Chemotaxis with directional sensing during Dictyostelium aggregation

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With an *in silico* analysis, we show that the chemotactic movements of colonies of the starving amoeba *Dictyostelium discoideum* are driven by a force that depends on both the direction of propagation (directional sensing) of reaction-diffusion chemotactic waves and on the gradient of the concentration of the chemoattractant. It is shown that the directional sensing of amoebae is due to the sensitivity of the cells to the time variation of the concentration of the chemoattractant combined with its gradient. It is also shown that chemotaxis exclusively driven by local concentration gradients leads to unstable local motion, preventing cells from aggregation. These facts show that the formation of mounds, which initiate multicellularity in *Dictyostelium discoideum*, is caused by the sensitivity of the amoebae to three factors, namely, to the direction of propagation of the chemoattractant, to its gradient, and to the spiral spatial topology of the propagating chemoattractant.

Chemotaxis is the phenomenon in which cells or microorganisms direct their movement as a response to the local variation of the concentration of some chemical substance. For example, in colonies of *Dictyostelium discoideum* (Dd), localized groups of starving amoebae initiate the production of cAMP (cyclic adenosine monophosphate) that spreads in space as reaction-diffusion travelling chemical waves. In the vicinity of these spontaneously formed cAMP diffusion centers, the amoebae sense cAMP and direct their chemotactic movements towards the initiation centers. Near these centers, amoebae rotate around a spontaneously formed hole (i.e., a zone depleted from cells), the cell density increases locally and, at a later stage, a new multicellular organism is formed, Fig. 1. This is one of the simplest known mechanisms of transition from colonies of unicellular to multicellular organisms. For a detailed description, see [9].

Experimental observations show that Dd cells move towards a region where cAMP chemical waves are produced, and the speed of cells is proportional to the slope or gradient of the concentration of cAMP, [15]. On the other hand, it has been observed that cells move as long as the gradient is positive, when measured along the direction of the wave source, [9, 17]. "When the slope reverses, Dd cells stop moving and await for the next wave", [9, p. 101], [17].

Here, we investigate whether the force that drives the chemotaxis of Dd cells is solely proportional to the gradient of the chemotactic substance or whereas it also depends on the direction of propagation (directional sensing) of the time varying chemotactic signal.

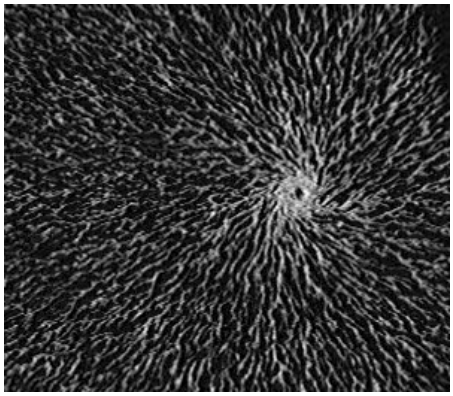

FIG. 1. Aggregation of a colony of *Dictyostelium discoideum* towards a spontaneously formed aggregation "center". Near the aggregation center, amoebae begin to rotate around a spontaneously formed hole. This hole is characterized by a depletion of cells. At a later stage, a new phase of the life cycle is reached, leading to the transition to a multicellular aggregate. (Data from Christiane Hilgardt, [6]).

If the chemotactic substance is produced at a localized source and disperses monotonically along space, the question of directional sensing is meaningless. However, if the chemoattractor propagates along space as an oscillatory wave, the hypothesis that the chemotactic motion of cells is driven by a local concentration gradient implies the existence of an oscillatory variation in time of the direction of motion of the cells. This simple gradient hypothesis induces motion of the amoebae in the direction opposed to the source of the chemotactic signal, leading, asymptotically in time, to dispersive motion, preventing the amoebae from aggregation (Appendix A). As far as we

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know, this oscillatory behavior in the aggregation pattern of *Dictyostelium discoideum* has never been observed.

In the experimentally oriented literature, it is implicitly assumed that Dd cells are sensitive to the direction of propagation of the cAMP wave [7, 13, 14, 16]. On the other hand, in simulation studies, it is generally assumed that cell chemotaxis is only driven by the sensitivity of the cells to the gradient of the chemotactic substance, [2, 8, 11, 12, 18], and directional sensing is not included in models.

Since we are interested in unravelling the importance of these two effects in the Dd aggregation phenomena, we will let the cells propagate in an external concentration field of cAMP. For simplicity, we will omit some well-known properties of Dd aggregation, like the fact that Dd amoebae produce and relay cAMP upon sensing of an external concentration of the chemoattractant, [5], and the phenomenon of streaming, [9].

a. Chemotaxis with directional sensing We consider that $X(x,y,t)$ represent the local concentration of some chemotactic substance. The equation of motion of an amoeba under the influence of a chemoattractant has the form,

$$m\ddot{\vec{r}} = -\lambda\dot{\vec{r}} + \vec{F}(X(x,y,t)) \tag{1}$$

where, as usual, the dots represent time derivatives, $\vec{r} = (r_x, r_y)$ are the spatial position coordinates of the amoeba, λ is a damping coefficient, m is the mass of the amoeba and $\vec{F}$ represents a generic chemotactic force field. The term $-\lambda\vec{r}$ describes the damped motion of the cells and has been measured experimentally, [1].

As it is shown in the Appendix A, the direction dependent chemotactic force field is,

$$\vec{F} = \begin{cases} \overrightarrow{\text{grad } X} & \text{if} \quad \text{sign}\left(\frac{\partial X}{\partial t}\right) > 0 \\ \vec{0} & \text{if} \quad \text{sign}\left(\frac{\partial X}{\partial t}\right) \leq 0\,. \end{cases} \tag{2}$$

Introducing (2) into (1), the equations of motion of an amoeba under the influence of the chemotactic signal $X(x,y,t)$ are:

i) If $\text{sign}\left(\frac{\partial X}{\partial t}\right) > 0$,

$$\begin{cases} m\ddot{r}_x &= -\lambda\dot{r}_x + \mu\dfrac{\partial X}{\partial x} \\ m\ddot{r}_y &= -\lambda\dot{r}_y + \mu\dfrac{\partial X}{\partial y}\,. \end{cases} \tag{3}$$

ii) If $\text{sign}\left(\frac{\partial X}{\partial t}\right) \leq 0$,

$$\begin{cases} m\ddot{r}_x &= -\lambda\dot{r}_x \\ m\ddot{r}_y &= -\lambda\dot{r}_y\,. \end{cases} \tag{4}$$

The equations of motion (3)-(4) have been derived under the assumption that amoebae are sensitive to the direction of propagation of the chemotactic wave, together with the condition that if the slope of the gradient reverses sign, there is no chemotactic motility.

To describe the spatial variation of the chemoattractant $X(x,y,t)$, we consider the Ginzburg-Landau reaction-diffusion equation, [4],

$$\begin{cases} \dfrac{\partial X}{\partial t} &= \nu\bar{X} - \beta\bar{Y} + (\bar{X}^2 + \bar{Y}^2)\left(a\bar{X} - b\bar{Y}\right) + D_X\Delta X \\ \dfrac{\partial Y}{\partial t} &= \beta\bar{X} + \nu\bar{Y} + (\bar{X}^2 + \bar{Y}^2)\left(a\bar{Y} + b\bar{X}\right) + D_Y\Delta Y \end{cases} \tag{5}$$

where $\bar{X} = X - X^*$, $\bar{Y} = Y - Y^*$, $\Delta = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2}$ is the two-dimensional Laplace operator, D_X and D_Y are diffusion coefficients, and ν, β, a, b, X^* and Y^* are parameters. The functions X and Y represent chemical variables. In bounded two-dimensional domains with zero flux boundary condition, if $\nu > 0$ and $a < 0$, the reaction-diffusion equation (5) has an homogeneous and unstable spatially extended steady state with constant values, $X(x,y,t) = X^*$ and $Y(x,y,t) = Y^*$. A local perturbation of this unstable spatial steady state leads to spatial oscillations of X and Y. For these parameter values, the ordinary differential equation part of equation (5) ($D_X = D_Y = 0$) has a limit cycle in the (X,Y) phase space with radius $r = \sqrt{-\nu/a}$.

For $\nu > 0$ and $a < 0$ and particular choices of the initial conditions, the numerical solutions of the the Ginzburg-Landau reaction-diffusion equation (5) in bounded domains and with zero flux boundary conditions produce wavelike target and spiral propagating patterns [4], similar to the ones observed in the early phase of aggregation of Dd colonies. Therefore, in order to investigate numerically the aggregation properties of the model equations (3)-(4), we have integrated numerically equation (5) in a circular region inside a square of side length $Ndx = 600 \times dx$. We have considered zero flux boundary conditions on the circular region, and the parameter values considered were $\nu = 1$, $\beta = 0.5$, $a = -1$, $b = 1$, $D_X = D_Y = 0.01042$, $X^* = Y^* = 1.5$, $dt = 0.005$ and $dx = \sqrt{6D_X dt} = 0.0177$, [3].

In Fig. 2, we show the time evolution of 1000 amoebae, calculated from (3)-(4), coupled with the chemotactic signal $X(x,y,t)$, propagating as a reaction-diffusion spiral wave emanating from the central region of the two-dimensional domain. The chemotactic signal $X(x,y,t)$ has been calculated from equation (5). The perturbation leading to a spiral wave has been introduced by perturbing four contiguous lattice sites with X and Y taking values on the limit cycle of the diffusion-free Ginzburg-Landau equation (5), [3, 4]. The values of the variables X and Y for these four sites have a phase advance in phase space of $\pi/2$. Initially, the amoebae move towards the central region of the circular domain and, after a transient time, all the cells rotate near the tip of the spiral, forming a ring as shown in Fig. 2d). The radius of the ring is related to the diffusion coefficients of X and Y, i.e., the radius augments with increasing diffusion coefficients.

Comparing the numerical simulation of Fig. 2d) with the aggregation patterns of Dd cells in Fig. 1, we con-

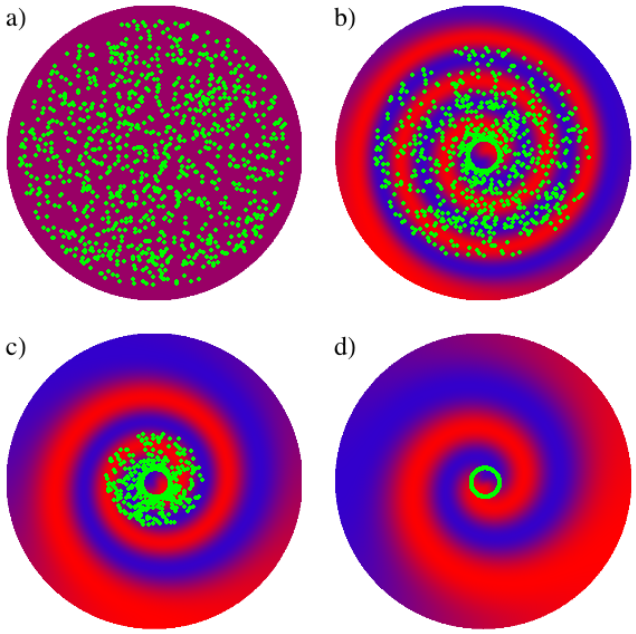

FIG. 2. (Color online) Time evolution of the position of 1000 amoebae in a two-dimensional circular domain, calculated with equations (3)-(4), for a) $t = 0$, b) $t = 40$, c) $t = 60$ and d) $t = 80$. Each green spot represents one amoeba. Red and blue correspond to regions of high and low concentrations of X, respectively. The chemotactic signal is a reaction-diffusion spiral wave originated at the central point of the two-dimensional domain. The parameters of the dynamics of the amoebae are $m = 0.0001$, $\lambda = 0.0001$ and $\mu = 0.00001$. The parameters associated with the dynamics of the chemotactic signal are described in the text.

clude that the rotating ring pattern of *Dictyostelium discoideum* colonies is similar to the rotating ring pattern obtained in these simulations. This suggests that the hole observed in Fig. 2d) is due to the rotation of the tip of the reaction-diffusion spiral wave associated with the dynamics of the chemoattractant X. Chemotactic models without the directional sensing condition introduced in (3)-(4), would lead to a non aggregative behavior as in the case of the simulation in Fig. 3 of the appendix A.

We have also tested the amoeba motion in the presence of a target waves, generated by the Ginzburg-Landau equation (5). In this case, the amoebae move towards the centre of the circular domain, accumulating near the wave initiation centre. After reaching this spotty region, all the amoebae stop moving.

b. Discussion From the model just described, we conclude that the typical circular ring pattern found in the aggregation of Dd colonies is explained by the sensitivity of the amoebae to the direction of propagation and to the gradient of the chemoattractant, together with the spiral shape of the propagating chemoattractant signal. In other models without directional sensing, [10], spiral chemoattractant waves do not produce this rotating motion of the Dd cells. This dynamic effect is at the origin of the multicellularity and of the formation of the mound, [9], prior to emergence of the Dd slug.

The sensitivity of amoebae to the gradient of concentration is due to the large number of cAMP receptors (of the order of 50,000) distributed along the cytoplasm membrane of amoebae, [9]. The density of occupation of the cAMP receptors along the amoeba's external membrane is a sensor for the gradient of chemoattractant, being in the origin of the motility of the cells against the chemotactic gradient. Dd amoebae can detect a 1% difference in concentration of the chemoattractant between the front and the back of the cells, [15, 19]. This spatial information together with the local sensitivity of the cells to the time variation of the concentration of chemoattractant, determines the directional sensing of amoebae. If the local concentration of chemoattractant decreases, the binding rate of cAMP to its receptors also decreases, justifying the sensitivity of the cells to the time variation of the concentration of chemoattractant.

These biological mechanisms explain why amoebae are sensitive to the temporal and spatial derivatives of the chemotactic field, explaining the stop moving condition, as observed in the Dd aggregation, [16, 17]. On the other hand, our simulations results are consistent with the polarization effects observed in the amoebae during aggregation, [14]. For the sake of clarity, we have concentrated this investigation on the roles of the cAMP gradient and directional sensing. Consequently, in the present approach, we did not consider other well-known effects observed in the aggregation of Dd colonies as streaming, [2, 9], and cAMP production by amoebae, [5].

Appendix A

We consider a chemotactic substance distributed along a one-dimensional domain. We assume that the concentration of this substance evolve in time and space as a one-dimensional travelling wave, symmetrically along the positive and the negative x-directions. This centrally symmetric wave is generated at the point $x = 0$ and has radial propagation speed $c > 0$. To simplify, we assume that the concentration of the chemotactic substance propagates according to the wave type law,

$$\phi(x,t) = \begin{cases} A(1-\cos(ct-|x|)), & \text{if } |x| \le ct \\ 0, & \text{if } |x| > ct \end{cases} \tag{A1}$$

where A is the concentration amplitude, and $t \ge 0$.

At time $t = 0$, we consider an immobile amoeba at position $x_0 > 0$. Assuming that the response of the amoeba to the chemotactic signal is solely proportional to its gradient, as it is generally assumed in the literature, [2, 11, 12], the one-dimensional equation of motion of the amoeba is,

$$m\ddot{x} = -\lambda\dot{x} + \frac{\partial\phi}{\partial x} \tag{A2}$$

where λ is a damping constant and $x(t)$ is the position of the amoeba at time t. Equation (A2) describes the chemotactic response of an amoeba without directional sensing.

To implement directional sensing in the model equation (A2), we now introduce vectorial notation. The effect of the chemoattractant $\phi(x,t)$ on the amoebae is assumed to be described by the force,

$$\vec{F} = \left(\frac{\partial \phi}{\partial \vec{n}}\right)\vec{n} = \left(\frac{\partial \phi}{\partial x}\right) n_x \vec{n} \tag{A3}$$

where $\vec{n} = n_x e_x$ is the direction of propagation of the chemical wave. In this one-dimensional case, $n_x = \pm 1$ and e_x is the usual direction versor. As the wave (A1) propagates radially from the origin of coordinates $x = 0$, if $x > 0$, then, $n_x = +1$. If $x < 0$, then $n_x = -1$. To introduce the directional sensitivity of the amoeba, we assume that the amoeba moves towards the origin $x = 0$ when it senses a positive slope of the gradient, measured in the negative x-direction ($\frac{\partial \phi}{\partial x} < 0$). The amoeba stops moving when the slope of the gradient reverses sign. Therefore, the response of the amoeba to the chemical signal with directional sensing is described by the effective force,

$$\vec{F} = F e_x = \begin{cases} \left(\dfrac{\partial \phi}{\partial x}\right) n_x \vec{n} = \left(\dfrac{\partial \phi}{\partial x}\right) n_x^2 e_x = \left(\dfrac{\partial \phi}{\partial x}\right) e_x, \\ \quad \text{if} \quad \left(\dfrac{\partial \phi}{\partial x}\right) n_x < 0 \\ 0 e_x, \quad \text{if} \quad \left(\dfrac{\partial \phi}{\partial x}\right) n_x \geq 0\,. \end{cases} \tag{A4}$$

In order to determine a closed form for the direction versor n_x, we consider the scalar wave field, $\phi(x,t) = \cos(ct - x)$, where c is a positive constant, $|x| \leq ct$ and $x, t \in \mathbf{R}$.

As $c > 0$, the scalar field $\phi(x,t)$ propagates in the positive x-direction and, for each fixed $t = t_1$, we can have two cases: i) If, $\frac{\partial \phi}{\partial x}|_{t=t_1} < 0$, then, $\frac{\partial \phi}{\partial t}|_{t=t_1} > 0$. ii) If, $\frac{\partial \phi}{\partial x}|_{t=t_1} > 0$, then, $\frac{\partial \phi}{\partial t}|_{t=t_1} < 0$. Therefore, in both cases, sign $\left(\frac{\partial \phi}{\partial x}\frac{\partial \phi}{\partial t}\right) < 0$, where sign$(x) = 1$ for $x > 0$, and sign$(x) = -1$ for $x < 0$.

Assume now that the scalar field propagates in the negative x-direction, that is, $c < 0$. i) If, $\frac{\partial \phi}{\partial x}|_{t=t_1} < 0$, then, $\frac{\partial \phi}{\partial t}|_{t=t_1} < 0$. ii) If, $\frac{\partial \phi}{\partial x}|_{t=t_1} > 0$, then, $\frac{\partial \phi}{\partial t}|_{t=t_1} > 0$. Therefore, in both cases, sign $\left(\frac{\partial \phi}{\partial x}\frac{\partial \phi}{\partial t}\right) > 0$.

So, the direction of propagation of the one-dimensional scalar field $\phi(x,t)$ is,

$$n_x = -\text{sign}\left(\frac{\partial \phi}{\partial x}\frac{\partial \phi}{\partial t}\right)\,. \tag{A5}$$

Introducing (A5) into (A4), the effective force on the amoeba is,

$$\vec{F} = F e_x = \begin{cases} \left(\dfrac{\partial \phi}{\partial x}\right) e_x, \quad \text{if} \quad -\text{sign}\left(\dfrac{\partial \phi}{\partial t}\right) < 0 \\ 0 e_x, \quad \text{if} \quad -\text{sign}\left(\dfrac{\partial \phi}{\partial t}\right) \geq 0\,. \end{cases} \tag{A6}$$

Hence, the equation describing the motion of the amoeba is,

$$m\ddot{x} = -\lambda \dot{x} + F \tag{A7}$$

where F is given by (A6). Equation (A7), together with (A6), describes the chemotactic force on the amoeba, taking into account directional sensing.

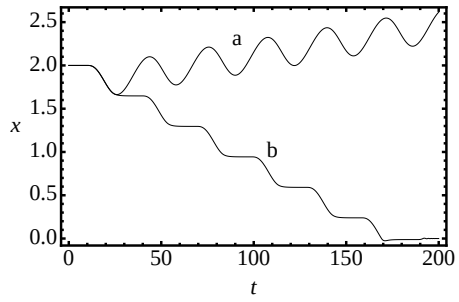

FIG. 3. Position of one amoeba as a function of time according to equations (A2) (a) and (A6)-(A7) (b). The initial position of the amoeba has been set to $x_0 = 2$ with zero initial speed. The parameters of the simulations are, $A = 0.02$, $c = 0.5$, $m = 1$ and $\lambda = 0.5$. From these numerical simulations, we conclude that the sensitivity to the gradient and to the direction of propagation (case b) explains cell movements towards the origin of coordinates, as observed in experiments.

In Fig. 3, we show the time evolution of the position of a cell with initial condition $x_0 = 2$ and $\dot{x} = 0$, calculated with equations (A2) and (A6)-(A7), respectively, and chemotactic field (A1). Initially, the cell is immobile. For $t \geq x_0/c = 2/c$, the movement of the amoeba is equally described by the two model equations and the amoeba starts to move in the direction of the point $x = 0$. For the case of simple chemotaxis, the motion is described by equation (A2). For larger values of t, the he slope of the chemoattractant concentration changes sign and the amoeba reverses the direction of motion. In this case, the motion of the amoeba is oscillatory and numerical simulations show that the cell slowly deviates from the origin of coordinates. Asymptotically in time, the position of the amoeba goes away from the source of the chemoattractant. A simple linear analysis shows that equation (A2) is linearly unstable around the phase space fixed point $(x = 0, \dot{x} = 0)$.

In contrast, using model equation (A7), with the force (A6), amoebae direct their motion towards the origin of coordinates, where the source of the chemoattractant is located.

The simulations in Fig. 3 show that a model based on equation (A6)-(A7) describes the observed motility in the aggregation of *Dictyostelium discoideum* colonies. On the other hand, the model based on equation (A2) shows that wave driven chemotaxis without directional sensing leads to divergent motion.

For wave propagation in two-dimensional domains, the directional sensing condition in (A6) and the direction versor (A5) are simply generalised.

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[1] H. U. Bödeker, C. Beta, T. Frank and E. Bodenschatz, Quantitative analysis of random ameboid motion, EPL 90 (2003) 28005.

[2] J. Dallon and H. Othmer, A discrete cell model with adaptive signaling for aggregation of *Dictyostelium discoideum*, Phil. Trans. R. Soc. Lond. B 352 (1997) 391-417.

[3] R. Dilão and J. Sainhas, Validation and Calibration of Models for Reaction-Diffusion Systems, Int. J. of Bifurcation and Chaos 8 (1998) 1163-1182.

[4] R. Dilão and A. Volford, Excitability in a Model with a Saddle-Node Homoclinic Bifurcation, Discrete and Continuous Dynamical Systems B 4 (2004) 419-434.

[5] A. Goldbeter and L. Segel, Unified mechanism for relay and oscillation of cyclic AMP in *Dictyostelium discoideum*, Proc. Natl. Acad. Sci. USA 74 (1977) 1543-1547.

[6] C. Hilgardt, Biologische Variabilität bei der Musterbildung von Dictyostelium discoideum, PhD Thesis, Otto-von-Guericke-Universität Magdeburg, 2010.

[7] C. Janetopoulos and R. Firtel, Directional sensing during chemotaxis, FEBS Letters 582 (2008) 2075-2085.

[8] E. Keller and L. Segel, Initiation of slime mold aggregation viewed as an instability, J. theor. Biol. 26 (1970) 399-415.

[9] R. H. Kessin, *Dictyostelium*, Evolution, Cell Biology and the Development of multicellularity, Cambridge University Press, 2001.

[10] D. Kessler and H. Levine, Pattern formation in *Dictyostelium* via the dynamics of cooperative biological entities, Phys. Rev. E 48 (1993) 4801-4804.

[11] J. Murray, Mathematical Biology, Springer-Verlag, 1989.

[12] S. Nagano, Diffusion-Assisted Aggregation and Synchronization in Dictyostelium discoideum, Phys. Rev. Lett. 80 (1988) 4826-4829.

[13] C. A. Parent, B. J. Blacklock, W. M. Froehlich, D. B. Murphy and P. N. Devreotes, G protein signaling events are activated at the leading edge of chemotactic cells, Cell 95 (1998) 81–91.

[14] A. Samadani, J. Mettetal and A. van Oudenaarden, 2006. Cellular asymmetry and individuality in directional sensing, Proc. Natl. Acad. Sci. USA 103 (2006) 265–289.

[15] L. Song, S. M. Nadkarnia, H. U. Bödeker, C. Beta, A. Bae, C. Franck, W. J. Rappel, W. F. Loomis and E. Bodenschatz, Dictyostelium discoideum chemotaxis: Thresholds for directed motion, Eur. J. Cell Biol. 85 (2006) 981–989.

[16] K. F. Swaney, C.-H. Huang and P. Devreotes, Eukaryotic chemotaxis: A network of signaling pathways controls motility, directional sensing, and polarity, Annual Review of Biophysics 39 (2010) 11549-11554.

[17] K. Tomchik and P. Devreotes, Adenosine 3'-5'-monophosphate waves in Dictyostelium discoideum: A demonstration by isotope dilution-fiuorography, Science 212 (1981) 443–446.

[18] C. van Oss, A. V. Panfilov, P. Hogeweg, F. Siegert and C. J. Weijer, Diffusion-Assisted Spatial pattern formation during aggregation of the slime mould Dictyostelium discoideum, J. theor. Biol. 181 (1996) 203-213.

[19] S. H. Zigmond, Ability of polymorphonuclear leukocytes to orient in gradients of chemotatic factors, J. Cell Biol. 75 (2) (1977) 606-616.