Trail networks formed by populations of immune cells

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Abstract

Populations of biological cells that communicate with each other can organize themselves to generate large-scale patterns. Examples can be found in diverse systems, ranging from developing embryos, cardiac tissues, chemotaxing ameba and swirling bacteria. The similarity, often shared by the patterns, suggests the existence of some general governing principle. On the other hand, rich diversity and system-specific properties are exhibited, depending on the type of involved cells and the nature of their interactions. The study on the similarity and the diversity constitutes a rapidly growing field of research. Here, we introduce a new class of self-organized patterns of cell populations that we term as ‘cellular trail networks’. They were observed with populations of rat microglia, the immune cells of the brain and the experimental evidence suggested that haptotaxis is the key element responsible for them. The essential features of the observed patterns are well captured by the mathematical model cells that actively crawl and interact with each other through a decomposing but non-diffusing chemical attractant laid down by the cells. Our finding suggests an unusual mechanism of socially cooperative long-range signaling for the crawling immune cells.

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1. Introduction

‘Self-organization’ is a ubiquitous phenomenon of any system that is composed of many interacting active particles, thermodynamically open and dynamic objects. Examples are abundant in nature, ranging from flocking animals [1, 2], swarming motor molecules [3, 4] to vibrating granular materials [5–7]. In particular, populations of biological cells have great potential to exhibit diverse patterns since they are inherently nonlinear and interact with each other through various chemical, mechanical and electrical means [8, 9]. However, the examples have been rather limited to regular static patterns [10–12], traveling waves [13–16], fingering undulation [17, 18] or large-scale fluctuations [19–22].

In this paper, a new class of patterns, namely, complex trail networks that are self-organized in dissociated cell cultures is discussed. These patterns were formed by the populations of rat microglia (MG), and the experimental evidence suggests that haptotaxis is the key element responsible for them. In haptotaxis, the cells are attracted (or repelled) toward the pattern (or gradient) of biochemicals that are expressed on a surface, in contrast to the classical model of the chemotaxis, in which the gradient is set in a soluble fluid. We also developed a system of mathematical model cells that actively crawl and interact with each other through a decomposing but non-diffusing chemical attractant laid down by the cells. Some of the key features of the observed patterns were well captured by the model population.

2. Results

2.1. Formation and evolution of the microglia trail network

MG are a type of glia that are the resident macrophages of the brain that act as the main form of active immune defense in the central nervous system [23]. We obtained dissociated MG cells from rat brains, seeded them on a culture dish and monitored their behavior by time-lapse imaging over several days (see figure 1(a)). About a half day after the initial seeding, the cells started to crawl around with a fan-shaped ruffling front and tail-like branch structures (see the inset of figure 1(a)). Then, the interacting cell population exhibited a very interesting network of migratory trails, as shown in figure 1(a) (day 1–3, video S1 (see the supplementary material, available from stacks.iop.org/NJP/16/023017/mmedia)). Indeed, the MG cells showed a strong preference for moving along the existing passages formed by other neighboring cells or even by them, as shown in figure 1(b) (see also video S2). The morphology of the network reflected the directional persistence of the freely crawling MG cells [24].

The cells seemed to leave some chemical markers behind their passages, similar to ants leaving pheromones or ameba excreting cyclic adenosine monophosphate behind their crawling passages. Then, the marker chemicals guided the cells to change their moving directions toward the existing passages. Note that the color-marked (e.g. green and blue) cells in figure 1(b) exhibit a strong inclination to move along the existing passages. Sometimes, the cells escaped from the existing trails: for example, the cell indicated by the red arrow has derailed from the existing track in the bottom only to rejoin a neighboring track extending along a vertical direction later.

The network evolved slowly, as illustrated in the pseudo-color images of figure 1(a). Some of the trails were enhanced or newly created (as in the red rectangular box), while some that were rarely visited disappeared (as in the red circle). In order to quantify the network evolution, we defined the trail as the area, where the standard deviation (SD) of the local
Figure 1. Trail networks formed by crawling rat MG cells. (a) Phase-contrast snapshot image of the MG cells and three pseudo-color images showing the evolution of their trails (for day 1–3) (scale bar: 100 µm). (b) A high magnification image, illustrating the passages of three different cells (for 12 h) in and around a trail (marked by a pair of dashed lines) (scale bar: 50 µm). (c) The time evolution of the trail network. The inset in (a) is a close-up view of a typical crawling MG cell (scale bar: 10 µm). The pseudo-color images in (a) are obtained based on the following procedure. Twelve hours after the initial cell seeding, phase-contrast images were acquired at every 5 min for 3 days. Then, for each pixel location, the SD of a 1 day time-series was computed and color-coded: the SD value is a good measure of the number of visits made by the MG cells, since every time when they pass through the site of interest they introduce a pulse-like signal in the time series. The color ‘blue’ (‘red’) represents 3 (15) counts of visits per day, approximately. The SD > 70 defines the trails. The seeding density was 200 cells mm\(^{-2}\).
2.2. Agents mediating cell-to-cell interaction

A recent study suggested that lipopolysaccharide (LPS) is a possible chemokine for the MG chemotaxis [25]. More recently, Dou et al [26] demonstrated that adenosine 5’-triphosphate disodium salt (ATP) mediated cell-to-cell interaction is essential for the long-range chemotaxis of the MG cells, as in the regenerative calcium waves of the astrocyte networks. With a well-controlled injection of (1 mM) micro droplet of ATP (Sigma) into a mineral-oil (Sigma) covered thin-layer (depth $\sim$ 1 mm, diameter 12 mm) sample preparation, we also confirmed that the rat MG cells could be attracted toward the region of the higher ATP concentration (see figure 2). However, these fluid-soluble chemoattractants were most probably not responsible for the formation of the trail networks, since there existed quite fast (mean velocity $\sim$500 $\mu$m min$^{-1}$), uncontrolled, convective flows inside the large-volume Petri dish (35 mm diameter, 10 mm height), where the MG cells were being monitored. The chemoattractants, even if they were released by the cells, would be quickly advected away by the flow, and the neighboring MG cells crawled just too slow (mean velocity $\sim$3 $\mu$m min$^{-1}$) to recognize the presence of them.

Alternatively, the haptotaxis seemed to be responsible for the collective cell dynamics; some substrate-bound chemoattractants, laid down by the crawling MG cells could be a guiding cue for the other cells. In fact, we found that MG cells leave a very faint, yet recognizable, trace of $\alpha 5$ integrins (see figures 3(a) and (b)). It is known that the integrins not only serve as adhesive molecules for traction but also work as signaling receptors that affect retrograde actin flows and actin polymerization [27]. Moreover, we found that the MG cells leave some fragments of needle-like microscopic structures (diameter 10–100 nm) behind their moving trajectories as shown in figures 3(c) and (d). These structures, presumably, bundles of F-actins and cell membranes, also seemed to guide the movements of the MG cells nearby. In other words, the MG cell haptotaxis could be driven both by chemical and physical cues. The landscapes of these cues could evolve in time since flows in the medium could wash them and because MG cells could degrade them [28].
Figure 3. Guiding cues for the MG cell haptotaxis. (a) An exemplary immunofluorescence staining image of α5 integrin. (b) A blown-up image of the boxed area in (a) showing a faint trace of laid out α5 integrin (marked by a yellow arrow). (c) An exemplary SEM image. (d) A close-up view of the boxed area in (c) revealing very thin needle-like structures (marked by white arrows). The scale bar of (a)–(d) represents 100, 20, 50 and 20 µm, respectively.

3. Trail network formed by a population of model cells

We could reproduce the observed trail patterns, including some of their key features, with a population of mathematical model cells (see section 7), which crawl on a two-dimensional space and interact with each other through a chemical attractant (see figure 4 and video S3 (see the supplementary material, available from stacks.iop.org/NJP/16/023017/mmedia)). The individual cell was a rule-based model, which was used earlier to understand the mechanism behind the directional persistence of the freely crawling MG cells [24] (see figure S3(a)). We extended the single cell model to an interacting population. Following our experimental observations, the attractant was assumed as substrate-bound molecules rather than fluid-soluble chemical agents; the model cells lay out the attractant q, which is not diffusing, but slowly decomposing in time (see figure S3(b)). We further assumed the production rate of the activator S+, an agent inducing the protrusion of a cell body, increased with the presence of q. This assumption was modeled after the so-called ‘clutch hypothesis’ integrating adhesion, the
Figure 4. Trail networks formed by a population of the mathematical model cells. (a) A snapshot image showing 250 model cells (top left) and three snapshot images showing the concentrations of the attractant \( q \) in pseudo-colors. (b) A blown-up image illustrating four neighboring model cells forming a trail (marked by a pair of dashed lines for a visual guide). (c) The time evolution of the trail network. The inset in (a) is a close-up view of a model cell. The circled (boxed) area in (a) is an exemplary case, showing the trails that have decayed (newly formed). Approximately, the color ‘blue’ (‘red’) represents 4 (25) counts of visits per day. The cell trajectories in (b) are for 3 h. \( K_{\text{decay}} = 0.03 \) and \( \tau_{\text{decay}} = 12 \) h. The scale bar of (a) and (b) represents 200 and 20 \( \mu \)m, respectively.

retrograde flow of the actins and actin polymerization [29]. Briefly, the linkage between the actin filaments to a fixed substratum increases the protrusion rate by shunting the forces that drive the retrograde flow and thereby inhibiting it. Hence, when a wandering model cell is encountered with a \( q \)-deposited trail, it could be guided along that trail to enhance it (see figure S3(b)).

Figure 4(b) illustrates four model cells that formed a vertical trail (see video S4, available from stacks.iop.org/NJP/16/023017/mmedia). Note that upon joining the trail, two of them (green and red) moved upward while the other two (blue and violet) traveled downward. The particular direction of the movement was, of course, dynamically determined by the cells’ past histories as well as the local landscape of \( q \). As a result of the \( q \)-mediated interaction, the model cells formed a large-scale complex network of trails (figure 4(a), 6–24 h) that was strikingly similar to that of the MG cells. In the absence of the \( q \)-mediated interaction, the traces of the freely crawling cells will simply fill the entire space (see figure S4).

This model network also evolved with time since the cells were able to escape from the portion of the trails that was less frequently visited, thus, thinned in \( q \) (for example, the red circled area in figure 4(a)). On the other hand, some of the trails became more pronounced with time because of frequent visits (see the red boxed area in figure 4(a)). Figure 4(c) well
recapitulates the development of the experimentally observed network of the MG trails shown in figure 1(c): the initial rises, followed by a maturing process, leading to a steady state are evident.

4. Factors governing the shape of the cellular trail network

Several different factors can govern the properties of a trail network; the obvious one is the cell density. With an MG culture sample of five times higher density, a network of trails (see figures 5(a) and (b), and see video S5 (see the supplementary material, available from stacks.iop.org/NJP/16/023017/mmedia)) that was similar to, but approximately twice as more compact (see figure 5(e)) than the lower density case of figure 1(a), was found. Subsequently, the correlation length of the network decreased approximately from 82 to 12 µm (see figure S5). A series of model simulations (see figures 5(c) and (d), video S6) also showed that the trail network became denser as a nonlinear function of increasing cell density, as shown in figure 5(f) and its inset. Its correlation length decreased accordingly as in the experiments (see figure S6). However, there was no dramatic change in the overall morphology or in the dynamic evolution of the network as the population density increased.

There are two important parameters that are critical for determining the morphology of a trail network, namely, \( \tau_{\text{decay}} \), which is the time constant for the decaying attractant \( q \), and \( K_{\text{decay}} \), which is one of the rate constants for the activator \( S^+ \). Figures 7(a) and (b) are two phase diagrams plotting the trail area fraction and the number of the nodes in the network, respectively. We found that \( K_{\text{decay}} \) is the most sensitive parameter that controls the directional persistence of a crawling model cell (see figure 6 and [24]). Trails will not form, either if \( q \) decomposes so quickly (e.g., \( \tau_{\text{decay}} \approx 2 \) h) that there is no cell-to-cell interaction whatsoever, or if the directional persistence of an individual cell is so low (e.g. when \( K_{\text{decay}} \geq 0.06 \) or \( K_{\text{decay}} \leq 0.02 \)) that it is trapped by the attractant released by itself and oscillates (see figures 8(a) and (b), video S7). This phenomenon is also very typical of that rat MG cell populations during the early stage of culture (see figures 8(c) and (d), video S8). Even if some trails can be formed in these cases, they are very localized and fragmented.

In the parameter regime of 0.03 \( \leq K_{\text{decay}} \leq 0.05 \), a well-connected network of trails can be formed. In this regime, the statistical properties of a network, such as the distribution of the network node-degrees and that of the shortest path lengths connecting two nodes, are significantly different from those of a random network having a similar number of nodes as shown in figures 7(c) and (d), respectively. We also find that as \( K_{\text{decay}} \) increases (with a fixed value of \( \tau_{\text{decay}} = 12 \) h) the overall network correlation length gradually increases, approximately from 30 to 40 \( \mu \)m, while the persistence length of a freely crawling cell trajectory was found to be gradually decreasing, approximately from 160 to 450 \( \mu \)m, according to our earlier report [24]. The inverse functional relationship between the network correlation length and the persistence length of the freely crawling cells is interesting but warrants an explanation.

5. Summary and conclusion

In summary, we report an experimental observation of the complex trail network patterns formed by the populations of crawling rat MG. For the network formation, the haptotaxis is believed to be a key mechanism. The experimental evidence strongly suggests that the substrate bound molecules such as integrins or some cell debris must have served as a guiding cue for the
Figure 5. Networks of trails formed by the high density cell populations. (a) Snapshot image of an MG cell population (1000 cells mm\(^{-2}\)). (b) Pseudo-color image of the SD for the MG cell population shown in (a) (based on 1 day time series image data beginning from 50 h in culture). (c) High density population of the model cells (1000 cells mm\(^{-2}\)). (d) Snapshot image of \(q\) at \(t = 24\) h. \(K_{\text{decay}} = 0.05\) and \(\tau_{\text{decay}} = 12\) h. (e) Trail area fraction versus days in vitro for two different MG cell densities. (f) Trail area fraction versus time for several different model cell densities. The inset of (f) plots the steady state values of the trail area fraction for the different cell densities. The scale bar of (a) and (c) represents 200 \(\mu\)m.
Figure 6. Some exemplary passages of a freely crawling model cell for $K_{\text{decay}} = 0.01$ (a), 0.03 (b) and 0.07 (c) showing different degrees of directional persistence. (d) Angular correlation function versus time. $\theta$ is the angle between two tangent vectors of the cell trajectory, separated by a time $T$. $\langle \rangle$ represents an ensemble average. The red, green and blue lines correspond to $K_{\text{decay}} = 0.01$, 0.03 and 0.07, respectively. The dashed line is a fit of $f(t) = D e^{-t/\tau_1} + (1 - D) e^{-t/\tau_2}$ to the green line: $\tau_1 (= 37 \text{ min})$ originates from the existence of small zigzag 'runs,' and $\tau_2 (= 159 \text{ min})$ measures the long-range persistence time. See [24] for more details. The scale bars in (a)–(c) represent 200 $\mu$m, and those in the corresponding insets are 20 $\mu$m.

haptotaxis. Liquid-soluble chemoattractants known for the MG cells, such as LPS and ATP, could have played some role in the trail network formation. However, even if they did, it would have been very limited since fast advective flows existed within the system where the MG cells were monitored. In a separate experiment (not shown), we also found that the MG cells can recognize the physical landscape around them and they incorporate the surrounding landscape into their motile behavior. Hence, a multitude of both direct and indirect cell-to-cell signaling pathways for the MG cells could have existed.

Some of the key features of the observed patterns were well reproduced by a population of mathematical model cells, interacting with each other through a chemoattractant. The attractant was assumed not diffusing but only degrading with time, reflecting our experimental observations. We assumed that the presence of the attractant increases the activator, which in turn, enhances the actin polymerization to guide the cell motility along the existing trail. In addition, three parameters were found critical in determining the shape of the network: the decay time constant of the attractant, the rate constant that controls the directional persistence of
the individual cells and the cell density. The experimental observations and the corresponding computer simulations reported in this paper have differences in the cell density, the level of directional persistence in the crawling cells, the overall crawling velocity and others. As such, any quantitative comparisons between them are perhaps meaningless. Of course, if the multi-dimensional parameter space of the model was explored in a systematic fashion, a detailed quantitative comparison between the two could have been possible.

We note that at a qualitative level the observed phenomenon reported in this paper is reminiscent of the behavior of the human trail systems in urban green spaces such as parks [30]. Pedestrians also tend to follow previous paths and create a path network in a similar way. Perhaps, the big difference is that humans receive and process much more complex feedback from the surrounding environment and have a multitude of different purposes with their travels.

In conclusion, the haptotaxis-mediated evolving networks, which were neither random nor regular, define a new class of self-organized patterns formed by the populations of the crawling cells. The observed collective phenomenon can be general for any other immune cells such as neutrophils or for some metastasizing tumor cells that interact with each other by a haptotactic signal.
Figure 8. ‘Self-trapped’ oscillatory cells observed in a model simulation and an experiment. (a) A snapshot image of the attractant $q$ of 500 oscillatory model cells (taken 24 h after the start of the simulation) (see video S7 (see the supplementary material, available from stacks.iop.org/NJP/16/023017/mmedia); scale bar: 100 µm). (b) A close-up view of a model cell (red line) (boxed area in (a)) and the trace of its centroid (black line) for a 4 h duration (see video S7 (close-up view); scale bar: 20 µm). The parameters were $K_{\text{decay}} = 0.01$ and $\tau_{\text{decay}} = 12$ h. (c) An image of SD (5 min duration from 12 h in vitro) (see video S8, scale bar: 100 µm). (d) A close-up view of an MG cell that oscillates back and forth (red line) (see video S8 (close-up view), scale bar: 10 µm).

6. Materials and methods

6.1. Microglia cell culture

Primary cultures of the MG cells were prepared from the cerebral cortex of post natal day 1–2 Sprague Dawley rat brains as discussed in [24]. Briefly, the brains were excised and the cerebral cortices were removed. After a papain (dissociating enzyme) treatment, the fragmented tissues were collected and dissociated mechanically. The separated cells were grown in T-75 culture flasks (BD Falcon) for several days, and then the culture flasks were shaken by following a special protocol to separate the MG cells from the other types of cells (e.g. neurons and astrocytes). Then, the harvested MG cells were re-plated onto poly-d-lysine coated Petri dishes at a desired density for time-lapse observation. During the entire observation period of 3–4 days, the samples were stored in a home-built incubation chamber (37 °C, 5% CO$_2$) mounted on an inverted microscope (Olympus IX71) stage. The MG cells rarely replicated during the course of our experimental observation.
6.2. Immunofluorescence staining

The MG were washed once with a phosphate-buffered saline (PBS) buffer and then they were fixed in 4% paraformaldehyde for 20 min at room temperature. After rinsing with PBS, the cells were stained with FITC-conjugated antibody CD49e/ITGA5 100 μl at 0.1 mg ml⁻¹ (SM2215F, Acris, Germany) for 24 h at room temperature. Finally, the cells were washed twice in a staining buffer, and the fluorescence intensity of the labeled MG was analyzed with a laser-scanning confocal microscope (FV500, Olympus, Japan).

6.3. Scanning electron microscopy

The MG were fixed in Karnovsky’s fixative (2% glutaraldehyde and 2% paraformaldehyde in a 0.05 M sodium cacodylate buffer; pH 7.2) for 2 h at 4°C. And then the cells were dehydrated in increasing concentrations of ethanol (30–100%). Subsequently, the samples were dried at 60°C for 24 h and Pt coated (Sputter E-1030, Hitachi, Japan). The scanning electron microscopy (SEM) (FE-SEM S-4700, Hitachi, Japan) imaging was performed at 15 kV.

6.4. Ethics statement

This study was approved by our institutional review board for animal research, the Korea University Animal Care and Use Committee (KUIACUC-2011-197).

7. Mathematical model

As for the individual model cell, we modified the crawling cell model, which was originally proposed by Satulovsky et al [31], so that it would display the chemoattractant mediated interaction. Basically, a freely crawling cell is viewed as a simply closed loop (see the inset of figures 3(a) and S3(a) (see the supplementary material, available from stacks.iop.org/NJP/16/023017/mmedia)), whose boundary is regulated by two scalar fields, activator \( S^+(\vec{r}, t) \) and inhibitor \( S^-(t) \), reacting together and diffusing inside each cell. In other words, the individual model cell is viewed as a bag of a nonlinear chemical reactor, and the shape and the movement of the active bag is regulated by the local concentration fields of the activator and the inhibitor.

The points along the cell perimeter are represented by the vector \( \vec{r} \) with the centroid of the cell being the origin (see figure S3(b)). The activator \( S^+(\vec{r}, t) \) is a local variable, whereas the inhibitor \( S^-(t) \) is a global variable, and their local concentrations regulate the movement of the cell boundary. For each iteration time step, the points along the perimeter can either advance, retreat or stay based on the following set of rules (see figure S3(a)). Retraction occurs when \( S^+(\vec{r}, t) \leq S^-(t) \), and the rate of retraction is governed by the following stochastic equation:

\[
\frac{\partial |\vec{r}|}{\partial t} = -\max(0, |\vec{r}| - r_{\text{min}}) R^-, \quad (1)
\]

where \( r_{\text{min}} \) is the constant minimum radius and \( R^- \) is the retraction rate constant. The function \( \max(x, y) \) selects the larger value out of \( x \) and \( y \). Protrusion occurs when \( S^+(\vec{r}, t) > S^-(t) \) at a rate governed by the following equation:

\[
\frac{\partial |\vec{r}|}{\partial t} = \max(0, G(R^+)), \quad (2)
\]
where $R^+$ is the average protrusion rate and $G(R^+)$ is a random number generated from a Gaussian distribution of the mean $R^+$ and the variance $R^+$.

The evolution of the activator $S^*(\vec{r}, t)$ is governed by the equation

$$\frac{\partial S^*(\vec{r}, t)}{\partial t} = K_{\text{diff}} \nabla^2 S^*(\vec{r}, t) - K_{\text{decay}} S^*(\vec{r}, t) + q(\vec{x}, t)$$

$$+ \max(G((f(S^*(\vec{r}, t) - S^-(t), \gamma, \lambda) + P_{\text{baseline}})N_{\text{burst}}), 0). \quad (3)$$

The first term accounts for the diffusion of $S^*$ along the cell perimeter and the second term renders a self-decomposition of $S^*$. The third term confers the attractant mediated cell-to-cell interaction, in which

$$q(\vec{x}, t) = q_0 \sum_i e^{-(t-t_i)/\tau_{\text{decay}}} \quad (4)$$

represents the total concentration of the attractant deposited at $\vec{x}$, a position vector with respect to the laboratory frame of reference. $t_i$ represents the specific time of the $i$th visit made by a cell in the past. The attractant decomposes by itself with a time constant $\tau_{\text{decay}}$ (see figure S3(b)).

The last term is a stochastic positive feedback loop accounting for both the local stimulation and the existence of a random signal. The function $f(x, \gamma, \lambda) = 0$ for $x < \lambda$ and $(x - \lambda)$ for $x \geq \lambda$, where $\lambda$ is a threshold value for the feedback. $P_{\text{baseline}}$ accounts for the rate of the random bursts caused by the internal baseline activities. The function $G$ again represents a random number generated from a Gaussian distribution.

The retraction signal is governed by the global inhibition rule $S^-(t) = C^- A \int S^*(\vec{r}, t) d\vec{r}$, where $C^-$ is the inhibition constant, $A$ is the total area of the cell and the integration is a line integral over the entire cell border, which is composed of 360 pixels (i.e. 1 pixel for 1° with respect to the centroid). Each pixel corresponds to 0.286 μm and one iteration time step is 1 s. At each iterative time step, the formations of the focal adhesions and their detachments are assigned stochastically to the points along the cell perimeter with probabilities $P_{fa}^+$ and $P_{fa}^-$, respectively. The retraction is inhibited when a perimeter point hits a focal adhesion.

Then, we consider a large population of these cells that interact with each other by recognizing the attractant $q$, continuously excreted by themselves as they crawl around. Therefore, when a model cell encounters a $q$-deposited trail, it will be guided along the trail since $q$ is a $S^*$ enhancing chemical agent. When the model cells crawl out of the view field, they are forced to reenter back to the system and the site of reentry is chosen randomly all around the system boundary.

The following fixed parameter values are used for our model cells throughout this paper: $R^+ = 0.103 \, \mu\text{m s}^{-1}$, $R^- = 0.02811 \, \text{s}^{-1}$, $K_{\text{diff}} = 11.9 \, \mu\text{m}^2 \text{s}^{-1}$, $N_{\text{burst}} = 13$, $P_{\text{baseline}} = 0.1811/(\text{s} \cdot \mu\text{m})$, $\lambda = 3.221/\mu\text{m}$, $\gamma = 29.11 \, \text{s}^{-1}$, $C^- = 1.93 \times 10^{-5} \, 1/\mu\text{m}^3$, $P_{fa}^+ = 0.0003$, $P_{fa}^- = 0.0058$, $r_{\text{min}} = 2.857 \, \mu\text{m}$ and $q_0 = 0.008$. Indeed, the single cell model has numerous parameters, and we have selected a set of values such that the model cell’s free crawling behavior is similar to that of a freely crawling real MG cell. In reality, the MG cells can show some significant variations in their motile properties (such as velocity, directional persistence, etc). However, we have assumed a homogeneous population in our simulation. The values of $K_{\text{decay}}$, $\tau_{\text{decay}}$ and the number of cells are specified in the corresponding figure legends.
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