HZI Workshop on Systems Biology
Modeling multicellular systems

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Yesterday you have learned how to model and analyze intracellular regulations in single cells. However, cells do not exist in isolation. They form tissues and organs *in vivo* and aggregates or colonies *in vitro*. Embedded in these structures, cells send and receive signals from neighboring or nearby cells that can affect gene expression and cell fate decisions. Through such local interactions, spatial patterns of gene expressions or cell type arrangements can arise.

The goal of this tutorial is to learn about spatial and multiscale models of cell fate decisions. You will analyze the behavior of several spatial models with Morpheus[1]. Morpheus is a modeling and simulation environment that facilitates the integration of ordinary differential equations, reaction-diffusion systems and cell-based models. It enables the development of multiscale models to investigate possible feedbacks between gene regulation, intercellular communication and morphogenetic mechanisms. Morpheus does not require programming expertise because models can be created, simulated and visualized using its graphical user interface.

This tutorial is structured into three parts. After a short introduction into the modeling environment, first, we look at the emergence of spatial patterns through **lateral inhibition** and study the effect of cell motility on patterning and cell fate stability. We then turn to a cell-based model of morphogenesis in which cells are sorted by **differential adhesion** between different cell types. Finally, we combine the two approaches in a multiscale model of mouse embryonic stem cells (ESCs). This model consists of an intracellular submodel that describes the **spatial expression of Nanog** and a cell-based model that accounts for cell division, adhesion and intercellular communication.

[1] Jörn Starruß, Walter de Back, Lutz Brusch and Andreas Deutsch. Morpheus: a user-friendly modeling environment for multiscale and multicellular systems biology. *Bioinformatics*, 30(9):1331-1332, 2014. *Website*: `http://imc.zih.tu-dresden.de/wiki/morpheus`

1 Lateral inhibition

1.1 Toggle switch

Before we introduce the models on lateral inhibition and spatial patterning, we first like to consider yesterday's toggle switch model (equations shown below) to familiarize ourselves with Morpheus.

$$\frac{dx}{dt} = \frac{a}{1+y^\beta} - x + \xi(0,\eta) \tag{1}$$

$$\frac{dy}{dt} = \frac{a}{1+x^\beta} - y + \xi(0,\eta) \tag{2}$$

Please start Morpheus and load the file `toggle_switch.xml` from the ... folder on your desktop. You can use the Open icon in the toolbar (see figure 1), File→Open, or drag and drop the file in the Morpheus window. After opening, the model can be browsed using the Document panel (figure 1).

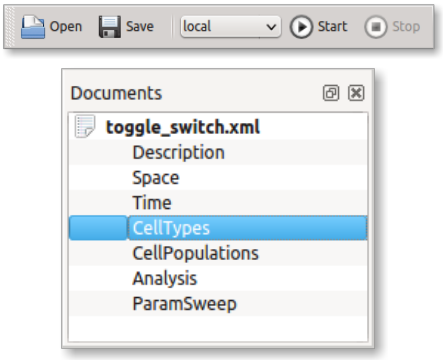

Figure 1: **Toolbar** and **Document panel**

The different elements in the Document panel specify all aspects required for model simulation, i.e.

- the model itself, i.e. the set of differential equations.
  See `CellTypes/CellType/System`.
- the initial conditions.
  See `CellPopulations/Population/InitProperty`.
- the spatial dimensions and duration of simulation.
  See `Space` and `Time`.

- the specification of data collection and visualization.
  See `Analysis/Logger`.

To simulate the model, use the Start icon in the toolbar (figure 1). Upon starting the simulation, Morpheus adds the job to the JobQueue panel (figure 2). The result browser shows the content of a folder that is created to store the results of this particular simulation. This folder contains a number of files that are created during simulation:

model.xml The XML file that specifies the model. This is created from the user-specified model upon starting a simulation and reflects the current state of the model.

model.xml.out The standard output file. The content of this file is shown in the panel at the bottom.

gnuplot_error0.log This file is created by Gnuplot (even if no errors occur). Gnuplot is the program that is used as a back-end to draw plots and graphics.

logger_*.log Plain text file created by the `Analysis/Logger`.

logger_*.png Graphics file that contains the plot created by the `Analysis/Logger`.

If you select a text or a graphics file, the content is shown in the Preview panel (figure 2). The containing folder can also be opened by an external file browser using the Folder icon. Alternatively, a command-line terminal can be opened using the Terminal icon.

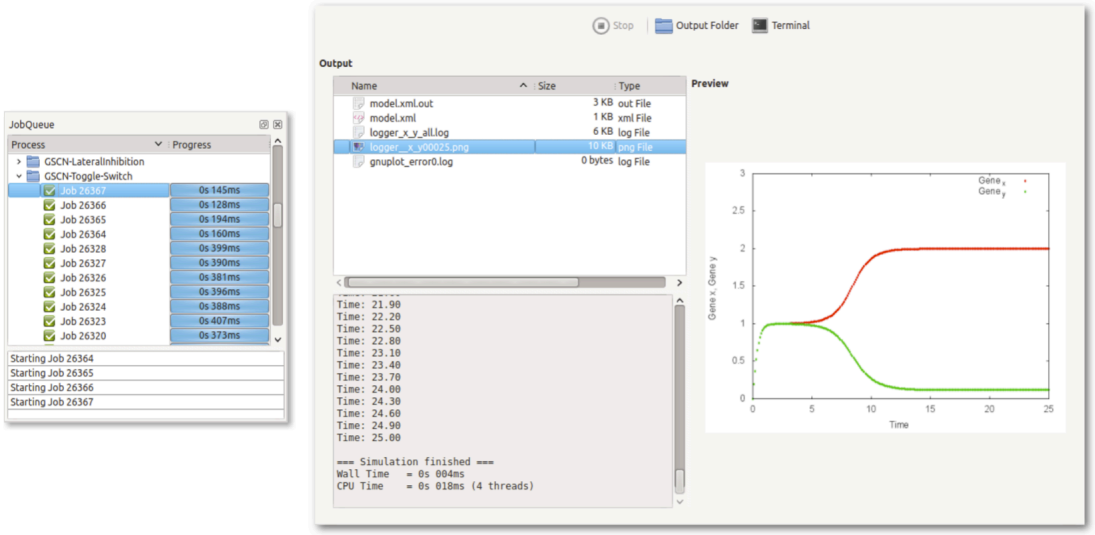

Figure 2: **JobQueue** (left) and **result browser** (right). The result browser shows the output folder (top), the simulation output (bottom) and a preview panel (right).

Looking at the plot, you will see that the expression of genes x and y diverge after a transient phase in which both are equally expressed.

Change one of the model parameters (a, β, η) and see how this affects the simulation result:

- Click on `CellTypes` in the Documents panel and then `CellType/System`.
- Select one parameter and change its value in the Attributes panel on the right side.
- Use the Start icon in the toolbar to run a new simulation.

Change the initial conditions (i.e. click on `CellPopulations` in the Documents panel and then `Population/InitProperty/Expression`) and see how this affects the result.

You can also check the effect of the integration time-step (i.e. click on `CellType/System` and then change `time-step` on the right side in the Attributes panel).

1.2 Lateral inhibition

With a few changes, we can turn the toggle switch model into a spatial model of lateral inhibition: instead of modeling mutual inhibition between two genes x and y within a single cell (as in the toggle switch), now we assume mutual inhibition between a single gene x in two adjacent cells. This has been done in the following cell couplet model in which x_n is the average expression of gene x in neighboring cells:

$$\frac{dx}{dt} = \frac{a}{1 + x_n^{\beta}} - x + \xi(0, \eta) \tag{3}$$

Please open the file `lateral_inhibition.xml` and run a simulation. If you run this model, you will see that the result, the time course plot (`logger_*.png`), looks very similar to the one obtained for the toggle switch. However, if you look at the other graphical result file (`plot_*.png`), you see the key difference (figure 3A).

In the plot, you can see the expression of gene x in two cells, represented as two squares (red is high expression, white is no expression). In the model description, the key differences between the toggle switch and lateral inhibition models are:

- Space now has size (x,y,z)=(2, 0, 0).
  See `Space`.
- Each cell now has only 1 differential equation, for the expression of gene x.
  See `CellTypes/Cellype/System`.
- Each cell gets information on the expression of gene x in the neighboring cell, and stores it in a property x_n.
  See `CellTypes/Cellype/NeighborReporter`.
- An analysis tool is added to draw the cells and their expression.
  See `Analysis/Gnuplotter`.

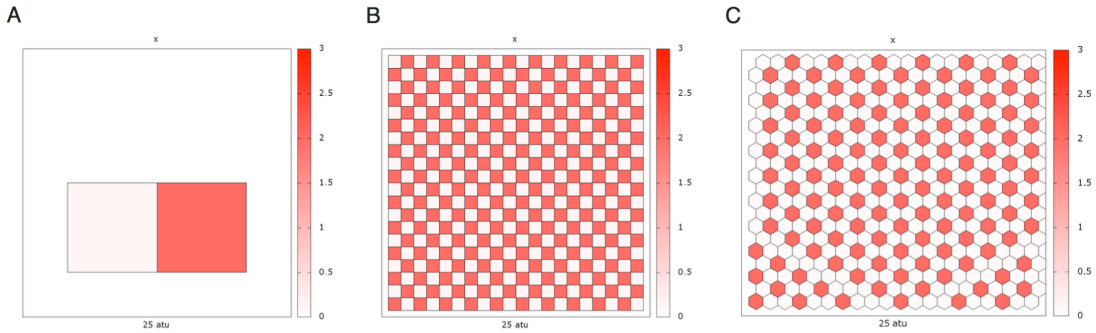

Figure 3: **Lateral inhibition** (A) in a cell couplet, (B) on a square lattice, and (C) on a hexagonal lattice.

1.2.1 Pattern formation in lattice simulation

To study pattern formation based on lateral inhibition in a tissue, we need to extend the two-cell couplet model into a tissue simulation. **Please change the model such that it simulates a tissue of approximately 400 cells in a regular square lattice (as shown in figure 3B).** Therefore, you only need to adjust `Space/Lattice`.

Try to answer the following questions:

1. What type of pattern results from this simulation? How is this pattern established?
    - **Tip:** Set `Analysis/Gnuplotter/endstate` to `false` to plot simulation results for intermediate states of the simulation.
2. Why is the pattern not perfectly regular? Formulate an hypothesis.
3. How can you test this hypothesis?
    - Can you think of a simulation model to test your hypothesis? If yes, changing the model accordingly.

1.2.2 Pattern formation in hexagonal lattice

The above simulations are based on a spatial representation of cells as squares. For epithelia, a somewhat more realistic representation is a hexagonal packing. **Change the model to use a regular hexagonal lattice (as shown in figure 3C) and try to answer the following questions:**

1. What differences do you observe? Can you think of a reason why these occur?
2. How can the original patterning be restored? (**Tip:** You might have to change the differential equation.)

2 Cell sorting

In the previous section, we have seen models of intercellular signaling with very abstract concepts for cell shape. In this section, we will focus on mechanical interactions using a model framework that more explicitly represents the irregular shapes of cells and their motility.

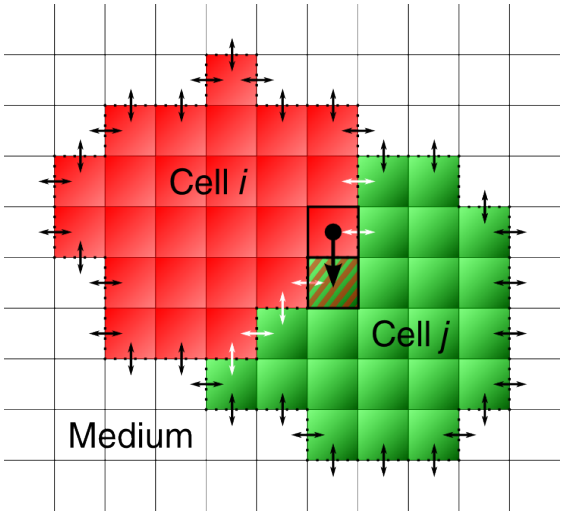

Figure 4: **Cellular Potts model**

The spreading of one embryonic tissue over another, the sorting out of their cells when intermixed and the formation of intertissue boundaries are reminiscent of the behavior of immiscible liquids like oil and water. Steinberg's differential adhesion hypothesis[2] (DAH) explains these liquid-like tissue behaviors as consequences of the generation of tissue surface and interfacial tensions arising from the adhesion energies between motile cells. Glazier and colleagues[3] have developed a lattice-based model known as the Cellular Potts model in which spatially extended cells rearrange as a result of membrane fluctuations to minimize their effective free energy (see figure 4). In this model, various morphogenetic mechanisms can be obtained through quantitative differences in the strength of surface adhesion (see figure 5).

Please open `cell_sorting.xml` and run a simulation. This model contains a few new elements:

- There are multiple cell types (displayed as red and yellow cells).
  See `CellTypes/Cellype`.

[2]Malcolm Steinberg, Differential adhesion in morphogenesis: a modern view, *Current Opinion in Genetics and Development*, 2007.

[3]François Graner and James Glazier, Simulation of Biological Cell Sorting Using a Two-Dimensional Extended Potts Model, *Physical Review Letters*, 69 (13): 2013–2016, 1992.

- Cells have a certain volume.
  See `CellTypes/Cellype/VolumeConstraint.`
- The element CPM contains the parameters for the cellular Potts model.
  See `CPM`.
- The surface adhesion strength between the various cell type and the medium are given as contact energies.
  See `CPM/Interaction/Contact`.

In the current state, the model defines two identical cell types (only their colors differ). However, by changing the contact energies, different cell configurations can be obtained including cell separation, mixing and demixing and engulfment. **Try to find the contact energy settings for each of the configurations in figure 5.**

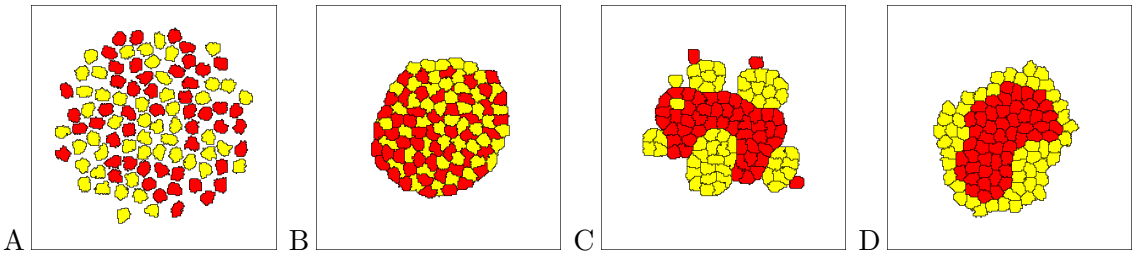

Figure 5: **Differential adhesion.** Different cell configurations resulting from differential adhesion between two cell types and medium (red, yellow, and white): (A) separation, (B) mixing, (C) demixing, and (D) engulfing.

3 Spatial expression of reporter genes in cell populations

Bistability is a characteristic feature of many molecular switches facilitating cell decision processes as they occur during differentiation and in response to external stimuli. ESCs are a typical example, in which a bistable situation is caught in an *in vitro* setting contributing to a heterogeneous, dynamically stabilized cell culture. In particular, flow cytometry measures of both Nanog and Rex1 reporter cell lines show a stable bimodal distribution with a fraction of Nanog/Rex1-high (about 70-80%) and a fraction of Nanog/Rex1-low cells (about 20-30%).

The molecular mechanisms underlying this intrinsic heterogeneity are still under debate. Yesterday, you got to know two potential explanations (noise-driven fluctuations vs. oscillations) and analyzed the fluctuations scenario on an intracellular level in detail. In the last part of this tutorial, we will integrate the simplified Nanog model into spatially extended cells and study the effect of Nanog-related cellular properties (such as proliferation and adhesion) on spatial patterns in growing colonies.

3.1 Single cell state transitions

First, we implement the intracellular Nanog model given by the following equation:

$$\frac{dN}{dt} = b + \frac{s \cdot N^2}{10 + N^2} - d \cdot N + \xi(0, \sigma) \cdot N \tag{4}$$

Please open `nanog_singlecell.xml` and look through this model. You will see that the structure is very similar to our first model of the toggle switch.

If you **run this model**, you will see a time course of the Nanog concentration in a single ESC, including transitions between low and high expression state, similar to the one shown in figure 6A. To obtain the characteristic bimodal distribution, however, we need a population of ESCs.

Try to extend the single cell model to a population model:

- Change the lattice class and its size (`Space/Lattice`)
- Add a `HistogramLogger` to plot the resulting Nanog distributions (in `Analysis`):
  - Click the right mouse button and then `Add` a HistogramLogger to store the data.
  - If you are only interested in the final distribution set `endstate = true` in the right Attributes panel.

- In `Binning` set `normalization = true`, `number_bins = 50`, `logarithmic_bins = true` (similar to flow cytometry measurements), `maximum = 100`and `minimum = 0.01`.
- In `Protery` set `celltype` to ESC and chose `symbol-ref = N`.
- To plot the histogram, you need to add a `Plot` by clicking with the right mouse button on the `HistogramLogger`. Set `terminal = png` in the Attributes panel.

Run the population model and look at the Nanog distribution at the end of the simulation.

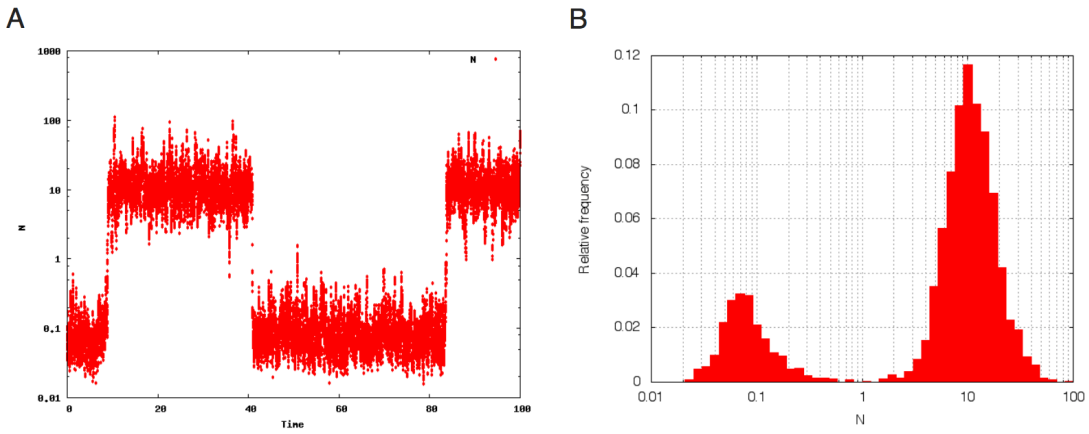

Figure 6: Transitions in Nanog expression on a **single cell level** (A) and the resulting bimodal distribution on a **population level** (B).

3.2 Bimodal distribution

Please open `nanog_population.xml`. This model simulates 2500 individual ESCs and plots the distribution of Nanog on a logarithmic scale (as shown in figure 6B). It also outputs a plot showing the numbers of high and low Nanog expressing cells through time.

We will use this model to do a little *in silico* experiment. Let's suppose we do not know whether the bimodal distribution is due to (a) the existence of two distinct ESCs populations or (b) due to the fact that cells undergo state transitions. This can be tested by removing one of the subpopulations and observing whether the bimodal distribution is restored.

To remove a particular subpopulation based on its Nanog expression from the simulation, we could use the `Apoptosis` plugin to forcefully remove cells. Alternatively, we can use

an `Event` to reset the expression of a cell in the subpopulation instead of removing them. In the open `nanog_population.xml` model, the `CellType/Event` is set up to do this.

Now, by enabling the disabled `Event` (right mouse button) you can **observe whether or not the bimodal distribution is restored.**

3.3 Multiscale model of ESC colonies

Populations of ESCs are not a collection of individual cells. They typically form colonies and thus establish a microenvironment that is vital for the maintenance of pluripotency. In these colonies, the expression of pluripotency factors does not appear to be random, but shows a spatial pattern with, on average, higher expression levels in the center of the colonies.[4] This suggests a form of spatial coupling among ESCs to coordinate this patterning. Although little is known about the intercellular communication that controls this patterning, mathematical and computational models can be used to test various hypotheses.

We have constructed a simulation model to investigate the spatial aspects of colonies of ESCs *in vitro*.

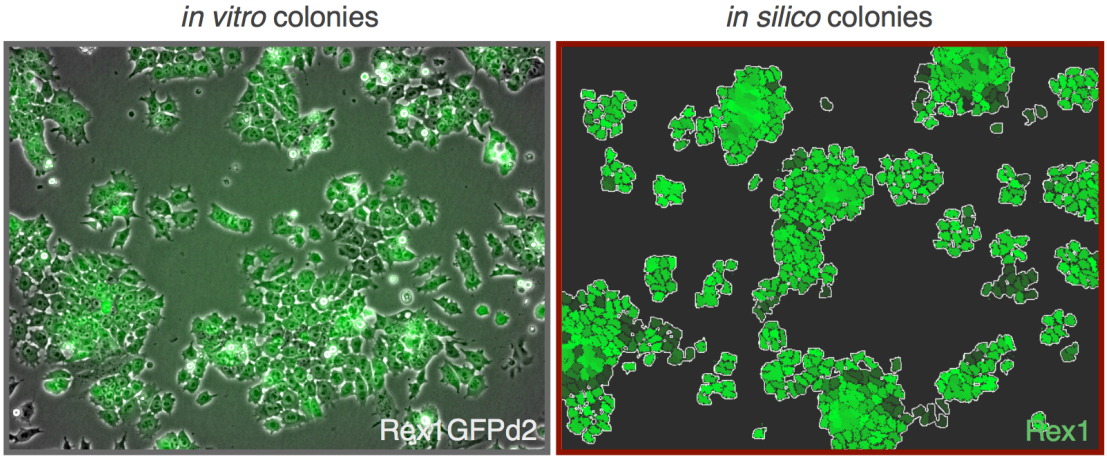

Figure 7: **ESCs colonies** with color indicating Rex1 expression.

Please open the file `nanog_colonies.xml`.

The example model used here combines the bistable Nanog model of equation 4 with the cellular Potts model (CPM). Since it represents and couples the intracellular scale with the cellular scale, this is a multiscale model. As before, the Nanog model describes

[4] Maria Herberg, Thomas Zerjatke, Walter de Back, Ingmar Glauche, Ingo Roeder, Image-based quantification and mathematical modeling of spatial heterogeneity in ESC colonies, *Cytometry: Part A*, 2015 (in press).

the dynamics of expression including state transitions between high and low levels. The CPM is used to model cells as shaped objects that are motile, adhere, divide and die.

Simulations of this model result in a bimodal distribution as in figure 6B (albeit with less cells) and a colony structure as shown in figure 8.

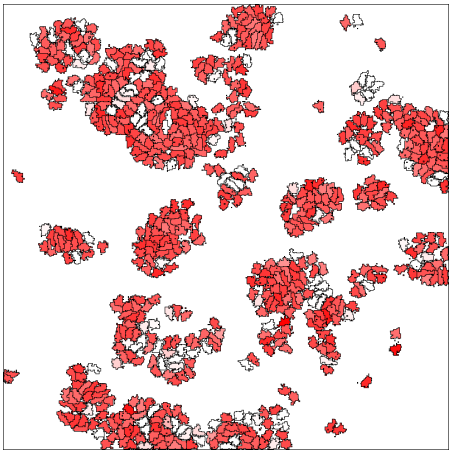

Figure 8: **Simulated ESCs colonies** with color indicating Nanog expression.

In the given model we did not assume any Nanog-related cellular properties (such as proliferation or adhesion) and Nanog expression is completely cell-autonomous.

It is now your final task to hypothesize and formulate mechanisms of cell-cell communication and Nanog-related cell properties that might influence spatial pattern formation.

To help you get started, here are some ideas:

1. The proliferation rate could be different between high and low expressing cells.

   You can implement this by changing the `Condition` for proliferation (`CellType/Proliferation`), into e.g. `if(`$N > 1$`,rand_uni(0,1)<` 0.001`,rand_uni(0,1)<` 0.0005`)`.

2. Cells with a high Nanog expression might adhere stronger to each other than cells with low Nanog levels.

   You can activate this relationship by enabling the disabled function `HomophilicAdhesion` in `CPM/Interaction/Contact`.

3. Nanog expression could depend on the expression state of neighboring cells.

   You could implement this by replacing N by Nn in the differential equation (`CellType/System/DiffEqn`). Here, Nn is the average expression in the neighboring cells as given by the `NeighborsReporter` (see Attributes panel).

4. Nanog expression could depend on both its own expression and that of the neighboring cells.

   You could model this by taking the average $(N + Nn)/2$ or the geometric mean $sqrt(N \cdot Nn)$. (What different molecular processes do these assume?)

5. Also think about the `NeighborsReporter`: Should we include or exclude the medium in averaging? Do you want to take the average over the cell-cell interfaces or over the neighboring cells?

6. ...

That's it! We hope that you can now see how mathematical and computational modeling can help in the formulation and testing of hypotheses.

If I cannot create it, I do not understand it
R. Feynman

All models are wrong, but some are useful
G. Box