RANKL-mediated cell-cell fusion in vitro

Problem 2

Team work

Pabel Shahrear, McGill University

Peter Pivonka, Melbourne University

Salwa Maria, McGill University

Spencer Moran, McGill University

Yongqiang Wang, Toronto University

Yue Zhao, Simon Fraser University

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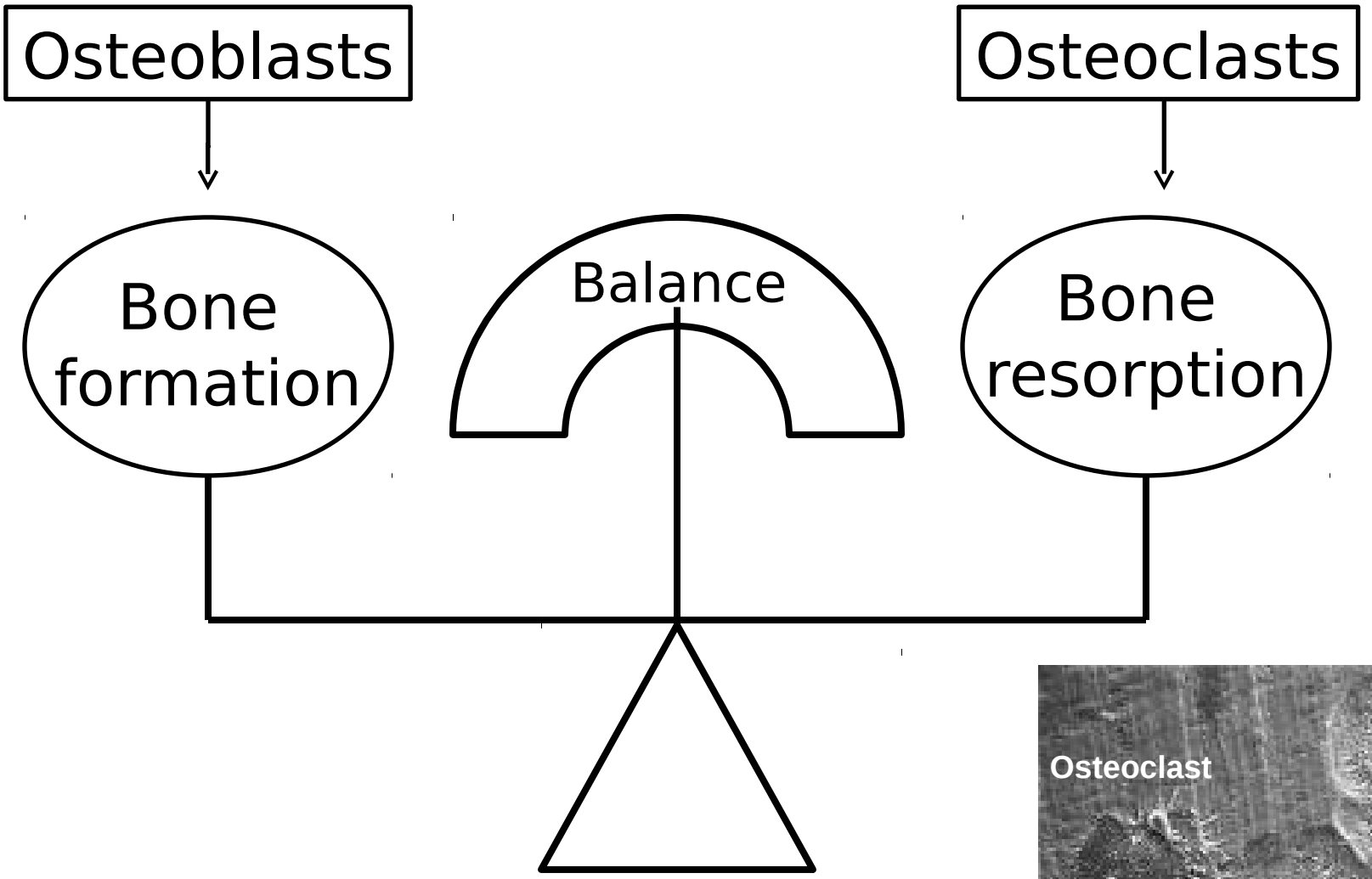
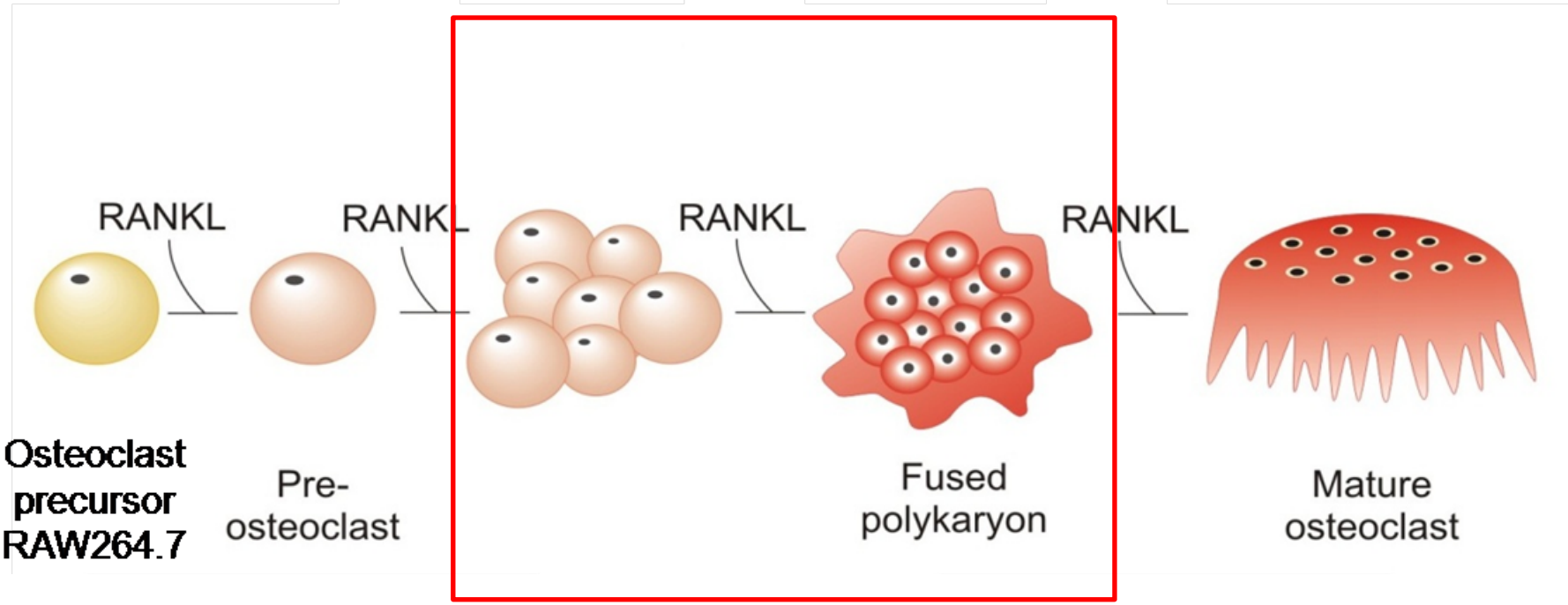
Bone remodeling

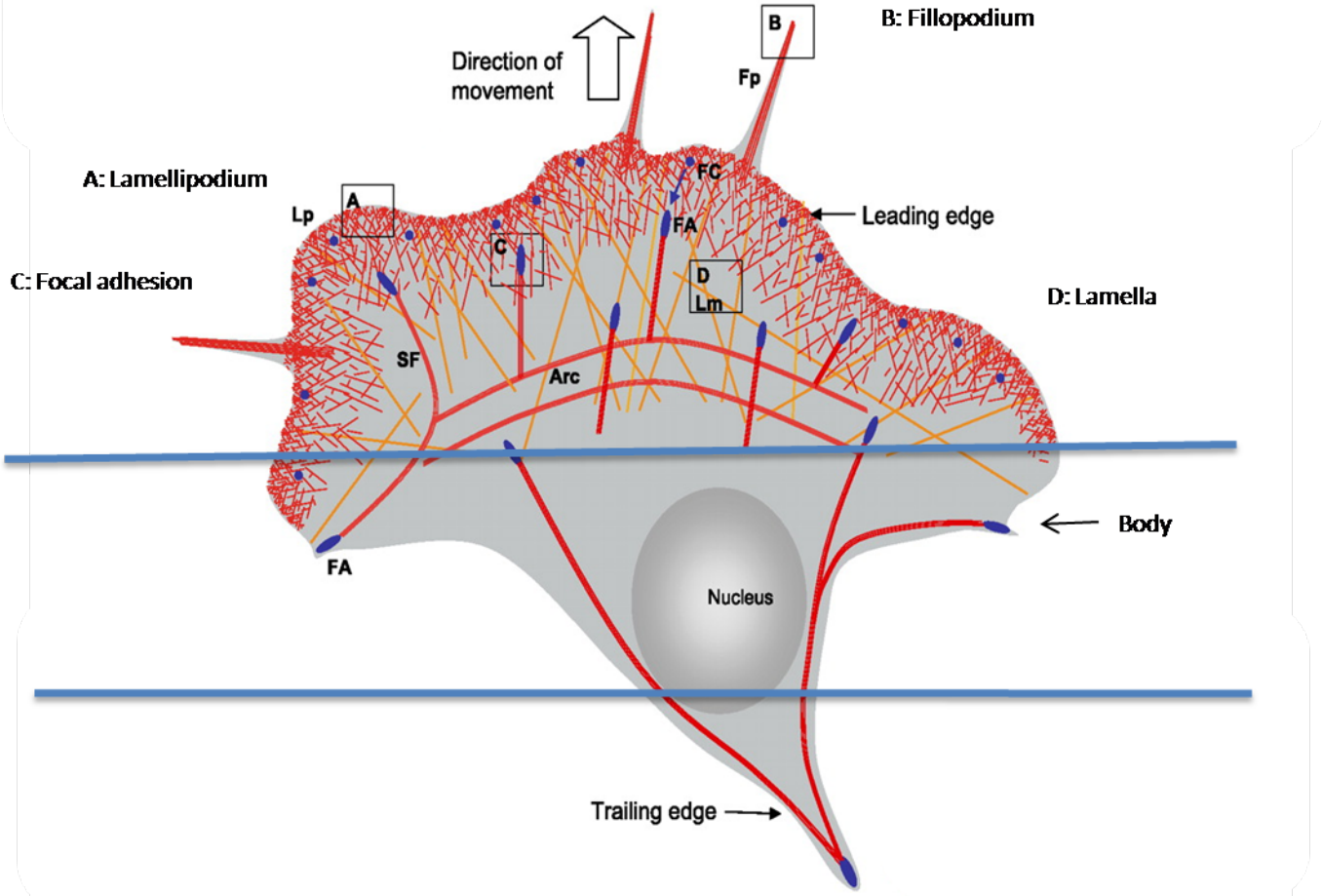


What is osteoclastogenesis?

Image Pro-Plus, cell tracking

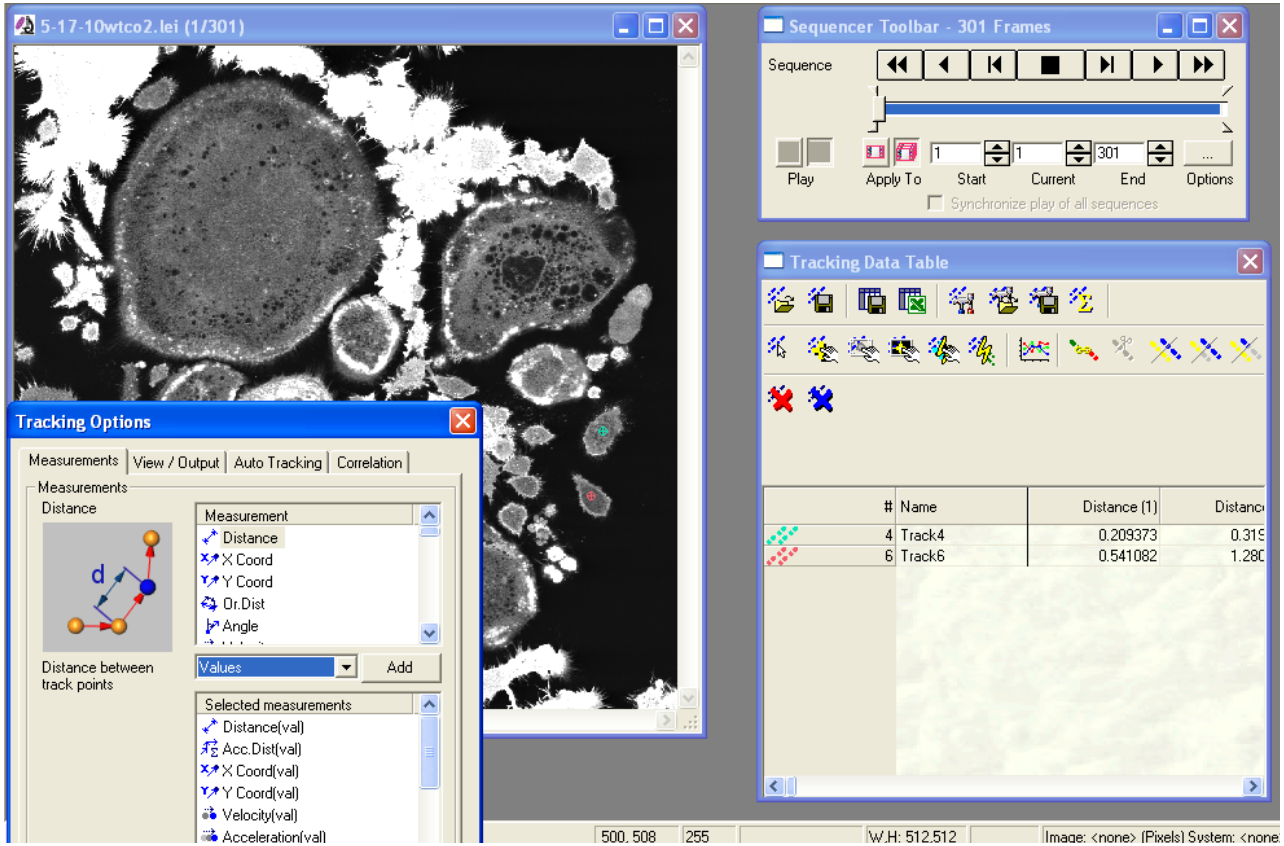


How does the cell migrate?



Physiol Rev 88: 489–513, 2008

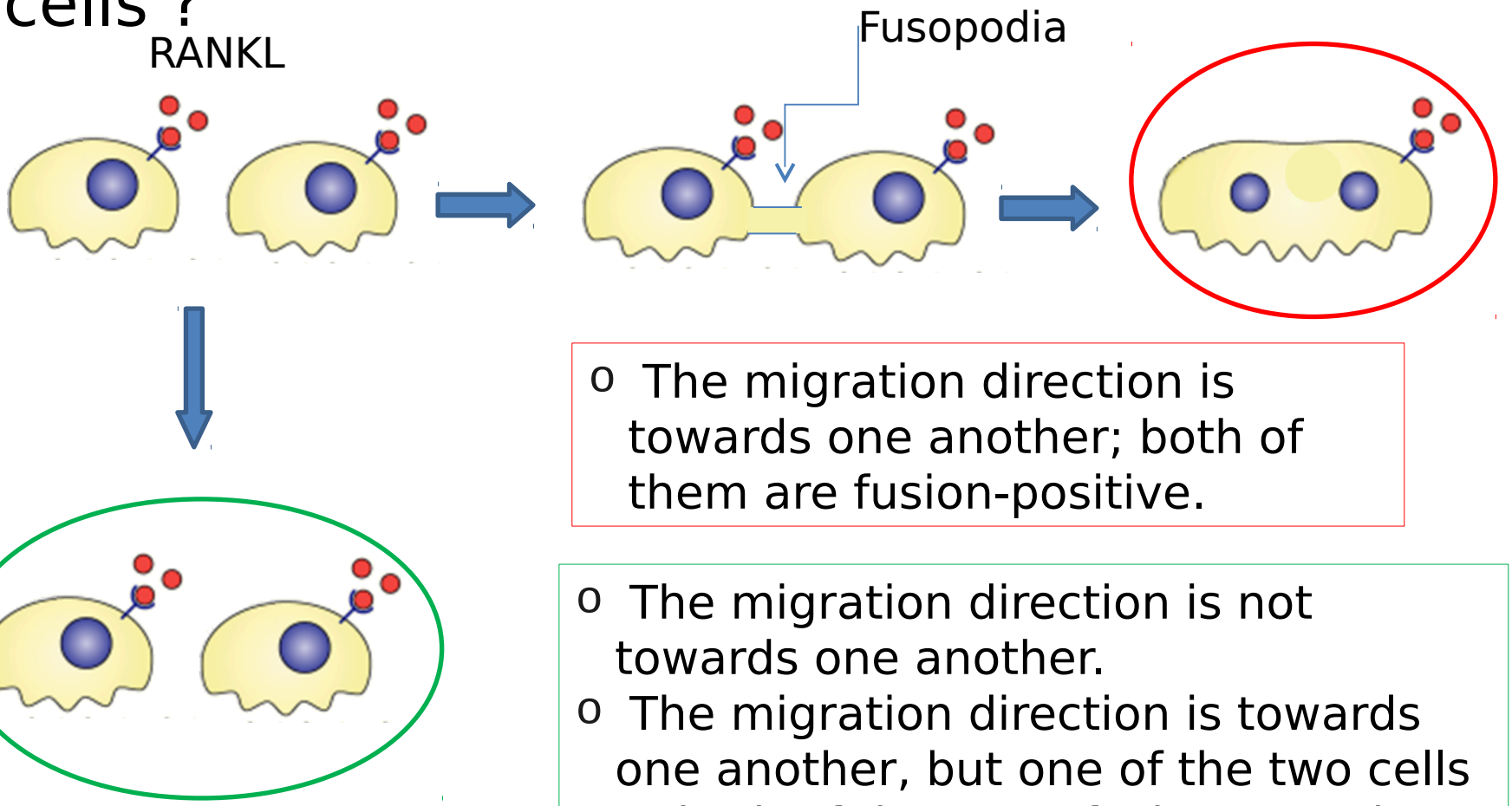
Software: Image Pro-Plus



Parameters

X Coord
Y Coord
Area (polygon)
Dendrites number
Dendritic length
Velocity
Distance
Acceleration
Diameter (mean)
Size (length)
Size (width)
Accumulated Distance
End points
Margination
Clumpiness
Rel. Time

What is the probability of fusion between two cells ?



- The migration direction is towards one another; both of them are fusion-positive.

- The migration direction is not towards one another.
- The migration direction is towards one another, but one of the two cells or both of them are fusion-negative.

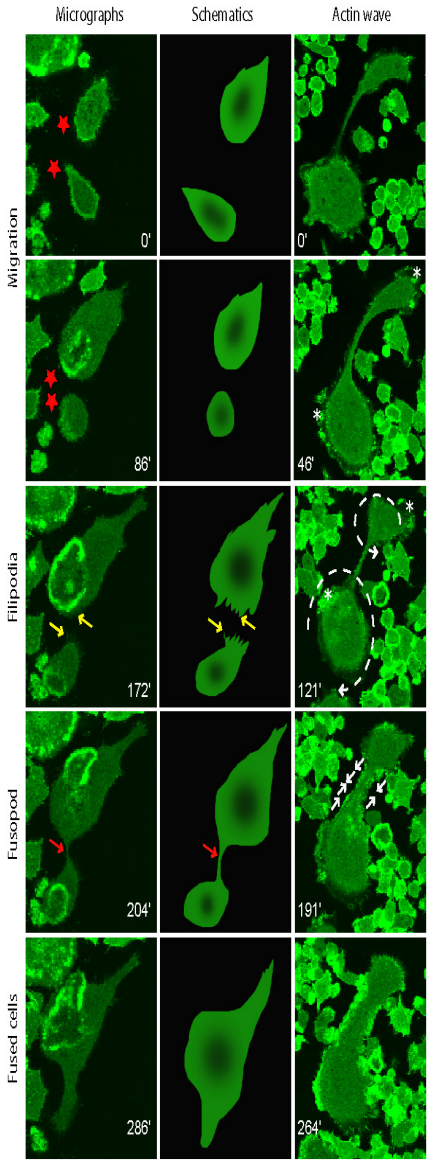
Micrographs Schematics of Fusion

Migration

Filopodia

Fusopod

Fused cells



The left panel shows a representative set of time-lapse images captured with a microscope.

The schematic shown beside the cells indicates the formation and importance of the fusopodia during cell fusion.

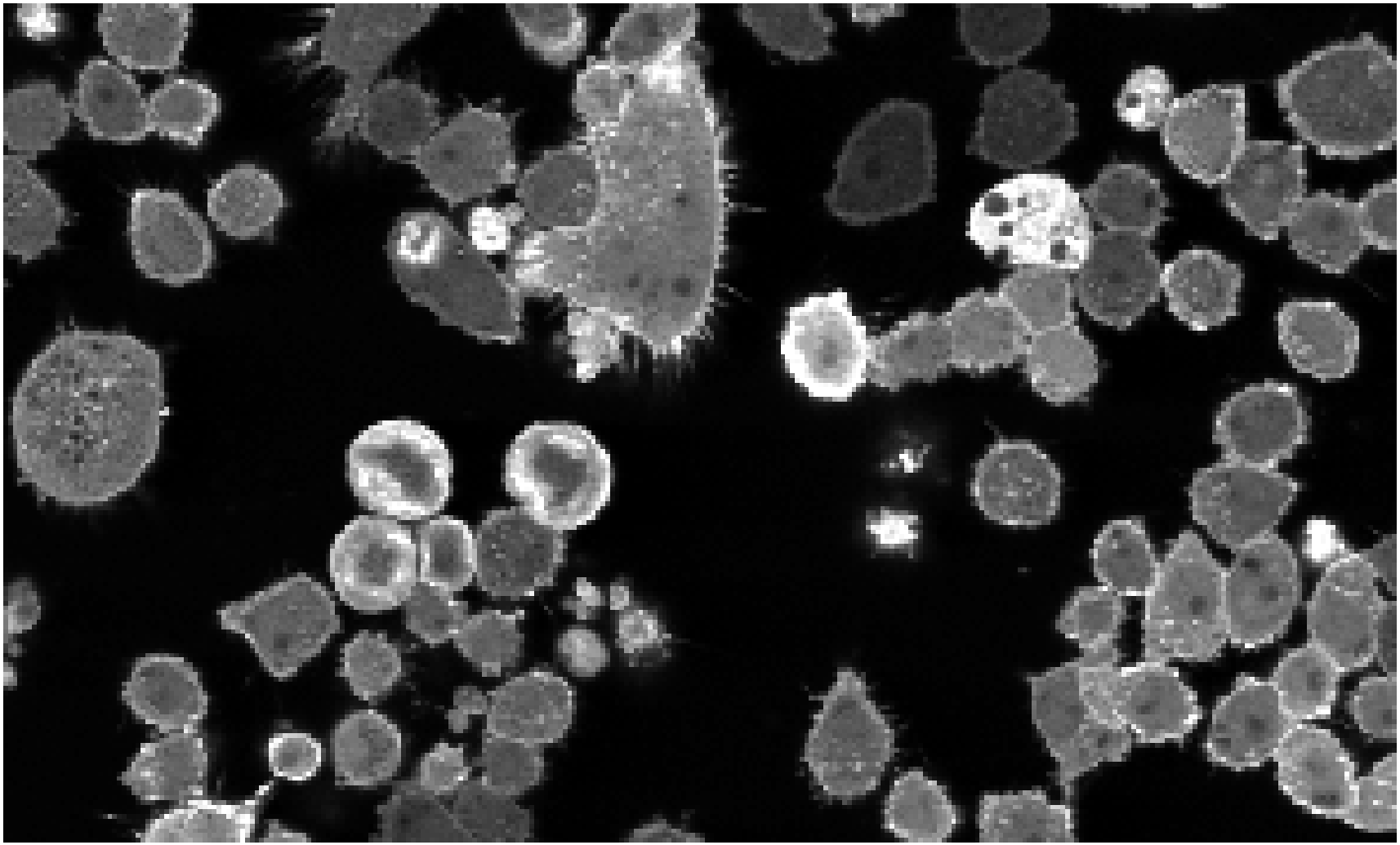
The Hypothesis

There are specific cytoplasmic structures that are important in RANKL-mediated cell-cell fusion in vitro.

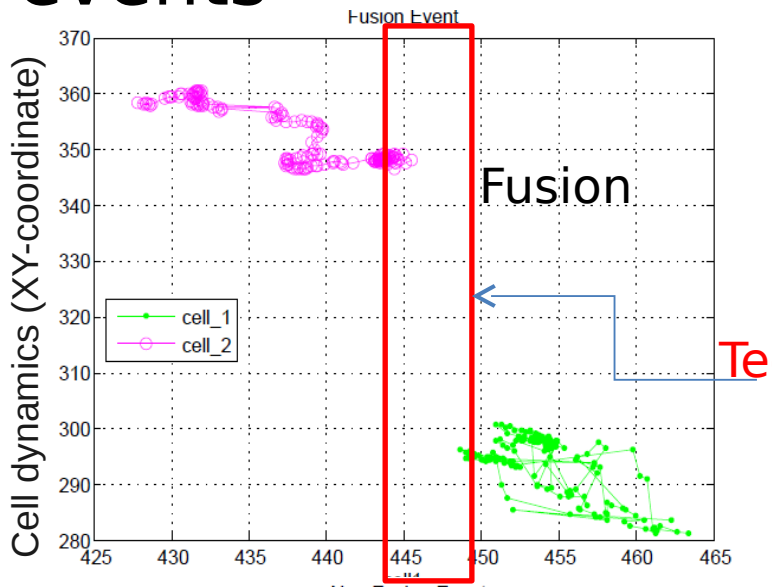
The Objectives

- Compare fusion events with non-fusion events.
- Characterize migrating cells: founder and follower.
- Develop a mathematical model that can characterize cells with fusion potential within the migrating ones.

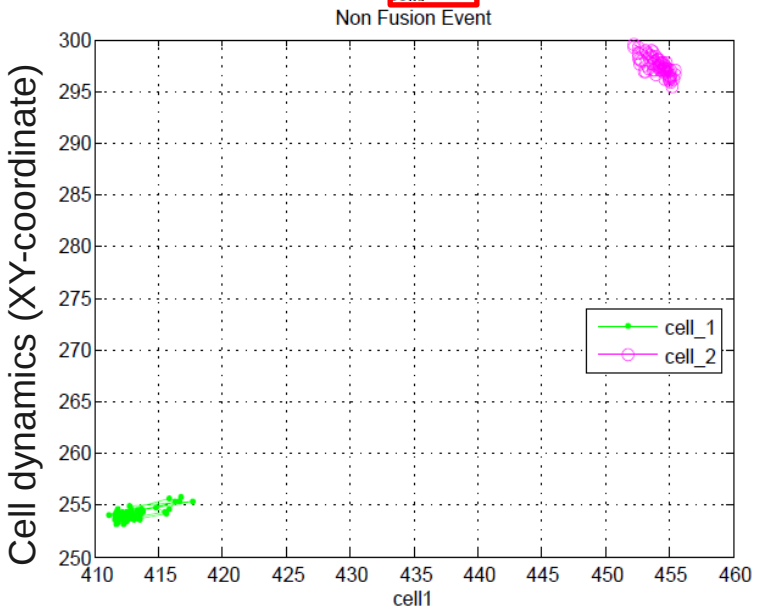
- Comparison between fusion and non-fusion events



❑ Comparison between fusion and non-fusion events

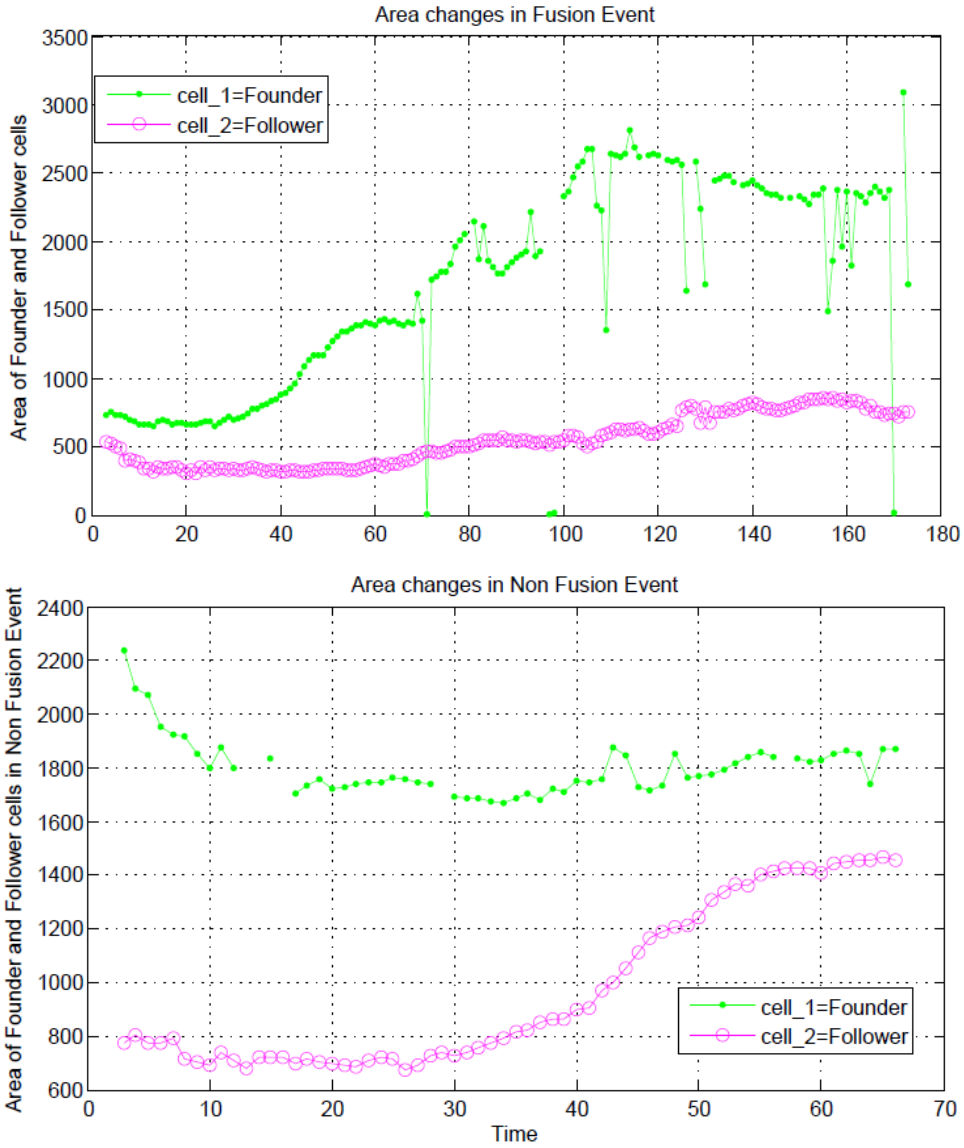


Technical Issue in the software



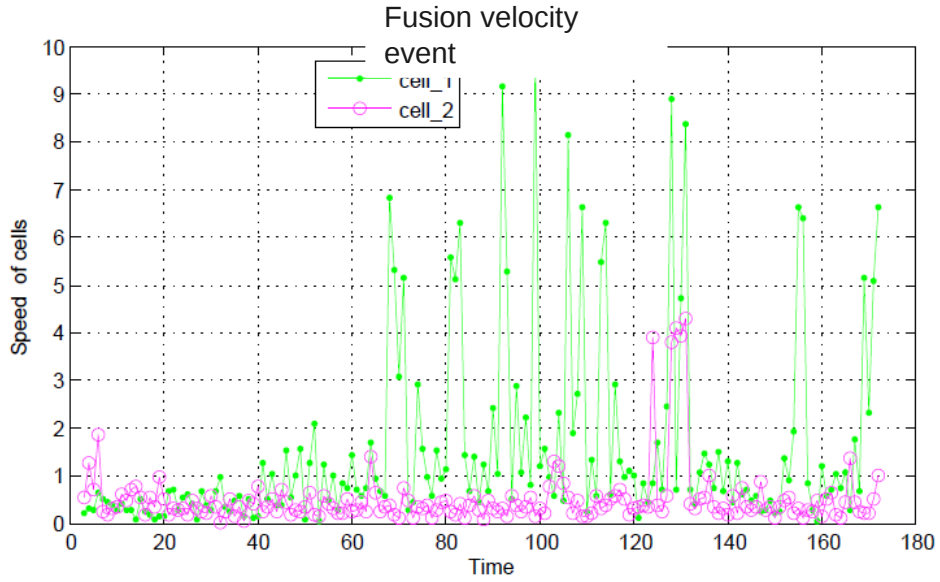
In a fusion event, the founder moves towards the follower forming fusopodia.

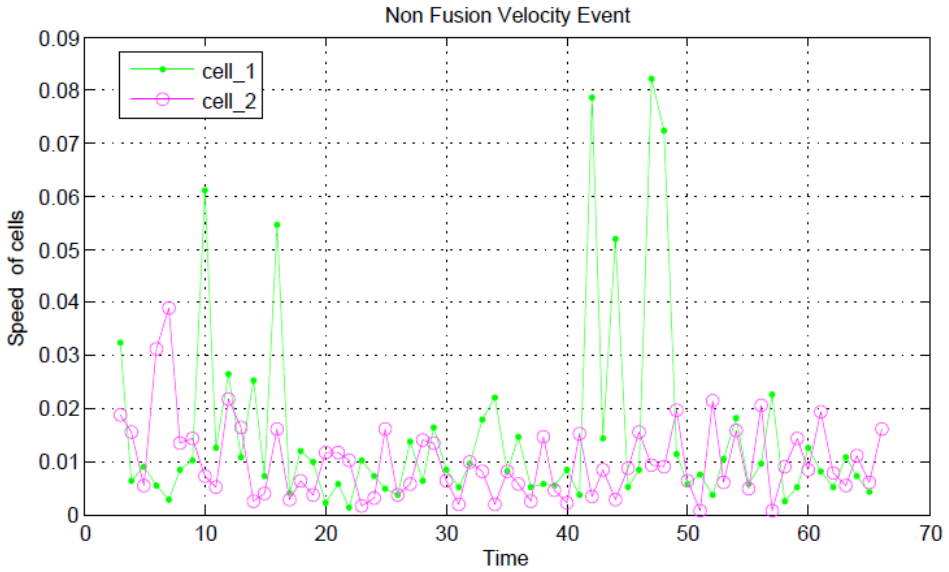
❑ Comparison between fusion and non-fusion events



In a fusion event, the founder cell body stretches to reach the follower.

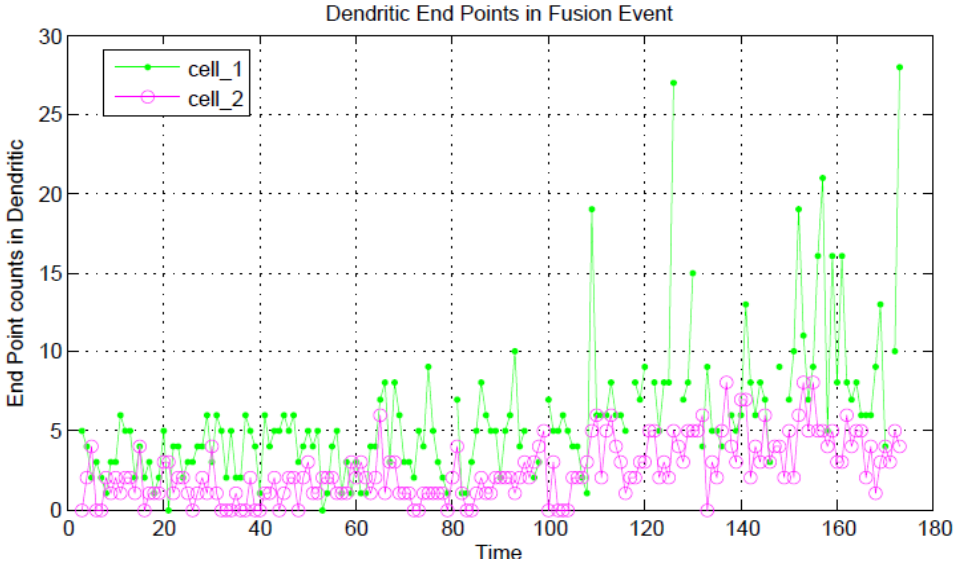
❑ Comparison between fusion and non-fusion events

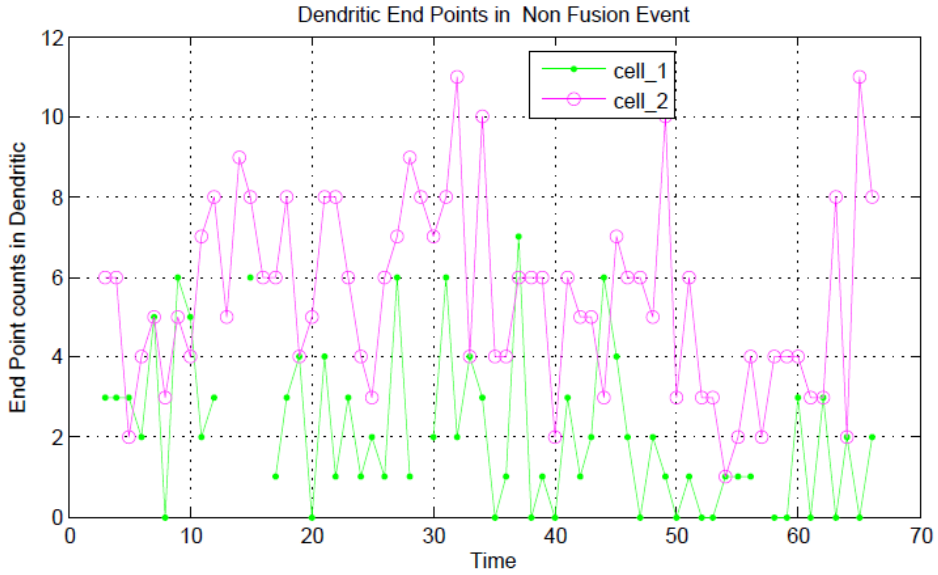




In a fusion event, the founder's velocity incerases more than the follower's.

Comparison between fusion and non-fusion events





In a fusion, the founder has more dendrites endpoints closer to the fusion.

❑ Characterizing migrating cells: founder and follower

Parameter	Founder	Follower
Cell Dynamics	Active and forward	Less active
Cell Size	Larger	Smaller
Velocity	Higher	Lower
Distance	Longer	Shorter
Dendrites number	Higher	Lower
Dendrites length	Longer	Shorter

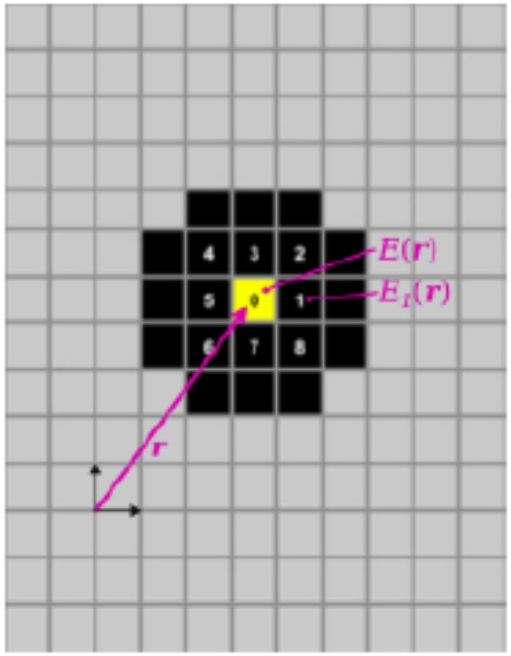
The red box indicates that the two parameter values are swapped when the osteoclast reaches a certain age and size; when the cell becomes larger the founder will move more slowly than the follower.

- Development of a mathematical model that can characterize cells with fusion potential within the migrating ones

Simulation/Model

- A significant amount of time was spent investigating how a stochastic, lattice based computational model could simulate osteoclast (OC) migration and fusion. The inspiration for this investigation was the paper *Investigation of bone resorption within a cortical basic multicellular unit using a lattice-based computational model* (Buenzli et al.)
- At each time step in the computation, an OC on the lattice has a probability P_i of migrating to a Moore neighbor i.
- P_i is dependent on the "interaction energies" between neighbouring OC's. Interaction energies are a convenient way to describe how neighbouring cells interact with each other. For example, the interaction energy between two OC's could depend on the difference in the number of nuclei of each OC.

Depicted below is a simple initial configuration of the lattice. The OC is located at Moore site $j = 0$. The numbered black boxes are the Moore sites $j = 1...8$ that the OC can migrate to.



- Q: How is the probability determined? A: Statistical mechnics, more specifically, Glauber dynamics: the OC moves to a configuration that minimizes the interaction energy.

$$P_j(\mathbf{r}) = \frac{exp(-E_j(\mathbf{r})/\lambda)}{\sum_{k=0}^{8} exp(-E_k(\mathbf{r})/\lambda)} \quad \text{(Boltzmann distribution)} \qquad (1)$$

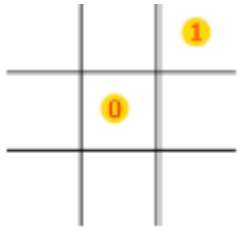
- λ is the metabolic energy of the cell. $E_i(\mathbf{r})$ is the total interaction energy at Moore site i.

$$E_i(\mathbf{r}) = \sum_{j=0}^{8} E_{ij}^{OC} N_j^{OC} \qquad (2)$$

$$N_j^{OC} = \begin{cases} 1 & \textit{if neigbour } j \textit{ is occupied by OC} \\ 0 & \textit{otherwise} \end{cases} \qquad (3)$$

$$E_{ij}^{OC} = \begin{cases} E_{OC-OC}^{fusion} & j = 0 \\ E_{OC-OC} & j = 1, 2, ..., 8 \\ 0 & otherwise \end{cases} \quad (4)$$

What do the interaction energies mean? E_{OC-OC}^{fusion} represents the "exclusion/repulsion energy". This means that if $E_{OC-OC}^{fusion} < +\infty$ then there is a possibility that another OC can occupy the site at $i = 0$ to attempt fusion (see example coming up). E_{OC-OC} is the energy of attraction between nearest Moore neighbours. Therefore, the key to setting up a useful simulation is determining the interaction energies as a function of some measureable quantity of the interacting cells. E.g. $E_{ij}^{OC} \propto |n_j - n_i|$, where n_i is the number of nuclei at site i.



- Example: Osteoclasts at positions 0 and 1

First calculate the energies $E_i(\mathbf{r}), i = 0, ..., 8$ that are required to compute the probability. Suppose for simplicity that $E_{ij}^{OC} = k > 0$ and $\lambda = 1$. Then,

$$E_{i\neq 0,1}(\mathbf{r}) = 0 \quad \text{(since there are no OCs at sites other than 0,1)} \tag{5}$$

$$E_{i=0,1}(\mathbf{r}) = N_0^{OC} E_{i0}^{OC} + N_1^{OC} E_{i1}^{OC} \tag{6}$$

$$= E_{i0}^{OC} + E_{i1}^{OC} \tag{7}$$

$$= k + k \tag{8}$$

$$= 2k \tag{9}$$

$$\implies P_{i\neq 0,1}(\mathbf{r}) = \frac{\exp\left(-E_i(\mathbf{r})/\lambda\right)}{Z} \tag{10}$$

$$= \frac{1}{7 + 2e^{-2k}} \tag{11}$$

$$P_{i=0,1}(\mathbf{r}) = \frac{\exp\left(-E_i(\mathbf{r})/\lambda\right)}{Z} \tag{12}$$

$$= \frac{e^{-2k}}{7 + 2e^{-2k}} \tag{13}$$

As a check, take the limits of P_i in the cases $k \to 0$ (no interaction energy) and $k \to \infty$ (absolute repulsion):

$$\underline{k \to 0:} \tag{14}$$

$$P_i(\mathbf{r}) \to \frac{1}{9}, i = 0, \ldots 8 \tag{15}$$

$$\underline{k \to \infty:} \tag{16}$$

$$P_{i=0,1}(\mathbf{r}) \to 0 \tag{17}$$

$$P_{i\neq 0,1}(\mathbf{r}) \to \frac{1}{7} \tag{18}$$

The previous checks make sense physically. For the case of no interaction energy, the OCs are free to migrate to *any* site with equal probability. In the case of absolute repulsion, an OC can only migrate to the unoccupied sites with equal probability.

Next Steps:

- How does the number of nuclei, n_i, in the cell affect the interaction energies? We hypothesize that larger cells (i.e. with more nuclei) have a tendency to remain stationary. Further, we guess that smaller cells (with less nuclei) have an affinity to larger cells. If we can express this relationship mathematically, then we can include them in the expression for total energy, which will in turn affect the probability of migrating to a neighbour site.
- Identify other characteristics of the cell that might affect interaction energy. Fusopods, fillapods?
- Investigate code that was provided to us to see how the CA model can be implemented using our set of rules.
- Investigate off-lattice models that are perhaps more realstic since they operate in continuous space (see the work of Chapman, Bruna, Flegg, Erban).

Thank you