A modelling framework for elucidating signal transduction underlying eukaryotic chemorepulsion

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Systems biology

- A systems approach to understanding the behaviour of biological processes
- A study of interaction between components of a biological system and how they give rise to function and behaviour of the biological system
- Distinct strands of modelling, computational, theoretical and systems work in biology

Systems theory and Biology: 1966 (Mesarovic)

A range of communities: biologists, mathematicians, physicists, engineers, computer scientists involved in tackling relevant problems

Modelling

- Develop models to explain quantitatively how networks give rise to specific processes
- How are signals organized and propagated temporally and spatially?
- Networks non-linear with strong feedback effects

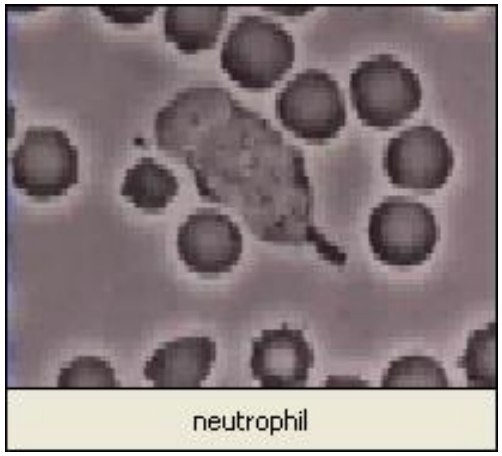
Sources of complexity and challenges:

- Unknown parameters, rate constants etc
- Missing biological steps
- Connection with and interpretation of experiments
- History dependence
- How does the model behaviour depend on many assumptions?
- How can the model make clear testable predictions?
- Multiple scales and interacting subprocesses

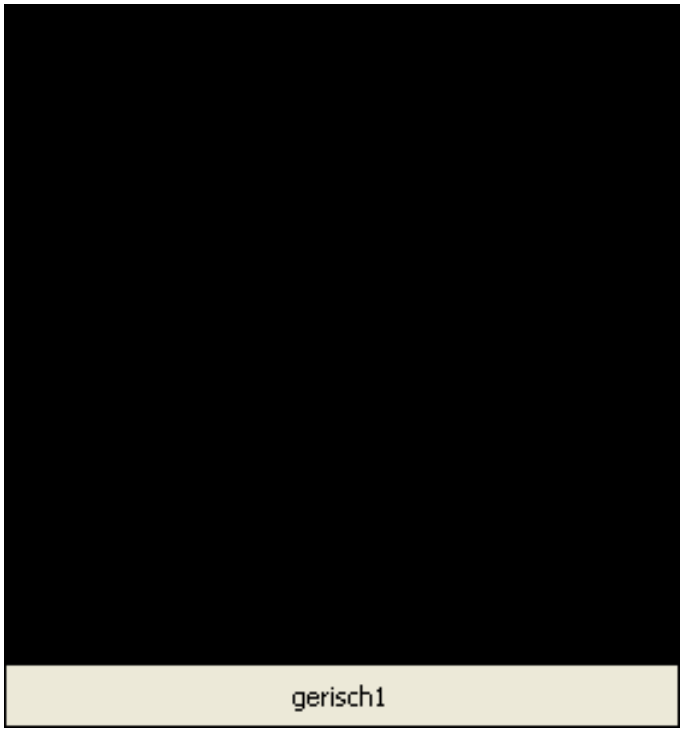
From a engineer's perspective

- Basic ingredients: reaction and transport
- Coupled with complex control regulation and certain special features
- Complex processes and their collective function
- A whole range of basic and application driven processes and systems
- Different questions!

Chemotaxis



Neutrophil chasing bacterium



Slime mold: Dictyostelium discoideum

Gerisch et al

Subprocesses involved in chemotaxis

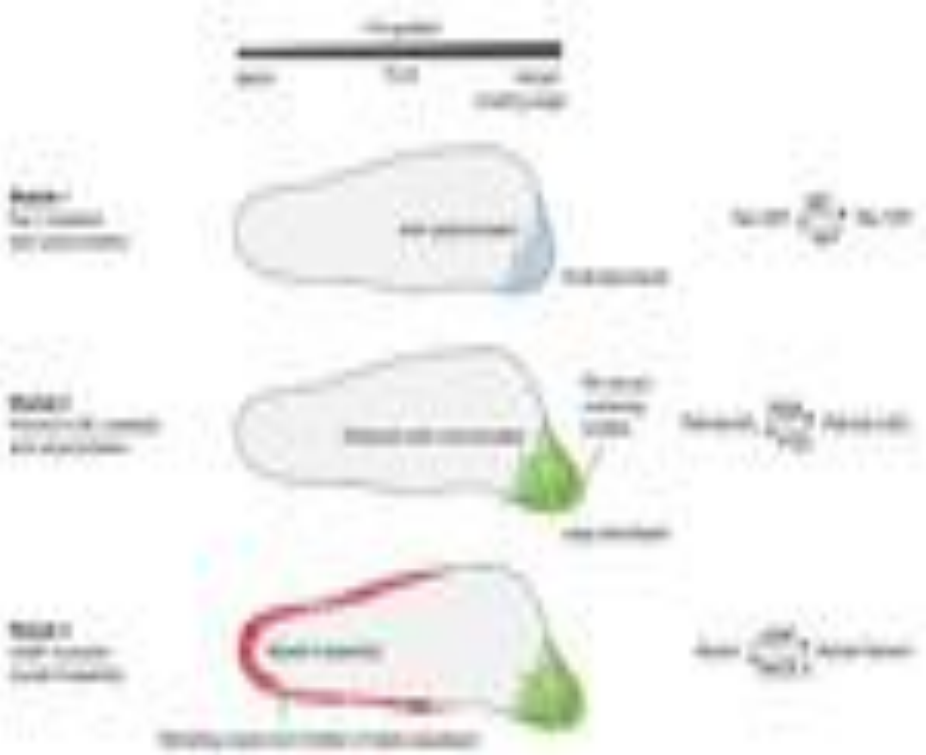
Gradient and concentration perception

Chemotaxis is firstly a sensory transduction process, activated by receptor-ligand binding and its spatial variation

This activates various downstream pathways, and other processes

Polarization of the motility apparatus

Formation of a clear front and back with associated signaling components

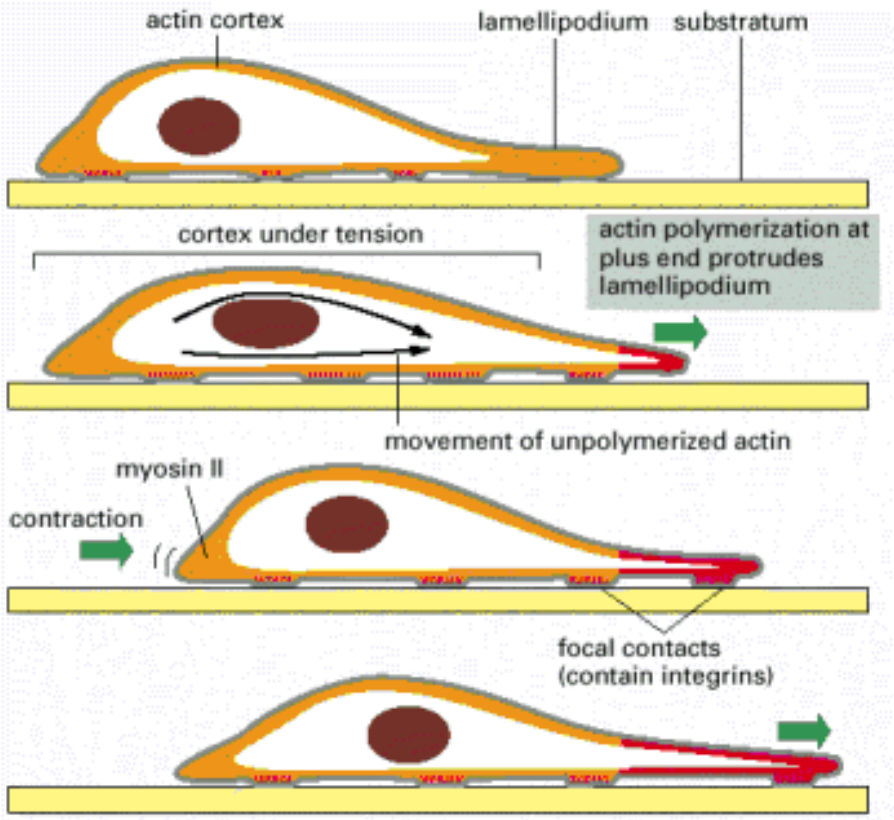


Van Haastert, 2004

Subprocesses involved in chemotaxis

Chemical regulation of the motility apparatus

Cell migrates by periodically extending the pseudopod, and retracting the uropod



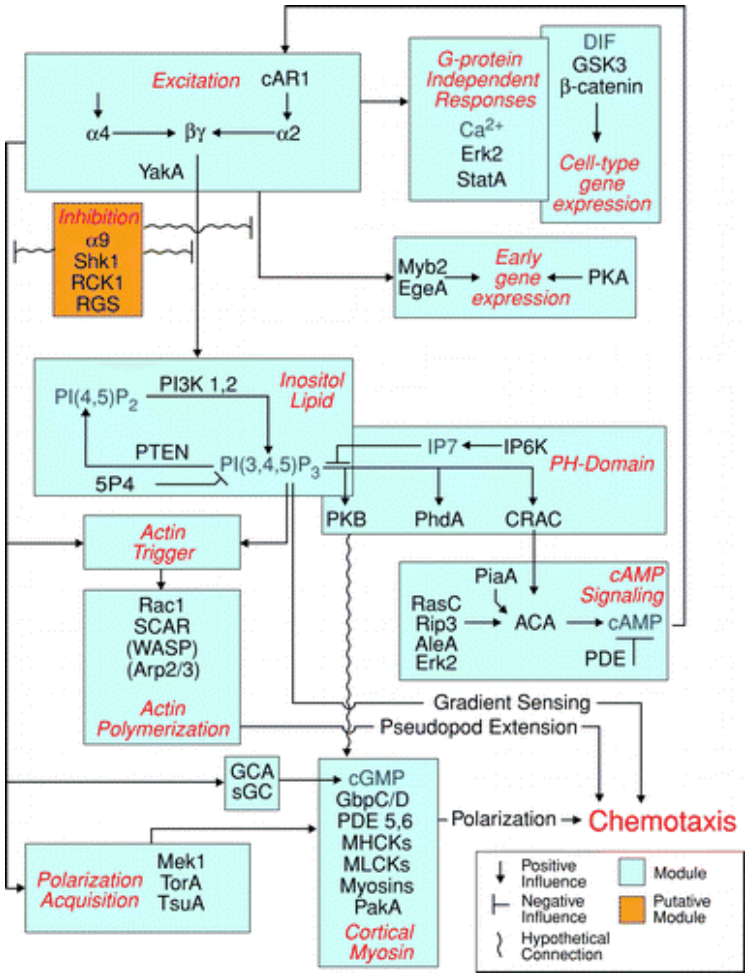
Alberts et al, 2002

Chemotaxis involves the interplay of various signaling pathways, and various physical processes

Spatial scales: cell size 10 microns; time scales– seconds to a few minutes

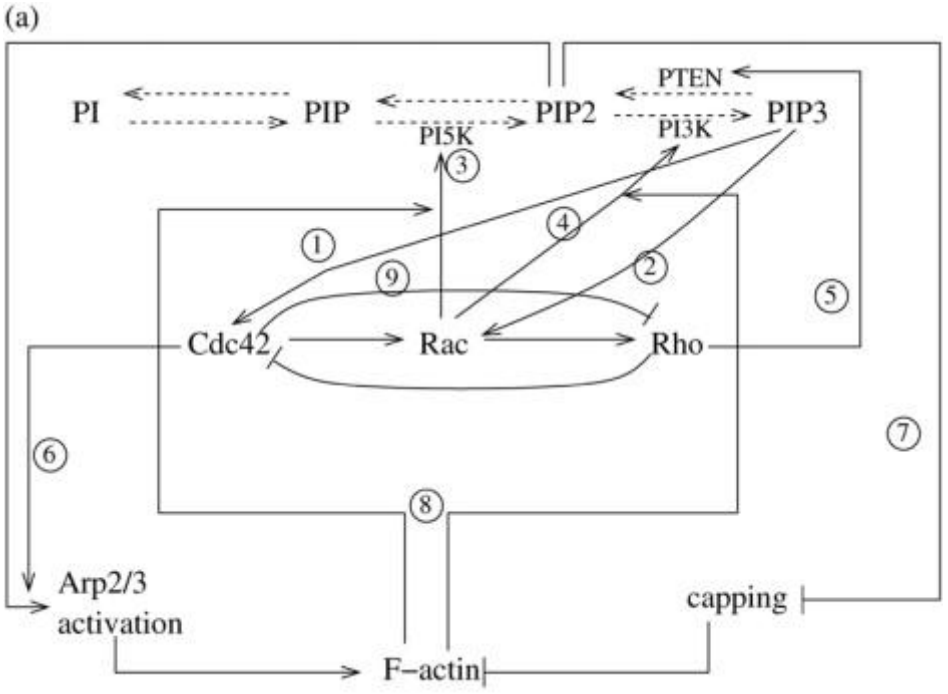
A modular perspective of some known biochemical pathways

Dictyostelium



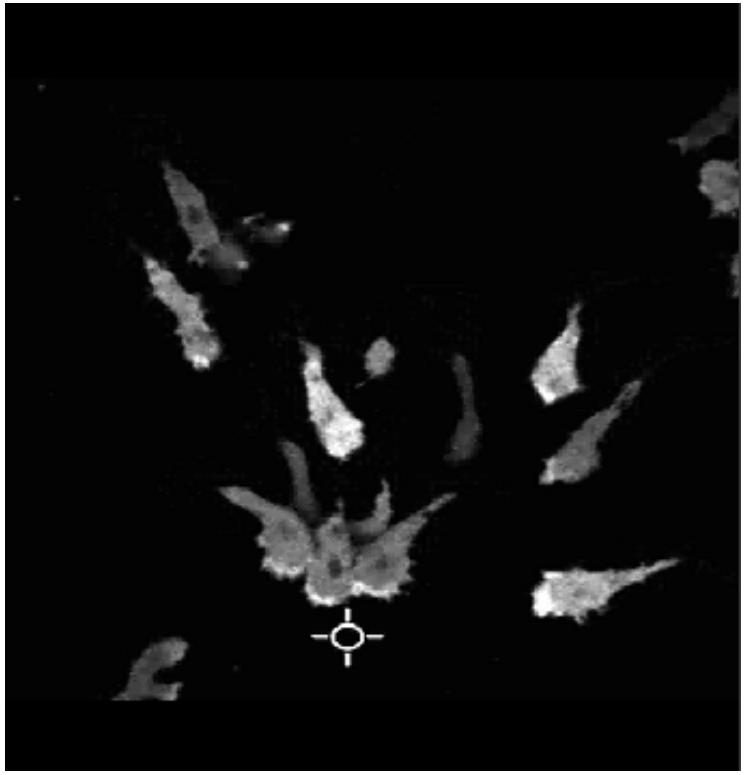
Manahan et al 2004

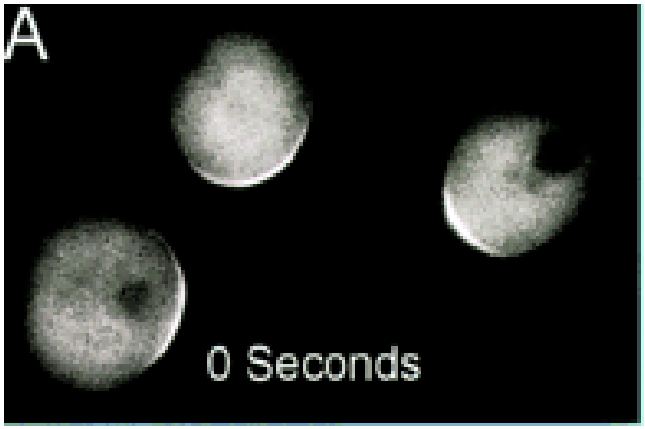
Sensing and polarization (leukocytes)



Dawes et al 2007

Gradient sensing





Parent and Devreotes, 1999

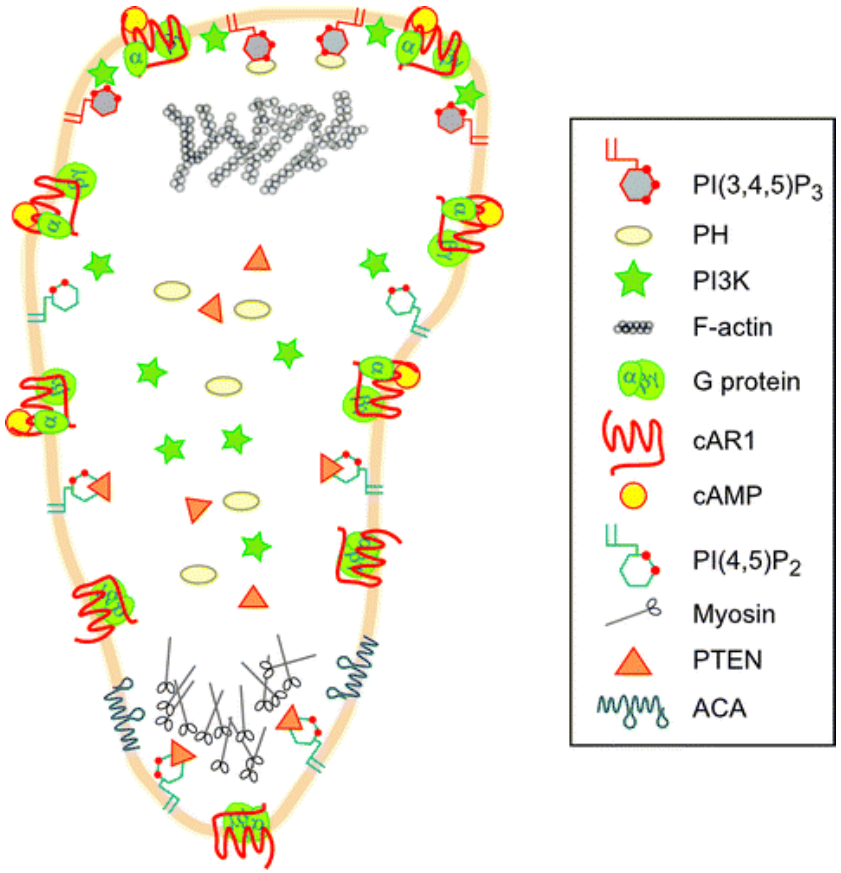
Cells immobilized with latrunculin A can still sense the gradient even if they cannot move

Gerrisch et al

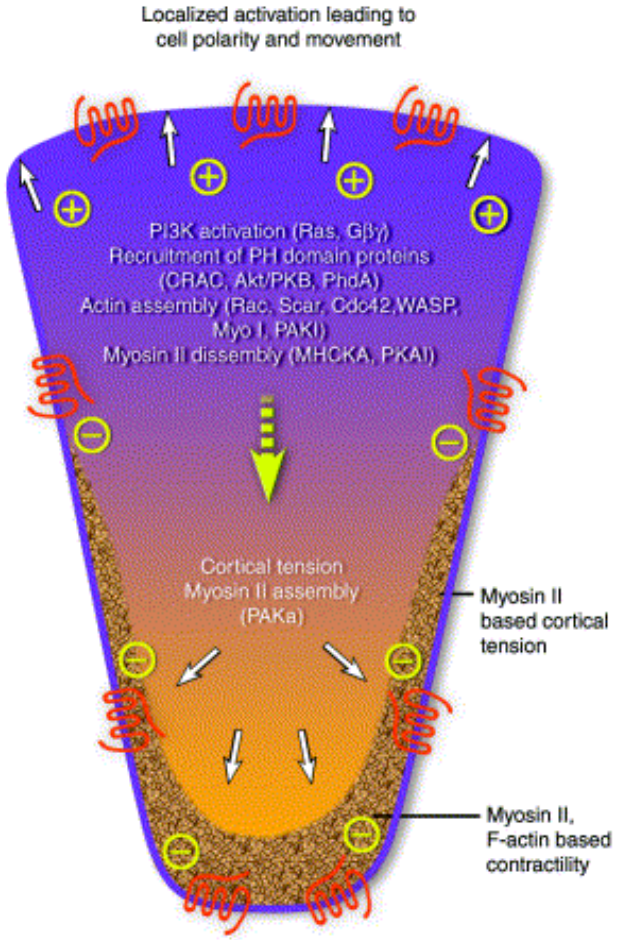
Latrunculin treated cells provide a simplified setting to study some important pathways responsible for chemotaxis

Polarization

The localization of various signalling and motility components to opposite ends of the cell, along with any shape change



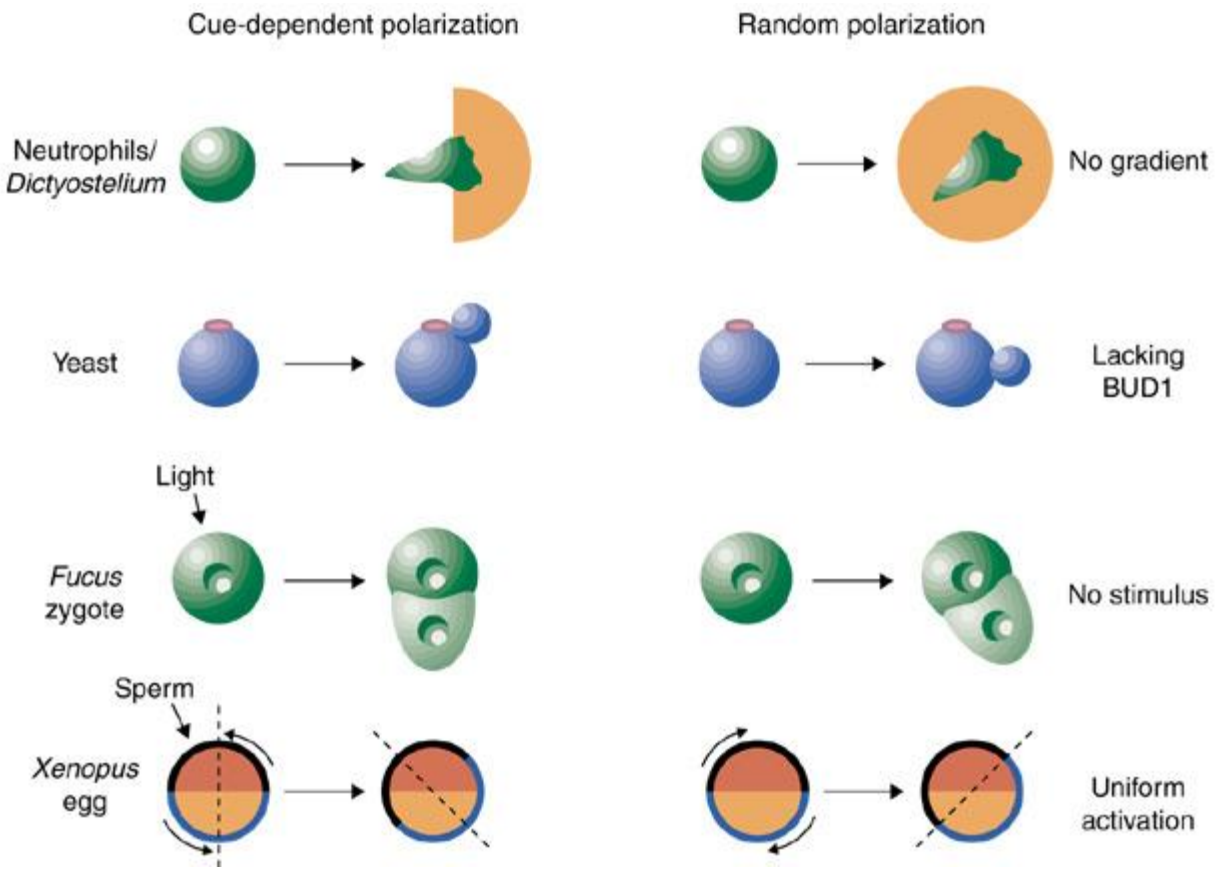
Manahan et al 2004



Chung et al 2001

Polarization

Important issues related to polarization



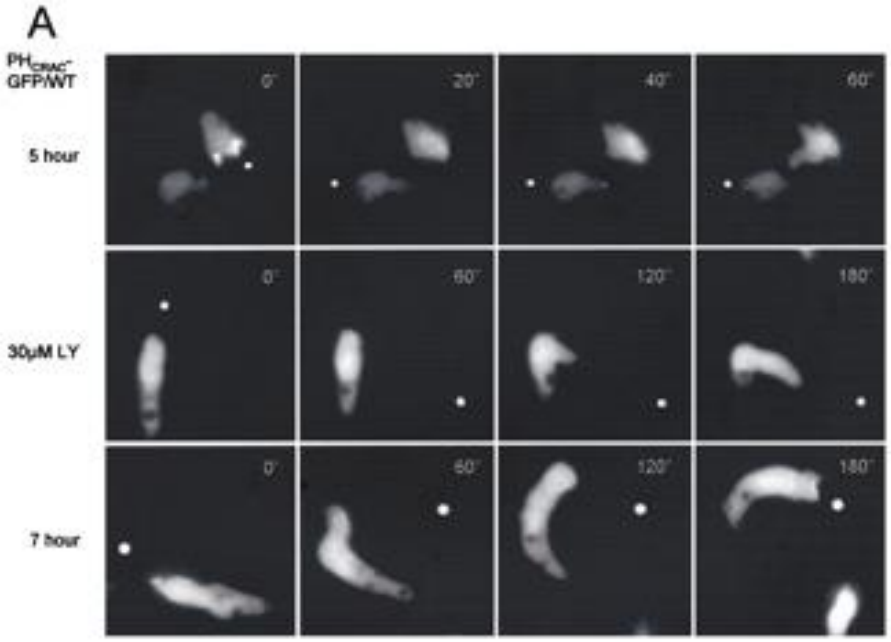
Rong Li et al, 2003

Cells are able to polarize in the absence of existing cues “random polarization”

How do cells use their intrinsic capacity for polarization, in chemotaxis?

Polarization modelling

Dictyostelium: basal state is such that it is always moving, extending pseudopods, even without externally imposed gradient



Cells 4-5 hours into development have receptors, but are weakly polarized at best

Cells 7 hours into development are distinctly polarized, without an externally imposed gradient

Chen et al 2003

How does chemotactic signalling affect/employ interact with this polarization process?

Directed cell migration

Critical roles in vital biological and physiological processes:

- Immune system response, wound healing, development
- Tumour metastasis
- Implicated in different pathologies such as chronic inflammatory diseases

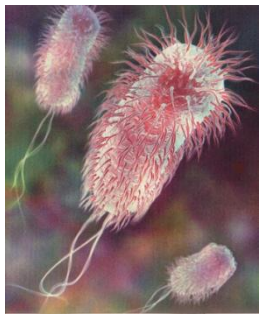
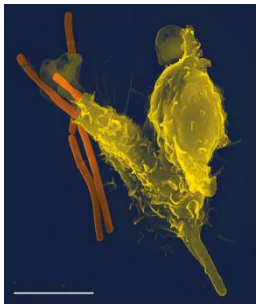
- An example of a complex sensor-actuator system

- ***Chemorepulsion:Process by which an organism or cell moves away from a chemical source or away from higher concentrations***

Video[1]

[1]Bielenberg et al, *J. Clin. Invest*., 2004
[1]Hutchinson, *Nature Reviews Cancer*, 2004

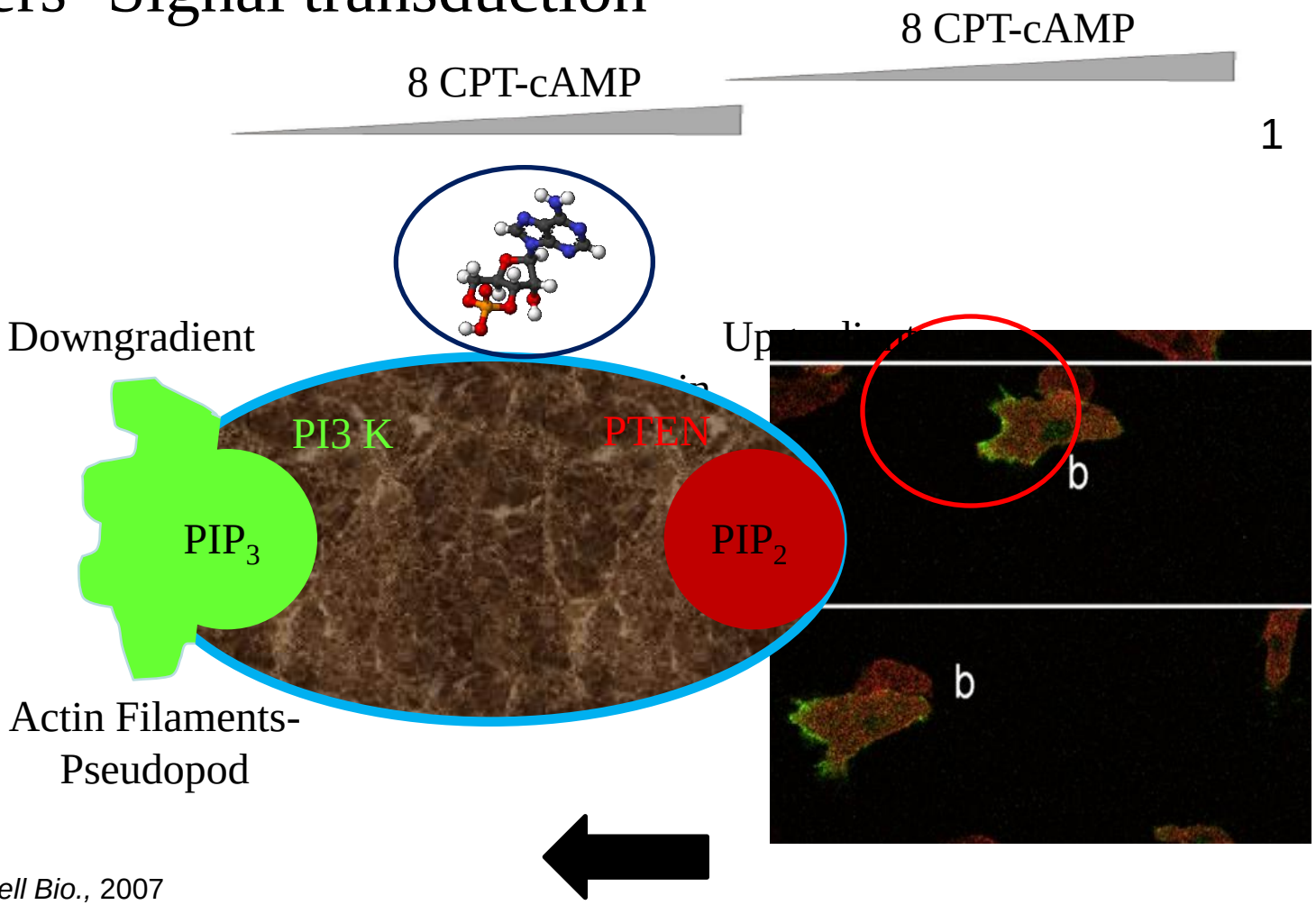
Where is Chemorepulsion Observed?

	Prokaryotes	Eukaryotes	
Organism	Bacteria[1]	Amoeba[2]	Mammalian Cells[3]
Example	*Escherichia coli*	*Dictyostelium discoideum*	White Blood Cells
Chemo-repellent	Hydrogen Peroxide	8 CPT- cAMP	IL-8
			

[1]Benov & Fridovich, *PNAS,* 1996
[2]Keizer-Gunnink et al., *J cell Bio, 2007*
[3]Tharp et al., *J. Leukocyte Bio*, 2006

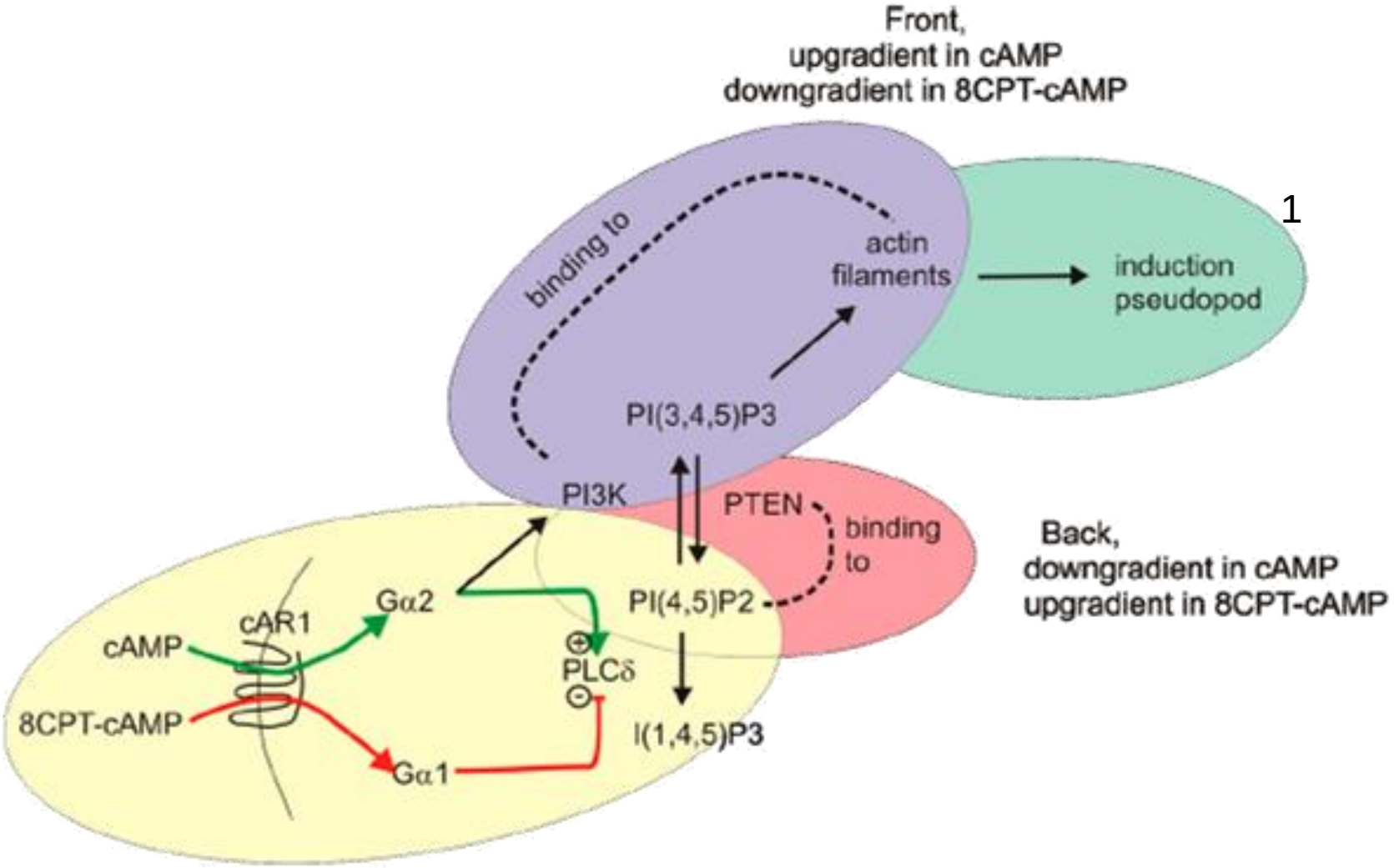
The Paradigm- *Dictyostelium discoideum* (Slime Mould)

- Standard model system
- Key Players- Signal transduction



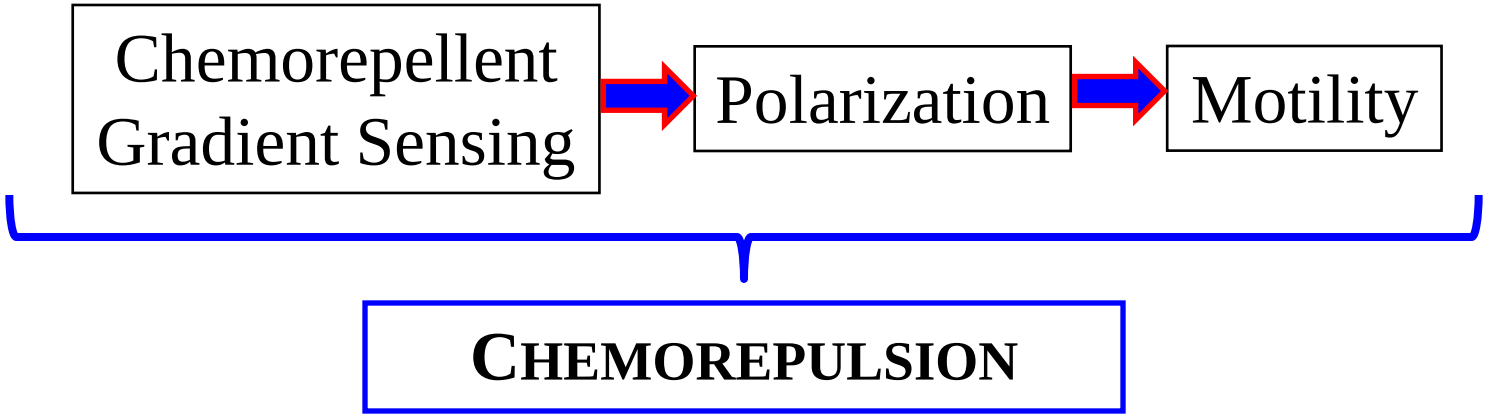
[1]Keizer-Gunnink et al., *J. Cell Bio.*, 2007

Goal- ***Achieve quantitative mechanistic systems based understanding of the biochemical signal transduction pathway of chemorepulsion in Dictyostelium discoideum.***



[1]Keizer-Gunnink et al., *J. Cell Bio.*, 2007

Modelling



- No chemorepulsion models
- Types of models
  - Use of both detailed biochemical and qualitatively simplified models
- Build in a modular fashion

Key modelling challenges

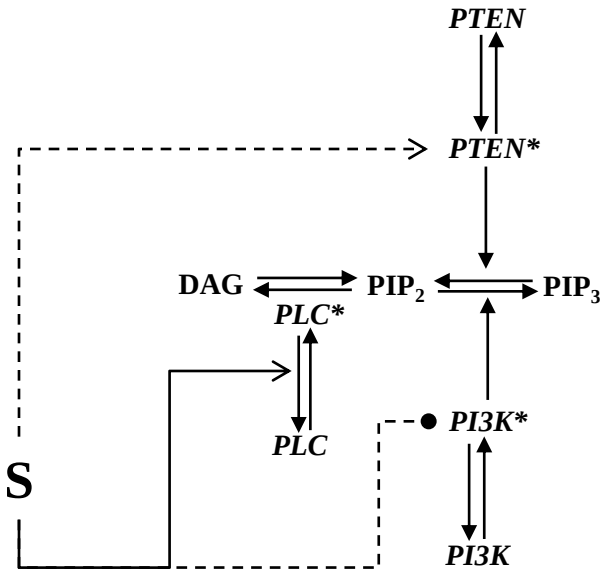
- Can the network and interactions give rise to desired behaviour?
- What is the role of PLC and the feedbacks (PIP3-PI3K) and(PTEN-PIP2)?
- What is the role of other receptor signalling to enzymes?
- What is the sensitivity of the network response to postulated interactions?

- A global approach, building around the core network of Keizer-Gunnink et al, and incorporating different possible additional receptor regulation
- Different hypotheses can systematically be examined
- Transparently connect hypotheses to predictions
- Aim to carefully characterize non-linear interactions as well spatial signal transduction, and role of feedback
- Investigate role of adaptation

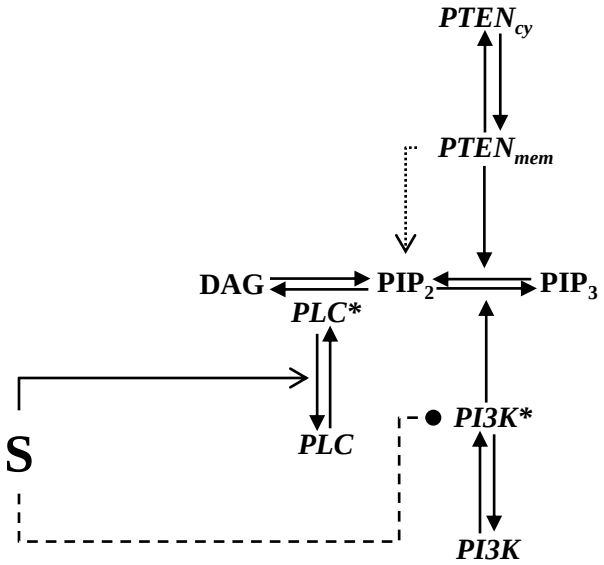
Modelling

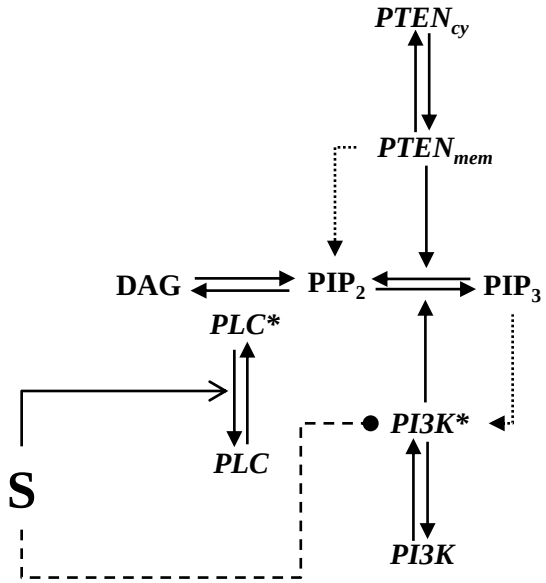
- A modelling framework, investigating different variants of regulation of key enzymes (feedforward and feedback)
- Model describes reaction and transport of key enzymes and lipids on the membrane
- Transport from cytosol to membrane described by additional cytosolic pool
- Distributed system with multiple feedback regulation
- Discretized by finite differences ~1200 ODEs
- Modelling using mass action kinetics

I: PI3K & PTEN are adaptively regulated by S

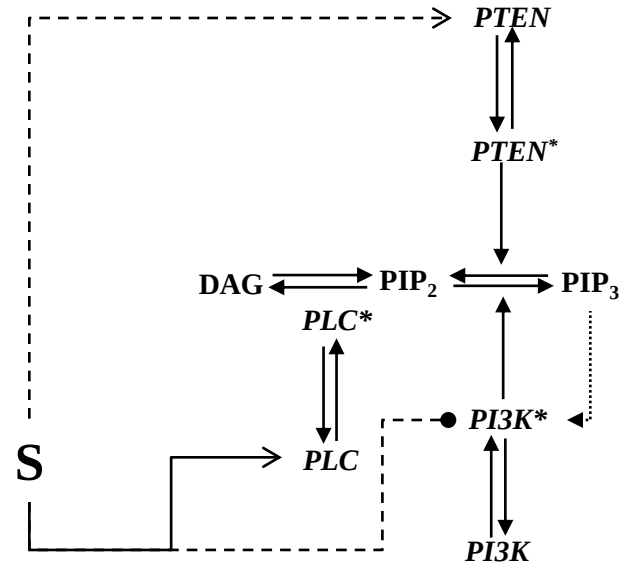


II: PI3K is adaptively regulated by S and PTEN via its binding to PIP_2





III: PI3K is locally regulated by S and PTEN via its binding to PIP_2. There exists a feedback between PIP_3 and PI3K.



IV: PI3K and PTEN are locally regulated by S and there exists a feedback between PI3K and PIP_3.

Model ingredients

PLC is locally regulated by signal

$$\partial[PLC^*]/\partial t = -k_{\_plc}[PLC^*] - k_s[S][PLC^*] + k_{plc}[PLC] + k_{dplc^*}\partial^2[PLC^*]/\partial\theta^2$$

Two kinds of feedforward regulation for PI3K considered: local and adaptive

Adaptive regulation of PI3K

$$\partial[A]/\partial t = K_a[S]^2 - K_{\_a}[A] + K_{da}\partial^2[A]/\partial\theta^2$$

$$\partial[I]/\partial t = K_i[S]^2 - K_{\_i}[I]$$

$$\partial[PI3K^*]/\partial t = K_f[A][PI3K] - K_r[I][PI3K^*]$$

Local regulation of PI3K by signal

$$d[PI3K^*]/dt = K_f[PI3K] - (K_r[S] + K_c)[PI3K^*]$$

PTEN regulation via feedforward and feedback regulation examined

$$\partial[PTEN^*]/\partial t = -K_{r'}[PTEN^*] + (K_{f'}[S] + K_{c'})[PTEN] + k_{dPTEN}\partial^2[PTEN^*]/\partial\Theta^2$$

Enzymes regulate the phosphoinositide lipids

$$\partial[PIP_2]/\partial t = k_{re}[PIP_3][PTEN^*] - k_{fo}[PIP_2][PI3K^*] - k_l[PIP_2][PLC^*]$$
$$+ k_{\_l}[DAG] + k_{dPIP_2}\partial^2[PIP_2]/\partial\theta^2$$

Feedback resulting from PTEN binding to PIP_2

$$[PIP_2]=[PIP_{2UB}]+[PIP_{2B}]$$

Binding of $PTEN_{cy}$ to PIP_{2UB}

$$[PTEN_{cy}]+[PIP_{2UB}]\Leftrightarrow[PTEN_{mem}]+[PIP_{2B}]$$

$$[PIP_{2B}]=[PTEN_{mem}]$$

$$[PIP_2]=[PIP_{2UB}]+[PTEN_{mem}]$$

$$\partial[PTEN_{mem}]/\partial t=-k_{-ten}[PTEN_{mem}]+k_{ten}[PTEN_{cy}]([PIP_2]-[PTEN_{mem}])+k_{dptenm}\partial^2[PTEN_{mem}]/\partial\theta^2$$

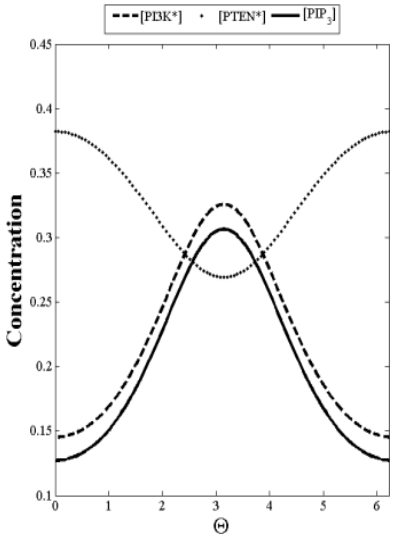
$$\partial[PTEN_{cy}]/\partial t=k_{-ten}[PTEN_{mem}]-k_{ten}[PTEN_{cy}]([PIP_2]-[PTEN_{mem}])+k_{dptenc}\partial^2[PTEN_{cy}]/\partial\theta^2$$

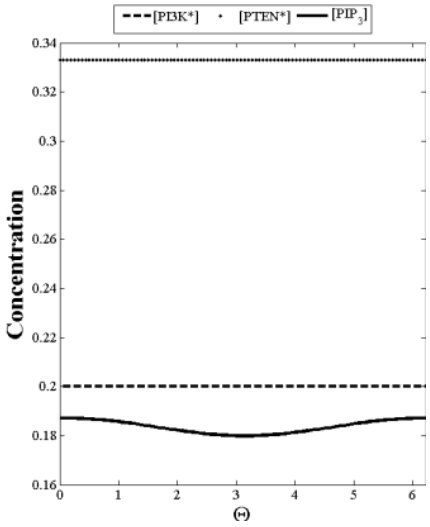
Equilibrium relationship between PIP_3 and PIP_2

$$[PIP_3]/[PI3K^*]=C[PIP_2]/[PTEN_{mem}]$$

Model variant 1

PI3K and PTEN are regulated by receptor in feedforward fashion (no other feedback mechanisms)





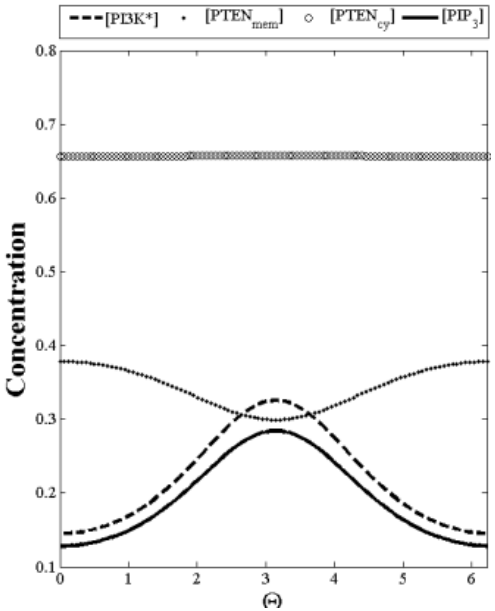
Similar results for local and adaptive feedforward regulation

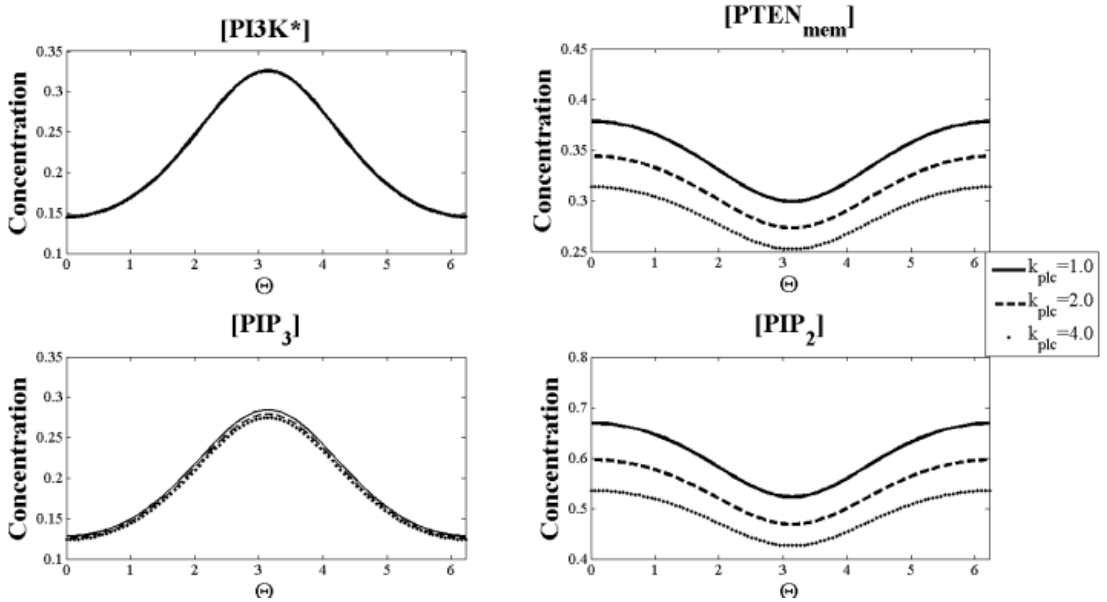
Results: Such receptor regulation of PI3K and PTEN can lead to a CR response. PLC plays a role to modulate that response. If no receptor regulation of PI3K, PTEN, then the regulation of PLC (alone) leads to a chemoattractive response!

Model variant 2

PI3K regulated in a feedforward manner by the receptor and PTEN by its binding to PIP2 (a double negative feedback)

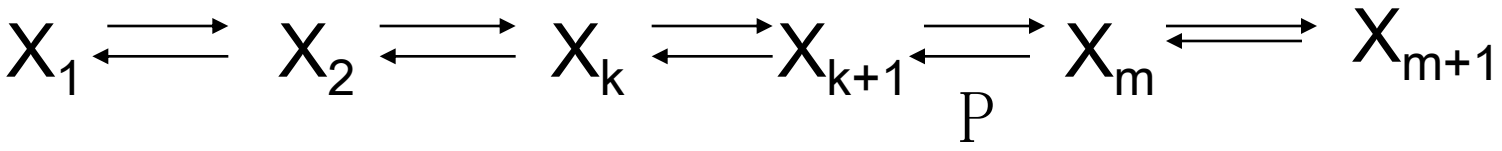
Variation of PLC activity





Results: Can get a chemrepulsive response (need the feedforward regulation of PI3K), but PIP3 response is extremely insensitive to PLC. Can be explained analytically, and crucially relies on PTEN binding to PIP2

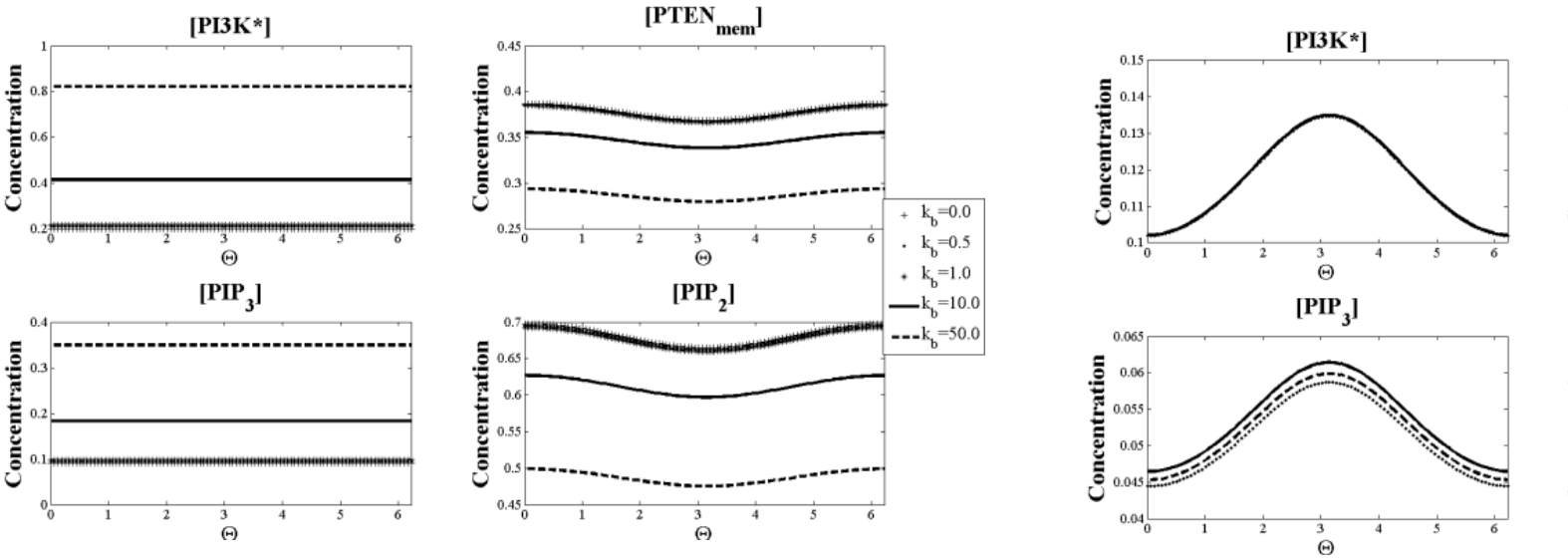
General effect of similar feedback in a chain of reversible reactions



Suppose P binds to X_k. Then X_m is insensitive to all enzyme signals to the left of X_k

Model variant 3

PTEN is regulated by its binding to PIP2 and PI3K locally by the receptor: the possible feedback from PIP3 to PI3K is also examined



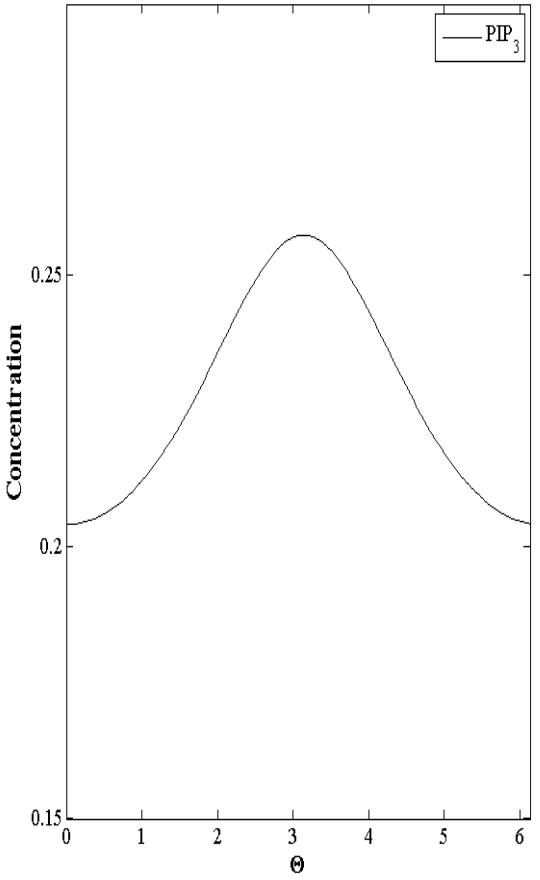
Results: A chemorepulsive response, where PLC regulation doesn't affect the PIP3 profile (as before). The feedback from PIP3 to PI3K plays a minor role in affecting the PIP3 profile

Is additional feedforward regulation necessary?

Possible role of enzymes acting at saturation:

Result: The key feature of the enzyme PI3K acting at saturation, along with the PTEN-PIP_2(4,5) feedback can generate repulsive biasing. Further enhanced by PLC acting at saturation

Alam-Nazki, Krishnan JTB (2010)



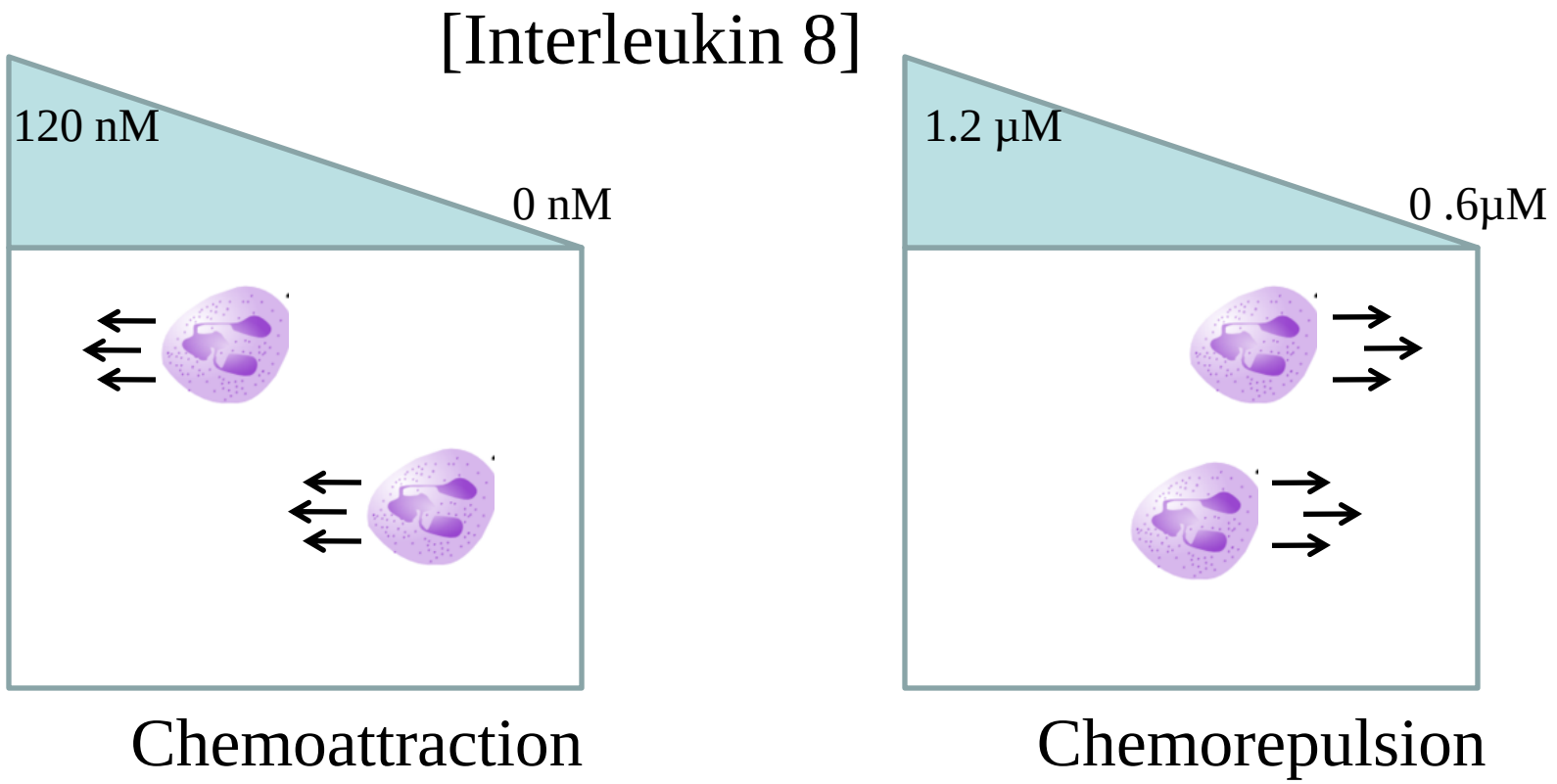
Various numerical results backed by analytical predictions

Attractive and repulsive biasing and their switching

- Does repulsive biasing result from a simple reversal of regulation of some key entity upstream of polarization pathways?
- Framework for understanding how gradient response may be switched:
- Switching due to changes in medium/alteration of pathways
- Switching due changes in the signal

Chemorepulsion in Neutrophils

Interleukin 8 signals to inflammation site[1]

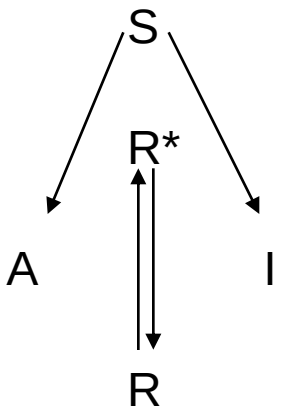


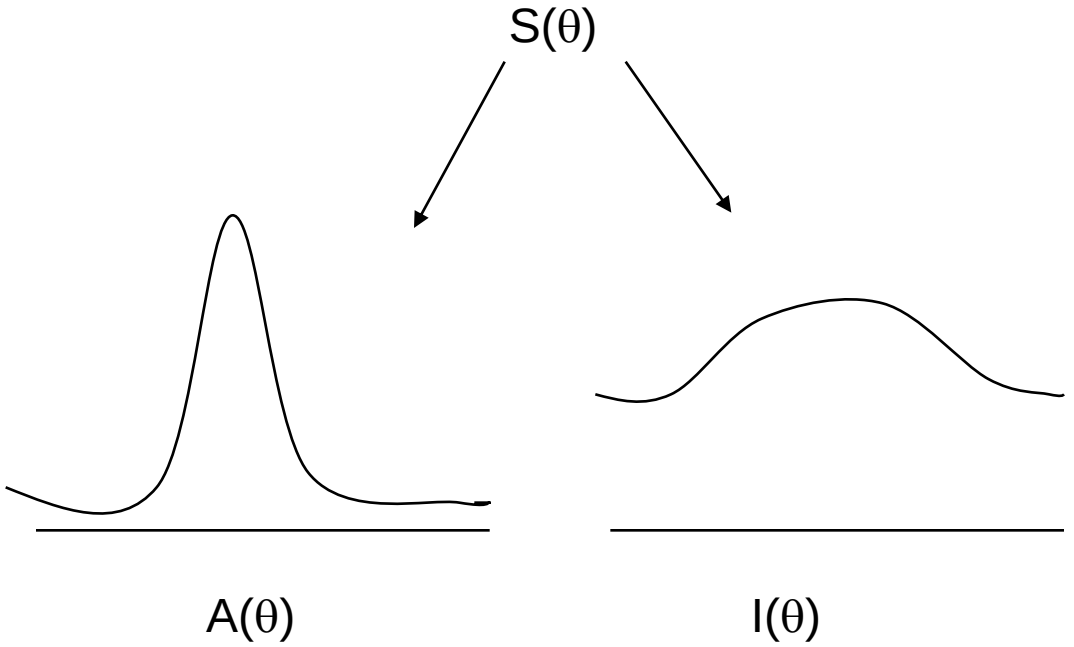
[1]Tharp et al., 2006, J. of Leukocyte Bio.

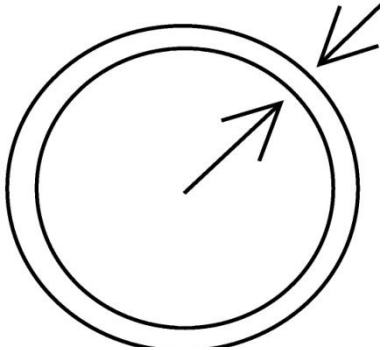
Conclusion

- Mechanistic modelling framework for investigating signalling from chemorepellent to regulators of motility
- Modelling involved construction of different variants
- Variants involved feedforward regulation of PLC by receptor but differed in whether feedforward or feedback mechanisms were involved in regulation of PI3K, PTEN; models built (and expanded) around network postulated (and studied in experiments)
- Distributed non-linear spatial system with potential multiple feedback
- Modelling predicts either than additional feedforward regulation is necessary, or else critical intermediate reaction acting close to saturation, along with PIP2-PTEN feedback to give biasing
- Can be accentuated by other feedback or feedforward effects

A feedforward module of adaptation and spatial signal transduction







$$dA/dt = k_a S - k_{-a} A + k_{da} \frac{\partial^2 A}{\partial \vartheta^2}$$

$$dI/dt = k_i S - k_{-i} I + k_{di} \frac{\partial^2 I}{\partial \vartheta^2}$$

$$dR^*/dt = k_f A(1-R^*) - k_r I R^*$$

Krishnan,JTB 2009

A generic feedforward adaptive module

Characterizing temporal, spatial and spatiotemporal behaviour

- How does adaptation affect signal processing?
- Transient increase if $k_{-a} > k_{-i}$, decrease otherwise

$$0 < R^* < (k_a/k_i)/ (k_r/k_f + k_a/k_i)$$

- Spatial response: positive bias towards signal if $k_{-a}/k_{da} > k_{-i}/k_{di}$, negative bias otherwise

Similar bias towards spatiotemporal signals if $k_{-a}+k_{da} < k_{-i} +k_{di}$

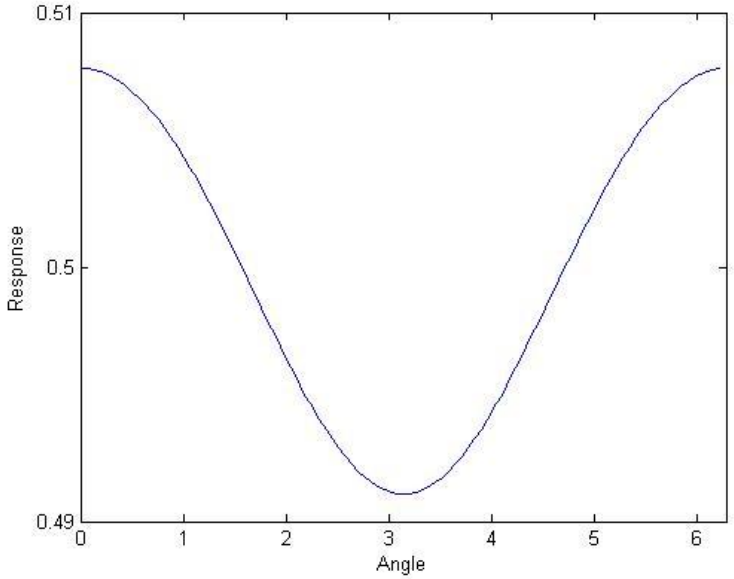
Various combinations of spatial temporal and spatiotemporal responses possible

Generalizeable insights

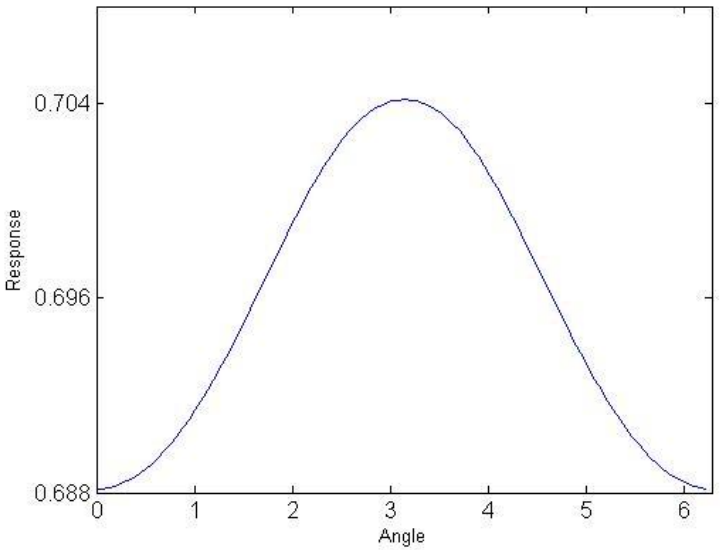
Dynamical systems, control

Plot showing transient reversal in biasing

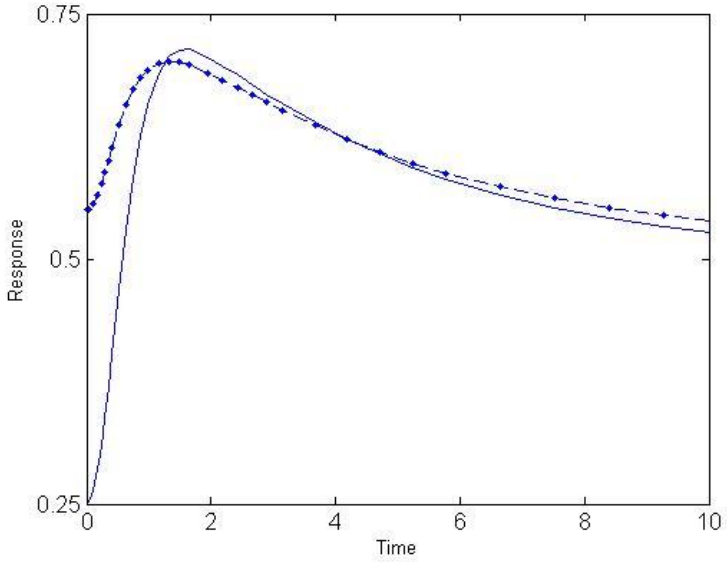
(a)



(b)

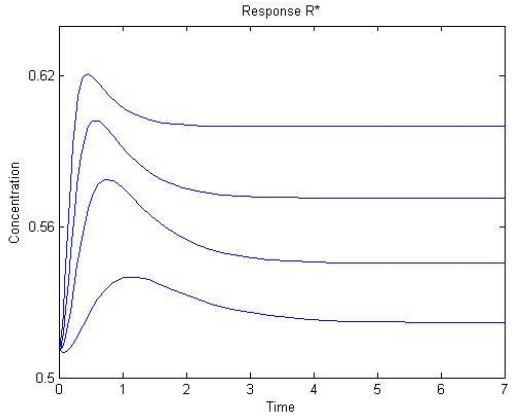


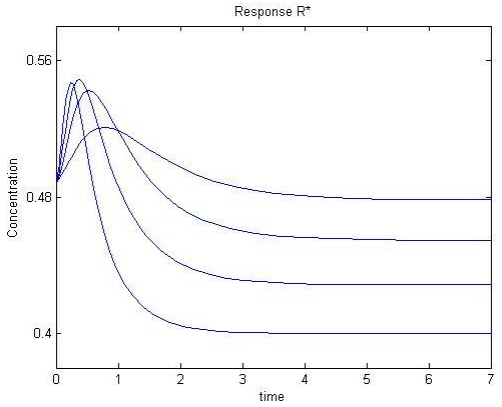
(c)



The role of saturating effects in feedforward adaptive regulation

- Conversion of exact adaptation to over/under-adaptation
- Linear → Bilinear control system
- Expands range of temporal regulation
- Possible to completely alter spatial behaviour
- Systematic study of activator/inhibitor limitation

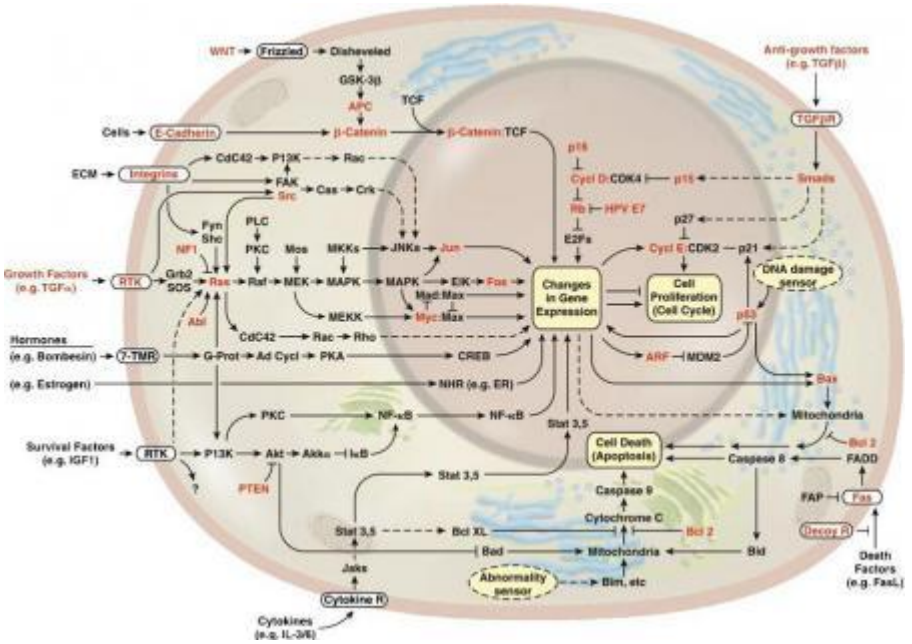




Krishnan, 2009, IET Syst. Bio (under revision)

Signal transduction property of interest	Module of adaptation without saturation	Module of adaptation with saturating effects
Steady state response to homogeneous input (i) $k_{-a} > k_{-i}$ (faster activator) (ii) $k_{-a} < k_{-i}$ (faster inhibitor)	Exact adaptation: Independent of parameters & signal size (i) Transient jump (ii) Transient dip	Inexact adaptation (i)Underadaptation if $A_{tot}/I_{tot} > k_a k_{-i}/k_i k_{-a}$ overadaptation if opposite inequality (ii) Underadaptation if $A_{tot}/I_{tot} < k_a k_{-i}/k_i k_{-a}$ overadaptation if opposite inequality
Range of dynamic signalling in homogeneous stimulus (i)$k_{-a} > k_{-i}$ (faster activator) (ii)$k_{-a} < k_{-i}$ (faster inhibitor)	(i) $0<R^*<k_a/k_i/(k_r/k_f+k_a/k_i)$ (ii) $1>R^*>k_a/k_i/(k_r/k_f+k_a/k_i)$	(i) If $A_{tot}/I_{tot} < k_a/k_i$, $0< R^* < k_a/k_i/(k_r/k_f + k_a/k_i)$ otherwise $0< R^*< A_{tot}/I_{tot}/(k_r/k_f+A_{tot}/I_{tot})$ (ii) If $A_{tot}/I_{tot} > k_a/k_i$, $1 > R^* > k_a/k_i/(k_r/k_f + k_a/k_i)$ otherwise $1> R^* > A_{tot}/I_{tot}/(k_r/k_f+A_{tot}/I_{tot})$
Response in a static linear gradient of mean value S_0	Attractive biasing if $k_{da}/k_{-a} < k_{di}/k_{-i}$ Repulsive biasing if $k_{da}/k_{-a} > k_{di}/k_{-i}$	Attractive biasing in a weak gradient if $k_{da}/k_{-a} + k_a S_0/k_{-a}A_{tot} < k_{di}/k_{-i} + k_i S_0/k_{-i} I_{tot}$ Repulsive biasing if $k_{da}/k_{-a} + k_a S_0/k_{-a}A_{tot} > k_{di}/k_{-i} + k_i S_0/k_{-i} I_{tot}$

- Signal transduction: how networks of proteins process signals and give rise to particular biological processes
- Modelling
- Systems approaches
- Dynamics and control



Dynamics is crucial!

- Are the processes interesting and important?
- Potential applications
- Are they suitable for modelling?