Reproducible computational modelling of Acute Promyelocytic Leukaemia resistance to Retinoic Acid therapy.

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DISEASE MODELLING

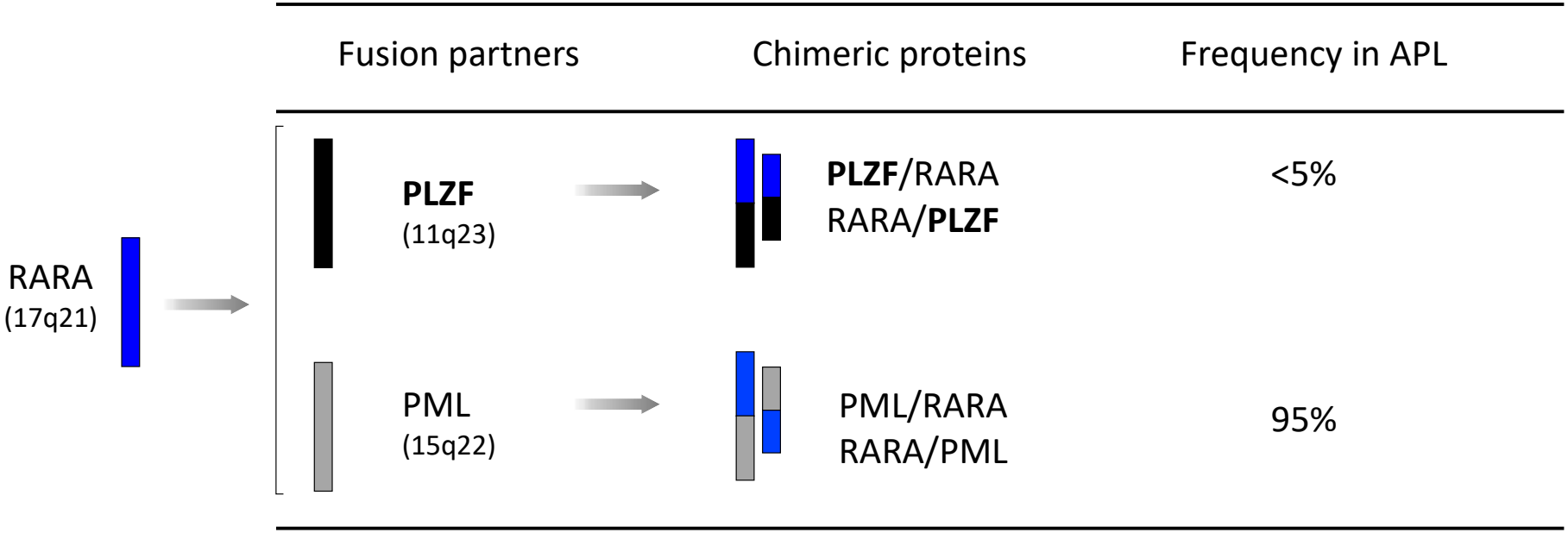
Modelling approaches for disease processes

May 6th, 2026.

Acute Promyelocytic Leukaemia (APL)

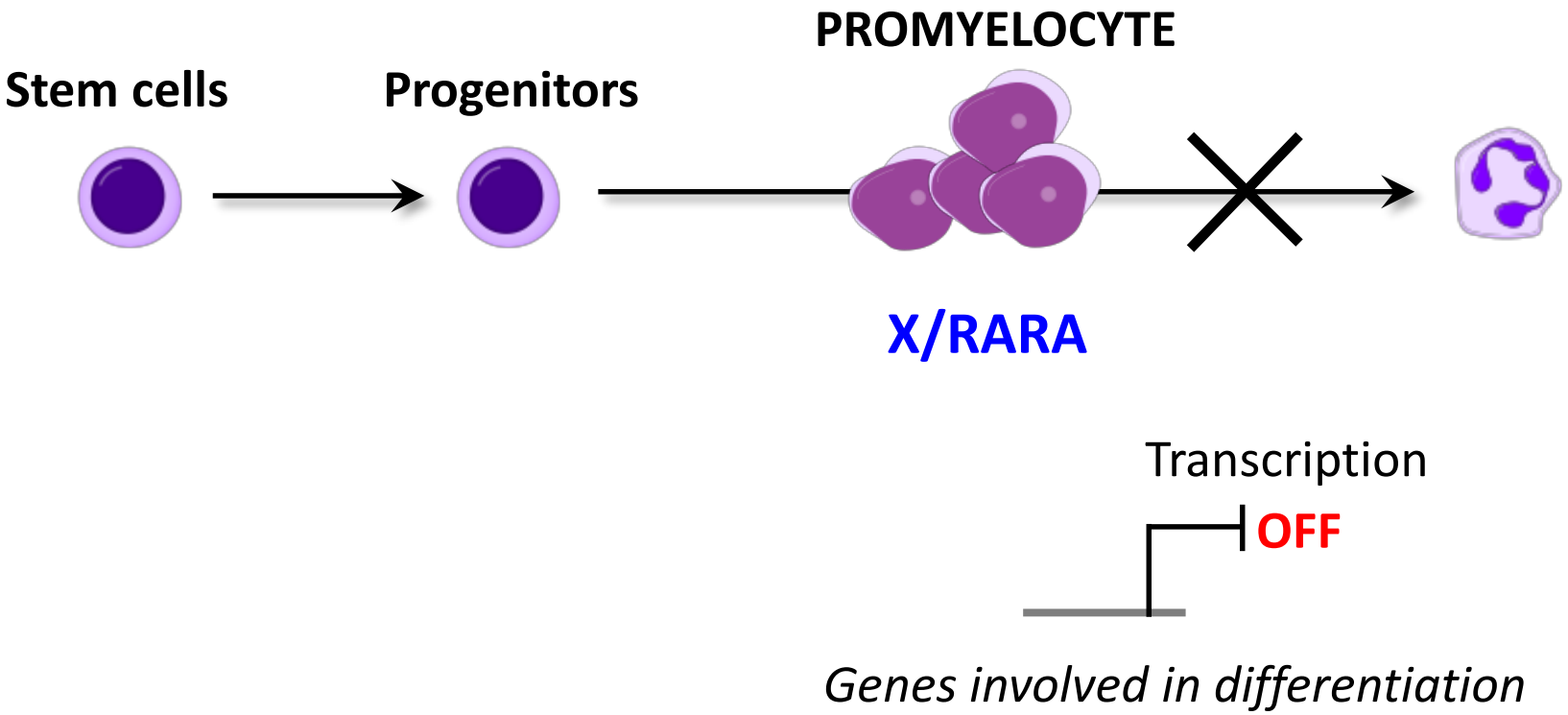
- **Acute promyelocytic leukemia** (APL) is a blood cancer characterised by a marked increase in a type of immature white blood cells known as promyelocytes.
- The bone marrow is filled by **malignant cells** and is unable to produce functional cells.
- The **treatment** of APL is based on **elimination of the malignant cells** and supportive care with transfusion of blood products to minimise the risk of bleeding or thrombosis.
- Treatment with all-trans **retinoic acid** (ATRA) and **arsenic trioxide** allow malignant promyelocytes to mature into neutrophils, which are unable to proliferate.

Acute Promyelocytic leukemia : M3 AML / Oncogenic Fusion X/RARA



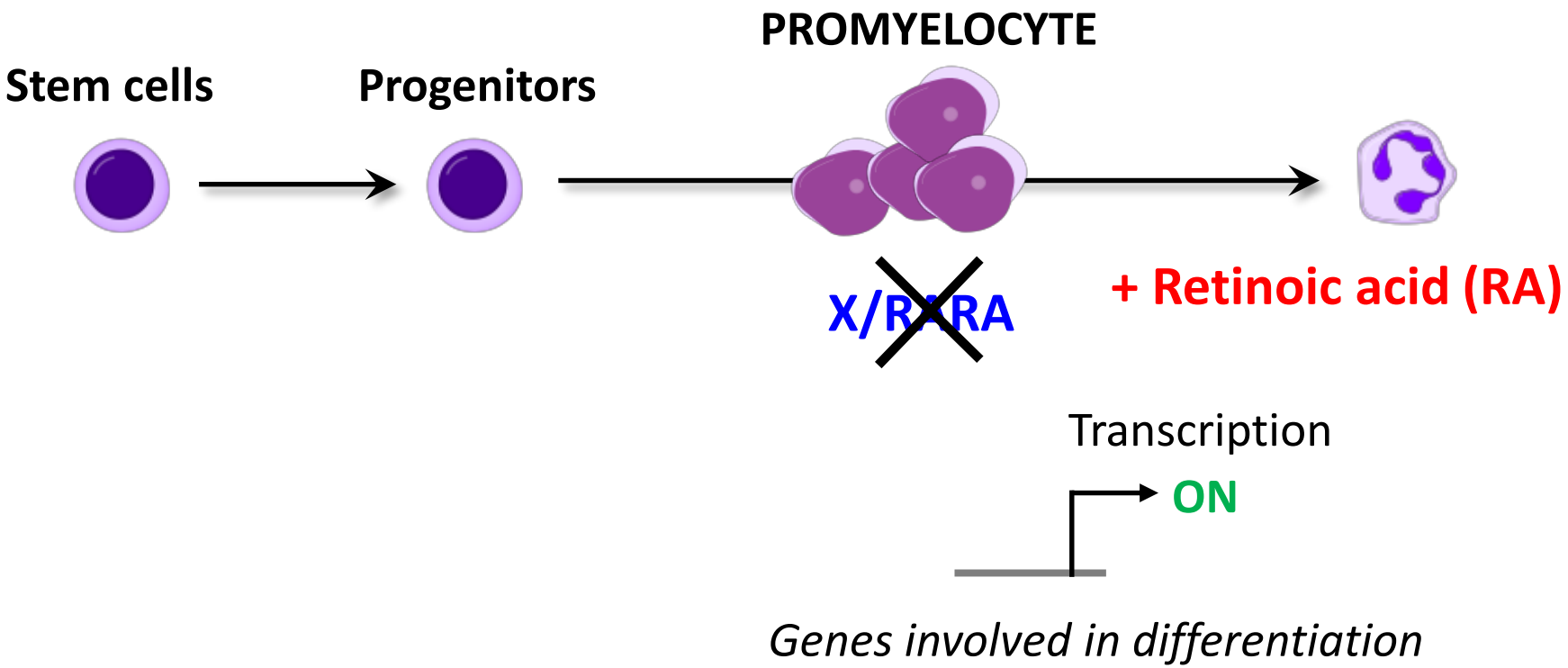
Two main gene **oncogenic fusions** have been reported, involving the **RARA** (nuclear Retinoic Acid Receptor alpha) gene fused with the **PLZF** or **PML** coding gene.

Acute Promyelocytic leukemia : M3 AML / Oncogenic Fusion X/RARA



The oncogenic fusion impairs promyelocyte differentiation

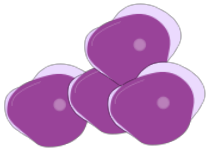
Acute Promyelocytic leukemia : M3 AML / Oncogenic Fusion X/RARA



Treatment with pharmacological levels of RA restores promyelocyte differentiation

Acute Promyelocytic leukemia : M3 AML / Oncogenic Fusion X/RARA

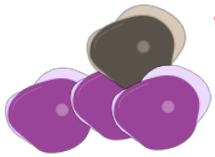
t(15;17)
PML/RARA
SENSITIVE APL



+ Retinoic acid (RA)

Survival
(90%)

t(11;17)
PLZF/RARA
RESISTANT APL



+ Retinoic acid (RA)

Relapse
(100%)

APL relapse occurs systematically in PLZF/RARA patients

Biological questions

- What are the **molecular mechanisms** underlying the apparition of **resistance** to RA + arsenic treatment ?
- How to explain the **different responses to RA treatment** observed in PLZF/RARA vs PML/RARA patients ?

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Regular Article
2022

MYELOID NEOPLASIA

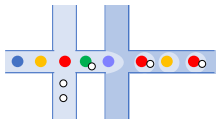
Noncanonical EZH2 drives retinoic acid resistance of variant acute promyelocytic leukemias

Mathilde Poplineau,[1-3] Nadine Platet,[1,3] Adrien Mazuel,[1,3,*] Léonard Hérault,[1,3,4,*] Lia N'Guyen,[1,3] Shuhei Koide,[2,5] Yaeko Nakajima-Takagi,[2,5] Wakako Kuribayashi,[2,5] Nadine Carbuccia,[6] Loreen Haboub,[1,3] Julien Vernerey,[1] Motohiko Oshima,[2,5] Daniel Birnbaum,[6] Atsushi Iwama,[2,5] and Estelle Duprez[1,3]

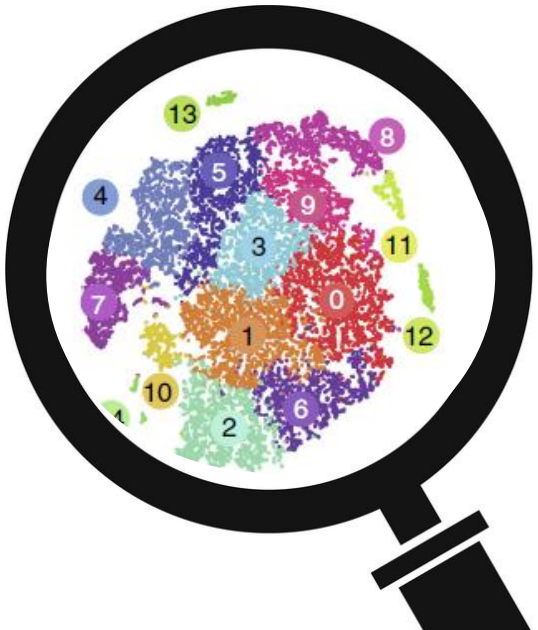
Single cell analyses & cell identity

Population heterogeneity

Single cell capture

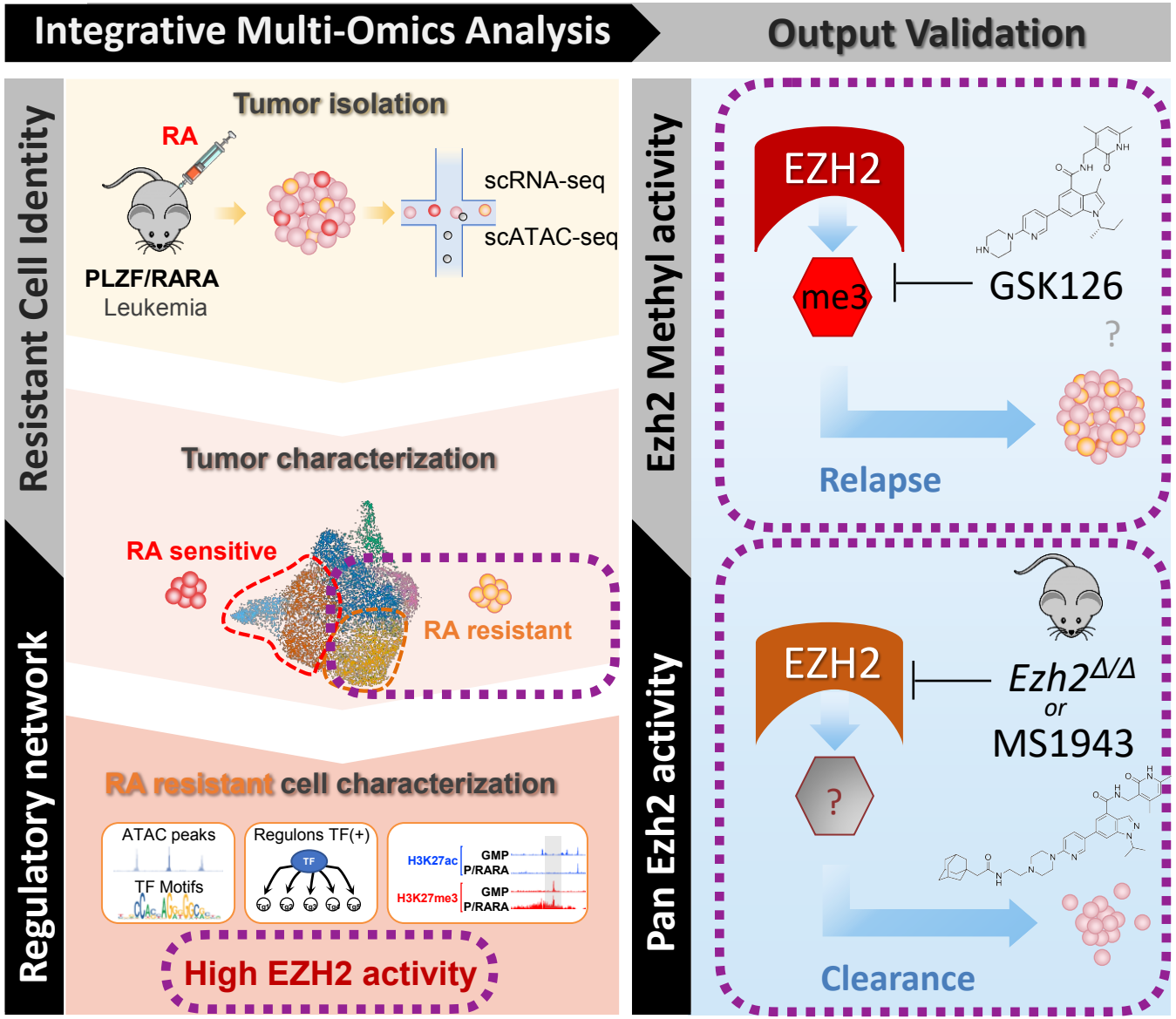


Information
Chromatin
Gene expression
Mutations …



Mathilde Poplineau

Non canonical / non-methyl activity of EZH2 involved in the resistance of APL to RA

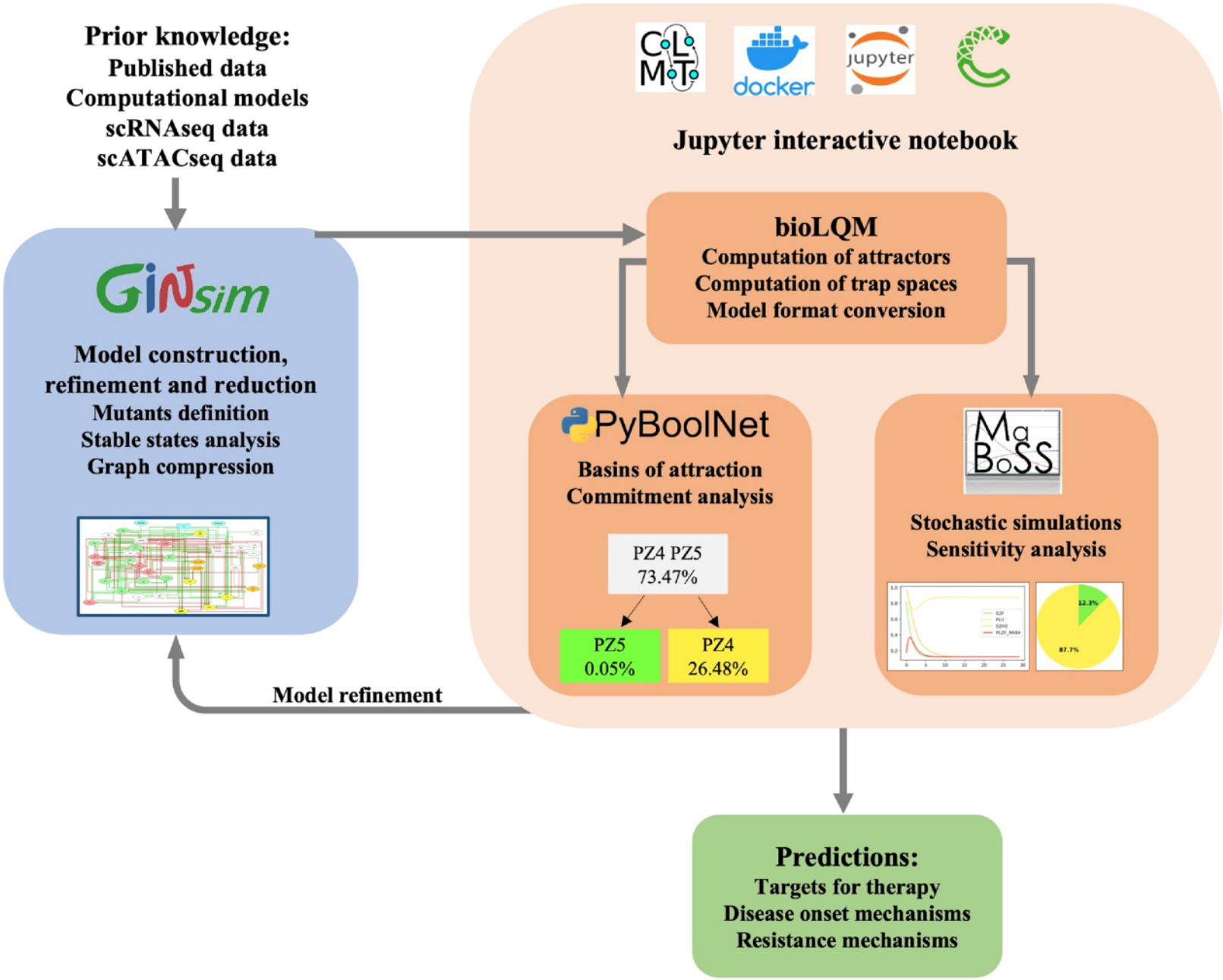


Further questions

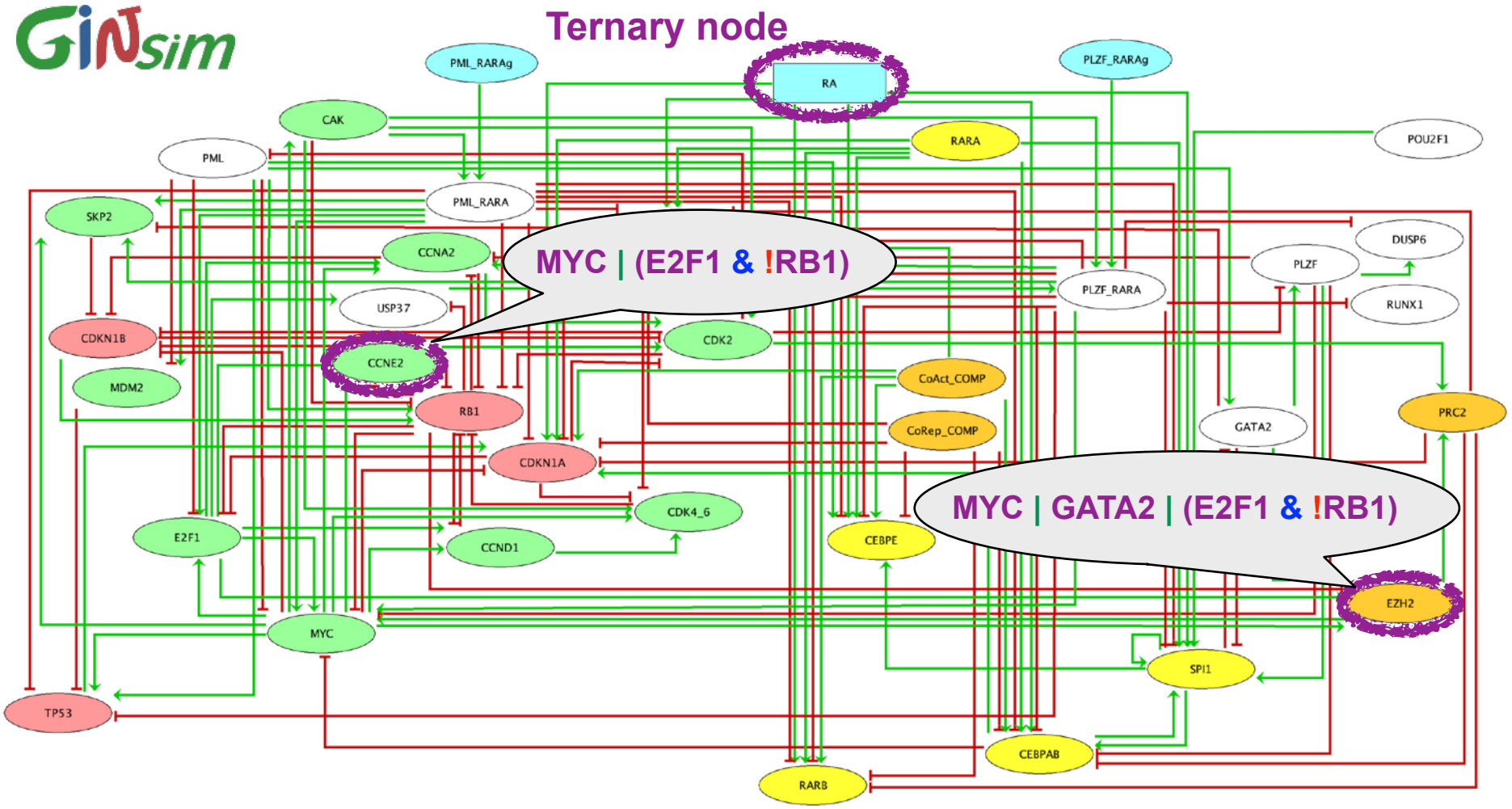
- How do **EZH2 activities** impact on RA signalling and cell fate decisions (i.e. proliferation vs differentiation) ?
- Can we build a **mechanistic model integrating** these different regulatory layers, i.e. **signalling, transcriptional regulation, and epigenetic remodelling** ?

José-Antonio Sánchez-Villanueva

Modelling workflow



APL logical model



The **APL regulatory graph** encompasses **38** nodes and **125** signed arcs. Internal nodes can be associated with four main functions: cell cycle arrest (red), cell cycle progression (green), granulocytic differentiation (yellow), and epigenetic regulation (orange). Oval nodes denote Boolean components, whereas the rectangular one denotes a ternary component (RA). Green arrows and red blunt arcs denote positive and negative regulatory influences, respectively. Three input nodes (light blue) denote the presence of translocations (PLM_RARAg or PLZF_RARAg), as well as the RA level considered (No RA: 0, physiological level 1, and pharmacological level 2).

Towards eproducible logical modelling analyses

frontiers in Physiology

METHODS
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Check for updates

The CoLoMoTo Interactive Notebook: Accessible and Reproducible Computational Analyses for Qualitative Biological Networks

Aurélien Naldi[1], Céline Hernandez[1], Nicolas Levy[2,3], Gautier Stoll[4,5,6,7,8], Pedro T. Monteiro[9], Claudine Chaouiya[10], Tomáš Helikar[11], Andrei Zinovyev[12,13,14,15], Laurence Calzone[12,13,14], Sarah Cohen-Boulakia[2], Denis Thieffry[1] and Loïc Paulevé[2]**

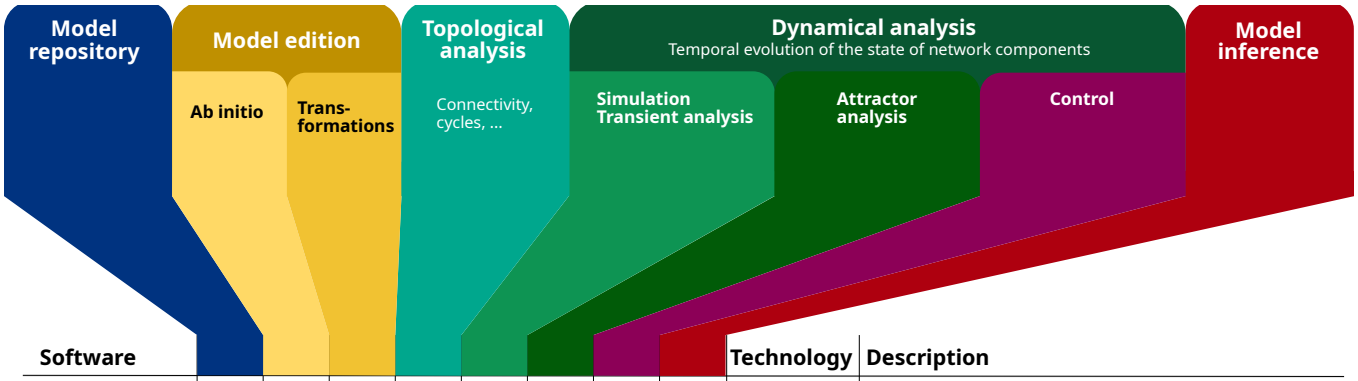
Complete, frozen virtual unix/linux computing environment

docker

+

jupyter Python Notebook

CoLoMoTo environment and notebook



Software	Technology	Description
CellCollective	REST API	Model repository and knowledge base
GINsim	Java	Boolean and multi-valued network modelling
bioLQM	Java, ASP	Logical Qualitative Modelling toolkit
CasQ	Python	Convert static interaction maps into BNs
MaBoSS	C++	Markovian Boolean Stochastic Simulator
BooleanNet	Python	Simulations with various update modes
minibn	Python	Simple Boolean network manipulation API
mpbn	Python, ASP	Analysis and simulation of Most Permissive BNs
NuSMV	C	General purpose symbolic model-checker
boolSim	C++	Attractors and reachable sets in (a)synchronous BNs
BNS	C++	Identification of synchronous attractors
ERODE	Java, SMT	Reduction of synchronous BNs
Pint	OCaml, ASP	Transient formal analysis, reduction, control
pyBoolNet	Python, ASP	Boolean network Python toolbox
ActoNet	ASP	Control of fixed points of BNs
BooN	Python	Analysis of dynamics, stable states, and control
CABEAN	C++	Control of asynchronous BNs
Caspo	Python, ASP	Control of signalling pathways
NORDic	Python	Network Oriented Repurposing of Drugs
pyStableMotifs	Python	Attractor and target-control
AEON.py	Rust	Symbolic analysis of partially specified BNs
BoNesis	OCaml, ASP	Verification, control, and synthesis
BoolNet	R	Simulation, attractors, network reverse-engineering
pyDrugLogics	Python	Automatic construction and in-silico perturbations

Currently
24 available tools

Preexisting Boolean models

Model	Focus	Regulatory layers	Nb of nodes
Krumsiek et al (2011)	Haematopoietic differentiation Myeloid fate commitment	Transcriptional	11
Yuan et al 2016	APL disease Leukemogenesis	Signalling Transcriptional	81
Collombet et al (2017)	Haematopoietic differentiation Transdifferentiation	Transcriptional	23
Wang et al (2021)	Haematopoietic differentiation Lineage commitment	Transcriptional	10
Hérault et al (2022)	Haematopoietic differentiation Impact of aging	Signalling Transcriptional	15

Stable states of the APL model

WT

	RA	PML	PLZF	EZH2	PRC2	CDKN1A	CDKN1B	TP53	E2F1	MYC	SPI1	CEBPAB	RARB
WT0	0	0	0	1	1	0	0	0	1	1	0	0	0
WT1	0	1	1	1	0	1	1	1	0	0	0	0	0
WT2	1	1	0	0	0	1	1	1	0	0	1	1	1
WT3	2	1	0	0	0	1	1	1	0	0	1	1	1

APL PML::RARA

	RA	PML	PLZF	EZH2	PRC2	CDKN1A	CDKN1B	TP53	E2F1	MYC	SPI1	CEBPAB	RARB
PM0	0	0	0	1	1	0	0	0	1	1	0	0	0
PM1	0	1	1	1	0	1	1	1	0	0	0	0	0
PM2	1	1	0	0	0	1	1	1	0	0	1	1	1
PM3	1	0	0	1	1	0	0	0	1	1	0	0	0
PM4	2	1	0	0	0	1	1	1	0	0	1	1	1

APL PLZF::RARA

	RA	PML	PLZF	EZH2	PRC2	CDKN1A	CDKN1B	TP53	E2F1	MYC	SPI1	CEBPAB	RARB
PZ0	0	0	0	1	1	0	0	0	1	1	0	0	0
PZ1	0	1	1	1	0	1	1	1	0	0	0	0	0
PZ2	1	1	0	0	0	1	1	1	0	0	1	1	1
PZ3	1	0	0	1	1	0	0	0	1	1	0	0	0
PZ4	2	1	0	0	0	1	1	1	0	0	1	1	1
* PZ5	2	0	0	1	1	0	0	0	1	1	0	0	0

Proliferation

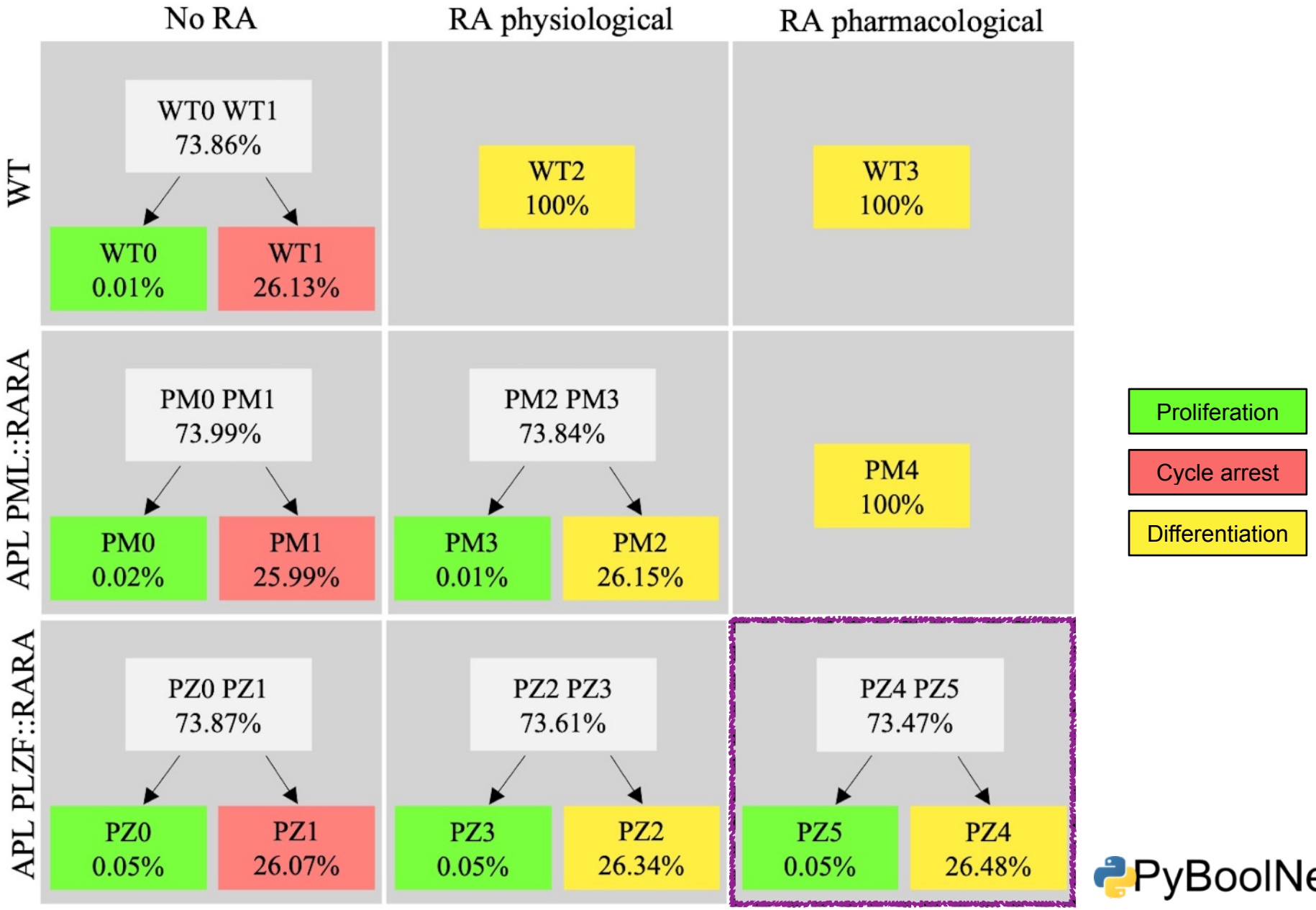
Cycle arrest

Differentiation

bioLQM

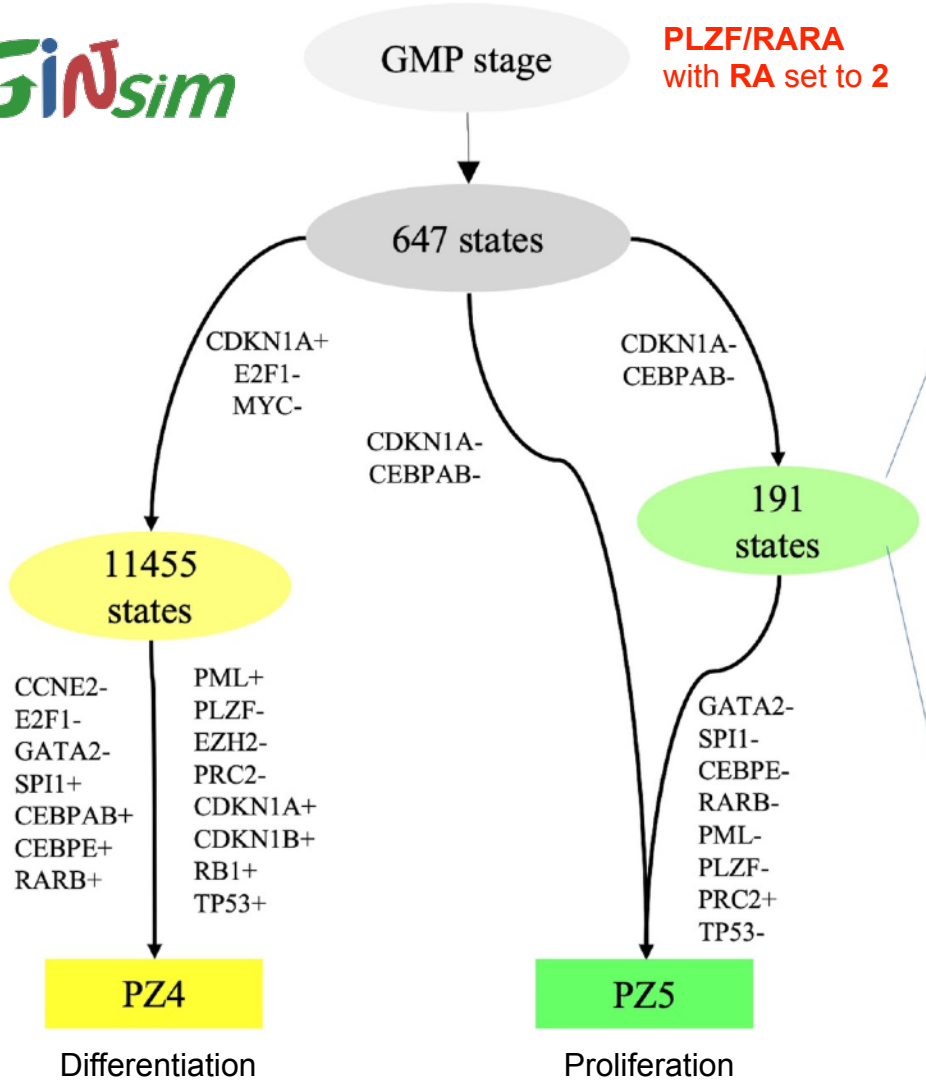
Stable states of the model for the wild-type, APL PML/RARA and APL PLZF/RARA (PZ, bottom) conditions. Each row represents a stable state characterised by the values of representative model components. The first column associates a label with each stable state.

Stable state commitment analysis



PyBoolNet

Hierarchical state transition graph (asynchronous simulation)



Representation of the 191 states in terms of 12 Boolean patterns

RARA	1	1	1	1	1	1	1	1	1	1	1	1
PML	0	0	0	0	0	0	0	0	0	0	1	1
PLZF	0	0	0	0	0	0	0	0	1	1	*	*
CoAct_COMP	1	1	1	1	1	1	1	1	1	1	1	1
EZH2	1	1	1	1	1	1	1	1	1	1	1	1
PRC2	0	0	1	1	1	1	1	1	*	*	*	*
CoRep_COMP	1	1	1	1	1	1	1	1	1	1	1	1
CDKN1A	0	0	0	0	0	0	0	0	0	0	0	0
CDKN1B	0	0	0	0	0	0	0	0	0	0	0	0
RB1	0	0	0	0	0	0	0	0	0	0	0	0
TP53	*	*	0	0	0	0	1	1	*	*	*	*
CCNE2	1	1	1	1	1	1	1	1	1	1	1	1
E2F1	1	1	1	1	1	1	1	1	1	1	1	1
MYC	1	1	1	1	1	1	1	1	1	1	1	1
POU2F1	1	1	1	1	1	1	1	1	1	1	1	1
GATA2	0	1	0	0	0	1	0	1	0	1	0	1
SPI1	*	0	0	0	1	0	*	0	*	0	*	0
CEBPAB	0	0	0	0	0	0	0	0	0	0	0	0
CEBPE	*	*	0	1	*	*	*	*	*	*	*	*
RARB	*	*	1	*	*	*	*	*	*	*	*	*

This hierarchical graph results from the compression of a state transition graph encompassing over 12000 states

Stochastic simulations

★ **Continuous-time Markov chain simulations**

Boolean translation of multilevel models

Specification of **Up** and **down rates** + simulation parameters (time step, number of iterations, etc.) => **Gillespie Algorithm**

★ **MaBoSS package (https://maboss.curie.fr/)**

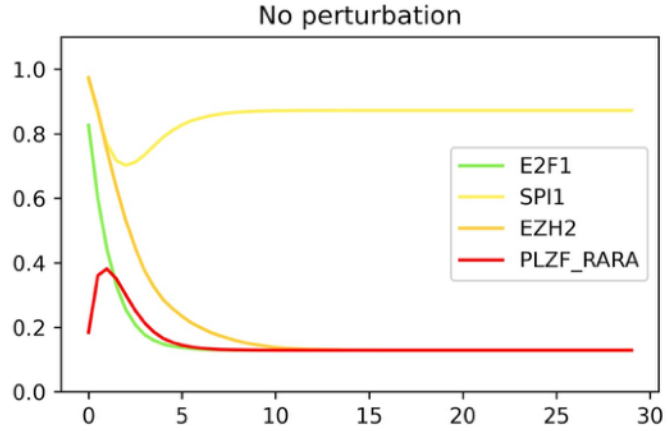
Automatic export of GINsim file to MaBoSS grammar

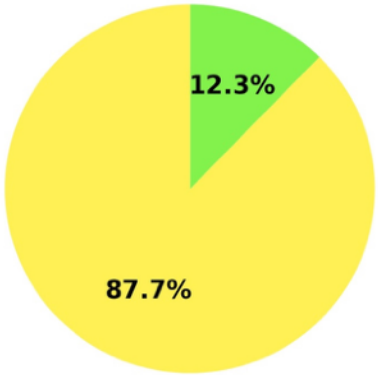
Perl and python scripts, C++ executableS

Laurence CALZONE

Gautier STOLL

Stochastic simulations of EZH2 perturbations

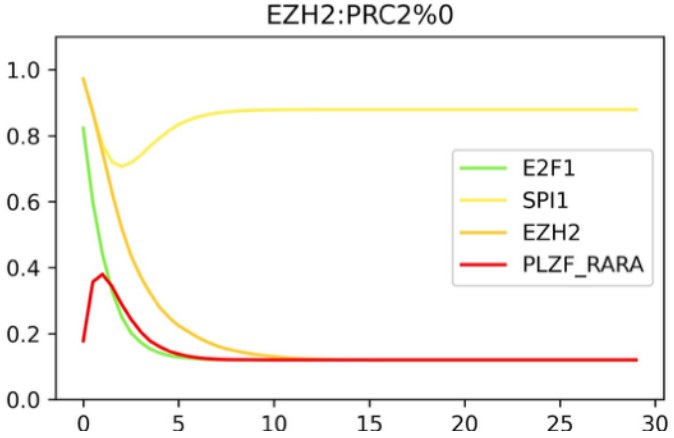


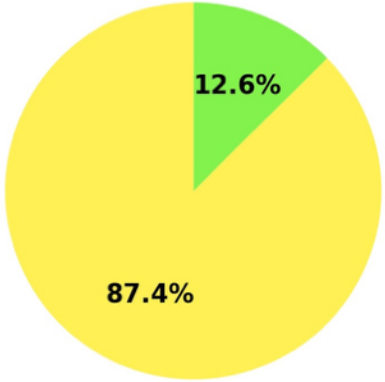


Initial state:
PLZF/RARA set to 1 and RA set to 2

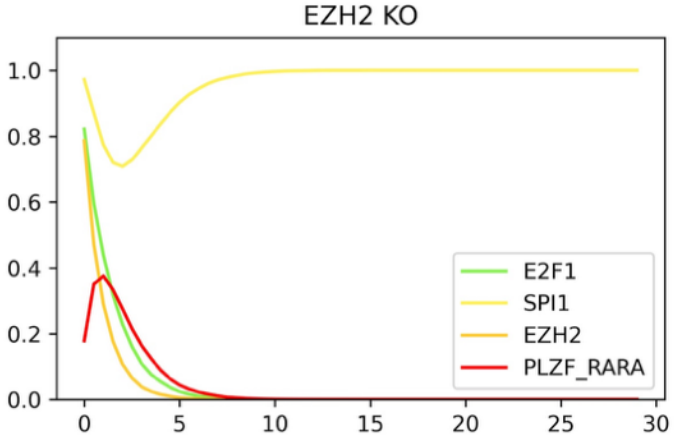
Proliferation

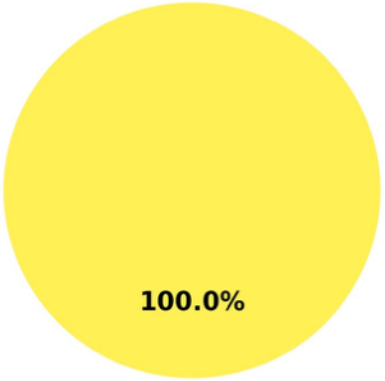
Differentiation





Blocking of EZH2 canonical activity (via PRC2)

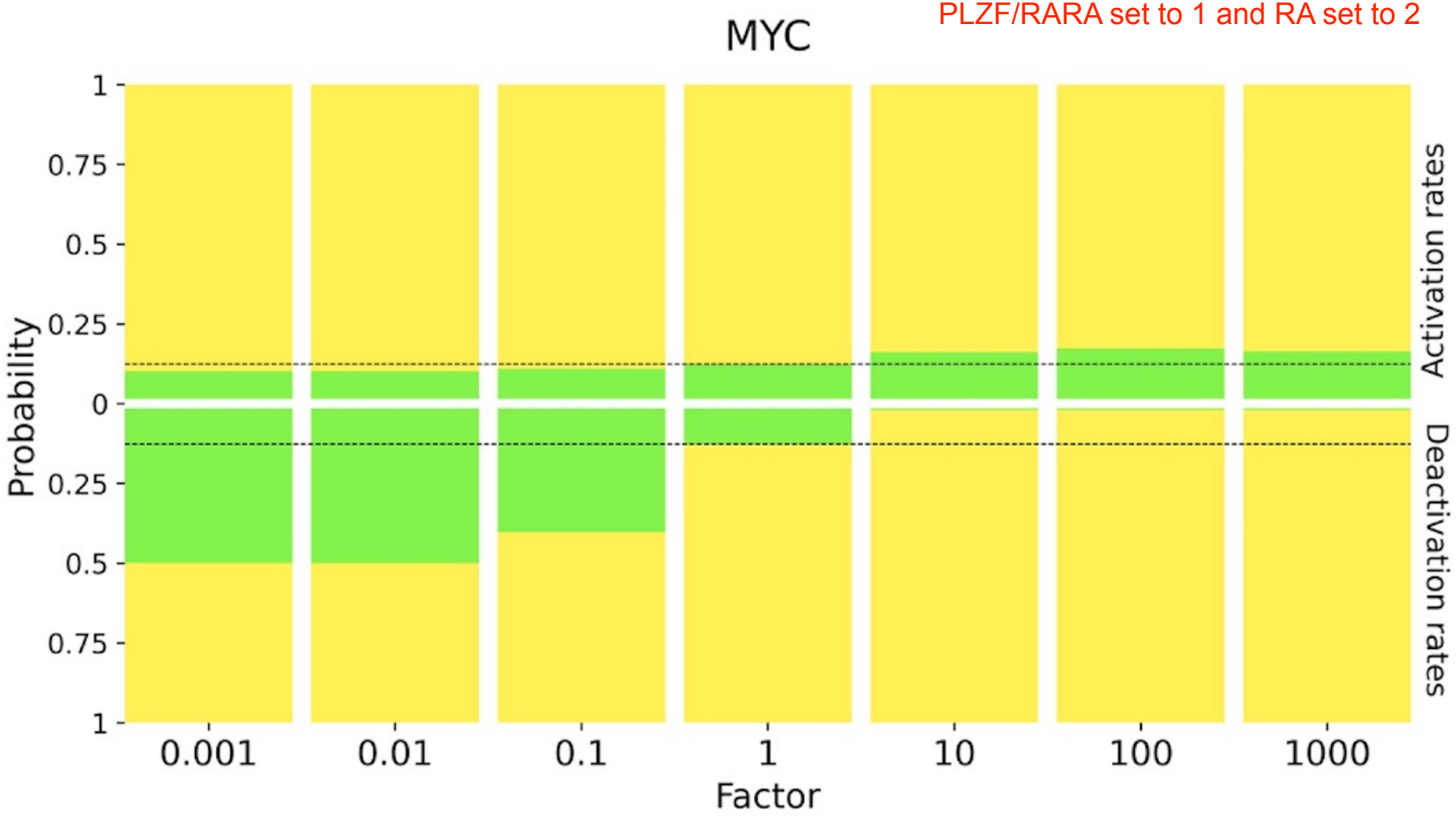




Full EZH2 KO

Up/down rates sensitivity analysis

Initial state:
PLZF/RARA set to 1 and RA set to 2

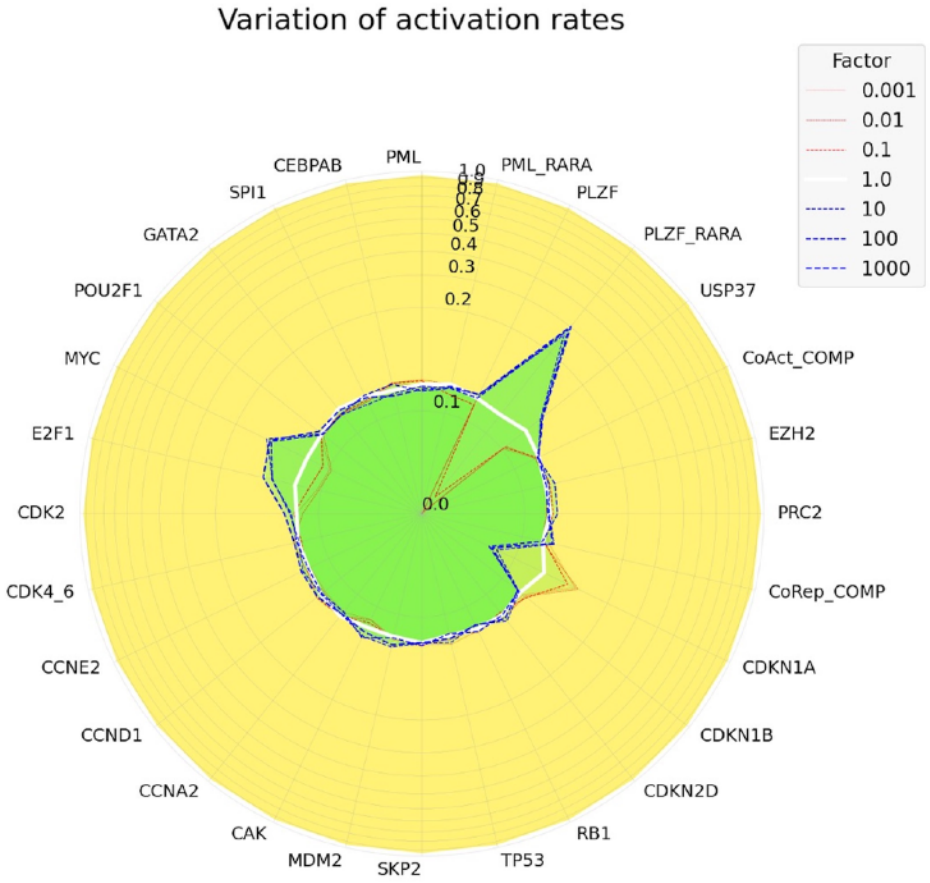


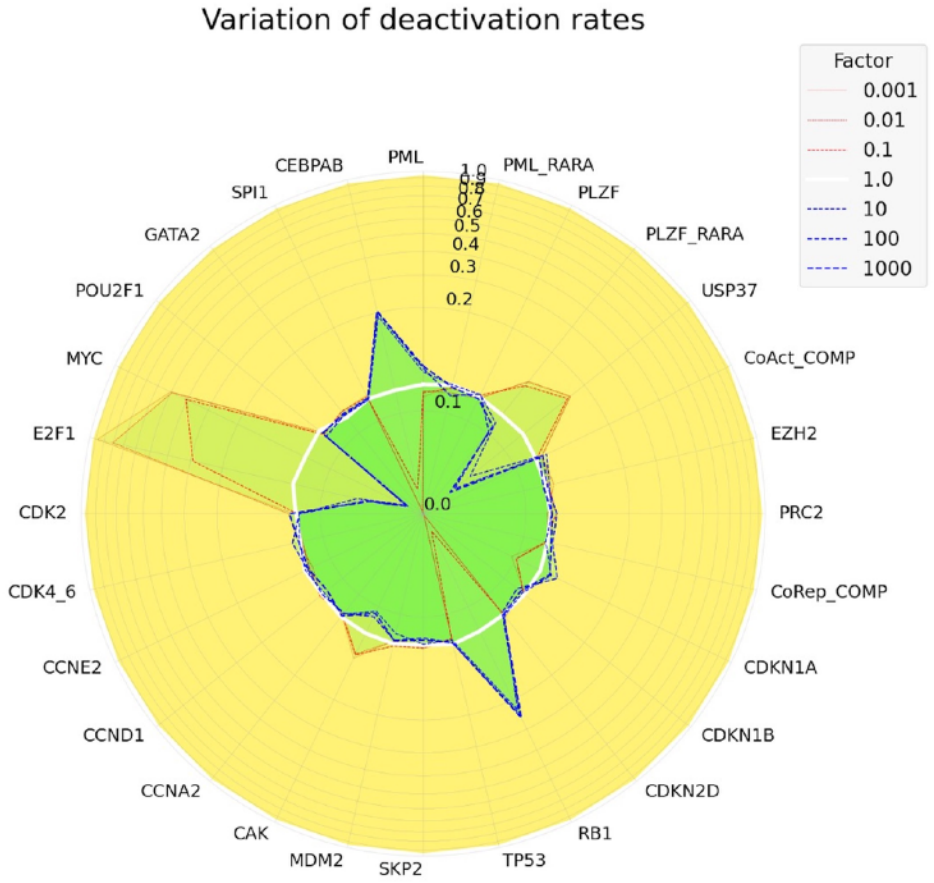
Differentiation

Proliferation

Up/down rates sensitivity analysis

Initial state: PLZF/RARA set to 1 and RA set to 2





Differentiation

Proliferation

MaBoSS

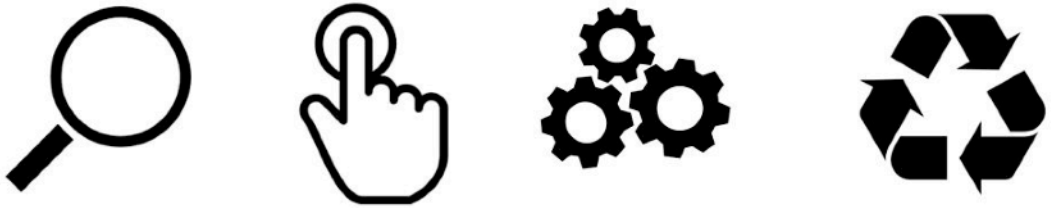
Outlook

- ★ Our **logical model** captures and explains **key features** of the responses of two main subtypes of Acute Promyelocytic Leukaemia (APL) cells to retinoic acid (RA) therapy.
- ★ The **stable states** of the model recapitulate the **phenotypes** of differentiated and aberrant proliferative cells induced by RA treatment for the different APL genetic backgrounds.
- ★ A **commitment analysis** identifies the crucial components underlying the decision between cell differentiation and aberrant proliferation.
- ★ Simulations of different **EZH2 perturbations** differentiate the **canonical versus non-canonical activities of EZH2** and highlight the key role of the non-canonical activity of EZH2 in the maintenance of the resistance to RA in APL PLZF::RARA cells.
- ★ The **mutant** and **parameter sensitivity analyses** further points to the components of the network underlying cell fate decisions, which constitute **potential targets for novel combinatorial therapy strategies**.
- ★ The computational analysis workflow has been made reproducible through the use of **CoLoMoTo Docker container & Jupyter notebook**.

All models are wrong

but some are useful

Findable **A**ccessible **I**nteroperable **R**eusable



George E.P. Box

1976

Selected bibliographical references

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- Noël V, Naldi A, Calzone L*, Paulevé L*, Thieffry D* (2025). Reproducible Boolean model analyses and simulations with the CoLoMoTo software suite: a tutorial. *Interface Focus*, in revision (manuscript currently available from bioRxiv: https://www.biorxiv.org/content/10.1101/2025.01.26.634971v1).

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