Systems Biology of cell death

Mathematical modeling of cell fate decision in response to death receptor engagement

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Outline

1. **Introduction**
   - General idea
   - The role of bioinformatics and systems biology
   - A model is…
   - Mathematical modelling pipeline

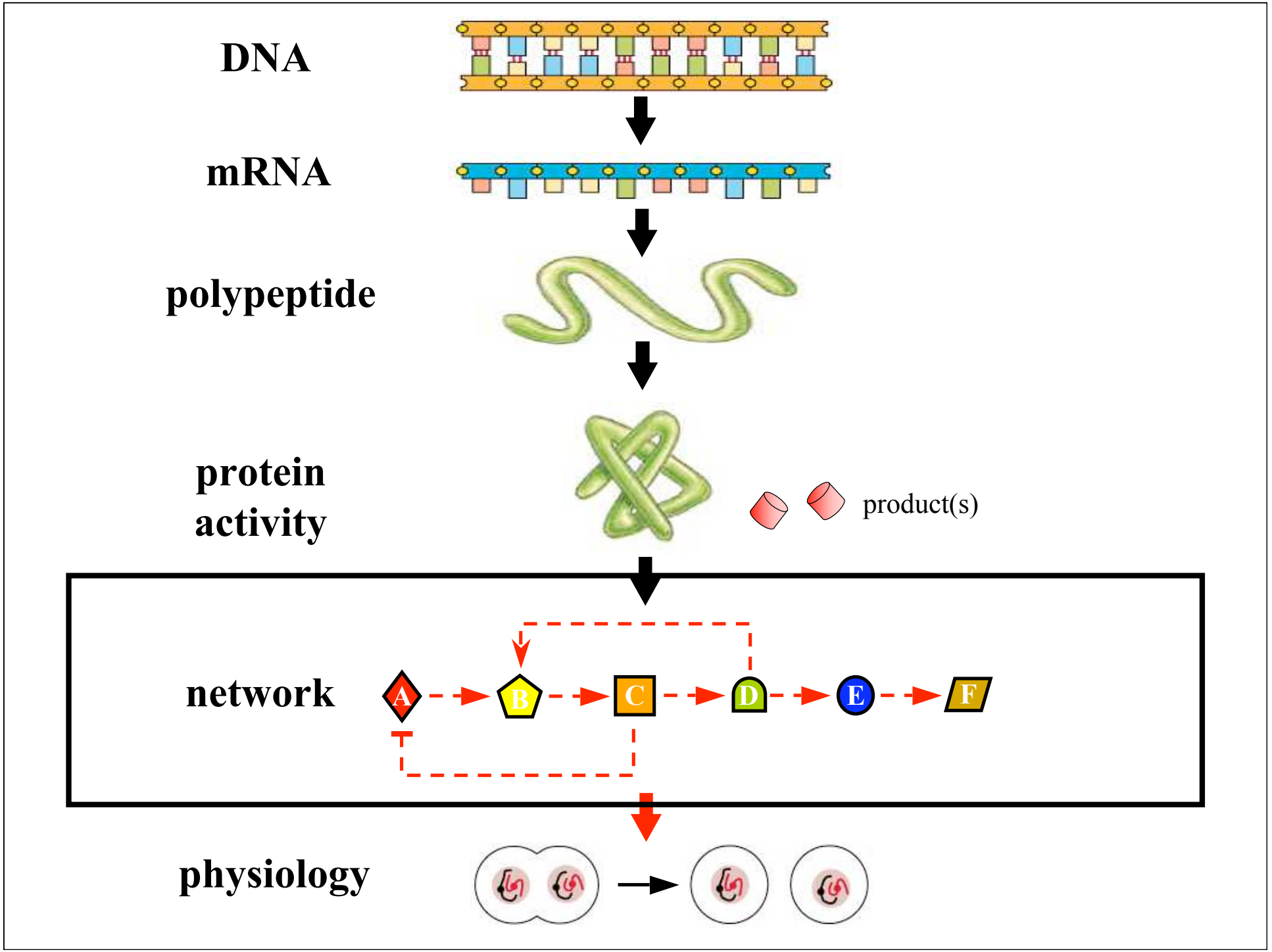
2. **Modelling cell fate decision process**
   - Motivations
   - Building a model
   - Scenarii of cell fate decision
   - Predictions

3. **Conclusion**

1. INTRODUCTION

General idea on mathematical modelling

We, systems biologists, try to understand the behaviour of a complex system as a whole, as opposed to the behaviour of its individual components


DNA
mRNA
polypeptide
protein
activity
product(s)
network
A
B
C
D
E
F
physiology

Establish a link between physiology and molecular interactions

Physiology of the cell

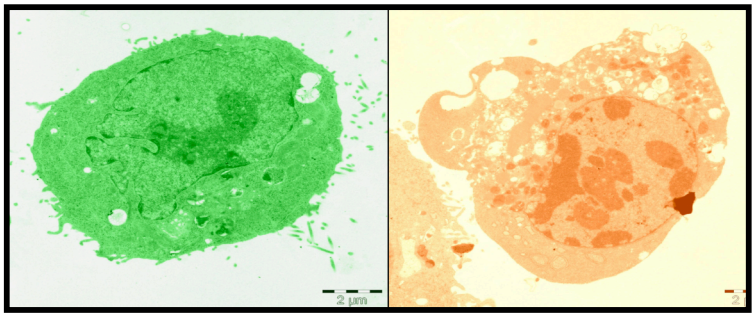
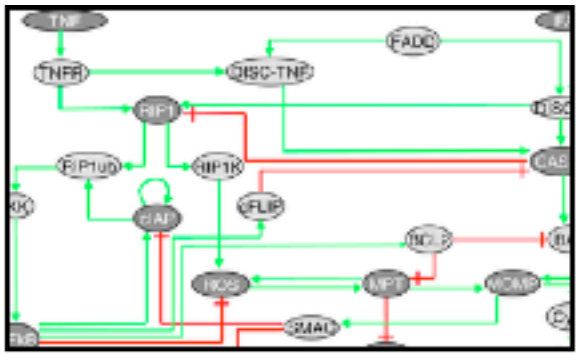
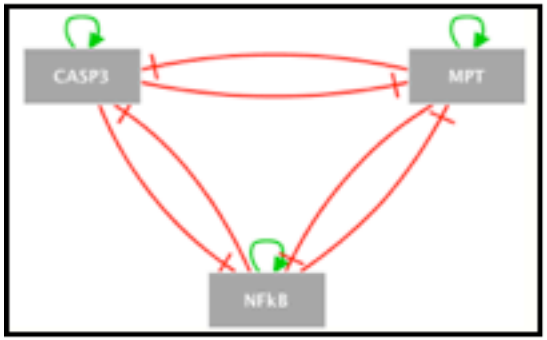
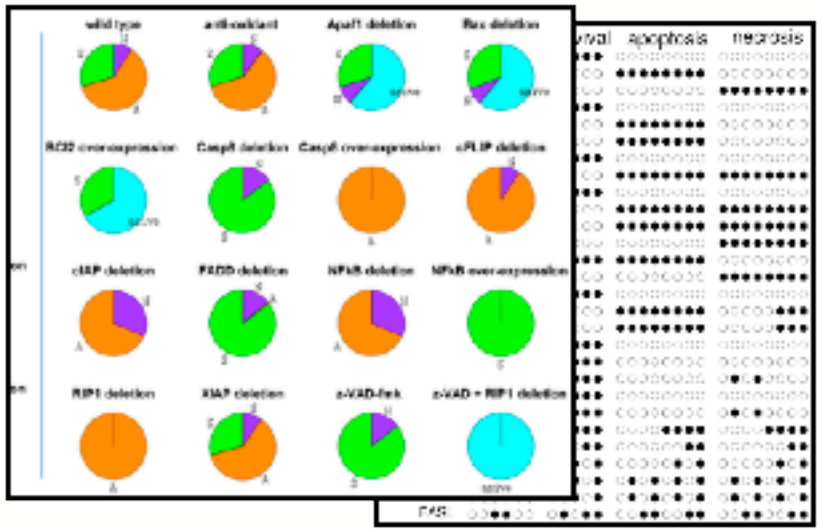


Diagram of protein interactions





Formulation of prédictions
Experimental validation

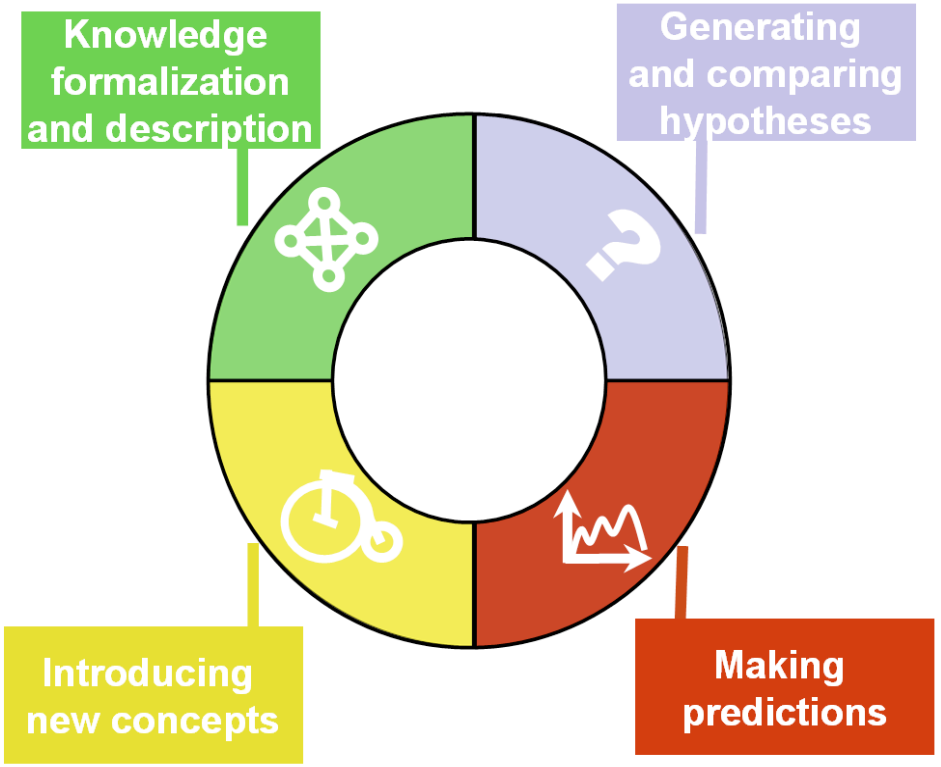


Translation in mathematical terms
Formal verification of what is known

The role of bioinformatics and systems biology

The goal is to:

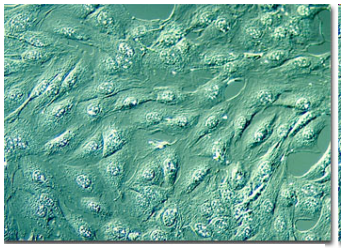
- provide a consensus picture of the cell functioning & integrate information from many experiments and publications
- help confirm or infirm hypotheses: check that the mechanism is correct
- propose experiments to experimentalists
- Introduce new concepts



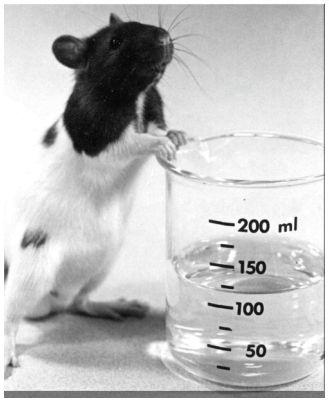
Four hallmarks of mathematical modelling

Models in molecular biology

Cell cultures

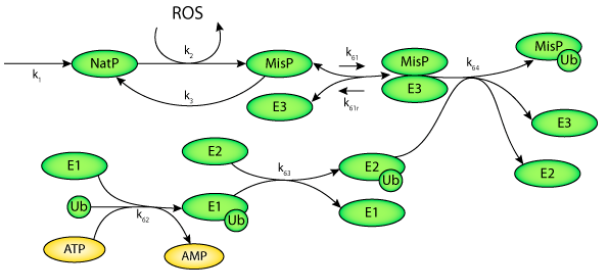


Animal models



Mathematical (in silico) models

$$\frac{dR}{dt} = \frac{1}{R^2} \int_{r=0}^{R} F(c) r^2 dr$$



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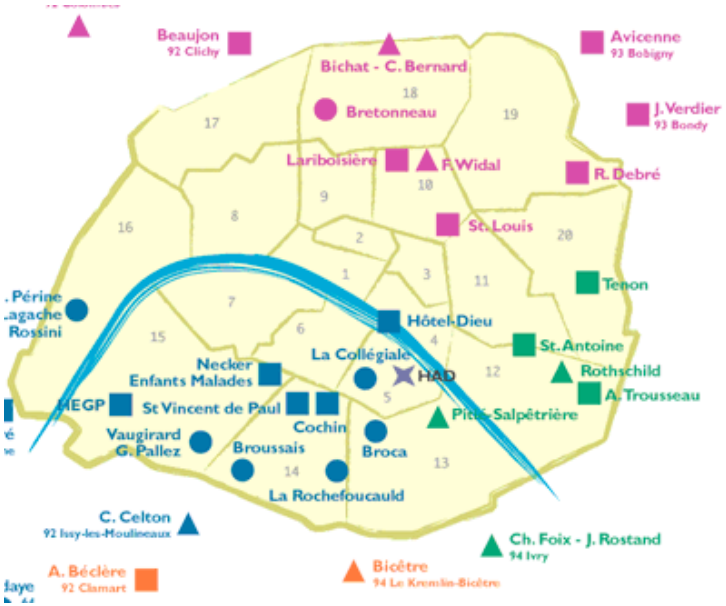
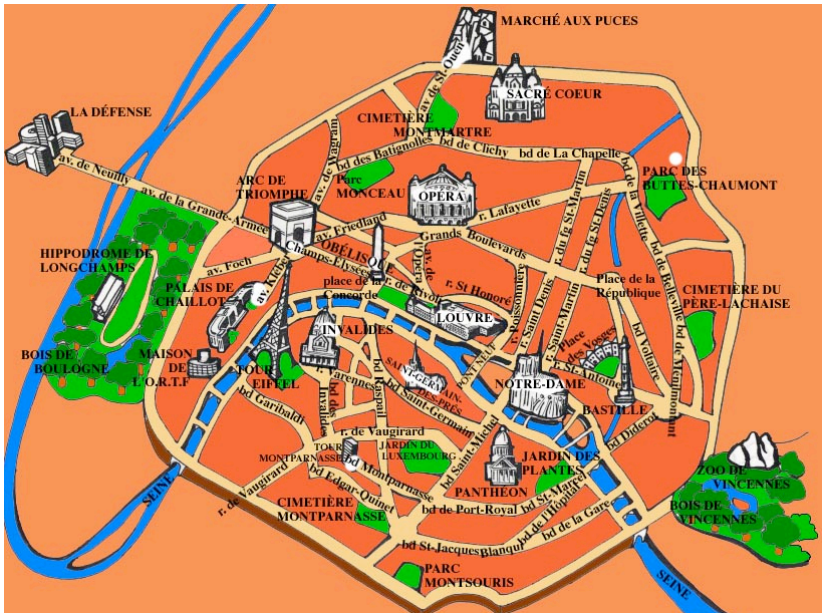
A model…

- is a set of laws that describe a more or less detailed biological process
- is a device that serves to :
  - formulate hypotheses,
  - test the coherence of disseminated and uncorrelated published data,
  - identify misunderstood zones or contradictory facts,
  - propose a logical functioning of a particular process,
  - establish predicting facts,
  - anticipate effects of a perturbation,
  - etc.
- needs to be comprehensive (capable of reproducing more than one single experiment)
- needs to be falsified, challenged
- is a transient object that assist the biologists' quests to understand complexity of life!
- has to constantly be refined (obsolete very fast)

A model is not a copy of reality.
It will take a different form depending on the subject of interest





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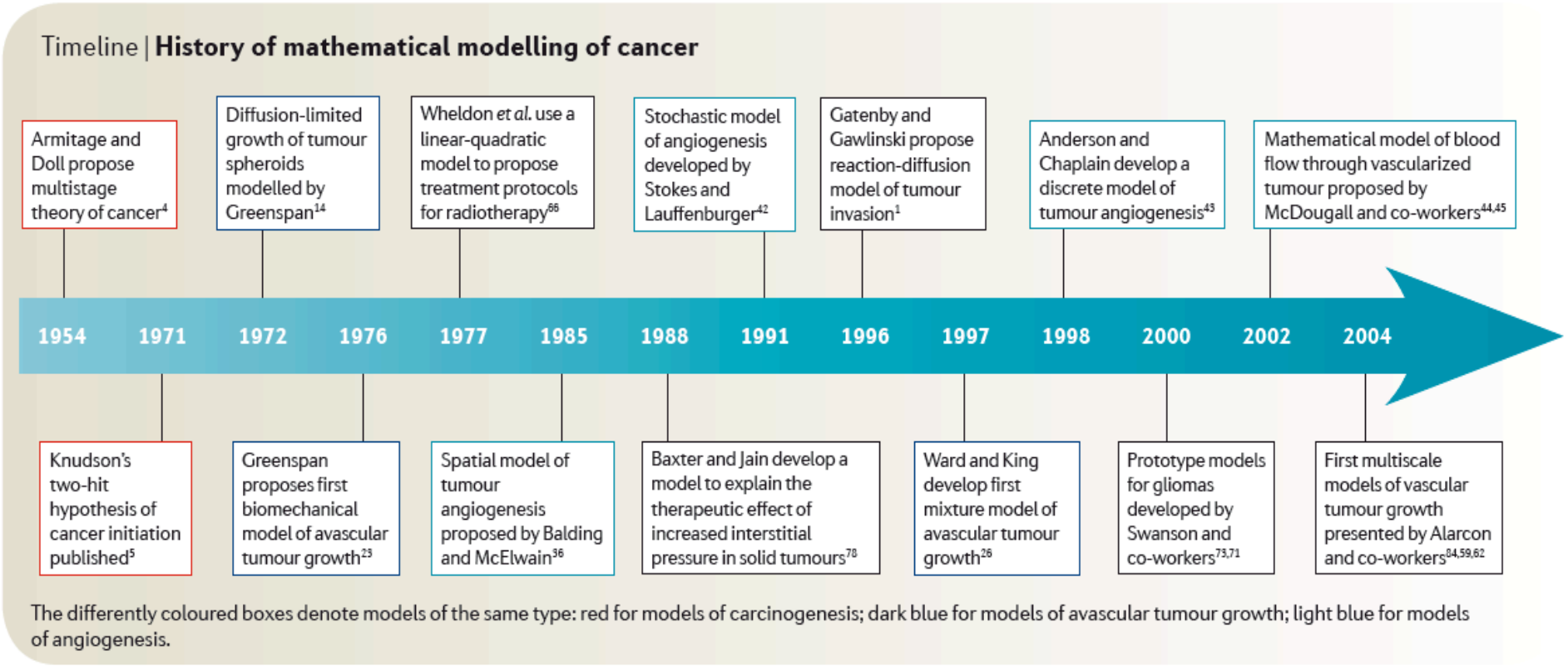
Mathematical modeling in cancer research

(Byrne, Nature Rev Cancer, 2010)



Cell population models: dynamic variables are numbers of cells of various types

Multiscale models: integrating intracellular biochemistry+cell behavior+tissue properties +organ functioning+organism physiology

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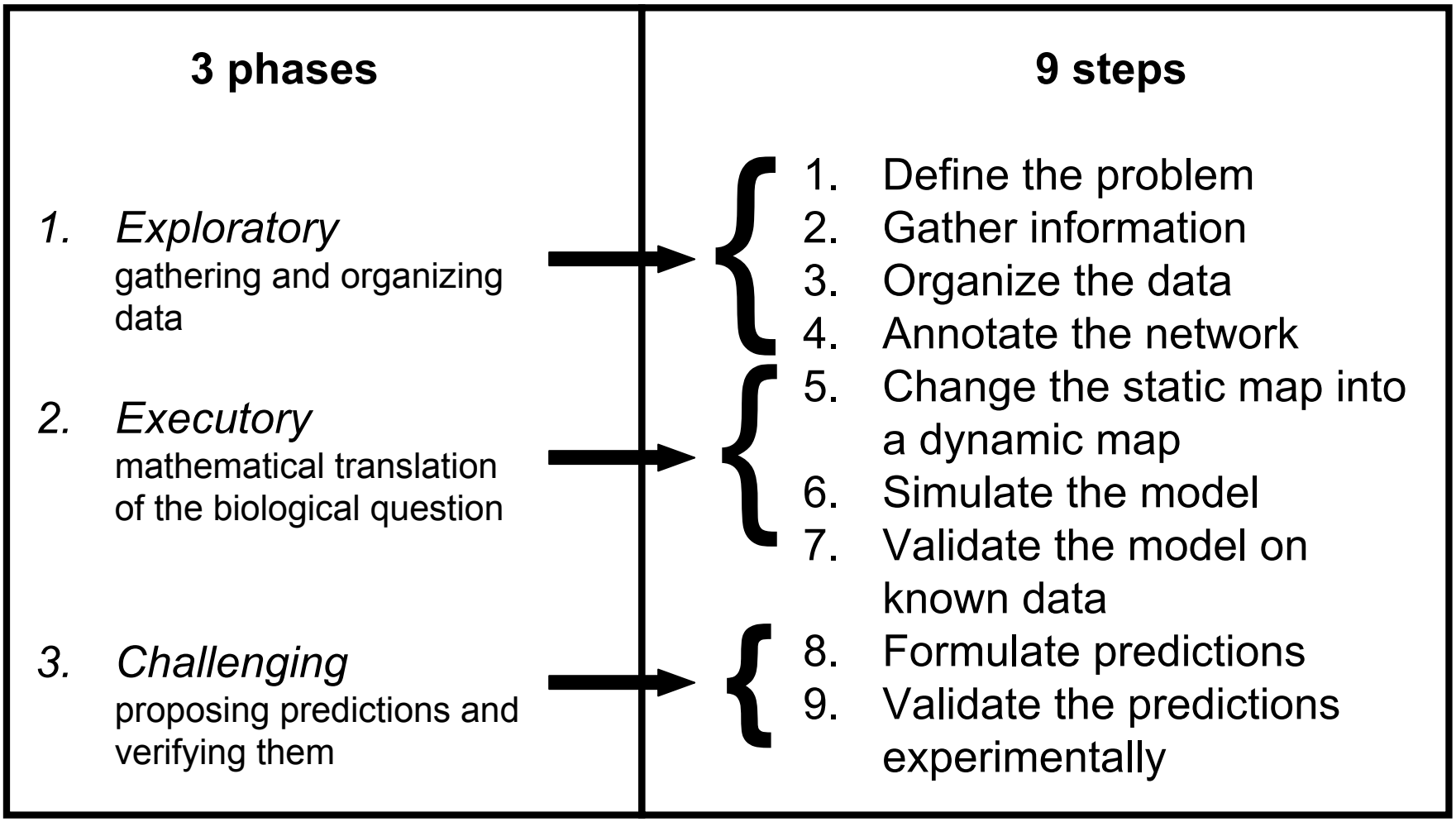
The modelling pipeline…



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2. MODELLING CELL FATE DECISION PROCESS

Apoptosis Systems Biology applied to cancer and AIDS

An integrated approach of experimental biology, data mining, mathematical modeling, biostatistics, system engineering and molecular medicine

http://www.apo-sys.eu/

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Motivations

- Identify key players and mechanisms involved in cell fate decision: cell death - apoptosis or necrosis - vs. survival
- Represent and integrate current knowledge about these processes
- Interpret heteregeneous biological data
- Predict novel behaviours (e.g. mutation phenotypes) and test hypotheses *in silico*
- Identify possible intervention points (e.g. for treatment of cancer)
- Anticipate effects of drugs

Step 1: Define the problem

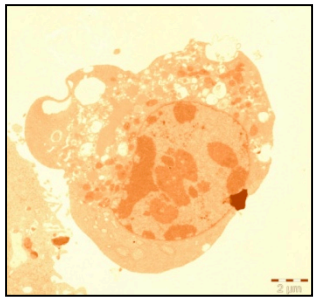
Problem:

Engagement of receptors such as TNFR1 or Fas can trigger cell death by apoptosis or necrosis, or lead to the activation of pro-survival signaling pathways such as NF-κB.

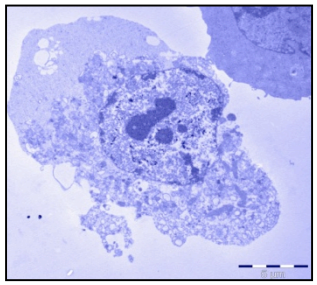
Cell response to death receptors

Two types of membrane-bound death receptors
- **TNF-R1** binds to TNFα
- **Fas** binds to Fas-L

- **Apoptosis**
  - Programmed cell death

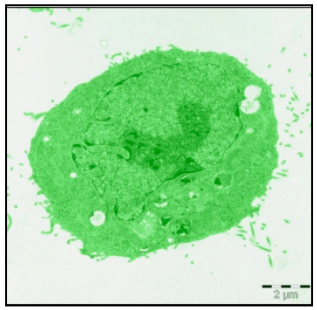


- **Necrosis**
  - Regulated and unregulated



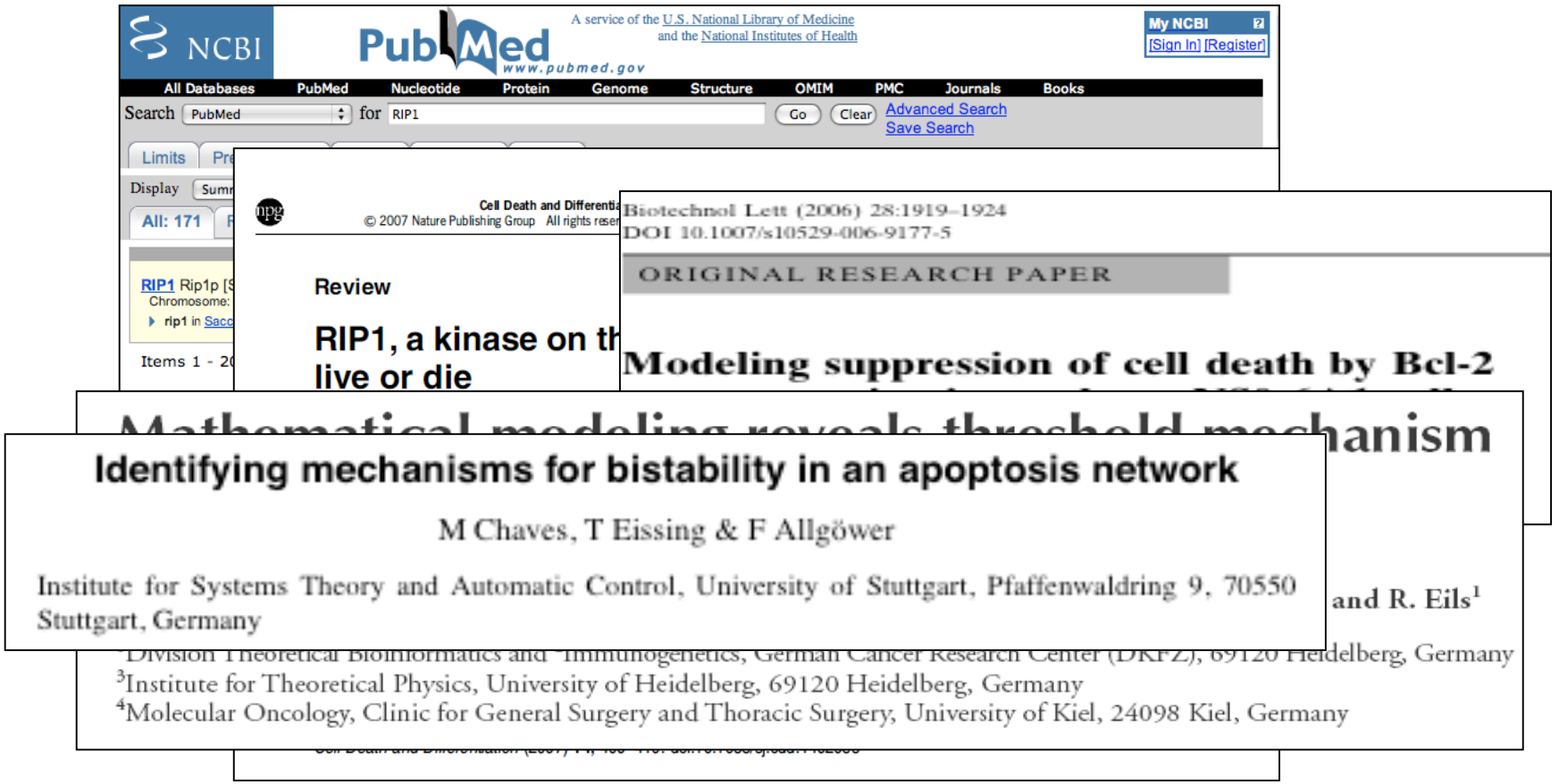
- Other death mechanisms: *e.g.* autophagy

- **Survival**
  - Passive survival (= absence of death)
  - "Controlled" survival → *e.g.* activation of NFκB pathway



Step 2: Gather information

How to find the proper information?



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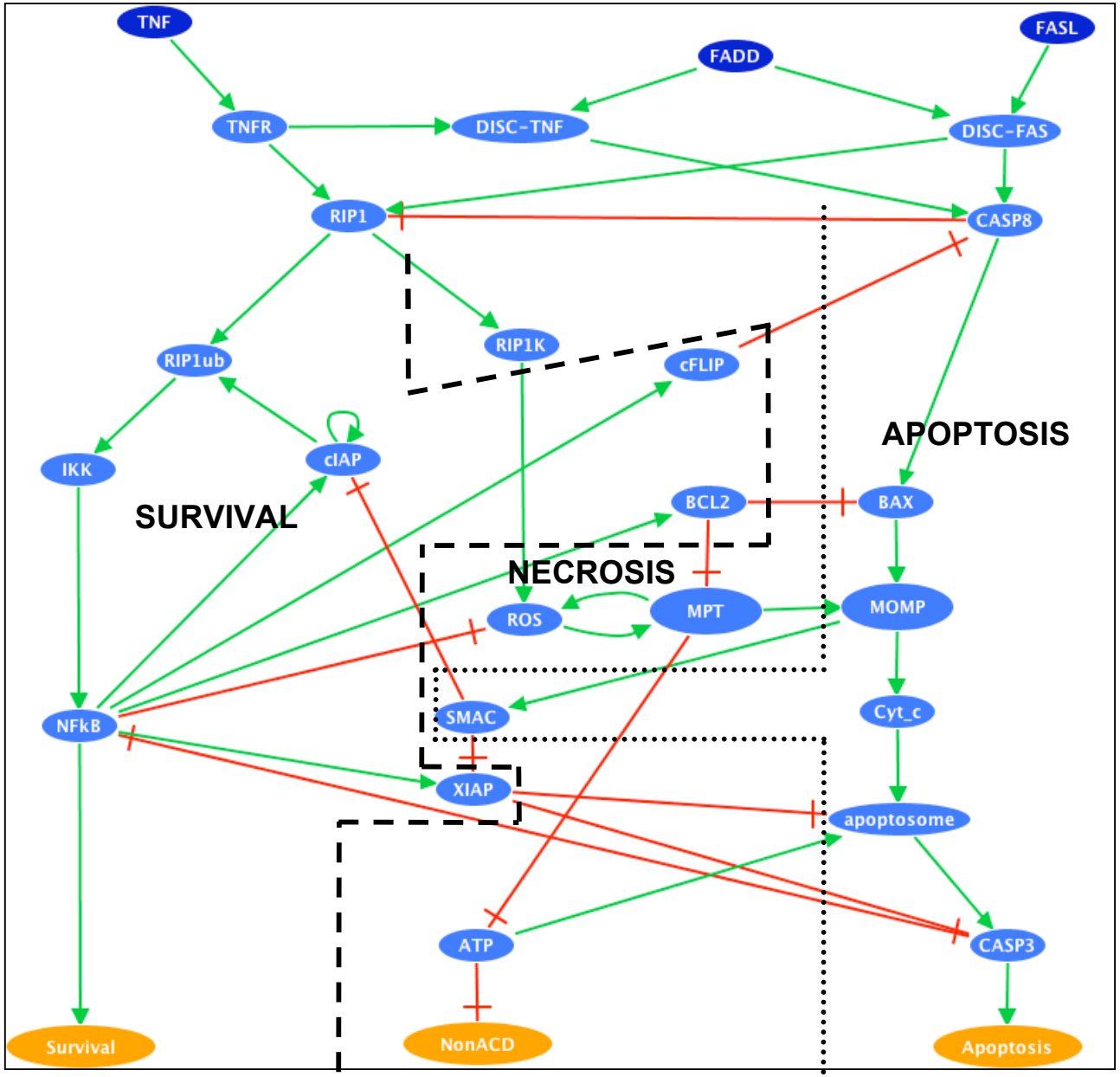
Step 3: Organize the data

After reading the literature and organizing the data…

In response to **TNF** or **FasL** stimulus



activation

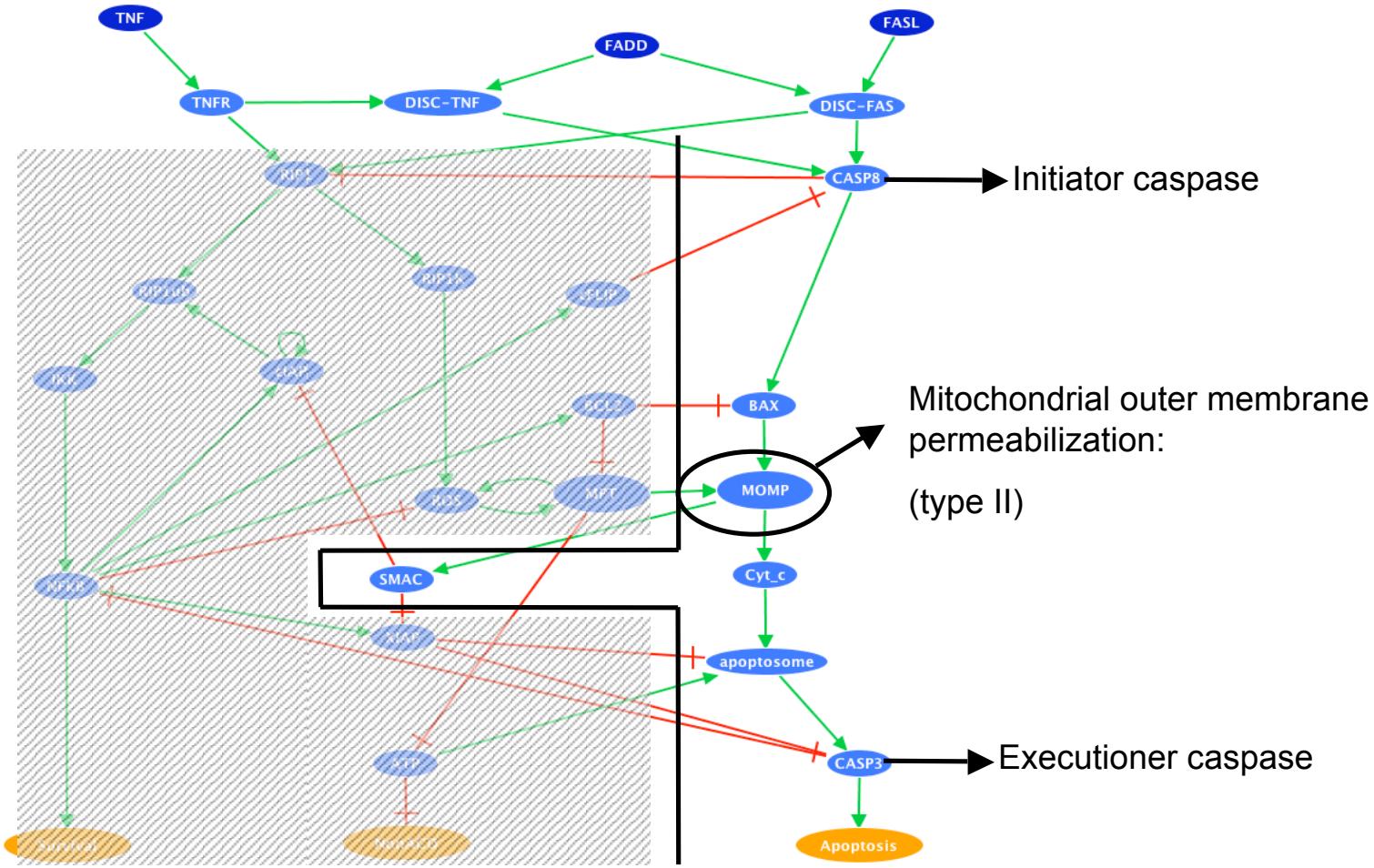
inhibition

3 cell fates:

Survival, **NonACD** (necrosis), **Apoptosis**

Software: GINsim

APOPTOSIS



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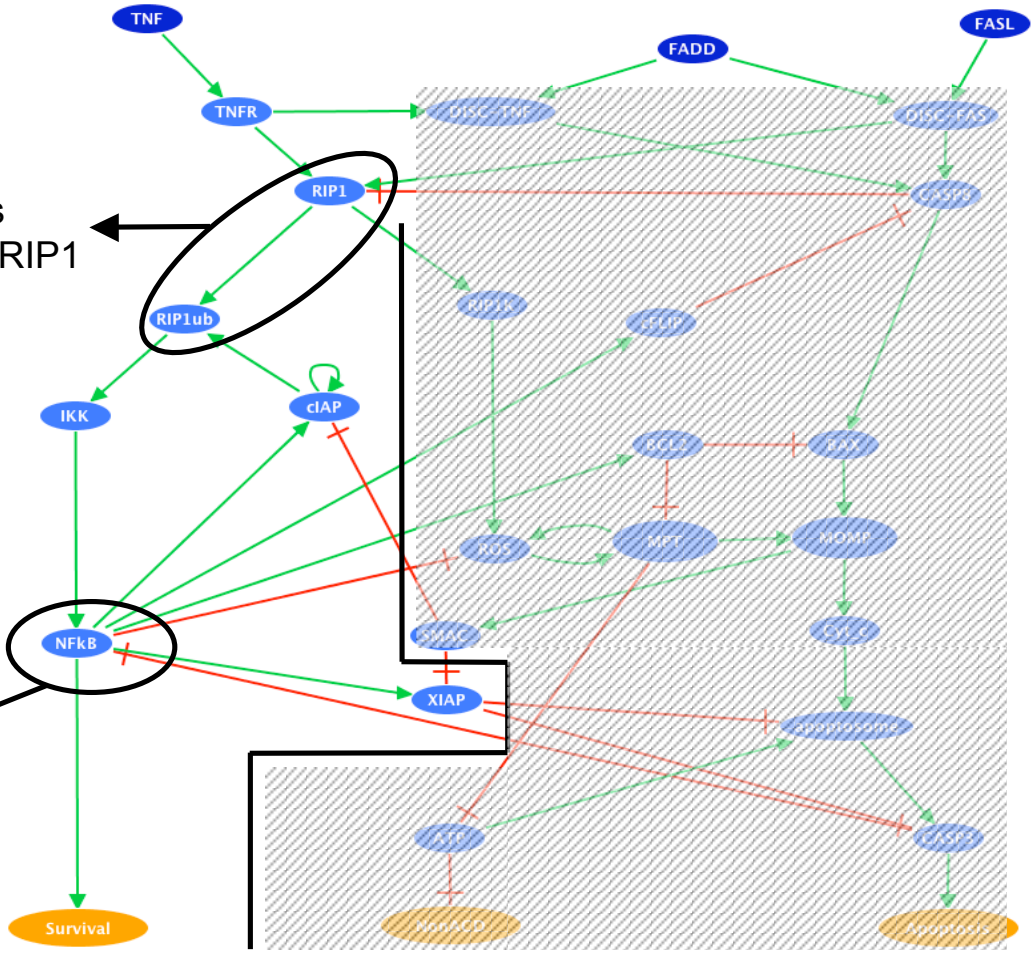
NFκB pathway

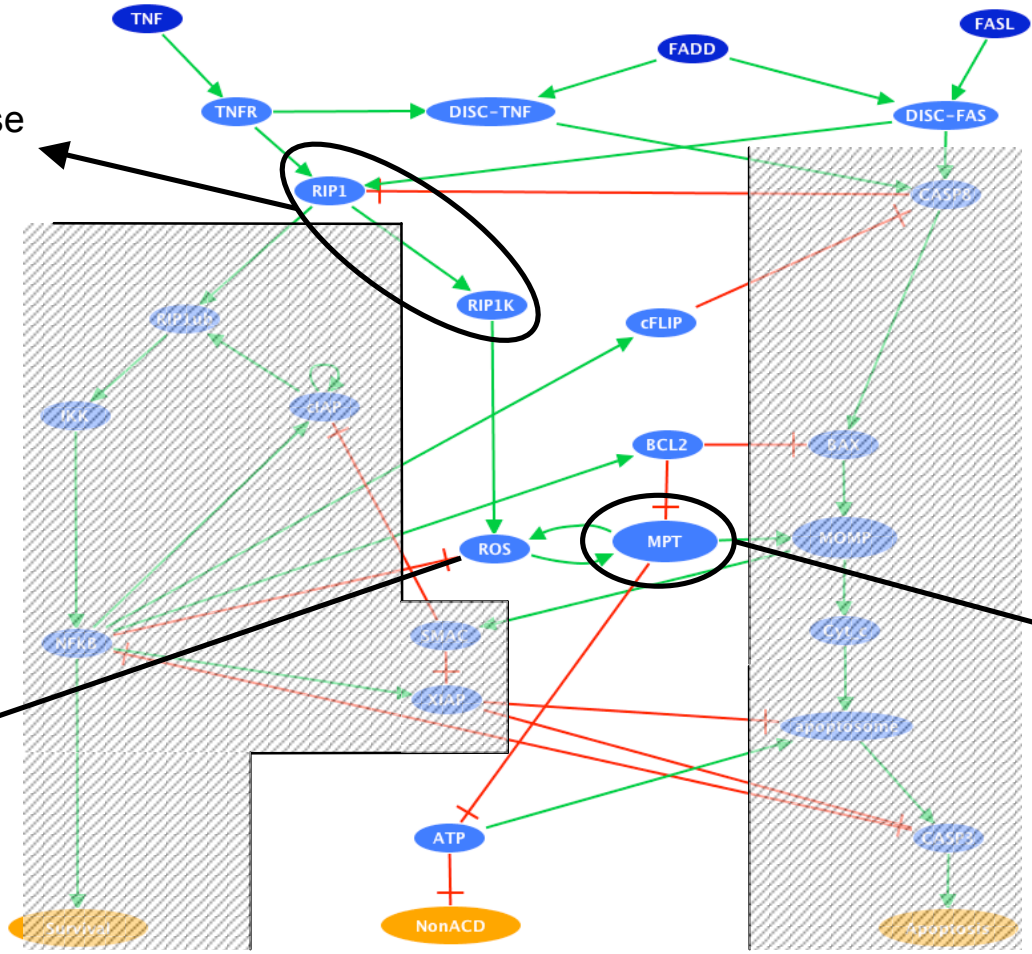
NFκB pathway needs ubiquitinated form of RIP1



No translocation of NFκB into the nucleus

NECROSIS

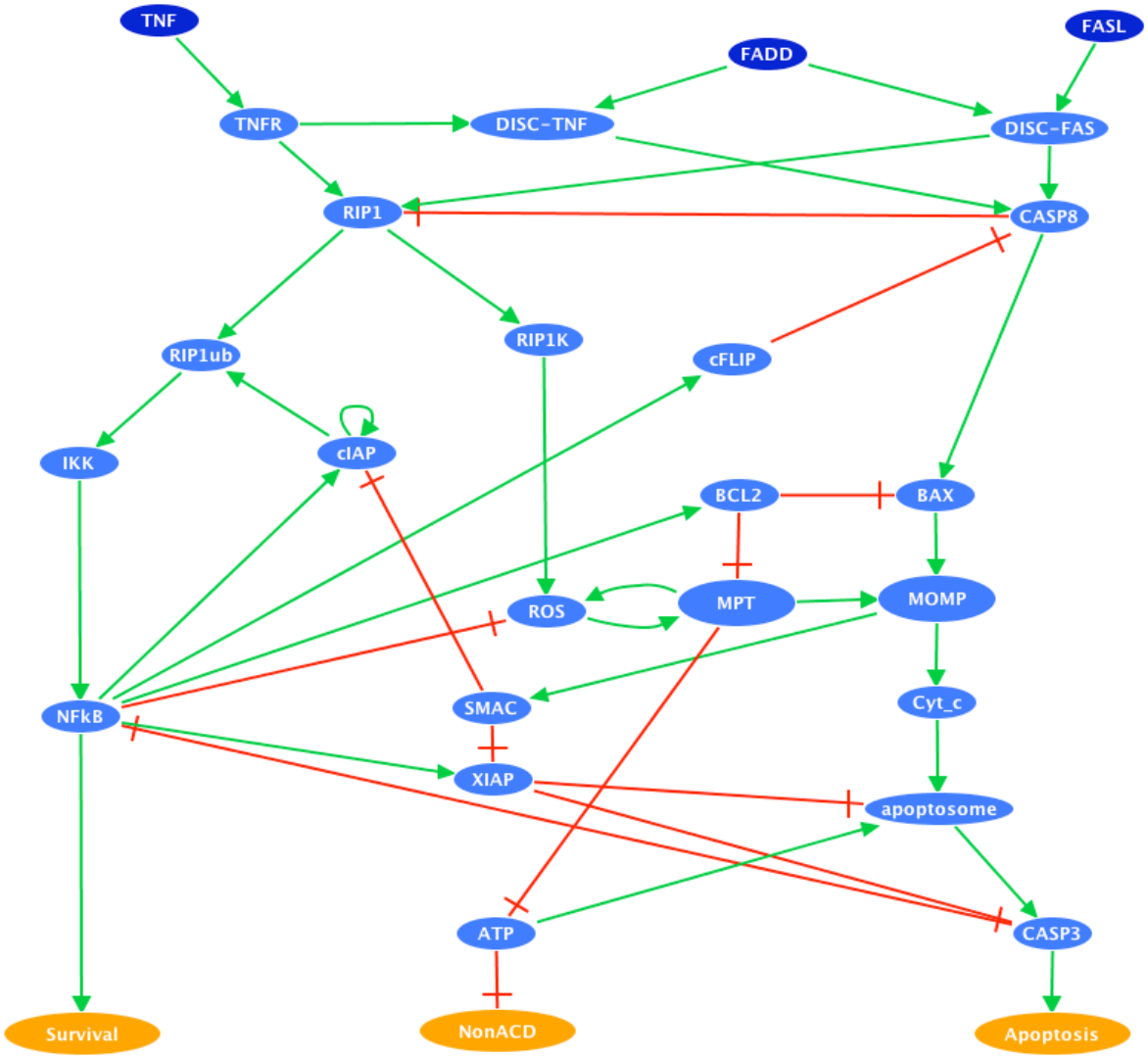
Necrosis needs kinase activity of RIP1



ROS : Reactive Oxygen Species

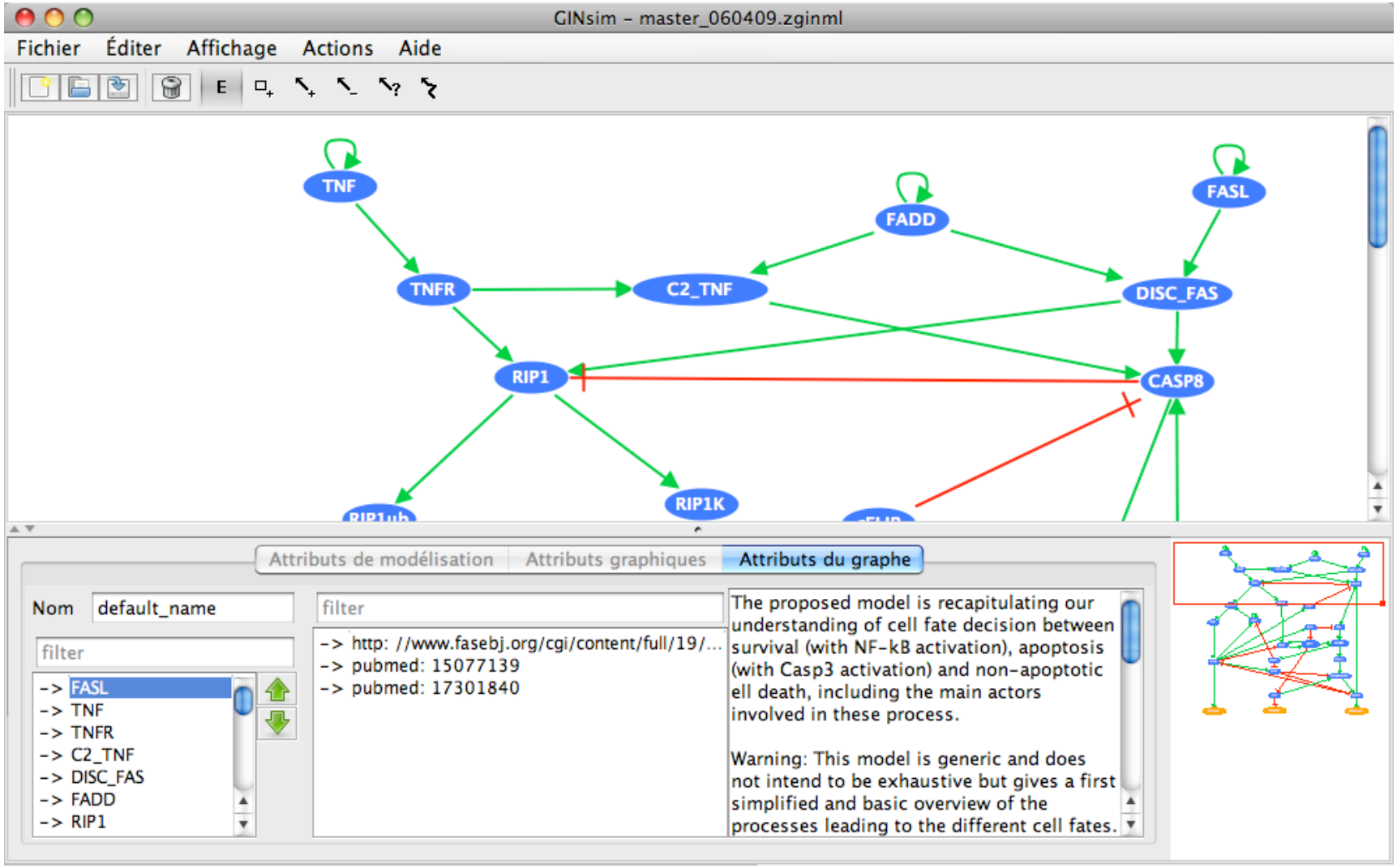
Mitochondria Permeability Transition

Integrated network



Step 4: Annotate the diagram

http://gin.univ-mrs.fr/ginsim



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Step 5: Model the network

Let's take a minute…

⇒ What is the question ?

How does the cell choose between death and survival?

⇒ What is the type of data?

Western Blots, CGH, transcriptome, etc.

⇒ What type of diagram can best describe the gathered information?

Influence diagrams, reaction networks…

⇒ **What do we want to do? Describe? Predict? What is the best formalism to use?**

Ordinary differential equations, Boolean modelling, …

(very much dependent on the type of data)

⇒ **What are the tools that are the most appropriate to build the diagram and simulate the model?**

CellDesigner, GINsim, Matlab, etc.

Modelling… ok… what do you mean?

GOAL: Find a way to verify that the structure is coherent with experimental evidence => use of mathematics

Questions that can be answered with modeling:

1. What are the possible solutions of the system?
i.e. can all phenotypes be observed in specific conditions (or cell types)?

2. What are the different possible successions of events from an initial signal to cell response?

Modelling… but how?

METHOD:

Data are qualitative!

1. Discrete or Boolean modelling

Assign logic to each node of the network

2. Dynamical analysis

Simulate the model in *normal* conditions

Identify all possible solutions (phenotypes) for all initial conditions

3. Validation

Validate the model on existing mutants

Boolean modeling

Assign logic to nodes

Example of CASP8

CASP8 = 1 when
- DISC-Fas=1 or/and DISC-TNF=1

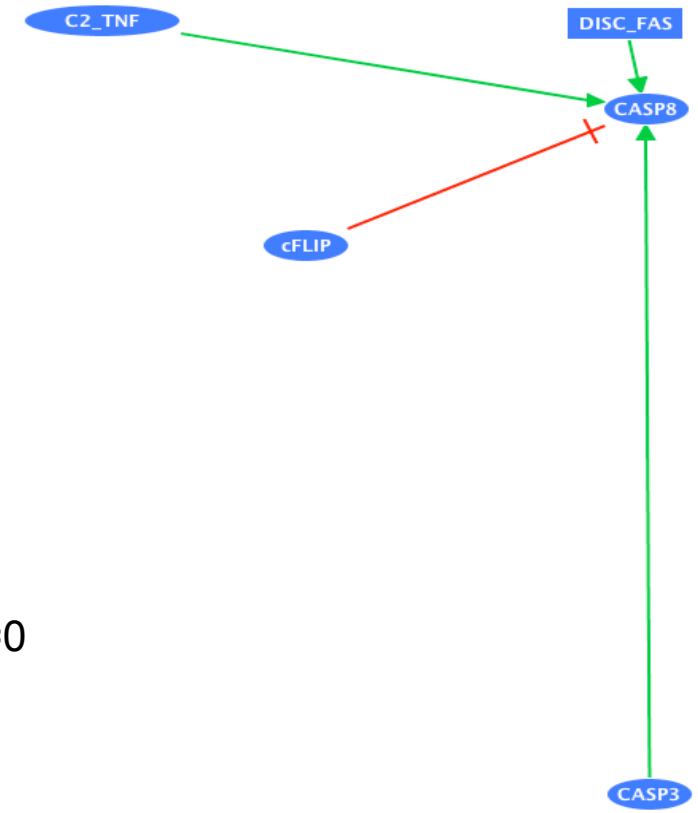
(signal from death receptors)
- CASP3=1

(amplification signal, feedback activation)
- AND no cFLIP

CASP8 = 0 when
- DISC-Fas=0 and DISC-TNF=0 and CASP3=0

(no external signals from death receptors
and no intracellular problems)
- cFLIP=1

(inhibition by the NFkB pathway)



One node = one species

Step 6: Simulate the model

Distribution of the possible solutions

FASL	TNF	TNFR	C2_TNF	DISC_FAS	FADD	RIP1	RIP1ub	RIP1K	IKK	NFkB	CASP8	Bax	Bcl2	ROS	ATP	MPT	MOMP	SMAC	cIAP	Cyt_c	XIAP	Apoptosome	CASP3	cFLIP	NonACD	Apoptosis	Survival
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
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1	1	1	1	1	1	0	0	0	0	0	1	1	0	1	0	1	1	1	0	1	0	0	0	0	1	0	0

Naïve survival | NFkB survival | apoptosis | necrosis

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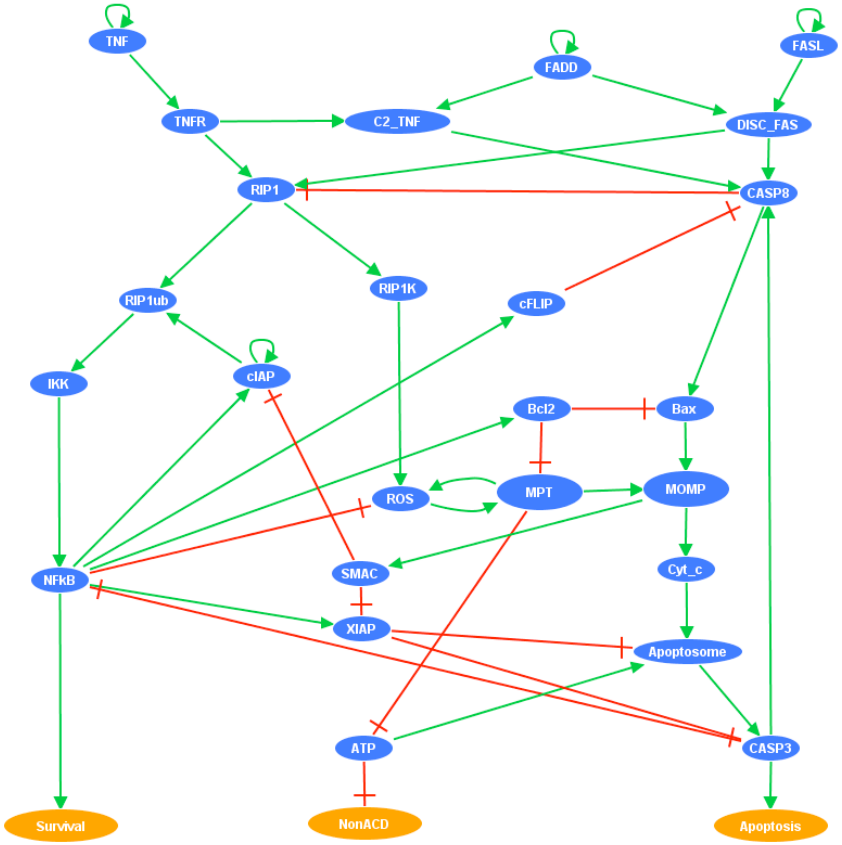
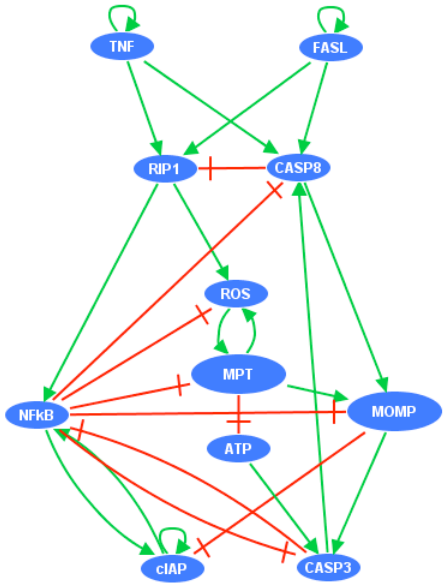
Dynamical simulation => Model reduction



- 9 inner variables
- 2 inputs: TNF and FASL (no FADD)
- Every “logical” interaction is conserved
- State space: $2^{11} = 2048$ states



Node	Logical update rule
TNF	*(INPUT NODE)*
FAS	*(INPUT NODE)*
RIP1'	!C8 & (TNF | FAS)
NFkB'	(cIAP & RIP1) & !C3
C8'	(TNF | FAS | C3) & !NFkB
cIAP'	(NFkB | cIAP) & !MOMP
ATP'	!MPT
C3'	ATP & MOMP & !NFkB
ROS'	!NFkB & (RIP1 | MPT)
MOMP'	MPT | (C8 & !NFkB)
MPT'	ROS & !NFkB

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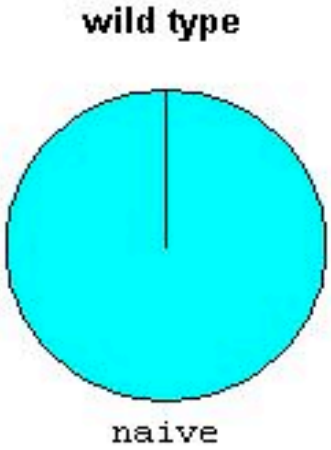
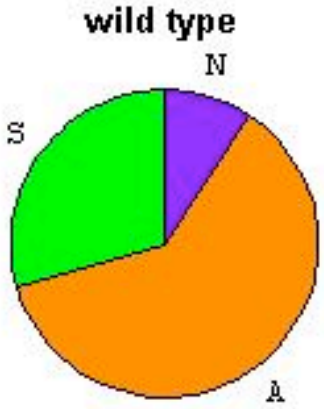
« Probabilities » of reaching alternative phenotypes from physiological initial conditions:

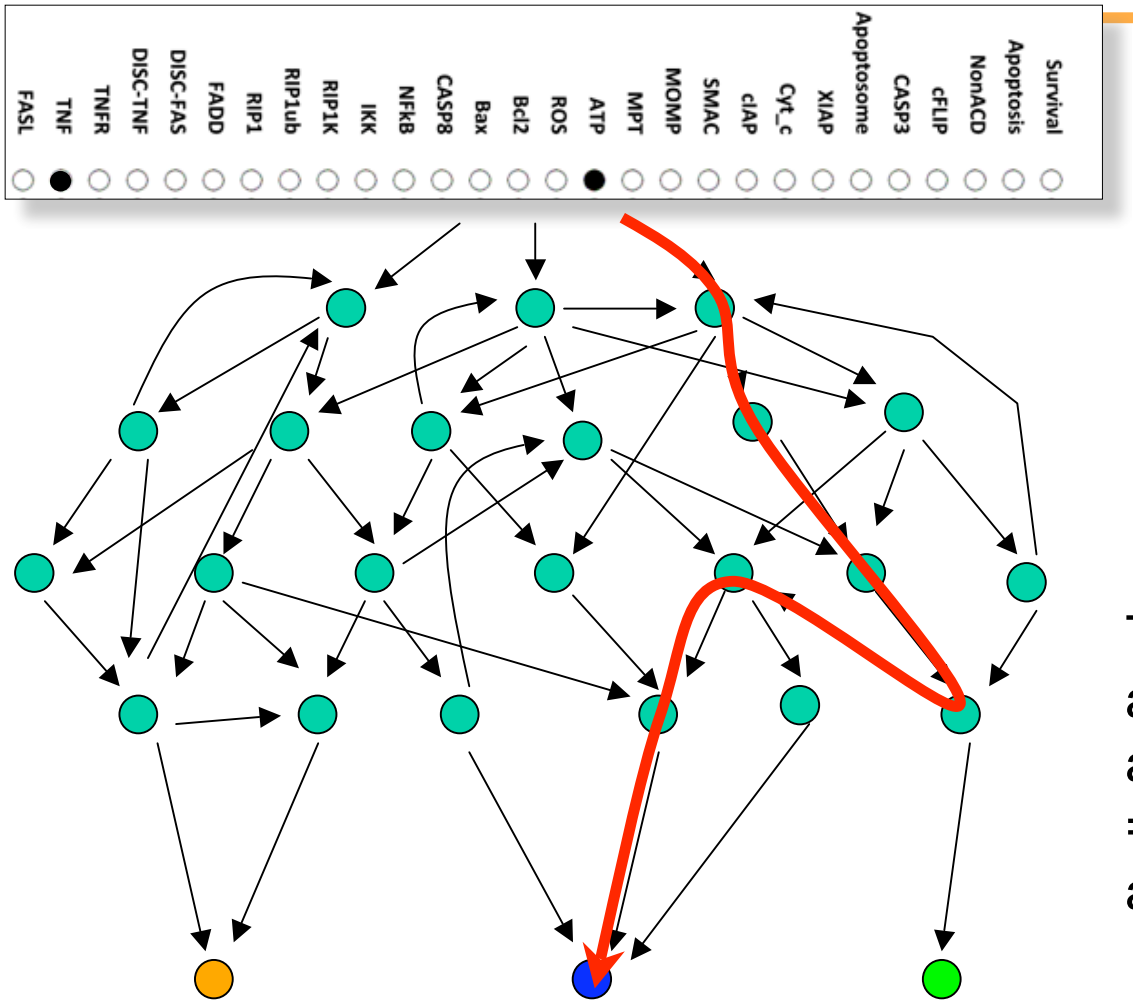


TNF=0



TNF=1

Asynchronous state transition graph



The probability to reach
a final state from
an initial state
= probability of observing
a phenotype in experiment

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Step 6: Validate the model

Confront the model to existing data

⇒ Simulations of mutants or drug treatments

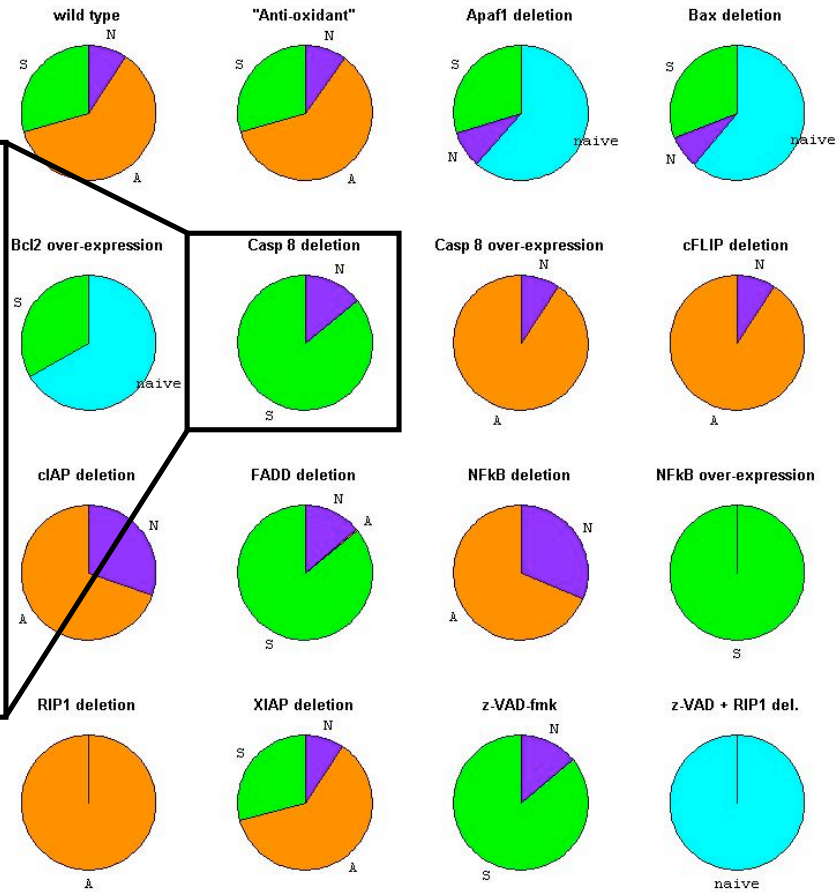
Name	Modified rules	Expected phenotypes	Qualitative results
Anti-oxidant	ROS'=(RIP1 OR MPT)		Suppression of NF_B anti-oxidant effect leads to no change in the decision process (the computed probabilities are noticeably close to the wild type).
APAF1 deletion	C3'=0	APAF1$^{-/-}$ mouse thymocytes are not impaired in Fas-mediated apoptosis (Yoshida *et al*, 1998)	Apoptosis disappears. Necrosis and survival are close to the wild type case. Lacking apoptosis is mainly replaced by the 'naïve' state
BAX deletion	MOMP'=MPT	BAX deletion blocks Fas or TNF+CHX - induced apoptosis in some cell lines, such as HCT116 (LeBlanc *et al*, 2002)	BAX deletion prevents apoptosis.
BCL2 over-expression	MOMP'=MPT MPT'=0	FAS induces the activation of NF_B pathway (Kreuz *et al*, 2001)	As expected, NF_B pathway is a reachable attractor. The second reachable attractor is the 'naïve' state, which means that both death pathways are inhibited.
C8 deletion	C8'=0	Caspase 8 deficient MEFs (Varfolomeev *et al*, 1998) or Jurkat cells (Kawahara *et al*, 1998) are resistant to Fas-mediated apoptotic cell death.	As expected, apoptosis is no longer reachable. Compared to the wild type, a slight increase of necrosis is observed, while NF_B survival becomes the main cell fate.
constitutively activated CASP8	C8'=1		Over-expression of caspase 8 leads to an increased disappearance of NF_B activation.
cFLIP deletion	C8'=TNF OR FAS OR C3	cFLIP-/- MEFs are highly sensitive to FasL and TNF α (Yeh *et al*, 2000)	The increase of apoptosis is effectively observed in the cFLIP mutant; however we also observe that NF_B pathway can no longer be sustained.
cIAP deletion	cIAP'=0	NFκB activation in response to TNF is blocked (Varfolomeev *et al*, 2008)	NFκB activation is impaired, and only the apoptotic or necrotic attractors are reached.
FADD deletion	C8'=C3AND NOT NF_B RIP1'=NOT C8 AND TNF	FADD-/- mouse thymocytes are resistant to Fas mediated apoptosis (Zhang *et al*, 1998) . FADD-/- MEFs are resistant to FasL and TNF α (Yeh *et al*, 1998) In Jurkat cells treated with TNFα+CHX, FADD deletion turns apoptosis into necrotic cell death (Harper *et al*, 2003)	In response to FasL, signalling is blocked, thus the 'naïve' attractor is the only reachable one. In response to TNF, apoptosis disappears.
NF_B deletion	NFkB'=0	TNFα induces both apoptosis and necrosis in NF- κB p65 $^{-/-}$ cells (Sakon *et al*, 2003) or in IKK β $^{-/-}$ fibroblasts (Kamata *et al*, 2005)	This mutant shows a strong increase of necrosis (to be related with concomitant apoptosis/necrosis)
constitutively active NF_B	NFkB'=1		Both death pathways are shut down in this mutant.
RIP1 deletion	RIP1'=0	RIPK1 $^{-/-}$ MEFs are hypersensitivity to TNF α, no TNFα-induced NF κB activation, (Kelliher *et al*, 1998)	Both NF_B and necrosis become unreachable. The effect of RIP1 silencing leads to a complete loss of the decision process (apoptosis becoming the only outcome).
XIAP deletion	C3'=ATP AND MOMP	No effect on TNF α-induced toxicity in XIAP $^{-/-}$ MEFs (Harlin *et al*, 2001)	S
z-VAD	C3'=0	FAS induced apoptosis is blocked	The simulation of z-VAD mutant is

TNF=1

Example : **Caspase 8 deletion**

- ≈ 85% survival (NFkB)
- ≈ 15% necrosis
- No apoptosis

Qualitatively consistent with the literature
"TNF-induced apoptosis is blocked though not necrosis"
[Kawahara, Ohsawa *et al., J Cell Biol* 1998]
(Jurkat cells, C8-/-)



Naïve survival | NFkB survival | apoptosis | necrosis

Step 8: Formulate predictions

Some predictions

-On the general structure of the network

e.g. Choice between two antagonist interactions:

cIAP -> NFkB *vs.* cIAP -| NFkB

-On the activity/behaviour of components of the system

Transient and maintained activity of RIP1 in necrosis and survival

-On the phenotype on novel mutants

e.g. *cIAP* del

-On the effect of length of TNF pulses on decision process (not shown)

- On points of intervention

e.g. *how to enforce death in cancerous cells evading apoptosis*

Step 9: Validate the predictions experimentally

In process…

- RIP1/3 transient or maintained activity
- How do different cell types choose their fate / Adapt the model to specific cell type:

  - cell conditions?
    => use high throughput data to adapt the model

  - speed of reactions?
    => test on appropriate cell types

Protagonists of cell fate decision model

Simon Fourquet

Laurent Tournier

Emmanuel Barillot

Denis Thieffry

Boris Zhivotovski

Andrei Zinovyev

OPEN ACCESS Freely available online

PLoS COMPUTATIONAL BIOLOGY

Mathematical Modelling of Cell-Fate Decision in Response to Death Receptor Engagement

Laurence Calzone[1,2,3*], Laurent Tournier[1,2,3], Simon Fourquet[1,2,3], Denis Thieffry[4,5], Boris Zhivotovsky[6], Emmanuel Barillot[1,2,3¶], Andrei Zinovyev[1,2,3¶]

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Concluding remarks

What we have provided:

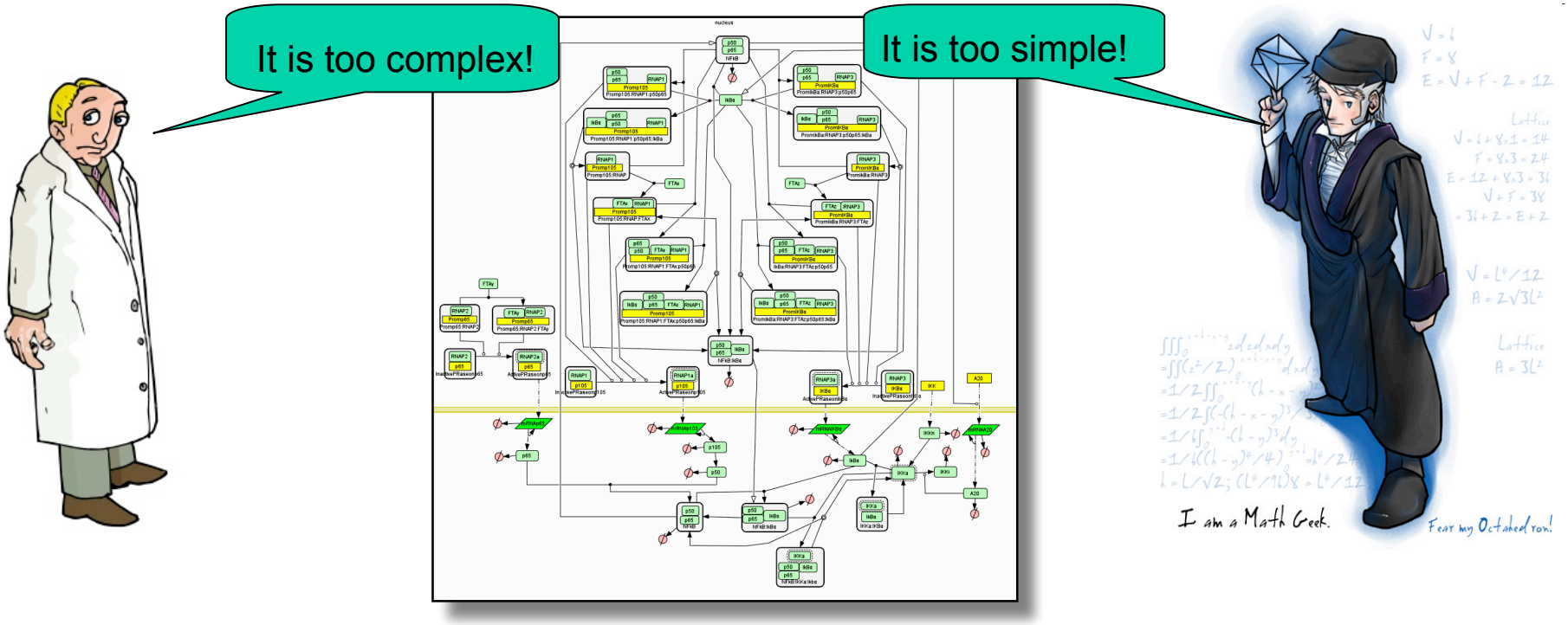
- A mathematical model of cell fate decision that recapitulates most known biological facts and that accurately predicts mutant conditions

- A way to formalise and test hypotheses

- A tool for *in silico* experiments (cell fate decision process has important implications for therapeutic treatment)

Biologists vs. Modelers
A matter of how to view complexity…

Dynamical model of NFkB pathway



Radulescu, Gorban, Zinovyev. BMC Syst Biol 2008

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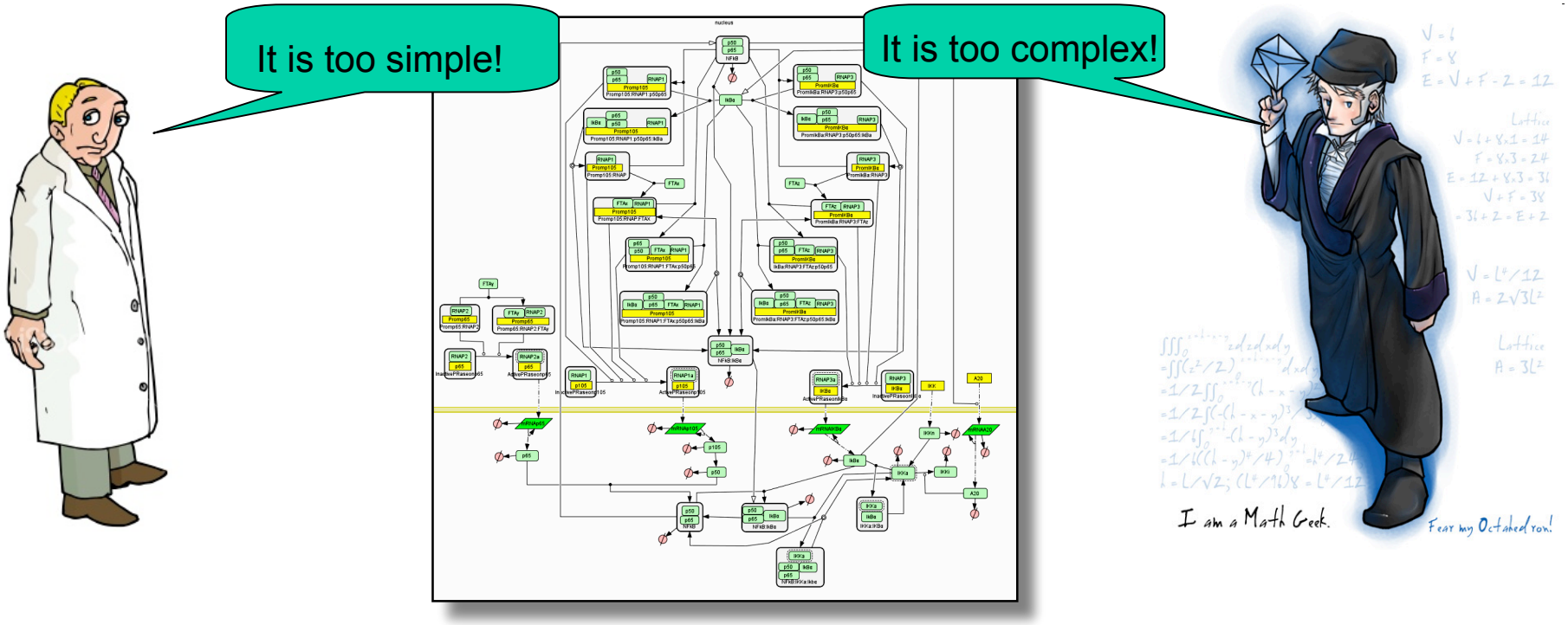

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