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PhysiBoSS 2.0: a sustainable integration of stochastic Boolean and agent-based modelling frameworks

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Abstract

Motivation: Cancer progression is a complex phenomenon that spans multiple scales from molecular to cellular and intercellular. Simulations can be used to perturb the underlying mechanisms of those systems and to generate hypotheses on novel therapies. We present a new version of PhysiBoSS, a multiscale modelling framework designed to cover multiple temporal and spatial scales, that improves its integration with PhysiCell, decoupling the cell agent simulations with the internal Boolean model in an easy-to-maintain computational framework.

Results: PhysiBoSS 2.0 is a redesign and reimplementation of PhysiBoSS, conceived as an add-on that expands the PhysiCell agent-based functionalities with intracellular cell signalling using MaBoSS having a decoupled, maintainable and model-agnostic design. PhysiBoSS 2.0 successfully reproduces simulations reported in the former PhysiBoSS and expands its functionalities such as using user-defined models and cells' specifications, having mechanistic submodels of substrate internalisation with ODEs and enabling the study of drug synergies.

Availability: PhysiBoSS 2.0 is open-source and publicly available on GitHub (https://github.com/PhysiBoSS/PhysiBoSS) under the BSD 3-clause license with several repositories of accompanying interoperable tools. Additionally, a nanoHUB tool has been set up to ease the use of PhysiBoSS 2.0 (https nanohub.org/tools/pba4tnf/).

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

PhysiBoSS (Letort *et al.*, 2019) is a standalone multiscale simulation software that was implemented by coupling an early version of PhysiCell (Ghaffarizadeh *et al.*, 2018), a multiscale multicellular agent-based modelling framework that allows the simulation of populations of cells in a defined microenvironment, together with MaBoSS (Stoll *et al.*, 2012), a continuous-time Markovian simulator for Boolean models that describe the cell's intracellular signalling and regulatory networks. This pioneering work allowed to bridge the modelling and simulation of processes at the intracellular level with those occurring at the cell population scale, a critical step toward the mechanistic description of complex systems such as tumours. Nevertheless, PhysiBoSS presented some design problems: it cannot be easily upgraded to use the current versions of any of those two pieces of software, hindering its long-term maintenance, and lacks a clear interface between these two tools, resulting in a software hard to modify and to extend without changing core functionalities.

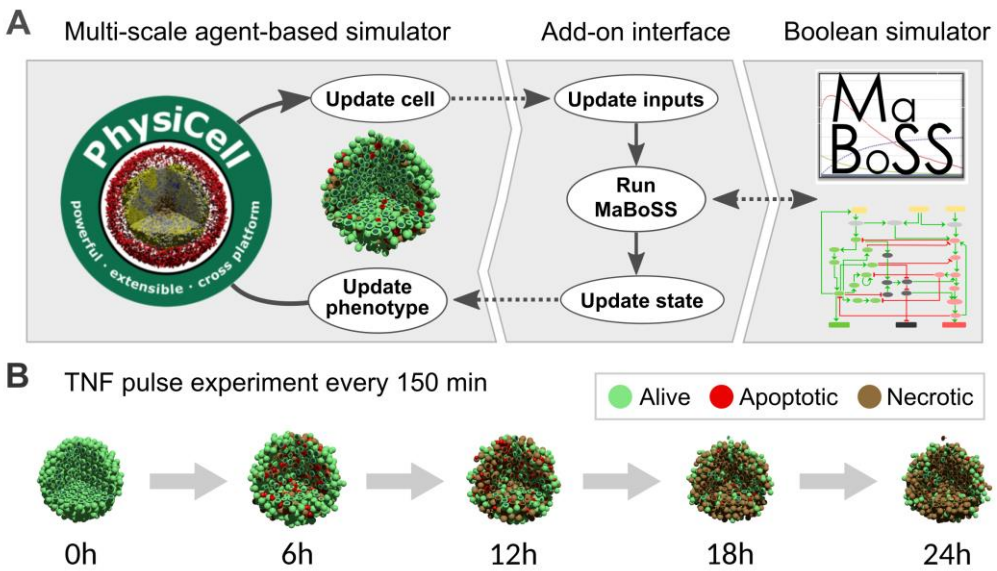

Fig. 1. PhysiBoSS 2.0 add-on-based design. A) Scheme of the add-on-based design of PhysiBoSS 2.0 that decouples PhysiCell and MaBoSS providing Boolean simulation functionality to individual cell agents in a maintainable manner. B) Example of a simulation in PhysiBoSS 2.0 that reproduces the complex simulations presented in Letort et al. (2019) work, where a tumour spheroid is supplied with periodic pulses of tumour necrotic factor (TNF) every 150 minutes (Supplementary Information and Fig. S13).

Herein, we present PhysiBoSS 2.0, a redesign and reimplementation of PhysiBoSS which solves the design problems of its early version and extends its functionalities. PhysiBoSS 2.0 is implemented as an add-on in PhysiCell that provides access to the MaBoSS simulator. In this new design, both PhysiCell and MaBoSS are decoupled and therefore can be upgraded independently. Furthermore, the code was designed and implemented following the best practices guidelines for bioinformatics software development, ensuring its long-term maintenance and prolonging its lifespan (Mangul *et al.*, 2019).

Additionally, PhysiBoSS 2.0 includes new functionalities like allowing the use of highly customisable settings such as user-defined Boolean models and cell types in the configuration XML, by easing the building of user-defined examples with different Boolean models in different cell types and by enabling the study of drug effects and synergies in multiscale simulations (Akasiadis *et al.*, 2022; Ponce-de-Leon *et al.*, 2022). PhysiBoSS 2.0 is a step towards the development of a PhysiCell add-on ecosystem of different types of models (Macklin *et al.*, 2018) that allows for the scaling-up of simulations in exascale high-performance computing clusters (Montagud, Ponce-de-Leon, *et al.*, 2021; Saxena *et al.*, 2021).

2 Methods

2.1 PhysiBoSS 2.0

PhysiBoSS 2.0 is a multiscale multicellular simulation framework that integrates PhysiCell (Ghaffarizadeh *et al.*, 2018) and MaBoSS (Stoll *et al.*, 2012), enabling the simulation of cell signalling in each cell agent. This allows the agents to integrate environmental and genetic signals and respond according to their internal Boolean model state. PhysiBoSS 2.0 is a redesign and reimplementation of PhysiBoSS as an add-on interface, which reduces their dependencies, facilitates its long-term maintenance and enables the extension of new functionalities. This design simplifies the tool's maintenance and allows for their independent upgrade, unlike the previous work of PhysiBoSS (Letort *et al.*, 2019).

The software is written in C++ and OpenMP is available to parallelise the execution. The implementation is described in further detail in the Supplementary Information together with a template project for new users. PhysiBoSS 2.0 code is open-source distributed under BSD 3-clause license (https://github.com/PhysiBoSS/PhysiBoSS). In addition, we also provide a nanoHUB GUI-based tool implementing an example model, to ease its use at https://nanohub.org/tools/pba4tnf/.

2.2 Boolean models

Several Boolean models were used in the present work. The first model is focused on the effect of TNF presence on cell fate decisions, was used previously in PhysiBoSS (Letort *et al.*, 2019) and is a modification of a published Boolean model of cellular fates (Calzone *et al.*, 2010). It has 31 total nodes, 3 input nodes (TNF, FADD, FASLG) and 3 output nodes (Survival, Non Apoptotic Cell Death (NonACD), Apoptosis). The model is available at https://maboss.curie.fr/.

The second model is a prostate cancer model (Montagud *et al.*, 2022). This model was used to identify druggable targets in prostate cancer that are personalised to patients and cell lines. The model has 133 total nodes, 9 inputs and 6 outputs nodes. This model is available as a Supplementary material of the corresponding paper (Montagud *et al.*, 2022).

The third model is a model of the gastric adenocarcinoma cell line (Flobak *et al.*, 2015), developed to predict drug synergies among seven drug-targeted genes part of the network (Fig. S11). The model has 90 total nodes with no input nodes and 2 output nodes (Pro-survival and Anti-survival). This model is available as a Supplementary material of the corresponding paper (Flobak *et al.*, 2015) and can be used to screen combinations of drugs (Fig. S12).

All these models are available in the dedicated repository: https://github.com/PhysiBoSS/boolean-models

2.3 Model exploration

Extreme-scale Model Exploration With Swift (EMEWS) (Ozik *et al.*, 2018) is a framework that combines model exploration with distributed simulations using the Swift/T language. Our model exploration strategy takes advantage of the DEAP package (De Rainville *et al.*, 2012; Fortin *et al.*, 2012), a python package that implements different evolutionary algorithms, including classic Genetic Algorithms, that can be used to perform model calibration or explore parameters that optimise a user-

defined goal (e.g. minimise cancer cells). We adapted the framework from PhysiCell-EMEWS (Ozik *et al.*, 2018) (available at https://github.com/MathCancer/PhysiCell-EMEWS) and expanded it by including a novel search strategy, Covariance Matrix Adaptation, as well as different sampling methods for performing parameter sweeps (Akasiadis *et al.*, 2022; Ponce-de-Leon *et al.*, 2022). Herein, we tested 3375 sets of parameter values for three kinetic constants of the TNF receptor model and selected the set that produces the results closest to those reported by Letort et al. (2019). The source code to reproduce this model exploration, as well as other experiments, is available at: https://github.com/PhysiBoSS/spheroid-tnf-v2-emews.

2.4 Personalisation of Boolean model using PROFILE methodology

The prostate Boolean model was tailored to different datasets using PROFILE methodology to obtain personalised models that capture the particularities of a set of patients (Béal *et al.*, 2019) and cell lines (Béal *et al.*, 2021). Proteomics, transcriptomics, mutations and copy number alteration (CNA) data can be used to modify different variables of the MaBoSS framework, such as node activity status, transition rates and initial conditions. The resulting ensemble of models is a set of personalised variants of the original model that can show great phenotypic differences. Different recipes (use of a given data type to modify a given MaBoSS variable) can be tested to find the combination that better correlates to a given clinical or otherwise descriptive data.

In the present case, prostate-cell-line-specific models were built using mutations, CNA and RNA expression data. More on PROFILE methodology can be found in its own work (Béal *et al.*, 2019) and at its dedicated GitHub repository: https://github.com/sysbio-curie/PROFILE.

2.5 Drug simulations in LNCaP-specific Boolean models

Boolean models can be used to simulate the effect of therapeutic interventions and predict the expected efficacy of candidate drugs on different genetic and environmental backgrounds by using our PROFILE_v2 methodology (Montagud, Béal, *et al.*, 2021). MaBoSS can perform simulations changing the proportion of activated and inhibited status of a given node. For instance, out of 5000 trajectories of the Gillespie algorithm, MaBoSS can simulate 70% of them with an activated AKT and 30% with an inhibited AKT node. The phenotypes' probabilities for the 5000 trajectories are averaged, and these are considered to be representative of a model with a drug that reduces the activity of AKT by 30%.

In the present work, the LNCaP model, a prostate cancer cell line, has been simulated with different levels of node activity, with 100% of node activity (no inhibition), 80%, 60%, 40%, 20% and 0% (proper knock-out). We simulated the inhibition of 6 nodes of interest. Drug synergies were studied using the Bliss independence model (Bliss, 1939) which is based on the idea that the two studied compounds are acting independently from each other, meaning that they are non-interacting (Greco *et al.*, 1996). Based on the effects of every single drug, a reference model was calculated as follows:

$$\hat{E}_{ab} = E_a + E_b - E_a * E_b$$

Where $0 \leq E_a, E_b \leq 1$. $\hat{E}_{ab}$ is the predicted combined effect of how the two drugs A and B act if no synergy or antagonism would exist; E_a and E_b are the single drug effects. If the observed combined drug effect is higher than the predicted effect, synergy is declared, and antagonism is concluded if it is lower. This can be expressed with the help of a Combination index (CI) (Foucquier and Guedj, 2015) as follows:

$$CI = \frac{\hat{E}_{ab}}{E_{ab}}$$

Where E_a and E_b are the efficiency of the single drug inhibitions and E_{ab} is the inhibition resulting from the double drug simulations. A Combination Index (CI) below 1 shows synergism while a value above 1 indicates antagonism. This methodology can be found in its own repository: https://github.com/PhysiBoSS/PROFILE_v2.

In PhysiBoSS 2.0, diffusing drugs in the microenvironment target and inhibit a specific node in the Boolean model. As modellers, the goal is to identify drugs that target candidate nodes that might be relevant in producing desired therapeutic outcomes such as increased apoptosis or reduced proliferation in LNCaP. We used a list of target nodes from Montagud et al. (2021) that have yielded interesting results using prostate Boolean models.

Suitable drugs were then identified using the DrugBank database (Wishart *et al.*, 2018) and matched with their availability on the Genomics of Drug Sensitivity in Cancer (GDSC) database (Yang *et al.*, 2013). The drug-target pairs of interest for LNCaP can be found in Tables 1 and S1.

Table 1. Drug-target pairs were used to perform the drug simulations on the LNCaP-specific Boolean model.

Node	Drug name	Drug GDSC ID
AKT	*Ipatasertib*	1924
EGFR	*Afatinib*	1032
ERK	*Ulixertinib*	2017 / 1908
HSPs	*Luminespib*	1559
MEK1_2	*Selumetinib*	1736
PI3K	*Pictisilib*	1058

The gene corresponding to the model nodes can be found in Table S1.

We have used standard pharmacodynamics methods to model the effect of various concentrations of drugs on the cell line growth. An important aspect in this field is the dose-response relationship, which has a direct translation to our modelling. The effect of the drug on the cell depends on the concentration of the drug and mostly exhibits a non-linear relation. Usually, sigmoidal shaped dose-response curves such as the Hill equation are used to model this behaviour. These dose-response curves provide insight into multiple drug characteristics such as potency or efficacy (Currie, 2018). The integration of dose-response curves into PhysiBoSS 2.0 allows us to better model the effect that the local concentration of a drug in the nearby surroundings of a cell has on the intracellular signalling model.

GDSC dose-response data was used to fit the raw cell viability data to a multi-level fixed effect model (Vis *et al.*, 2016) with which a specific sigmoidal dose-response curve was obtained for each drug and cell line pair. The code to reproduce this can be found in the *gdscIC50* R package (https://github.com/CancerRxGene/gdscIC50)

We integrated the specific dose-response curves for the selected drugs (Table S1) on LNCaP into the drug mapping function that governs the inhibition of a Boolean target node upon drug availability in the cell's surroundings. A cell is informed of the drug concentration for each simulated substrate at its nearest voxel, then the respective target node is inhibited with a probability based on the dose-response curve.

3 Results

In this section, we first introduce the redesign of PhysiBoSS together with the newly implemented features and functionalities. Secondly, we present novel results as examples of the kind of experiments possible with PhysiBoSS 2.0.

3.1 The new design of PhysiBoSS 2.0 allows for extended functionalities

The original PhysiBoSS embedded MaBoSS in PhysiCell and adapted several core classes of the latter, burdening the updatability of these pieces of software. PhysiBoSS 2.0 new design solves this maintainability issue by decoupling PhysiCell and MaBoSS with an interfacing add-on between them (Fig. 1A, Supplementary Information and Fig. S1).

The decoupling is obtained by encapsulating the functionalities used to simulate the Boolean model in a dedicated *MaBoSSNetwork* class that uses the MaBoSS library to store the current Boolean state of the cell and perform the simulations. This class is then used in a specialised implementation of PhysiCell's *Intracellular* interface (*MaBoSSIntracellular*), which describes how to import and simulate a MaBoSS intracellular model. This modularisation allows our code to be more easily maintainable. In addition, this add-on connection has been incorporated into the main PhysiCell code for this and other intracellular models (Macklin *et al.*, 2018) (Supplementary Information and Fig. S1).

This decoupling design facilitates the use of the latest versions of MaBoSS and PhysiCell, allowing PhysiBoSS 2.0 to incorporate new features that enable studies that were not possible with the former PhysiBoSS. For instance, by using PhysiCell 1.9, we can track internalised substrates in cells, use configuration XML files to define different cell types with their associated intracellular model, and have cell-cell contact behaviours. Also, by using MaBoSS 2.4.0, we can work with Boolean models with more than 64 nodes, use OpenMP to parallelise the simulation, be able to run under Microsoft Windows OS, run different Boolean models in the same simulation and define these models in the SBML-qual standard (Supplementary Information). Additionally, our design allows PhysiBoSS 2.0 to ease the integration of new Boolean models by using custom modules that connect intracellular variables to agent-based ones and configuration XML files with custom data for different cell types and environments.

Together with PhysiBoSS 2.0, we have developed the PhysiCell ToolKit (PCTK) a python-based package that includes a library and command-line scripts to process and analyse simulation outputs. Although there are already available tools for handling PhysiCell outputs (such as https://github.com/PhysiCell-Tools/python-loader), with PCTK we aim to gather and organise different pieces of python code that have been recurrently useful in different projects involving PhysiCell and PhysiBoSS. Currently, the package implements a simple module to parse and handle the MultiCellDS standard and uses an efficient schema to process the output files containing the cells and microenvironment data. Moreover, on the top of this module we have implemented different command-line tools for processing and creating basic plots, including time courses of the number of alive and dead cells and generating POV files used as inputs for the 3D rendering of the multicellular models using POV-Ray (Persistence of Vision Raytracer, PovRay 2004). PCTK can be used both as a callable library and as a stand-alone command-line tool and can be found at https://github.com/PhysiBoSS/pctk.

3.2 PhysiBoSS 2.0 enables multiscale modelling that uncovers mechanisms of drug treatments and synergies

PhysiBoSS 2.0 new implementation and extended functionalities provide a much more flexible framework that enables the study of more complex multiscale models. We showcase here two examples: the integration of a transport module for environmental substrates and the integration of pharmacodynamics and Boolean models to study drug combinations.

We incorporated an ODE-based transport module into an already published Boolean model that studies the cells' response to different tumour necrosis factor (TNF) dosage recipes (Calzone *et al.*, 2010). We replicated work reported by Letort, *et al.* (2019) using this model that showed complex dynamics and emergent behaviours: continuous exposures to TNF resulted in cells becoming resistant to the effect of the cytokines, whereas short pulses of a certain frequency caused the reduction of the tumour spheroid (Fig. 1B and S11). The TNF transport module focuses on the substrate's receptor dynamics allowing for the explicit modelling of the transport of environmental substrates such as nutrients and drugs inside the agents using ODEs and studying their effects on the Boolean model. This transport module can be user-defined to capture the particularities of different nutrients and drugs and it can be used to find optimal drug regimens (Ponce-de-Leon *et al.*, 2022).

As the parameters of the new TNF example in PhysiBoSS 2.0 (based on PhysiCell 1.9) do not have a one-to-one mapping to those of the former PhysiBoSS example (based on PhysiCell 1.0), we used a model exploration method (Ozik *et al.*, 2018) to find parameters that reproduce the dynamics previously reported (Supplementary Information, Fig. S13). The PhysiBoSS 2.0 results are consistent with those of the original PhysiBoSS, as evidence of the correctness of our reimplementation (Supplementary Information and Fig.S11-S13). Finally, we have deployed a PhysiBoSS 2.0 instance running this example to facilitate the study of different parameter sets that is publicly available at https://nanohub.org/tools/pba4tnf/.

In addition, PhysiBoSS 2.0 enables the performance of drug studies such as synergy predictions in cell populations while considering heterogeneity among the cells and the environment. By using personalised Boolean models (Béal *et al.*, 2019) and GDSC dose-response profiling data (Vis *et al.*, 2016), we can portray the typical characteristics of cell lines and drugs (Fig. 2). Then, with PhysiBoSS 2.0, we can perform multiscale simulations and find synergistic drug combinations for a cell line of interest. We hereby provide an example study on the LNCaP prostate cell line (Montagud *et al.*, 2022) with six different drugs (Supplementary Information).

We identified two combinations of drugs as interesting: *Pictilisib* (targeting PI3K) with *Ipatasertib* (targeting AKT) and *Pictilisib* with *Luminespib* (targeting HSPs) (Table S2). In both drug pairs, we see that the Growth Index (a measure of the relative growth of treated cells vs untreated cells, see Supplementary Information) for LNCaP is much lower when using both drugs than when using individual drugs (Fig. 2B, 2C and S3). In addition, we studied the Bliss independence of these combinations (Fig. S6 and S7) and found complex synergies in both cases (Supplementary Information, Section 3.5).

The complete study of LNCaP and six drugs (*Ipatasertib* targeting AKT, *Luminespib* targeting HSPs, *Pictilisib* targeting PI3K, *Afatinib* targeting EGFR, *Ulixertinib* targeting MAPK1, *Selumetinib* targeting MAP2K1 and MAP2K2) can be found in the Supplementary Information together with the same analysis for the AGS gastric cell line (Flobak *et al.*, 2015) and seven drugs of interest (Fig. S11 and S12).

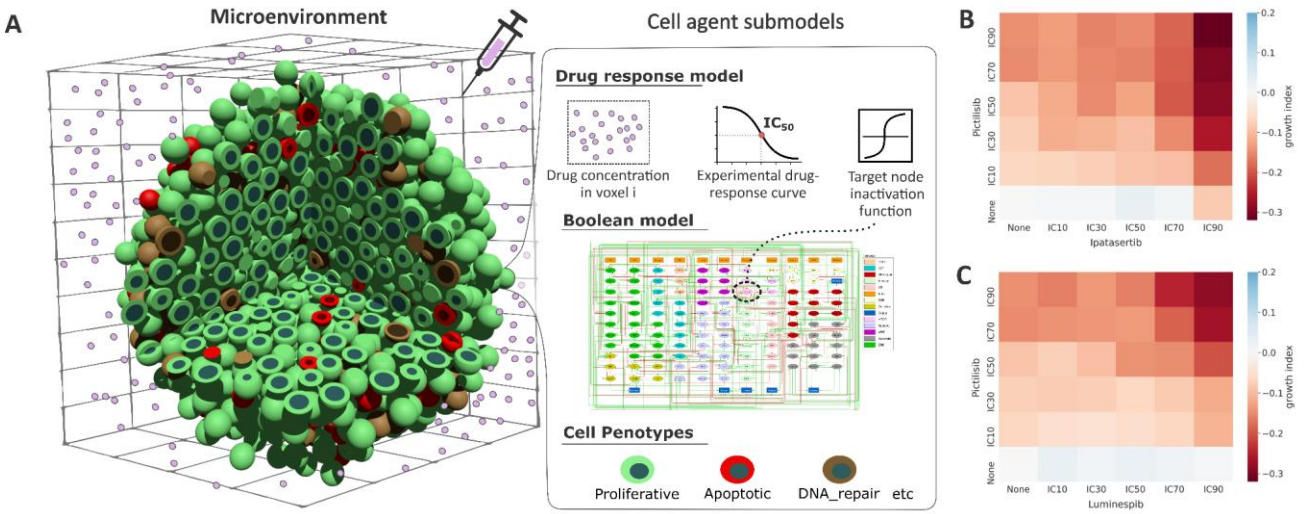

Fig. 2. Multiscale simulation of LNCaP prostate cancer cell line and combinations of drugs. A) Overview of PhysiBoSS 2.0 simulation framework. Drugs in the microenvironment affect the cells' behaviour according to an experimental drug-response curve. Depending on how a specific drug affects a specific cell line, the node targeted by the drug is inhibited at a given rate affecting the cell's phenotype probabilities, allowing for a tailored simulation of drugs and cell lines. B and C) Growth index of a simulation with *Pictilisib + Ipatasertib* (B) and *Pictilisib + Luminespib* (C) with respect to the untreated LNCaP. Each simulation was replicated 10 times. A positive growth index (blue) means the drug increased the growth and a negative growth index (red) means that the drug diminished the growth of the cells.

With these capabilities, PhysiBoSS 2.0 takes an important step towards the study of drug treatments and effective drug synergies, a needed step towards reaching a truly personalised medicine and helping improve treatments for cancer patients. Implicitly, this also showcases the flexibility of PhysiBoSS 2.0 to run with any user-provided Boolean model in MaBoSS or SBML-qual format, as opposed to the original PhysiBoSS.

4 Conclusions

PhysiBoSS 2.0 is a redesign and reimplementation of PhysiBoSS to be a reusable, extensible and updatable add-on for the PhysiCell framework. Our tool follows a modular design that decouples PhysiCell and MaBoSS codes, minimising the dependencies between these tools and ensuring long-term maintainability and is, therefore, more respectful of the single responsibility principle and the façade pattern software design (Gamma *et al.*, 1995).

We show added functionalities that enable PhysiBoSS 2.0 to study disease mechanisms that cause malfunctions in personalised models while being flexible to handle many different models and consistent with the results of the former PhysiBoSS. Altogether, the new design and implementation allow PhysiBoSS 2.0 to be model-agnostic, easily customisable by users and provides a simple framework of custom modules and custom settings to use with any Boolean model of interest in MaBoSS or SBML-qual format.

We have made efforts to provide full accessibility to PhysiBoSS 2.0 code as well as to several accompanying interoperable tools that make the full software bundle much more reusable at https://github.com/PhysiBoSS/.

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Conflict of Interest: none declared.

References

Akasiadis,C. *et al.* (2022) Parallel model exploration for tumor treatment simulations. *Comput. Intell.*

Béal,J. *et al.* (2019) Personalization of logical models with multi-omics data allows clinical stratification of patients. *Front. Physiol.*, **9**, 1965.

Béal,J. *et al.* (2021) Personalized logical models to investigate cancer response to BRAF treatments in melanomas and colorectal cancers. *PLOS Comput. Biol.*, **17**, e1007900.

Bliss,C.I. (1939) The Toxicity of Poisons Applied Jointly. *Ann. Appl. Biol.*, **26**, 585–615.

Calzone,L. *et al.* (2010) Mathematical modelling of cell-fate decision in response to death receptor engagement. *PLoS Comput. Biol.*, **6**, e1000702.

Currie,G.M. (2018) Pharmacology, Part 1: Introduction to Pharmacology and Pharmacodynamics. *J. Nucl. Med. Technol.*, **46**, 81–86.

De Rainville,F.-M. *et al.* (2012) DEAP: a python framework for evolutionary algorithms. In, *Proceedings of the 14th annual conference companion on Genetic and evolutionary computation*, GECCO '12. Association for Computing Machinery, New York, NY, USA, pp. 85–92.

Flobak,Å. *et al.* (2015) Discovery of Drug Synergies in Gastric Cancer Cells Predicted by Logical Modeling. *PLOS Comput. Biol.*, **11**, e1004426.

Fortin,F.-A. *et al.* (2012) DEAP: evolutionary algorithms made easy. *J.*

Mach. Learn. Res., **13**, 2171–2175.
Foucquier,J. and Guedj,M. (2015) Analysis of drug combinations: current methodological landscape. *Pharmacol. Res. Perspect.*, **3**.
Gamma,E. *et al.* (1995) Design patterns: elements of reusable object-oriented software Addison-Wesley, Reading, Mass.
Ghaffarizadeh,A. *et al.* (2018) PhysiCell: An open source physics-based cell simulator for 3-D multicellular systems. *PLOS Comput. Biol.*, **14**, e1005991.
Greco,W.R. *et al.* (1996) The search for cytotoxic synergy between anticancer agents: a case of Dorothy and the ruby slippers? *J. Natl. Cancer Inst.*, **88**, 699–700.
Letort,G. *et al.* (2019) PhysiBoSS: a multi-scale agent-based modelling framework integrating physical dimension and cell signalling. *Bioinformatics*, bty766.
Macklin,P. *et al.* (2018) Open source tools and standardized data in cancer systems biology. *bioRxiv*, 244319.
Mangul,S. *et al.* (2019) Improving the usability and archival stability of bioinformatics software. *Genome Biol.*, **20**, 47.
Montagud,A., Béal,J., *et al.* (2021) Patient-specific Boolean models of signaling networks guide personalized treatments. *bioRxiv*, 2021.07.28.454126.
Montagud,A. *et al.* (2022) Patient-specific Boolean models of signalling networks guide personalised treatments. *eLife*, **11**, e72626.
Montagud,A., Ponce-de-Leon,M., *et al.* (2021) Systems biology at the giga-scale: Large multiscale models of complex, heterogeneous multicellular systems. *Curr. Opin. Syst. Biol.*, **28**, 100385.
Ozik,J. *et al.* (2018) High-throughput cancer hypothesis testing with an integrated PhysiCell-EMEWS workflow. *BMC Bioinformatics*, **19**, 483.
Persistence of Vision Raytracer Persistence of Vision Pty. Ltd.
Ponce-de-Leon,M. *et al.* (2022) Optimizing dosage-specific treatments in a multi-scale model of a tumor growth. *Front. Mol. Biosci.*, **9**.
Saxena,G. *et al.* (2021) BioFVM-X: An MPI+OpenMP 3-D Simulator for Biological Systems. In, Cinquemani,E. and Paulevé,L. (eds), *Computational Methods in Systems Biology*, Lecture Notes in Computer Science. Springer International Publishing, Cham, pp. 266–279.
Stoll,G. *et al.* (2012) Continuous time Boolean modeling for biological signaling: application of Gillespie algorithm. *BMC Syst. Biol.*, **6**, 116.
Vis,D.J. *et al.* (2016) Multilevel models improve precision and speed of IC50 estimates. *Pharmacogenomics*, **17**, 691–700.
Wishart,D.S. *et al.* (2018) DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res.*, **46**, D1074–D1082.
Yang,W. *et al.* (2013) Genomics of Drug Sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells. *Nucleic Acids Res.*, **41**, D955-61.