From Crawlers to Swimmers- Mathematical and Computational Problems in Cell Motility

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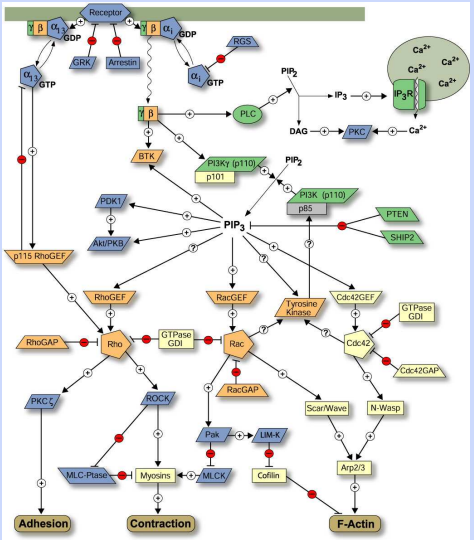
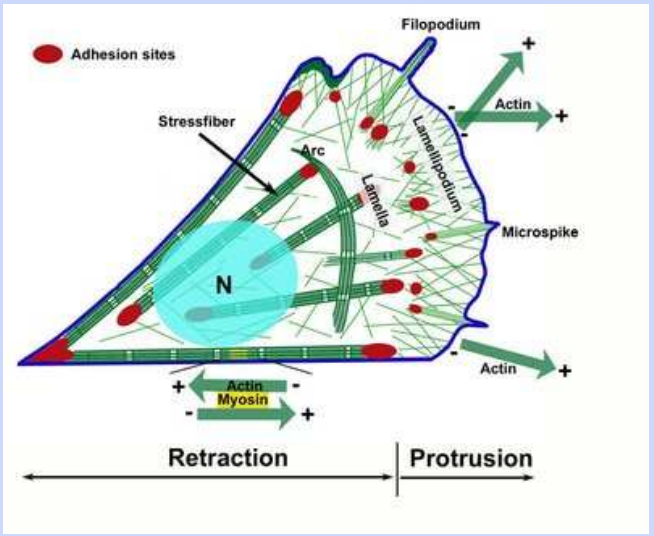
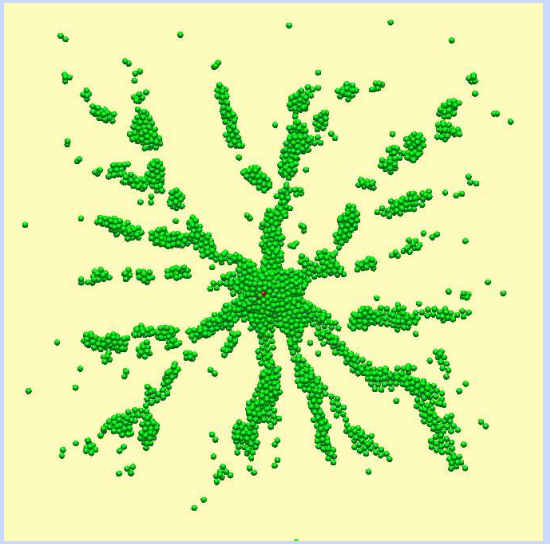






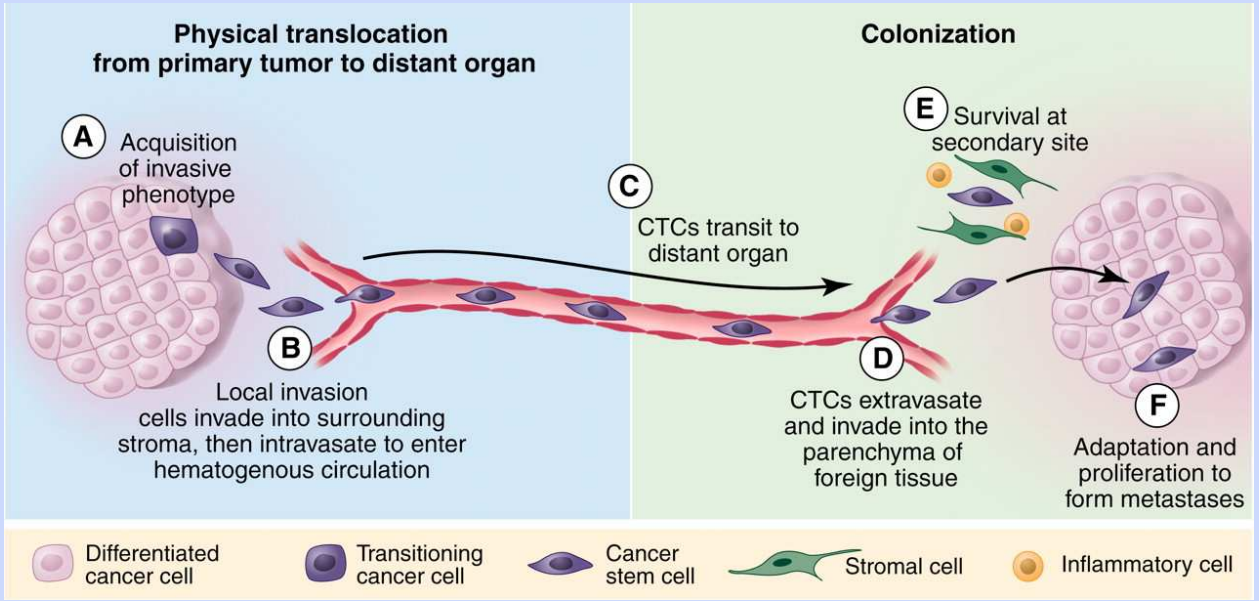
Edinburgh – Oct. 2017 – Lecture 1

Overview

- Why cell motility is important and how cells move
- Description of a model system - Dictyostelium discodeum
- Signal transduction and direction sensing
- How cells swim by shape changes
- Actin dynamics
- Stochastic modeling of actin waves
- Mechanics of multicellular aggregates and tissues

Why understanding cell motility is important ...

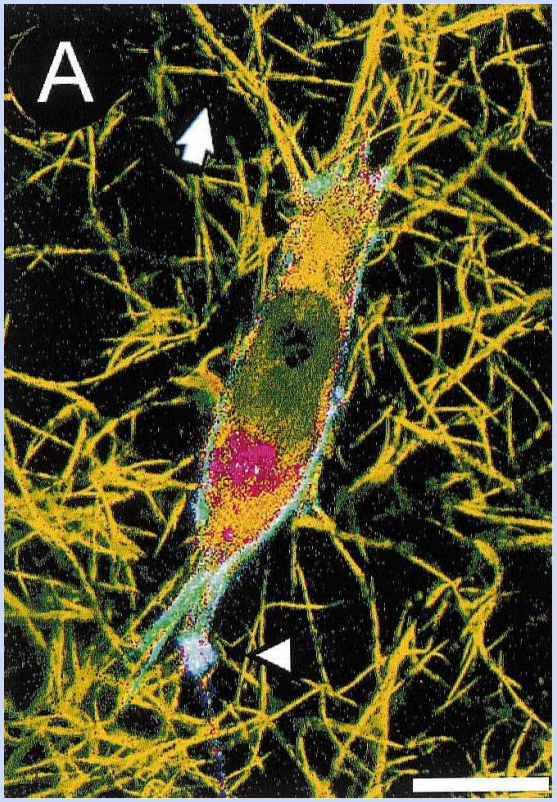
- Development – we start as a single cell but ultimately have ~ 250 cell types correctly located in the adult. Morphogenetic movements occur at both the single cell and tissue levels.
- The immune system – e.g., neutrophils respond to bacterial invasion.
- Wound healing – some cells move into a wound to fight infection, others to close the wound and rebuild the tissue.
- Metastasis in cancer – invasion of new sites by active migration and passive transport in the circulatory system

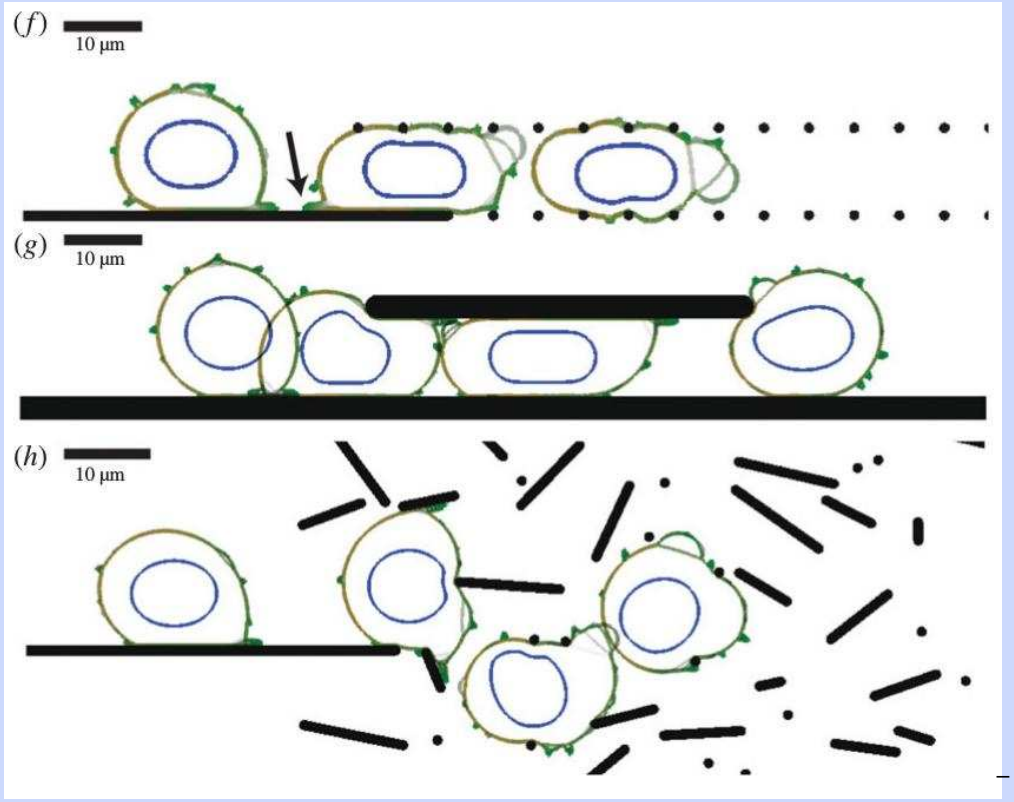


Various modes of cell motility

1. Swimmers
   - Bacteria – individually and as swarms, Sperm, ...

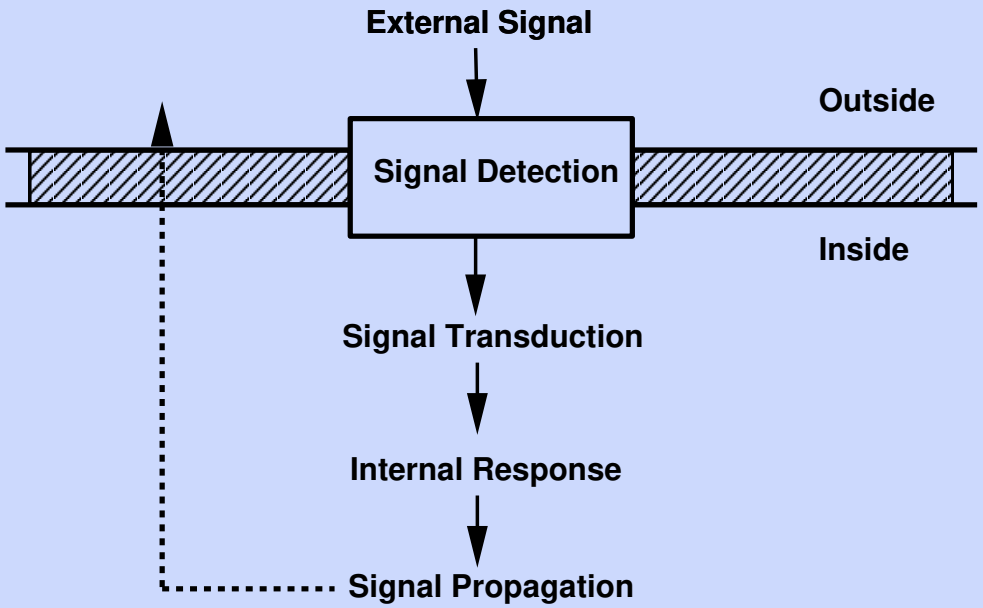
2. Crawlers
   - Lot of examples – neutrophils, fibroblasts, macrophages, leukocytes, Dictyostelium —

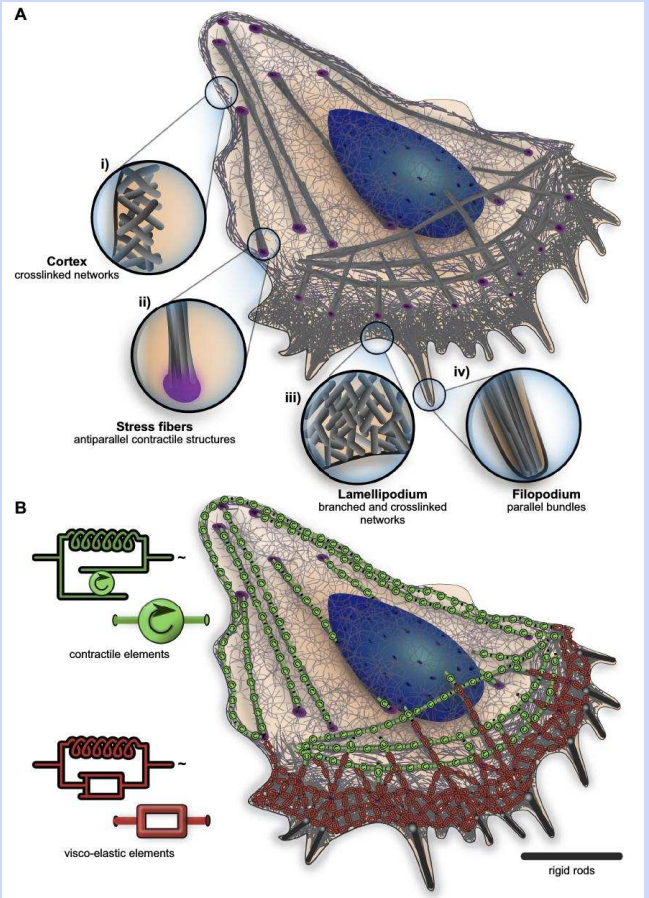




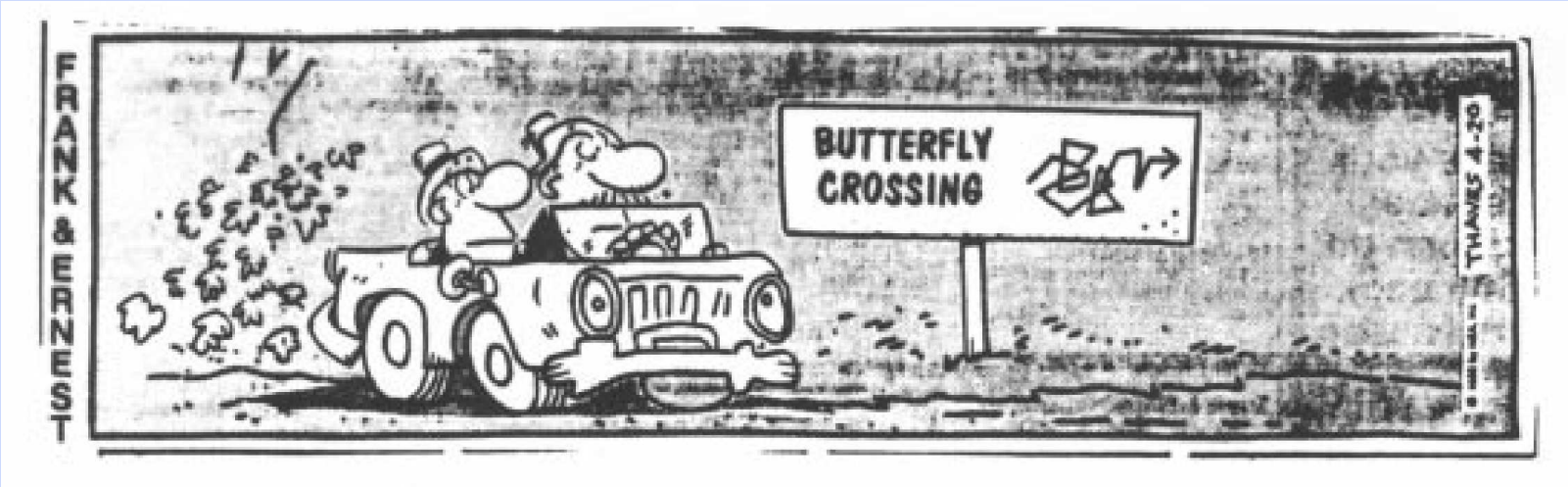
The basic processes in movement at the cell level

- An external signal and transduction of it into ‘information’ for movement
- Orientation toward the signal
- Movement – which involves directed force generation and frequently some level of remodeling of the internal structure
- Generation of the energy required to produce force and movement





The basic problem and terminology .....



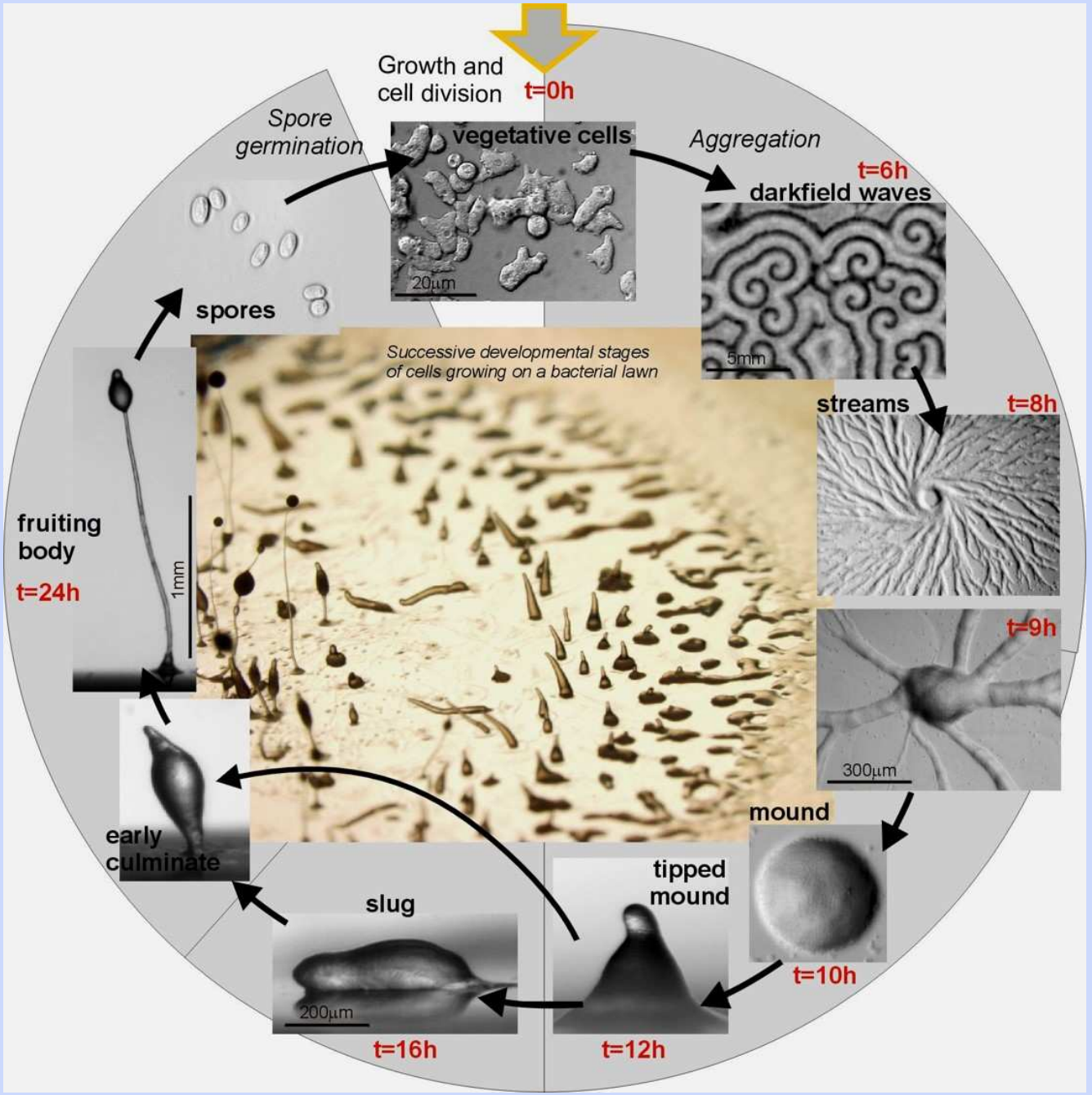
Taxis: A behavioral response in which a motile cell or organism alters its direction of motion in response to an external stimulus, without changing its speed or turning rate.

Examples: Chemotaxis, geotaxis, aerotaxis, haptotaxis, ...

Kinesis: A behavioral response in which a motile cell or organism changes its rate of locomotion or turning in response to the intensity, but not the direction, of an external stimulus.

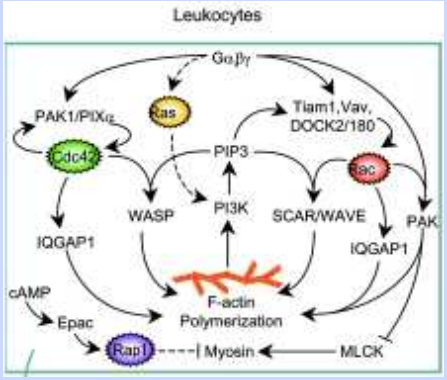
In many systems the external stimulus is a scalar field, but stresses can play a role.

A model system – *Dictyostelium discoideum*

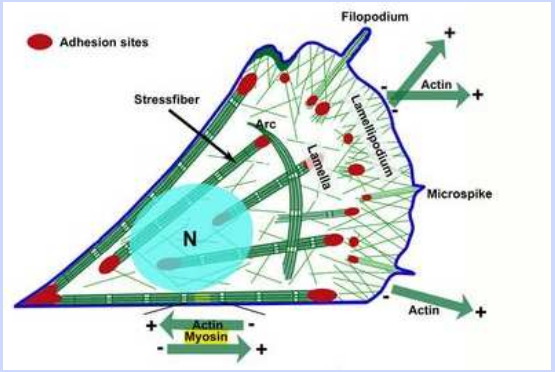


This leads to three major problems ...

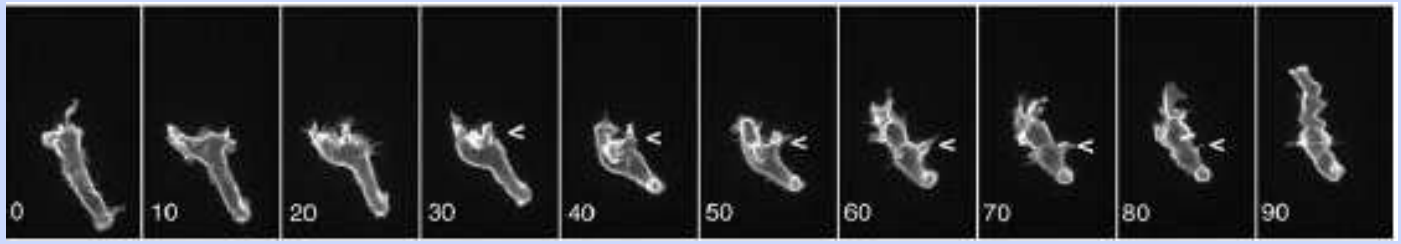
• The transduction problem — how are extracellular signals transduced into intracellular signals that can be used to control the shape changes. (Module I)



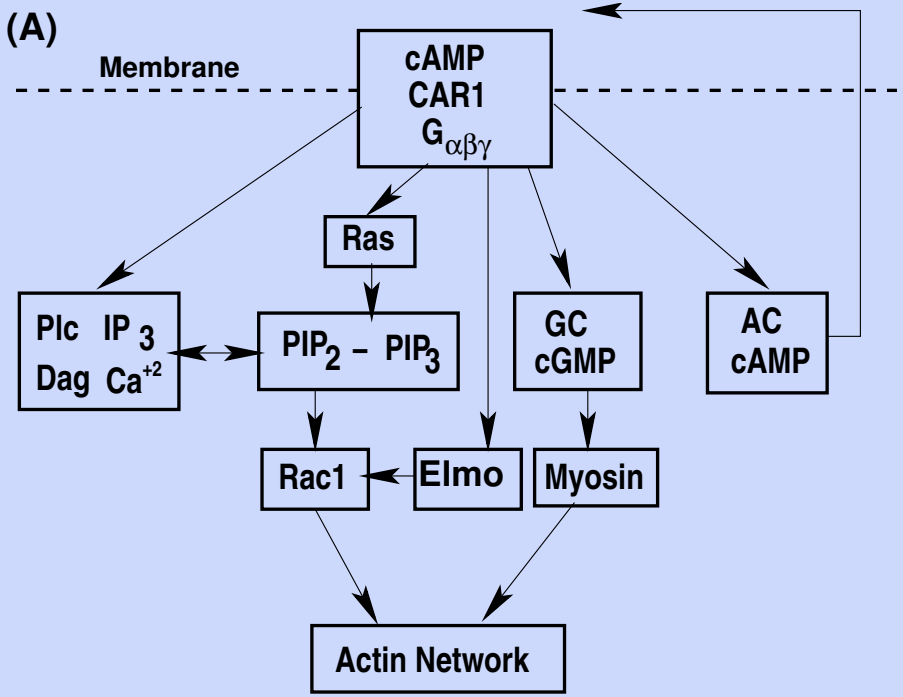
• The interior problem — how does the cell control the shape changes that give rise to motion. (Module II)

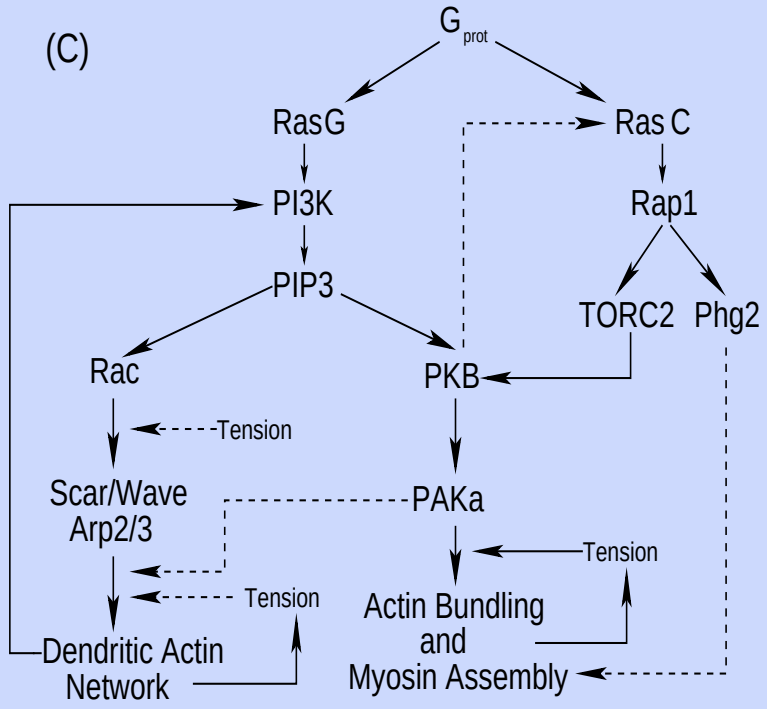


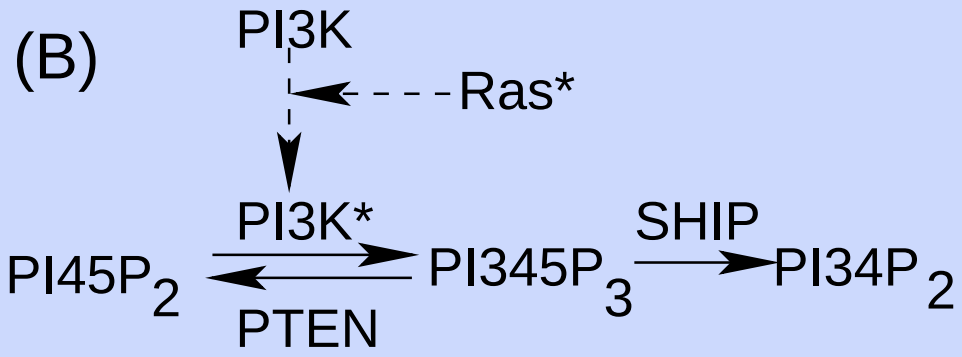
• The exterior problem — how do the shape changes give rise to motion, how fast do they move, and how efficient is the motion. (Module III)



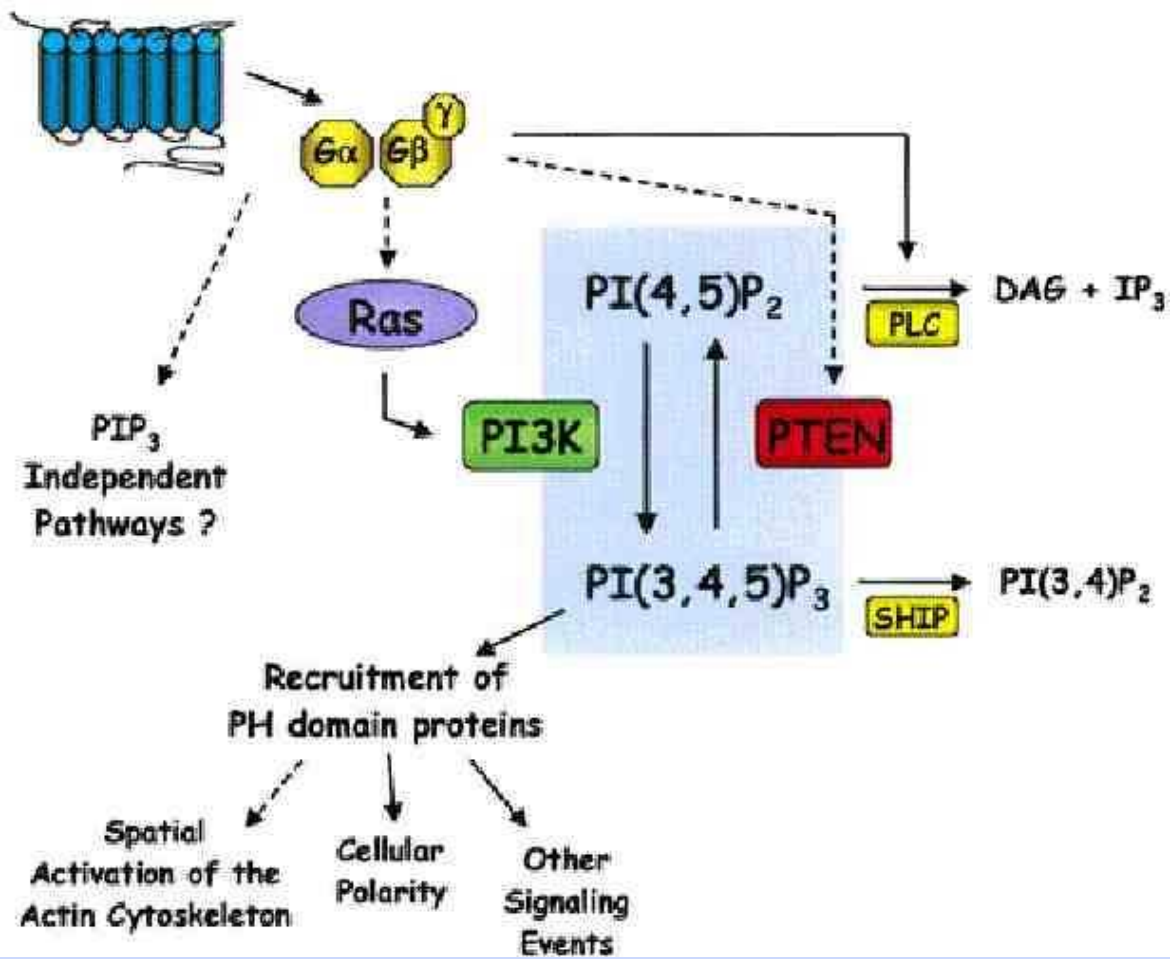
The control of all pathways

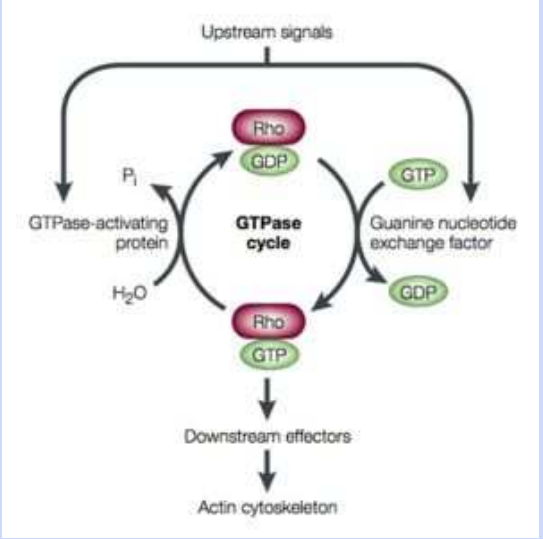




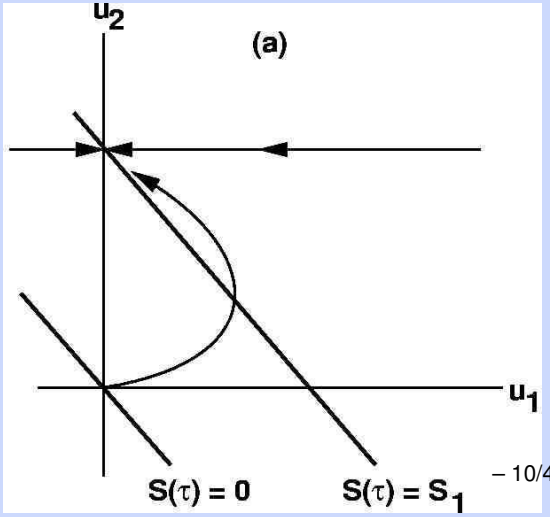


First the orientation component

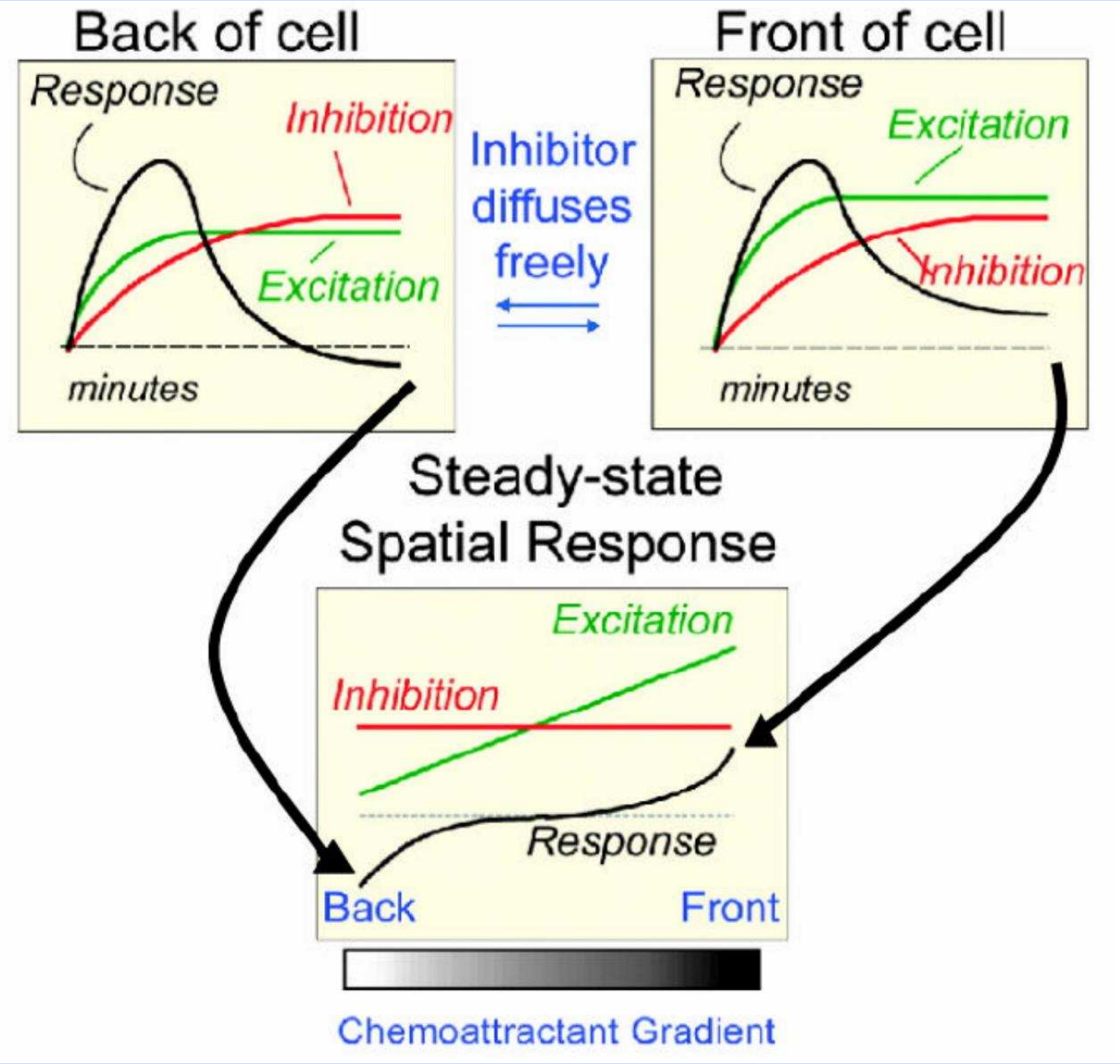




$$\frac{dy_1}{dt} = \frac{S(t) - (y_1 + y_2)}{t_e}$$
$$\frac{dy_2}{dt} = \frac{S(t) - y_2}{t_a}.$$

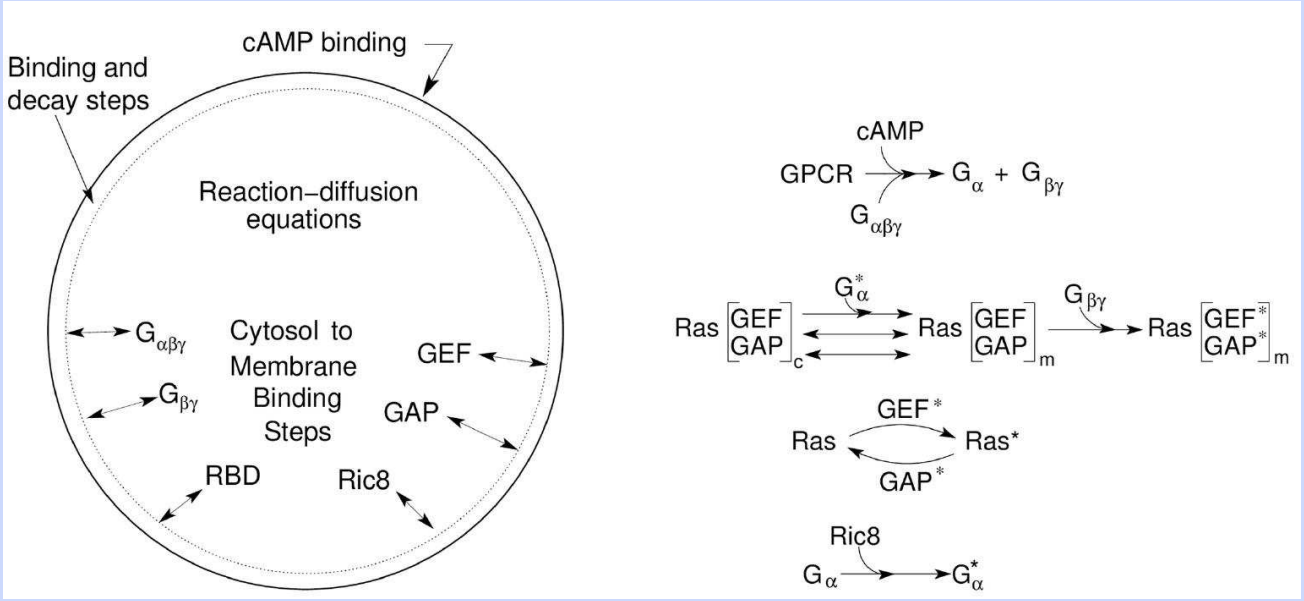


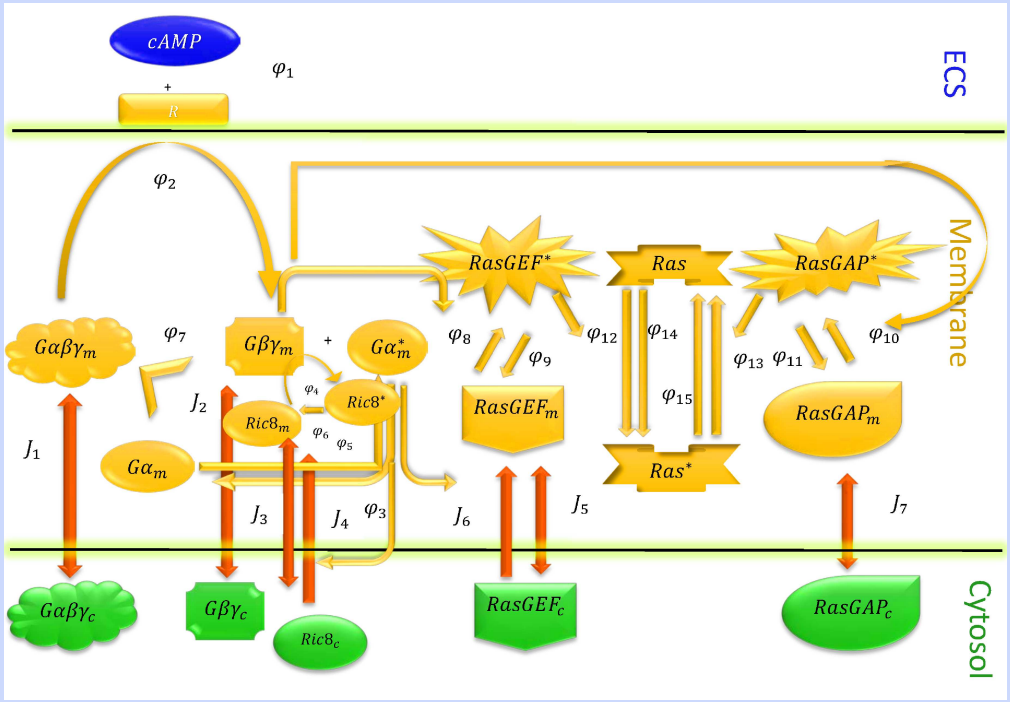
How do cells orient to a signal - the LEGI model?



See Meinhardt, Devreotes, Iglesias

The major components of the signal transduction step





The governing equations

What processes are involved?

- Reaction and diffusion in the interior of the cell (the cytosol)
- Reaction on the boundary
- Exchange between the boundary and the interior

This leads to a nonlinear system of reaction-diffusion equations in the interior and nonlinear boundary conditions.

• Most parameters can be estimated by matching a subset of the experimental observations.

• The model differs from existing LEGI models in that ALL species diffuse at equal rates. Thus inclusion of details shows where simplified models break down.

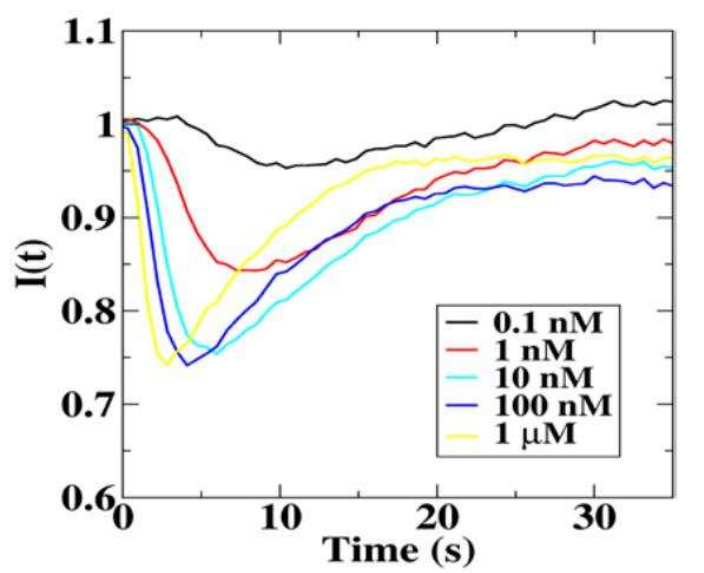
Theorem The system works!

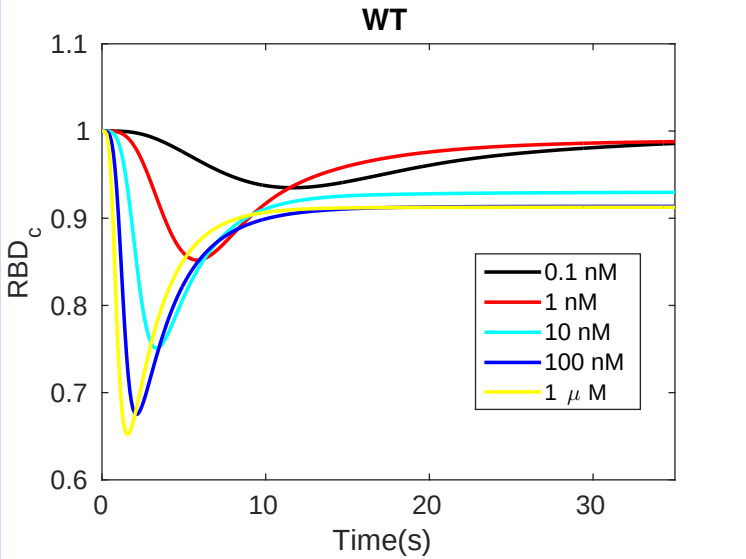
Sketch of the proof

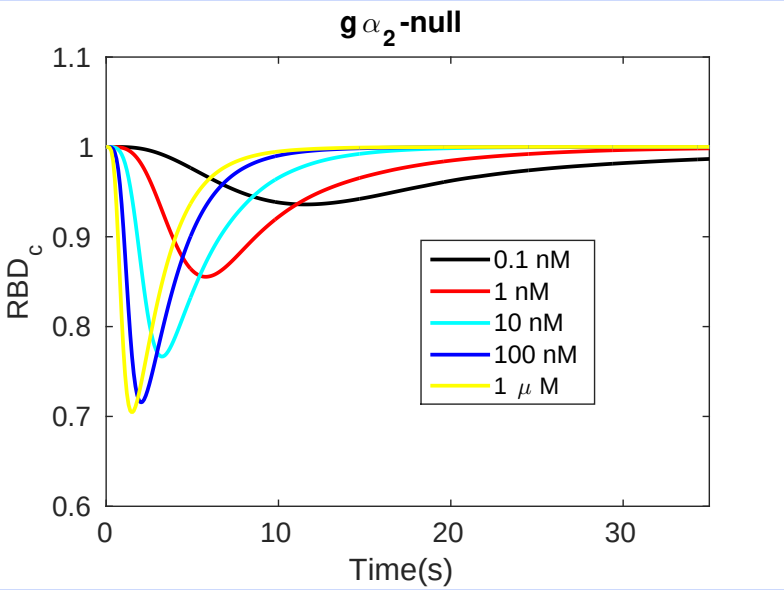
The new experimental results that have to be captured by the model are ...

- Under a spatially uniform stimulus, Ras is transiently activated, and adapts imperfectly.
- Under a graded simulus the response is biphasic – uniform followed by symmetry breaking, independent of the cytoskeleton.
- The system has a well-defined refractory period as shown by the response to repeated stimuli .
- There is a persistent memory in the system.

The response under uniform stimuli

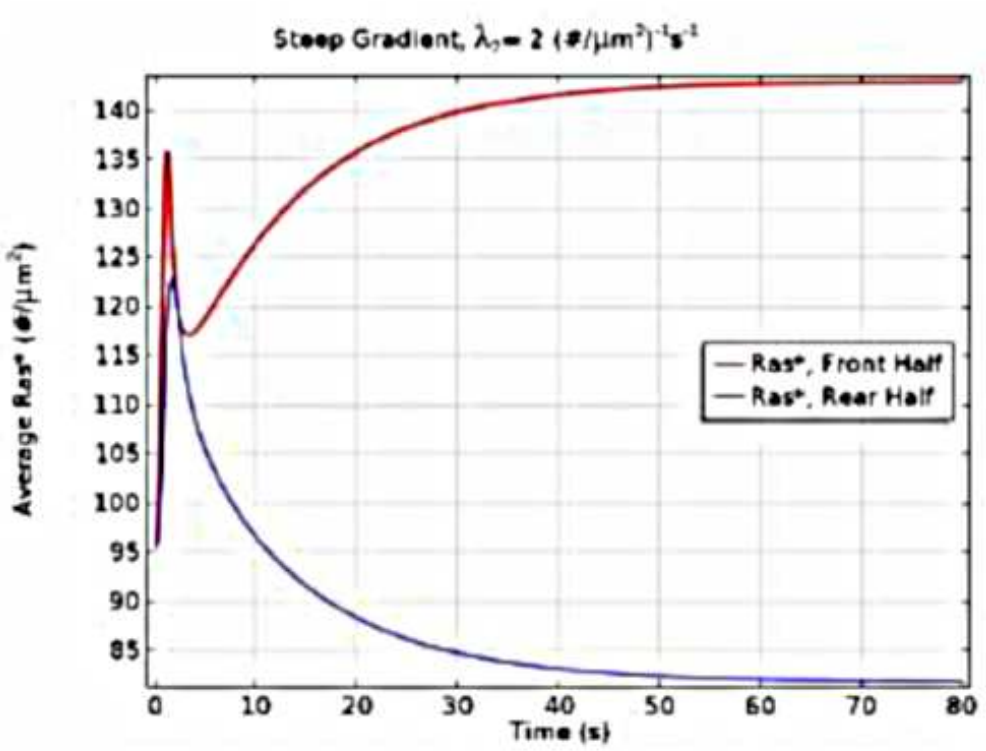


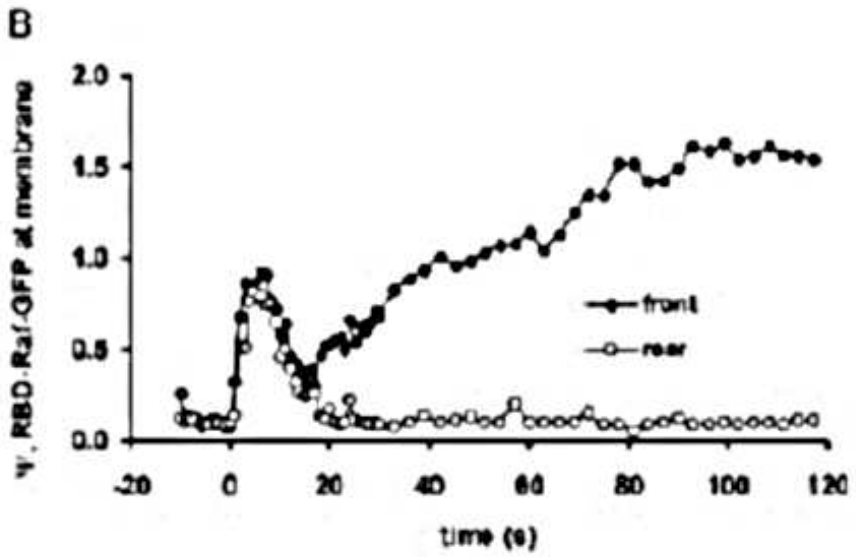




Takeda K, Shao D, Adler M, Charest PG, Loomis WF, Levine H, et al. Incoherent feedforward control governs adaptation of activated Ras in a eukaryotic chemotaxis pathway. Cell Biology. 2012.

Symmetry-breaking under a gradient

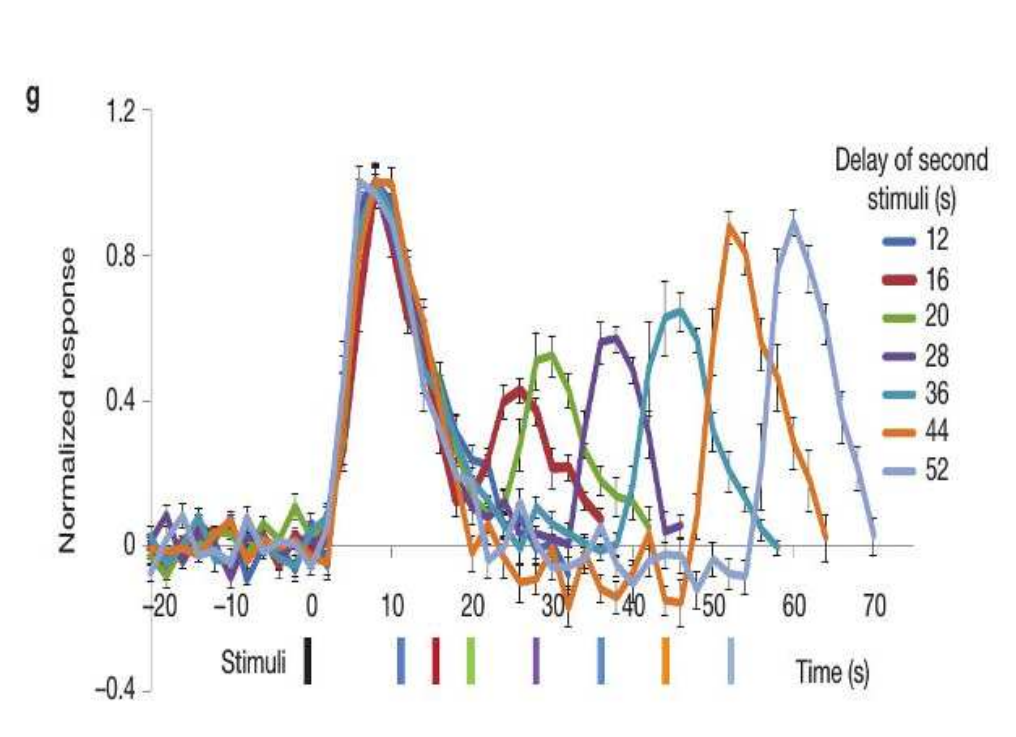


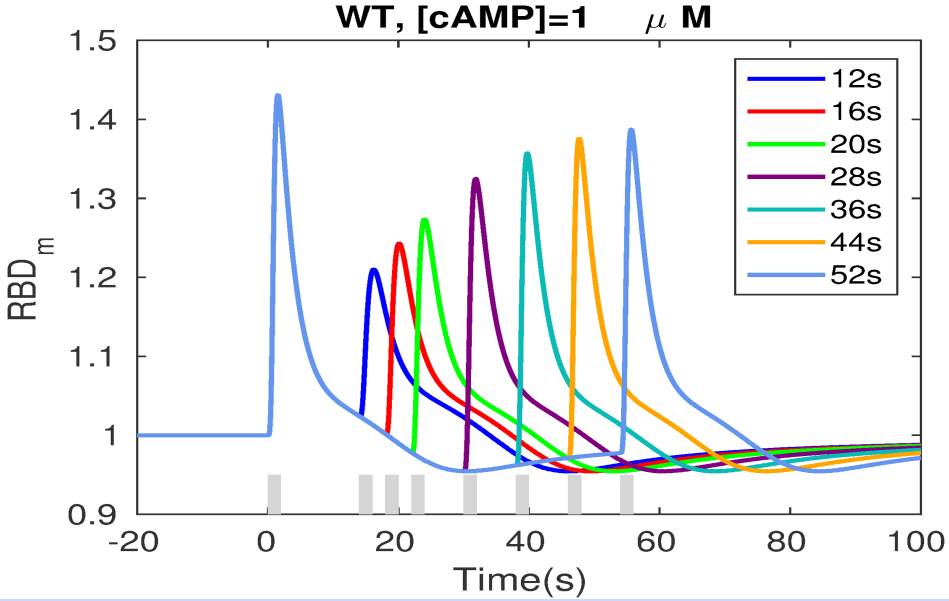


Thus the response is biphasic – first uniform around the periphery and then higher at the high end of the gradient

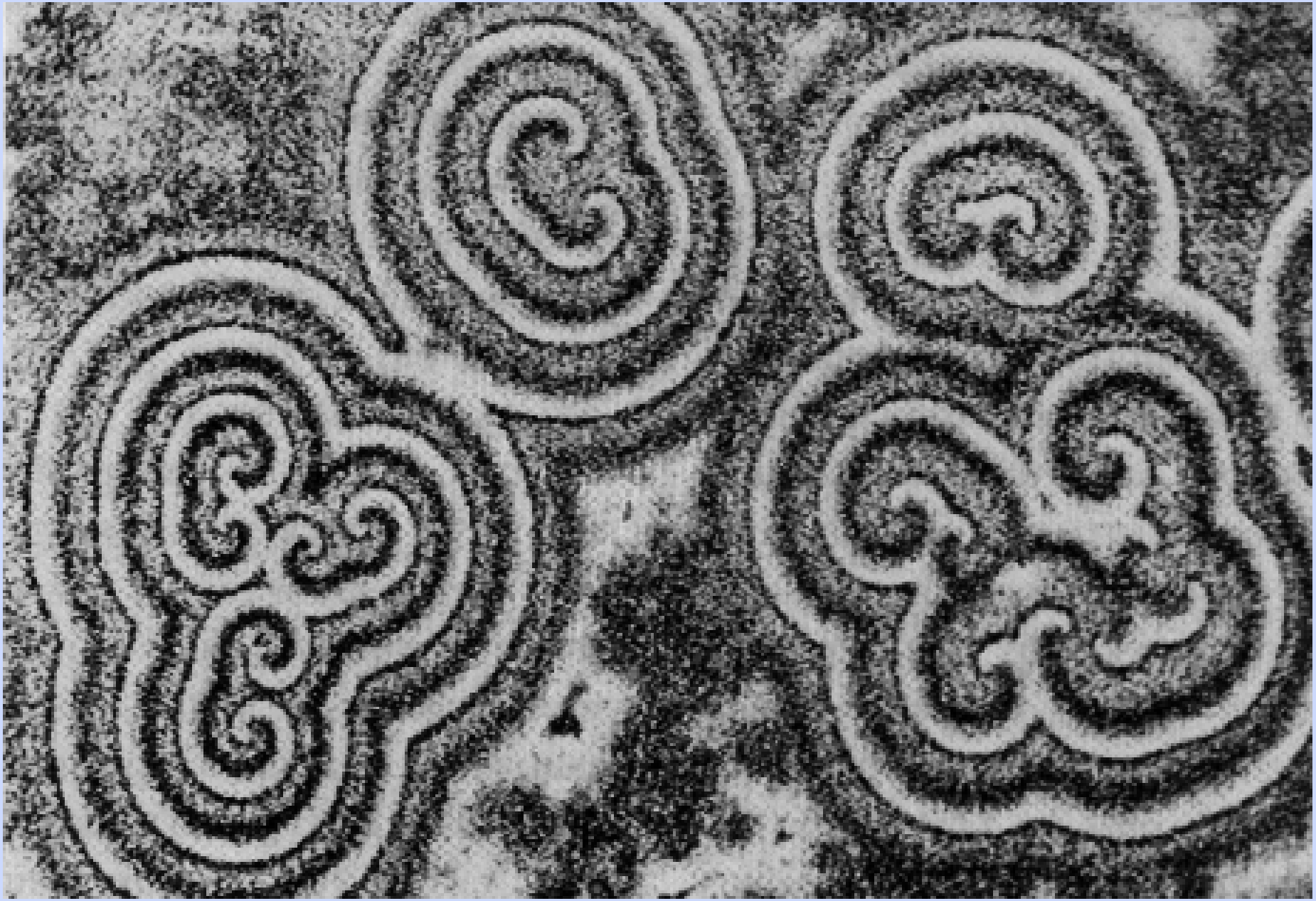
Kortholt A, Keizer-Gunnink I, Kataria R, van Haastert PJM. Ras activation and symmetry breaking during Dictyostelium chemotaxis. J Cell Sci. 2013

There is a refractory period – the system is excitable

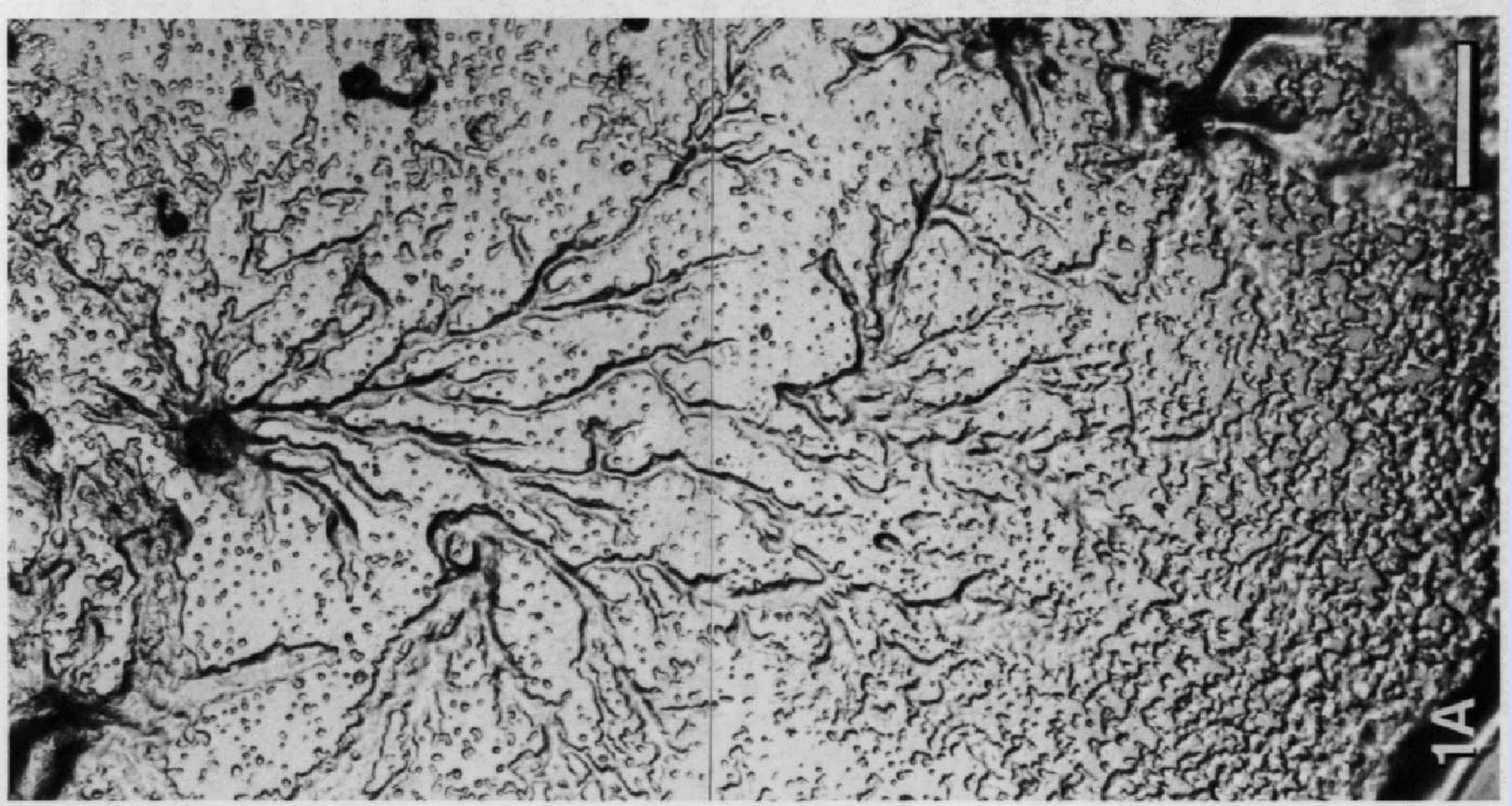




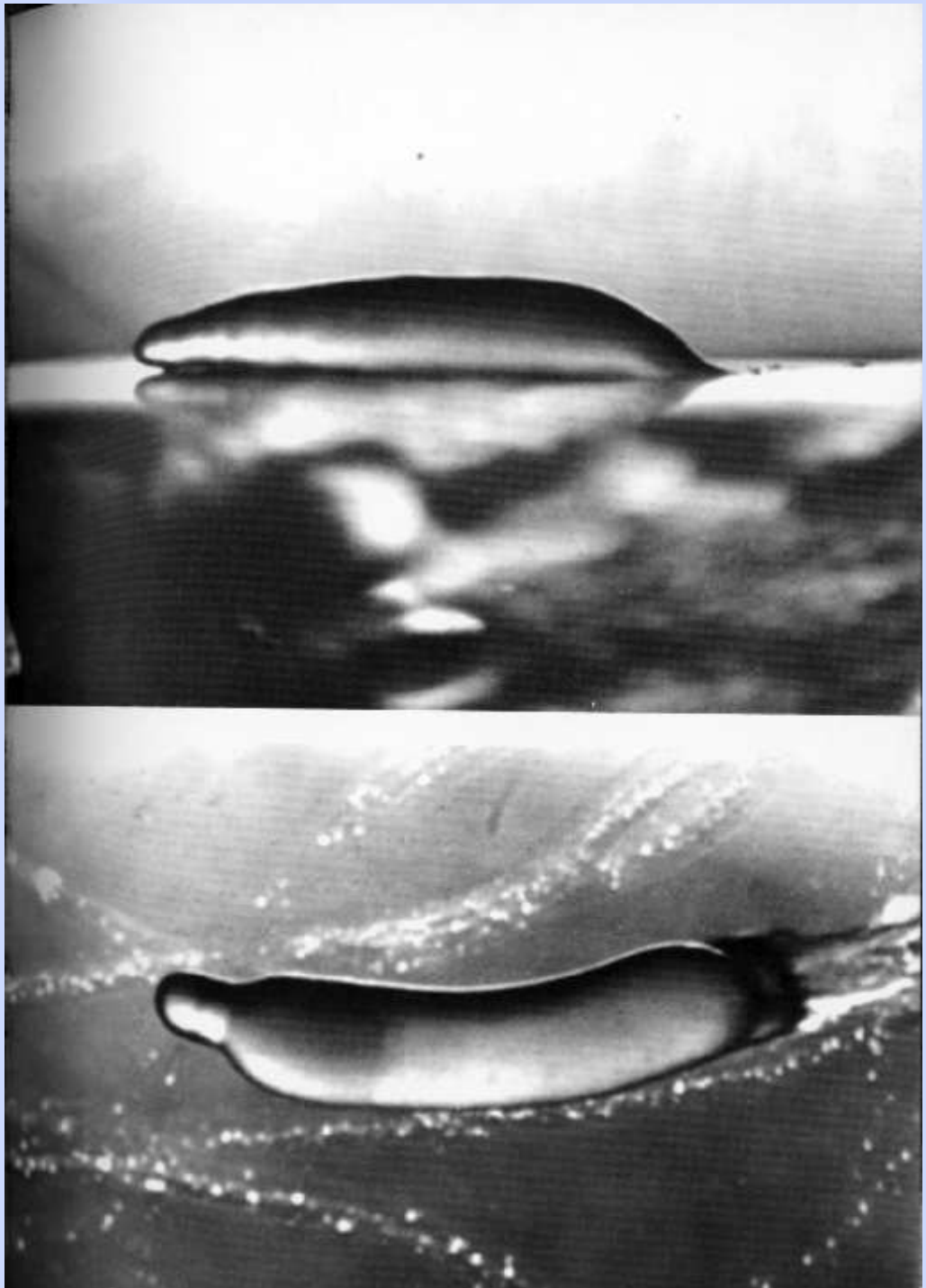
Wave patterns in early aggregation



Aggregation patterns



Movement of the slug

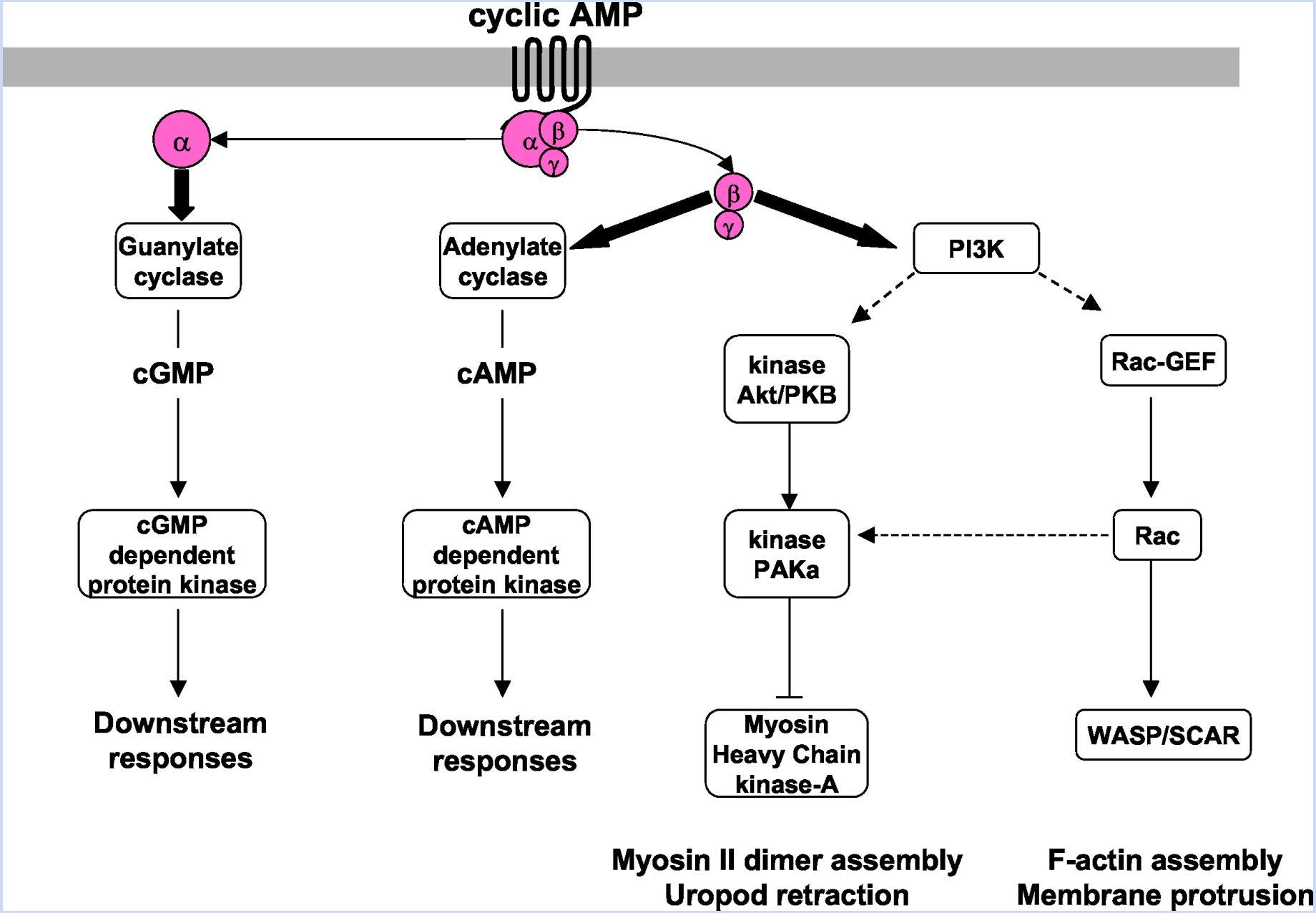


How do we model and analyze these behaviors, and what do we learn from that process?

What cellular-level processes are involved in producing the population-level aggregation patterns? Said otherwise, what must a cell do to have a chance of passing on its genes?

- Some cells (or small groups of cells) must become pacemakers
- A cell must detect the external signal cyclic adenosine 3',5'-monophospahte (cAMP)
- It must choose a direction in which to move
- Cells must amplify and relay the signal, and adapt to the ambient signal
- They must move for an appropriate length of time
- Eventually a cell interacts with its neighbors and moves collectively, first in pairs, then in streams, ..
- Slightly later it has to 'decide' what type of cell to become in the final fruiting body. This is a collective decision reached by the community (absent cheaters!).
- The entire aggregate has to stop migrating and erect the fruiting body

Signal transduction in *Dictyostelium discoideum*



The steps in cAMP production and release

(I) The Stimulus Pathway

$$
\begin{aligned}
H + R_s &\underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} HR_s \\
HR_s + G_s &\underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} HR_sG_s \\
HR_sG_s + GTP &\xrightarrow{\hat{k}_3} HR_s + G'_s \\
&\qquad + GDP + \beta\gamma \\
G'_s + UC &\underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} G'_sAC \\
G'_s &\xrightarrow{k_5} \alpha_sGDP + P_i \\
G'_sAC &\xrightarrow{k_5} \alpha_sGDP + UC \\
&\qquad + P_i \\
\alpha_sGDP + \beta\gamma &\xrightarrow{k_6} G_s
\end{aligned}
$$

(II) The Inhibitory Pathway

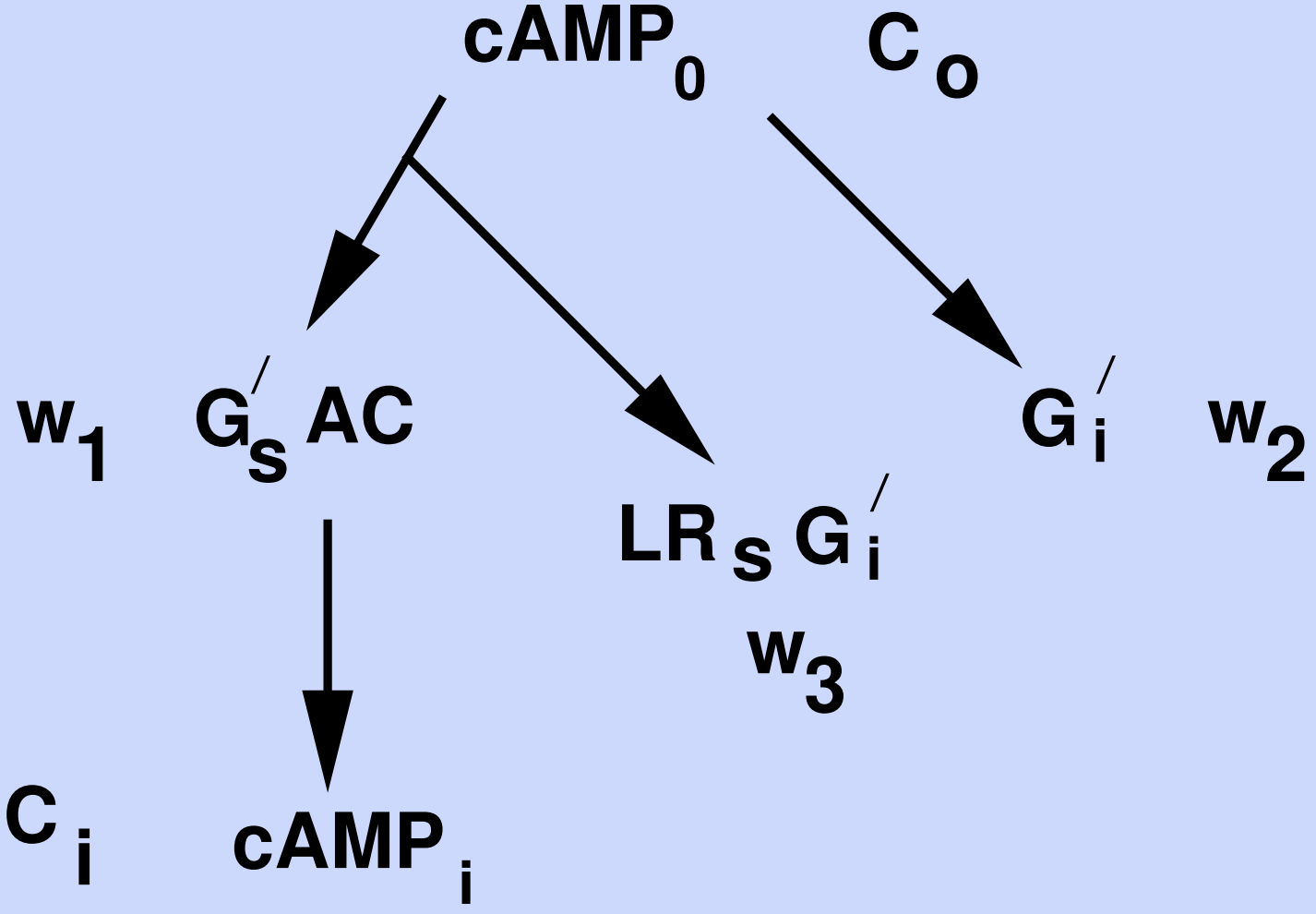
$$
\begin{aligned}
H + R_i &\underset{h_{-1}}{\overset{h_1}{\rightleftarrows}} HR_i \\
HR_i + G_i &\underset{h_{-2}}{\overset{h_2}{\rightleftarrows}} HR_iG_i \\
HR_iG_i + GTP &\xrightarrow{\hat{h}_3} HR_i + G'_i \\
&\qquad + GDP + \beta\gamma \\
G'_i + HR_s &\underset{h_{-4}}{\overset{h_4}{\rightleftarrows}} HR_sG'_i \\
G'_i &\xrightarrow{h_5} \alpha_iGDP + P_i \\
HR_sG'_i &\xrightarrow{h_6} H + R_s + G'_i \\
HR_sG'_i &\xrightarrow{h_5} HR_s + \alpha_iGDP \\
&\qquad + P_i \\
\alpha_iGDP + \beta\gamma &\xrightarrow{h_7} G_i
\end{aligned}
$$

Y. Tang and H. G. Othmer, Excitation, oscillations and wave propagation in a G-protein-based model of signal transduction in Dictyostelium discoideum, Phil Trans Roy Soc, 349, 179-195, (1996).

(III) The production and secretion of intracellular cAMP

$$
\begin{aligned}
G'_sAC + ATP &\underset{l_{-1}}{\overset{\hat{l}_1}{\rightleftarrows}} G'_sAC - ATP \\
G'_sAC - ATP &\xrightarrow{l_2} cAMP_i + G'_sAC \\
cAMP_i + iPDE &\underset{l_{-3}}{\overset{l_3}{\rightleftarrows}} cAMP_i - iPDE \\
cAMP_i - iPDE &\xrightarrow{l_4} AMP + iPDE \\
cAMP_i &\xrightarrow{l_5} cAMP^*_o
\end{aligned}
$$

The reduced local dynamics



The governing equations for perfusion experiments

$$\frac{dw_1}{d\tau} = \alpha_4 u_2 - w_1 - \alpha_4 u_2 w_1$$

$$\frac{dw_2}{d\tau} = \beta_2\beta_3 c_2 u_4 - \beta_5 w_2 + \beta_6 c_3 w_3 - c_3\beta_4 u_1 w_2 - \beta_2\beta_3 c_2 u_4 (w_2 + c_3 w_3)$$

$$\frac{dw_3}{d\tau} = -(\beta_5 + \beta_6) w_3 + \beta_4 u_1 w_2$$

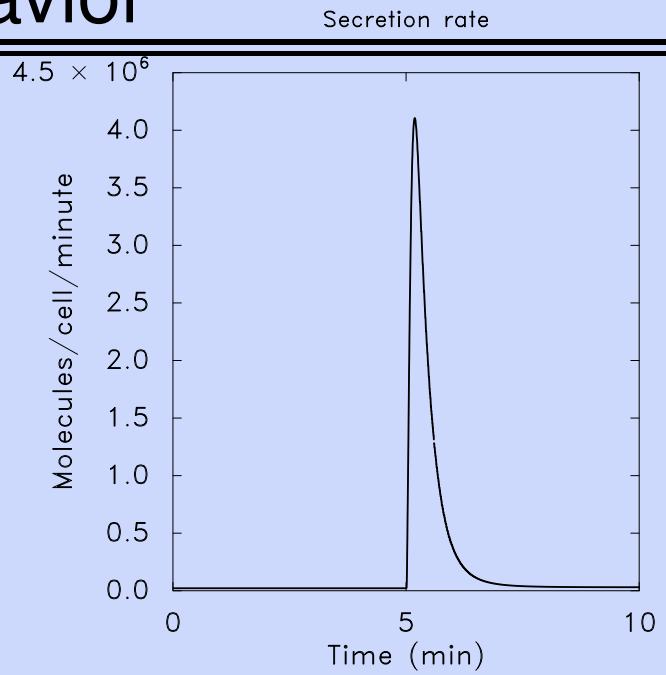
$$\frac{dw_4}{d\tau} = \gamma_1\gamma_2 w_1 + \Gamma_5(1 - \Gamma_7 w_1) - \gamma_4 \frac{w_4}{w_4 + \gamma_3} - sr(w_4)$$

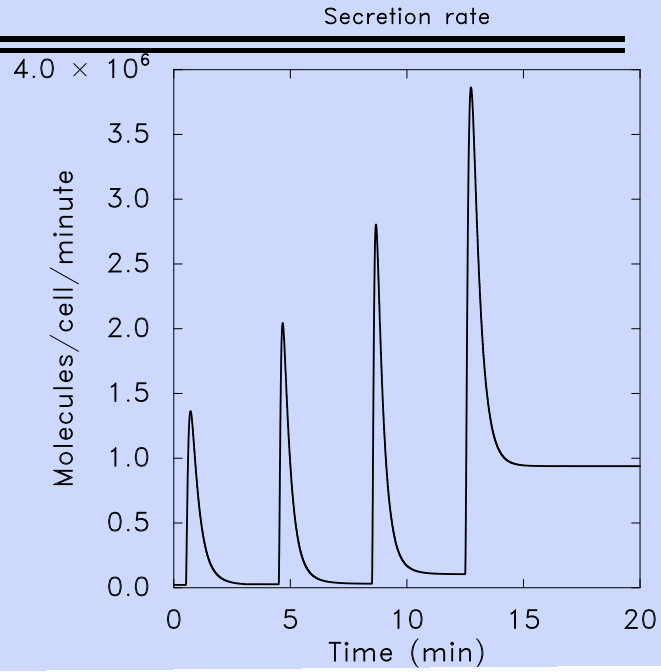
$$u_1 = \frac{\alpha_0 w_5 + (\beta_5 - \alpha_0 w_5) w_3}{\alpha_1 + \alpha_0 w_5(\mathbf{x}_i) + \beta_4 w_2} \qquad u_2 = \frac{\alpha_2\alpha_3 c_1 u_1 (1 - w_1)}{1 + \alpha_4 + \alpha_2\alpha_3 c_1 u_1 - \alpha_4 w_1}$$

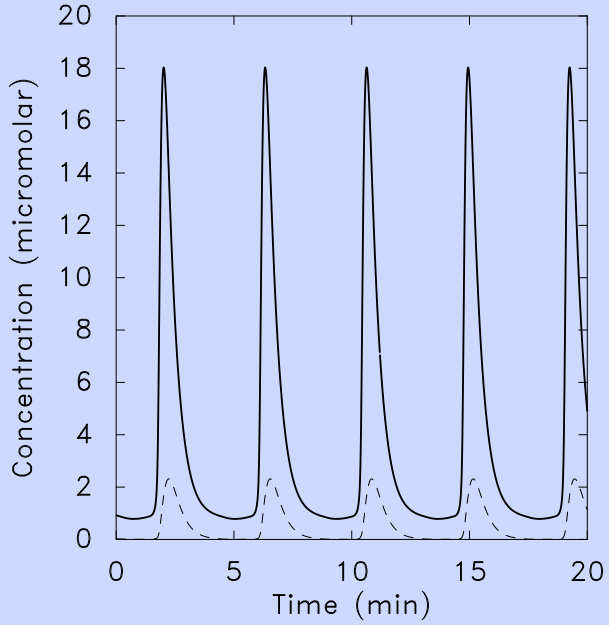
$$u_4 = \frac{\beta_0 w_5}{\beta_1 + \beta_0 w_5}$$

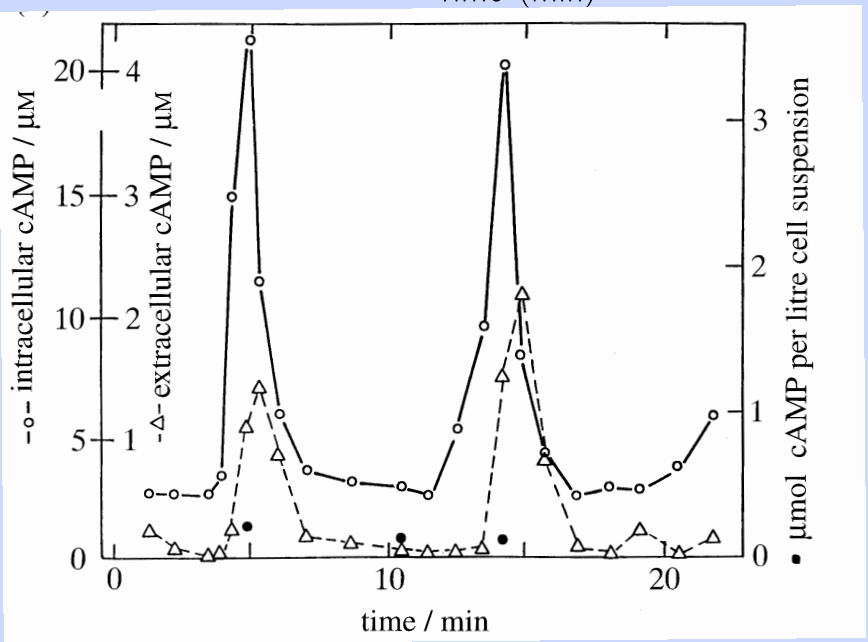
w_5 is a specified function of time

Response to repeated steps in cAMP and oscillatory behavior









The governing reaction-diffusion equations for early aggregation

$$\frac{dw_1^i}{d\tau} = \alpha_4 u_2^i - w_1^i - \alpha_4 u_2^i w_1^i$$

$$\frac{dw_2^i}{d\tau} = \beta_2\beta_3 c_2 u_4^i - \beta_5 w_2^i + \beta_6 c_3 w_3^i - c_3\beta_4 u_1^i w_2^i - \beta_2\beta_3 c_2 u_4^i (w_2^i + c_3 w_3^i)$$

$$\frac{dw_3^i}{d\tau} = -(\beta_5 + \beta_6) w_3^i + \beta_4 u_1^i w_2^i$$

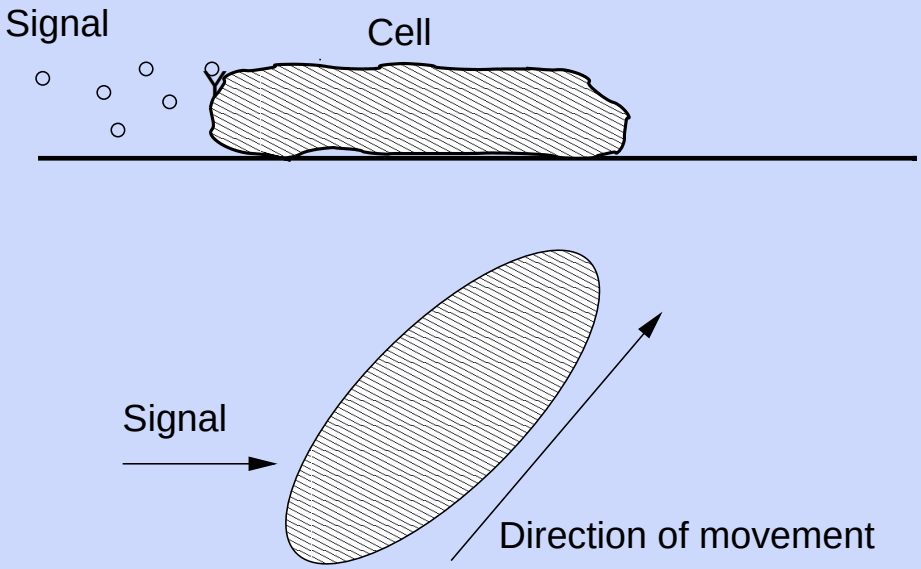
$$\frac{dw_4^i}{d\tau} = \gamma_1\gamma_2 w_1^i + \Gamma_5(1 - \Gamma_7 w_1^i) - \gamma_4 \frac{w_4^i}{w_4^i + \gamma_3} - sr(w_4^i)$$

$$\frac{\partial w_5}{\partial \tau} = \Delta_1 \nabla^2 w_5(\mathbf{x}) - \hat{\gamma_9} \frac{w_5(\mathbf{x})}{w_5(\mathbf{x}) + \gamma_8} + \sum_{i=1}^{N} \frac{V_c}{V_o} \delta(\mathbf{x} - \mathbf{x}_i) \left(sr(w_4^i) - \gamma_7 \frac{w_5(\mathbf{x})}{w_5(\mathbf{x}) + \gamma_6} \right)$$

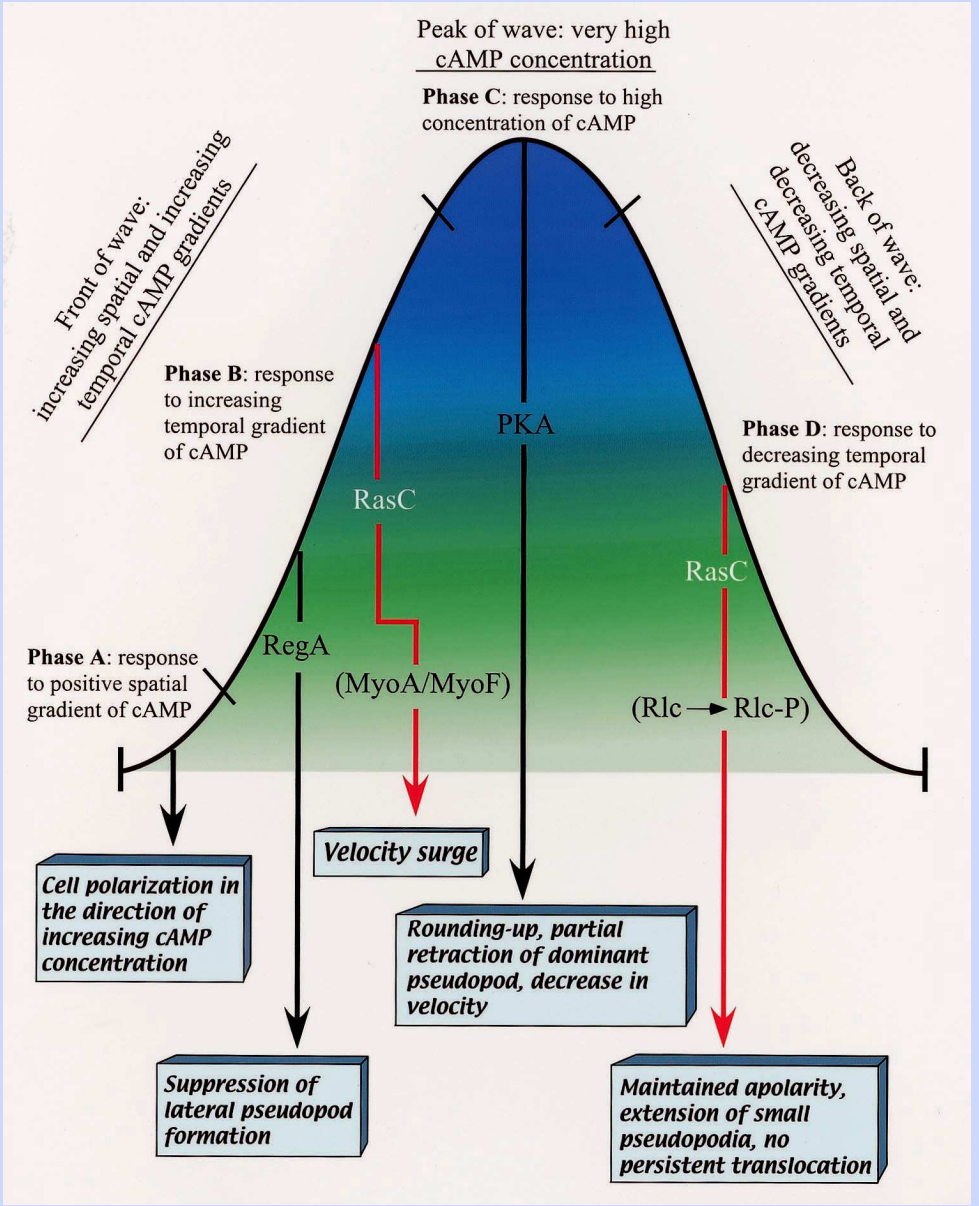
$$u_1^i = \frac{\alpha_0 w_5(\mathbf{x}_i) + (\beta_5 - \alpha_0 w_5(\mathbf{x}_i)) w_3^i}{\alpha_1 + \alpha_0 w_5(\mathbf{x}_i) + \beta_4 w_2^i} \qquad u_2^i = \frac{\alpha_2\alpha_3 c_1 u_1^i (1 - w_1^i)}{1 + \alpha_4 + \alpha_2\alpha_3 c_1 u_1^i - \alpha_4 w_1^i}$$

$$u_4^i = \frac{\beta_0 w_5(\mathbf{x}_i)}{\beta_1 + \beta_0 w_5(\mathbf{x}_i)}$$

Orientation and movement in a wave

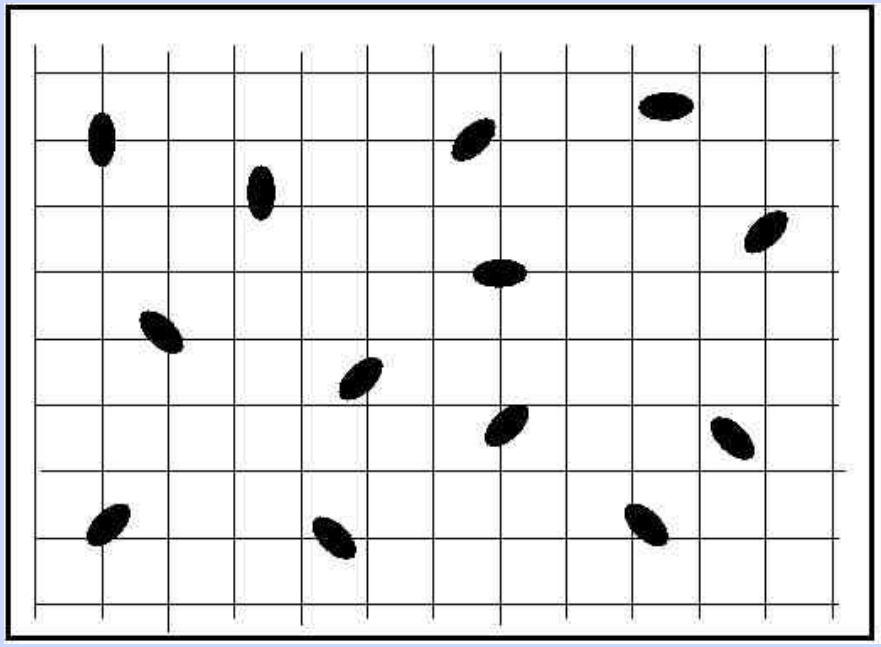


In an aggregation wave ...



AND ... A cell also stimulates itself when it signals!

The computational algorithm



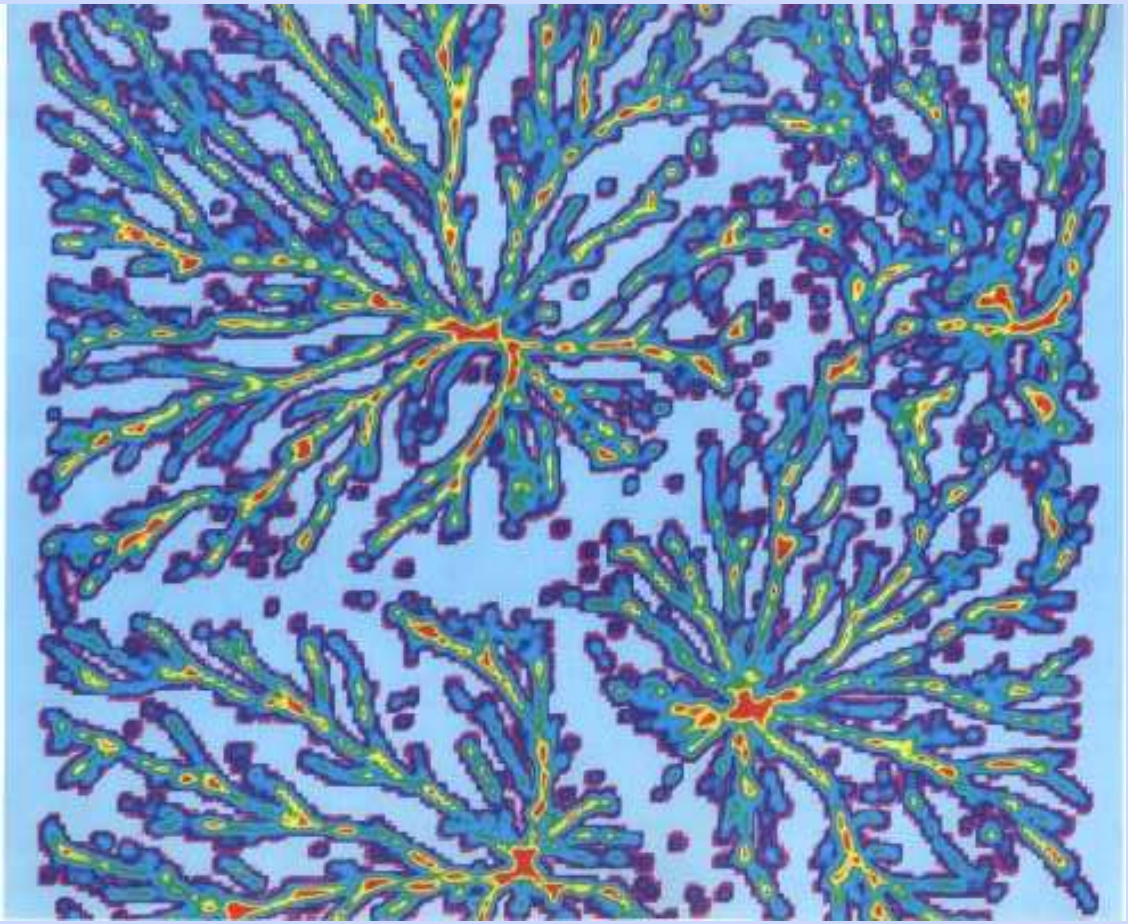
(i.) Solve the extracellular equation on a regular grid, using an Alternating-Direction Implicit (ADI) method for the partial differential equation, lagging the secretion term.

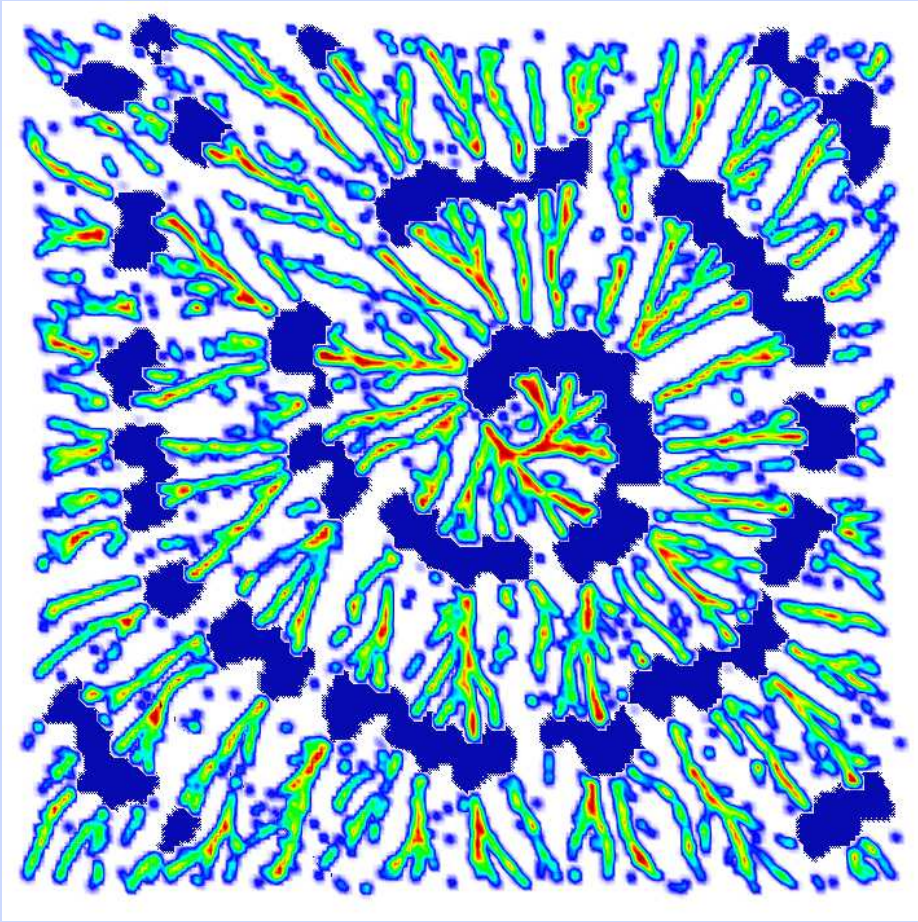
(ii.) Interpolate cAMP from the grid to the cell positions and update the intracellular variables and the secretion by an implicit scheme.

(iii.) Update cell movement. If a cell is not moving, should it begin to move? If so, compute the direction and start movement. If it is moving, should it continue?

(iv.) Transfer the secreted cAMP to the grid and repeat the cycle.

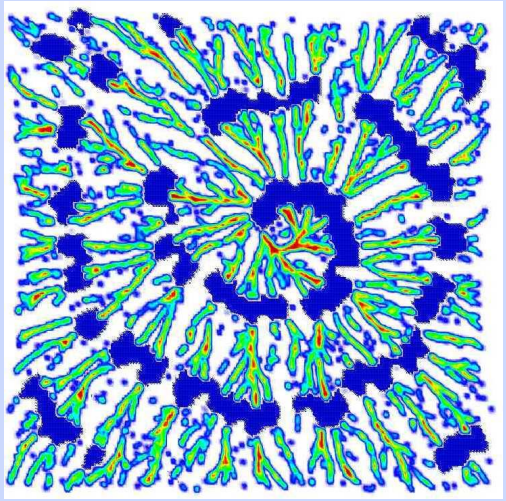
Wave patterns in early aggregation...

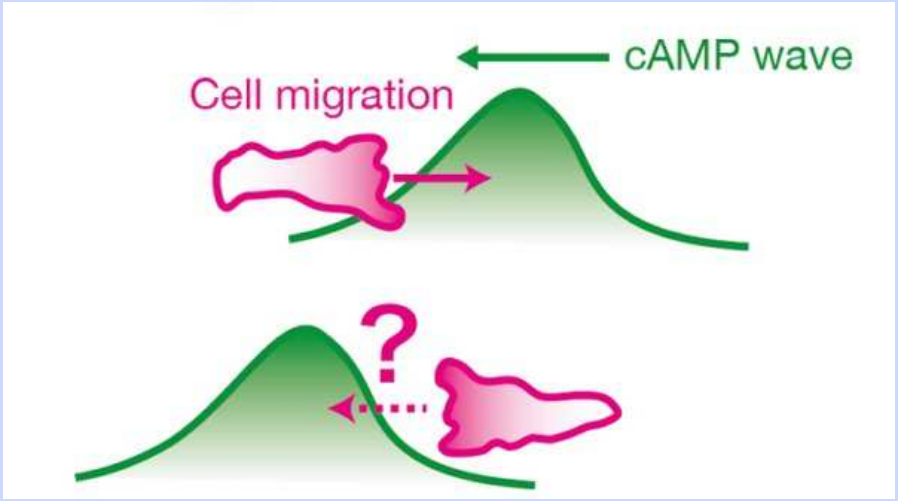


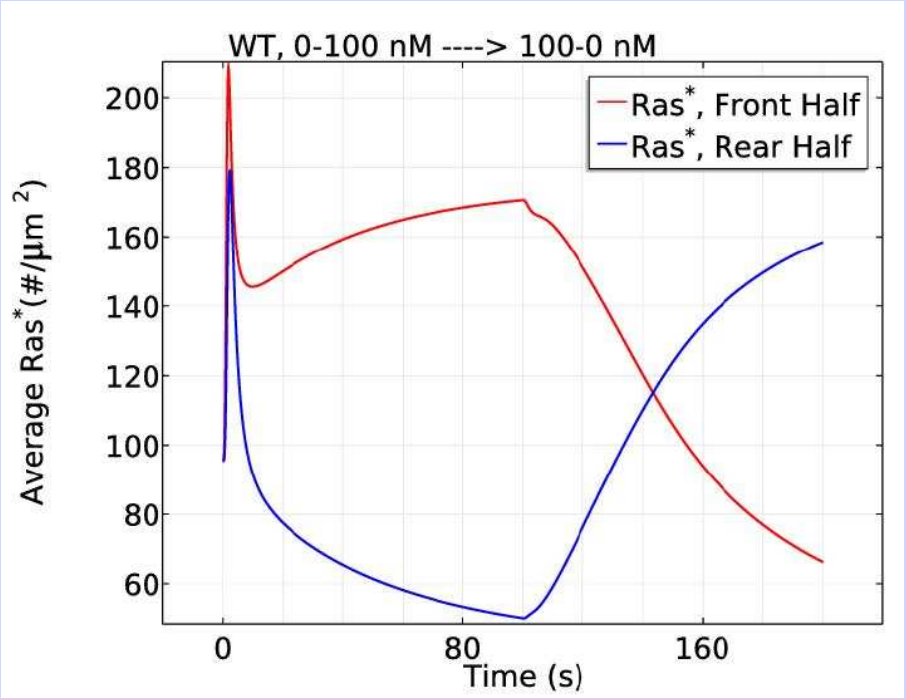


J. Dallon and H. G. Othmer, Phil. Trans. Roy. Soc. (Lon.), 352, 391-418, (1997).

What cells see in a wave and how they ’remember’

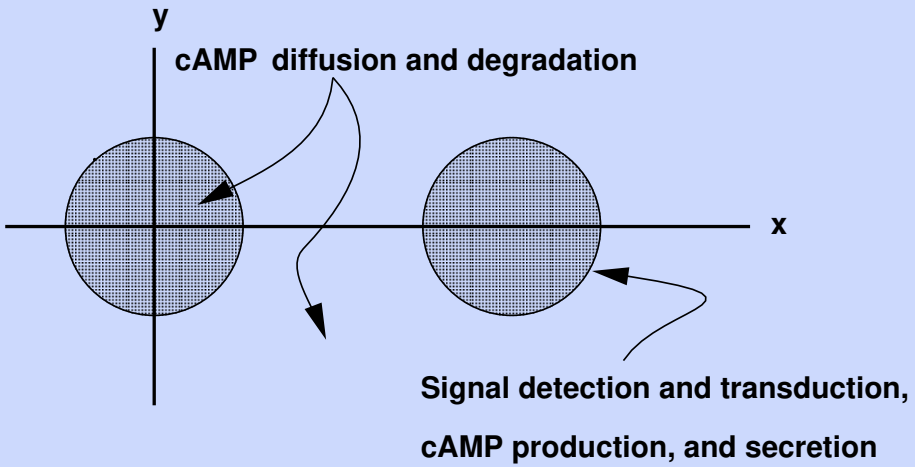


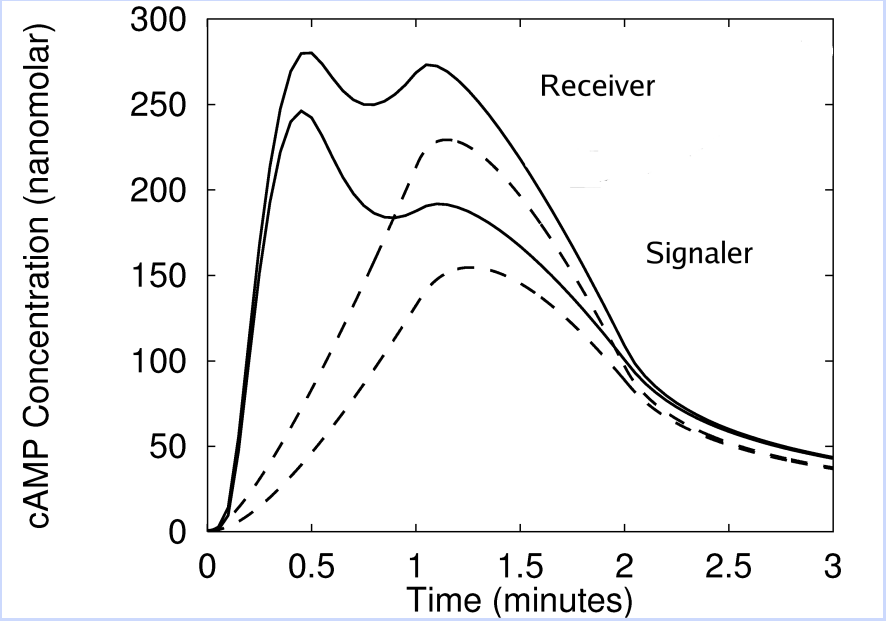


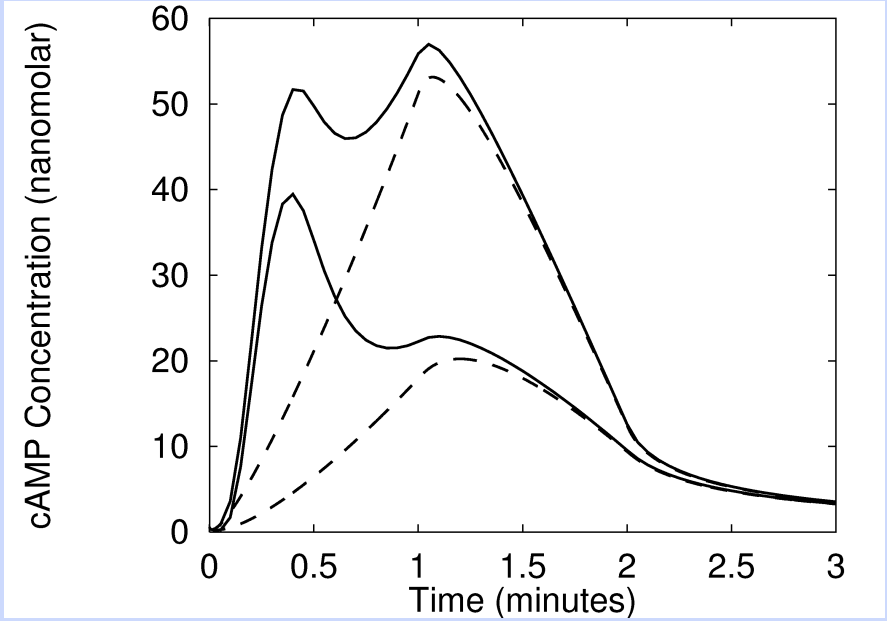


This may be part of a solution for the ’back-of- the- wave’ problem.

What is the time available for deciding how to move?

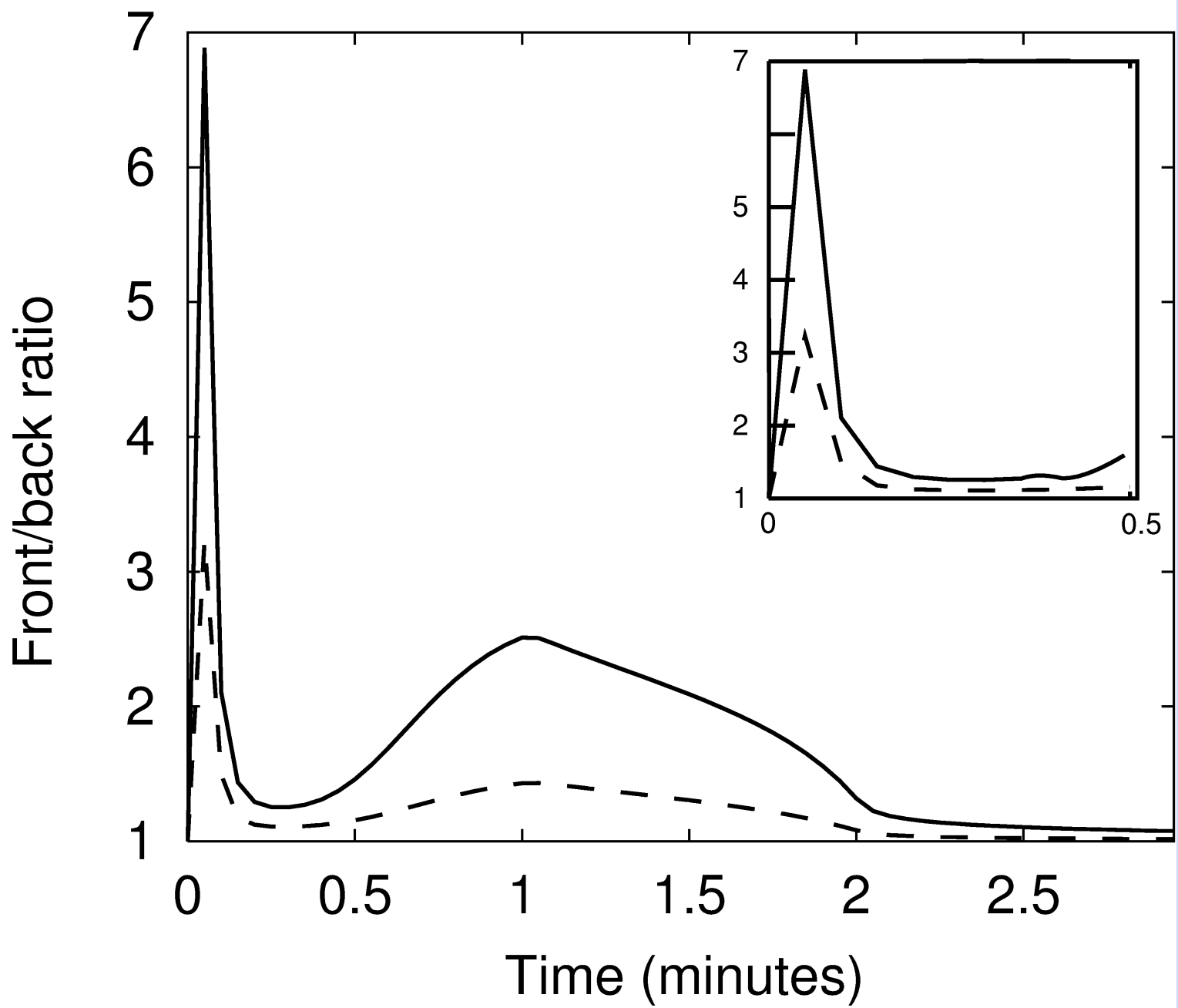






J. C. Dallon and H. G. Othmer, J. Theor. Bio., 194, 461-483, (1998).

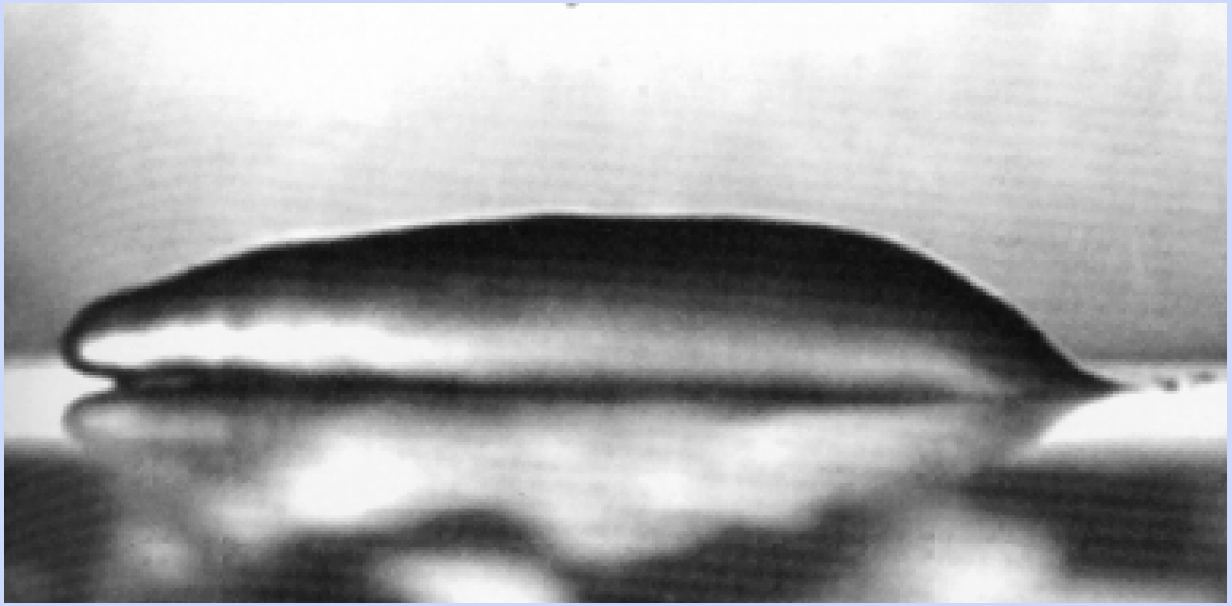
What's the best signal ratio a cell sees?

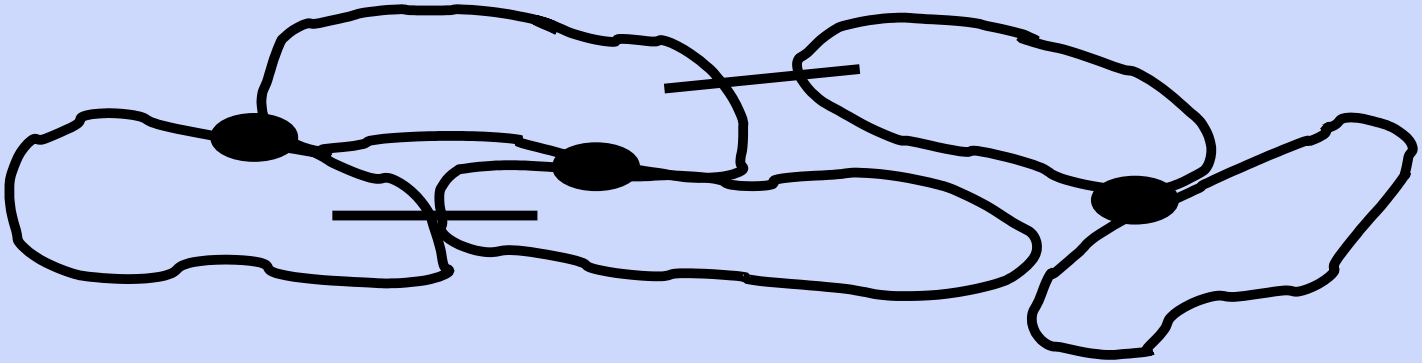


What do the analysis and computations of the cell-based model explain and predict?

- A single cell can be a pacemaker.
- The signal transduction model shows how adaptation to the signal is used to solve the ‘back-of-the-wave’ problem.
- The model predicts the effect of density and cell excitability on the frequency of oscillation, the wave speed, and the size of the central core.
- The cell-based model provides an explanation for the origin of streaming and the origin of spiral waves: computations show that cell movement and random variations in cell density are necessary for streaming and facilitate the generation of spirals.
- The model predicts that cells must choose a direction within 10-15 seconds in order to orient to the local gradient.
- Computations show that aggregation is very robust with respect to the choice of direction of movement.

How do cells move in dense aggregates?





Theoretical analysis of the multicell problem

Suppose there are N cells in the slug. The forces on a cell in the slug are classified as follows.

- Active forces $\mathbf{T}_{i,j}$ a cell exerts on neighboring cells or the substrate: the reaction force to this is denoted $\mathbf{M}_{j,i}$
- The reaction to forces exerted by other cells on it
- Dynamic drag forces that arise as a moving cell forms and breaks adhesive bonds with neighboring cells
- Static frictional forces that exist when cells are rigidly attached to each other or to the substrate

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J. C Dallon and H. G. Othmer, How cellular movement determines the collective force generated by the *Dictyostelium discoideum* slug, J. Theor. Biol., **231**, 203-222, (2004).

Let $\mathcal{N}_i^a$ denote the neighbors, including the substrate, of i upon which it can exert traction. The 'neighbor' relation is symmetric for all cells; if cell i can exert traction on cell j, then cell j can exert traction on cell i. This is not true for the substrate, which is assumed to be passive in that it does not generate stress. The total motive force generated by i is

$$\mathbf{M}_i = \sum_{j \in \mathcal{N}_i^a} \mathbf{M}_{j,i}.$$

The total traction force which other cells exert on cell i is

$$\mathbf{T}_i = \sum_{j \in \mathcal{N}_i^a} \mathbf{T}_{j,i}.$$

The *dynamic friction force* on cell i due to interaction with j is defined as

$$\mathbf{D}_{j,i} = \mu_{ij}(\mathbf{v}v_j - \mathbf{v}_i),$$

where μ_{ij} is the friction coefficient. This satisfies $\mathbf{D}_{i,j} = -\mathbf{D}_{j,i}$, which implies that $\mu_{ij} = \mu_{ji}$.

If $\mathbf{D}_i$ is the total dynamic friction force on cell i, and $\mathcal{N}_i^d$ is the set of cells that interact with i via a frictional force, then

$$\mathbf{D}_i = \sum_{j \in \mathcal{N}_i^d} \mathbf{D}_{j,i} = \sum_{j \in \mathcal{N}_i^d} \mu_{ij}(\mathbf{v}_j - \mathbf{v}_i).$$

In general $\mathcal{N}_i^d \subseteq \mathcal{N}_i^a$ since cells may extend pseudopods to cells at some distance, but not be in contact with them in the sense of giving rise to a frictional force.

Lastly is a *static friction force* on some cells, which is the means for constructing a network that can transmit stress to the boundary.

Statically-bound cells by definition function as one rigid object. Let $\mathcal{N}_i^s$ denote the set of cells that statically bind to cell i. This is a transitive relation in that if i is bound to j and j is bound to k, then i is bound to k. In particular, if i is statically bound to the substrate either directly or indirectly then it can transmit forces applied to it to the substrate. These are the only type of chains that can transmit stress from the interior; a chain of say four cells statically bound to each other but not bound to the substrate simply functions as a larger unit. If S_{ji} denotes the static binding force on the i^{th} cell when bound to the j^{th}, then $S_{ji} = -S_{ij}$ and the cell-cell forces cancel on all but those cells attached to the substrate.

The total force on the i^{th} cell is

$$\begin{aligned} \mathbf{F}_i &= \mathbf{M}_i + \mathbf{T}_i + \mathbf{D}_i + \sum_{j \in \mathcal{N}_i^s} \mathbf{S}_{ji} \qquad (1) \\ &= \sum_{j \in \mathcal{N}_i^a} \mathbf{M}_{j,i} + \sum_{j \in \mathcal{N}_i^a} \mathbf{T}_{j,i} + \sum_{j \in \mathcal{N}_i^d} \mu_{ij}(\mathbf{v}_j - \mathbf{v}_i) + \sum_{j \in \mathcal{N}_i^s} \mathbf{S}_{ji}. \end{aligned}$$

If we sum these over all cells we see that the sum of the tractions and motive forces, as well as sum of the dynamic friction force, vanish for all cell-cell interactions in the interior of the slug.

The only surviving terms are those due to direct interaction of a cell with the substrate, or the indirect interaction via a chain of statically-connected cells that is connected to the substrate. The latter takes the form of a force on an interior cell equal and opposite to the traction force it exerts on a statically-connected chain of cells. Thus in the absence of static binding there is no mechanism by which an interior cell can transmit stress to the boundary, and accordingly, there can be no volumetric forces on the slug in a continuum description in the absence of other mechanisms.

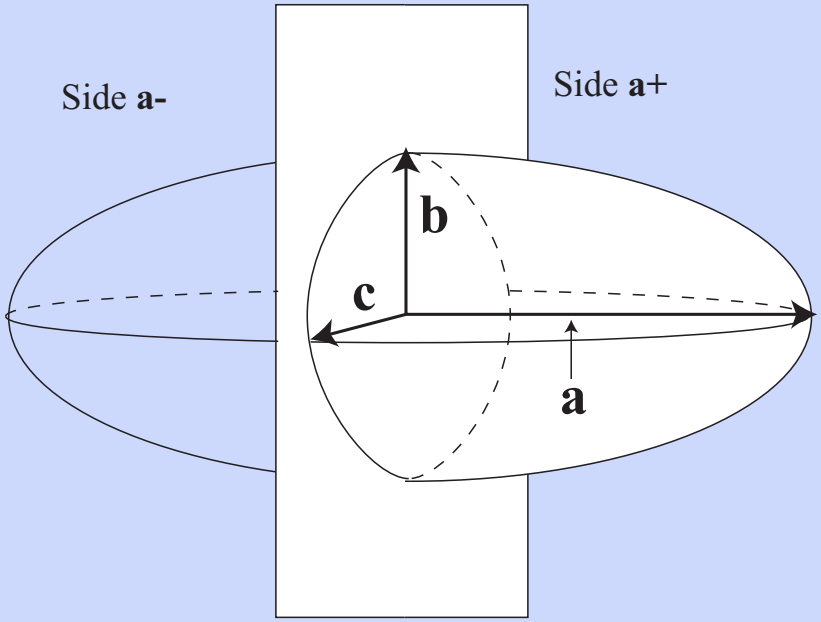
The ‘bedspring’ model ...

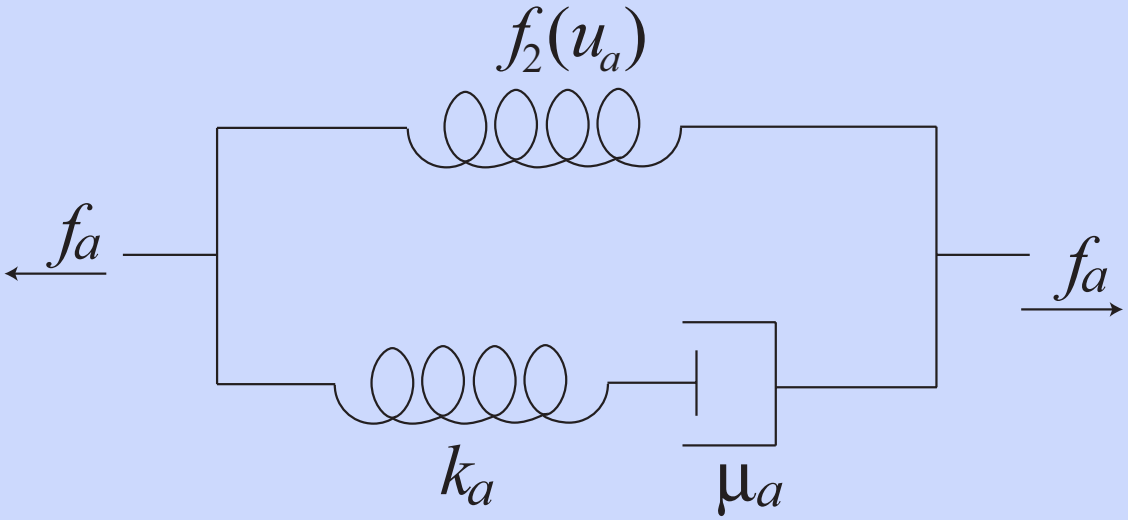
A cell i is said to be ‘frozen’ if it statically binds to the substrate directly or indirectly, that is, if there exists a sequence j_1, $j_2 \cdots j_k$, such that i statically binds to j_1, j_1 statically binds to j_2, $\cdots\cdots$, and j_k statically binds to the substrate. The set of all frozen cells is called the frozen network of the slug.

The foregoing can be summarized as follows: in the absence of a frozen network in the slug, the total force on the substrate is the active force exerted by cells in direct contact with the substrate, and for a slug translocating at a constant velocity, this exactly balances the dynamic frictional interaction with the substrate.

Said otherwise, a cell in the interior can contribute to the motive force if the slug functions as a dynamic ‘bedspring’ in which cells cycle between the motile and frozen states.

The assumptions on cell mechanics





The governing equations and the computational algorithm

- Treat cells as ellipsoids of constant volume but with arbitrary positive semiaxes
- Incorporate intracellular rheology, active forces, cell-cell adhesion, etc
- Use the previously discussed model for cAMP dynamics

$$\mu_s A_{is} \frac{d\mathbf{x}_i}{dt} = \mathbf{F}^{act}_{i(j/s)} + \sum_{j \in \mathcal{N}(i)} \mathbf{F}^{pass}_{ij} + \sum_{j \in \mathcal{N}(i)} \mathbf{F}^{act}_{ij} - \mu_c \sum_{j \in \mathcal{N}(i)} \frac{A_{ij}}{A} (\mathbf{v}_i - \mathbf{v}_j) \qquad (2)$$

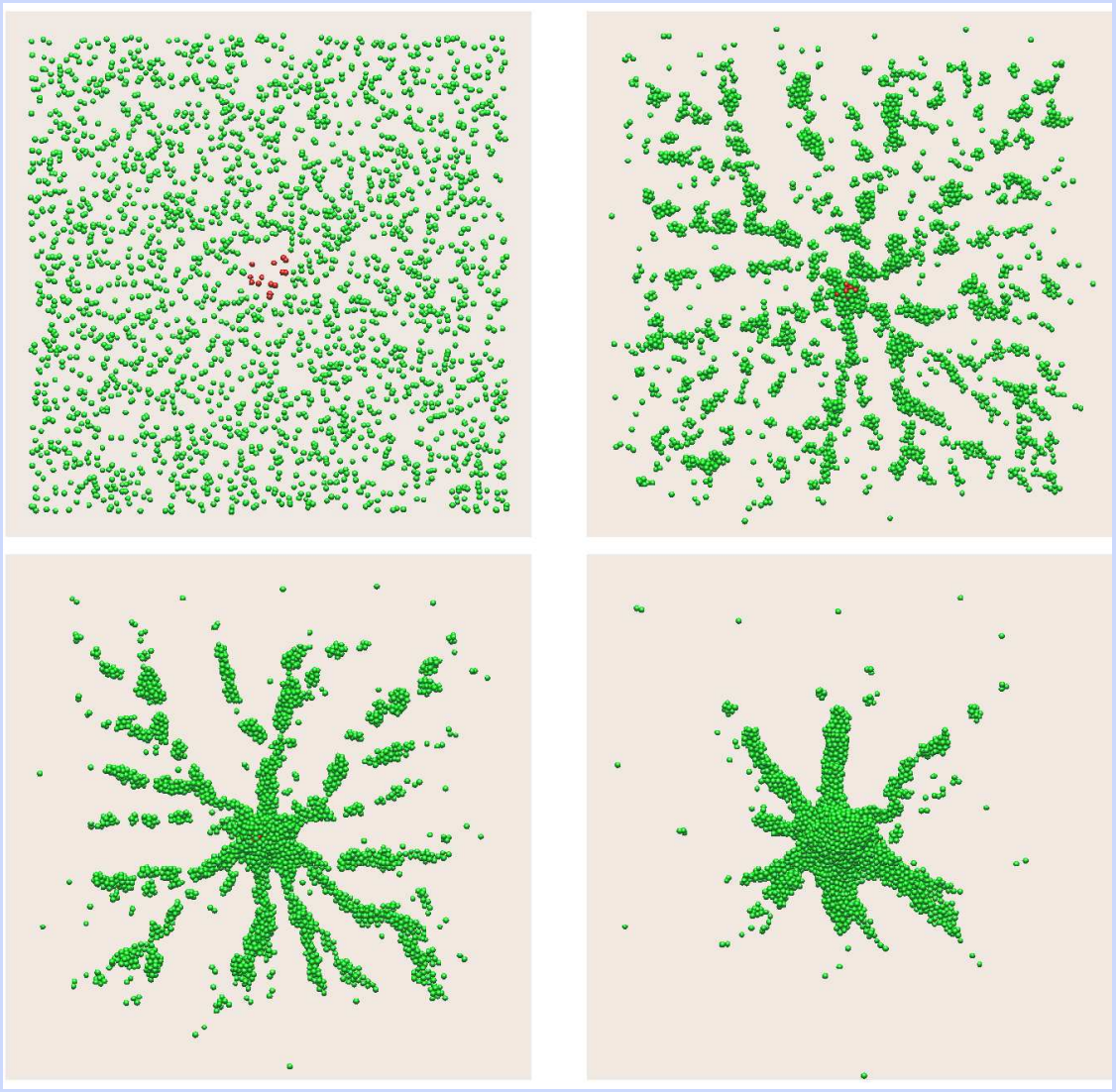
Step 1. Locate all cells that are within a given distance from cell i.

Step 2. Search the cell's neighborhood to determine if the cAMP levels are above threshold, and if so find the direction of the highest cAMP concentration.

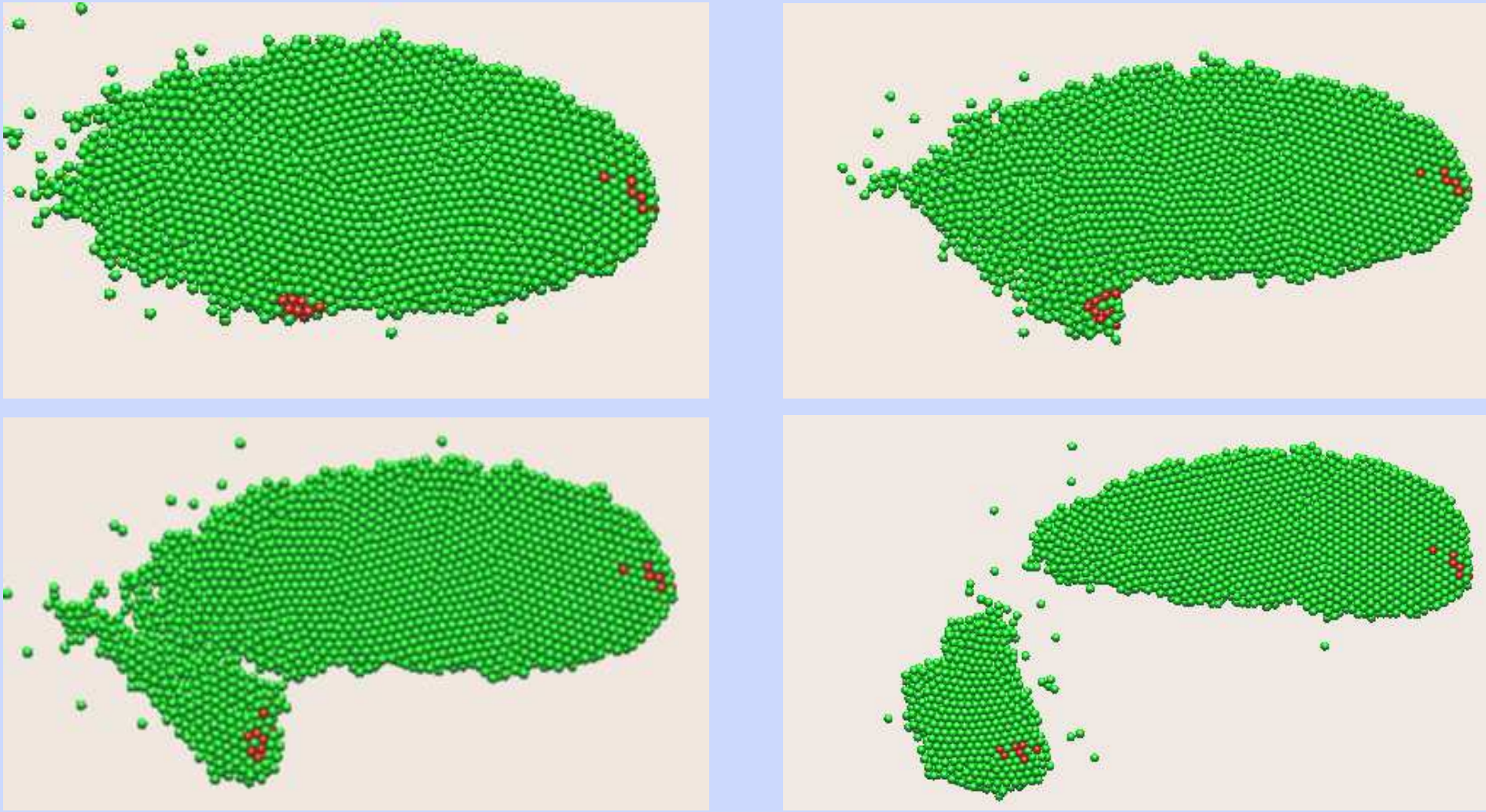
Step 3. If necessary, orient the cell towards the direction of the highest cAMP concentration.

Step 4. Find all the forces that act on the cell, $\mathbf{F}^{net}$ from each of the neighbor cells found in Step 1, deform the three axes of the ellipsoid, and move the cell according to (2).

Collective cell movement in Dd –aggregation

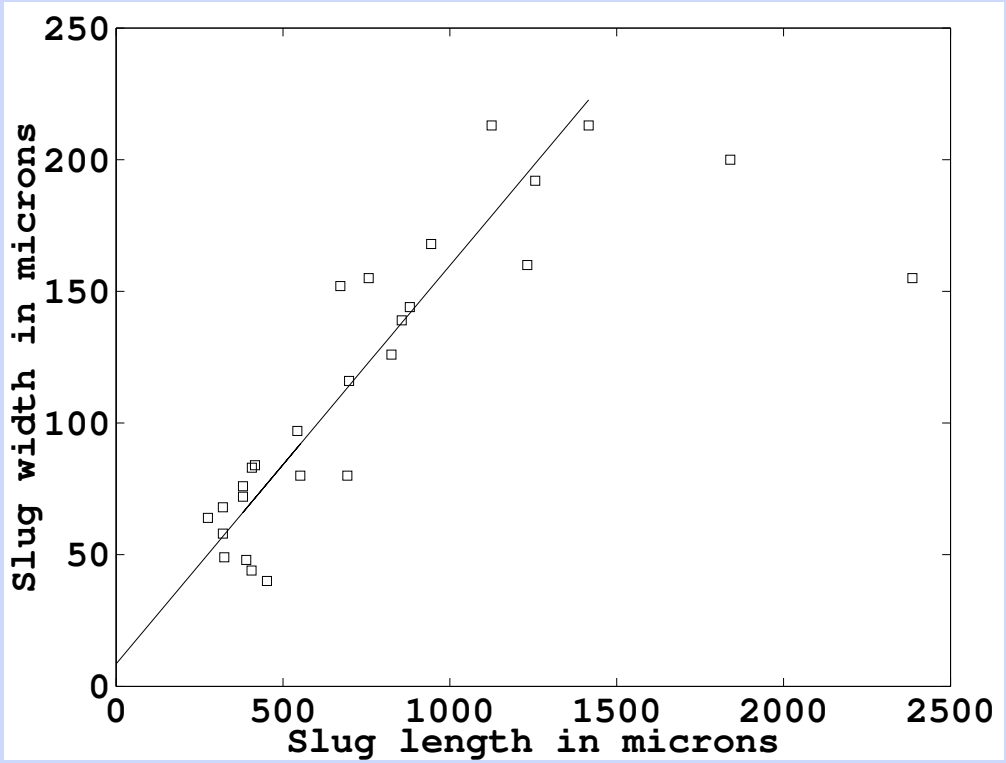


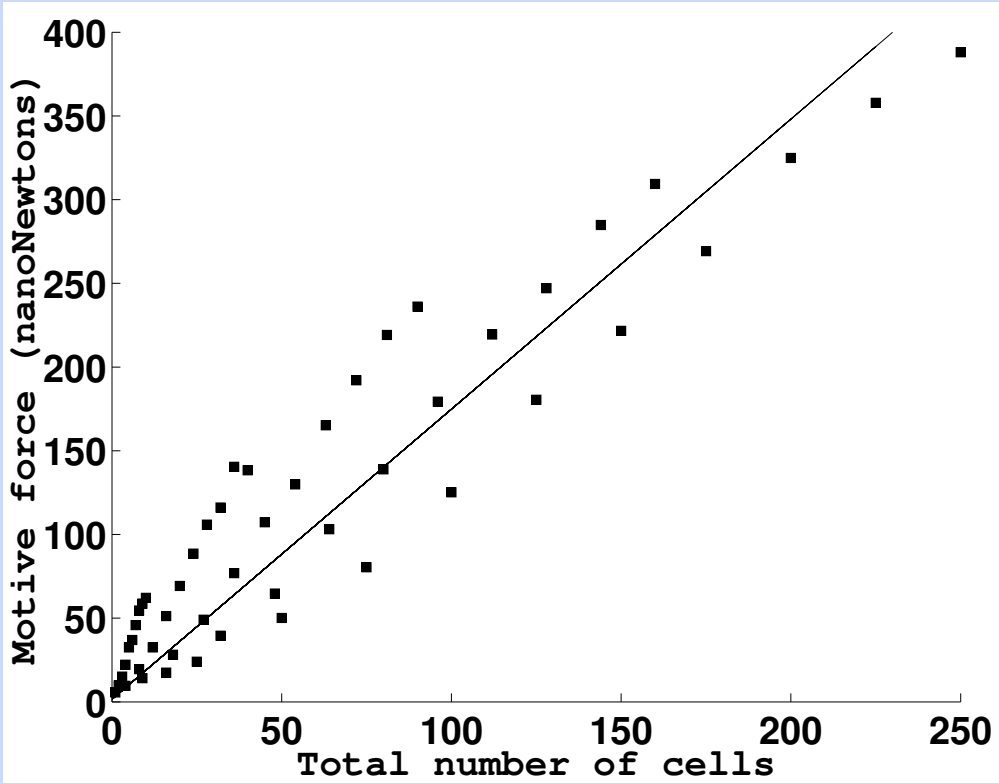
Collective cell movement in Dd – the slug



Eirikur Palsson and Hans G. Othmer, A model for individual and collective cell movement in Dictyostelium dicoideum, PNAS, **97**, 10448-10453, (2000).

What do we learn from 3D computations?



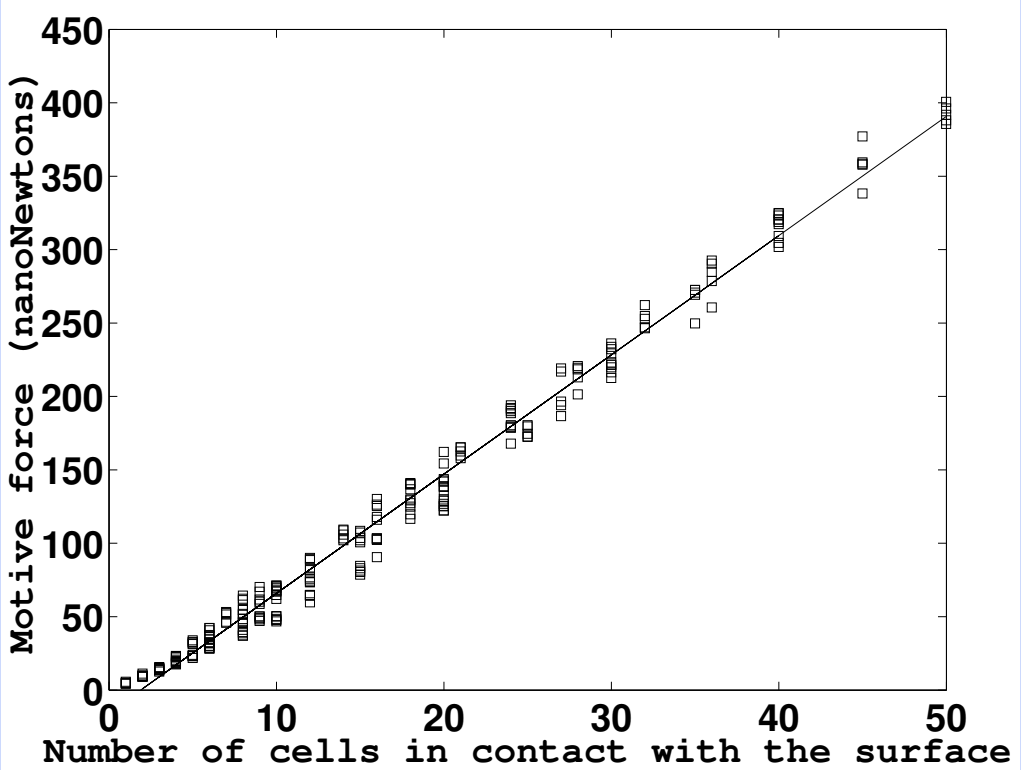


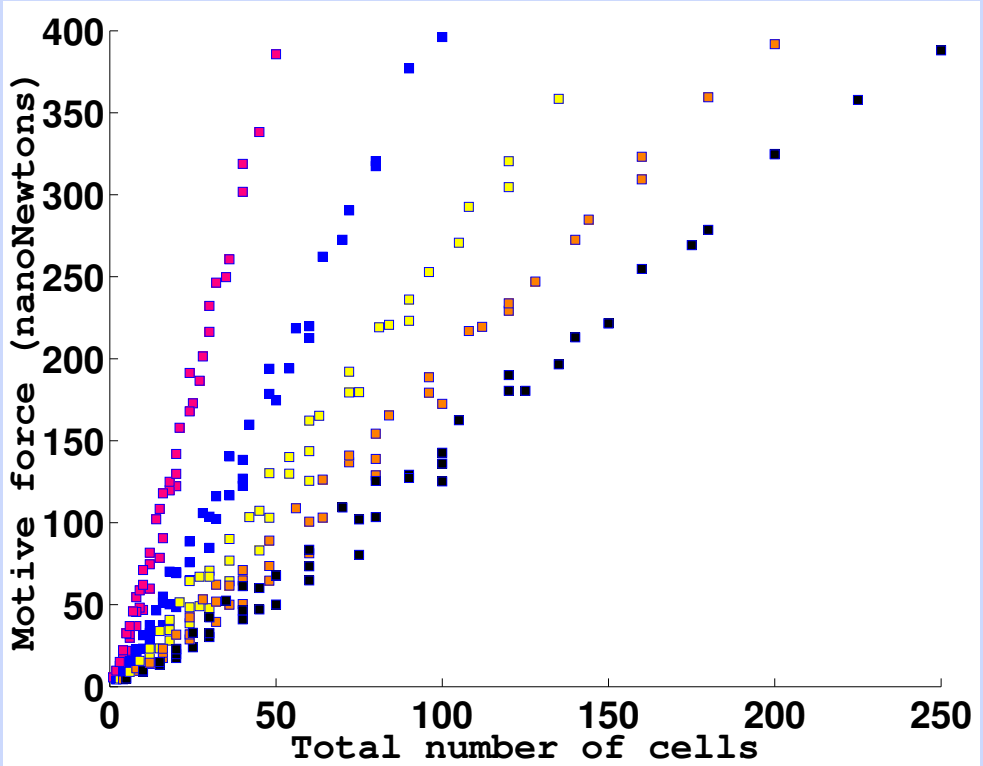
Inouye and Takeuchi, Protoplasma, 1978

Dallon and Othmer, J Theor. Biol., 2004

It appears as though the force scales with the number of cells, but if we look a bit closer ...

we see that ...

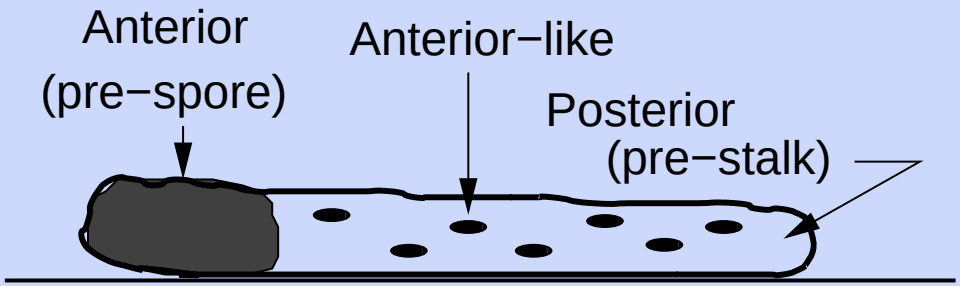




- Motive force scales with the number of cells in contact with the surface
- If the aspect ratio of the slug remains constant, then the force *appears* to scale with the volume

Open problems

Biological



- Better measurements of the length-velocity relations for crawling slugs are needed (in progress)?
- Can one explain the proportioning in the mound and later?
- Can one explain the caterpillar and other motions of the slug?

Mathematical and computational

- How does one adequately describe the cell-cell signaling in the slug?
- Is the free-boundary problem for the continuum description of the slug using a complex fluid model well-posed ?
- What are efficient and accurate computational methods for solving the governing equations for cell signaling and response, cell motion, and pattern formation in the slug?