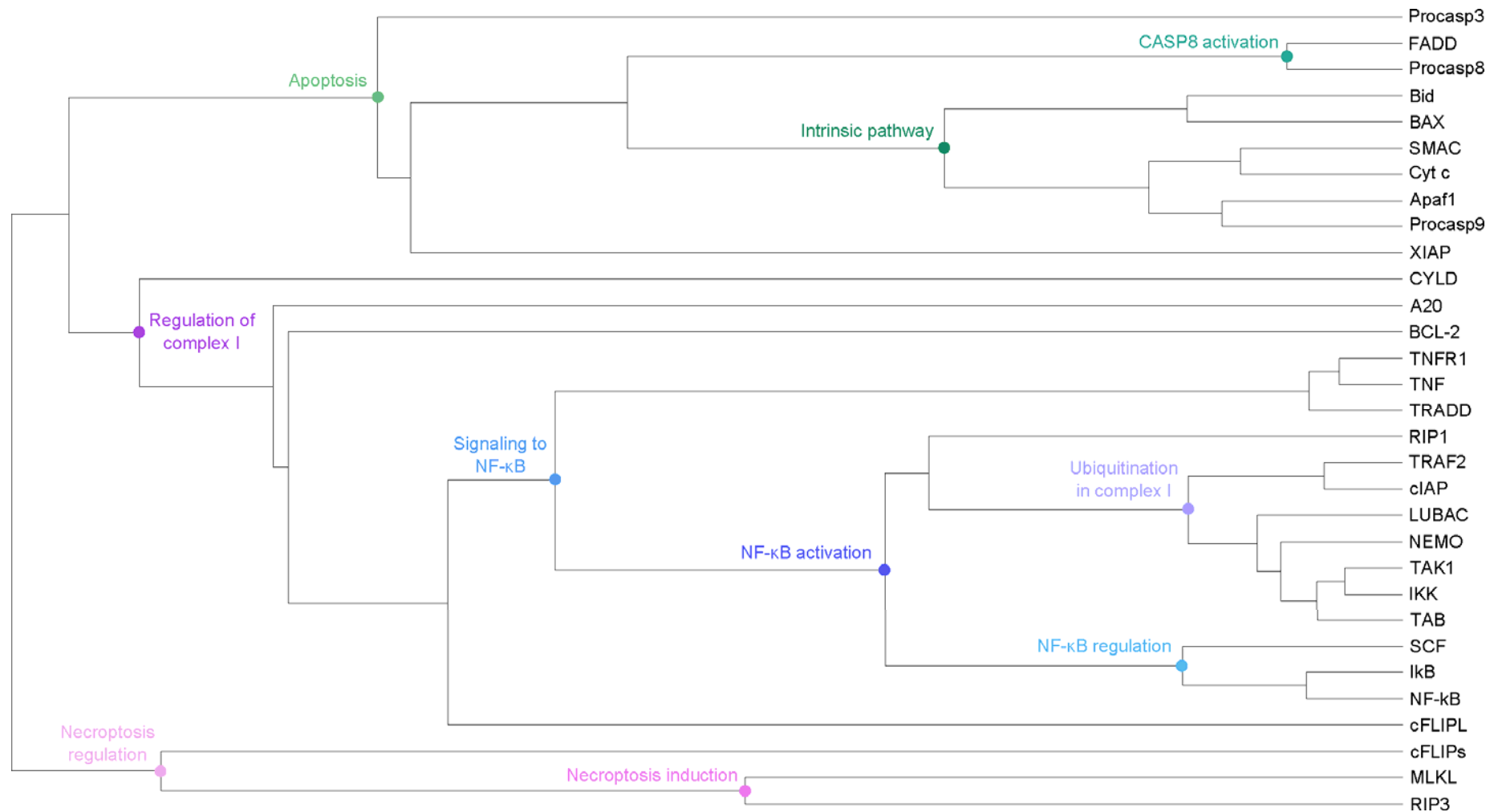# The complete knockout matrix

Ranking of the proteins

unpublished

Cluster tree of all proteins of the TNFR1 PN

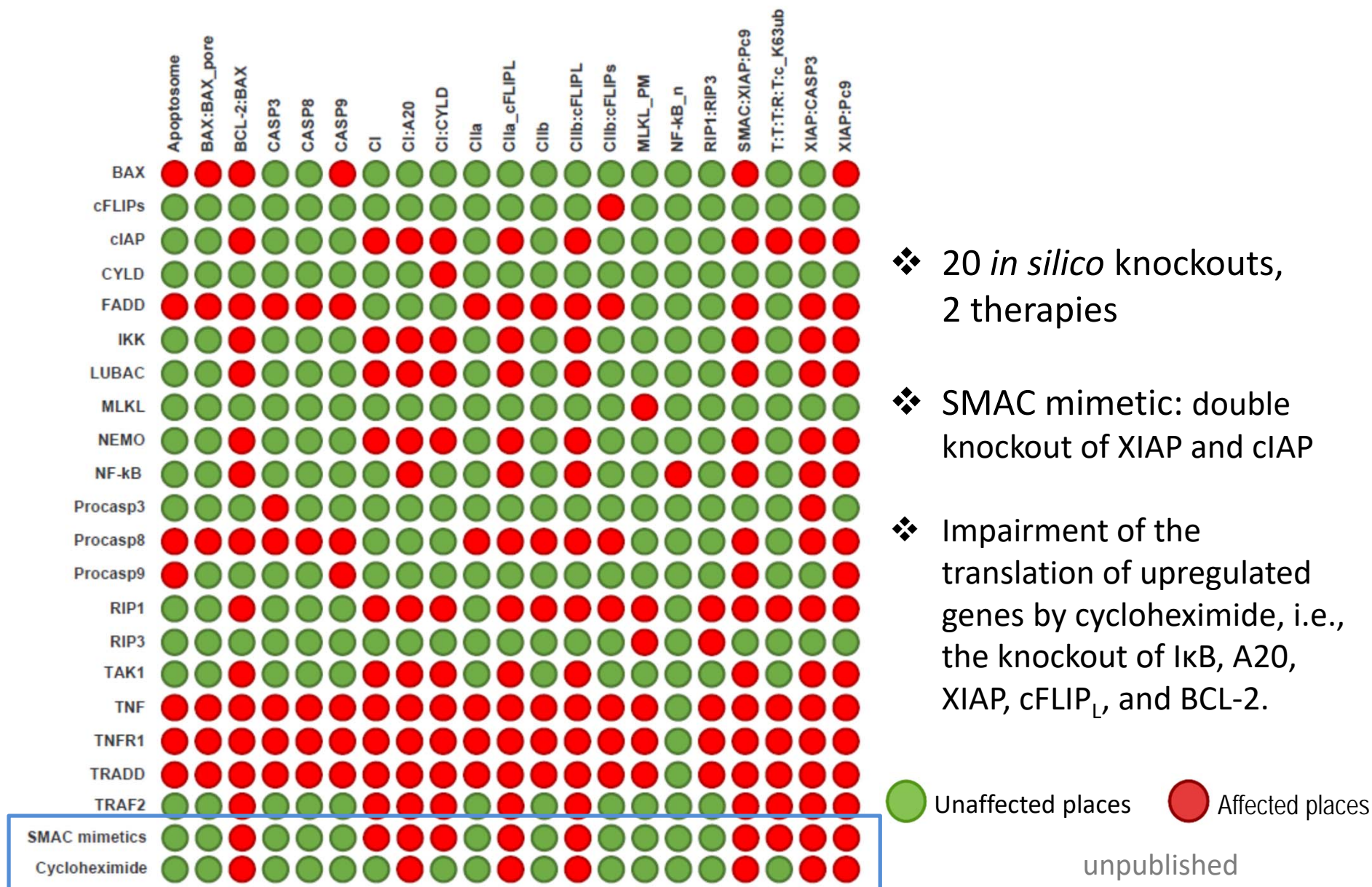UPGMA with Pearson correlation distance NF-B activation, apoptosis induction, necroptosis induction

unpublished

In silico knockouts in the TNFR1 Petri net

- ❖ 20 *in silico* knockouts, 2 therapies

- ❖ SMAC mimetic: double knockout of XIAP and cIAP

- ❖ Impairment of the translation of upregulated genes by cycloheximide, i.e., the knockout of IκB, A20, XIAP, cFLIP$_L$, and BCL-2.

Unaffected places   Affected places

unpublished

In silico knockouts in the TNFR1 Petri net

Effect of SMAC mimetic (XIAP and cIAP): 10 red entries. All refer to the formation of complex I, NF-κB-dependent gene expression and XIAP regulation. Only apoptosis and necroptosis induction remain functional.

Effect of cycloheximide (IκB, A20, XIAP, cFLIP$_L$, BCL-2): **7** red entries. Only the cell death pathways remain unaffected. It results in enhanced cell death.

Knockout of NF-κB: **8** red entries. All red entries refer to NF-κB regulation via IκB and the regulation of NF-κB-dependent genes

Knockout of RIP1: **14** red entries. The knockout affects the formation of complex I and the induction of necroptosis. Only apoptosis processes are still functioning at steady-state conditions since RIP1 is a major player in the TNFR1 signal transduction pathway

Knockout of RIP3: **2** red entries. The formation of the necrosome and the activation of MLKL are affected by the knockout of RIP3 (RIP1:RIP3, MLKL_PM).

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Summary and conclusions
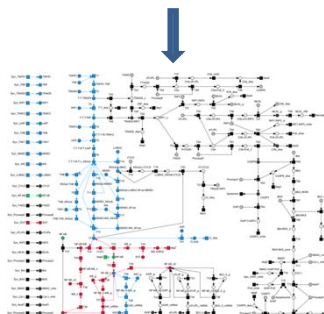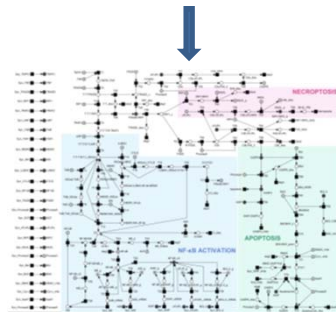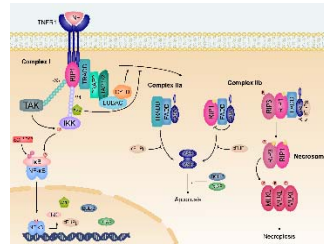
❖ The TNFR1 signaling pathway to model the switching behavior of cell survival, apoptosis and necroptosis

❖ The Petri net model
❖ First exhaustive theoretical model of the molecular processes, including cell survival, apoptosis and necroptosis
❖ Verification of the model by transition invariants and Manatee invariants

❖ Manatee invariants as linear combination of transition invariants
❖ Identification of complete signal flows in models with cyclic structures

❖ In *silico* knockout: systematic analysis of the knockout behavior
❖ Analysis of the system regarding robustness and vulnerability

Disease-related projects

Comparison and cross-talks of the canonical and non-canonical NF-κB pathways

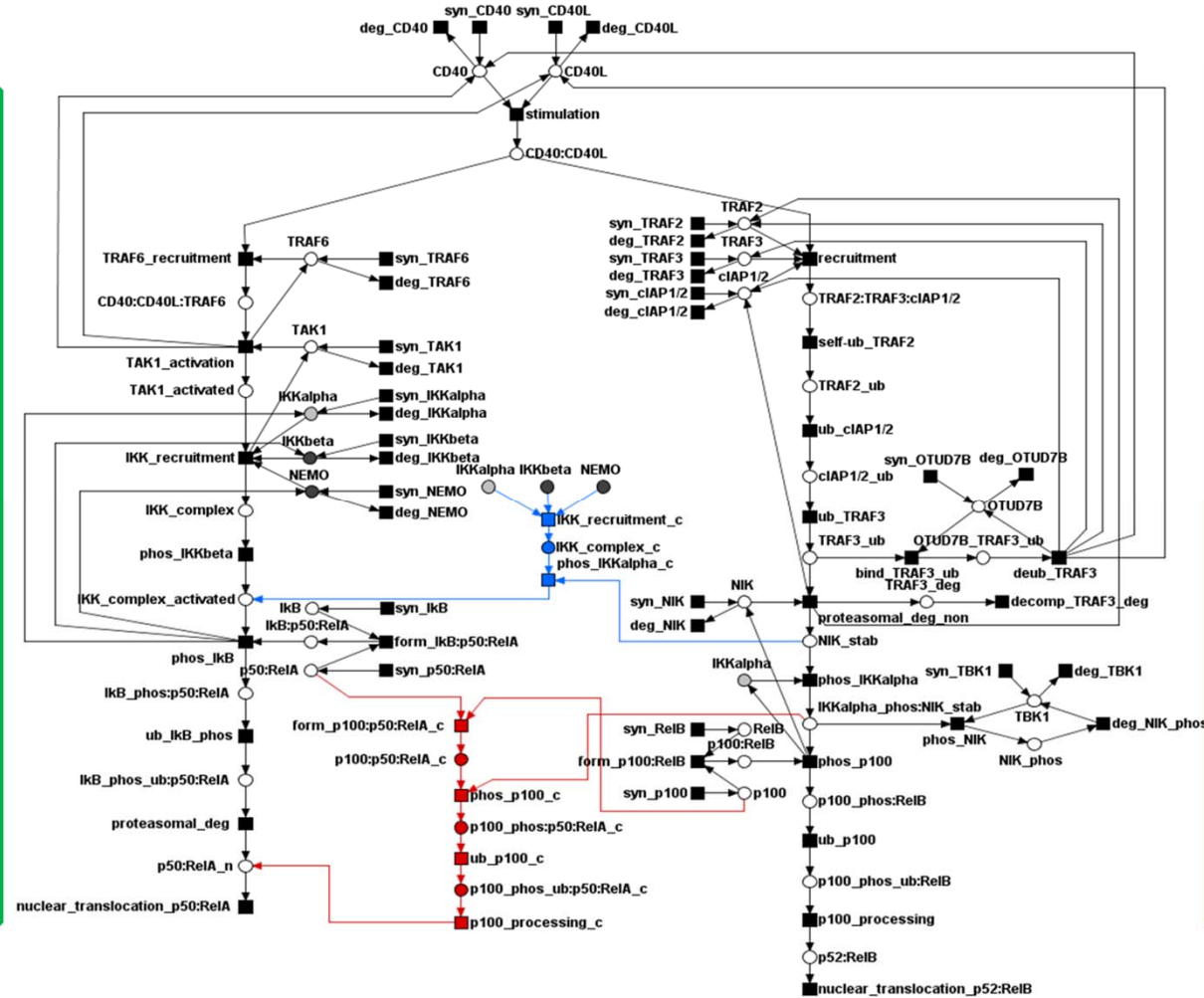Kira Trares

Franziska Krämer
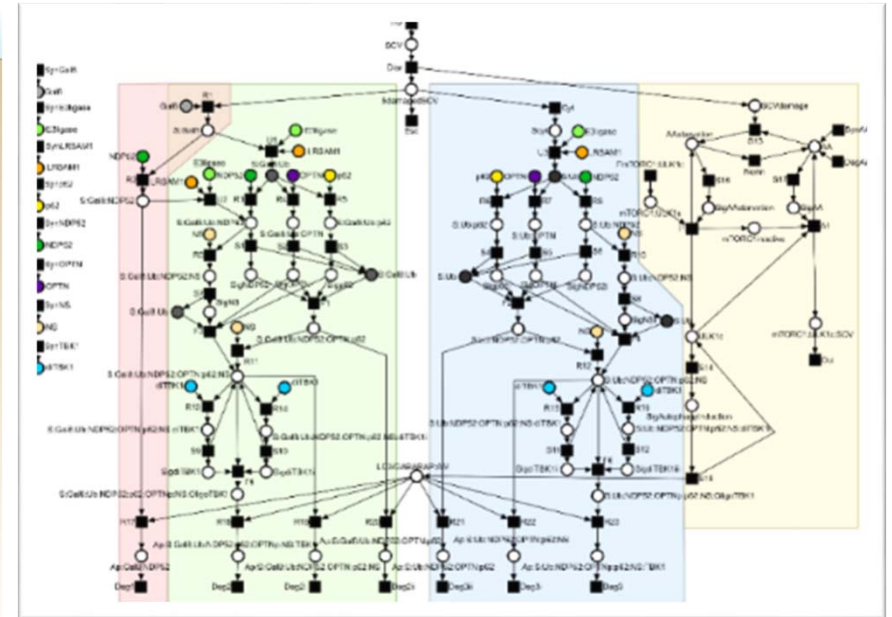
Canonical pathway

Non-canonical pathway

unpublished
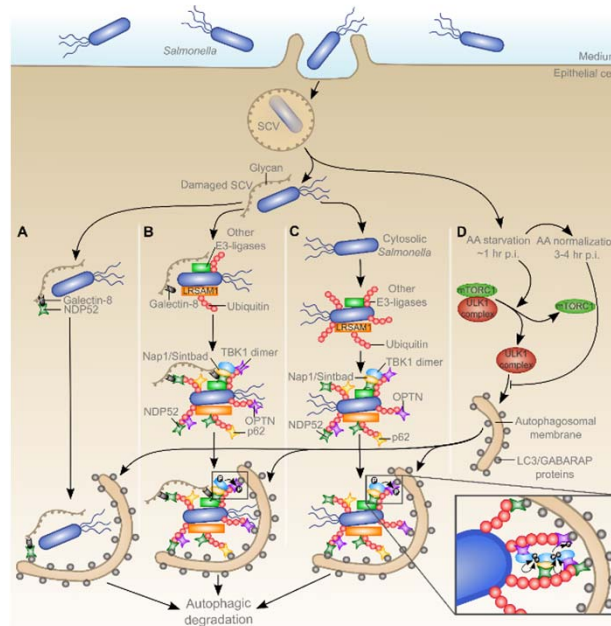
Xenophagy in epithelial HeLa cells after *Salmonella* Typhimurium infection

Jennifer Hannig

Ivan Dikic

❖ Classical Petri net
❖ Stochastic Petri net

Hannig et al. (2018)*Bioinformatics* 5:892

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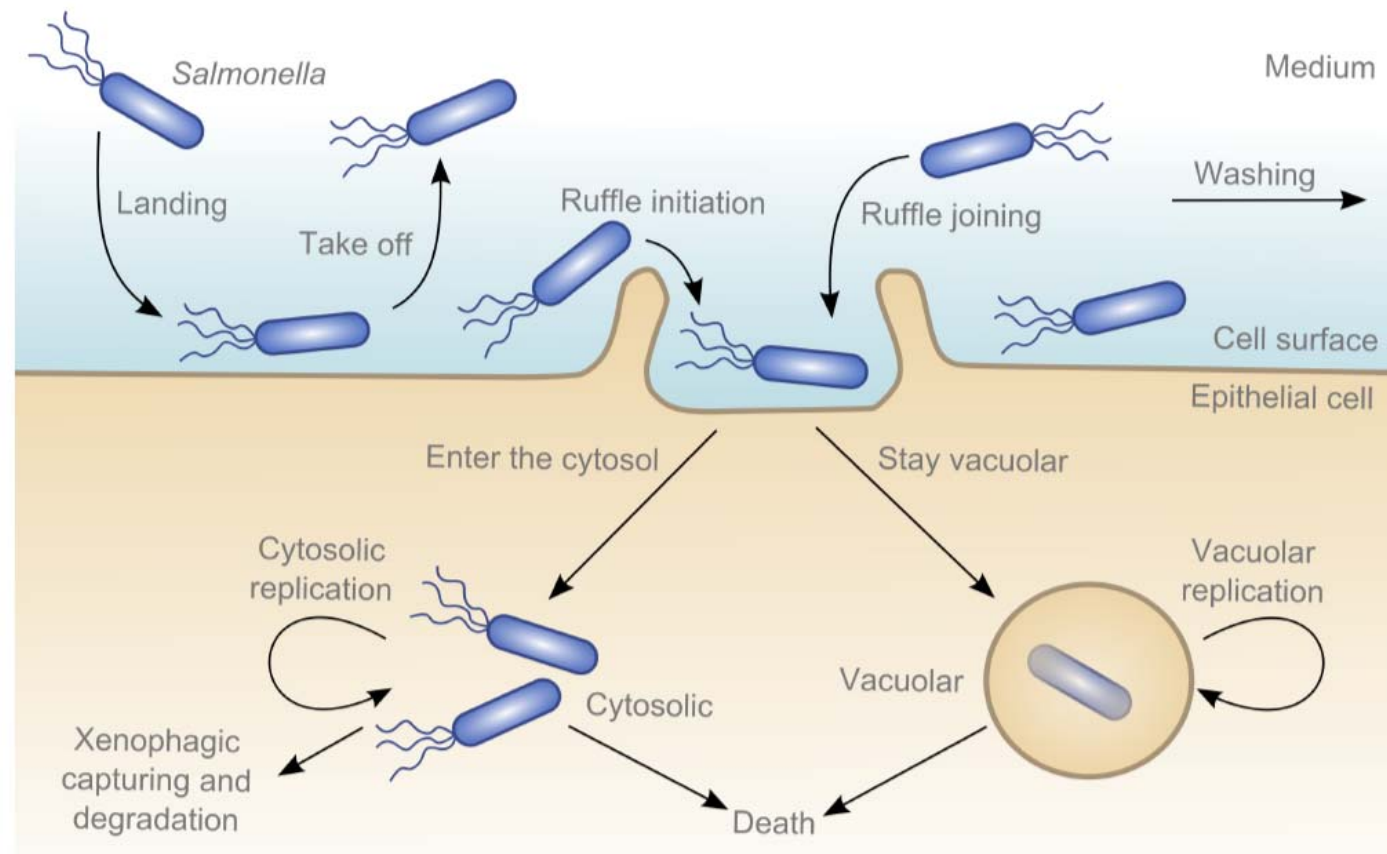Biology, 12(12), e1005200

Agent-based model of *Salmonella* movement on the cell surface
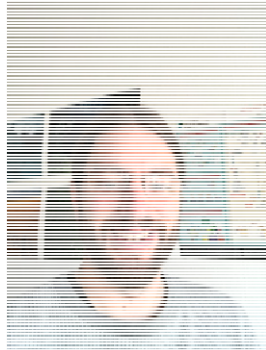

Nasrin Alikhani Chamgordani

Jennifer Hannig

Protein-protein interaction networks of *Salmonella*-infected and of *Shigella*-infected HeLa cells
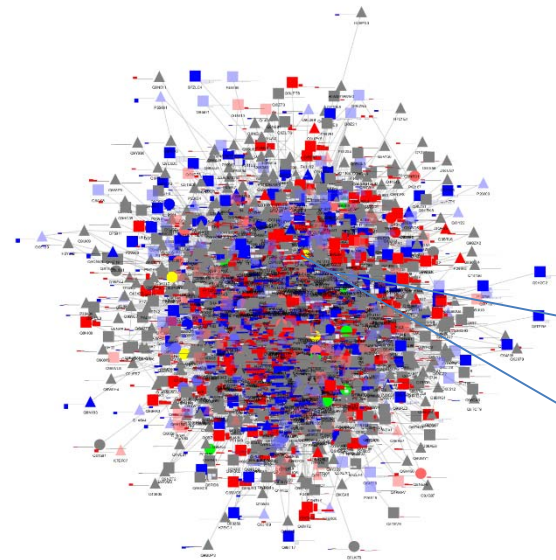
Jens Rieser

❖ Merged network: all proteins from experimental, ubiquitinated proteins and phosphorylated proteins
❖ 1,704 proteins (vertices) and 18,974 interactions (edges)

Ioana Popa

UBP24_HUMAN

Ubiquitinated   Phosphorylated

Lucas Fein   IvanDikic

Topological clustering

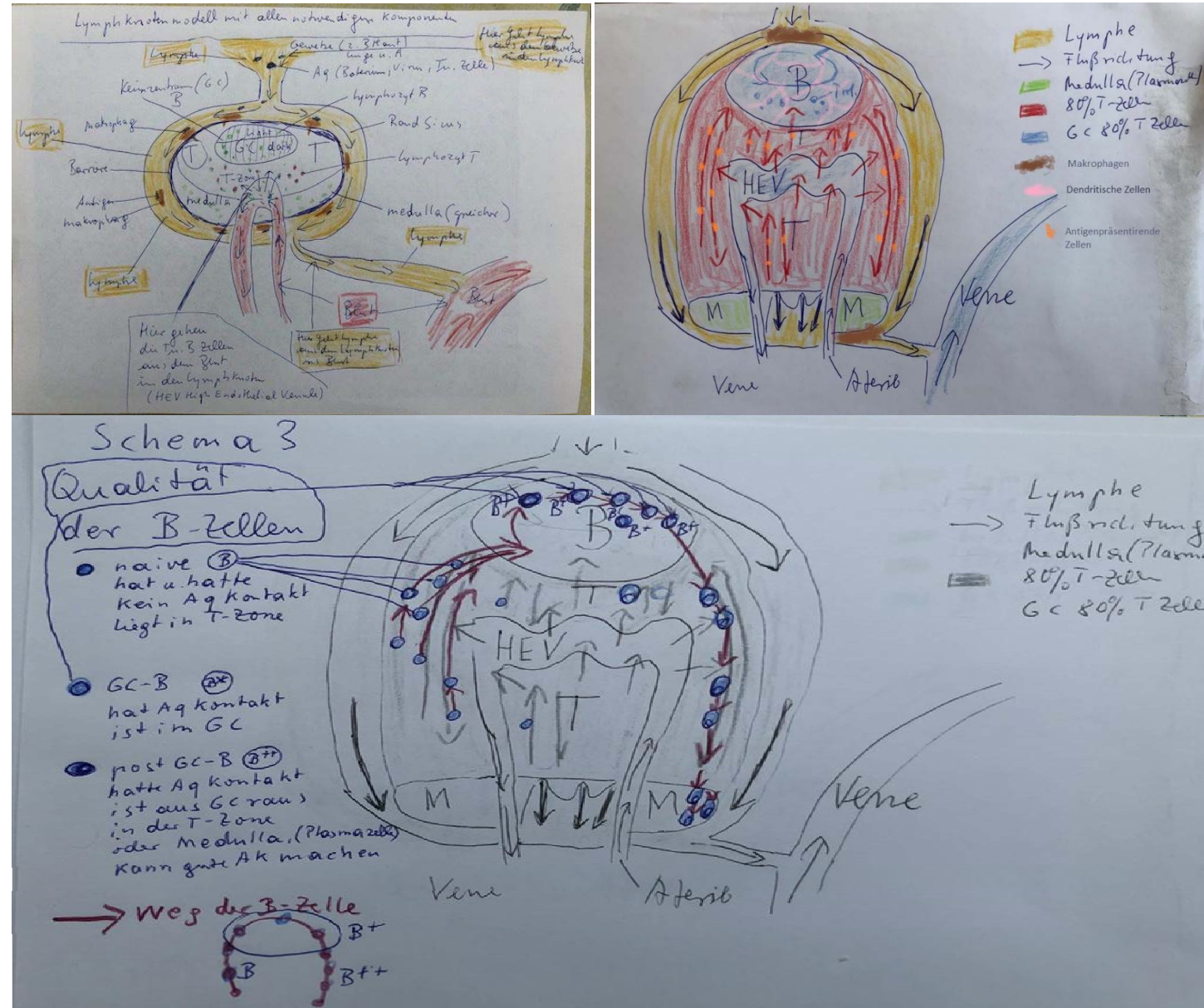| Annotation data from		Phosphorylation/Ubiquitination (extreme value)	
Phosphorylation		blue downregulated	Ubiquitin down, Phosphor up
Ubiquitination		red upregulated	Ubiquitin up, Phosphor down
Both		Neither up- nor downregulated	
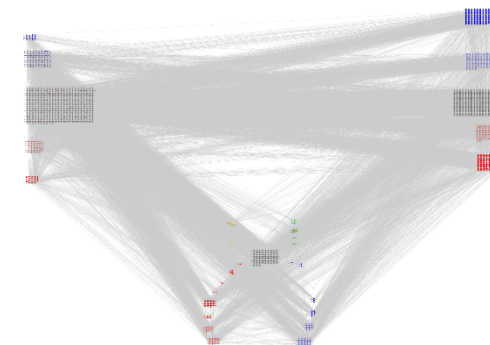
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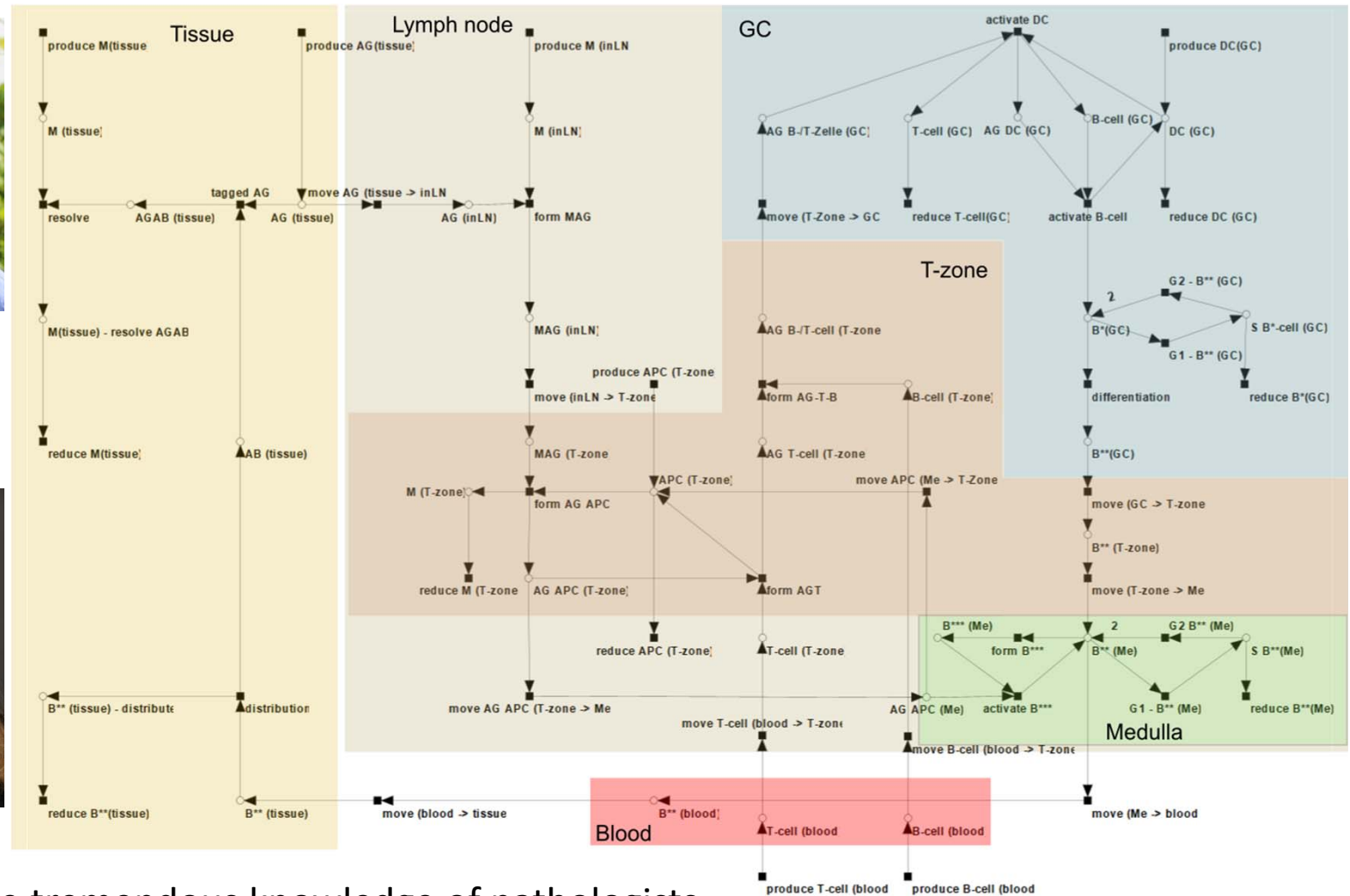# A Petri net model of the human lymph node

Martin-Leo Hansmann

A Petri net model of the human lymph node

Martin-Leo Hansmann

Sonja Scharf

❖ Based on the tremendous knowledge of pathologists and on own experimental 2D and 3D data

unpublished

Acknowledgments

Goethe-University Frankfurt
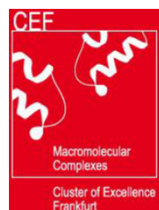
Group of Ivan Dikic
Group of Simone Fulda
Group of Ilka Wittig
Group of Heinz Osiewacz:
Andrea Hamann
Group of Martin-Leo Hansmann

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Radboud University Nijmegen
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Selina Post
Sonja Scharf
Brigitte Geiß
Patrick Wurzel
Ina Koch
Nasrin Alikhani
Katja Korolew
Zheng Song

Master students   E   **Alumnis**

Thank you!

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Ioana Popa

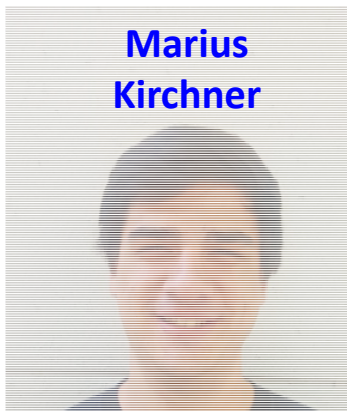**Marius Kirchner**

Yauheniya Zhadanovic   **Nils Nover**   **Mariella Zunker**   **Isra Nurhassen**   **Melanie Mößer**