Multiscale extensions of the cellular Potts models: toward a nested-hybrid approach

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Communicated by Roberto Natalini

Abstract

Multiscale problems are ubiquitous in all biological phenomena, which emerge from the complex interaction between processes happening at various levels and in particular from the relationship between the subcellular genomic and proteomic dynamics and the behavior of the cell. In order to deal with such an intricate network of organization, this work discusses some innovative extensions of the cellular Potts model (CPM), a discrete, lattice-based, technique able to reproduce and analyze innumerable biological phenomena. The aim is to increase the accuracy of the method and to create a multilevel framework able to deal with the multiscale complexity typical of biological development. The proposed CPM extensions are finally tested with sample applications, that show their potential and biological realism.

Keywords: Cellular Potts model, multiscale model, hybrid model.

AMS Subject Classification: 92B05, 92C05, 92C17

1. Introduction.

In order to deal with the multilevel complexity typical of biological systems, the *experimental sciences* need an increasing collaboration with the *applied mathematics*. A computational approach is in fact able to simplify the biological problem, offering both a concise description of its essential features and the possibility of highlighting the most relevant mechanisms and parameters. It can also be used, in a predictive manner, to determine the consequences of experimental manipulations, providing a useful guide for possible future experiments.

Among the different mathematical approaches used to describe the evolution of a biological system, the class of *discrete* models closely focuses on the cell-level (i.e., the mesoscopic scale) of abstraction (for comprehensive reviews see [1,2]). Indeed, they represent biological cell-scale individuals as...
one or a set of discrete units, with rules describing their movements and interactions. In particular, the morphology of the elements is restricted according to some underlying discretization of the simulation domain, which can be either regular (such as square or cubic grids) or irregular (Voronoi tessellation). These approaches can be further classified into two categories: those for which each individual is correlated to a single spatial unit of the domain, and those for which each element can be formed by a collection of spatial units. Discrete models can therefore naturally capture the biophysical properties of each individual, such as shape, movement or adhesion, and handle their interactions. In these methods, the cell-scale elements obey to a set of prescribed rules, that may depend on their type and on the signals they receive from the neighbors and from the environment. In particular, these techniques are able to analyze the mechanisms by which relatively simple behaviors and interactions of individuals collectively direct macroscopic pattern formation and development, and, vice versa, to infer how phenomena occurring at the macroscopic level feed back to the phenomenology of single elements.

In the last decades, purely discrete methods have been increasingly integrated with continuous approaches suited to describe the evolution of microscopic variables (i.e., ion or molecules). The aim is to create hybrid environments able to span both the mesoscopic and the microscopic scale with a sufficient level of accuracy, see as an example [3,4]. One of these computational environments is the Cellular Potts Model (CPM, also called Glazier-Graner-Hogeweg model developed in [5,6] and reviewed in [7,8]). As a generalization of the Ising model, the CPM is a grid-based, Monte Carlo method, whose core is an iterative energy minimization, which drives the evolution of the simulated system.

In this paper, we present some important developments of the method, detailed in Sections 2 and 3. In particular, we first give a plausible procedure to interface the basic CPM with accurate models of microscopic biochemical pathways. Then, we introduce alternative laws for the Boltzmann transition function, the core of the Metropolis algorithm, and we propose a more accurate representation of biological individuals, which can be realistically compartmentalized into reasonable subunits. A couple of sample applications are finally given to illustrate how the proposed model extensions can be applied to specific biological problems with a significant accuracy and experimental usefulness. In the last part of the work, cf. Section 4, the conclusions are drawn.
2. Uncompartmentalized CPM.

All CPMs include a list of objects, a description of their interactions and the identification of a generalized energy whose minimization drives the evolution of the system. The CPM domains are $d$-dimensional lattices $\Omega \subseteq \mathbb{R}^d$, where $d = 2, 3$ (for the sake of completeness, it is useful to underline that the original Ising approach can be employed also in one dimension: in this case, lattice reduces to a string of points on a line, as illustrated in [9]). The term lattice defines a regular repeated graph, formed by identical $d$-dimensional closed grid sites $x \in \mathbb{R}^d$, which therefore represent the basic unit of length of the system. Each site $x \in \Omega$ is labeled by an integer number, $\sigma(x) \in \mathbb{N}$, which can be interpreted as a degenerate spin coming from the statistical physics [10,11]. The border of $x$ is denoted by $\partial x$, one of its neighbors by $x'$, while its overall neighborhood by $\Omega'_x = \{x' \in \Omega : x'$ is a neighbor of $x\}$. Objects in the CPM are finite, spatially-extended lattice subdomains of contiguous sites denoted with the same spin $\sigma$, i.e. $\Sigma_{\sigma}$ is defined by $N$ integer spins $\sigma = 1, 2, \ldots, N$, see Figure 1. The borders between sites with different spins, that are thus shared between a couple of objects, define their membranes, i.e. $\partial \Sigma_{\sigma} = \bigcup_{x \in \Sigma_{\sigma}, x' \in \Omega'_x, x' \notin \Sigma_{\sigma}} (\partial x \cap \partial x')$.

Each unit $\Sigma_{\sigma}$ has a set of attributes (both geometrical, such as volume and surface, and biophysical, such as velocity and elasticity), and an associated type $\tau(\Sigma_{\sigma})$ (e.g. endothelial cell, fibroblast, or ECM fiber). Mesoscopic, cell-level objects rearrange their boundaries to realistically reproduce shape changes and motion. Moreover, they can grow, die, duplicate and carry a set of possible rules for transitions between types.
The discrete CPM can be coupled with continuous models that describe the spatio-temporal evolution of microscopic entities, that may reside within the discrete objects (e.g., DNA, RNA, cytosolic ions, or proteins), or in the external environment (e.g., growth factors, matrix proteins or matrix metalloproteinases). These fields are represented as variable concentrations with standard reaction-diffusion (RD) equations, which are numerically solved on grids that exactly match the CPM domain. Specific interactions between discrete cell-level objects and continuous molecular-level elements can be characterized either by reaction terms in diffusion equations, as in the case of cell absorption and secretion of chemical diffusants, or, as we will explain hereafter, by constitutive laws relating the mesoscopic properties of discrete individuals to the evolution of specific microscopic variables. Indeed, the coexistence between the discrete CPM and selected continuous models provides to the computational environment a hybrid characteristic.

The CPM core principle consists of an iterative stochastic minimization of a system free energy, which is described by the Hamiltonian functional $H$, that will be defined in detail below. Simulated objects in fact rearrange to gradually reduce such a pattern energy. The energy minimization is implemented by a Metropolis algorithm for Monte Carlo methods, which evolves through repeated attempts of site identification spin updates, that are accepted with Boltzmann-like probabilities [6,8,12]. Procedurally, at each time step, $t$, a lattice site $x$, belonging to object $\Sigma_\sigma$, is randomly selected (source voxel), and proposed to copy its spin $\sigma(x)$ into an unlike neighbor $x' \not\in \Sigma_\sigma$ (target voxel), also randomly selected. It is useful to underline that, in the applications proposed in this article, we will set to select the target site from the first-nearest neighborhood of the source site (i.e., $x' \in \Omega'_{x}$). This choice is made since, in our models, the simulated objects are cells, that typically move with continuous membrane rufflings or gradual extensions/retractions of pseudopods. However, in order to take into account longer-range interactions, it would be indeed possible to opt for more extended $n^{th}$-nearest neighborhoods (where $n > 1$). To accept such a change in the lattice configuration (also called spin flip), we propose the use of this specific family of Boltzmann transitional functions:

$$P(\sigma(x) \rightarrow \sigma(x'))(t) = \begin{cases} p(T_{\Sigma_\sigma(x)}(t))e^{-\Delta H|\sigma(x)\rightarrow\sigma(x')|/T_{\Sigma_\sigma(x)}(t)} & \Delta H|\sigma(x)\rightarrow\sigma(x')| > 0 ; \\ p(T_{\Sigma_\sigma(x)}(t)) & \Delta H|\sigma(x)\rightarrow\sigma(x')| \leq 0 , \end{cases}$$

where $\Delta H|\sigma(x)\rightarrow\sigma(x')|$ is the net variation in the total energy of the system as a consequence of the spin update and $T_{\Sigma_\sigma(x)} \in \mathbb{R}^+$ is a Boltzmann temperature which characterizes each single individual, assuming therefore the role
of its intrinsic motility. Indeed, the nomenclature of $T$ originates from the fact that membrane agitation rates in biological individuals play an analogous role to real temperatures in ordinary thermodynamics, as explained in [13]. $p(T_{\Sigma_{\sigma}}(t)) : \mathbb{R}^+ \mapsto [0, 1]$ is instead a sort of maximum transition probability, a continuous and increasing function of $T_{\Sigma_{\sigma}}$ characterized by

\[
\begin{align*}
\left\{ \begin{array}{l}
p(0) = 0; \\
\lim_{T_{\Sigma_{\sigma}} \to +\infty} p(T_{\Sigma_{\sigma}}) = 1.
\end{array} \right.
\]
\]

The probability (1) differs from the classical Boltzmann functions used in literature [5–7,11] for two reasons. First, the temperature $T$ is no longer a constant neither for the entire system (as, for example in [14–17]), nor for all individuals belonging to a given type (see [18]). Then, the proposed model takes into account of the specific object motility also in the case of energetically favorable displacement attempts: this allows to consider and realistically reproduce the cases of "frozen" elements, which do not move even if they sense external stimuli to do that (i.e., resulting in $\Delta H \ll 0$), as commented in [19].

After the discrete object $\Sigma_{\sigma}$ has evolved through a spin flip, both equations that describe the variation of continuous fields, and the attributes of all the objects are rederived, on the basis of new lattice configuration. The basic step of the Metropolis algorithm is than iterated until the whole system reaches an energetic global minimum or, if this is not obtained, until a given observation time, defined a priori. In particular, the global minimum configuration could not exist because of the presence of specific terms in the Hamiltonian, such as chemotactic energies, or of periodic boundary conditions in the domain: in these cases, if the observation time is long enough, the system may stabilize around configurations characterized by local energetic minima. The unit of time of the CPM is the Monte Carlo Step (MCS). A MCS corresponds to a fixed number of trial lattice updates, which is usually set equal to total number of sites of the domain [8]. However, in the proposed applications, we will define a MCS as a single spin flip attempt. A direct correspondence between a MCS and the actual time scale is not straightforward, giving rise to one of the main criticisms of the method. A realistic correspondence is usually set by fitting a posteriori the temporal dynamics of the simulated phenomenon with the relative experimental counterparts.

The effective energy of the system, given by the Hamiltonian $H$, contains a variable number of terms, which can be grouped as:

\[
H(t) = H_{\text{adhesion}}(t) + H_{\text{constraint}}(t) + H_{\text{force}}(t).
\]
$H_{\text{adhesion}}$ describes the adhesive/repulsive interfacial energy between all the couples of discrete objects that interact across their common membrane. $H_{\text{adhesion}}$ is based on Steinberg’s Differential Adhesion Hypothesis (DAH) [6,20,21]. The DAH proposes that individuals in the same aggregate adhere to each other with different strengths, according to their type, and that such a hierarchy of contact forces determines the specific configuration of the aggregate, which evolves in order to maximize the interface lengths corresponding to the strongest adhesions (indeed, minimizing its overall free energy). Specific hierarchies of contact strengths therefore lead to specific configurations of the cellular ensemble. The DAH says nothing about the dynamics of moving individuals: differential adhesion itself, in fact, only helps to select the most favorable configuration among the different possibilities that have been explored. Evidence supporting DAH has been observed in a wide array of biological systems, especially at the embryonic stage of life. From the late 50s, it is in fact widely noticed that during embryonic development the behavior of cell aggregates resembles that of viscous fluids, as a random mixture of two types of embryonic cells is able to spontaneously organize and to establish coherent homogeneous tissues. Similar processes are key steps also in the regeneration of normal animal from aggregates of dissociated cells, as in the case of adult hydra.

All these phenomena, commonly called cell sorting, involve neither cell division nor differentiation, but are entirely caused by spatial rearrangements of cell positions due to differences in the specific adhesivities, see [5,6,22] and references therein. The typical formulation of DAH-derived $H_{\text{adhesion}}$ is:

$$H_{\text{adhesion}}(t) = \sum_{x,x' \in \Omega'_x,(\partial x \in \partial \Sigma_\sigma) \cap (\partial x' \in \partial \Sigma_{\sigma'}) \neq \emptyset} J_{\tau(\Sigma_{\sigma}(x)),\tau(\Sigma_{\sigma'}(x'))}(t), \quad (4)$$

where, as seen, $x$ and $x'$ are two neighboring sites and $\Sigma_\sigma$ and $\Sigma_{\sigma'}$ the relative two neighboring objects. The coefficients $J_{\tau(\Sigma_{\sigma}),\tau(\Sigma_{\sigma'})} \in \mathbb{R}$ are the binding forces per unit area, the first type of the so-called Potts parameters, and are obviously symmetric w.r.t. the indices. In the case of cells, such contact strengths give a qualitative measure of the expression of the different types of adhesion molecules (e.g., integrins or cadherins) characterizing a specific cell line.

The term $H_{\text{constraint}}$, whose use also comes from the physics of classical mechanics, sums the energetic components that describe the object attributes. They are written as energetic penalties that increase as the objects deviate from a designed state:

$$H_{\text{constraint}}(t) = \sum_{\Sigma_\sigma} \sum_{i-\text{constraint}} \lambda^i_{\Sigma_\sigma}(t) U(a^i_{\Sigma_\sigma}(t), A^i_{\Sigma_\sigma}(t)), \quad (5)$$
where \( a^i_{\Sigma_\sigma}(t) \) is the actual and \( A^i_{\Sigma_\sigma}(t) \) the target value of the \( i \)-attribute of individual \( \Sigma_\sigma \), that can vary in time, and \( U(a^i_{\Sigma_\sigma}, A^i_{\Sigma_\sigma}) \geq 0 \) is a potential with the property that

\[
U(A^i_{\eta,\Sigma_\sigma}, A^i_{\eta,\Sigma_\sigma}) = 0.
\]

The Potts parameters \( \lambda^i_{\Sigma_\sigma} \in \mathbb{R}^+ \) take the role of elastic moduli, which determine the weight of the relative energetic constraint, and thus the importance of the relative attribute. Low values of \( \lambda^i_{\Sigma_\sigma} \), in fact, allow the discrete unit \( \Sigma_\sigma \) to deviate more from the configuration that satisfies the constraint. Indeed, the Metropolis algorithm automatically drives any configuration of the system towards one that let the energetic contribution given in Equation (5) decreases. In principle, an energetic minimum is obtained when \( H_{\text{constraint}} = 0 \), i.e., when each and every constraint \( i \) is satisfied with \( a^i_{\Sigma_\sigma} = A^i_{\Sigma_\sigma} \) for each element \( \Sigma_\sigma \). However, multiple constraints may be in conflict: in this cases, the system is not able to exactly satisfy all of them and therefore it will fluctuate around configurations characterized by some residual stresses. Among others, the energetic components relative to geometrical attributes of discrete objects, such as their volume and surface, are of particular relevance. In most published CPMs, the author use a simple quadratic potential

\[
U(a^i_{\Sigma_\sigma}(t), A^i_{\Sigma_\sigma}(t)) = \left(a^i_{\Sigma_\sigma}(t) - A^i_{\Sigma_\sigma}(t) \right)^2.
\]

However, the form of (7) has the disadvantage that a finite energy is sufficient by a discrete unit to achieve a vanishing value of one of its constraints, a situation that should be avoided and that would in principle require an infinite energy. For this reason we propose the use of potentials that blow up in the case of \( a^i_{\eta,\Sigma_\sigma} \to 0 \) as

\[
U(a^i_{\Sigma_\sigma}(t), A^i_{\Sigma_\sigma}(t)) = \left| \frac{a^i_{\eta,\Sigma_\sigma}(t) - A^i_{\eta,\Sigma_\sigma}(t)}{a^i_{\eta,\Sigma_\sigma}(t)} \right|^p,
\]

with \( p \in \mathbb{R}^+ \). In this way, in addition to the just stated advantages, all the components of \( H_{\text{constraint}} \) are non-dimensional, and thus all the relative Potts coefficients are coherently scaled to units of energy.

The last term in Equation (3) includes the energetic counterparts of the forces (both effective and generalized) that act on the simulated individuals and are described in the following form:

\[
H_{\text{force}}(t) = - \sum_{x \in \Sigma_\sigma} \sum_{k - \text{force}} \mu^k_{\Sigma_\sigma(x)}(t) \mathbf{F}^k(t) \cdot \mathbf{r}_x,
\]
where \( \mathbf{r}_x = (i_x, j_x, k_x)^T \) is the position vector of lattice site \( x \), which is the application point of force \( F^k \), and \( \mu^k_{\Sigma} \) is the relative Potts parameter, which measures the effective strength of the force on object \( \Sigma_\sigma \). The most diffused examples in CPM applications, introduced in [23], are the forces that are exerted by extracellular chemical substances (which are described as continuous elements) on a population of cells (which are typical discrete objects):

\[
H_{\text{force}}(t) = -\sum_{\Sigma_\sigma} \sum_{x \in \Sigma_\sigma} \mu^\text{chem}_{\Sigma_\sigma}(x)(t) c(x,t),
\]

where \( c(x,t) \) is the concentration of the chemical sensed by cell site \( x \) (which can be modeled as the concentration of the substance in site \( x \) itself [14, 15, 24, 25] or in its neighborhood [26, 27]) and the Potts coefficient \( \mu^\text{chem}_{\Sigma_\sigma} \) is, in this case, an effective chemical potential of cell \( \Sigma_\sigma \). The net energy difference caused by such a chemical force is:

\[
\Delta H_{\text{force}}^{\text{chemical}}|_{\sigma(x)\rightarrow\sigma(x')} = \mu^\text{chem}_{\Sigma_\sigma}(x)(c(x,t) - c(x',t)),
\]

where \( x \in \Sigma_\sigma \) and \( x' \notin \Sigma_\sigma \) are the two neighboring lattice sites randomly selected during the trial update at time \( t \). In particular, \( \mu^\text{chem}_{\Sigma_\sigma} > 0 \) yields to a motion up the gradient of \( c \) (which is thus a chemoattractant), while \( \mu^\text{chem}_{\Sigma_\sigma} < 0 \) yields to a motion in the opposite direction (and \( c \) is a chemorepellent). Moreover, if \( c \) is a non-diffusive fixed substrate, Equation (10) is a representation of a haptotactic force, as in [27–29].

The importance of each term in the Hamiltonian (i.e., of each included biological mechanism) is defined by the magnitude of the relative Potts parameter, which acts as a sort of penalty coefficient. It is indeed possible to easily comprehend the importance of each mechanism involved in the simulated phenomenon by only altering the relative Potts parameter, so that the other terms in the Hamiltonian scale accordingly. In particular, by equating all the other terms to zero, it is possible to understand whether such a mechanism is individually capable of producing the process of interest, or whether it requires cooperative processes. In this respect, a crucial role in determining the evolution of the system is therefore played by the hierarchy of the Potts coefficients, and not by their exact values.

2.1. Nested approach.

The key benefits of the CPM energetic formalism are its simplicity and extensibility. Almost any biological mechanism can in fact be included in the model, simply by adding an appropriate generalized potential term in the Hamiltonian \( H \), as suggested in the main reviews of the method [7,8].
However, the standard CPM approach is not free of some serious drawbacks, which limit a good quantitative comparison between \textit{in silico} and \textit{in vitro} results and thus the predictive value of the method. First, most Potts parameters (as well as the target states of most attributes) are common for all objects of the same type, despite their individuality. Furthermore, they are generally static over the whole simulations or have heuristic variations, as commented in \cite{30}. Finally, by treating biological elements with only a cell-level phenomenological approach, basic CPM applications do not consider (or, in some cases, only approximately describe) the molecular scale of biological organisms, as reviewed in \cite{8}. All these considerations lead to one of the main criticisms of the CPM: most simulated phenomena emerge from quite strong \textit{a priori} assumptions. The behavior of the simulated individuals is in fact typically imposed by qualitative rules that do not easily adapt during the evolution of the system: this is not completely accurate since real biological elements continuously change their biophysical and biomechanical properties (and therefore their behavior) as a consequence of continuous internal and external stimuli, see again \cite{30}.

In order to overcome these issues, we here propose the use of a \textit{nested} approach, based on the assumption that the internal state (i.e., the microscopic level) of a biological individual regulates its biophysical properties (described by mesoscopic Potts coefficients) which, as seen, in turn direct its evolution (described by the relative term in the \textit{Hamiltonian}).

Procedurally, let $\Sigma_{\sigma}$ denote a certain discrete object: we define its internal state vector $s_{\Sigma_{\sigma}} \in \mathbb{R}^n$. The length $n$ of $s_{\Sigma_{\sigma}}$, defined by the number of internal factors considered in the microscopic model, represents a sort of internal degree of freedom of $\Sigma_{\sigma}$. Each component $s_{\Sigma_{\sigma},l}$, where $l = 1, \ldots, n$, is typically represented as a continuous object and can be local (i.e., per site) and/or time-dependent (i.e., linked to a specific regulatory pathway, which needs to be modeled, as it will be explained hereafter). Hence, in general, $s_{\Sigma_{\sigma}} = s_{\Sigma_{\sigma}}(x, t)$, where $x \in \Sigma_{\sigma}$. The spatial localization of $s_{\Sigma_{\sigma}}$ is mandatory to accurately represent internal inhomogeneities of $\Sigma_{\sigma}$, while its time-dependence to reproduce its microscopic evolution. For any $\Sigma_{\sigma}$, let us consider a generic Potts coefficient $\alpha \in \{ \lambda_{\Sigma_{\sigma}}^{l}; T_{\Sigma_{\sigma}}; \mu_{\Sigma_{\sigma}}^{k}, \ldots \}$. We now define $s_{\alpha}^{\Sigma_{\sigma}} \in \mathbb{R}^m$, where $m \leq n$, the subvector of $s_{\Sigma_{\sigma}}$ whose components influence the biophysical property of $\Sigma_{\sigma}$ described by $\alpha$. Therefore the spatio-temporal evolution of $\alpha$ can be expressed as

\begin{equation}
\alpha(x \in \Sigma_{\sigma}, t) = f(s_{\alpha}^{\Sigma_{\sigma}}(x, t)),
\end{equation}

where $f : \mathbb{R}^m \rightarrow \mathbb{R}$ is a continuous function, which obviously needs to be appropriately defined in relation to the case of interest. According to the same notation, if $\gamma \in \{ J_{\gamma}(\Sigma_{\sigma}), \tau(\Sigma_{\sigma'}) \}$, for each local interface between

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neighboring objects (i.e., $\partial x \in \partial \Sigma_\sigma \cap \partial x' \in \partial \Sigma_{\sigma'}$ or $\partial x \in \partial \eta \cap \partial x' \in \partial \eta'$ in the case of compartmentalized individuals), we have:

$$\gamma((\partial x \cap \partial x'), t) = \gamma(s_{\Sigma_\sigma}(\mathbf{x}, t), s_{\Sigma_{\sigma'}}(\mathbf{x}', t)) = g(s_{\Sigma_\sigma}(\mathbf{x}, t), s_{\Sigma_{\sigma'}}(\mathbf{x}', t)),$$

where $g : \mathbb{R}^m \times \mathbb{R}^m \mapsto \mathbb{R}$. The local adhesive strengths are indeed determined by the local internal state of both elements, as they are not only a property of each single individual.

Indeed, the Potts parameters which can locally vary (such as the adhesive interactions or the effective strengths of specific forces) require that the relative functions of the internal state vector $\mathbf{s}$ are local (i.e., they need to take into account of the local concentration of the internal factors of interest), whereas the Potts parameters characterizing an entire individual (such as the motility or the elasticity) require that the relative functions of $\mathbf{s}$ are global (i.e., they need to take into account of the overall level of the internal factor of interest). Summing up, Equations (12) and (13) state that the variation of the Potts coefficients of an element (either an entire individual or one of its compartments) is due to the evolution of its internal state: in this way its mesoscopic biophysical properties are no longer given a priori (or varied with prescribed rules) but are autonomously and continuously inherited from the flow of information coming from its microscopic level.

The application of the new approach to biological cells is of particular interest. In this context, each component of the internal state vector represents the spatio-temporal variation of the concentration of intracellular ions and molecules, which can be represented as continuous objects and whose quantity regulates the cell phenomenology. In particular, for any component $s_{\Sigma_\sigma, l}$ of $\mathbf{s}_{\Sigma_\sigma}$, given a well-characterized (although simplified) biochemical pathway, it is always possible to set a suitable model (such as a reaction-diffusion (RD) system, which specializes in several coupled differential equations, whose outcome is $s_{\Sigma_\sigma, l}$ itself.

2.2. Motility of individuals.

The description of the motility of individuals is one of the most attractive features of the CPM. The Metropolis algorithm is in fact able to represent the exploratory behavior of biological organisms through biased extensions and retractions of their boundaries. It also allows to differentiate the isotropic intrinsic motility of each element, which is described by its Boltzmann temperature $T$ (which can be approximately compared to a diffusion coefficient with a continuous point of view), and the directional, force-based component of its motion. Indeed, since a difference in a potential energy is the work done by a force, for each object $\Sigma_\sigma$ we can write
\[
\frac{\Delta H}{\Delta t} = - \prod = - \sum_{x \in \Sigma} F_{x \in \Sigma} \cdot \frac{\Delta x}{\Delta t} = - \sum_{x \in \Sigma} F_{x \in \Sigma} \cdot v_{x \in \Sigma},
\]

where \( \prod \) is the power of force \( F_{x \in \Sigma} \) and \( v_{x \in \Sigma} \) the local velocity. In extremely viscous regimes, such as biological environments, the local force is proportional to the local velocity, and not to the acceleration. This leads to the so-called *overdamped force-velocity response* which is characteristic of some Individual Cell-Based Models (IBMs) \cite{4} and writes

\[
F_{x \in \Sigma} = \alpha_{x \in \Sigma} v_{x \in \Sigma} = \frac{1}{k_{x \in \Sigma}} v_{x \in \Sigma} \propto \frac{1}{p(T_{\Sigma}) T_{\Sigma}} v_{x \in \Sigma},
\]

As analytically demonstrated in \cite{8,19}, the coefficient \( k_{x \in \Sigma} \) is the *net rate of transition* of site \( x \) (i.e., the difference between its probability of moving and staying still, \( P(\sigma(x) \rightarrow \sigma(x')) - P(\sigma(x) \rightarrow \sigma(x')) \)) and is proportional to the Boltzmann temperature of the element \( T_{\Sigma} \) scaled by the value of \( p(T_{\Sigma}) \). However, w.r.t. those published results, we here prefer to use a proportional dependence and not an equation, since the exact relation between the Monte Carlo spin copy attempts and the continuous time, as well as the kinetics application of the Metropolis-like algorithm, are still debated and a persistent sources of criticism. Putting (15) in (14), we obtain

\[
\frac{\Delta H}{\Delta t} \propto - \sum_{x \in \Sigma} \frac{1}{p(T_{\Sigma}) T_{\Sigma}} v_{x \in \Sigma}^2 = - \frac{1}{p(T_{\Sigma}) T_{\Sigma}} \sum_{x \in \Sigma} v_{x \in \Sigma}^2,
\]

given that \( T_{\Sigma} \) is a global property of the entire object. Let us now decompose the velocity \( v_{x \in \Sigma} \) as

\[
v_{x \in \Sigma} = v_{x \in \Sigma}^{CM} + w_{x \in \Sigma},
\]

where \( v_{x \in \Sigma}^{CM} \) is the velocity of the object center of mass and \( w_{x \in \Sigma} \) a local fluctuation. Simple calculations lead to

\[
\sum_{x \in \Sigma} v_{x \in \Sigma} = \sum_{x \in \Sigma} v_{x \in \Sigma}^{CM} + \sum_{x \in \Sigma} w_{x \in \Sigma} = v_{x \in \Sigma}^{CM} a_{\Sigma}^{volume} + \sum_{x \in \Sigma} w_{x \in \Sigma},
\]

where the second term of the sum vanishes. Therefore, substituting in (14), we obtain

\[
\frac{\Delta H}{\Delta t} \propto - \frac{1}{p(T_{\Sigma}) T_{\Sigma}} \left[ (v_{x \in \Sigma}^{CM})^2 a_{\Sigma}^{volume} + \sum_{x \in \Sigma} w_{x \in \Sigma}^2 \right].
\]
Given that $\frac{\Delta H}{\Delta t} = \frac{\Delta H}{\Delta x} v^{CM}_{\Sigma\sigma} \Sigma\sigma$, we can finally conclude that

$$\frac{\Delta H}{\Delta x^{CM}_{\Sigma\sigma}} \propto -\frac{1}{p(T_{\Sigma\sigma})T_{\Sigma\sigma}} \left[ v^{CM}_{\Sigma\sigma} a^{volume}_{\Sigma\sigma} + \frac{1}{v^{CM}_{\Sigma\sigma}} \sum_{x \in \Sigma\sigma} w^{2}_{x \in \Sigma\sigma} \right]$$,

or, with another view point,

$$a^{volume}_{\Sigma\sigma} v^{CM}_{\Sigma\sigma} \propto -p(T_{\Sigma\sigma})T_{\Sigma\sigma} \frac{\Delta H}{\Delta x^{CM}_{\Sigma\sigma}} - \frac{1}{v^{CM}_{\Sigma\sigma}} \sum_{x \in \Sigma\sigma} w^{2}_{x \in \Sigma\sigma}.$$ 

Some comments on the consequences of relations (20) and (21):

- they are a definitive confirmation that discrete objects move in order to minimize the total energy;
- the modulus of the velocity of the center of mass of $\Sigma\sigma$ depends on the magnitude of the energy difference due to the proposed spin flip, as well as on its intrinsic motility $T_{\Sigma\sigma}$, which, in our extended approach, is coherently a variable property of each unit $\Sigma\sigma$ and is determined by its microscopic state: different individuals therefore have different velocities even if they experience the same energy difference;
- it is straightforward to evaluate the contribution to the velocity of unit $\Sigma\sigma$ of each term of the Hamiltonian. In fact, for any mechanism $i$, by equating all the other terms to zero, we obtain:

$$a^{volume}_{\Sigma\sigma} v^{CM}_{\Sigma\sigma} |_{i=mechanism} \propto -p(T_{\Sigma\sigma})T_{\Sigma\sigma} \frac{\Delta H^{i=mechanism}}{\Delta x^{CM}_{\Sigma\sigma}}.$$ 

2.3. Test simulation: in vitro tubulogenesis.

We now present a model reproducing a classical tubulogenic assay. A similar model has been already developed in [26] to analyzed biomedical therapies able to disrupt tumor vascular transition. However, we here simplify and adapt it in order to show how the CPM extensions can be applied, and to clarify the complex notation used in the previous sections. In particular, differently from that work, we here will treat the cells as uncompart-mentalized elements and we will neglect the inertial term in the energy of the system. Moreover, we will explicitly define the cell internal state vector and we will closely refer to the above-introduced notation.

As reviewed in [31,32], a tubulogenic experiment classically consists in the incubation on a gel substrate (analogous of a natural basement mem-brane matrix) of a dispersed population of endothelial cells (ECs), which
are able to autonomously organize in a bidimensional tubular network, even in the absence of other cell types or positional cues. More in details, the ECs initially undergo an isotropic motion around their initial position, maintaining a round shape. Then, they start to polarize and to undergo a persistent motion, until they collide with their closest neighbours (3-6 hours) [33]. After collision, ECs attach to their neighbours eventually forming a continuous multicellular network, which can be represented as a collection of nodes connected by capillary chords and resembles a primitive in vivo capillary-like plexus, see Figure 2(A). The network slowly moves as a whole, undergoing a slow thinning process, which however leaves the structure mainly unaltered (7-10 hours). During this stabilization phase the cells downregulate their migratory capacity. Later, they will start fold up to origin the lumen of the capillary (i.e., its internal zone where the blood flows), so that the resulting vascular network forms along the lines of the previous structure, as described in [31,34]. The overall process, which is complete within almost 12 hours (a characteristic time sufficiently distant from critical events, such as cell mitosis or death) is largely mediated by the activity of an autocrine chemical morphogen (such as the VEGF isoforms), which not only acts as a chemoattractant, as widely demonstrated in the experimental literature [31,35,36], but also initiates a series of downstream pathways involving intracellular messengers nitric oxide (NO) and arachidonic acid (AA) and culminating in calcium signals, as characterized in [37–40], see Figure 2(B). Such a variation in the intracellular calcium level regulates selected biophysical properties of the ECs, such as their motility, adhesive capability, chemotactic strength and elasticity, as described more in details below.

In the nested environment, we use a CPM to represent the phenomenology of ECs and a continuous method to approach the VEGF-induced calcium-dependent cascades. These levels are interfaced by a set of constitutive relations for the evolution of the Potts coefficients. Each RD equation describing the evolution of a molecular variable is here solved with a finite element scheme on a grid discretized at the same resolution of the CPM lattice. The ECs are modeled as discrete objects $\Sigma_{\sigma=1,...,200}$ of type $\tau = C$. They reside in an experimental medium, which is represented as a generalized substrate $\Sigma_{\sigma=0}$ of type $\tau = M$. The internal state vector of each cell is

\begin{equation}
\mathbf{s}_{\Sigma_{1,...,200}}(\mathbf{x},t) = (a(\mathbf{x},t), n(\mathbf{x},t), c(\mathbf{x},t)) \in \mathbb{R}^3,
\end{equation}

where $a(\mathbf{x},t)$ corresponds to the local concentration of AA, $n(\mathbf{x},t)$ of NO and $c(\mathbf{x},t)$ of Ca$^{2+}$. The system Hamiltonian is given by:

\begin{equation}
H(t) = H_{adhesion}(t) + H_{constraint}(t) + H_{chemotaxis}(t).
\end{equation}
$H_{\text{adhesion}}$ considers the intercellular adhesive interactions. In particular, $J_{C,C}$ gives a measure of the formation of local intercellular VE-cadherin-VE-cadherin complexes, which depends on the quantity of active exposed molecules on either sides of the interface. Indeed, since VE-cadherin activity is enhanced by calcium ions, which generate clusters of activated molecules [41–43], for any cell $\Sigma_{\sigma=1,...,200}$:

$$J_{C,C}(\partial x \in \partial \Sigma_{\Sigma(x)}, \partial x' \in \partial \Sigma_{\Sigma(x')}, t) = g(c(x, t), c'(x, t)) = J_0 \exp \left(-p c(x, t)c'(x', t)\right)$$

$H_{\text{constraint}}$ takes into account of cell shape changes (i.e., of area and perimeter, since we are in 2D), using potentials of type (8). The target values $A_{\text{surface}, \text{perimeter}}^{\Sigma_{\sigma=1,...,200}}$ are EC initial dimensions. Cell volume fluctuations are indeed kept negligible by high constant values for $\lambda_{\text{surface}}^{\Sigma_{\sigma=1,...,200}} = 20$ (as done in similar theoretical works [15,24]), whereas the deformability is a characteristic of each cell, being regulated by its intracellular level of calcium, which has been demonstrated to facilitate cytoskeletal reorganizations (refer to [44] and to [45], where the authors have specifically analyzed the influence of calcium signals on the actin dynamics in a model of tumor derived endothelial cells). Therefore for each $\sigma = 1,...,200$, we set:

$$\lambda_{\text{perimeter}}^{\Sigma_{\sigma}}(x, t) = f(c(x, t)) = \lambda_{\text{per}}^0 \exp \left(-k \sum_{x \in \Sigma_{\sigma}} c(x, t)\right)$$

Finally, the movement of ECs along gradients of VEGF concentration is implemented by a linear-type chemotaxis term of the form (10):

$$\Delta H_{\text{chemotaxis}} = \mu_{\Sigma_{\sigma}}^{\text{chem}}(x, t) \left[v(x, t) - v(x', t)\right],$$

where the chemical strength is also calcium-dependent: the local redistribution of VEGF receptors from the Golgi apparatus to the plasma-membrane, and their following activation, is in fact caused by a local elevation of the calcium level, as provided in [46]. Indeed, for each cell $\Sigma_{\sigma=1,...,200}$, we assume:

$$\mu_{\Sigma_{\sigma}}^{\text{chem}}(x, t) = f(c(x, t)) = \mu_{\text{chem}}^0 c(x, t)$$

The cell culture evolves following the Boltzmann probability function (1), with $p = \tanh$. In particular, each cell is characterized by its own variable...
motility which is mediated by its overall calcium level in a dose dependent manner, as provided in [47], where wound healing assays are performed on several types of vascular cells stimulated by different agonists (such as AA and NO themselves). Indeed, for \( \sigma = 1, \ldots, 200 \), we set

\[
\mathbf{s}^T_{\Sigma_\sigma}(x, t) = \left( c(x, t) \right)
\]

(32)

\[
T_{\Sigma_\sigma}(t) = f(c(x, t)) = T_0 \sum_{x \in \Sigma_\sigma} c(x, t)
\]

(33)

The evolution of the microscopic variables is regulated by a set of standard reaction-diffusion equations:

\[
\begin{align*}
\frac{\partial v}{\partial t} &= D_v \Delta_v - \lambda_v \delta_t(\Sigma_\sigma(x), M) + (\varepsilon_v - \chi_v(x, t)) \delta_t(\Sigma_\sigma(x), C) \quad \text{in } x \in \Omega; \\
\frac{\partial a}{\partial t} &= D_a \Delta_a - \lambda_a a + \varepsilon_a v + \varepsilon_a c \quad \text{in } x \in \Sigma_{1, \ldots, 200}; \\
\frac{\partial n}{\partial t} &= D_n \Delta_n - \lambda_n n + \varepsilon_n v + \varepsilon_n c \quad \text{in } x \in \Sigma_{1, \ldots, 200}.
\end{align*}
\]

(34)

Notice that the equation of the intracellular messengers AA and NO work only within the ECs. All the coefficients of diffusivity, degradation or decay, and production of the chemicals are constant in time, homogeneous in space and derived from previous experimental determinations [48]. The local concentration of calcium is determined by a balance between the AA- and NO-activated fluxes, its extrusion from the cell cytosol and its buffered diffusion. Its evolution therefore satisfies:

\[
\begin{align*}
\frac{\partial c}{\partial t} &= K_{buff} \frac{D_c \Delta c}{\text{diffusion}} \quad \text{in } x \in \Sigma_{1, \ldots, 200}; \\
\left. n \partial_x \right|_{\partial \Sigma_\sigma} \cdot \nabla c &= f_a a + f_n n - f_e c \quad \text{at } \partial x \in \partial \Sigma_{1, \ldots, 200}; \\
\frac{\partial c}{\partial t} &= \frac{D_c \nabla^2 c}{\text{diffusion}} \quad \text{in } x \in \Sigma_0,
\end{align*}
\]

(35)

where \( n \partial_x \) is the unit outward normal to the external boundary of site \( x \in \Sigma_{\sigma = 1, \ldots, 200} \). The coefficient of diffusion, \( D_c \), is assumed to be homogeneous, while the scaling factor \( K_{buff} < 1 \) models the activity of endogenous buffers (proteins and mitochondria), which bind the ion [49].
In vitro tubulogenesis. (A) Experimental image of the final configuration of a HUVEC network. (B) Schematic representation of VEGF-induced calcium-dependent pathways. VEGF activates a series of intracellular events inducing the intracellular production of arachidonic acid (AA) and nitric oxide (NO). Both intracellular messengers are able to activate the entry of extracellular calcium, which, with a feedback mechanism, enhances their biosynthesis. The increment in calcium level triggers cell motility, adhesion, chemical responses and cytoskeletal reorganization. (C) Representative images showing different stages of tubule organization at time intervals of 3 hours.

As represented in Figure 2(C), the proposed multilevel environment is able to reproduce the kinetics of the patterning, as well as its final configuration in close comparison with experimental analysis performed with a large number of endothelial cell lines (e.g., human umbilical vein endothelial cells (HUVEC), human dermal microvascular endothelial cells (HDMEC), human capillary endothelial cells (HCEC), human marrow microvascular endothelial cells, bovine aortic endothelial cells (BAEC), bovine capillary endothelial cells (BCEC), bovine retin endothelial cells (BREC), rat capillary endothelial cells (RCEC), embryonic stem cells (ESC), calf pulmonary aortic endothelial cells (CPAEC), adrenal capillary endothelial cells (ACEC)) seeded on different specific matrix substrate (e.g., Matrigel, fibronectin, collagen, fibrin, semisolid methylcellulose), as reviewed in [32]. In particular, in the resulting structured network, vascular branches, which are typically 1-2 cells wide, enclose lacunae, which in turn are almost uniform in size (i.e., they range from 120 µm to 150 µm). Such natural length
scales have been demonstrated to be dictated by the effective range of cell-to-cell interactions (that is mediated by the release of the soluble VEGF, in particular by its diffusion coefficient and decay rate [48]) and to be functional and instrumental for an optimal metabolic exchange. A coarser capillary pattern would be in fact unable to differentiate to form the lumen, while an immature and finer structure would be obviously useless [50]. The geometrical description of the emerging structure is consistent also with the measures performed both with human umbilical vein ECs, see [48] and with a culture of endothelial cells derived from human breast carcinomas (B-TECs) [37].

In our model, the different phases of the pattern formation are realistically driven by the complex and coordinated interplay of the multiscale mechanisms taken into account, i.e., the calcium-dependent increase in cell motility, adhesion and cytoskeletal remodeling and the VEGF-mediated chemotactic migration, employed by the proposed nested approach. In fact, given that the VEGF-evoked calcium signals are clearly detectable in the early phases of the patterning, when ECs are not well connected in a mature network, while when the tubules are more structured they are significantly downregulated (as observed elsewhere both experimentally [37] and theoretically [26]), the cell behavior is let to continuously change. In particular, the initial dramatic increment in intracellular calcium levels enhances the cell migratory properties, fundamental in the first stages of the process, as the ECs have to efficiently move and interact. The subsequent decrease of the cytosolic calcium then partially inactivate the cells, as their positions are now stabilized in the structure and the overall network is mature. It is useful to notice that the use of constant values for the Potts parameters describing the cell biophysical properties, as typically done in classical CPMs [15,24], would have led to a less realistic stabilization of the structure, as the cells would have continued to be elongated and to significantly fluctuate.

3. Compartmentalization approach.

Most CPM models, as the one presented above, represent discrete object as isotropic objects, formed by equivalent and undifferentiated sites. This representation provides a useful level of abstraction, but also hides relevant inhomogeneous properties that characterize all biological individuals and are important to keep in several applications. For example, in the case of cells, the cytoskeleton, the plasmamembrane, the nucleus and other internal organelles are not distinguished.

The simplest and most realistic way of reproducing such complex morphologies is to introduce a compartmentalization technique. According to
this approach, a collection of standard CPM objects can be clustered to form a compartmentalized element, which can more accurately reproduce a real individual. In other words, if in the basic CPM a single discrete object represented an entire individual, it now represents one of its compartments. Technically, with the new procedure, the discrete units $\Sigma_\sigma$ share an additional attribute, a cluster id $\eta(\Sigma_\sigma) \in \mathbb{N}$, which defines the compartmentalized individual they belong to. Obviously, discrete units without $\eta$ are not part of a compartmentalized entity, but represent, on their own, an entire element (as in the basic CPM). Apart from the type already defined for the discrete units, $\tau(\Sigma_\sigma)$, we can now introduce a type for the entire clusters, $\theta(\eta)$. The borders between subunits belonging to the same individual represent internal membranes, while its external membrane is defined as $\partial \eta = \bigcup_{x \in \Sigma_\sigma \subseteq \eta, x' \in \Omega_\sigma' \cap \partial \eta} \chi(x \cap \partial x')$. Here and in the following, we will use the simplified notations $x \in \eta$ to identify a site $x$ belonging to a compartmentalized individual $\eta$ (i.e., it would write $x \in \Sigma_\sigma \subseteq \eta$) and $\partial x \in \partial \eta$ to identify that $\partial x$ belongs to the external membrane of $\eta$.

The new representation of individuals requires a redefinition of the characteristic terms of the CPM. First, it is necessary to differentiate the contributions of $H_{\text{adhesion}}$, due either to the contact between couples of discrete units belonging to the same element, namely $H_{\text{int}}^{\text{adhesion}}$, or to the contact between the membranes of couples of units belonging to different elements, namely $H_{\text{ext}}^{\text{adhesion}}$:

\begin{equation}
H_{\text{adhesion}}(t) = H_{\text{int}}^{\text{adhesion}}(t) + H_{\text{ext}}^{\text{adhesion}}(t).
\end{equation}

$H_{\text{int}}^{\text{adhesion}}$, which indeed models contact forces within the same individual (e.g. between the nucleus and the cytosol in a cell), writes as

\begin{equation}
H_{\text{int}}^{\text{adhesion}}(t) = \sum_{x \in \Omega_\sigma, x' \in \Omega_\sigma'} \delta_{\eta(\Sigma_\sigma(x)), \eta(\Sigma_\sigma'(x'))}(t).
\end{equation}

The form of Equation (37) is analogous to that of Equation (4) and $\delta$ is the Kronecker delta. $J_{\tau(\Sigma_\sigma), \tau(\Sigma_\sigma')}^{\text{int}}(t) \in \mathbb{R}^-$ account for a high cohesion between the internal compartments, fundamental for preventing the single individuals from splitting. The use of negative surface energies was first introduced in [51] to reproduce the behavior of highly cohesive cells, which reduce their energy by binding, while maintaining constant their total membrane area. Although this has no longer been developed in CPM applications, we here employ it to the case of the contact between units belonging to same element, since the underlying rationale is the same.

$H_{\text{ext}}^{\text{adhesion}}$ is formed instead by the effective adhesion energies between different compartmentalized individuals, that interact with their external...
membranes:

$$H_{adhesion}(t) = \sum_{x, x' \in \Omega' x \cap (\partial x \cap \partial x' \cap (\partial x' \cap (\partial x') \neq \emptyset \quad J_{\theta(\eta), \theta(\eta')}^{ext}(t).$$

The strengths $J_{\theta(\eta), \theta(\eta')}^{ext} \in \mathbb{R}^+$ depend, as a simple extension of the basic CPM, on the types of the respective interacting clusters. It is worth to notice that, if the objects in contact represent standard non-compartmentalized individuals, the relative energetic contributions are in the classical form of Equation (4).

The compartmentalized approach requires then to specify both the attributes and the experienced forces (i.e., and the relative energetic contributions) for each and every subunit that formed the compartmentalized individuals.

The proposed technique is clearly flexible, since it allows the level of details to be tuned by only increasing or decreasing the number of units that form the clustered individual, or the number of lattice sites per functional unit. However, it is obviously computationally expensive, and often such a level of detail is neither required nor relevant for a good simulation of a number of biological processes. The optimal strategy depends on the phenomenon of interest, which therefore requires a preliminary analysis.

It is useful to underline that the compartmentalization approach is not entirely new in the CPM: it was in fact first introduced in [52], where the authors have subdivided a Myxococcus Xanthus into strings of subcellular domains, in order to give the bacterium a particular geometry and to control its overall length. Moreover, in [53] a keratocyte has been represented with a set of undifferentiated hexagonal subunits, which have allowed to reproduce the polarization of its cytoskeleton during motion. These approaches have indeed represented a first step towards a more accurate representation of biological individuals in the CPM. However, the subcellular compartments introduced in those works do not have an immediate or direct correspondence with real subcellular elements: according to us, the best way of reproducing different and extremely complex cell morphologies is instead to compartmentalize them according to the compartmentalization suggested in nature, and thus to explicitly represents the plasmamembrane (PM), the cytosolic region, the nucleus, and other intracellular organelles (mitochondria, ribosomes, Golgi apparatus, secretory granules, and so on.

Finally, the specific microscopic models of intracellular dynamics can be used together with the compartmentalized approach described in the previous section (if $\Sigma_\sigma$ represents a subregion of a compartmentalized individual $\eta$, the internal state vector is $s_{\eta, \Sigma_\sigma}$). The biochemical processes can in fact be localized within a well-defined subcellular compartment, as
occurs in reality, allowing to handle several biological mechanisms, which are difficult to reproduce with the basic CPM.

3.1. Test simulation: cell movement in a fibrous matrix.

In order to provide the usefulness of the compartmentalization approach, we model the migration of an individual cell within a dense fibrous scaffold. The cell, initially a sphere, is differentiated in two compartments: the central nucleus \((\Sigma_{\sigma=1}, \tau(\Sigma_{\sigma=1}) = N)\) and the surrounding cytosol \((\Sigma_{\sigma=2}, \tau(\Sigma_{\sigma=2}) = C)\), see Figure 3(A, right panel). The cell cluster id is \(\eta = 1\), of type \(\theta(\eta) = E\). The extracellular environment is composed of a network of 125 collagenous fibers, which are standard, non compartmentalized, CPM objects \(\Sigma_{\sigma=3,...,125}\), of type \(\tau(\sigma) = F\), and of a special generalized object \((\Sigma_{\sigma=0}, \tau(\sigma) = M)\), which represents the extracellular medium, homogenously distributed throughout the simulation domain. The matrix threads form a regular cubic mesh characterized by pores of subnuclear dimensions, see again Figure 3(A, left panel).

The \textit{Hamiltonian} \(H\) is formed by two terms. \(H_{\text{adhesion}}\) is differentiated as in Equation (36) in the contribution due to either the generalized contact tension between the nucleus and the cytoplasm within the cell, or the effective adhesion between a cell and a matrix component. \(J_{\text{int}N,C} \ll 0\) implicitly models the forces exerted by intermediate actin filaments and microtubules to anchor the nucleus to the cell cytoskeleton. \(J_{\text{ext}E,F}\) and \(J_{\text{ext}E,M}\) are instead a measure of the affinity between cell surface adhesion complexes (i.e. sugar-binding receptors or integrins) to either non-solid (i.e. glycosaminoglycans in medium) or solid (i.e. fibrillar collagen) extracellular ligands, respectively: indeed, we assume \(J_{\text{ext}E,F} < J_{\text{ext}E,M}\) since most cell lines in standard conditions adhere more strongly with the fibrous part of the extracellular matrix rather than with its soluble component.

\(H_{\text{constraint}}\) models the geometrical attributes of the simulated objects (both the cell subunits and the matrix threads) where the target values \(A_{\text{volume}}^{\Sigma_{1,...,125}}\) and \(A_{\text{surface}}^{\Sigma_{1,...,125}}\) are their initial dimensions. Indeed, assuming that the cell does not significantly grow during migration, the fluctuations of their volumes are kept negligible with high constant values of \(\lambda_{1,\Sigma_{1,2}}^{\text{volume}}\). Moreover, cells moving in matrix environments are typically deformable, but their nuclei show a higher rigidity w.r.t. the cytoplasm region: therefore, we set \(\lambda_{1,\Sigma_{2}}^{\text{surface}} < 1\) and \(\lambda_{1,\Sigma_{1}}^{\text{surface}} \gg 1\). The matrix fibers are instead assumed inelastic by high \(\lambda_{\Sigma_{3,...,125}}^{\text{volume}} = \lambda_{\Sigma_{3,...,125}}^{\text{surface}} \gg 1\).

Given the \textit{Hamiltonian}, the evolution of the system is driven by the Boltzmann probability function (1), with \(p = \tanh\). In particular, the temperature \(T\) is assumed to characterize each single object: indeed, \(T_{1,\Sigma_{1}}\) is
Figure 3. Migration of a single compartmentalized cell within a fibrous matrix. (A, left panel) Domain $\Omega$, a $20 \times 20 \times 20$ lattice (1 lattice site $\approx 4 \mu m^3$). The cell is initially a 16.5 $\mu$m-diameter sphere, with a nucleus with a diameter of 7.5 $\mu$m. The fibers are standard objects, 28 $\mu$m long and 1.5 $\mu$m width, which form a network with subnuclear dimensions (7 $\mu$m). (A, right panel) $xy$ section of the compartmentalized cell. It is possible to see the different subunits: the nucleus ($\Sigma_1$, $\tau(\Sigma_1) = N$) and the cytosol ($\Sigma_2$, $\tau(\Sigma_2) = C$). (B) Wind-rose graphs show 10 cell tracks over 9 h. Cell movement is only permitted by an enhancement in nucleus elasticity. In the inset, the nucleus deforms to allow the entire individual to pass through the dense fiber network. The nucleus is encircled manually. Values of the parameters: $J_{int}^{N,C} = -20$, $J_{ext}^{E,F} = 4$, $J_{ext}^{E,M} = 8$ $\lambda_{vol}^{\Sigma_1} = \lambda_{vol}^{\Sigma_2} = \lambda_{vol}^{\Sigma_3, \ldots, 125} = \lambda_{surf}^{\Sigma_1, \ldots, 125} = 20$, $\lambda_{surf}^{\Sigma_1} = 0.5$; $T_{1, \Sigma_1} = 0.5$, $T_{1, \Sigma_2} = 15$, $T_{\Sigma_3, \ldots, 125} = 0$. $\lambda_{surf}^{\Sigma_1}$ is varied from 20 to 1 to model the enhanced nucleus elasticity.

A low value reproducing the passive motion of the nucleus, which, unable to have an autonomous movement, is dragged by the surrounding cytosol, characterized instead by a high $T_{1, \Sigma_2} \gg 1$ (that gives a measure of the "real" cell intrinsic motility, i.e. the frequency of cell PM ruffles). Finally, the collagenous threads are fixed by $T_{\Sigma_3, \ldots, 125} = 0$.

The analysis of cell movement (captured in a time span of 9 hours) shows that any cell migration over long distances is prohibited. An even complete stretch of cell cytosol is in fact not sufficient to pass through the steric hindrances, as the nucleus can not significantly deform, causing the overall individual to be confined in a small area, see Figure 3(B, left panel). Interestingly, an enhancement in the elasticity of the nuclear cluster, with a low value of $\lambda_{surf}^{\Sigma_1}$, results in an appreciable cell movement in such a dense matrix, as the cell is allowed to completely squeeze and stretch through...
the existing mesh, as captured in Figure 3(B, right panel) and in the inset therein. It is straightforward to notice that these considerations would have not been pointed out by the basic, non-compartmentalized CPM.

The outcomes of our model are consistent with the relative observations provided in the experimental literature. In 3D environments, neutrophil migration (both velocity and directional coefficient) has been reported to be partially inhibited by low gel pore size [54], while mouse fibroblasts have been observed to migrate more significantly in collagen-glycosaminoglycan (CG) scaffolds featuring pore dimensions somewhat smaller than cellular dimensions, whereas they have exhibited less dispersion in matrices with larger pores [55]. Moreover, in [56–58], the authors have provided that cell migration efficiency, which decreases with matrix density, is associated with nuclear deformation. Finally, in [59], glioma cell lines have been demonstrated to significantly deform their nucleus (upon recruitment of non-muscle myosin II, NMMII) in order to squeeze through narrow locations in a brain model in vivo, thereby increasing their metastatic potential.


Over the last decade, the CPM method has become a standard technique for cell-to-tissue level in silico biology, first replicating, then guiding in vitro experiments, and eventually leading to new experimental discoveries [8,30,60]. We have here discussed some improvements of the method, which aim at overcoming its main limitations. Along the text, we have also illustrated some sample simulations, i.e. of a typical tubulogenic assay and of the migration of an individual cell within a dense fibrous scaffold. In both cases, the results obtained by the model have agreed with the published experimental observations, thus showing the consistency of our CPM extensions.

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