Supplementary Informations

Supplementary informations for PhysiBoSS: a multi-scale agent based modelling framework integrating physical dimension and cell signalling

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Contents

1 Figure S1: PhysiBoSS features 2
2 Figure S2: Cell fate decision Boolean model 3
3 Figure S3: Population response to TNF injection 4
4 Figure S4: Effects of simulation parameters in spheroids 5
5 Figure S5: Genetically heterogeneous population under TNF treatment 7
6 Figure S6: Genetically heterogeneous population under TNF treatment and oxygen-limited regime 8
7 Table S1: Different multi-scale modelling frameworks 10
8 Table S2: Simulation run times 12
1 Figure S1: PhysiBoSS features

Initial and final snapshots of the simulations presented in main text, Figure 2.
A: Boolean network used. Pathway related to *Survival* (green), related to *Apoptosis* (red) and related to *NonACD* (black). Green arrows represent activation, red arrows inhibitions. B: Schematic representation of how cell cycle is simulated. Cells are initially in *Proliferative* state (growing and dividing; green). At frequent interval, their internal signalling network is updated by running MaBoSS (orange) given its environmental conditions (for instance, internalization of TNF). These conditions affects the fate of the cell: to continue proliferating or commit to *Apoptosis* (red) or *NonACD* (black).
3 Figure S3: Population response to TNF injection

Supporting informations for main text, Figure 3. A: Simulations without TNF. Snapshots of a simulation at t = 0, 4, 8 and 12h. B: Simulations under a low dose injection of TNF (1 ng/mL during 5 min). Snapshots of a simulation at t = 0, 4, 8 and 12h. C: Final fate of the active cells in the simulation for each 'stimulus area' tested. A-C: Colour code: green, Proliferative cells; red, cells committed to Apoptosis; black, cells committed to NonACD.
Figure S4: Effects of simulation parameters in spheroids.

A: Growth without TNF. Snapshots of a simulation of a spheroid growth at t = 0, 8, 16 and 24 h.
B: Growth under a 0.5 ng/mL continuous dose of TNF. Snapshots of a simulation at t = 0, 8, 16 and 24 h.
C: Growth under a pulsed injection of TNF, 0.5 ng/mL for 5 min every 300 min.
D: Variation of secretion rate value. Time evolution of the cell population in each fate (three leftmost panels) for a secretion rate of: 0 (1st panel), 0.1 (2nd) and 10 (3rd). TNF was injected at the beginning of the simulation for 5 min at a dose of 1 ng/mL. Note that when secretion rate
is 10, TNF accumulates in the environment (grey shading). Final number of cells in each fate according to the secretion rate used for individual simulations (rightmost panel). E: Variation of the initial spheroid radius. Time evolution of the cell population in each cell fate for an initial population radius of 50 (left panel), 150 (middle) and 200 (right) µm. TNF is injected continuously during the simulations at a concentration of 0.5 ng/mL. In order to better show population dynamics, Y-axes are different among panels. F: Variation of the transcription rate used in the Boolean network transitions. Time evolution of the cell population in each cell fate for a transcription rate of 1/48 (1st panel), 1/24 (2nd, the one used in all other results), 1/12 (3rd) and 1 (4th panel). Transitions rates for other nodes described in the note below are 1. A-F: Colour code: green, Proliferative cells; red, cells committed to Apoptosis; black, cells committed to NonACD. Grey shading indicates TNF presence. Except in D, right panel, 5 simulations are presented for each condition. Simulation time is 24 h, initial spheroid radius is 100 µm, which accounts for roughly 1000 cells, except explicitly stated.

**Note on the different rates of transition**

Regarding the different rates of transition used in our model, two sets of nodes were established: first, most of the nodes present in the model represent protein-to-protein interactions and signal transduction such as Apoptosis pathway activation, TNF activation of Survival pathway and ROS activation. The value for these nodes was 1 MaBoSS rate that corresponds roughly to 10 minutes of PhysiBoSS time. Additionally, a subset of nodes, the ones that represent the activation of BCL2 and cFLIP as well as translation of different mRNAs (mROS, mclAP and mXIAP) (all of them activated by NFkB) and the translation of cIAP from mclAP, were considered to be activated in much more time (1/24 MaBoSS rate that corresponds roughly to 4 hours of PhysiBoSS time).

We considered that protein-to-protein interactions and signal transduction happened 24 times faster than the transcriptional and translation steps. This was an educated guess based in previous works: Hirata et al. (2002) and Shimojo et al. (2008) found that transcription is 12 times faster than translation in mouse cells (and Lewis (2003) considered this as well for zebrafish). In our case, most interactions are protein activations, and those are faster than translation, therefore considering a factor of 24 between protein activations and translation.

**Note on spheroid size comparison to experiments**

In Figure 4A, we present simulations without TNF injections: spheroids with an initial radius of 100 µm with ≈1000 cells reached a size of approximately 150 µm radius with ≈4500 cells in 24h, a growth of 4.5x factor. In this case, these results consider that oxygen is freely available to cells, even in the centre of the spheroid. Additionally, in Figure S6A in Supplementary Information we present results without TNF injection and with oxygen and nutrients limitations, a closer set-up to experimental data. In this case, an initial 9000-cells spheroid has a 200 µm radius and after 24 hours grows to a final ≈16500 cells spheroids with a 245 µm radius, a growth of 1.8x factor.

Experimentally, comparable population growths have been observed in Freyer et al. (1984) work, which measured a fivefold increase in two spheroids (from 1000 to 5000 cells in one and from 600 to 3000 cells in another). Nonetheless, the number of cells in the spheroids are quite different, which is likely due to the different cell strains we are comparing: V-79 Chinese hamster fibroblasts in Freyer et al. (1984) and 3T3 Swiss albino mouse embryo fibroblasts simulated in present work with data taken from Tay et al. (2010) and Kellogg et al. (2015). This density discrepancy is also a probable cause for the differences we see in terms of volume changes. Freyer et al. (1984) 1000-cells spheroid’s radius goes from 210 µm to a final 230 µm for the 5000-cells spheroid (9.5% radius increase) and 600-cells spheroid’s radius goes from 150 µm to a final 220 µm for the 3000-cells spheroid (32% radius increase), while our simulated spheroid has a 50% radius increase without oxygen limitation. However, in simulations with oxygen and nutrient limitations, we observed that these are impeding factors for growth once the spheroid goes...
beyond a threshold volume. Indeed, considering these limitations, we measured a radius increase of approximatively 25%, which is in range of the experimental values. These considerations highlight the importance of multi-scale frameworks that take into account both environmental and genetic information.

5 Figure S5: Genetically heterogeneous population under TNF treatment.

Simulations of heterogeneous population composed of 75% of WT cells (orange) and 25% of mROS+ and cIAP- mutated cells (blue) or with CASP3+ and Cytc+ mutated cells (light blue). A: Snapshots of a simulation for each case at initial and final time (24 h), with cells coloured by cell type (left and middle) or by cell fate commitment (right). B: Time evolution of the number of cells of each strain (WT and mutated) for 10 simulations. C: Ratio of final number of surviving cells against initial number of cells for each cell line (WT or mutated). No significant differences were found under Kolmogorov-Smirnov test. D: Time evolution of the number of cells in each strain (WT and mutated) for 10 simulations for the 3 different mutants with different initial proportion of WT cells compared to the total population: 25% (top) and 50% (bottom). A-C: Colour code: green, Proliferative cells; red, cells committed to Apoptosis; black, cells committed to NonACD. Grey shading indicates presence of TNF in continuous injection at 0.5 ng/mL. Initial spheroid radius is 200 µm, which accounts for roughly 9000 cells, + stands for...
over-expression and - stands for knock-out.

6 Figure S6: Genetically heterogeneous population under TNF treatment and oxygen-limited regime.

A: Simulation of WT-only population without TNF. Snapshots of a simulation (left) and oxygen levels (middle) at 24 h. Time evolution of the number of cells in each cell fate (right). B: Simulation of WT-only population for a low-dose injection of TNF. Snapshots of a simulation (left) and oxygen levels (middle) at 24 h. Time evolution of the number of cells in each cell fate (right). C-E: Simulations of heterogeneous population and TNF treatment composed of 75% of WT cells (orange) and 25% of mROS+ and cIAP- mutated cells (blue) or with CASP3+ and Cytc+ mutated cells (light blue). C: Snapshots of a simulation for each case at initial and final
time (24 h), with cells coloured by cell type (left and middle) or by cell fate commitment (right). D: Time evolution of the number of cells in each strain (WT and mutated) for 10 simulations. E: Ratio of final number of surviving cells against initial number of cells for each cell line (WT or mutated). F: Time evolution of the number of cells in each strain (WT and mutated) for 10 simulations for the 3 different mutants with different initial proportion of WT cells compared to the total population: 25% (top) and 50% (bottom). A-F: Oxygen levels, represented from dark blue (injected level) to white (lowest level), are measured in the z=0 plane of the simulated space. Colour code: green, Proliferative cells; red, cells committed to Apoptosis; black, cells committed to NonACD. Grey level background in graphs indicates presence of TNF in continuous injection at 0.5 ng/mL. All simulations are in oxygen-limited regime, initial spheroid radius is 200 µm, which accounts for roughly 9000 cells, + stands for over-expression and - stands for knock-out.
7 Table S1: Different multi-scale modelling frameworks

Examples of different multi-scale modelling frameworks that could be used to address in different manners problems similar to the ones presented in this work. Intracellular framework can be differential equations, ordinary (ODE) or partial (PDE), or logical modelling (Boolean or multilevel). Extracellular framework can be based on a lattice (cellular automata (CA) or Cellular Potts (CP)) or independent from a lattice description (agent-based (AB) or vertex-based (VB)). Dimensions (Dim.) can be 1: 1D (lines), 2: 2D (areas), 3: 3D (volumes) or 3m: 3D objects in a monolayer. OS compatibility can be Linux (L), Mac OS (M) or Windows (W).
| Name, ref.                  | Intracellular framework | Extracellular framework | Cell shape          | Dim. | OS | Parallelization | Code license (and link) |
|----------------------------|-------------------------|-------------------------|---------------------|------|----|-----------------|------------------------|
| Athale and Deisboeck (2006)| ODE                     | off-lattice AB          | squares             | 2    | L  | Not available   | Not available          |
| CellSys                    |                         |                         |                     |      |    |                 |                        |
| Hoehme and Drasdo (2010)   |                         |                         |                     |      |    |                 |                        |
| Chaste                     |                         |                         |                     |      |    |                 |                        |
| Mirams et al. (2013)       | ODE, PDE                | off-lattice (AB, VB)    | spheres             | 1,2, 3,3m | L | OpenMP, MPI | Open                  |
| EmbryoMaker                |                         |                         |                     |      |    |                 |                        |
| Marin-Riera et al. (2016)  | ODE                     | off-lattice AB          | spheres, cylinders  | 3,3m | L | Not available | Open                  |
| EpiLog                     | Logical                 | on-lattice CA           | hexagons            | 2    | L,M,W | Not available | Not available          |
| MecaGen                    | ODE                     | off-lattice AB          | spheres             | 3,3m | L  | OpenMOLE compatible | Open                  |
| Orchestral                 |                         |                         |                     |      |    |                 |                        |
| Coulier and Hellander (2018)| ODE                    | depends on single-cell modelling | depends on single-cell modelling | L,M,W | Dask | Open            |                        |
| PhysiBoSS                  | Logical                 | off-lattice AB          | spheres             | 3,3m | L,M, W(docker) | OpenMOPE compatible | Open                  |
| Prokopiou et al. (2014)    | ODE                     | on-lattice CP           | squares can be used to form other shapes | 2 | L,M,W | CompuCell3D is OpenMP compatible | Uses CompuCell3D |
| Schluter et al. (2014)     | ODE                     | off-lattice AB          | spheres             | 3m   | -  | Not available | Not available          |
| VirtualLeaf                | ODE                     | off-lattice VB          | irregular           | 2    | L,M,W | Not available | Open                  |

Note: Dim. refers to the dimensionality of the simulations.
8 Table S2: Simulation run times

Representative examples of run time necessary for different kind of simulations. Simulations were run on one node of a Linux cluster (with 16 OpenMP threads). The processes used around 300 MB of memory while running (Proportional Set Size measured, from 284 MB for 1000 cells to 295 MB for 10000 cells).

| Cell number (init-final) | Simulated time | Remark | Wall time |
|-------------------------|----------------|--------|----------|
| 1022 - 1969             | 720 min        | no TNF | 2 min    |
| 1024 - 1443             | 720 min        | cont. TNF | 1.5 min |
| 1131 - 4772             | 1440 min       | no TNF | 10.5 min |
| 1134 - 2211             | 1440 min       | cont. TNF | 25.75 min |
| 1136 - 3544             | 1440 min       | one pulse injection | 9 min |
| 3843 - 7284             | 1440 min       | cont. TNF | 42 min |
| 16297 - 23227           | 1440 min       | 7192 passive cells | 45.5 min |

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