Cell-cell adhesion and 3D matrix confinement determine jamming transitions in breast cancer invasion

Supplementary Information: Model definition and simulation study

Olga Ilina[1], Pavlo G. Gritsenko[1], Simon Syga[2], Jürgen Lippoldt[3], Caterina A. M. La Porta[4, 5, 6], Oleksandr Chepizhko[7], Steffen Grosser[3], Manon Vullings[1], Gert-Jan Bakker[1], Jörn Starruss[2], Peter Bult[8], Josef A. Käs[3], Stefano Zapperi[4, 9, 10], Andreas Deutsch[2], and Peter Friedl[1, 11, 12]

[1]Department of Cell Biology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands
[2]Department of Innovative Computing, Centre for Information Services and High Performance Computing, Technische Universität Dresden, Dresden, Germany
[3]Peter Debye Institute for Soft Matter Physics, Leipzig University, Leipzig, Germany
[4]Center for Complexity and Biosystems, University of Milan, via Celoria 16, 20133 Milano, Italy
[5]Department of Environmental Science and Policy, University of Milan, via Celoria 26, 20133 Milano, Italy
[6]CNR - Consiglio Nazionale delle Ricerche, Istituto di Biofisica, via Celoria 26, 20133 Milano, Italy
[7]Institut für Theoretische Physik, Leopold-Franzens-Universität Innsbruck, Technikerstrasse 21a, A-6020 Innsbruck, Austria
[8]Department of Pathology, Radboud University Medical Center, Nijmegen, The Netherlands
[9]Department of Physics, University of Milan, via Celoria 16, 20133 Milano, Italy
[10]CNR - Consiglio Nazionale delle Ricerche, Istituto di Chimica della Materia Condensata e di Tecnologie per l'Energia, Via R. Cozzi 53, 20125 Milano, Italy
[11]David H. Koch Center for Applied Genitourinary Cancers, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA
[12]Cancer Genomic Centre, 3584 CG Utrecht, The Netherlands

Correspondence to: peter.friedl@radboudumc.nl; pfriedl@mdanderson.org

1 Model definition

1.1 The lattice-gas cellular automaton

We develop a stochastic, spatio-temporal cell-based model to study the plasticity of cancer cell invasion under conditions of varying E-cadherin expression and varying confinement by the extracellular matrix (ECM). This allows us to incorporate single cell behavior that depends on the local, spatial micro-environment and to account for microscopic fluctuations that reflect cellular and micro-environmental heterogeneity. In particular, we define a spatially and temporally discrete model, namely a lattice-gas cellular automaton (LGCA). LGCA were originally introduced to simulate fluid flow [1–3], but have since been proven useful to study biological systems [4]. LGCA models are particularly well-suited to investigate cell-cell and cell-ECM interactions orchestrating collective cell migration [5–7]. Further, LGCA facilitate a rigorous parameter analysis.

The LGCA model is defined on a d-dimensional regular lattice $\mathcal{L} \subset \mathbb{R}^d$ with reflecting boundary conditions. Each lattice node $\mathbf{r} \in \mathcal{L}$ is connected to its b nearest neighbors by unit vectors $\mathbf{c}_i, i = 0, \dots, b-1$, called *velocity channels*. The distance between nearest neighbors is given by the *lattice spacing* $\varepsilon > 0$. Here, we choose a two-dimensional hexagonal lattice, representing the interface plane in the in vitro experiments. Therefore the velocity channels are given by

$$\mathbf{c}_j = \begin{pmatrix} \cos\frac{2\pi j}{b} \\ \sin\frac{2\pi j}{b} \end{pmatrix}, \quad j = 0, \dots, b-1. \tag{1}$$

In addition to the velocity channels, there are a channels with zero velocity called *rest channels*. Each channel can be occupied by at most one cell at a time. The total number of channels per node is called the capacity $K := a + b$. In this work we choose $a = 3$. We denote the state of a node $\mathbf{r}$ by a vector $\mathbf{s}(\mathbf{r}) = \big(s_0(\mathbf{r}), s_1(\mathbf{r})\big), \dots, s_{K-1}(\mathbf{r})) \in \{0,1\}^K$, where s_i are Boolean variables with $s_i = 1$ if channel $\mathbf{c}_i$ is occupied and $s_i = 0$ if $\mathbf{c}_i$ is not occupied. Consequently, the local cell number is given by

$$n(\mathbf{s}(\mathbf{r})) := \sum_{i=0}^{K-1} s_i(\mathbf{r}). \tag{2}$$

The dynamics of a state at node $\mathbf{r} \in \mathcal{L}$ is influenced by the nodes in the *interaction neighborhood* $\mathcal{N}(\mathbf{r})$. The neighborhood $\mathcal{N}(\mathbf{r})$ of all nodes is defined using a *neighborhood template* $\mathcal{N} := \mathcal{N}(0)$ denoting the interaction neighborhood of node $\mathbf{r} = 0$, so that $\mathcal{N}(\mathbf{r}) := \mathcal{N} + \mathbf{r} = \{\mathbf{r}' + \mathbf{r} | \mathbf{r}' \in \mathcal{N}\}$. Here, we choose an R-radial neighborhood $\mathcal{N}_R$, defined as

$$\mathcal{N}_R := \mathcal{N}_R(0) = \left\{\mathbf{r}' \in \mathcal{L}, ||\mathbf{r}'||_2 \leq R\right\}, \quad R > 0, \tag{3}$$

where the radius is set to double the lattice spacing $R = 2\varepsilon$.

The time evolution is defined in discrete time steps $k \in \mathbb{N}$, which we map to real time

by introduction of a time step length $\tau > 0$. In each time step a sequence of *rearrangement* and *propagation* rules is applied at all nodes simultaneously. The stochastic rearrangement rule accounts for the relevant cell-cell and cell-ECM interactions, and updates the state $\mathbf{s} \to \mathbf{s}'$ with a probability of the form

$$P(\mathbf{s} \to \mathbf{s}'|\mathbf{s}_\mathcal{N}, \rho_{\mathrm{ECM}}) = \frac{\delta\left(n(\mathbf{s}), n(\mathbf{s}')\right)}{Z} e^{E(\mathbf{s}', \mathbf{s}_\mathcal{N}, \rho_{\mathrm{ECM}})}, \tag{4}$$

where $\mathbf{s}_\mathcal{N} = \big(\mathbf{s}(\mathbf{r})\big)_{\mathbf{r}\in\mathcal{N}}$ denotes the states in the neighborhood, the Kronecker delta assures mass conservation and $Z = \sum_{\mathbf{s}'} e^{E(\mathbf{s}', \mathbf{s}_\mathcal{N}, \rho_{\mathrm{ECM}})}\delta(n, n')$ is a normalization constant. The term $E(\mathbf{s}', \mathbf{s}_\mathcal{N}, \rho_{\mathrm{ECM}})$ weights the states $\mathbf{s}'$ according to the chosen interactions that depend on cells in the neighborhood $\mathcal{N}$ and the local ECM confinement ρ_{ECM}, see next section. The definition of the chosen interaction rule can be found in subsection 1.3. Finally, during the propagation step, cells residing in velocity channels are deterministically transported to the respective next-nearest neighbor node,

$$s_i(\mathbf{r} + \mathbf{c}_i, k + 1) = s_i'(\mathbf{r}, k). \tag{5}$$

In summary the time evolution from time k to time $k + 1$ is given by

$$\mathbf{s}(\mathbf{r}, k) \xrightarrow{\text{Rearrangement}} \mathbf{s}'(\mathbf{r}, k) \xrightarrow{\text{Propagation}} \mathbf{s}(\mathbf{r}, k + 1). \tag{6}$$

1.2 The ECM

The aim of this work is to study the effect of a varying ECM density on tumor cell invasion. To be able to compare model predictions to experiments, we mimic the interface invasion assay, in which tumor cells migrate into the gap between a collagen-coated glass surface and a collagen network of varying density. Therefore, we introduce the ECM as a scalar field $\rho_{\mathrm{ECM}}(\mathbf{r}) > 0$ defined on the LGCA lattice, representing the local confinement by the collagen network. As invading cancer cells open up the gap between collagen-coated interface and the overlying collagen network, we assume that during the LGCA interaction step, confinement is decreased on lattice nodes that are occupied by cells. In particular, we assume a linear relationship between the change in confinement and the cell number at node $\mathbf{r}$

$$\Delta\rho_{\mathrm{ECM}}(\mathbf{r}, k) := \rho_{\mathrm{ECM}}(\mathbf{r}, k + 1) - \rho_{\mathrm{ECM}}(\mathbf{r}, k) = -\alpha\rho_{\mathrm{ECM}}(\mathbf{r}, k)\rho_{\mathrm{cells}}(\mathbf{r}, k), \tag{7}$$

where $0 \leq \alpha \leq 1$ is a parameter that determines how fast cells can open up the gap, and $\rho_{\mathrm{cells}}(\mathbf{r}, k) := \frac{n(\mathbf{r},k)}{K}$ is the local cell density.

1.3 Interactions

In this subsection the rearrangement rule is defined. Cells are stochastically rearranged within channels on a node with a probability that accounts for cela new mechal-cell and

cell-ECM interactions according to Equation 4. Thereby, the weight $E(\mathbf{s}', \mathbf{s}_\mathcal{N}, \rho_{\text{ECM}})$ of a new configuration $\mathbf{s}'$ is determined by the sum of three independent interactions: steric interactions between cells, adhesive interactions between cells, and cell-ECM interactions,

$$E(\mathbf{s}', \mathbf{s}_\mathcal{N}, \rho_{\text{ECM}}) = E_{\text{steric}}(\mathbf{s}', \mathbf{s}_\mathcal{N}) + E_{\text{adhesion}}(\mathbf{s}', \mathbf{s}_\mathcal{N}) + E_{\text{ECM}}(\mathbf{s}', \rho_{\text{ECM}}). \tag{8}$$

1.3.1 Steric interactions

We mimic steric interactions using a term that repels cells if the local cell density exceeds the homeostatic cell density ρ_0. This is obtained by the following configuration weight

$$E_{\text{steric}}(\mathbf{s}', \mathbf{s}_\mathcal{N}) := -\beta_{\text{steric}} \mathbf{j}(\mathbf{s}') \cdot \mathbf{g}_{\text{steric}}(\mathbf{s}_\mathcal{N}), \tag{9}$$

where $\beta_{\text{steric}} > 0$ is a positive parameter, setting the strength of the steric interaction,

$$\mathbf{j}(\mathbf{s}') := \sum_i \mathbf{c}_i s'_i \tag{10}$$

is the cell flux after rearrangement, and

$$\mathbf{g}_{\text{steric}}(\mathbf{s}_\mathcal{N}) := \sum_{i=0}^{b-1} \mathbf{c}_i \tilde{\rho}\left(\mathbf{s}(\mathbf{r} + \mathbf{c}_i)\right) \tag{11}$$

is the cell density gradient after subtracting the homeostatic density ρ_0,

$$\tilde{\rho}(\mathbf{s}) := \begin{cases} \frac{n(\mathbf{s}) - \rho_0}{K - \rho_0} & \text{for } n(\mathbf{s}) > \rho_0, \\ 0 & \text{else.} \end{cases} \tag{12}$$

1.3.2 ECM confinement

We assume that confinement by the ECM increases the friction for moving cells, thereby slowing them down. This is realized by an increased weight for cells residing in rest channels,

$$E_{\text{ECM}}(\mathbf{s}', \rho_{\text{ECM}}) := \sum_{i=b}^{K-1} \rho_{\text{ECM}} s'_i. \tag{13}$$

1.3.3 Cell-cell adhesion

We assume that there are two main effects of cell-cell adhesion. First, we assume that there is an optimal number of neighbors n_{crit} that is energetically favored by the

physical interactions between cells. Therefore we apply a rearrangement weight that maintains this optimal number of neighbors, corresponding to the homeostatic cell density. Additionally, we assume that cells that interact adhesively are mechanically coupled, effectively resulting in cells synchronizing their movement. Even though both mechanisms are a result of adhesive cell interactions, we introduce them independently in the model as

$$E_{\text{adhesion}}(\mathbf{s}', \mathbf{s}_{\mathcal{N}}) = E_{\text{aggregation}}(\mathbf{s}', \mathbf{s}_{\mathcal{N}}) + E_{\text{alignment}}(\mathbf{s}', \mathbf{s}_{\mathcal{N}}). \tag{14}$$

The first term $E_{\text{aggregation}}$ maintains the homeostatic cell density ρ_0, and corresponds to an adaption of a continuous model for cell-cell adhesion [8]. It is defined as

$$E_{\text{aggregation}}(\mathbf{s}', \mathbf{s}_{\mathcal{N}}) = \beta_{\text{ag}} \mathbf{j}(\mathbf{s}') \cdot \mathbf{g}_{\text{ag}}(\mathbf{s}_{\mathcal{N}}), \tag{15}$$

where β_{ag} is a positive parameter, $\mathbf{j}(\mathbf{s}')$ is the post-rearrangement flux, defined in Equation 10,

$$\mathbf{g}_{\text{ag}}(\mathbf{s}_{\mathcal{N}}) := \sum_{i=0}^{b-1} \mathbf{c}_i u(\mathbf{s}_{\mathcal{N}}), \tag{16}$$

is the discrete gradient of a logistic potential

$$u(\mathbf{s}_{\mathcal{N}}) := \frac{1}{2n_{\text{crit}}} n_{\text{nb}}(\mathbf{s}_{\mathcal{N}}) \left(1 - \frac{n_{\text{nb}}(\mathbf{s}_{\mathcal{N}})}{n_{\text{crit}}}\right)^{+}, \tag{17}$$

that depends on the number of neighbors,

$$n_{\text{nb}}(\mathbf{s}_{\mathcal{N}}) = \sum_{i=0}^{b-1} n(\mathbf{s}(\mathbf{r} + \mathbf{c}_i)). \tag{18}$$

Here, n_{crit} is the optimal number of neighbors, that depends on the homeostatic cell density ρ_0,

$$n_{\text{crit}} := (b+1)\rho_0, \tag{19}$$

and the bracket denotes clipping to positive values

$$(x)^{+} := \begin{cases} x & \text{if } x > 0, \\ 0 & \text{else.} \end{cases} \tag{20}$$

Lastly, we assume that mechanical coupling via cell-cell adhesion effectively leads to synchronized movement. This is obtained using the following configuration weight

$$E_{\text{alignment}}(\mathbf{s}', \mathbf{s}_{\mathcal{N}}) := \beta_{\text{align}} \left(\frac{1}{2b} \mathbf{j}(\mathbf{s}') \cdot \mathbf{g}_{\text{flux}}(\mathbf{s}_{\mathcal{N}}) + \frac{1}{b\rho_0} n_{\text{rest}}(\mathbf{s}') n_{\text{rest}}(\mathbf{s}_{\mathcal{N}}) \right). \tag{21}$$

Here, β_{align} is another positive parameter, $\mathbf{j}(\mathbf{s}')$ is the post-rearrangement flux,

$$\mathbf{g}_{\text{flux}}(\mathbf{s}_{\mathcal{N}}) := \sum_{i=0}^{b-1} \sum_{j=0}^{b-1} \mathbf{c}_{\mathbf{j}} s_j(\mathbf{r} + \mathbf{c}_i) \tag{22}$$

Table 1. Simulation parameters

Parameter	Description	Value
β	Adhesion strength	$[0, 0.2, \ldots, 10]$
$\bar{\rho}_{\mathrm{ECM}}$	Initial ECM density	$[0, 0.2, \ldots, 10]$
β_{steric}	Strength of steric interaction	5
α	ECM degradation rate	1
ρ_0	Homeostatic cell density	3
r_b	Cell influx	0.05
a	Number of rest channels	3
L	Lattice size	50 $[\varepsilon]$

is the total flux in the neighborhood, and $n_{\mathrm{rest}}(\mathbf{s}'), n_{\mathrm{rest}}(\mathbf{s}_{\mathcal{N}})$ are the number of resting cells after rearrangement and in the neighborhood, respectively. This interaction rule corresponds to a classical LGCA interaction rule for collective motion and aggregation [9]. The prefactors normalize the terms, so that both effects are equally strong.

2 Simulation study

To obtain a "phase diagram" of cancer cell invasion modes, we vary the cell-cell adhesion strength $\beta = \beta_{\mathrm{align}} = \beta_{\mathrm{ag}}$ and the initially homogeneous ECM density $\bar{\rho}_{\mathrm{ECM}}$, and fix all other parameters, see Table 1. As initial condition, a line of cells of width $w = 2$ nodes at homeostatic density $n = \rho_0 = 3$ is placed at the $y \in \{0, 1\}$ line of a 50×50 hexagonal lattice with reflecting boundary conditions, surrounded by homogeneous ECM. To mimic the inflow of cells from the tumor spheroid that is residing outside of the interface plane, we add cells at a constant rate $r_b = 0.05$ cells per channel and time step at nodes at $y \in \{0, 1\}$. The time evolution was monitored for 200 time steps after an initial transient time of 50 time steps. To determine the type of invasion, we measure two observables: the cumulative number of single cells N_{single}, and the average next-neighbor velocity correlation $\langle c_v \rangle$, defined below. We regard single cells as cells that do not have another cell in their neighborhood $\mathcal{N}$. We define the next-neighbor velocity correlation as

$$c_v(\mathbf{s}, \mathbf{s}_{\mathcal{N}}) := \frac{\mathbf{j}(\mathbf{s}) \cdot \mathbf{g}_{\mathrm{flux}}(\mathbf{s}_{\mathcal{N}})}{\big|\mathbf{j}(\mathbf{s})\big|\big|\mathbf{g}_{\mathrm{flux}}(\mathbf{s}_{\mathcal{N}})\big|}, \quad \text{for} \quad \big|\mathbf{j}(\mathbf{s})\big|, \big|\mathbf{g}_{\mathrm{flux}}(\mathbf{s}_{\mathcal{N}})\big| > 0, \tag{23}$$

where $\mathbf{j}(\mathbf{s})$ is the local flux, defined in Equation 10, and $\mathbf{g}_{\mathrm{flux}}(\mathbf{s}_{\mathcal{N}})$ is the total flux in the neighborhood, defined in Equation 22. We vary both the adhesion strength and the ECM density from 0 to 10 in steps of 0.2, and average 5 independent realizations for each parameter combination $(\beta, \bar{\rho}_{\mathrm{ECM}})$.

3 Scaling

In this section we assign the lattice spacing ε and the time step length τ physical values, so that the homeostatic cell density ρ_0, and the maximum cell speed $v_{\max} = \frac{\varepsilon}{\tau}$ match experimental values. We find ε by considering that at ρ_0 the node area A_{node} should match the cumulative cell area

$$A_{\text{node}} = \rho_0 A_{\text{cells}}. \tag{24}$$

Since we consider a hexagonal lattice, the node area is given by the area of a regular hexagon with an inradius $\frac{\varepsilon}{2}$

$$A_{\text{node}} = A_{\text{hexagon}} = \frac{\sqrt{3}}{2}\varepsilon^2. \tag{25}$$

Solving for ε we obtain

$$\varepsilon = \sqrt{\frac{2\rho_0 A_{\text{cells}}}{\sqrt{3}}}. \tag{26}$$

Assuming a circular cell shape, the cell area is given by $A_{\text{cells}} = \pi R_{\text{cells}}^2$. With an approximate cell radius $R_{\text{cells}} \approx 5\,\mu\text{m}$ we then obtain $\varepsilon \approx 20\,\mu\text{m}$.
For the time step length τ we choose $\tau = 15\,\text{min}$. This means that the maximum cell speed is given by $v_{\max} = \frac{\varepsilon}{\tau} \approx 1.3\,\frac{\mu\text{m}}{\text{min}}$, which matches well with experimental observations [10].

References

1. Hardy, J., Pomeau, Y. & de Pazzis, O. Time Evolution of a Two-Dimensional Classical Lattice System. *Phys. Rev. Lett.* **31,** 276–279. doi:10.1103/PhysRevLett.31.276 (1973).
2. Frisch, U., Hasslacher, B. & Pomeau, Y. Lattice-Gas Automata for the Navier-Stokes Equation. *Phys. Rev. Lett.* **56,** 1505–1508. doi:10.1103/PhysRevLett.56.1505 (1986).
3. Gao, Y. & Sharma, M. M. A LGA model for fluid flow in heterogeneous porous media. *Transp. Porous Media* **17,** 1–17. doi:10.1007/BF00624047 (1994).
4. Deutsch, A. & Dormann, S. *Cellular Automaton Modeling of Biological Pattern Formation* doi:10.1007/978-1-4899-7980-3 (Birkhäuser Boston, Boston, MA, 2018).
5. Böttger, K. *et al.* An emerging Allee effect is critical for tumor initiation and persistence. *PLOS Comput. Biol.* **11,** e1004366. doi:10.1371/journal.pcbi.1004366 (2015).

6. Reher, D., Klink, B., Deutsch, A. & Voss-Böhme, A. Cell adhesion heterogeneity reinforces tumour cell dissemination: novel insights from a mathematical model. *Biol. Direct* **12,** 18. doi:10.1186/s13062-017-0188-z (2017).

7. Talkenberger, K., Cavalcanti-Adam, E. A., Voss-Böhme, A. & Deutsch, A. Amoeboid-mesenchymal migration plasticity promotes invasion only in complex heterogeneous microenvironments. *Sci. Rep.* **7,** 9237. doi:10.1038/s41598-017-09300-3 (2017).

8. Armstrong, N. J., Painter, K. J. & Sherratt, J. A. A continuum approach to modelling cell–cell adhesion. *J. Theor. Biol.* **243,** 98–113. doi:10.1016/j.jtbi.2006.05.030 (2006).

9. Deutsch, A. A new mechanism of aggregation in a lattice-gas cellular automaton model. *Math. Comput. Model.* **31,** 35–40. doi:10.1016/S0895-7177(00)00019-4 (2000).

10. Wolf, K. *et al.* Physical limits of cell migration: Control by ECM space and nuclear deformation and tuning by proteolysis and traction force. *J. Cell Biol.* **201,** 1069–1084. doi:10.1083/jcb.201210152 (2013).