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# A biomechanical model for cell sensing and migration

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## Abstract

We developed an original computational model for cell deformation and migration capable of accounting for the cell sensitivity to the environment and its appropriate adaptation. This cell model is ultimately intended to be used to address tissue morphogenesis. Hence it has been designed to comply with four requirements: (1) the cell should be able to probe and sense its environment and respond accordingly; (2) the model should be easy to parametrize to adapt to different cell types; (3) the model should be able to extend to 3D cases; (4) simulations should be fast enough to integrate many interacting cells. The simulations carried out focused on two aspects: first, the general behaviour of the cell on a homogeneous substrate, as observed experimentally, for model validation. This enabled us to decipher the mechanisms by which the cell can migrate, highlighting respective influences of the adhesions lifetimes and their sensitivity to traction; second, it predicts the sensitivity of the cell to an anisotropic patterned substrate, in agreement with recently published experiments. The results show that mechanosensors simulated by the model make it possible to reproduce such experiments in terms of migration bias generated by the substrate anisotropy. Here again, the model provides a biomechanical explanation of this phenomenon, depending on cell-matrix interactions and adhesion maturation rate.

**Keywords:** cell morphology, focal adhesion, mechanosensor, patterned substrate, stress fibres.

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# 1 Introduction

Cell motility, *i.e.* cell deformation and migration has been studied for many decades both on two-dimensional substrates (Abercrombie et al. 1970, Abercrombie 1980, Theriot & Mitchison 1991) or in 3D (Yamada et al. 2022). Indeed the cell ability to migrate in complex media underpins a number of fundamental biological processes including embryogenesis, wound healing, angiogenesis, cell invasion and metastasis (Peschetola et al. 2013, Kulesa et al. 2021, Bouchalova & Bouchal 2022). But the mechanisms of motility are difficult to understand because they involve the complex interplay between actin dynamics at the cell edge (Theriot & Mitchison 1991), contractility through acto-myosin dedicated assembly/disassembly (Chi et al. 2014), retrograde flow (Kruse et al. 2006), as well as coordinated links between the cytoskeleton and focal adhesions (Galbraith et al. 2007). Finally, the modes of migration used by cells (*i.e.* mesenchymal or ameboid) can be strongly influenced by confinement (Liu et al. 2015), in particular in 3D rich-filamentous networks (Starke et al. 2013).

To decipher the various mechanisms at play, many theoretical models, either mathematical or computational, have been developed at the scale of the single cell. The cytoskeletal actin dynamics in relation with the formation of adhesions is at the heart of many studies (Gardel et al. 2008, Chandra et al. 2022), although some models focus more on the membrane, hydrostatic pressure and intracellular contractility-generated fluxes (Petrie et al. 2014, Woolley et al. 2017). Other theoretical models have allowed to clarify the roles, regulatory activities and relationships of key proteins such as Cdc42, Rac (Marée et al. 2006), rho GTPases (Holmes et al. 2016, Rens & Edelstein-Keshet 2019) and Arp2/3 (Garner & Theriot 2022). They have also helped identify the physical mechanisms required for cell elongation, translocation and motion as a combination of focal adhesions formation and stress fibre maturation and turnover (Franco et al. 2010, Vernerey & Farsad 2014).

More recently, the necessity to integrate cell interactions with its environment in the models has been highlighted (Eichinger et al. 2021). Chemoattraction used to be considered as a major driver of cell migration, but the focus is now more specifically on the cell-matrix biomechanical interactions. A plethora of models have stressed the importance of these bidirectional interactions (Muntz et al. 2022, Yamada et al. 2022, Akbarzadeh Solbu et al. 2023). On the one hand cell sensing allows to detect the properties of the matrix in terms of surface ligands and bulk mechanical properties leading to the formation of adhesions and the build up of traction forces (Gardel et al. 2008). On the other hand the matrix can be remodeled, *i.e.* deformed (Schluter et al. 2012, Reinhardt et al. 2013), degraded (Zhu & Mogilner 2016, Heck et al. 2020) or synthesized by the cell. Those dynamically changing properties reciprocally affect the interaction with the cell (Stéphanou et al. 2015). That is why understanding this dynamical relationship is considered a major therapeutic challenge (Yamada et al. 2022).

A thorough review of the literature shows that there are many existing models, but none of them answer all the questions emphasized above. Models by Zhu & Mogilner (2016) and Satulovsky et al. (2008) allow to gener-

53 ate a great diversity of cell shapes and behaviours, but they are computationally costly. Models that address the  
54 cell cytoskeleton with nodes and springs are usually more efficient and well adapted for sensing the environment  
55 stiffness (Kim et al. 2013, Bangasser et al. 2017), but lack the probing ability. Kim et al. developed a model for  
56 filopodia sensing in 3D but at the cost of an increased complexity (Kim et al. 2015, 2018). The agent-based model  
57 by Heck et al. (2020) and the cellular Potts model by Rens & Merks (2020) are well adapted to probe and sense the  
58 extracellular matrix, however they cannot be easily generalized to address cell migration in a 3D environment.

59 We note that the paper by Merino-Casallo et al. (2022) could fit most of our requirements. The model describes  
60 mesenchymal migration as a 3-step process including probing, pathways activation and cell translocation. The  
61 probing phase is based on chemosensing that activates a signaling network involving cytosolic PI3K regulating  
62 protrusion dynamics. One of the highlight of this model is to take into account the steric constraints, that is the  
63 ability of the cell to squeeze through the extracellular matrix (ECM) pores. For that the plasma membrane is  
64 represented to delineate the space occupied by the cell. This allows to calculate a friction term which is used to  
65 represent the steric hindrance, *i.e.* the difficulty encountered by the cell to migrate through the dense 3D ECM.  
66 Although appealing, this model takes into account the plasma membrane and molecular dynamics that we wished  
67 to avoid to reduce the computational cost.

68 In this paper our goal was to develop an efficient model for cell sensing with in mind the future idea to ad-  
69 dress many cell-cell interactions (up to a hundred cells) in a 3D matrix. This will allow to investigate cell-matrix  
70 remodelling in connection with cell auto-organization and applications to wound healing, angiogenesis or cell in-  
71 vasion. To achieve this objective, we had to comply to the following requirements: (1) cell ability to probe and  
72 sense the environment and respond accordingly; (2) efficient parametrization to adapt to different cell types; (3)  
73 easy extension to 3D cases; (4) fast simulations to integrate many interacting cells.

74 The model proposed is directly inspired from a previous one describing the synchronized maturation of actin  
75 stress fibres and focal adhesions (Stéphanou et al. 2008, Stéphanou 2009, Franco et al. 2010). The hybrid model was  
76 coupling PDEs for the actin dynamics and force generation with an algorithm describing the formation of discrete  
77 independent adhesion sites and their maturation. However the model was strictly limited to 2D and simulations  
78 were slow. We reconsidered this initial model, assuming that the cytoskeletal fibres and adhesion proteins were  
79 not a limiting factor (always available in sufficient amount). As a consequence, the cell membrane that delineates  
80 the intracellular space was not required. The actin cytoskeletal fibres could radiate from the cell centre and form  
81 adhesions at their ends. We retained from the original model the reciprocal maturation of the cytoskeletal fibres  
82 and their associated adhesions as the fundamental key for cell sensing. Three levels of maturation were considered.  
83 The first one corresponds to an exploratory phase with dynamic adhesions progressively maturing by increasing  
84 the adhesion strength while reducing their dynamic turnover (2nd phase). In the last phase the adhesion is anchored

85 and the fibre can reciprocally develop higher forces required for cell translocation hence migration.

86 The model strength relies on the dynamic adaptation of the motile cell to the environment. Its ability to detect  
87 an adhesive environment was first tested in 2D on a homogeneous substrate, then on an anisotropic one. The  
88 simulations reproduced the well-known experimental behaviours regarding the relationship between velocity and  
89 adhesion strength (Palecek et al. 1997, Caballero et al. 2014). Then migration biases were introduced through an  
90 anisotropic environment – as observed in a recent experimental setup (Lo Vecchio et al. 2020) – and could also be  
91 reproduced and explained on a qualitative basis. The model appears to be a reliable simulation tool to further tackle  
92 more complex cell-matrix interactions involving matrix remodelling.

## 93 **2 Cell model**

### 94 **2.1 Biological motivation and main assumptions**

95 Our aim was to develop a relatively simple cellular model to subsequently address cell auto-organization and  
96 different processes of morphogenesis. Although simple, the model should capture the main mechanisms responsible  
97 for cell migration. Moreover, in order to attain its future goal, the model developed had to comply to a number of  
98 constraints. Those constraints are:

- 99 1. the cell should be able to probe and sense its environment and to respond adequately to it;
- 100 2. the model should be easy to parameterize to adapt to different cell types context (since we envisaged to model  
101 different processes of morphogenesis);
- 102 3. the model should be easy to extend to 3D;
- 103 4. the simulation should not be costly in term of calculation time, since we intend to subsequently integrate  
104 numerous interacting cells (from several hundred to thousands of cells).

105 There is a consensus on how mesenchymal migration proceed: the cell exhibits membrane protrusions sus-  
106 tained by actin fibres; makes adhesions to the extracellular matrix through integrins; actomyosin fibres contract to  
107 allow the cell to move forward by taking support on the adhesions, that ultimately break. Many different protein  
108 are involved and adhesions and cytoskeleton turnover is well orchestrated to allow the cell to renew the all process  
109 to migrate (Mavrakis & Juanes 2023). As in most biomechanical models, we made the choice not to describe the  
110 complex protein regulations. On the other hand, we integrated coupled adhesions and fibres reinforcement through  
111 three maturation stages to reproduce the protrusion-adhesion-translocation sequence in a dynamical way.

113 Although the shape that a cell can take can be complex, it is common to represent it with radial fibres. Those  
114 fibres are given (visco)elastic properties and generate forces on the nucleus at one end, and an adhesion at the  
115 other end (Bangasser et al. 2017, Isomursu et al. 2022, Kim et al. 2013). The force competition between the fibres  
116 will ultimately condition the cell displacement. We chose to consider radiating elastic fibres that stand for actin  
117 cables that later mature, with the recruitment of myosin, into stress fibres. The sensing property of the cell is due  
118 to filopods, that are short actin fibre extensions. We integrate this structure into the model which develops where  
119 adhesions form.

120  
121 Currently there is no extracellular matrix in the model. The environment is considered as a substrate – whose  
122 nature is not specified – on which the cell can form adhesions. The modulation of the adhesion strength is used as  
123 a proxy to simulate different matrices characteristics (it can account for different concentrations of ligands and/or  
124 a differential cell-matrix affinity).

## 125 2.2 Overall cell structure

126 The cell model is constructed to account for the overall cell dynamics based on the interactions between cytoskeletal  
127 fibres and focal adhesions. It is composed of branches and nodes that form a hierarchical tree like structure meant to  
128 represent the mechanical structure of the cell by emulating its centre (nucleus), membrane protrusions, adhesions  
129 and mechanosensors. Each node  $N_i$  has an order  $i$  (from 0 to 2) that indicates its degree of separation from the  
130 central node  $N_0$ . The parent node of  $N_i$  is node  $N_{i-1}$  for  $i > 0$ . Several  $N_i$  nodes can radiate from a node  $N_{i-1}$  and  
131 each branch  $B_i$  connects the two nodes  $N_{i-1}$  and  $N_i$ .

- 132 • The  $N_0$  node represents the cell centre, (*i.e.* the cell nucleus). This point is unique and it is used to locate the  
133 cell.
- 134 • The  $N_1$  node represents a cell adhesion as the tip of a  $B_1$  branch, *i.e.* a protrusion emanating from the cell  
135 centre.  $N_1$  nodes have the potential to mature and exist under three different forms of increased maturity  
136 (described below) in order to account for the evolving nature of the cell adhesions.
- 137 • The  $N_2$  node represents a mechanosensor at the extremity of a  $B_2$  branch. Those typically represent membrane  
138 extensions, such as filopodia, probing the cell environment.

139 The number, position and properties of nodes and branches are dynamically regulated, such that the cell gener-  
140 ates or deletes nodes and branches. The latter transmit and generate forces thus enabling nodes to move and pull on  
141 the substrate. The cell can consequently spread, adhere, pull and move. The behaviour of each node is dependent  
142 on its order, thus enabling the emergence of a coherent system. The rules regulating each node type are summarized

143 in Figure 1 and are detailed below.

## 144 **2.3 Nodes generation**

### 145 *N<sub>1</sub> nodes*

146 *N<sub>1</sub>* is the main adhesion that undertakes a maturation process eventually leading to a focal adhesion able to sustain  
147 cell translocation (higher force) so that the cell migrates. Several *N<sub>1</sub>* nodes can be generated during the cell's life  
148 time. The frequency and the location at which they are generated are regulated in a probabilistic manner. Therefore,  
149 to determine how many *N<sub>1</sub>* nodes should be generated during the current time step, we first need to calculate the  
150 occurrence of independent events that will attempt to generate them. To do so, we define the mean frequency  $\nu_1$   
151 at which these independent events occur around the cell. But, to avoid overcrowding of *N<sub>1</sub>* nodes in one area, an  
152 exclusion angle  $\delta\theta_1$  is defined around the *B<sub>1</sub>* branch, so as to prevent new *N<sub>1</sub>* nodes from forming too close to their  
153 already existing neighbours (Fig. 2). From there we can then calculate  $\lambda_1$ , the expected rate of occurrence during a  
154 time step  $dt$ :

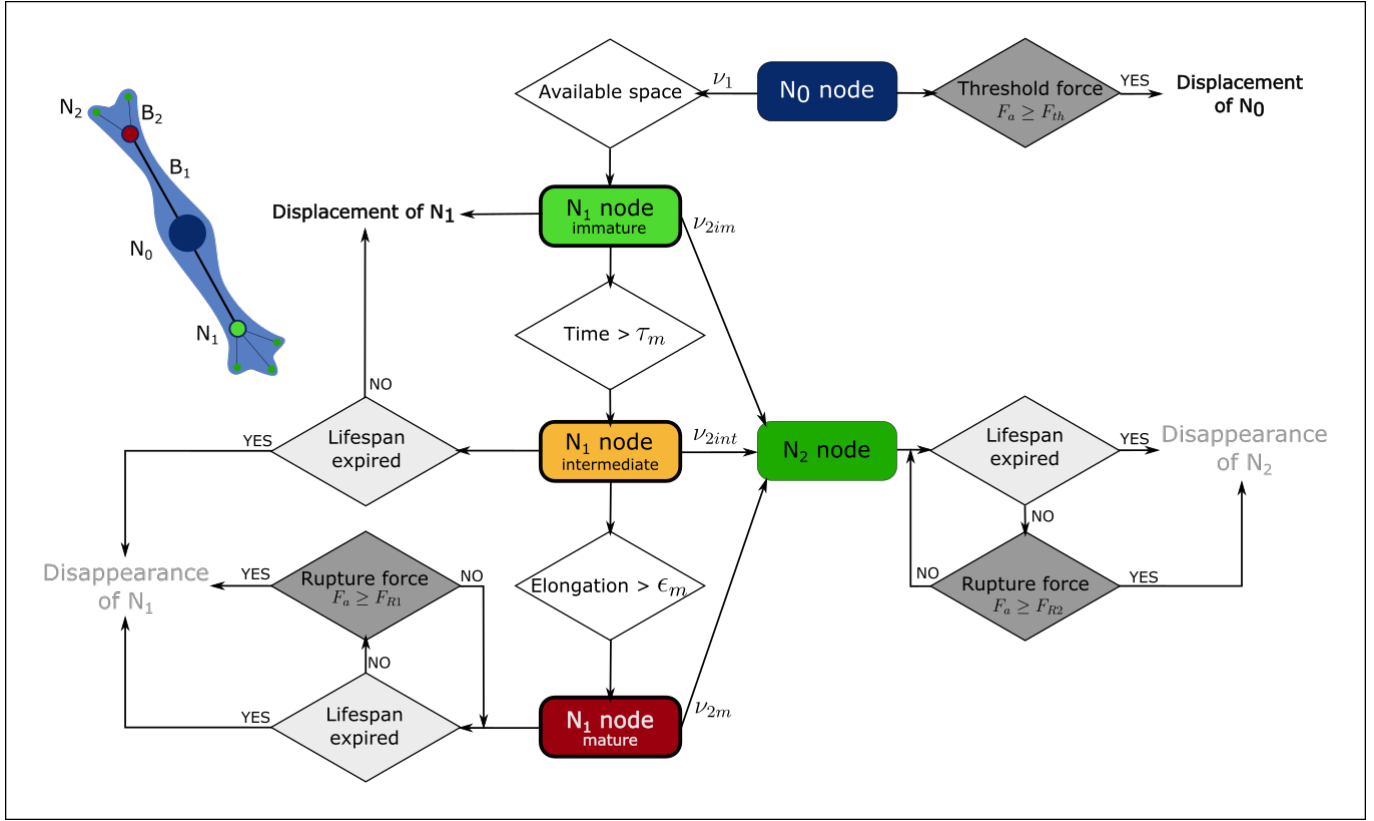
$$\lambda_1 = \nu_1 dt \quad (1)$$

155 Then we use a pseudo-random variate generator that follows the Poisson's distribution with  $\lambda_1$  as input. The  
156 obtained random variate integer  $j_1$  is the number of events that will attempt to create a *N<sub>1</sub>* node during the current  
157 time step. If  $j_1$  is greater than zero and if there is room to create a new node, then a new node is created at a fixed  
158 initial distance  $l_{10}$  from *N<sub>0</sub>* at a randomly generated angle  $\theta_1$  (Fig. 2). This angle is a random variate pulled from a  
159 uniform distribution over the angular domain that remains available. This process is repeated  $j_1$  times or until there  
160 is no more room to create another node because the angular domain is fully inhibited.

### 161 *N<sub>2</sub> nodes*

162 *N<sub>2</sub>* nodes can be considered as membrane spikes probing the cell environment. *N<sub>2</sub>* nodes are stochastically emitted  
163 by the parent *N<sub>1</sub>* node with a mean frequency  $\nu_2$  depending on the maturation level of *N<sub>1</sub>*. An immature *N<sub>1</sub>*  
164 emits *N<sub>2</sub>* nodes with a higher frequency to actively probe the environment and orient the cell displacement. As it  
165 matures, the *N<sub>1</sub>* node reinforces its adhesion strength to the substrate and emits *N<sub>2</sub>* nodes with a lower frequency  
166 to progressively reduce the probing activity and stabilize the adhesion. The expected rate of occurrence  $\lambda_2$  of a new  
167 *N<sub>2</sub>*, during a time step  $dt$ , is:

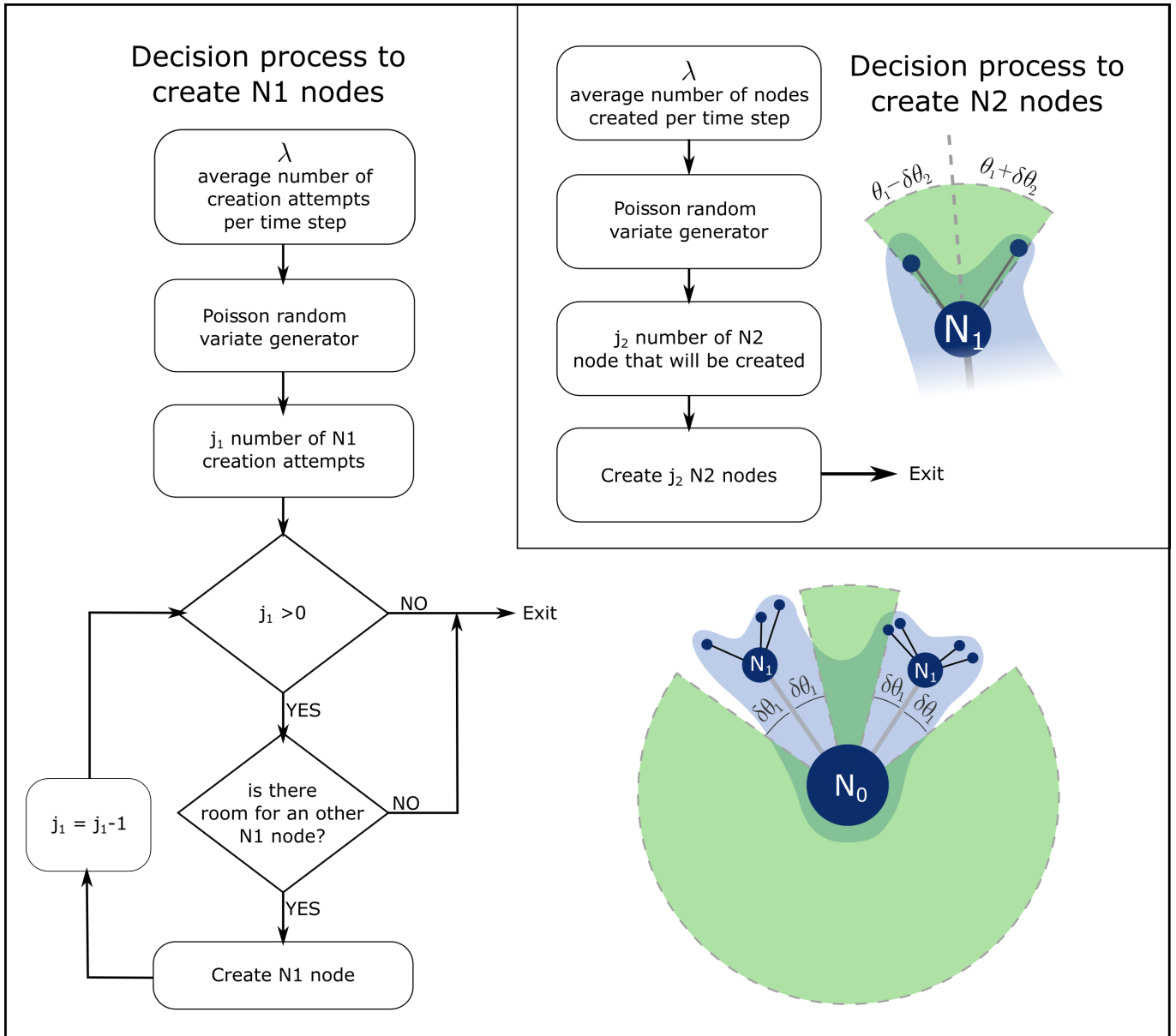
$$\lambda_2 = \nu_2 dt \quad (2)$$



**Figure 1:** The cell structure is composed of nodes and branches,  $N_0$ , the root node is at the centre,  $B_1$  branches connect the root node to  $N_1$  nodes and  $B_2$  branches connect the latter to  $N_2$  nodes. The flowchart shows how nodes evolve in time. The  $N_0$  node is bound to the substrate and moves if the adhesion force  $F_a$  is greater than the threshold force  $F_{th}$ . It generates  $N_1$  nodes if space is available.  $N_1$  nodes are initially immature, then intermediate and finally mature. The respective function of each  $N_1$  state is to: explore (if immature), assess if it can become mature (if intermediate) and bind to the substrate (if mature). During each maturation state a  $N_1$  node respectively generates  $N_2$  nodes at an average stochastic rate of  $\nu_{2im}$ ,  $\nu_{2int}$  and  $\nu_{2m}$ . The function of the  $N_2$  nodes is to probe the environment and contribute to the movement of the cell's leading edge. Therefore, immature and intermediate  $N_1$  nodes move, while mature  $N_1$  nodes are bound to the substrate. Immature  $N_1$  nodes transit to an intermediate state after reaching the age  $\tau_i$ , while the mature state is reached once the  $B_1$  branch is strained above  $\epsilon_m$ . When the adhesion force  $F_a$  of a  $N_1$  or  $N_2$  node is greater than the respective rupture force  $F_{R1}$  or  $F_{R2}$ , there is rupture and the node disappears and its child nodes with it.



169 A Poisson's variate generator is used to define the number of  $N_2$  nodes that are created during the time step with  $\lambda_2$   
 170 as input. Then the angle with which each new  $N_2$  appears, is defined using a uniform-random variate generator that  
 171 follows a uniform distribution. The sample space is defined between  $\theta_1 \pm \delta\theta_2$ . The new  $N_2$  node is thus created at  
 172 the angle  $\theta_2$  and fixed at an initial distance  $l_{20}$  from the position of the parent  $N_1$  node (Fig. 2).



**Figure 2:** Nodes creation. *Left and bottom:* decision process to create  $N_1$  nodes at each time step. A  $N_1$  node located at an angle  $\theta_1$  will inhibit the formation of new  $N_1$  nodes in the angular domain surrounding the  $B_1$  branch i.e.  $\theta_1 \pm \delta\theta_1$ . *Top right:* Decision process to create  $N_2$  nodes at each time step in an angular domain surrounding the  $B_1$  branch i.e.  $\theta_1 \pm \delta\theta_2$ . Areas in green represents the zone where a new node can be created.

## 2.4 Nodes displacement

Our model aims at describing the cell movement and displacement dynamics. Therefore the nodes adhering to the substrate with various degrees, that depend on their type and maturation level, can be displaced by the mechanical forces applied on them through the branches.

Each branch (of index  $i$ ) connected to a node exerts a force  $\mathbf{F}_i$  on the node such that the net force applied by the  $n$  branches on the node is the sum of these forces. If the node is bound to the substrate the adhesion force  $\mathbf{F}_a$  prevents the node from moving such that:

$$\mathbf{F}_a = - \sum_{i=1}^n \mathbf{F}_i \quad (3)$$

On the other hand, when the node is able to move, according to the quasi-static approximation the acceleration is negligible. Thus using Newton's second law, and by stating that the friction force  $\mathbf{F}_v = -\alpha\mathbf{v}$  is always opposed to the node's displacement with velocity  $\mathbf{v}$ , we have:

$$\alpha\mathbf{v} = \sum_{i=1}^n \mathbf{F}_i \quad (4)$$

where  $\alpha$  is the friction coefficient of the node with the substrate. It depends on the node type and maturation level. The node's new position at the next iteration noted  $\mathbf{x}_N^{t+dt}$  is thus given by:

$$\mathbf{x}_N^{t+dt} = \mathbf{x}_N^t + \mathbf{v}dt \quad \text{with} \quad \mathbf{v} = \frac{1}{\alpha} \sum_{i=1}^n \mathbf{F}_i \quad (5)$$

In our model,  $N_2$  nodes play the role of sensors interacting with the substrate to probe its mechanical and/or adhesive properties in order to orient the cell displacement. Several  $N_2$  nodes are simultaneously linked by  $B_2$  branches to their parent node  $N_1$  on which they pull.  $B_2$  branches are assumed to be short elastic actin spikes with elastic coefficient  $\kappa_2$ . They are emitted with an initial length  $l_{20}$ , chosen to correspond to the length of a filopod and they are characterized by a predefined strain  $\epsilon_{20}$  such that the resting length  $l_{2r}$  of the spike is smaller than its initial length, *i.e.* :

$$\epsilon_{20} = \frac{l_{20} - l_{2r}}{l_{2r}} > 0 \quad (6)$$

Parameters are chosen so that the spike is initially stretched and will attempt to return to its rest length. This will cause the branch to pull on both nodes, giving rise to a positive elastic force  $F_{el}$  along the  $\overrightarrow{N_1N_2}$  axis since the  $N_2$  node is fixed (bounded to the substrate) and the  $N_1$  node is free to move (at least in its non mature states):

$$F_{el} = \max(0, \kappa_2(l_2(t) - l_{2r})) \quad \text{with} \quad l_{2r} = \frac{l_{20}}{1 + \epsilon_{20}} \quad (7)$$

The resulting force on the parent node  $N_1$  is calculated as the sum of the elastic contributions of each  $B_2$  branches with equation (4). The  $N_1$  node is then displaced to its new position according to the vector  $v$  given in equation (5).

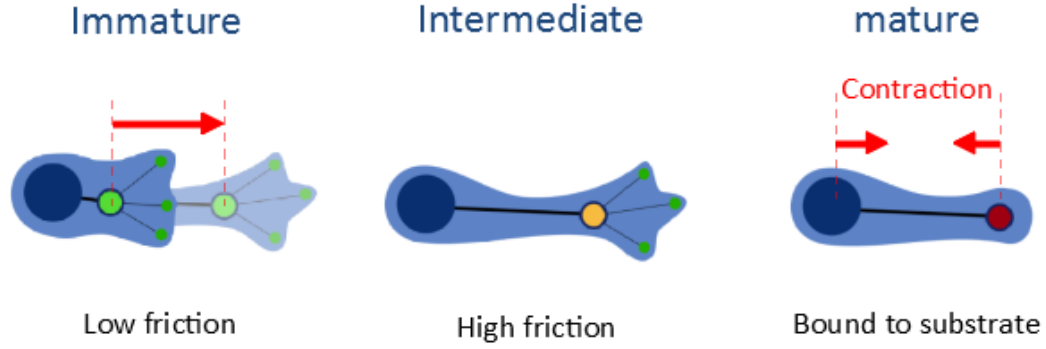
Since we neither take into account actin polymerisation dynamics nor the cell membrane in our model, actin retrograde flow - which is driven by the resistance of the membrane to actin polymerization at the leading edge (Parsons et al. 2010) - is not represented. However, the actin fibers corresponding to the  $B_1$  branches pull on the  $N_1$  adhesions creating a backward movement towards the cell center. At the immature state, this backward movement is counterbalanced by the probing activities of the  $N_2$  nodes that allow to oriente the cell B1 branch extension towards the most favorable locations where the  $N_1$  adhesion will stabilize.

## 2.5 Nodes maturation

The  $N_1$  nodes represent the adhesion points on which the cell actin fibres, represented by the  $B_1$  branches, take support. Before the cell is able to move, the adhesion initially composed of integrins, should be reinforced by the recruitment of new integrins via binders. These binders are recruited for the nucleation and binding of actin fibres, including talin, paxillin, vinculin, FAK, VASP, Arp2/3 (Legerstee & Houtsmuller 2021). The fibres can then generate increasing forces on the  $N_1$  adhesion that reaches maturation under the form of a focal adhesion through the recruitment of zyxin and tensin (Zaidel-Bar et al. 2003). Reciprocally the mature  $N_1$  adhesions can resist higher tensions from the cytoskeletal fibres. This bi-directional maturation process between adhesion and cytoskeletal fibres is important to realistically describe the cell sensing ability and its evolving biomechanics. Indeed, depending on the mechanical nature of the substrate the maturation process will be more or less efficient depending on the level of forces reached by the cell fibres ( $B_1$  branches).

We consider in the model three levels of maturation for the  $N_1$  nodes and its associated  $B_1$  branch: immature, intermediate and mature states. Each successive state is characterized by an enhanced adhesiveness to the substrate and by an enhanced potential of force generation (Fig. 3 and Fig. 4).

- *Immature state*: the  $N_1$  node is free to move and is displaced by the forces exerted by the  $N_2$  nodes emitted at a higher rate  $v_{2im}$  in this first state. Consequently its friction with the substrate is initially small with coefficient  $\alpha_{i0}$  and we propose that it increases linearly with time - as integrins and actin-bound proteins are progressively recruited with a typical timeframe (Parsons et al. 2010) - to reach the value  $\alpha_{int}$  at time  $\tau_t$  which characterizes the friction of the intermediate state. The friction evolution between the immature and



**Figure 3:** Illustration of the maturation stages of the  $N_1$  node. The immature node, characterized by a low friction with the substrate, is displaced by the traction of the  $N_2$  nodes. After some pre-defined time the  $N_1$  node reaches the intermediate maturation state characterized by an increased friction with the substrate. It finally reaches maturation if the  $B_1$  branch is sufficiently elongated by the tensions from the  $N_2$  nodes. The mature adhesion is bound to the substrate and the  $B_1$  branch becomes contractile to generate higher forces on the  $N_0$  node in order to allow the cell to translocate by taking support on the  $N_1$  node to move forward.

223 intermediate states is thus given by:

$$\alpha_1(t) = \frac{\alpha_{int} - \alpha_{10}}{\tau_t} t + \alpha_{10} \quad (8)$$

224 As a concomitant event, the  $B_1$  branch is progressively reinforced by actin fibres recruitment, that leads to a  
225 linearly increasing stiffness from  $\kappa_{10}$  (immature state) to  $\kappa_{11}$  (intermediate state):

$$\kappa_1(t) = \frac{\kappa_{11} - \kappa_{10}}{\tau_t} t + \kappa_{10} \quad (9)$$

226 The branch initially corresponds to an unstretched spring of length  $l_{10}$  that can bear tension and compression  
227 depending on the node displacement  $l_1(t)$ . The elastic restoring force in the branch is then given by:

$$F_{el} = \kappa_1(t)(l_1(t) - l_{10}) \quad (10)$$

228 • *Intermediate state:* it is reached when the  $N_1$  node reaches the age  $\tau_t$  for which the values of the friction  
229 coefficient and branch stiffness are kept constant as:

$$\alpha_1(t) = \alpha_{int} \quad \text{and} \quad \kappa_1(t) = \kappa_{11} \quad (11)$$

230 The increased friction coefficient of the node  $N_1$  makes it more resistant to the displacement from the pulling  
231  $N_2$  nodes. Moreover, the production rate of  $N_2$  nodes is concomitantly reduced with  $v_{2int} < v_{2im}$ . This reduces  
232 significantly the exploration potential of the branch. At this stage, the  $B_1$  branch corresponds to a bundle of

actin fibres for which the rest length is reassessed and given by  $l_1(\tau_t)$ . The elastic restoring force in the branch is now given by:

$$F_{el} = \kappa_{11}(l_1(t) - l_1(\tau_t)) \quad (12)$$

- *Mature state*: it corresponds to the local culminating cell protrusion concomitant to the formation of a stable focal adhesion. The transition to this state only occurs if the  $B_1$  branch elongation from its new resting length  $l_1(\tau_t)$  reaches the elongation target  $\epsilon_m$ , *i.e.* if:

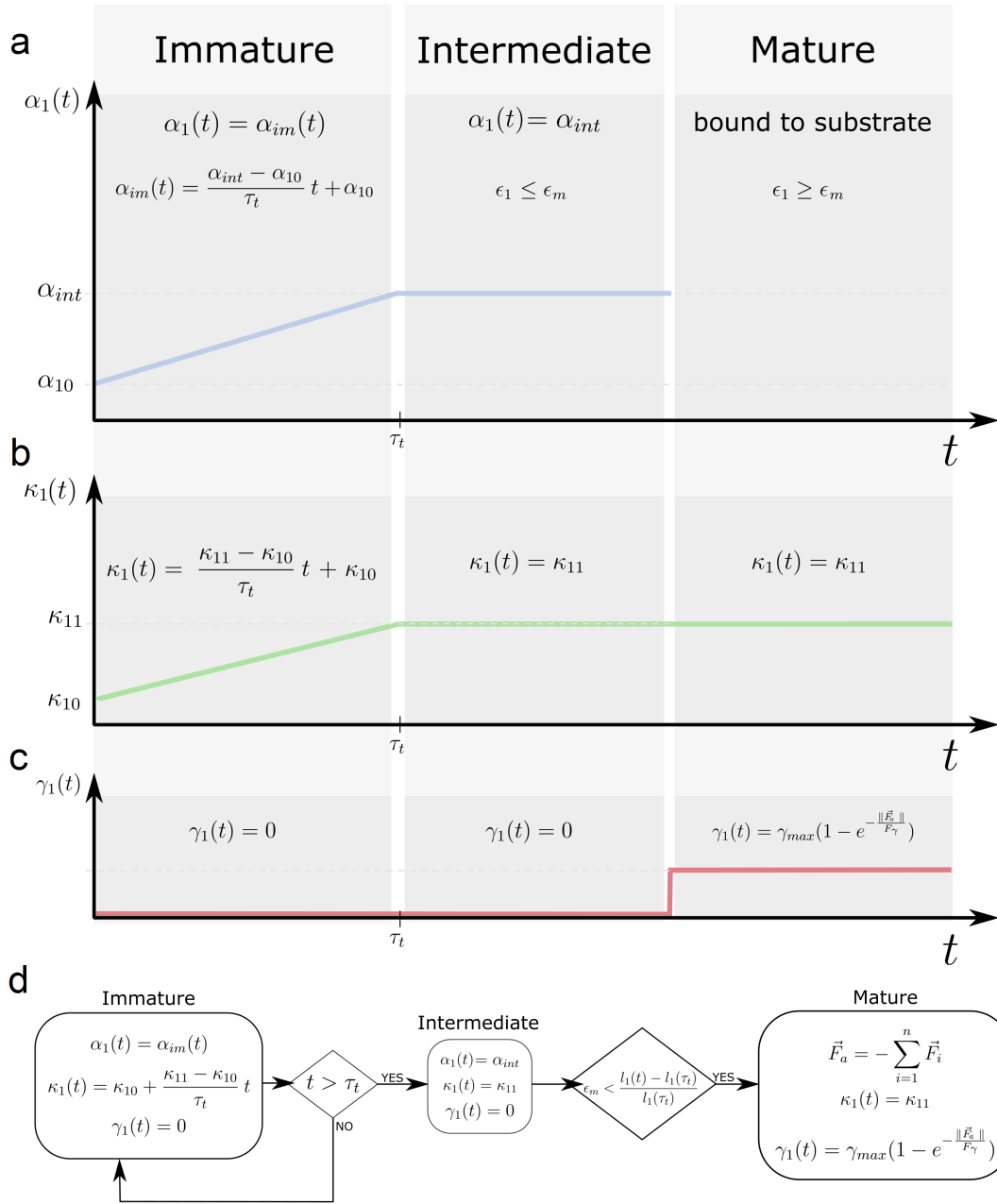
$$\frac{l_1(t) - l_1(\tau_t)}{l_1(\tau_t)} \geq \epsilon_m \quad (13)$$

This condition is not necessarily reached, so not all  $N_1$  mature to their final state. If they do, two major transitions affect the node and branch. First, the node is fixed, bound to the substrate, in order to be able to sustain cell translocation that is required for the cell displacement. The production rate of  $N_2$  is further reduced with  $v_{2m} < v_{2int}$ . Second, the branch becomes contractile, through myosin recruitment, and corresponds to a stress fibre which is able to generate the force required for the cell translocation, *i.e.* the force that will allow the  $N_0$  node to move. The contractile force  $F_c$  grows concomitantly with the increased resistance of the adhesion force  $F_a$ . We propose to represent it by mean of the following exponential function:

$$F_c = \gamma_{max}(1 - e^{-\frac{\|\mathbf{F}_a\|}{F_\gamma}}) \quad (14)$$

where  $\gamma_{max}$  is the stall force of the adhesion,  $F_\gamma$  is a characteristic force constant, and  $\|\mathbf{F}_a\|$  is the norm of the adhesion force. The total force in the branch is the sum of the passive elastic contribution  $F_{el}$  (Eq. (12)) and active contractile one  $F_c$  (Eq. (14)). The cell translocation occurs if the adhesion force  $F_a$  (Eq. (3)) is big enough to support the tearing of the cell, corresponding to the threshold force  $F_{th}$ .

Regarding the  $N_1$  branch maturation, we assume that the actin fiber is an elastic rod under tension at the initial stage. As the fibers mature, fibers are recruited to form a bundle of fibers still possessing an elastic property. The contractile contribution on the other hand is an active process and involves the recruitment of myosin. The actomyosin fibers are responsible for the cell contractility. This process comes into play at the end of the maturation process of the actin fibers, which correspond to stress fibers at this stage of maturation. The active contractile force is much higher than the passive elastic force and dominates to generate the traction force required for cell translocation leading to migration.



**Figure 4:** Summary of the evolution of the model parameters along the maturation process: (a) friction coefficient  $\alpha_1(t)$ , (b) branch stiffness  $\kappa_1(t)$ , (c) contractile force  $\gamma_1(t) = F_c$ , (d) associated flowchart.

## 2.6 Nodes disappearance

Nodes can disappear in two different ways. First they have a limited lifespan and they spontaneously disappear when this time limit is reached. The lifespan depends on the node type (see Table 1). Second the nodes that are

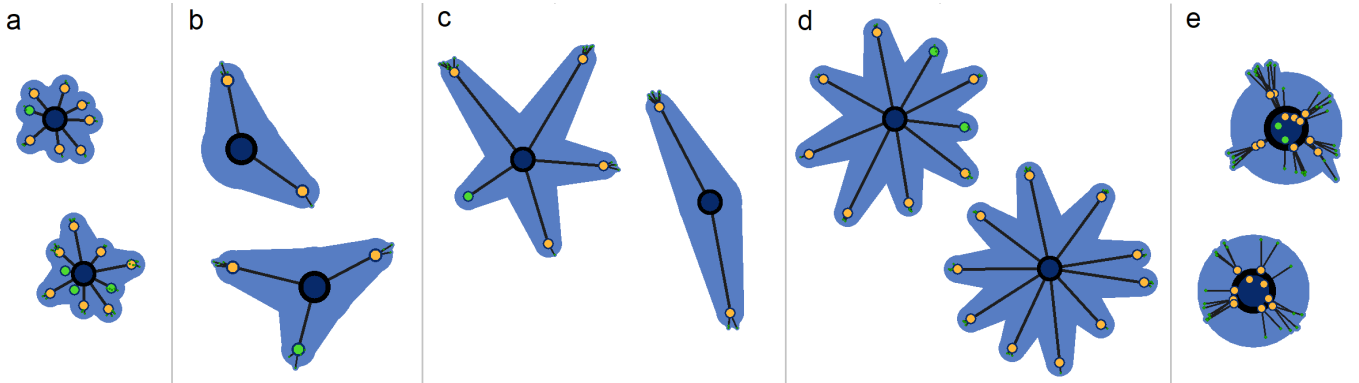
bound to the substrate can be broken if the total tension force exerted on the node exceeds the rupture force. In both cases, when the node disappears, the connecting branch and child nodes also disappear instantly.

### 3 Results

#### 3.1 General cell behaviour

The model aims at genericity, *i.e.* at representing potentially any cell types. A particular cell type can be generated by adjusting the parameters in order to obtain a specific cell shape (from round shape to stellar shape). Specifically, changing the inhibition angle  $\delta\theta_1$  for cell protrusion allows to control the number of simultaneous protrusions that a cell can generate. The size  $l_{10}$  of the  $B_1$  branch controls the size of the actin fibres (bundles or stress fibres) that generate the protrusions. The size  $l_{20}$  of the  $B_2$  branch controls the size of the spikes length to probe the environment. Those are lighter and more motile actin fibres (higher turnover) for faster exploration.

Figure 5 shows some examples of cell shapes that can be generated by the model by changing those parameters (a-e). Increased protrusion number and protrusion length leads to glial-like phenotypes (d), whereas suppressing the formation of actin stress fibres leads to rounder keratocyte-like phenotype (e).



**Figure 5:** Examples of cell shapes that can be generated by the model by changing the inhibition angle  $\delta\theta_1$  for the formation of protrusions and the initial lengths  $l_{10}$  and  $l_{20}$  of the  $B_1$  and  $B_2$  branches respectively. Two snapshots in a sequence of the deforming cell are presented in each case. (a)  $\delta\theta_1 = \pi/6$ ,  $l_{10} = 4\mu\text{m}$ ,  $l_{20} = 2\mu\text{m}$ ; (b)  $\delta\theta_1 = \pi/2$ ,  $l_{10} = 7\mu\text{m}$ ,  $l_{20} = 4\mu\text{m}$ ; (c)  $\delta\theta_1 = \pi/3$ ,  $l_{10} = 14\mu\text{m}$ ,  $l_{20} = 4\mu\text{m}$ ; (d)  $\delta\theta_1 = \pi/6$ ,  $l_{10} = 14\mu\text{m}$ ,  $l_{20} = 2\mu\text{m}$ ; (e)  $\delta\theta_1 = \pi/6$ ,  $l_{10} = 1\mu\text{m}$ ,  $l_{20} = 4\mu\text{m}$ .

For the simulations presented, we will consider an average unspecified cell (fig. 5.b) that we defined based on the following set of predefined constraints:

1. *cell shape:* the observation of isolated cells in two-dimensional cell culture shows that the cell shapes usually exhibit a limited number of main protrusions, rarely exceeding 4 branches (endothelial cells or fibroblasts).

277 We fixed this limit as a first constraint with  $\delta\theta_1 = \pi/2$ . The ability to form membrane spikes to probe the  
278 environment is on the other hand defined by the parameter  $\delta\theta_2$ . We set  $\delta\theta_2 = \pi/6$  to allow a large perimeter  
279 of exploration.

- 280 2. *cell size*: the branches length, used to define  $B_1$  and  $B_2$ , were set to match a protrusion and spike lengths  
281 respectively so that the maximum size of the cell does not exceed  $50\mu m$ . The values taken to remain within  
282 the values of an average cell size are  $l_{10} = 7\mu m$  and  $l_{20} = 4\mu m$ , the latter corresponds to the length of large  
283 filopodia (Husainy et al. 2010).
- 284 3. *cell force generation*: the cell mechanical properties defined by the cell stiffness coefficients ( $\kappa$ ) and adhesion  
285 coefficients ( $\alpha$ ) were set so that the cell can develop the required range of forces, typically around  $50nN$   
286 (Lekka et al. 2021). The progressive increase in force generation associated to the maturation of the adhesions  
287 and fibres is obtained by making these parameters evolve along the 3 maturation states.
- 288 4. *cell motile dynamics*: time parameters such as adhesion production rates ( $v$ ) and adhesion lifespans ( $\tau$ ) con-  
289 tribute to the dynamics of the cell. But more importantly, the adhesion rupture forces ( $F_R$ ) and the threshold  
290 force required for cell translocation ( $F_{th}$ ) determine the level of interaction of the cell with its substrate and  
291 its ability to easily detach in order to move.

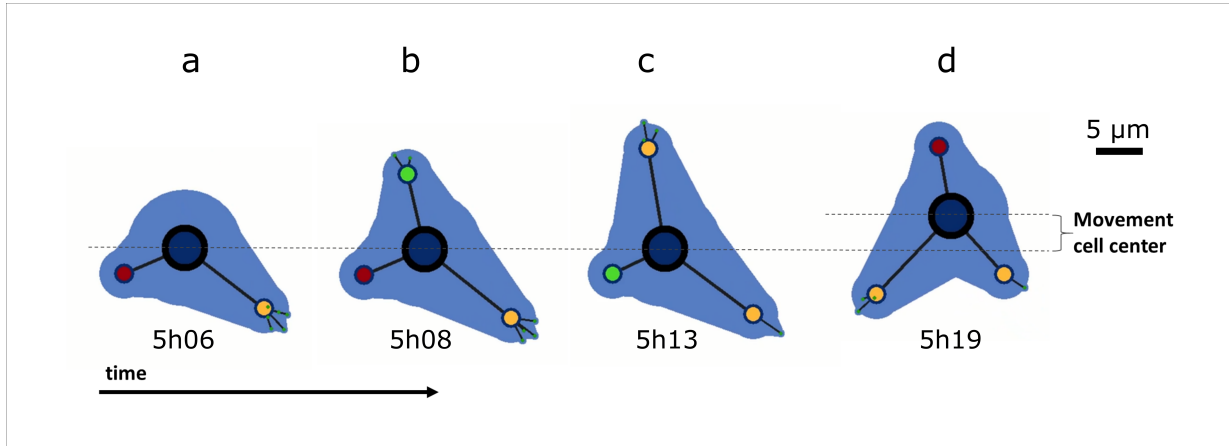
292 A suitable set of parameters that responds to these constraints and to the admissible range of values from the liter-  
293 ature has been determined semi-empirically (see Table 1). The simulation carried out with the above parameters is  
294 presented in figure 6.

295  
296 Figure 6 shows a typical sequence of cell shape changes, in relation with the maturation of the nodes, and  
297 leading to the cell displacement. The simulation exhibits the three different outcomes for the  $N_1$  nodes: (i) a mature  
298 node disappears and is instantly replaced by an immature node (node on the left of the cell, from  $b$  to  $c$ ), (ii) a  
299 node is displaced, but the branch is not sufficiently elongated to reach the maturation criterion (node on the right of  
300 the cell, from  $a$  to  $d$ ), (iii) a node progressively and successfully evolves through the three maturation stages and  
301 eventually the contraction of the mature branch leads to the cell displacement (node on the top of the cell, from  $b$   
302 to  $d$ ).

### 303 *Adhesion dynamics*

304 Cell deformations and migration have been simulated over 72 hours. The different events related to the nodes  
305 dynamics, including maturation and turnover, have been recorded. Figure 7a shows the cause of the  $N_1$  nodes  
306 disappearance: among the 642  $N_1$  nodes generated, 366 (57%) disappear at the intermediate stage as their lifespan  
307 expired and 273 (43%) reach the mature state. Only 20% of the mature  $N_1$  nodes attain their time limit, all the other  
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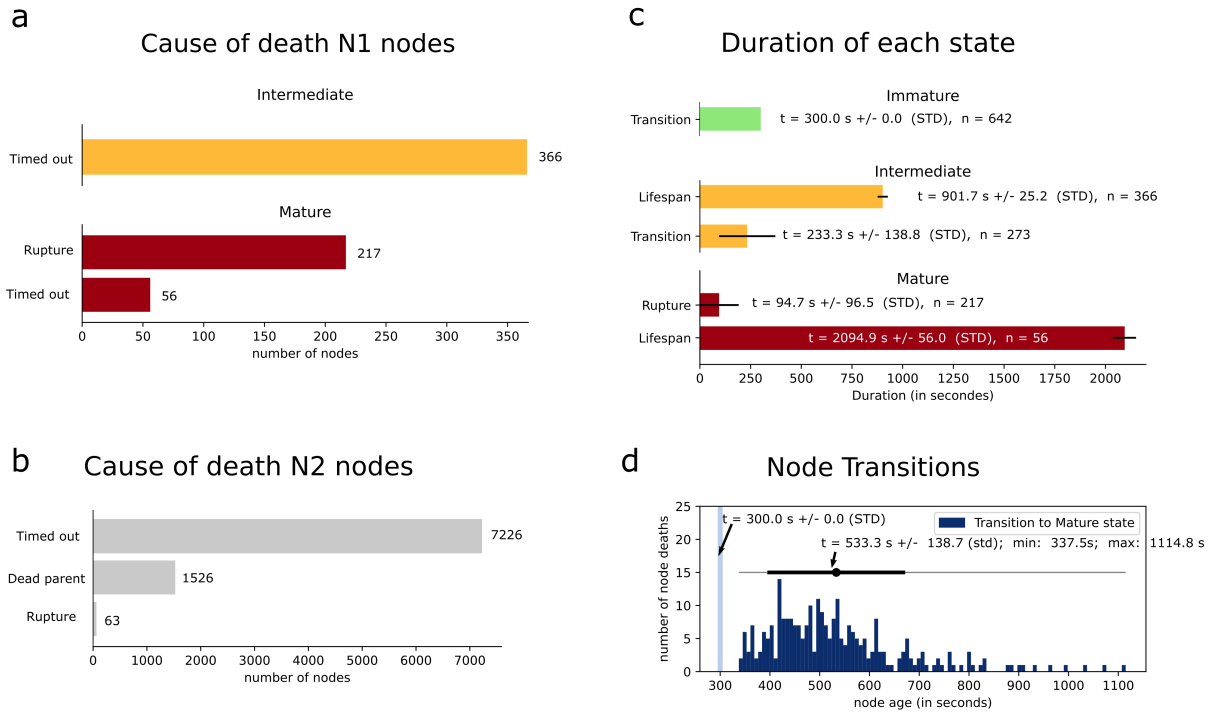


**Figure 6:** Cell deformation and migration observed over a 13 minute period of a long lasting simulation of 72 hours. The cell mainly exhibits a characteristic triangular shape, with dynamical movements of extension/retraction of its protrusions (branches). Colour code for  $N_1$  node states: green for immature, yellow for intermediate and red for mature. The cell envelop in blue - made of connected circles and lines - is represented for cosmetic purpose only, since this model does not describe the cell membrane nor its cytoplasm.

309 mature nodes broke because of the tension forces applied on them. This relatively high contribution of the rupture  
 310 force reveals that only a limited amount of mature adhesions  $N_1$  are strong enough to resist the branch contractility  
 311 as focal adhesions would. If the adhesion is strong enough to support cell translocation the cell will move, if it is not  
 312 strong enough the adhesion will break (rupture). On the other hand, the main cause of death of the 8815 generated  
 313  $N_2$  nodes is the expiration of their lifespan that accounts for 82% of the disappearances (Fig. 7b). Indeed, the role  
 314 of  $N_2$  nodes is to probe the environment dynamically. Their lifespan has been fixed short enough to favour this  
 315 rapid dynamics, but long enough for the  $B_2$  branches to exert tension forces to relocate the  $N_1$  node in the region of  
 316 interest (in the case of a heterogeneous substrate).

317  
 318 The maturation dynamics of the  $N_1$  node is highlighted in figures 7c and 7d. All the 642  $N_1$  nodes formed  
 319 during the 72 hour simulation mature to the intermediate state since this transition is unconditional as soon as the  
 320 node is 300 seconds old. At the intermediate state, the lifespan of the  $N_1$  nodes is around 900 seconds. However, if  
 321 the branch  $B_1$  elongates sufficiently, the node matures and this can occur over a vast period of time (as shown in  
 322 fig 7d) with an average time of  $233 \pm 139s$ , *i.e.* well before reaching the lifespan limit. This ensures that a sufficient  
 323 amount (about 43%) of  $N_1$  nodes will mature. Once maturation is reached, an enhanced force competition - with  
 324 the addition of a contractile force component - takes place in the branch. Rupture of the  $N_1$  nodes occurs on a  
 325 relatively short window period of  $95 \pm 96s$  after the node reaches maturation. The remaining nodes reach their time  
 326 limit of about 2000 seconds.

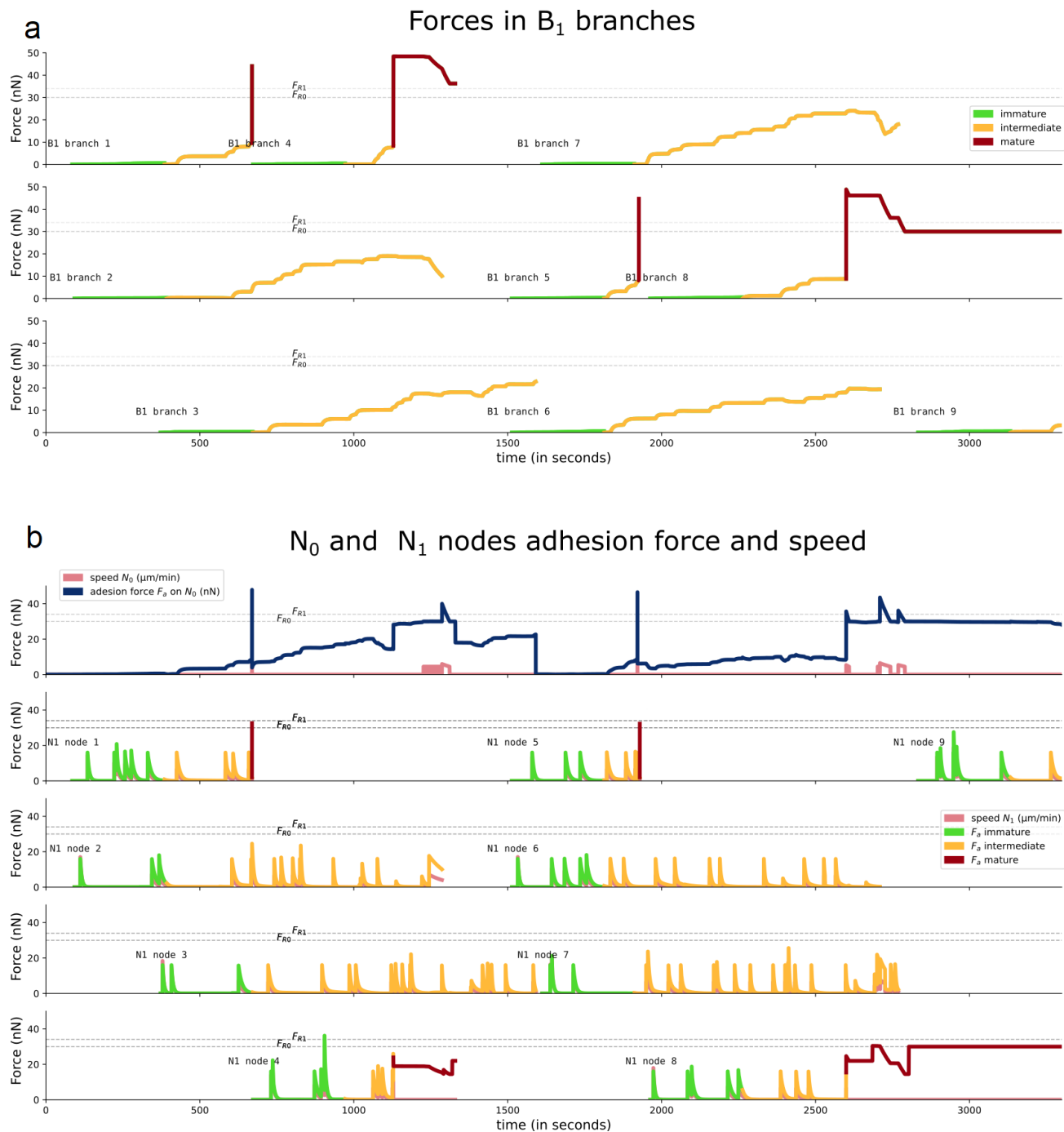
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**Figure 7:** Nodes dynamics for a single cell over a 72 hour simulation. (a) cause of death for the intermediate and mature  $N_1$  nodes; (b) cause of death of the  $N_2$  nodes; (c) duration at which the transitions to the more mature states occur, effective durations of the nodes lifespan and mean duration at which a mature node breaks (STD stands for standard deviation of the mean); (d) recorded  $N_1$  node transition events in function of the node age. All immature to intermediate transitions happened at the age of 300s, while all intermediate to mature transitions happened afterwards. The horizontal grey line spans from the earliest to the latest transition, while the black spot and bar show mean transition age and its standard deviation. We note that the node age is the sum of the times spent in each state. Colour code for  $N_1$  node states: green for immature, yellow for intermediate and red for mature.

### Force generation

Adhesions and branches mature concomitantly. The maturation of the branch is characterized by the progressive increase of the force it can generate on its nodes. Figure 8 displays the evolution of the forces generated in the branches and exerted on the nodes during a sequence of a single cell movement of about one hour in order to highlight how the forces in the branches drive the nodes displacement dynamics and cell migration. In figure 8, the upper graph shows that at the immature stage, the forces in the  $B_1$  branches remain very small despite the linear increase of the elasticity coefficient with time. After 300s the elasticity coefficient of the branch stops evolving and the branch resting length is redefined to its acquired length. These new conditions define the intermediate state of the branch where the force progressively increases, often above 20nN, because of the branch elongation due to the  $N_1$  node displacement under the traction of the  $B_2$  branches (not represented). If the  $B_1$  branch elongation reaches the elongation threshold  $\epsilon_m$  then the  $B_1$  branch attains the mature state and becomes contractile. This acquired contractility generates a jump of about 40nN in the branch force. This value was defined so as to generate a level of force on  $N_0$  sufficiently high to displace this node, *i.e.* for the cell to migrate. In figure 8, the lower graph shows the resulting forces applied by the branches on the nodes. Forces applied on the  $N_1$  nodes appear as a succession of spikes of about 20nN in intensity. This spike profile is explained by the  $N_1$  node displacement that dissipates the resulting force from the  $B_1$  and  $B_2$  branches. Force dissipation is possible as long as  $N_1$  is free to move, *i.e.* when it is in its immature or intermediate states. At the mature state, the  $N_1$  node is bounded to the substrate to resist the contractile force. If the force on the node reaches the threshold  $F_{R1}$  then the node breaks and instantly dissipates the force (two occurrences for node 1 and node 5 in the figure). In both cases the sudden force increase in the branch is sufficient to reach the threshold force  $F_{th}$  on the  $N_0$  node required for the cell to move (*i.e.* non-zero speed) just before the times 750s and 2000s. All other cases of cell displacements (*i.e.* non-zero speed) occurred when at least one branch became contractile to increase the resulting force on the  $N_0$  node above the  $F_{th}$  threshold.



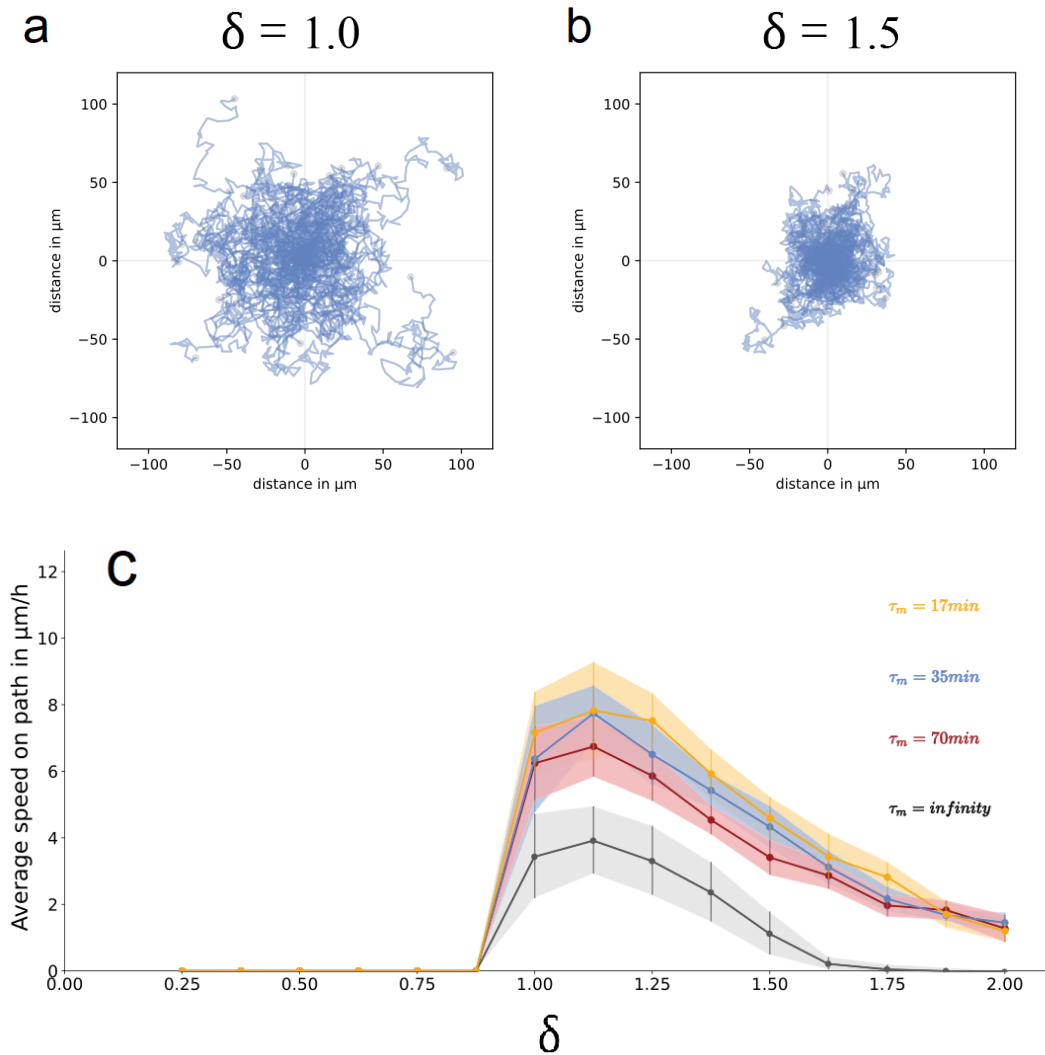
**Figure 8:** Upper graph (a): evolution of the force intensity in the different  $B_1$  branches. The branches are displayed on 3 graphs to avoid overlapping of the co-existing branches. During the one-hour observation period 9 branches are observed with in average 3 co-existing branches. The colour code represents the maturation state of the branch: immature (green), intermediate (yellow), mature (red). Lower graph (b): evolution of the intensity of the resulting forces applied on the  $N_0$  (first row) and  $N_1$  nodes (rows 2 to 4). The  $N_1$  nodes are displayed on 4 graphs to avoid overlapping of the co-existing nodes. During the one-hour observation period 9,  $N_1$  nodes were observed. The colour code represents the maturation state of the  $N_1$  node: immature (green), intermediate (yellow), mature (red). The horizontal grey dotted lines in each graph represent the force thresholds  $F_{R0} = F_{th}$  for  $N_0$  to move and  $F_{R1}$  for  $N_1$  to break.

### 3.2 Cell migration on a homogeneous substrate

To test the model we first perform cell migration simulations on a homogeneous substrate. Figure 9 (upper left graph) presents the superimpositions of 50 single cell trajectories recorded over 72 hours. The homogeneous distribution of the trajectories is coherent with a typical random migration behaviour, meaning that our model does not introduce any migration bias. We then tested the influence of the attachment force of the cell to the substrate. The cell attachment force corresponds to the threshold forces triplet  $(F_{th}, F_{R1}, F_{R2})$  that can change depending on the biochemical nature of the substrate, *i.e.* on the cell matrix fibres composition, characterized by the amount of ligands and/or the strength of the adhesive bonds with which the cell can interact. To change the cell-substrate affinity, *i.e.* the cell attachment force, we introduce the dimensionless parameter  $\delta$  to modulate the force magnitude as  $\delta \times (F_{th}, F_{R1}, F_{R2}) = (\delta F_{th}, \delta F_{R1}, \delta F_{R2})$ . We then compare  $\delta = 1$  which is the reference simulation with  $\delta = 1.5$  which means a 50% increase of the attachment force. Figure 9 (upper right graph) shows that the cell exploration zone is significantly reduced while maintaining its random migration characteristic.

If  $\delta$  is changed from 0.25 to 2.0, the average cell speed evolves with a bell shape (Fig.9, lower graph) which is in agreement with experimental facts (Palecek et al. 1997). For  $\delta < 1$  the cell remains unable to move since the attachment force is too weak for the cell to adhere to the substrate. The level of forces developed by the branches systematically break the adhesions that cannot strengthen and reach maturation to allow the cell to move. Once the attachment force is strong enough for  $\delta \geq 1$  then the cell can move. For  $\delta = 1.125$  the average cell speed reaches its maximum close to  $8\mu\text{m}/\text{h}$ , and decreases progressively for increasing values of  $\delta$ . When the attachment force is higher, then the cell adhesions reach maturation however the forces developed in the branches are not high enough to reach the cell translocation threshold which limits the cell migration.

Since the level of force for cell translocation can only be reached once the  $N_1$  adhesion is mature, we further tested the influence of the mature  $N_1$  adhesion lifespan  $\tau_m$  (Fig.9, lower graph). As expected, when the adhesion lifespan is shorter ( $\tau_m/2 = 17\text{min}$ ), there is a higher turnover of the adhesions which allows the cell to move more often, thus increasing the average migration speed. On the other hand when the lifespan is longer ( $2 \times \tau_m = 70\text{min}$ ), then the cell adhesion and its associated branch which does not reach the translocation threshold force remains stuck until the adhesion is released as it reaches its time limit. This is slowing down the cell migration speed. In the extreme case where the adhesion lifespan  $\tau_m$  is infinite, the only way to break the adhesion is for the associated branch to reach the translocation threshold. The lack of adhesion turnover reduces drastically the average migration speed by a factor two (the maximum speed is  $4\mu\text{m}/\text{h}$ ), however the cell remains able to migrate.



**Figure 9:** Upper graphs: Cell trajectories on a homogeneous substrate for two cell detachment conditions  $\delta = 1.0$  (a) and  $\delta = 1.5$  (b). Fifty trajectories of a single cell moving for 72 hours are superimposed in each graph. Lower graph (c): Average cell speeds as a function of the parameter  $\delta$  representing the cell detachment condition. Each point of the curves corresponds to 10 trajectories of a single cell moving for 12 hours. Each coloured curve corresponds to a different value for  $\tau_m$ , the lifespan of mature  $N_1$  nodes.  $\tau_m = 35$  min is the reference simulation used in the two upper graphs.

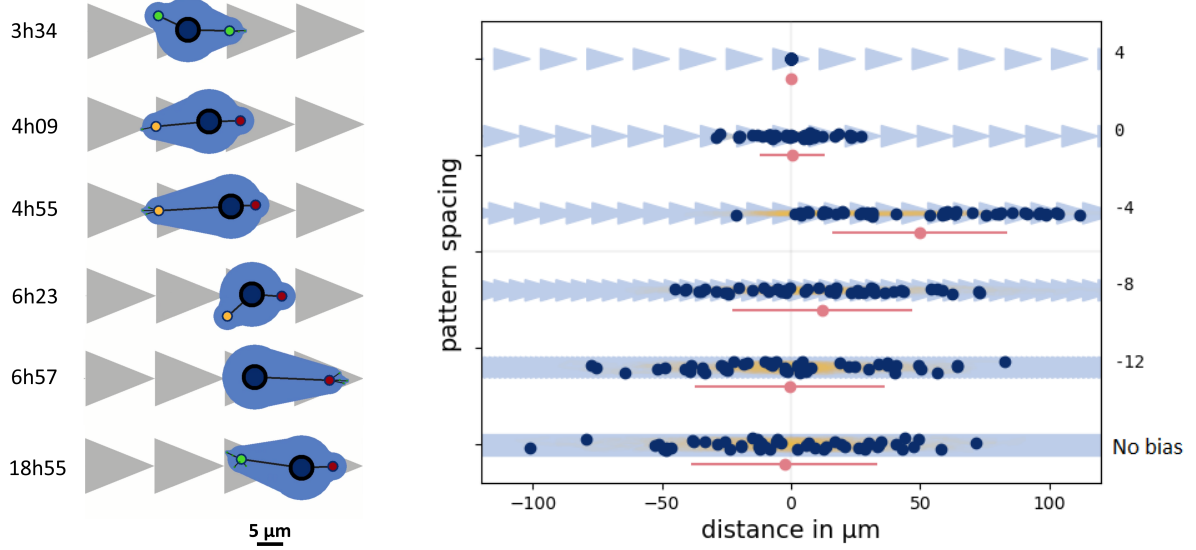
### 3.3 Constrained cell migration on adhesive patterns

Engineered adhesive patterns, typically coated with fibronectin, are often used to constrain the cell shape in order to study the resulting cytoskeletal organization. They can also be used to investigate the factors influencing the cell displacements. Inspired by the study of Lo Vecchio et al. (2020), we challenged our cell model by constraining the cell migration on a stripe of adhesive triangular patterns. By varying pattern spacing, the aim was to determine if the pattern can favour a migration direction on our virtual cell. The triangular shape presents an adhesive asymmetry for the cell surface adherence between the left and the right side of the patterns. The question is *will the cell follow the arrows ?*

For the simulations, the nodes  $N_1$  and  $N_2$  can only form if in contact with the pattern. Each triangle of the pattern is  $10\mu\text{m}$  high and  $20\mu\text{m}$  long (Fig. 10). The gap distance between consecutive triangles is set for each simulation. It varies from  $-12\mu\text{m}$  to  $4\mu\text{m}$  with an increment of  $4\mu\text{m}$  from one simulation to another. A negative value of the gap distance means that the triangles overlap with this length. We also considered a pattern of reference with no bias, corresponding to a continuous adhesive stripe.

Figure 10 (left) exhibits a sequence of cell movements and displacements on the adhesive pattern with a gap distance between consecutive triangles that is equal to zero. The cell forms branches that can reach the triangles on the left or on the right. However the inhibition condition for node and branch formation implemented in the model (see Fig. 2), limits the formation of a single branch per triangle (the triangle is not big enough to accommodate two  $N_1$  nodes). As a consequence, the direction for the cell displacement is not the resultant of the force competition between left and right, since the forces tend to equilibrate with one branch on each side. On the other hand it mostly depends on the probability to form some adhesions, which is directly related to the differential length of the arcs corresponding to the intersection of the circle of radius  $l_{01}$  with the adhesive surface at each side of the cell. This is a strong difference with the experiments of Lo Vecchio *et al.* (2020) where the differential quantities between both sides of the cell is the adhesion area. Indeed the longer the arcs does not mean/correspond to the largest adhesion area.

To compare our theoretical results with the experimental results of Lo Vecchio *et al.* (2020) we used the same representation where the respective percentage of cells ending their trajectories on the left (-) or right side (+) from their initial position is plotted as a function of the gap distance between the triangular patterns (Fig. 11.a). As in the experiments, the bias towards the positive direction tends to increase with the gap distance. But whereas in the experiments, the negative direction is selected for small gap distances, it is never the case in our simulations. The



**Figure 10:** Left graph: cell displacement on triangular adhesive patterns. Right graph: each single cell was left to move on the pattern and its position after 72 hours is represented by a dark blue dot. The positions of 50 cells are displayed for each pattern spacing value. The average position and standard mean displacement are respectively represented by a red dot and line. The simulations are reproduced for different triangular pattern spacing from  $4\mu\text{m}$  until there is a continuous adhesive strip (meaning no direction bias).

414 main reason is that our model is less sensitive to the substrate since, as explained above, the  $N_1$  nodes form at a  
 415 fixed distance from the cell centre and do not exploit the whole adhesion area available.

416  
 417 To quantify the cell migration properties, Lo Vecchio et al. (2020) proposed the calculation of a coefficient  $p$   
 418 informing on the direction bias of the cell trajectory. This coefficient is calculated from the quantities  $N_+$  and  $N_-$   
 419 that correspond respectively to the number of steps made by the cell in the positive direction (following the tips of  
 420 the triangles)  $N_+$  and in the negative direction (opposite direction)  $N_-$  during a 48 hour sequence of migration:

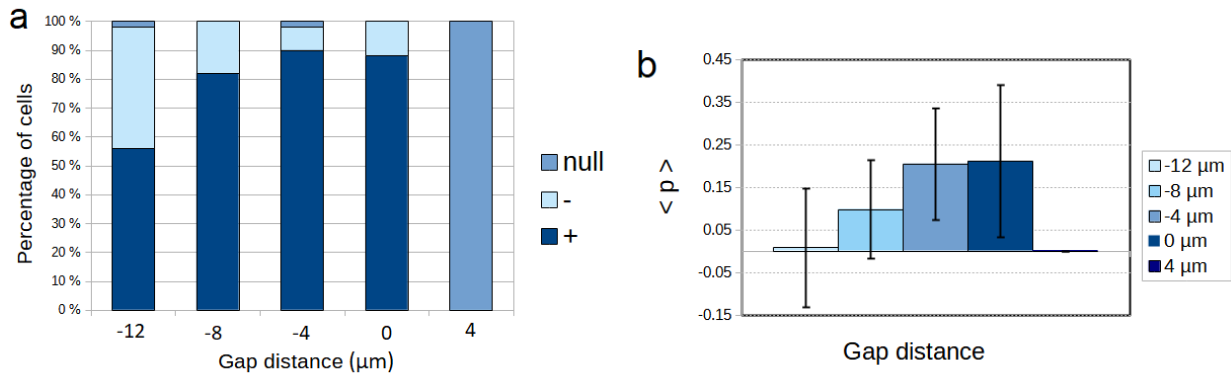
$$p = \frac{N_+ - N_-}{N_+ + N_-} \quad (15)$$

421 The difference with the previous graph is that all the migration steps are exploited and not just the terminal  
 422 position of the cell (Fig 11.b). The figure confirms that the bias increases with the gap distance, whereas in the  
 423 experiments the increase is initially small and gets bigger with the gap distance, our simulations show the opposite  
 424 with an initially high increase that saturates. Again this is due to the fact that experimentally the cell is able to  
 425 stretch itself to attain the next distant pattern. In the model the protrusion is emitted at a constant length hence there  
 426 is a saturation effect.



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As a consequence, the model is qualitatively valid but is not refined enough to be quantitatively accurate. The limitation related to the constant length of emitted protrusions, can however be easily addressed by allowing the formation of  $N_1$  nodes at distances from the cell centre determined by a normal distribution in a future implementation of the model.



**Figure 11:** Left graph (a): percentage of cells ending their trajectories in the left side (-), right side (+) or same position (null) from their initial position, as a function of the gap distance. Right graph (b): average direction bias of the cell trajectories ( $\langle p \rangle$ ) as a function of the gap distance. Measurements in both graphs were made from 50 cells migrating for 48 hours.

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## 4 Discussion

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The model developed was aimed to address cell sensing of the substrate and how this influences cell migration dynamics. The model was designed to be generic enough in order to be adjusted to match the characteristics of a large variety of cell types in terms of morphology and migration velocity. It can thus potentially describe any type of cell including fibroblasts, macrophages, keratocytes, glial cells and can also be tuned to match tumor cells.

For this, a number of key parameters need to be defined. The model was designed so as to keep the number of parameters as small as possible, by identifying dependencies between the parameters of a same type (temporal parameters, force parameters, *etc.*) It is then relatively easy, to determine the parameters in a semi-empirical way, based on the admissible range of values from the literature and on a set of observables that defines the cell type of interest.

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In the simulations presented we chose to model an unspecified cell to better highlight the full model potential. The structural and mechanical model parameters were defined based on the following observables that are used as model constraints : (1) the cell size should not exceed  $50\mu\text{m}$  ; (2) the number of cell branches was limited to 4; (3) the fibres elasticity coefficient and adhesion coefficient were set so that the cell can develop a range of forces

446 around  $50nN$ ; (4) the adhesion lifespans and rupture forces depend on the interaction of the cell with its substrate.  
447 They were determined so that the cell dynamical behaviour is compatible with available experimental observations  
448 regarding the cell velocity.

449 The first two constraints determine the cell morphology. The size is set by the branch length parameters, and  
450 the shape by the exclusion angle between branches. If the exclusion angle is large, the branches formed will be  
451 spaced and the cell will take a more starlike shape compatible with glial cells or fibroblasts. On the other hand  
452 if the exclusion angle is small the cell will have a more rounded shape compatible with keratocytes for example.  
453 The third and fourth constraints defined the adhesion turnover, i.e. the cell dynamics where the migration velocity  
454 highly depends on the nature of the substrate (adhesivity and rigidity in our example).

455 The cell model was first tested by considering a homogeneous substrate. As expected, no directional bias was  
456 observed. However, the adhesion conditions clearly impacted the cell movements. More specifically the adhesion  
457 velocity evolves with the increase of the adhesion strength in a biphasic manner, thus reproducing the well known  
458 experimental bell-shape curve (Palecek et al. 1997). The velocity first increases with the adhesion strength to  
459 reach an optimum above which the cell migration is refrained. We showed that this is additionally modulated by  
460 the adhesion lifespan ( $\tau_m$ ). This needs to be finely tuned so that maturation has time to develop while ensuring  
461 adequate adhesion turnover.

462 The model was then challenged with a non-homogeneous substrate based on the experiments by Lo Vecchio  
463 et al. (2020) that uses asymmetrical adhesive patterns. As in the experiments, the model reproduces the observed  
464 migration bias. However quantitative differences were obtained and exhibit some model limitations. Indeed in  
465 the current model implementation, the cell protrusions (branches) are emitted with a constant length that do not  
466 allow to explore the entire adhesive surface offered to the cell. However this limitation can easily be addressed by  
467 relaxing this condition and by defining the branch length in a normal distribution.

468 The strength of the model resides in its simplicity which allows to describe a wide range of cell morphologies  
469 and dynamics in relation with the environmental properties thanks to the implementation of cell sensing. The  
470 dynamic nature of the probing filopods associated to the maturation of focal adhesions makes this model particularly  
471 well adapted to explore further the cell-matrix interactions through more complex coupling including matrix rigidity  
472 gradient, deformable visco-elastic matrices, anisotropic matrix topography through matrix fibre orientations, *etc.*  
473 Moreover the model was designed to be easily generalizable in 3D. Structurally, this only requires the addition of an  
474 azimuthal angle to define the branch position in the 3D space. Cell migration in a 3D environment requires the cell  
475 to move through a dense network of fibres. In order to overcome the steric hindrance, the cell can either squeeze  
476 through the pores of the matrix network, or degrade the matrix to create the space needed for its migration. Our  
477 model can easily address cell squeezing since the only physical part of the cell in the model is the cell nucleus, the

478 membrane is not explicitly described. The deformation of the nucleus from a sphere to an ellipsoid of equivalent  
479 volume is straightforward and only involves setting a threshold for the allowed deformation associated with the  
480 mechanical properties of the nucleus, as a first approximation. Regarding matrix degradation, the cell secretion of  
481 matrix degrading enzymes such as matrix metalloproteases (MMPs) and their diffusion are necessary. This implies  
482 including a partial differential equation to address this aspect, which we have already considered in a previous 2D  
483 model (Stéphanou et al. 2015).

484 In its current implementation the model allows for fast simulations. One hour of a single cell computation  
485 is performed in 0.2 second without visualization. The biggest performance hits were caused by recording the  
486 amount of data onto the disk. However this can be optimized. In addition, parallel optimization could be further  
487 implemented to process high cell count simulations in real time or even faster. The recorded computation speed  
488 suggests that the model could be implemented to study various multi-cellular phenomena such as morphogenesis,  
489 tissue patterning and angiogenesis, which were previously performed via grid dependent modelling schemes. These  
490 simulations could therefore include a few hundred or even a few thousand cells, while delivering results within a  
491 reasonable time frame. In addition, the simplicity of the modelling paradigm makes it flexible enough to be adapted  
492 to various scenarii, which makes it a promising modelling framework for the future.

Parameter	Description	Value	unit	Ref.
<i>Cell shape</i>				
$\delta\theta_1$	inhibition angle for $N_1$	$\pi/2$	-	
$\delta\theta_2$	apparition angle for $N_2$	$\pi/6$	-	
$l_{10}$	initial length of $B_1$	7	$\mu\text{m}$	
$l_{20}$	initial length of $B_2$	4	$\mu\text{m}$	
<i>Adhesion dynamics</i>				
$v_1$	production rate of $N_1$	0.01	$s^{-1}$	(1)
$v_{2im}$	production rate of $N_2$ from immature $N_1$	0.015	$s^{-1}$	
$v_{2int}$	production rate of $N_2$ from intermediate $N_1$	0.015	$s^{-1}$	
$v_{2m}$	production rate of $N_2$ from mature $N_1$	0.00001	$s^{-1}$	
$\tau_t$	duration of $N_1$ immature state	5	min	
$\tau_{int}$	lifespan of intermediate $N_1$	15	min	
$\tau_m$	lifespan of mature $N_1$	35	min	(2)
$\tau_2$	lifespan of $N_2$	200	s	
<i>Substrate interaction</i>				
$\alpha_0$	friction coefficient of $N_0$	400	$nN \cdot s / \mu\text{m}$	
$\alpha_{10}$	initial friction coefficient of $N_1$	50	$nN \cdot s / \mu\text{m}$	
$\alpha_{int}$	friction coefficient of intermediate $N_1$	150	$nN \cdot s / \mu\text{m}$	
$\alpha_m$	friction coefficient of mature $N_1$	165	$nN \cdot s / \mu\text{m}$	
$\alpha_2$	friction coefficient of $N_2$	8	$nN \cdot s / \mu\text{m}$	
$F_{th}$	Threshold force for $N_0$ displacement	30	nN	(3)
$F_{R1}$	Rupture force of $N_1$	34	nN	
$F_{R2}$	Rupture force of $N_2$	17	nN	
<i>Actin branch property</i>				
$\epsilon_m$	maturation Cauchy strain of $B_1$	0.5	-	
$\epsilon_{20}$	initial Cauchy strain of $B_2$	0	-	
$\kappa_{10}$	initial stiffness of $B_1$	0.1	$nN / \mu\text{m}$	
$\kappa_{11}$	stiffness of $B_1$	3.5	$nN / \mu\text{m}$	(1)
$\kappa_2$	stiffness of $B_2$	12	$nN / \mu\text{m}$	
$\gamma_{max}$	contractility of $B_1$	40	nN	(4)
$F_\gamma$	force constant	30	nN	

**Table 1:** Model parameters. Cell shape parameters:  $\delta\theta_1$  and  $\delta\theta_2$  allow to define the maximum number of cell protrusions and the width of the exploration area by the filopods respectively;  $l_{10} + l_{20}$  define the maximum length of the cell membrane extension. The adhesion dynamics is defined from the production rates  $v$  and lifespans  $\tau$  of each adhesion types. They are hierarchically define depending on the adhesion type and maturation level. Since the extracellular matrix is not explicitly represented, the cell interaction with the substrate is defined through the friction coefficients  $\alpha$  and rupture forces  $F$ . The actin branch properties are defined by the Cauchy strains  $\epsilon$ , stiffnesses  $\kappa$  and contractility  $\gamma$ . The parameter values have been semi-empirically defined to generate a given cell shape and migration dynamics in agreement with range of values from the literature: (1) Zhu & Mogilner (2016); (2) Stehbens & Wittmann (2014), Oakes & Gardel (2014); (3) Lekka et al. (2021); (4) Boys & Owens (2021).

## 493 **Declarations**

### 494 **Ethical Approval**

495 Not applicable.

### 496 **Competing interests**

497 Not applicable.

### 498 **Authors' contributions**

499 All authors contributed in the model development. I.M. made the simulations and prepared the figures. A.S. wrote  
500 the manuscript. A.C., C.V., G.C. and I.C. reviewed the manuscript.

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### 507 **Availability of data and materials**

508 The code is publicly available at the following GitLab repository :

509 <https://gricad-gitlab.univ-grenoble-alpes.fr/stephana/cell-motility>

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