Computational and Structural Biotechnology Journal 20 (2022) 5661–5671

COMPUTATIONAL ANDSTRUCTURAL BIOTECHNOLOGY JOURNAL

journal homepage: www.elsevier.com/locate/csbj

Review

Modeling signaling pathways in biology with MaBoSS: From one single cell to a dynamic population of heterogeneous interacting cells

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ARTICLE INFO

Article history:
Received 5 July 2022
Received in revised form 30 September 2022
Accepted 2 October 2022
Available online 7 October 2022

Keywords:
Stochastic modeling
Cancer
Population dynamics
Boolean approach

ABSTRACT

As a result of the development of experimental technologies and the accumulation of data, biological and molecular processes can be described as complex networks of signaling pathways. These networks are often directed and signed, where nodes represent entities (genes/proteins) and arrows interactions. They are translated into mathematical models by adding a dynamic layer onto them. Such mathematical models help to understand and interpret non-intuitive experimental observations and to anticipate the response to external interventions such as drug effects on phenotypes. Several frameworks for modeling signaling pathways exist. The choice of the appropriate framework is often driven by the experimental context. In this review, we present MaBoSS, a tool based on Boolean modeling using a continuous time approach, which predicts time-dependent probabilities of entities in different biological contexts. MaBoSS was initially built to model the intracellular signaling in non-interacting homogeneous cell populations. MaBoSS was then adapted to model heterogeneous cell populations (EnsembleMaBoSS) by considering families of models rather than a unique model. To account for more complex questions, MaBoSS was extended to simulate dynamical interacting populations (UPMaBoSS), with a precise spatial distribution (PhysiBoSS). To illustrate all these levels of description, we show how each of these tools can be used with a running example of a simple model of cell fate decisions. Finally, we present practical applications to cancer biology and studies of the immune response.

Contents

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https://doi.org/10.1016/j.csbj.2022.10.003

1. Introduction

With the advent of experimental technologies, the amount of data and knowledge accumulated over the years and the variety of studies have offered the possibility to describe molecular processes with great detail highlighting the complexity of the processes that are deregulated in various diseases. Some of the newest experimental devices facilitate the identification of gene or protein expression, number of molecules, cell types, or both the intra- and extracellular location of biological entities. Some examples include transcriptomics or proteomics and single-cell RNA-sequencing, spatial transcriptomics, flow cytometry, etc. [1] gathering and integrating multilevel understanding of the studied processes.

One way to represent knowledge is to recapitulate the acquired information in the form of networks bringing into light some signaling or metabolic pathway crosstalks in the context of gene or protein expression data. Many pathway databases with data extracted from the literature, and manually curated, exist and can be used as a source to infer a specific type of networks from a list of genes [2–6]. A real community effort is conducted to create the networks in standard format in order to ensure their readability, their classification, their homogeneity, their exchange and their analysis by making them understandable to machines (Systems Biology Graphical Notations, SBGN [7]). Many of these databases have joined this effort and provide the networks in one of the standard formats.

Most pathway databases include annotations about the nature of the interactions, where they take place, in which cell type and for which types of experimental conditions the data were generated. Nonetheless, one issue when constructing these networks is that they can very easily reach a large dimension with a high number of nodes and numerous (sometimes heterogeneous types of) interactions on which it becomes more and more difficult to reason intuitively.

One solution to overcome this difficulty is to study the dynamic properties of these networks. When choosing an appropriate mathematical formalism, the static network shows dynamic properties and more formal studies can be performed. By means of mathematical models, it becomes then possible to predict behaviors of biological processes in various conditions. It is important to note, though, that a model is not a strict representation of reality. Its purpose is to extract and abstract the essential molecular knowledge in its sleekest and simplest form, by making some strong assumptions and approximations, and converting physical situations into mathematical terms.

The use of mathematical models to describe biological observations is not new and has been applied for decades in biology with very famous models such as the Hodgkin–Huxley model of action potentials in neurons in 1952 [8], or the follow-up models of Denis Noble of the heart from 1962 on [9]. In cellular biology, the first models of cell cycle exhibiting oscillatory behaviors were published in the 90s [10–12]. With a simple mathematical description of the known biology, they proved that the dynamical properties of the cell cycle could be reproduced and challenged with a minimal number of variables. If the current models of cellular and molecular biology are based on these ideas, their size has significantly increased because of the possibilities that computational techniques offer today with optimized codes and high performance computing technologies.

The model that describes crosstalks between signaling pathways and studies cell fate decision in response to the activation of receptors focuses on one cell or, depending on the formalism, on a population of identical cells with the same molecular profile. These pathways can be activated by intracellular signals and extracellular stimuli, such as a stressor or the presence of extracellular entities, and trigger a cascade of events ultimately leading to the transcription of important genes of the cell cycle, cell death or cell phenotypes, linking an extracellular signal to a phenotype through a network. The most common formalisms for such models are based on ordinary differential equations (ODEs) that allow following the concentration of the molecular entities participating in the processes over time. In this context, the initial conditions of the model are fixed and represent the status of the cell and its environment at a particular time. These initial inputs can be varied, though, to show other environmental conditions. The difficulty of this formalism is to find the proper parameters that reproduce the observed behaviors, knowing that these parameters are difficult to identify in real life experiments. A more coarse-grain approach consists in translating the information into a logical model where variables are discrete and there are no parameters to fit. The approach is simpler and more versatile but the conclusions that can be drawn from this type of models are not quantitative and not all questions can be answered (dosage of drug treatments, precise timing of events, etc.).

We present, here, an alternative approach for mathematical modeling of these signaling pathways and its implementation in a software, MaBoSS (Markovian Boolean Stochastic Simulator) [13,14]. The main hypothesis of MaBoSS relies on the definition of genes or proteins' activities by discrete levels, within a continuous time dependency framework. Initially, MaBoSS was designed to model signaling pathways in a single cell type, but over time and to answer the evolution of the biological questions that were formulated, MaBoSS was extended to tackle a broader biological context, including heterogeneous and dynamic cell populations. There remain limitations of MaBoSS, though. Among them, we can list the possibility to address spatial description as it is understood in developmental biology, or to address questions related to precise biochemical mechanisms as it is the case for pharmacodynamics / pharmacokinetics perspectives or for metabolism. For these types of applications, other approaches are more appropriate than MaBoSS and related tools.

In this review, we introduce MaBoSS tool suite that consists of a primary tool, MaBoSS, and a set of extensions: EnsembleMaBoSS, UPMaBoSS and PhysiBoSS. We first define MaBoSS grammar and applications. We then present its extensions that tackle more complex levels of descriptions: EnsembleMaBoSS for handling families of models, UPMaBoSS for interacting and dynamic cell populations

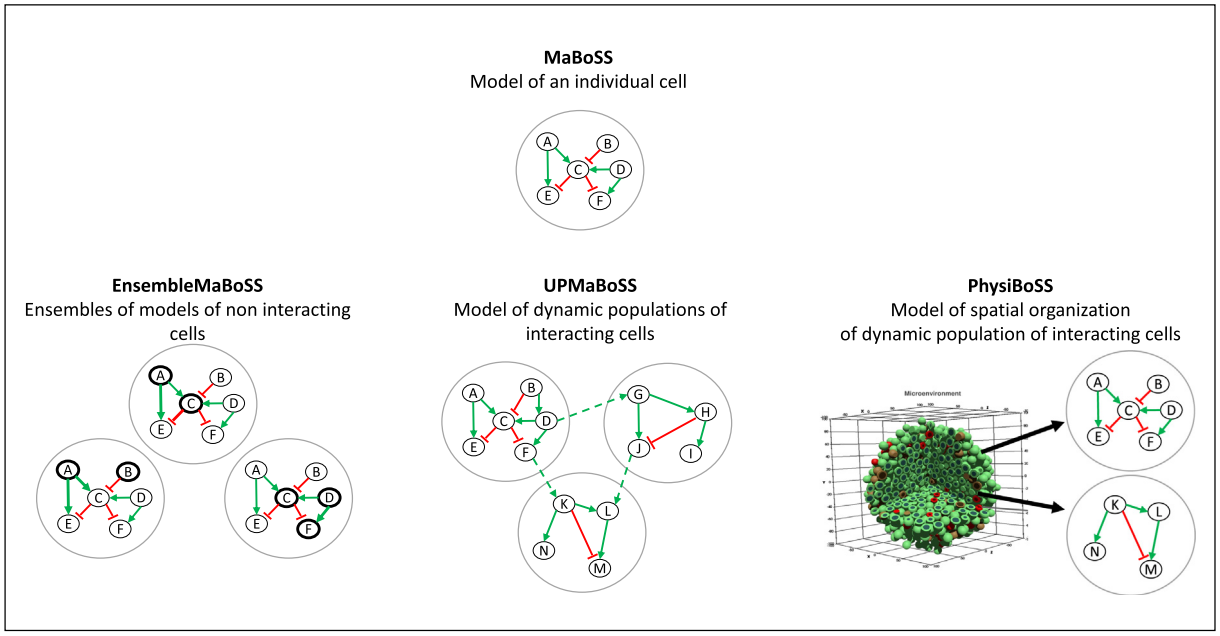

Fig. 1. MaBoSS tool suite: MaBoSS, EnsembleMaBoSS, UPMaBoSS and PhysiBoSS.

and PhysiBoSS for a dynamic spatially-organized population of heterogeneous cells. Finally, we illustrate the tools with some practical examples in cancer and immune biology.

2. Rationale for a new modeling framework

Over the last 20 years, a lot of research effort has been put into building comprehensive predictive models such as the digital twins [15,16] or virtual objects for clinical purposes (cf. the virtual liver [17], the virtual heart [18,19], the virtual tumor [20], or the virtual patient [21]). Some real challenges were also met when developing mathematical models of the whole cell, capturing molecular species, physical compartments, genetic alterations, and metabolic variations, with a mechanistic description to allow the formulation of predictions [22–24]. In the whole cell modeling approach, the construction of these comprehensive models has been addressed with hybrid modeling to account for multiple scales, different timing, and various types of data to integrate.

The aim of these models is not only to reproduce experimental observations but also to anticipate the system's evolution in time and space when placed in different contexts and cellular conditions. These cell decisions depend on how molecules interact, diffuse and fluctuate in an environment without ignoring the fact that these processes can occur with a certain degree of randomness. The mathematical formalisms to represent these processes can be chosen according to the desired level of simplification of such random and stochastic events. In biological applications, they can range from pure statistical approaches [25]) to very complex models spanning all scales [26], from molecular interactions (genes and proteins' dynamics with ODEs, rule-based or logical models [27,28]), cell evolution (focused on cell fate, such as proliferation or death with cellular automata [29]), tissue evolution and the interaction with the environment (with agent-based modeling framework [30–32]), organ specificity (with mechanical considerations [33,34]), to the patient itself, as mentioned for the virtual patient.

Over the past decade, our contribution towards more comprehensive and complex approaches has been extended in several directions. We first started modeling the signaling pathways of a single cell with MaBoSS (https://github.com/sysbio-curie/MaBoSS-env-2.0) [13,14] and WebMaBoSS, a user-friendly web interface (https://github.com/sysbio-curie/WebMaBoSS) [35]. We then explored the possibility to consider families of resembling models that fit biological constraints with the simulation of ensembles of MaBoSS models [36]. We further allowed the cells to interact with each other and other cell types, die and divide with UPMaBoSS (https://github.com/sysbio-curie/UPMaBoSS-docker) [37]. Finally, we included spatial considerations in the interactions among cells and cell types and with the microenvironment with PhysiBoSS (https://github.com/PhysiBoSS/PhysiBoSS) [30] (Fig. 1).

3. Logical formalism

The biological knowledge spread in different sources can be gathered and recapitulated in the form of a *network*, where nodes represent variables and arrows account for the interactions between nodes. *Variables* refer to genes and proteins but can also account for protein complexes, metabolites, cell types and even cell position. As already mentioned, the network can take several forms [7]. The choice for the most appropriate type of networks and the corresponding mathematical formalism will depend on: (1) which mathematical objects are associated to variables (nodes): discrete numbers, continuous numbers, vector of numbers, etc.; (2) how the interactions are interpreted: are they chemical or physical interactions, influences, correlations, etc.; (3) what the parameters of the model represent: chemical events (e.g., synthesis, degradation, phosphorylation, transport), affinity (e.g., ligand-receptor interactions, cooperation or competition between two ligands on a receptor), or initial conditions (e.g., initial concentration or amount of species, initial extracellular status).

Our approach is based on the construction of an influence (or regulatory) network, where nodes are connected by directed and signed arrows representing influences of one node onto another (Fig. 2A), and the corresponding mathematical model uses a logical formalism where variables can take two values 0 (for absent or inactive and written as −) and 1 (for present or active and written as +) (Fig. 2B). The variables are updated according to the status of the input nodes linked by logical connectors OR, AND, and NOT

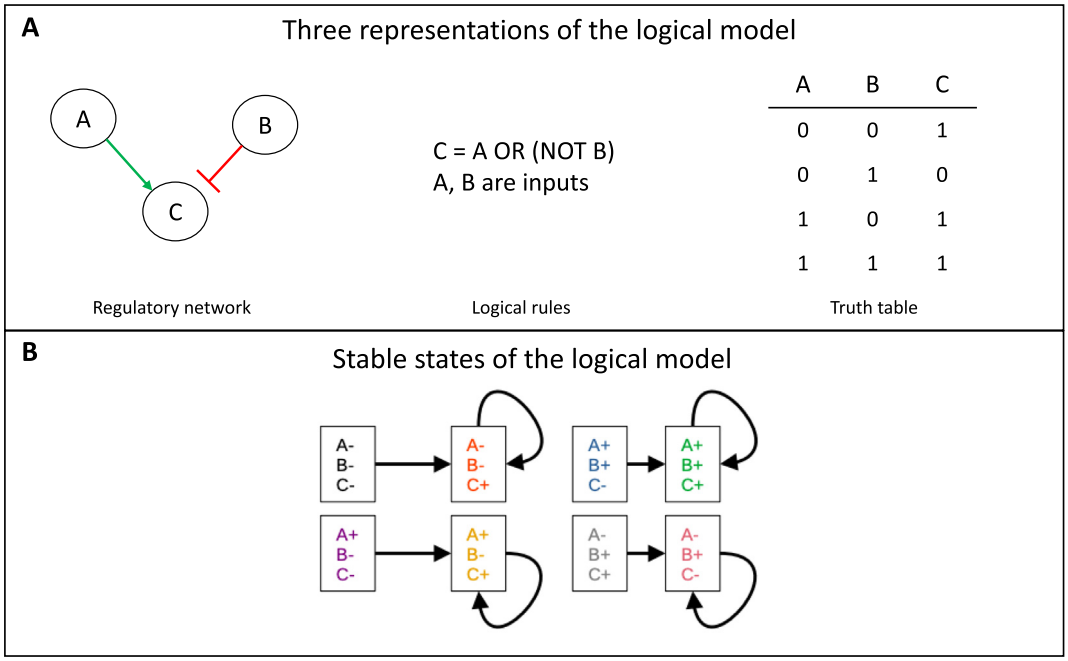

Fig. 2. Logical formalism: (A) Three representations of the logical model: the regulatory network depicting the influence of a node on the others, the logical rule for C where A and B are inputs, the truth table; (B) A graph recapitulating the possible transitions from one model state to another, with four stable states (or fixed points): (A−,B−,C+), (A+,B−,C+), (A+,B+,C+), (A−,B+,C−) where A− denotes its absence (or value = 0) and A+ its presence (or value = 1). A model state is a vector of all nodes of the model informing on their status at each step.

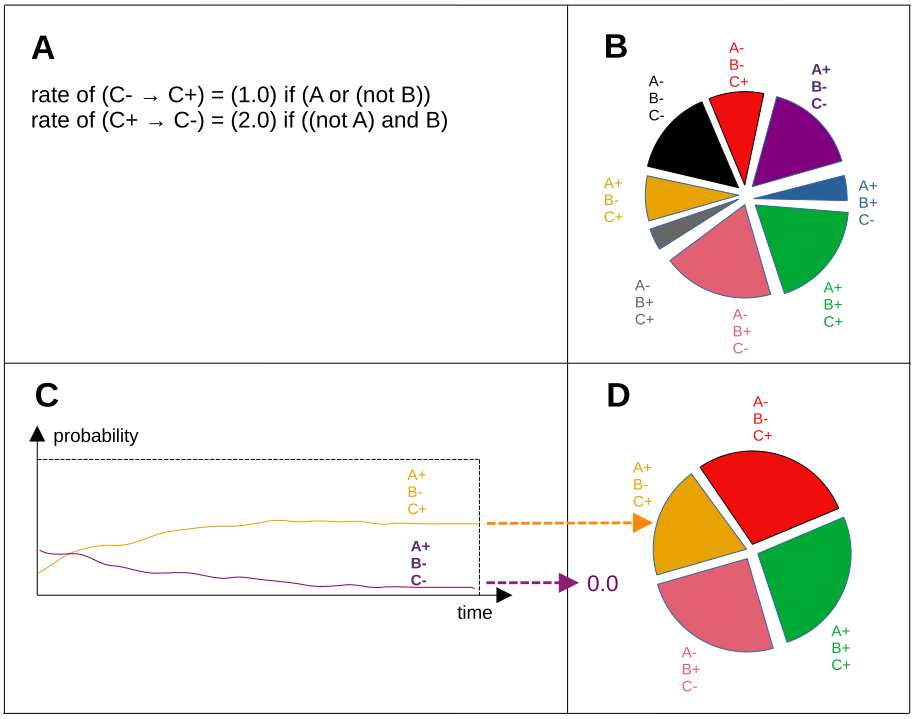

Fig. 3. Logical modeling framework of MaBoSS: (A) transition rates for node activation and inhibition, which can be read as follows: for C to be activated (from C− to C+), if the rule "A or not B" is verified, then the rate of activation is 1; for C to be inhibited (from C+ to C−), if the rule "not A and B", then the rate of inhibition is 2; (B) a set of initial conditions per model state. There are two possible outputs of MaBoSS simulations in the form of (C) time-dependent model state probabilities for (A+,B−,C+) in orange and (A+,B−,C−) in purple only, and (D) a pie chart representing the model states when the system has reached its asymptotic solution (it corresponds to the model states of the last point of the simulations in C).

(Fig. 2A). For instance, a gene C can be activated by a gene A and inactivated by gene B. The activation rule for C can be written as follows: C = (A OR (NOT B)), which means that C will go from − to + when either A is present or B is absent. When the rule is not verified, C will be set to 0 (C−). We provide a slightly more complex example of a model in the Supplementary materials, written and analyzed in MaBoSS with a positive and a negative feedback loop, leading to one stable state, and a simulation of single mutants.

It is important to note that the writing of the logical rules may not be unique and this issue is common to all logical tools. The rules should reflect the knowledge that can be extracted from publications or experimental observations. In the case that a node has two inputs, i.e., a gene can be activated by two different transcription factors, it can reflect two scenarios. If the two transcription factors were identified in two different publications, they can be connected with an OR gate; if the two transcription factors form a complex (e.g., EWS/FLI1), then they are connected by an AND connector. If no information is provided, we usually opt for an OR connector.

A recent review describes in great detail the formalism, the modes of updating schemes and the available modeling tools [38].

4. Qualitative modeling with MaBoSS and its extensions

MaBoSS primary tool is a C++ software. It requires a *.bnd* file (Boolean Network Descriptor) and a *.cfg* file (configuration) as inputs. These files can be written and edited manually, but very often, models are constructed with a different software (BoolNet or GINsim), imported, handled, modified and simulated within a python interface called PyMaBoSS (https://github.com/colomoto/pyMaBoSS) developed by the CoLoMoTo consortium [39]. All the model files and the python notebooks used to simulate the models in this review are provided in the Supplementary material and in the dedicated GitHub available at https://github.com/sysbio-curie/MaBoSS-Review/.

4.1. MaBoSS

MaBoSS applies the logical formalism within a continuous time window by applying continuous Markov chains over the Boolean network.

To the list of logical rules (Fig. 2A) common to many logical model frameworks, transition rates are associated to each variable of the model, setting the time it takes for activation (rate_up) and for inactivation (rate_down) (Fig. 3A). Similarly, some probabilities can be set for the initial conditions corresponding to the probability to be active (1) or inactive (0) at the beginning of a simulation (Fig. 3B). It is thus possible to follow the probability for a model state (vector of Boolean values), or for a model variable (gene or protein of the model), over time until it reaches its asymptotic solution (Fig. 3C, D). Therefore, a MaBoSS model has a set of parameters encompassing rates of transition and initial conditions. The rates are set to 1 by default. Ideally, these parameters would be measured experimentally, but in practice, they are often estimated from various experimental contexts. To cope with the issue of parameter identification, parameters are often given a value that represents a proper order of magnitude, that can be evaluated with a sensitivity analysis.

As a running example, we present a published model of cell fate decision in response to death receptor activation [40] (Fig. 4A). In this model, there are three inputs, TNF, FAS and FADD, and three outputs or read-outs, *Survival* that is activated by the NFkB pathway, *Apoptosis* triggered by caspases, and *NonACD* for non-apoptotic cell death corresponding to programmed necrosis,

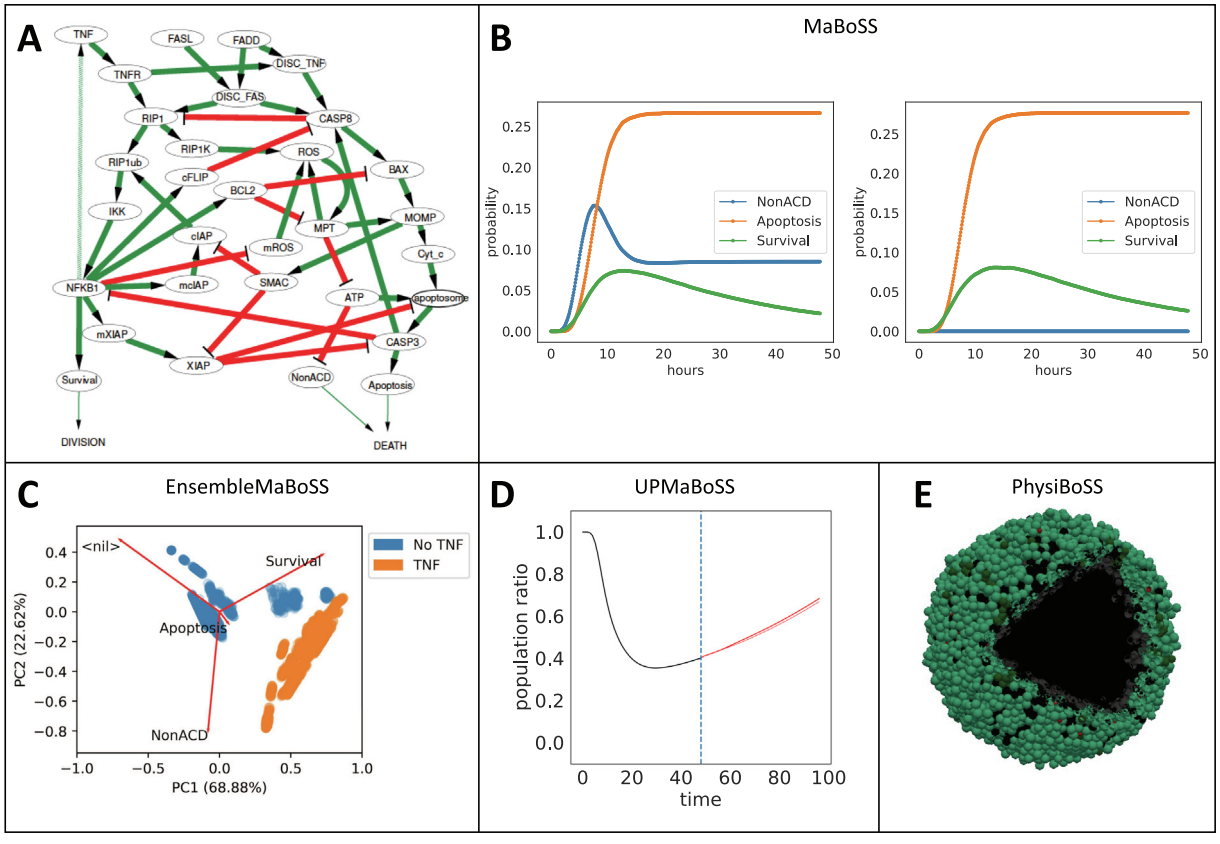

Fig. 4. Cell Fate model, network and simulations. (A) Influence network of the cell fate model (light green arrow represent ligand-receptor interactions), (B) Probability of Non-Apoptotic Cell Death, Apoptosis and Survival, obtained by MaBoSS simulation for wild-type model (top) and ROS knock-down (bottom), (C) PCA plot of the models generated and simulated by EnsembleMaBoSS with respect to the model phenotypes in the case where TNF is OFF (blue) and TNF is ON (orange), (D) Cell population ratio computed by UPMaBoSS, with one dose of TNF treatment (black curve), and after 48 h, a second TNF treatment (right curve, with its control in a red dashed line that almost coincides with TNF-treated simulations), (E) PhysiBoSS simulation of TNF-treated cells marked with proliferative cells (in green), apoptotic cells (in red, but rare on the figure) and necrotic cells (in black).

also called "necroptosis". When none of the phenotypes are active, the obtained solution is referred to as naive state in the initial publication and is noted as <nil> in MaBoSS simulations. It corresponds to the case when the genes encoding FAS, TNF or FADD are OFF, and as a result, none of the signaling pathways can be activated.

For simplificity, we set FAS to 0 as an initial condition and focus only on TNF-induced pathway activation. It is initially assumed that FADD is random (present or not), cIAP and ATP are active and all other components of the model are inactive. When TNF is ON, *Survival* can be activated with a probability of 1.4%, *Death* with a probability of 42.8%, the rest corresponding to the naive case (not shown on the graph). When a gene is mutated (the corresponding variable is set to 0 or 1 according to the type of mutation), these probabilities change. For instance, a mutation in ROS-related genes (overactivation of the node ROS (reactive oxygen species) in the model) strongly affects the proportion of surviving versus dying cells to 1.6% and 28.1%, respectively, highlighting the role of ROS-related genes in cell death (Fig. 4B).

The same simulations can be performed using a user-friendly web interface, WebMaBoSS [35] available at this address: https://maboss.curie.fr/webmaboss/). The user, after login in, can import any model from public databases or from local MaBoSS files and simulate models without any extensive knowledge of the software.

4.2. EnsembleMaBoSS

As previously mentioned, when building a Boolean model, some choices about the logical formulae have to be made: for each node of the regulatory network, their input nodes are linked by logical rules (see section on logical formalism) to define the conditions for updating the value of the corresponding variable. The number of combinations for these choices can become extremely large, and as such, the number of possible Boolean models for a regulatory graph can become exponential. If some choices are directed by experimental evidence (when two genes participate in the activation of a third by complexation, for instance), most of the rules are not known and these choices can bring an unavoidable bias to the model. One way to address this issue is to automatically build a large ensemble of Boolean models using Bonesis [36], which generates models compatible with a set of constraints. Typically, these constraints would translate biological observations, which could consist in a list of fixed points that correspond to discretized data of cell conditions or known interactions among proteins.

We developed such an ensemble of models for the example of Fig. 4A. We used the regulatory graph, as well as constraints on the reachable phenotypes: models must be able to reproduce *Survival*, *Apoptosis*, *NonACD* phenotypes and a naive state where none of the phenotypes are active (written <nil> in the simulations). In other words, there must be one steady state of the model with each of these phenotypes that must be reachable. Note that this does not prevent other phenotypes (for example combinations of phenotypes, such as *Survival* and *Apoptosis*, which would be identified as aberrant in this model).

We generated an ensemble of 2000 models compatible with these constraints using Bonesis. Interestingly, we did not obtain any aberrant phenotypes in the ensemble, suggesting that the constraints present in the regulatory graph are enough to enforce the mutual inhibition of these phenotypes. We then used principal component analysis (PCA) to plot the distribution of the probabilities of the fixed points of these models in a 2D space. This allows us to study the variability of the distribution of fixed points within the ensemble (Fig. 4C).

The points in blue show the positions of the steady state distribution of each model, when simulated without the TNF. While the published model of Fig. 4A exhibits only the naive (<nil>) state in this context, here we can observe that many models can have completely different probabilities, just based on an alternative choice of formulae. A subset of models clearly shows that the *Survival* phenotype can be favored depending on the rules. The points in orange represent the distribution of steady states of models when simulated with TNF, and show a shift away from the <nil> state, with variability in the distribution of the active phenotypes (*Apoptosis*, *NonACD*, *Survival*).

With these results, we are able to refine our selection of models in our ensemble, to fit more closely to experimental data. For example, if an experiment shows that without TNF, no cells become resistant nor die, whereas with TNF 5% of cells become resistant and 95% die, with these two simulations, we can select an ensemble of models with these characteristics.

In some cases, clusters of aberrant models can appear, e.g., where apoptosis and proliferation occur concomitantly in one fixed point. These models could be dismissed by adding some constraints on the selection of models or by translating different conditions of the wild type or of reported mutants into a more complete list of constraints using MaBoSS_test [41]. This procedure reduces the list of possible models to those that are able to pass all the tests.

4.3. UPMaBoSS

MaBoSS simulates a population of asynchronous and independent cells which do not communicate. UPMaBoSS was developed not only to account for cell–cell interactions but also to consider the fact that cells die and divide throughout the time of the simulations. This dynamic feature of the model affects the final proportion of living cells and may better match experimental observations. Moreover, UPMaBoSS is able to simultaneously simulate a mixture of different cell types that have their own signaling pathways and that communicate through a ligand-receptor interaction.

In UPMaBoSS, there are no spatial considerations as every cell can interact with each other no matter where they are situated. It is possible to account for simple spatial position by adding nodes that describe spatial states. For instance, for a tumor model, a node "tumor" can be active only if the cell is inside the tumor and not in its periphery, both situations will be determined by different initial conditions.

As it is the case in MaBoSS, UPMaBoSS provides probabilities of model states over time. In addition, UPMaBoSS produces a time-dependent *population ratio*, which corresponds to the relative change in the population size. Note that the population size is set to the value 1 at time 0, by default, but can be modified by the modeler to fit experimental data. The size of a given cell type population, where the cell type can be represented by a node in the network, e.g., *Type_1*, is the product of three numbers: 1) the population ratio, 2) the probability of *Type_1,* and 3) the initial population size.

The cell fate model has been adapted to account for cell–cell interactions by adding an extra-cellular influence (light green arrow in Fig. 4A), a division node and a death node. The TNF treatment is modeled as TNF being ON (present) at the beginning of the simulation. The activity of TNF is further maintained by the paracrine loop NFκB → TNF creating a feedback loop that favors death (as shown in [37]). Nonetheless, following the treatment, a heterogeneous population of cells is obtained as a proportion of cells is killed by both apoptosis and necrosis, and another one proliferates through NFκB activation. When a second treatment is imposed 48 h after the first one, a resistance mechanism against TNF treat-

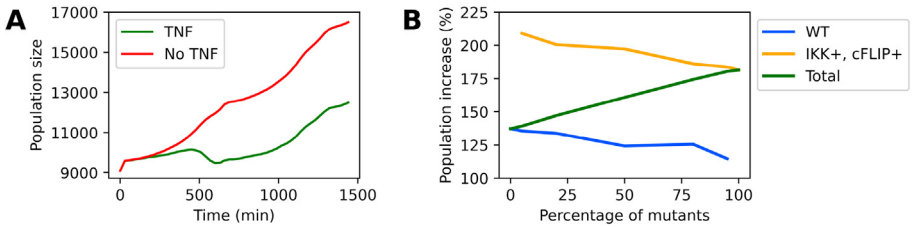

Fig. 5. Simulation of spheroids. (A) Simulations of homogeneous population of wild type cells, without TNF treatment (red), and with TNF treatment (green). (B) Population increase after 24 h of treatment for heterogeneous populations of wild type cells (blue) and IKK+ and cFLIP+ mutated cells (orange), according to the percentage of mutated cells in the global population (green).

ment can be observed. Indeed, if one dose of TNF kills 80% of the cells, the second dose is unable to kill the remaining cells and is equivalent to no treatment at all (Fig. 4D). Note that this resistance mechanism is not due to a genetic selection because the treatment is simulated on the wild type model. Other responses can be observed *in silico* in a system in which mutations are introduced (see [37]).

4.4. PhysiBoSS

When spatial considerations are needed to address the biological question, the model must be adapted in a new framework. One natural choice is to use an agent-based modeling approach such as PhysiCell [31] or CompuCell3D [42], where agents (cells in our case) have a position, can move and interact with each other and the environment. The cell environment can be described by a gradient of species (e.g., of oxygen) which can diffuse and be taken up or secreted by cells. What is lacking in these tools, though, is the intracellular description of these agents. PhysiBoSS [30,43] was developed to address this aspect, by allowing the representation of signaling pathways within each cell of PhysiCell using a Boolean model. The input nodes of the Boolean models are activated according to signals from PhysiCell, integrating the various external environmental conditions (signal from neighboring cells, presence of chemical signals, etc.). Cell phenotypes are triggered according to output nodes of the intracellular model, which leads to a response in the environment. MaBoSS is especially suited to perform this task as it simulates Boolean networks in continuous time, allowing to integrate MaBoSS notion of time within PhysiCell notion of time. Moreover, the stochasticity inherent of MaBoSS simulations allows to represent heterogeneity in cell response.

The cell fate decision model is a good example of a MaBoSS model that can be used in PhysiBoSS: two of the input nodes (TNF, Fas) can be directly linked to the presence of these molecules in the proximity of the cells, and its three output nodes can trigger behaviors in PhysiCell: *Survival* can trigger proliferation, *Apoptosis* and *NonACD* can trigger cell death. Such a PhysiBoSS model was developed in [30] to study TNF treatment in multi-cell spheroids (Fig. 4E). A link between the oxygen present around the cell and the node *NonACD* was also added to this model to account for oxygen dynamics, and reproduce the necrotic cells present at the core of the tumor. When stimulated with TNF, most of the peripheral cells undergo apoptosis. However, a small part of these cells activate NFκB and become resistant to TNF. After 24 h of treatment, most of the cells are resistant and the lethal effect of TNF vanishes (Fig. 5A).

This type of frameworks facilitates the simulations of clones in a tumor and the impact of treatments on a heterogeneous cell population. Clones are subsets of cells with similar sets of mutations. In the initial publication, we explored the effect of the treatment on the population that carried 25% of a clone with cFLIP and IKK overexpressed. These mutations were selected because they showed resistance in the MaBoSS simulation of a cell. We studied the impact of the size of two populations, one mutated and one wild type, on the growth rate of the population. We were able to predict that increasing the size of the clonal population would globally increase the growth rate of the whole population (from 130% increase with 0% mutated cells to 180% increase with 100% mutated cells). However, when looking at each clonal population particularly, their growth rate would be slightly decreased by the introduction of a more resistant clone, competing with them for access to oxygen (Fig. 5B).

While this type of models is a lot more complex and computationally demanding, it allows the exploration of many aspects of the influence of the microenvironment on the individual cell fate decision process and its spatial characteristics. In the context of studying cancer and its microenvironment, requiring the simulation of cellular migration through the extracellular matrix, structures of heterogeneous populations inside a tumor, or the action of the immune system, we believe this framework can provide unique insights.

4.5. Applications in cancer and immune biology

The logical approach has already been successfully applied to cancer biology [44–47] or immune biology [48–51] and has provided significant insights on drug identification [52,53,28].

4.5.1. Application of MaBoSS in cancer

Cancer is often referred to as a network or systems biology disease, with diverse and unexpected responses to chemotherapeutic treatments. Each cancer could be represented as a network modified by specific genetic and epigenetic alterations that are found in individual patients, leading to different possible outputs [54–56]. Not two patients will have the same molecular profiles and, as a consequence, will not respond to therapy in the same way. This heterogeneity between patients can be expanded to the intra-patient heterogeneity where the primary and the secondary tumors differ in their mutational profile. Similarly, inside a tumor, there might exist multiple clones with clusters of cells with similar (although not identical) genetic alterations. Many reviews and publications have addressed these aspects of heterogeneity [57]. When modeling tumorigenesis, these challenges should be tackled.

The stochasticity of MaBoSS tool suite can partly solve this question: with MaBoSS, each trajectory is a cell and each cell can follow its own path. It is possible to simulate a certain percentage of cells with different sets of mutations, reproducing to a certain extent the clonality reported in the tumor. We developed further a methodology to integrate omics data into these models and create a model per patient with different mutations and transition rates inferred from discretized data (mutations of copy number variation data), and continuous data (RNAseq or proteomics data), respectively [58]. As we already showed, PhysiBoSS is particularly suited for modeling and simulating clonality and the impact that a

treatment may have on the composition and proportion of different clones inside a tumor. In [30], we showed that two alterations of the NFκB pathway in the cell fate model (Fig. 5A) would lead to tumor growth with or without a TNF treatment, suggesting that patients with these mutations would not respond well to the treatment. The experiment to show this effect is not straightforward, though. Assessing the impact of this double mutation in patients by computing the correlation between their co-occurrence and the response to a TNF-related treatment could confirm or infirm the model observations, provided that this type of data is available. In 2015, a model of bladder cancer was constructed to explain the co-occurrence and the mutual exclusivity in a subset of gene alterations that had been derived from a statistical analysis[44]. In this example, the model was able to support some results of the statistical analysis by providing a mechanistic explanation of the findings but with this model, a co-occurrence could be refuted. In that case, we showed that a third alteration was needed for high grade tumors and these triplets of alterations were confirmed in data obtained from patients with severe disease.

Depending on the Boolean model and the genes included in the signaling pathways, specific questions can be studied with their own *in silico* experiments. Some models have focused on the interplay between signaling pathways with a generic approach [47,59], while other focused on specific cancers: breast [46], colon [60–63], bladder [44], gastric [64], or prostate cancer [28]. For each of these studies, some analyses could be made on the search for drug synergies, mutation associations, personalized treatments, and the consequences of combinations of mutations on the phenotypes, showing the wide panels of questions that can be explored with these models. One recent model of prostate cancer helped prioritize some drug targets for a sensitive cell line that were further validated experimentally. The model was personalized to the prostate cell line, LNCaP, using the methodology PROFILE [58], and drugs that significantly decreased proliferation and increased apoptosis were selected. Two of them were tested experimentally and their effects on cell viability were confirmed. The experimental validation of the prediction of the models is not always easy as such predictions are often made on cancer patients with heterogeneous profiles. Thus, they cannot be straightforwardly translated into an experiment. In these cases, the model can still be used as a strong support for reasoning.

4.5.2. Applications of MaBoSS and UPMaBoSS in immune response studies

A considerable effort has been invested into the modeling of immune response in the context of cancer but also to study and better understand autoimmune diseases. Some models have been published that explore T cell differentiation and clonal expansion [48,65–71], macrophage polarization [72–74], dendritic cell differentiation [75] and some have simulated the effect of checkpoint inhibitors for targeted cancer treatments [76,51]. Autoimmune disorders have also been characterized to better understand the signaling pathways that could be responsible for the diseases. Among them, models of Rheumatoid arthritis (RA) [77,78] or of psoriasis [79] have provided insights into the mechanisms at stake.

Most of these models focus on individual cell types and their intracellular regulation. However, the interactions among distinct cell types might provide additional insights and understanding on how to attack and revert the altered phenotypes. With UpMaBoSS, we constructed a model including several cell types and interacting through ligand-receptor dynamics. The population model described the immunogenic cell death (ICD): this type of cell death, induced in particular by some anti-cancer treatments, is able to recruit the immune system through the release of danger signals (DAMPs) by dying cells. ICD amplifies the killing effect of ICD inducers, through a complex mechanism resulting in cytotoxic effect of activated T-cells. The mathematical model includes tumor cells, dendritic cells and different T-cell subsets. It is able to reproduce the amplification of chemotherapy by anticancer immune responses. The model provides a tool to select some proteins as potential targets to enhance ICD and could also be used as a basis to explore the reasons why some chemotherapeutic drugs are good ICD inducers (e.g., oxaliplatin, mitoxantrone) and some are not (e.g., cisplatin).

4.6. Relevance and limitations of MaBoSS modeling approach

The logical framework is mainly chosen for its simplicity and versatility over more complex approaches such as chemical kinetic models. Even though the analyses and the predictions may appear limited compared to continuous models, the MaBoSS approach is highly appropriate in several situations. The first one is when a high number of genes (i.e., nodes) are required to answer the question. The lack of parameters is an advantage in this case. The second situation is when pairwise conditions are compared, e.g., wild type vs. mutation of a gene. In this case, the difference of probabilities to reach a phenotype or to activate a node (gene) in these two conditions can inform on the downstream impact of the mutation. In our example of the cell fate model, ROS-related genes reduced the non-apoptotic cell death phenotype to 0 compared to wild type. This type of effects could be observed experimentally with an increase of surviving cells after activation of an oncogene, or as the result of a statistical analysis which identified some genes as significantly differentially expressed in two experiments. Another interesting situation is related to population sizes: UPMaBoSS can compute the proportions of sub-populations defined by over/under-expression of surface markers, such as CD4 or CD8 markers for T cells, and this output could be compared to flow cytometry data if available.

As a general comment, the comparison between logical model simulations and data outputs is not straightforward and remains challenging (see tentative table in the Supplementary material summarizing some possible correspondence).

Finally, on top of the technical difficulties of writing the logical rules, the choice of the parameters (transition rates) can be mentioned. By default, the rates are set to 1. If they are modified, they can represent the inverse of the half-life of a protein, the inverse of the length of a process, or can simply separate the fast and slow processes by choosing rough ranges of parameter values.

5. Conclusion

Appropriate tools of the MaBoSS tool suite can address a wide variety of questions, in particular related to cancer and immune-related diseases. A MaBoSS mathematical model is based on discrete levels of entities (genes, proteins, cell types, compartments) and predicts time-dependent state probabilities from a user-defined initial condition. There are a number of documents that facilitate the construction of models with MaBoSS and its extensions, including online presentations of the tools, training materials, dedicated GitHub repositories and tutorials. In addition, all the published models using MaBoSS modeling tools have accompanying Jupyter notebooks to reproduce published results. These materials can all be easily reused with new models and are gathered in a GitHub repository: https://github.com/sysbio-curie/MaBoSS-Review.

Therefore, MaBoSS modeling framework is particularly suited when signaling pathways are described as a directed influence network (a set of logical influences between entities), with a limited number of cell types. MaBoSS evolved over time to answer new questions that emerged with new data in order to represent more

complex models: from a single cell approach with MaBoSS tool, to a more heterogeneous tissue within EnsembleMaBoSS, to a dynamical heterogeneous population of interacting cells, and finally to a full spatial modeling approach with PhysiBoSS.

In a given biological context, other frameworks than MaBoSS may be better fit to address a question, or could be included in a more thorough study incorporating different data types. For this purpose, MaBoSS is part of the CoLoMoTo consortium [39] that allows the exchange, the composition and the reuse of logical models and tools. MaBoSS can be used in a routine with tools that perform mutant analyses (cf. PINT [80]) or more formal stable state analyses [81–83].

The most efficient studies should be conducted with the most appropriate mathematical formalism, which may require, depending on the data and on the type of questions, to simulate a model with partial or ordinary differential equations to suggest ranges of drug doses or frequencies of treatments. In this case, MaBoSS models can be seen a first approximation of more quantitative analyses.

Conflicts of interest

GK has been holding research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Osasuna Therapeutics, Samsara Therapeutics, Sanofi, Sotio, Tollys, Vascage and Vasculox/Tioma. GK has been consulting for Reithera. GK is on the Board of Directors of the Bristol Myers Squibb Foundation France. GK is a scientific co-founder of everImmune, Osasuna Therapeutics, Samsara Therapeutics and Therafast Bio. GK is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis and metabolic disorders.

Acknowledgments

GK is supported by the Ligue contre le Cancer (équipe labellisée); Agence National de la Recherche (ANR) – Projets blancs; AMMICa US23/CNRS UMS3655; Association pour la recherche sur le cancer (ARC); Cancéropôle Ile-de-France; Fondation pour la Recherche Médicale (FRM); a donation by Elior; Equipex Onco-Pheno-Screen; European Joint Programme on Rare Diseases (EJPRD); Gustave Roussy Odyssea, the European Union Horizon 2020 Projects Oncobiome and Crimson; Fondation Carrefour; Institut National du Cancer (INCa); Institut Universitaire de France; LabEx Immuno-Oncology (ANR-18-IDEX-0001); a Cancer Research ASPIRE Award from the Mark Foundation; the RHU Immunolife; Seerave Foundation; SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); and SIRIC Cancer Research and Personalized Medicine (CARPEM). This study contributes to the IdEx Université de Paris ANR-18-IDEX-0001. LC, EB, VN, and GS are part of the ModICeD project from MIC ITMO 2020: Approches interdisciplinaires des processus oncogéniques et perspectives thérapeutiques: apports á l'oncologie des mathématiques et de l'informatique. Part of the research leading to these results has received funding from the European Union's Horizon 2020 Programme under the PerMedCoE Project (www.permedcoe.eu), grant agreement n° 951773. The development of PhysiBoSS is partly funded by a grant for promoting emerging research from Inserm.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.csbj.2022.10.003.

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