Temporal aspects of chemotactic signaling
What can theoretical models tell us?

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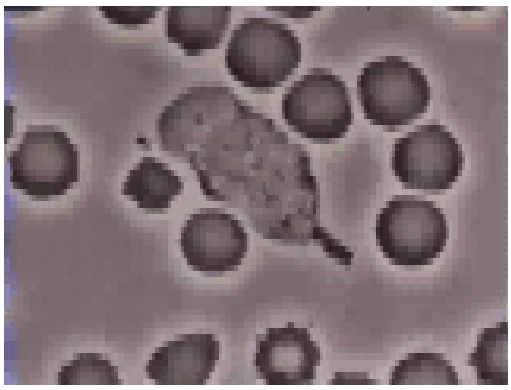
Chemotaxis

- Ability to respond to spatial and temporal gradients of chemoattractants/repellants
- Observed in many eukaryotic cell types
- Gradients determine direction of motion

Examples

- Wound healing
- Embryogenesis
- Neuronal patterning
- Angiogenesis



Neutrophil chasing a bacterium (*Staphylococcus aureus*)
Movie made by David Rogers, taken from the website of Tom Stossel (expmed.bwh.harvard.edu)

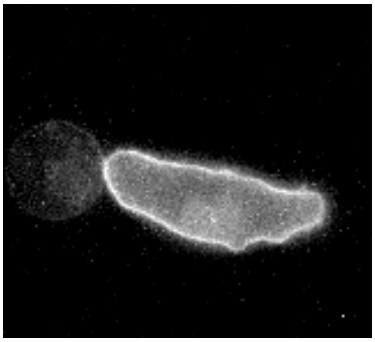
Chemotaxis in Dictyostelium discoideum

•In Dicty, cells display strong chemotactic response to cAMP

•Dicty cells move up the gradient

From S. Lee, Firtel lab, UCSD

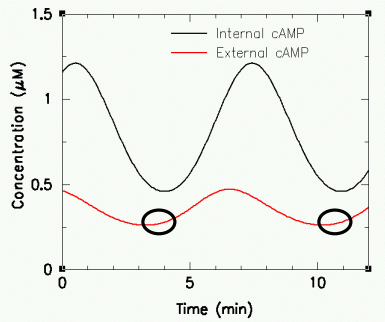
cAMP binds to cell membrane via ~10,000 uniformely distributed receptors (CAR1)

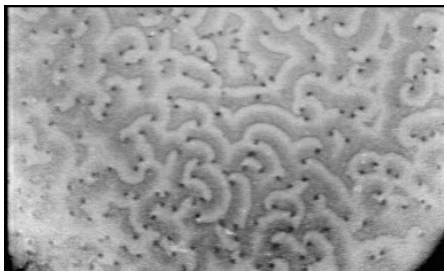


cAMP waves in developing populations

Waves have a periodicity of 6-8 minutes

Cells move during first half of the wave period





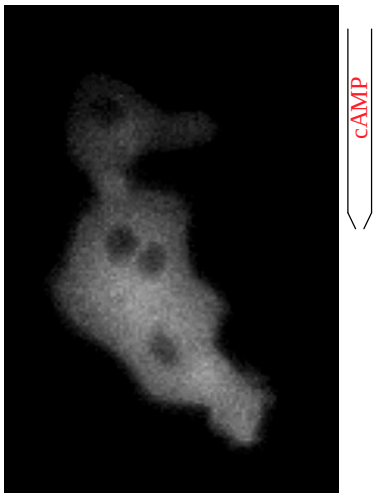
Our immediate goal:

To determine how the cell establishes its direction/polarity

Once direction is established, it can be amplified or stabilized

Hence, we focus on the first few seconds after signal is received

Recent experiments using GFP-tagged PH (Pleckstrin Homology) domain proteins



Asymmetric cAMP stimulus

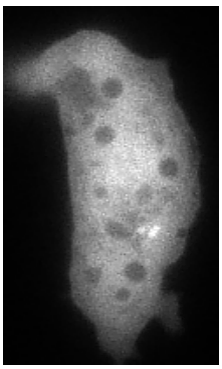
Frames every 2 s

Three pulses from the right

6 s duration

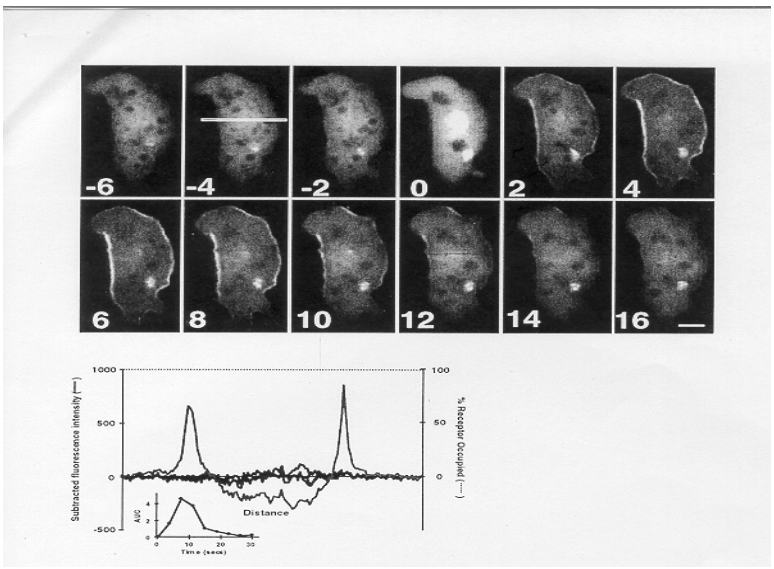
http://www.med.jhu.edu/devreotes

Uniform stimulus

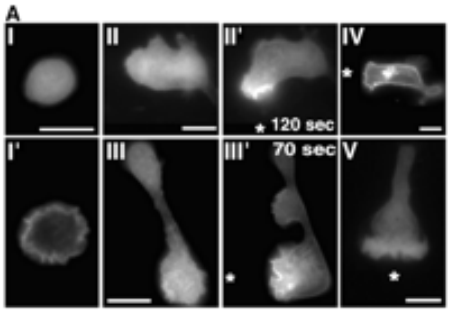


Response to a uniform increase in chemoattractant concentration. Frames were taken every 2 seconds. The chemoattractant was added just before the cell goes out of focus. From C.A. Parent and P.N. Devreotes, Johns Hopkins.

C.A. Parent *et al.*, Cell **95**, 81 (1998)

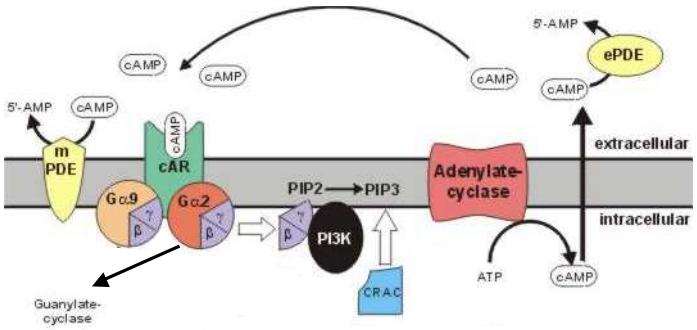


Asymmetric response of PH domain proteins also found in Neutrophils



G. Servant *et al.*, Science 2000

Relay model for Dicty



Basic fact from experiments:

Asymmetry is established in very short time

SINCE

- Applied signal is well above threshold
- CAR1 receptors uniformly distributed over cell membrane
- cAMP diffuses rapidly around cell

THUS

It is likely that there is an inhibitory intracellular mechanism which suppresses the localization of PH domain proteins at the back of the cell.

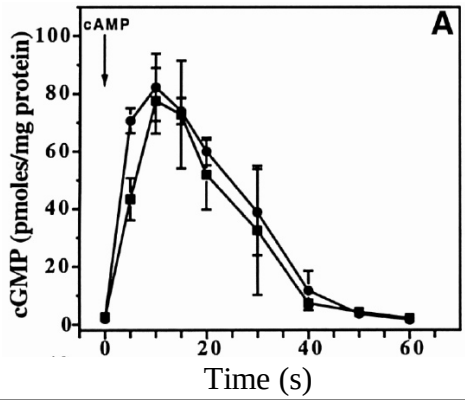
We propose:

Inhibitory process delivered via an intracellular messenger

This messenger diffuses in the interior of the cell and competes with the external signal

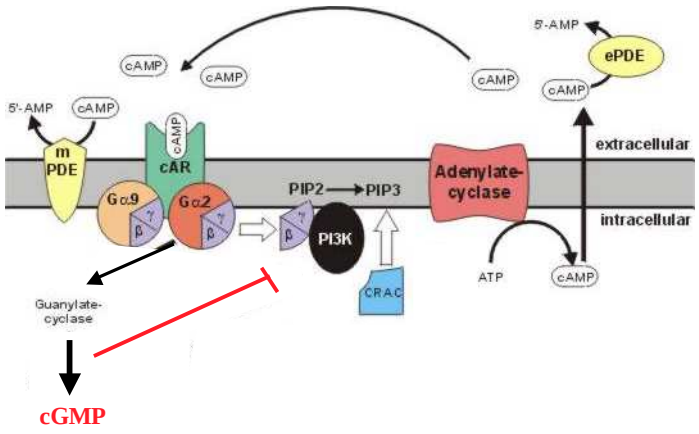
cGMP is a good candidate

It is produced rapidly after cAMP stimulus



D. Traynor *et al.*, EMBO J. **19**, 4846 (2000)

Relay model



Our model

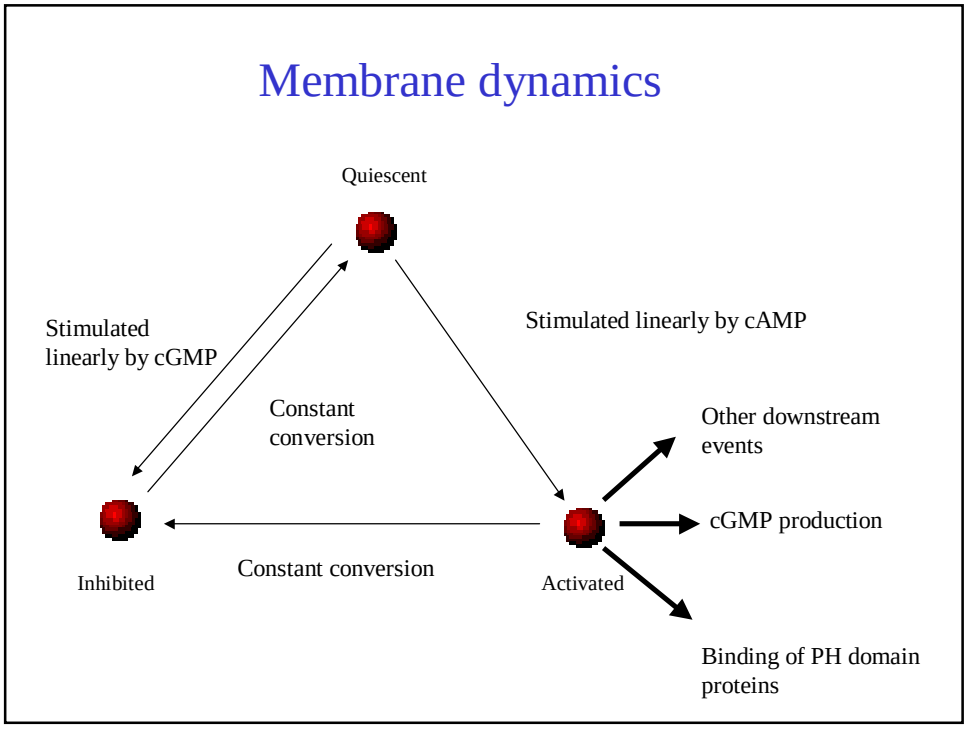
Membrane can be in three states: Quiescent, Activated or Inhibited

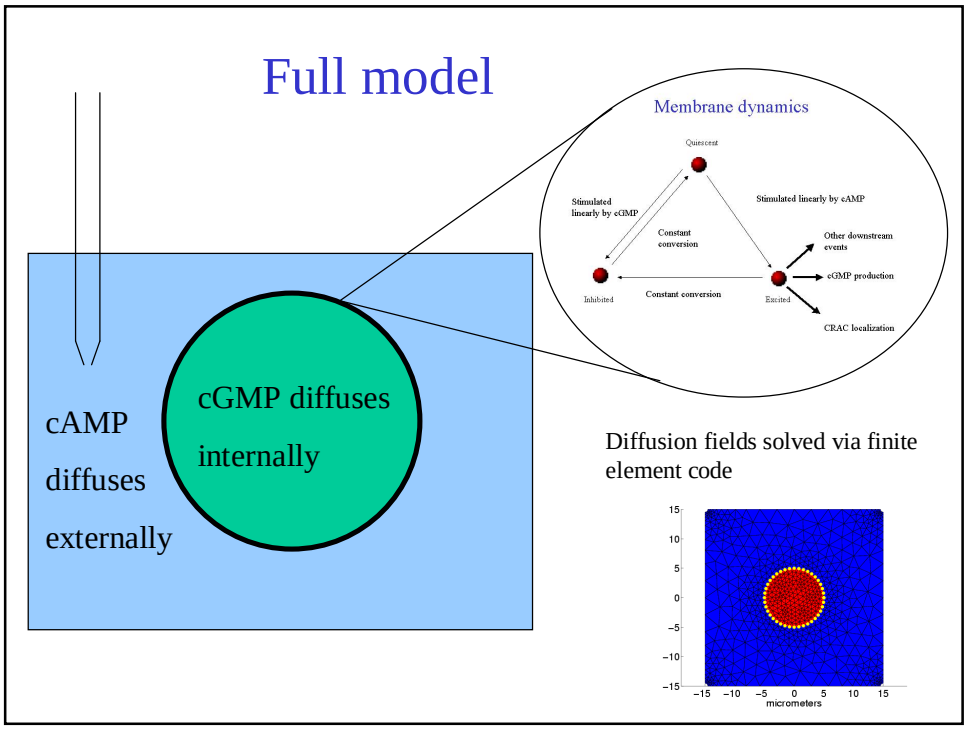
The transition rates between these states are dependent on the extracellular cAMP concentration and the intracellular cGMP concentration

The activated state of the membrane produces the localization of PH domain proteins and subsequent downstream events

cAMP and cGMP diffuse in the exterior and interior repectively

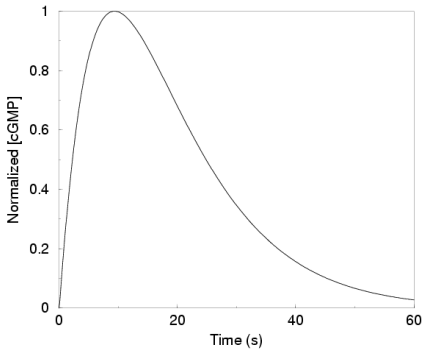
Cells are treated as two dimensional ellipsoids

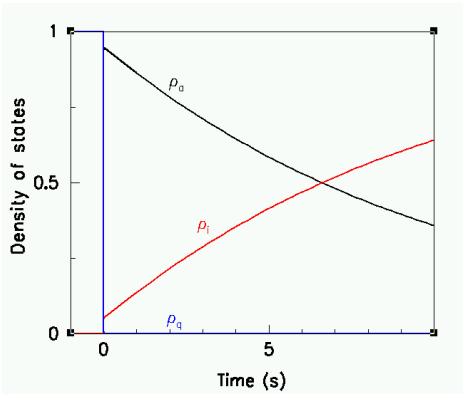

Membrane dynamics
Quiescent
Stimulated linearly by cGMP
Stimulated linearly by cAMP
Constant conversion
Other downstream events
cGMP production
Inhibited
Constant conversion
Activated
Binding of PH domain proteins


Full model
Membrane dynamics
Quiescent
Stimulated linearly by cGMP
Stimulated linearly by cAMP
Constant conversion
Other downstream events
cGMP production
Inhibited
Constant conversion
Excited
CRAC localization
cAMP diffuses externally
cGMP diffuses internally
Diffusion fields solved via finite element code
micrometers

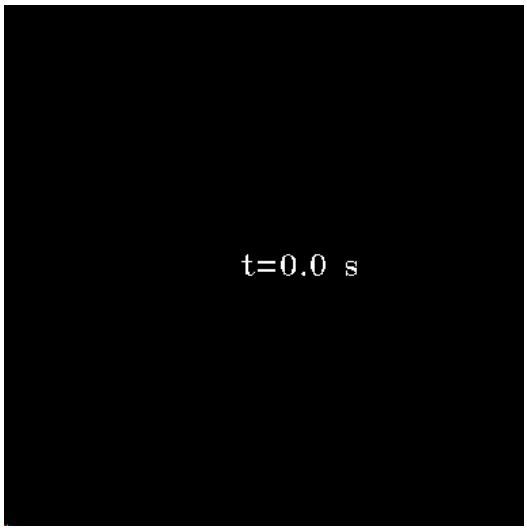
RESULTS

Uniform pulse of cAMP

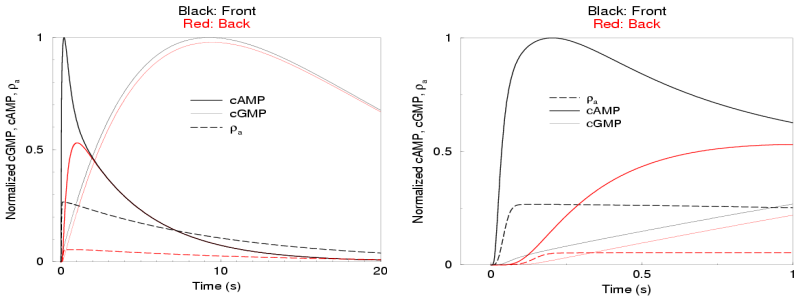




Asymmetric pulse



Asymmetric pulse, cont.



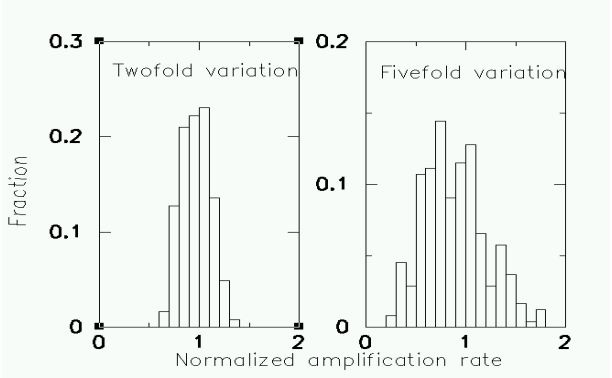
Amplification ratio AR= $\frac{\text{Peak } \rho_a, \text{front}}{\text{Peak } \rho_a, \text{back}}$

Here: AR=5

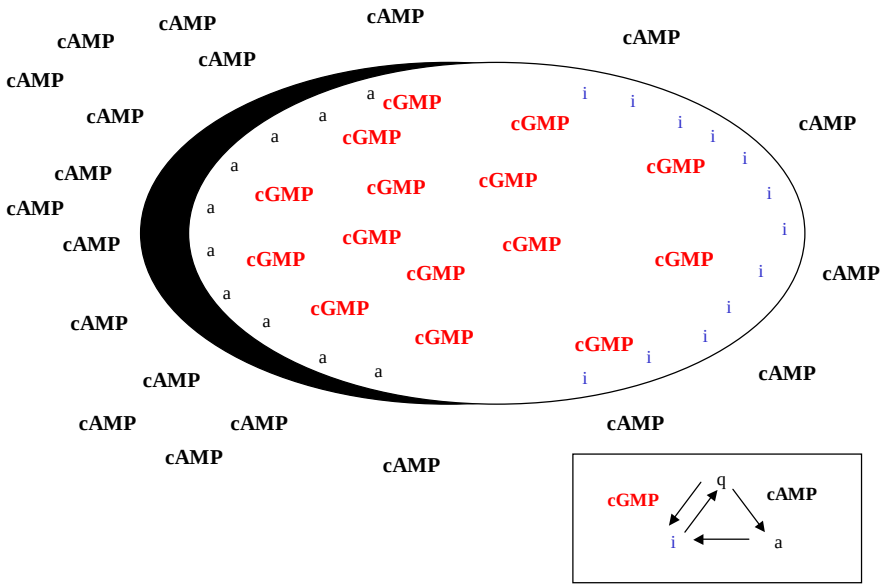
Sensitivity to parameter values

Changed five parameters ($\alpha, \beta_f, \beta_r, \delta, \sigma_g$) two or five fold up and down

Investigated amplification ratio for 243 combinations of parameter values



Model in cartoon form



Predictions fall in two catagories

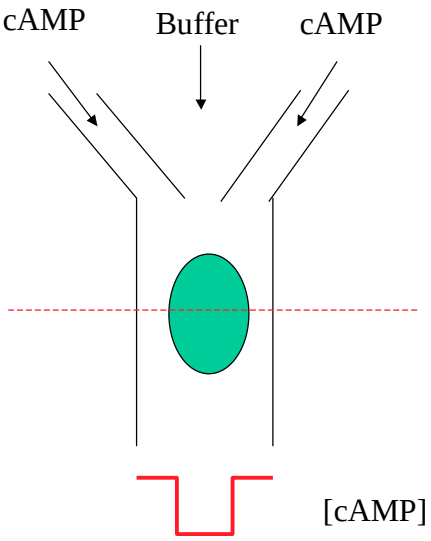
1. Predictions resulting from the mechanism

1. We propose the following experiment

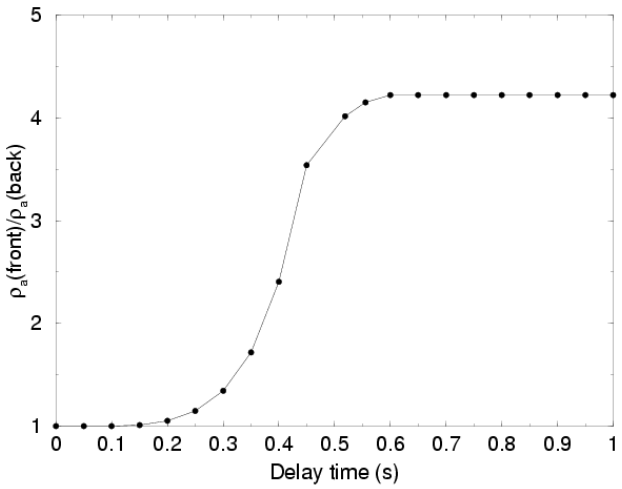
Pulse from the left (front) followed by pulse from the right (back)

This will give insight in the time scales of the inhibition and activation

Timing experiment



Result from the numerical model:



2. Spatio-temporal signal is needed for directional sensing

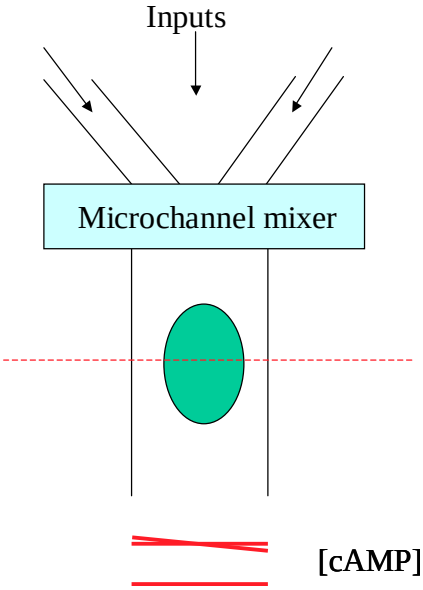
Dicty chemotaxes in very shallow *static* gradients (~2%)

Difficult to establish static gradient without a temporal signal

Our model predicts that directional sensing should be absent in purely static spatial gradients

Several experiments have hinted at this (Vicker *et al*. 86, Vicker 86, Korohoda 02)

New timing experiment on single cells should be able to clarify this issue



Predictions, cont.

2. Predictions specific to cGMP

If cGMP is the inhibitor, then cGMP mutants should have radically altered PH domain protein localization patterns. To be verified.

These mutants should not chemotax properly. Recently verified (Bosgraaf *et al*., EMBO 2002).

Cells “pretreated” with cGMP should not show translocalization

Is finite element modeling necessary?

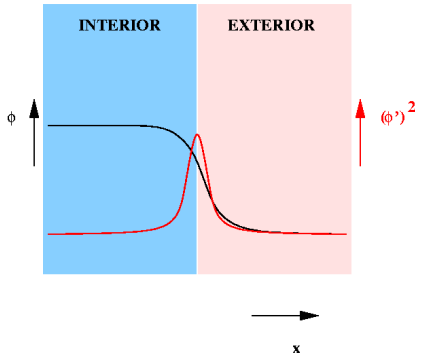
- In higher dimensions, finite element codes can become computationally costly
- Shape changes are hard to implement

Alternative: Phase-field modeling

Motivated by successful work in free-boundary problems, including dendritic growth and fracture

Basic Idea:

Introduce an auxiliary field (the phase field) that takes on one value in the interior and another in the exterior and that varies smoothly from one to the other across a narrow diffuse interface.



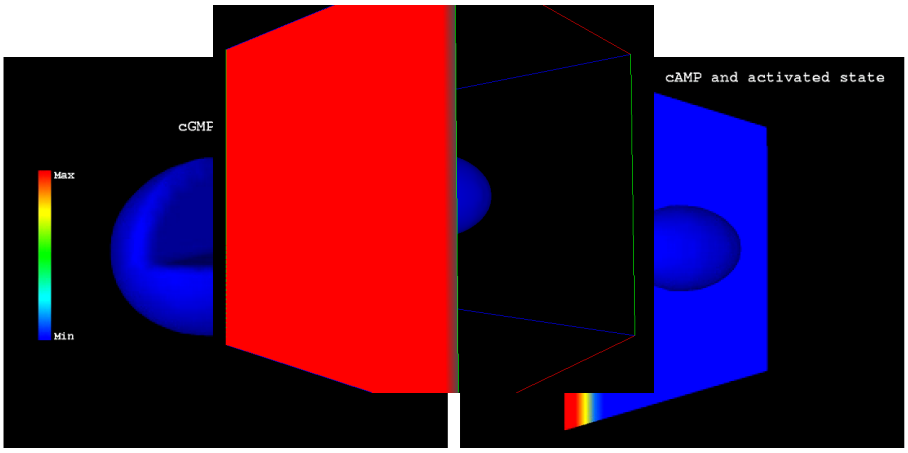
Diffusion equation becomes

$$\frac{\partial([cGMP])}{\partial t} = D\nabla(\phi\nabla[cGMP])$$

Membrane fields can also be defined in a natural way

$$\frac{\partial \rho_e}{\partial t} = (\phi')^2\{\alpha c \rho_q - \delta \rho_e\}$$

Example of 3D code: prolate spheriod (radii of 2.5 μm, 2.5 μm and 5 μm) in cube. cAMP stimulus from one face of computational boundary.



Summary

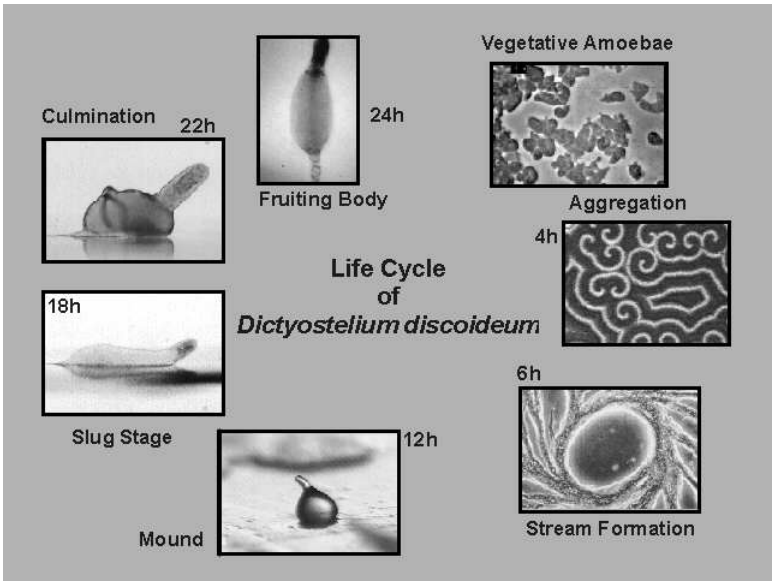
- Model can produce significant asymmetry within a few seconds
- Requires rapidly diffusing internal messenger
- Likely candidate: cGMP (mutant data)
- Specific predictions can verify model

Future work

- Perform proposed dual injection experiment
- Verify prediction for mutant experiments
- Extend model past initial response (include adaptation)

What Is Dicty?

- Unicellular amoeba (~ 10 μm).
- Live as separate cells on forest floor; feed on bacteria.
- Upon starvation cells interact by chemical signals, adhesion, etc. and aggregate (50,000 cells).
- Differentiate into 20% stalk and 80% spore cells.
- Form slug (~ mm) and fruiting body.



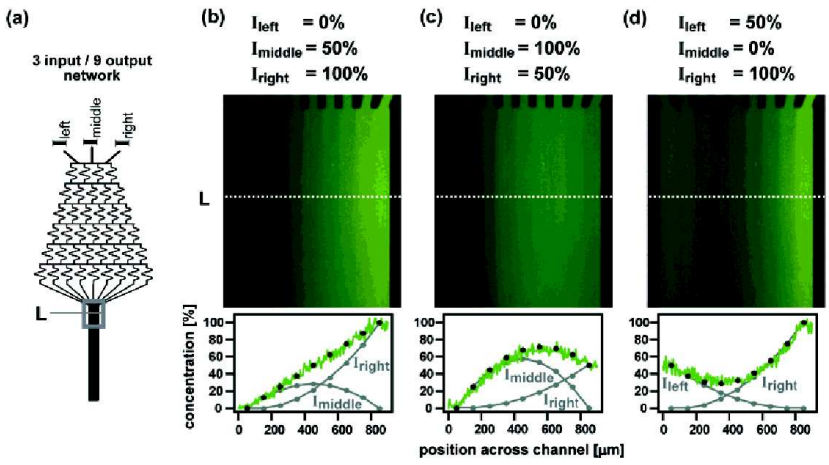
Why study anything in Dicty?

- Short life cycle (24 hours).
- Easy to grow.
- Many mutants developed.
- Exhibits many important biological processes.

Why study chemotaxis in Dicty?

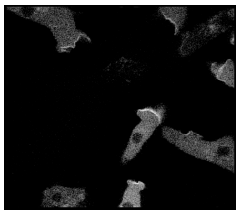
- Genetic manipulations have revealed large part of the architecture of the signaling network.
- Library of strains with GFP-fused proteins.
- These strains can be used in subcellular fluoresence microscopy.

Experimental set-up in Eberhard Bodenschatz's lab

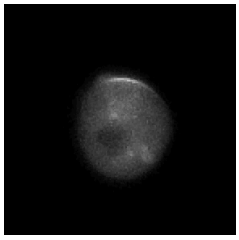


S.K.W. Dertinger et al. Anal. Chem. 2001, 73, 1240

Response to pulse from micro capillary



cAMP micro capillary near right upper corner.



cells lacking actin filament formation.
Micro capillary moves around

Images taken every 5 seconds. From C.A. Parent and P.N. Devreotes.

Some more remarks

Our model addresses first few seconds

Does not include: cAMP production, PH domain protein localization, cAMP/cGMP adaptation, establishment of polarity

Cannot account for behavior in long lasting spatial gradients