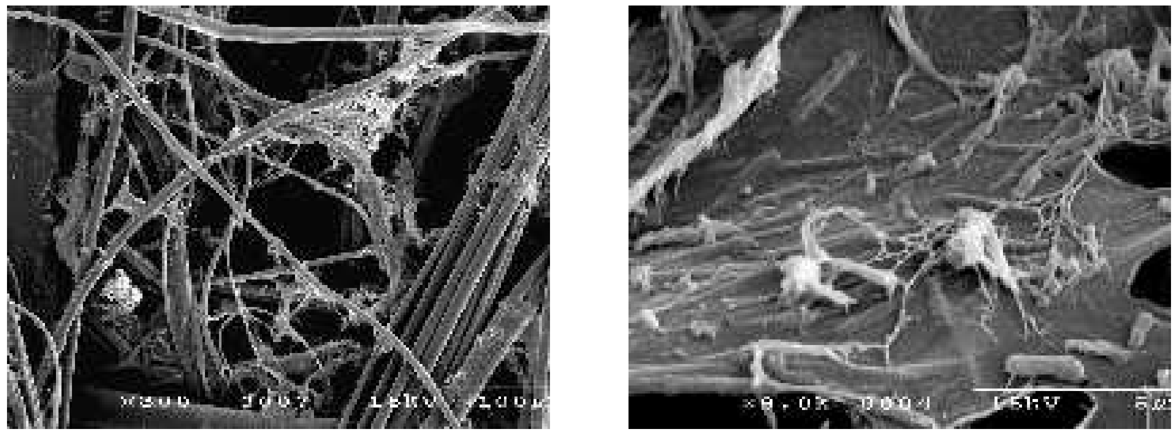
The Dynamics of Growing Biofilm

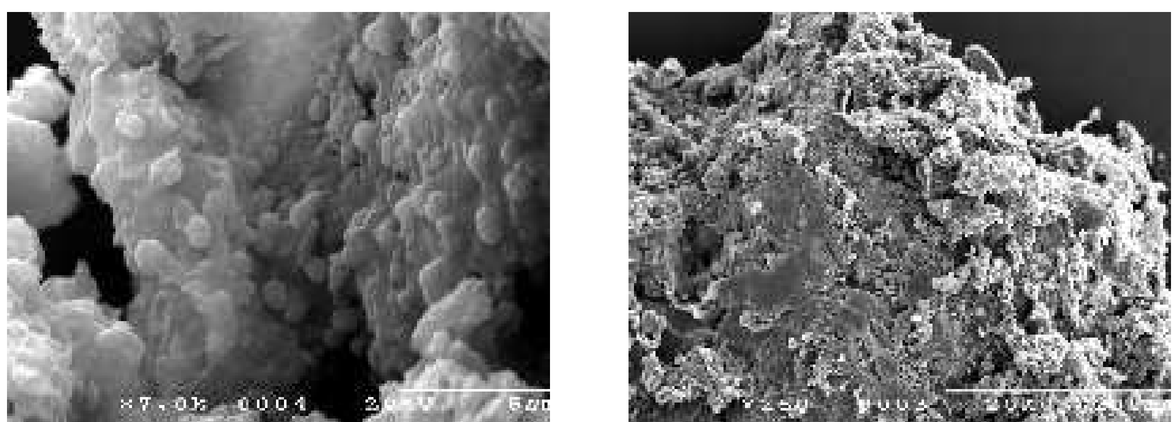
J. P. Keener

Department of Mathematics
University of Utah

Biofilms



biofilm fouling of filter fibers



Placque on teeth

Some Interesting Questions

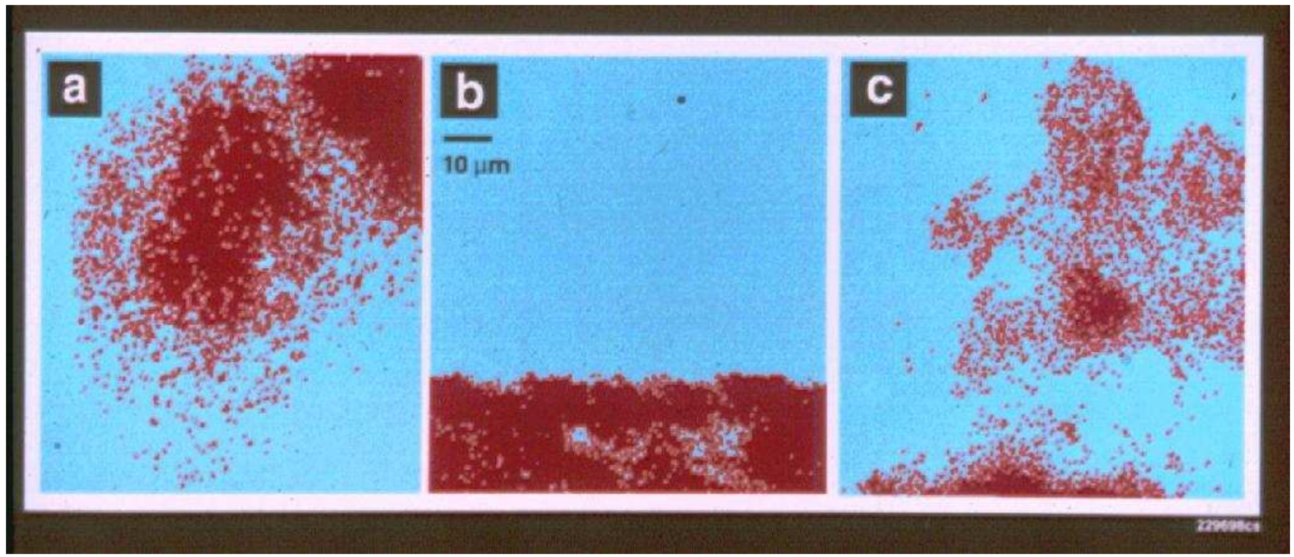
How do gels grow?

- *P. aeruginisa* (on catheters, IV tubes, etc.)
- Mucus secretion (bronchial tubes, stomach lining)
- Colloidal suspensions, cancer cells
- Gel morphology (the shape of sponges)

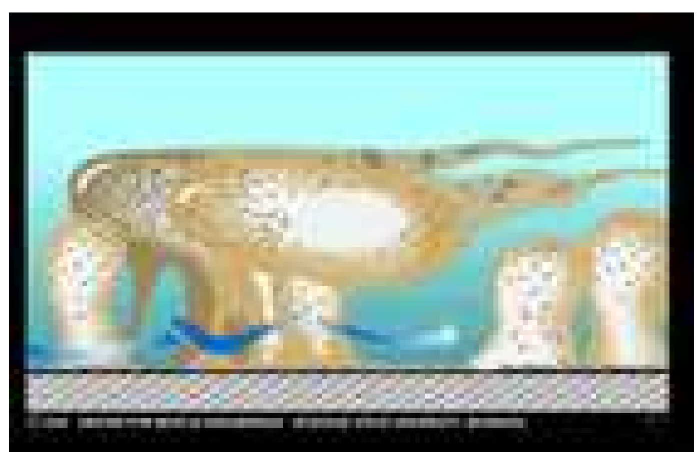
Why are gels important?

- Protective capability
- Friction reduction
- High viscosity (low washout rate) for drugs
- Acid protection

Biofilm Formation in *P. Aeruginosa*



Wild Type Biofilm Mutant Mutant with autoinducer



Dynamics of Growing Biogels

I: Quorum sensing:

- What is it?
- How does it work?

II: Heterogeneous structures

- How do cells use polymer gel for locomotion?
- What are the mechanisms of pattern formation?

I: Quorum Sensing in P. *aeruginosa*

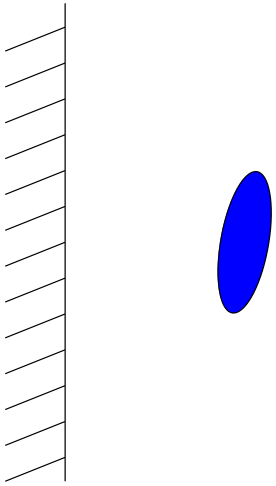
Quorum sensing: The ability of a bacterial colony to sense its size and regulate its activity in response.

Examples: *Vibrio fisheri*, *P. aeruginosa*

P. Aeruginosa:

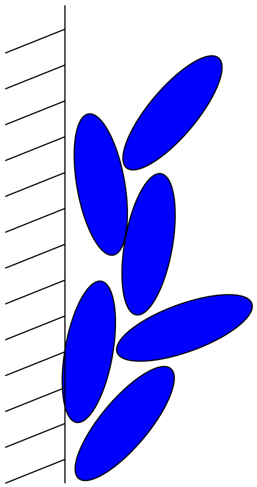
- Major cause of hospital infection in the US.
- Major cause of death in intubated Cystic Fibrosis patients
- In planktonic form, they are non-toxic, but in biofilm they are highly toxic and well-protected by the polymer gel in which they reside. However, they do not become toxic until the colony is of sufficient size, i.e., quorum sensing.

Stages of Growth



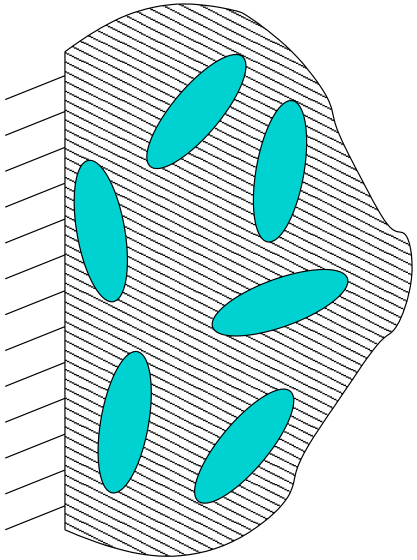
Planktonic

Stages of Growth



Small Dense Colony

Stages of Growth



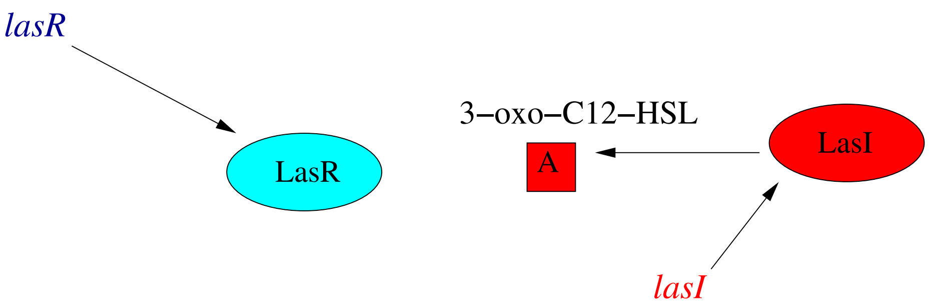
Biofilm Colony

Biochemistry of Quorum Sensing

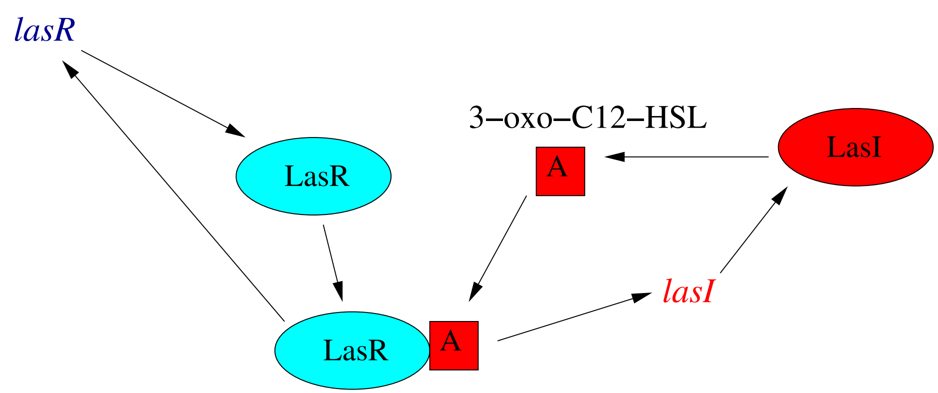
lasR

lasI

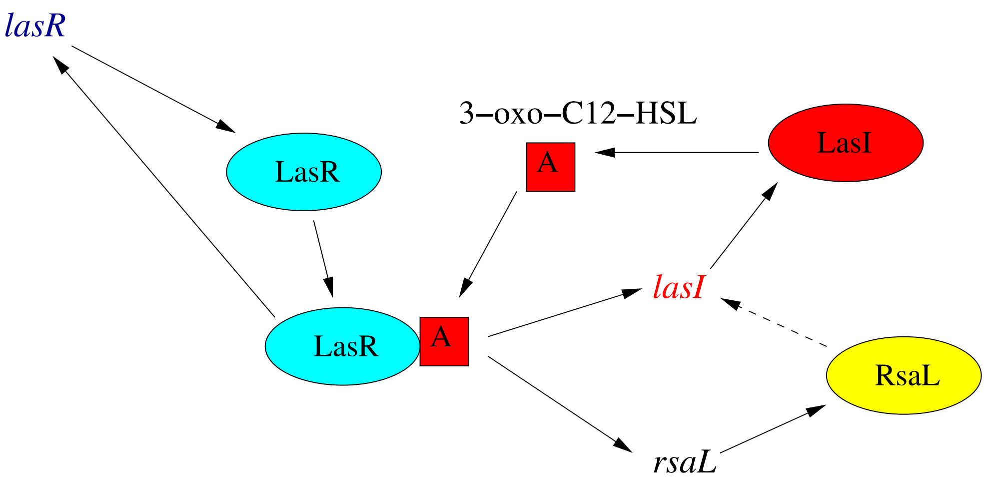
Biochemistry of Quorum Sensing



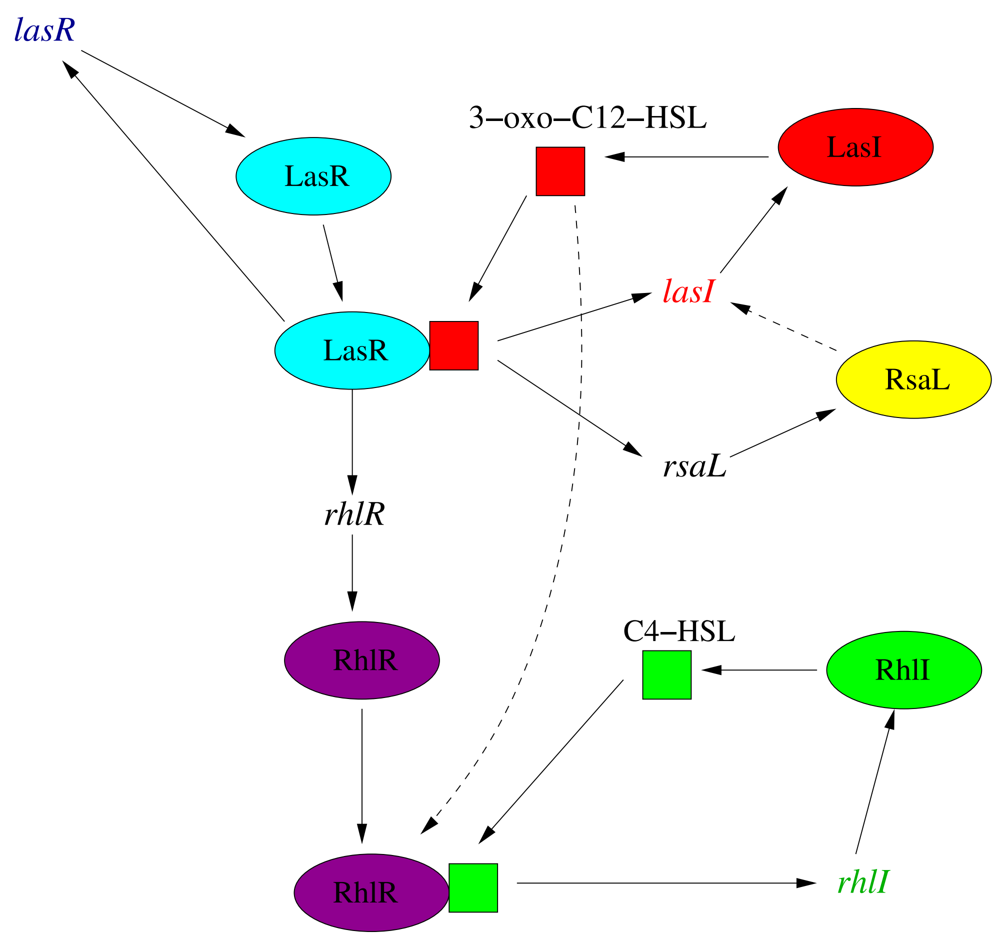
Biochemistry of Quorum Sensing



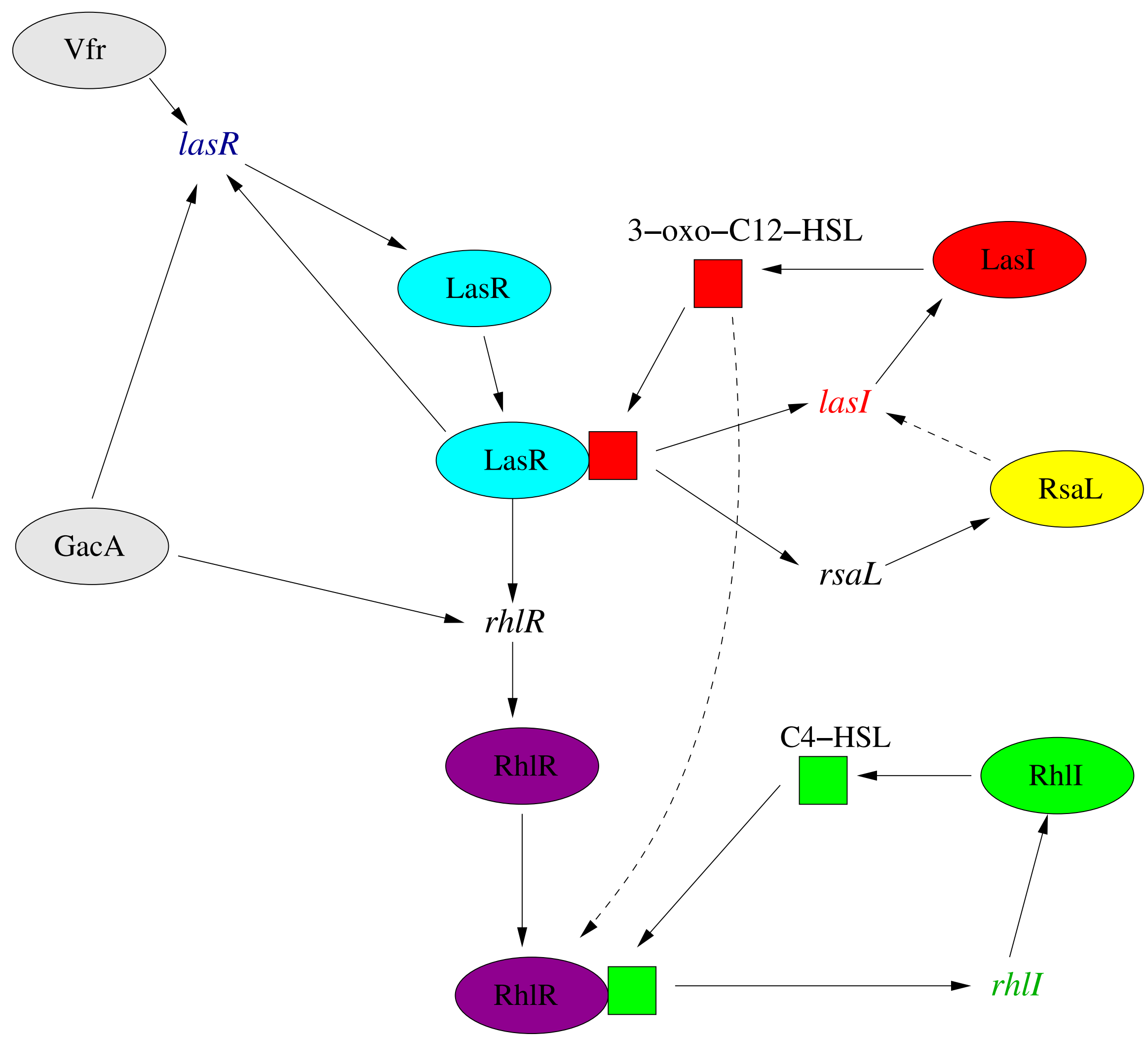
Biochemistry of Quorum Sensing



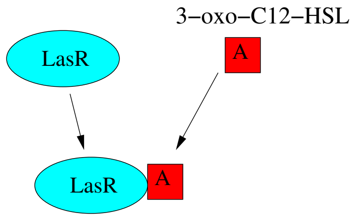
Biochemistry of Quorum Sensing



Biochemistry of Quorum Sensing



Modeling Biochemical Reactions

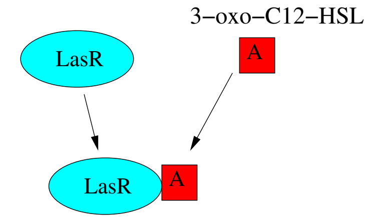


Bimolecular reaction $A + R \longleftrightarrow P$

$$\frac{dP}{dt} = k_+ AR - k_- P$$

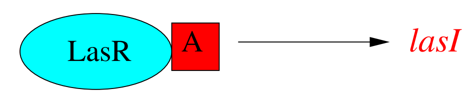
Modeling Biochemical Reactions

Bimolecular reaction $A + R \longleftrightarrow P$



$$\frac{dP}{dt} = k_+ AR - k_- P$$

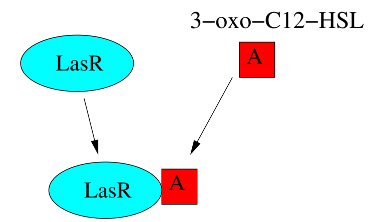
Production of mRNA $\xrightarrow{P} l$



$$\frac{dl}{dt} = \frac{V_{max} P}{K_l + P} - k_{-l} l$$

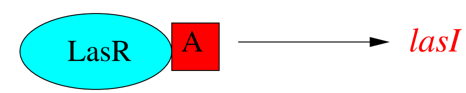
Modeling Biochemical Reactions

Bimolecular reaction $A + R \longleftrightarrow P$



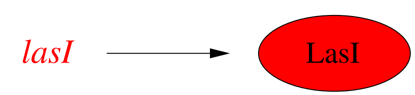
$$\frac{dP}{dt} = k_+ AR - k_- P$$

Production of mRNA $\xrightarrow{P} l$



$$\frac{dl}{dt} = \frac{V_{max} P}{K_l + P} - k_{-l} l$$

Enzyme production $l \rightarrow L$



$$\frac{dL}{dt} = k_l l - K_L L$$

Full system of ODE's

$$\frac{dP}{dt} = k_{RA}RA - k_P P$$

$$\frac{dR}{dt} = -k_{RA}RA + k_P P - k_R R + k_1 r,$$

$$\frac{dA}{dt} = -k_{RA}RA + k_P P + k_2 L - k_A A,$$

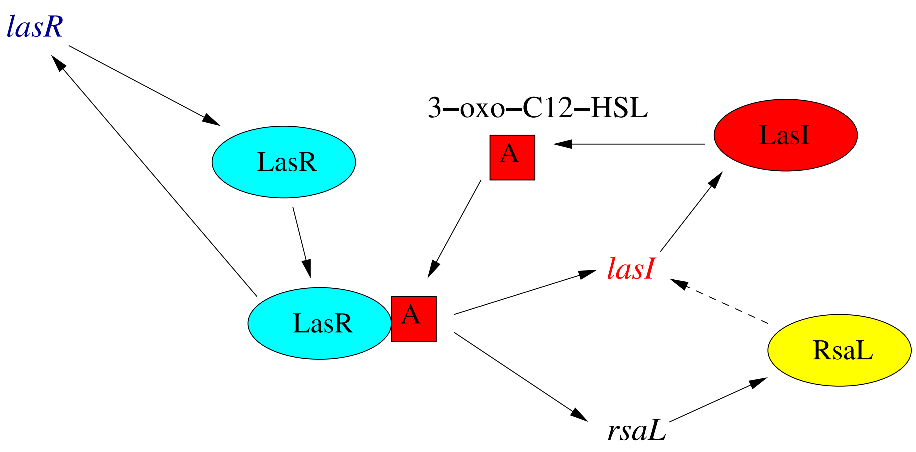
$$\frac{dL}{dt} = k_3 l - k_l L,$$

$$\frac{dS}{dt} = k_4 s - k_S S,$$

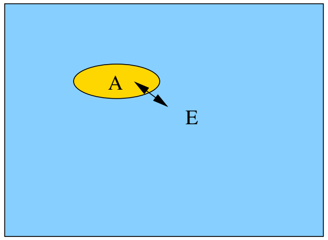
$$\frac{ds}{dt} = V_s \frac{P}{K_S + P} - k_s s,$$

$$\frac{dr}{dt} = V_r \frac{P}{K_r + P} - k_r r + r_0,$$

$$\frac{dl}{dt} = V_l \frac{P}{K_l + P} \frac{1}{K_S + S} - k_l l + l_0$$



Diffusion



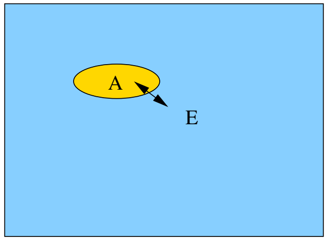
$$\frac{dA}{dt} = F(A,R,P) + \delta(E - A)$$

$$\frac{dE}{dt} = - k_E E + \delta(A - E)$$

Imagine the Possibilities

Mathematical Biology
University of Utah

Diffusion

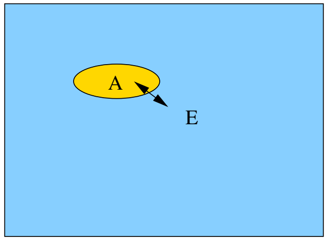


$$\boxed{\frac{dA}{dt}} = F(A, R, P) + \delta(E - A)$$

$$\boxed{\frac{dE}{dt}} = - k_E E + \delta(A - E)$$

rate of change,

Diffusion

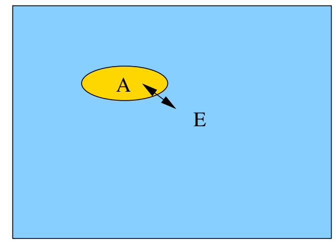


$$\frac{dA}{dt} = \boxed{F(A,R,P)} + \delta(E-A)$$

$$\frac{dE}{dt} = -\boxed{k_E E} + \delta(A-E)$$

rate of change, production or degradation rate,

Diffusion

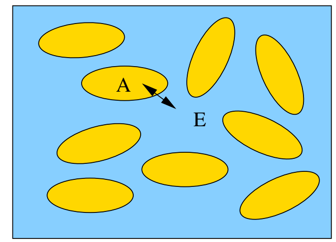


$$\frac{dA}{dt} = F(A,R,P) + \boxed{\delta(E-A)}$$

$$\frac{dE}{dt} = -k_E E + \boxed{\delta(A-E)}$$

rate of change, production or degradation rate, diffusive exchange,

Diffusion



$$\frac{dA}{dt} = F(A,R,P) + \delta(E-A)$$

$$\boxed{(1-\rho)}\left(\frac{dE}{dt} + K_E E\right) = \boxed{\rho}\,\delta(A-E)$$

rate of change, production or degradation rate, diffusive exchange, density dependence.

Model Reduction

Two (possible) ways to proceed:

- Numerical simulation (but few of the 22 kinetic parameters are known),
- Qualitative analysis (QSS reduction)

Imagine the Possibilities

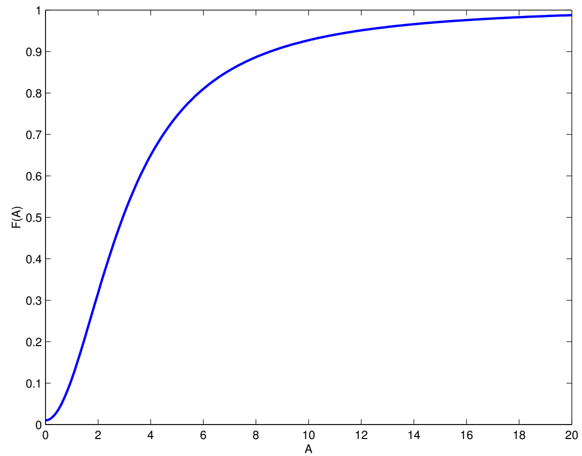
Mathematical Biology
University of Utah

Model Reduction

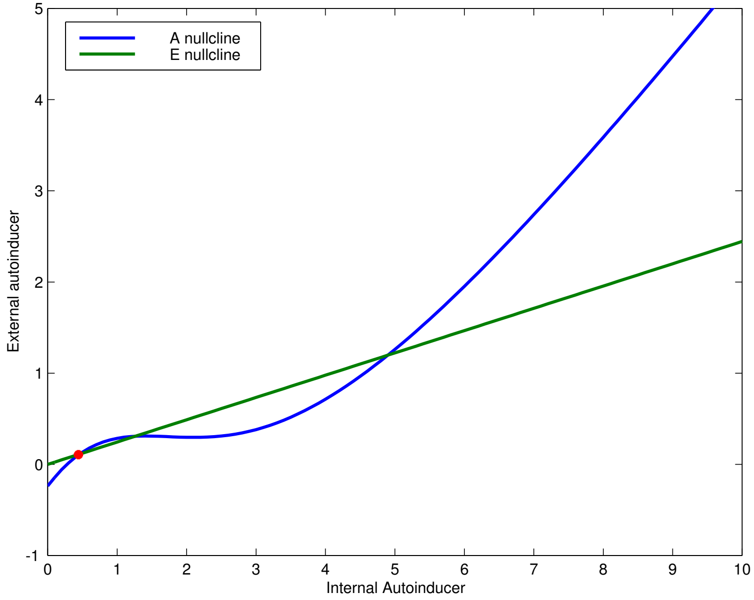
Two (possible) ways to proceed:

- Numerical simulation (but few of the 22 kinetic parameters are known),
- Qualitative analysis (QSS reduction)

$$\frac{dA}{dt} = F(A) + \delta(E - A), \qquad (1-\rho)\left(\frac{dE}{dt} + k_E E\right) = \rho\delta(A - E)$$



Two Variable Phase Portrait



A PDE Model

Suppose cells are immobile, so internal variables do not diffuse, but extracellular autoinducer E diffuses

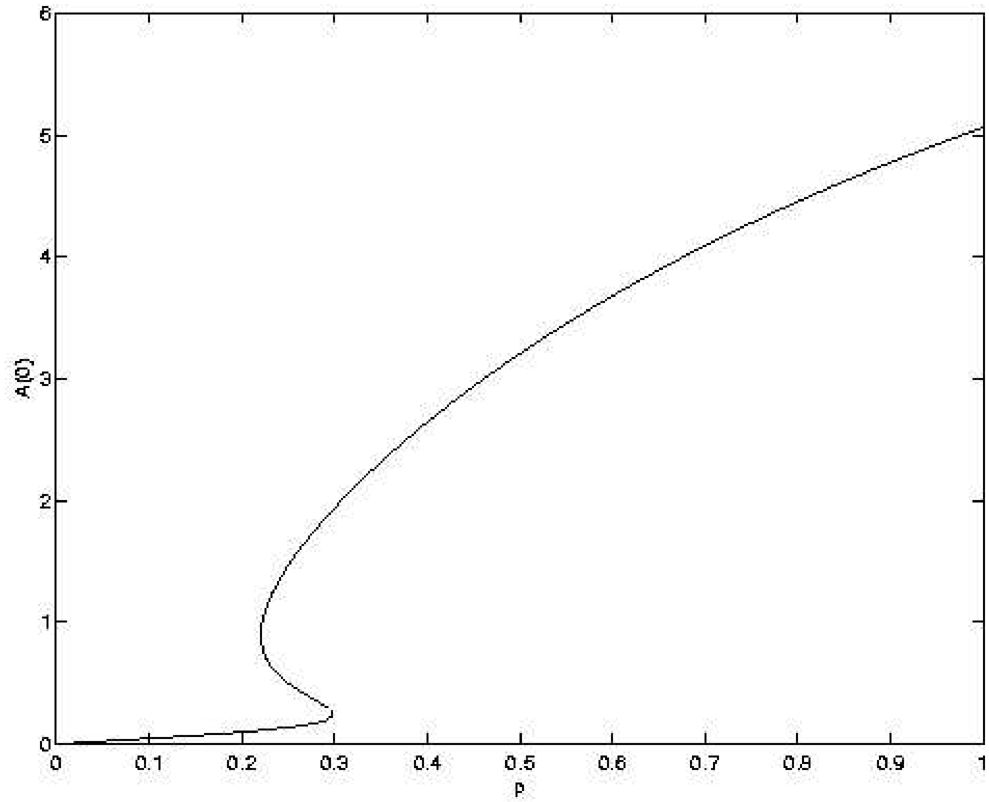
$$\begin{aligned} \frac{\partial A}{\partial t} &= F(A,U) + \delta(E - A), \\ \frac{\partial U}{\partial t} &= G(A,U), U \in R^7, \\ \frac{\partial E}{\partial t} &= \nabla \cdot (D_E \nabla E) - k_E E + \frac{\rho}{1-\rho}\delta(A - E) \end{aligned}$$

in Ω with Robin boundary conditions

$$n \cdot D_e \nabla E + \alpha E = 0$$

on $\partial\Omega$.

Autoinducer as function of cell density



A Hydrogel Primer

- What is a hyrogel?
  A tangled polymer network in solvent.
- Examples of biological hydrogels
  Micellar gels
  Jello (a collagen gel $\approx 97\%$ water)
  Extracellular matrix
  Blood clots
  Mucin - lining the stomach, bronchial tubes, intestines
  Glycocalyx
  Sinus secretions

A Hydrogel Primer - II

Functions of a biological hydrogel

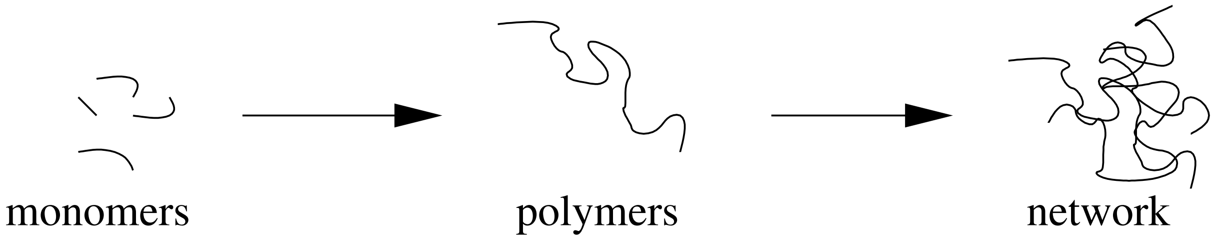
- Decreased permeability to large molecules
- Structural strength (for cell walls)
- Capture and clearance of foreign substances
- Decreased resistance to sliding/gliding
- High internal viscosity (low washout)

Important features of gels

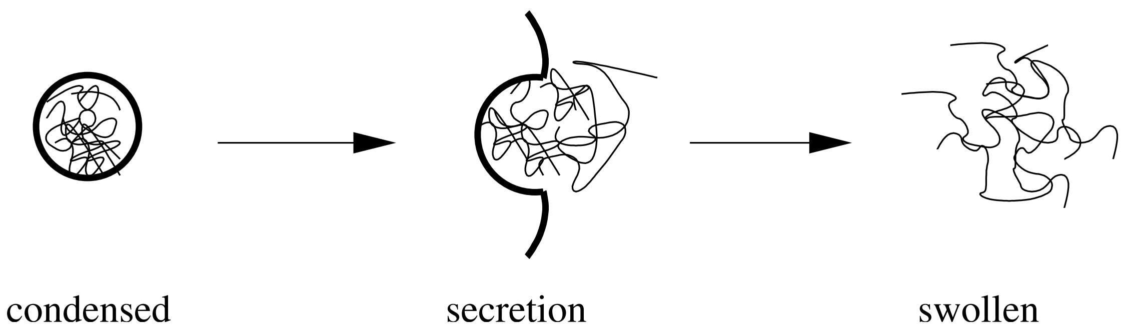
- Usually comprised of highly polyionic polymers
- Can undergo volumetric phase transitions in response to ionic concentrations, temperature, etc.
- Volume is determined by combination of forces (entropic, electrostatic, hydrophobic, cross-linking, etc).

How gels grow

- Polymerization/deposition



- Secretion



Modelling Biofilm Growth

A two phase material with polymer network volume fraction θ

$$\frac{\partial\theta}{\partial t} + \nabla\cdot(V_n\theta) = g_n$$ Network Phase (EPS)

$$\frac{\partial\theta_s}{\partial t} + \nabla\cdot(V_s\theta_s) = 0$$ Solute Phase

$$\frac{\partial b}{\partial t} + \nabla\cdot(V_n b) = g_b$$ Bacterial concentration

$$\frac{\partial\theta_s u}{\partial t} + \nabla\cdot(\theta_s(V_s u - D_u\nabla u) = g_u$$ Resource Concentration

where $\theta + \theta_s = 1$,

Modelling Biofilm Growth

A two phase material with polymer network volume fraction θ

$$\frac{\partial \theta}{\partial t} + \nabla \cdot (V_n \theta) = g_n$$ Network Phase (EPS)

$$\frac{\partial \theta_s}{\partial t} + \nabla \cdot (V_s \theta_s) = 0$$ Solute Phase

$$\frac{\partial b}{\partial t} + \nabla \cdot (V_n b) = g_b$$ Bacterial concentration

$$\frac{\partial \theta_s u}{\partial t} + \nabla \cdot (\theta_s (V_s u - D_u \nabla u) = g_u$$ Resource Concentration

where $\theta + \theta_s = 1$, solute volume fraction,

Modelling Biofilm Growth

A two phase material with polymer network volume fraction θ

$$\frac{\partial \theta}{\partial t} + \nabla \cdot (V_n \theta) = g_n$$ Network Phase (EPS)

$$\frac{\partial \theta_s}{\partial t} + \nabla \cdot (V_s \theta_s) = 0$$ Solute Phase

$$\frac{\partial b}{\partial t} + \nabla \cdot (V_n b) = g_b$$ Bacterial concentration

$$\frac{\partial \theta_s u}{\partial t} + \nabla \cdot (\theta_s (V_s u - D_u \nabla u) = g_u$$ Resource Concentration

where $\theta + \theta_s = 1$, solute volume fraction, network velocity,

Modelling Biofilm Growth

A two phase material with polymer network volume fraction θ

$$\frac{\partial \theta}{\partial t} + \nabla \cdot (V_n \theta) = g_n$$ Network Phase (EPS)

$$\frac{\partial \theta_s}{\partial t} + \nabla \cdot (V_s \theta_s) = 0$$ Solute Phase

$$\frac{\partial b}{\partial t} + \nabla \cdot (V_n b) = g_b$$ Bacterial concentration

$$\frac{\partial \theta_s u}{\partial t} + \nabla \cdot (\theta_s (V_s u - D_u \nabla u) = g_u$$ Resource Concentration

where $\theta + \theta_s = 1$, solute volume fraction, network velocity, solute velocity,

Modelling Biofilm Growth

A two phase material with polymer network volume fraction θ

$$\frac{\partial\theta}{\partial t} + \nabla\cdot(V_n\theta) = g_n + \epsilon\nabla^2\theta$$ Network Phase (EPS)

$$\frac{\partial\theta_s}{\partial t} + \nabla\cdot(V_s\theta_s) = 0$$ Solute Phase

$$\frac{\partial b}{\partial t} + \nabla\cdot(V_n b) = g_b$$ Bacterial concentration

$$\frac{\partial\theta_s u}{\partial t} + \nabla\cdot(\theta_s(V_s u - D_u\nabla u) = g_u$$ Resource Concentration

where $\theta + \theta_s = 1$, solute volume fraction, network velocity, solute velocity, artificial network diffusion.

Imagine the Possibilities
Mathematical Biology
University of Utah

Force Balance

Solute Phase (an inviscid fluid)

$$\boxed{h_f\theta\theta_s(V_n - V_s)} - \theta_s\nabla p = 0,$$

solute-network friction

Force Balance

Solute Phase (an inviscid fluid)

$$h_f \theta \theta_s (V_n - V_s) - \boxed{\theta_s \nabla p} = 0,$$

solute-network friction pressure

Force Balance

Solute Phase (an inviscid fluid)

$$h_f \theta \theta_s (V_n - V_s) \; - \; \theta_s \nabla p \; = 0,$$

solute-network friction   pressure

Network Phase (a viscoelastic material)

$$\eta \nabla(\theta(\nabla V_n + \nabla V_n^T)) - \; h_f \theta \theta_s (V_n - V_s) \; - \; \nabla \psi(\theta) \; - \; \theta \nabla p \; = 0$$

network viscosity

Force Balance

Solute Phase (an inviscid fluid)

$$h_f \theta \theta_s (V_n - V_s) \; - \; \theta_s \nabla p \; = 0,$$

solute-network friction pressure

Network Phase (a viscoelastic material)

$$\eta \nabla (\theta (\nabla V_n + \nabla V_n^T)) \; - \boxed{h_f \theta \theta_s (V_n - V_s)} - \; \nabla \psi(\theta) \; - \; \theta \nabla p \; = 0$$

network viscosity solute-network friction

Force Balance

Solute Phase (an inviscid fluid)

$$h_f \theta \theta_s (V_n - V_s) \; - \; \theta_s \nabla p \; = 0,$$

solute-network friction pressure

Network Phase (a viscoelastic material)

$$\eta \nabla (\theta (\nabla V_n + \nabla V_n^T)) \; - \; h_f \theta \theta_s (V_n - V_s) \; - \; \boxed{\nabla \psi(\theta)} \; - \; \theta \nabla p \; = 0$$

network viscosity solute-network viscosity osmosis

Force Balance

Solute Phase (an inviscid fluid)

$$h_f \theta \theta_s (V_n - V_s) \; - \; \theta_s \nabla p \; = 0,$$

solute-network friction pressure

Network Phase (a viscoelastic material)

$$\eta \nabla (\theta (\nabla V_n + \nabla V_n^T)) \; - \; h_f \theta \theta_s (V_n - V_s) \; - \; \nabla \psi(\theta) \; - \; \boxed{\theta \nabla p} = 0$$

network viscosity solute-network viscosity osmosis pressure

Force Balance

Solute Phase (an inviscid fluid)

$$h_f \theta \theta_s (V_n - V_s) \; - \; \theta_s \nabla p \; = 0,$$

solute-network friction pressure

Network Phase (a viscoelastic material)

$$\eta \nabla (\theta (\nabla V_n + \nabla V_n^T)) \; - \; h_f \theta \theta_s (V_n - V_s) \; - \; \nabla \psi(\theta) \; - \; \theta \nabla p \; = 0$$

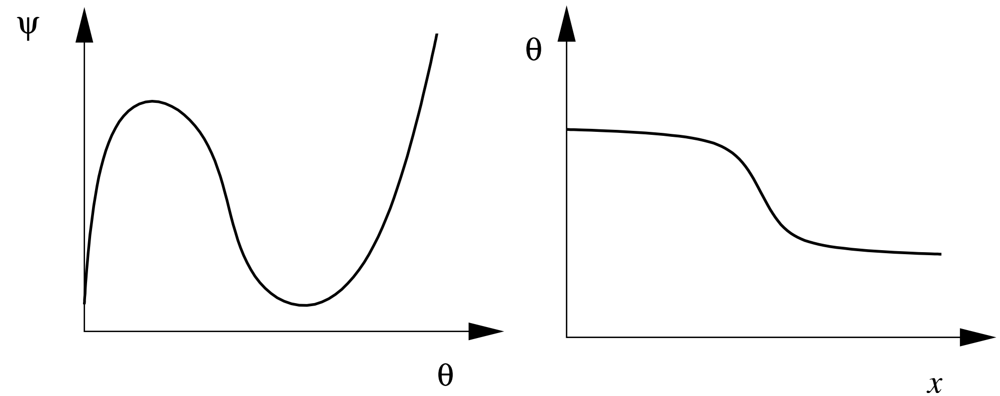
network viscosity solute-network viscosity osmosis pressure

Imcompressibility

$$\nabla \cdot (\theta V_n + \theta_s V_s) = g_n$$

Osmotic Pressure

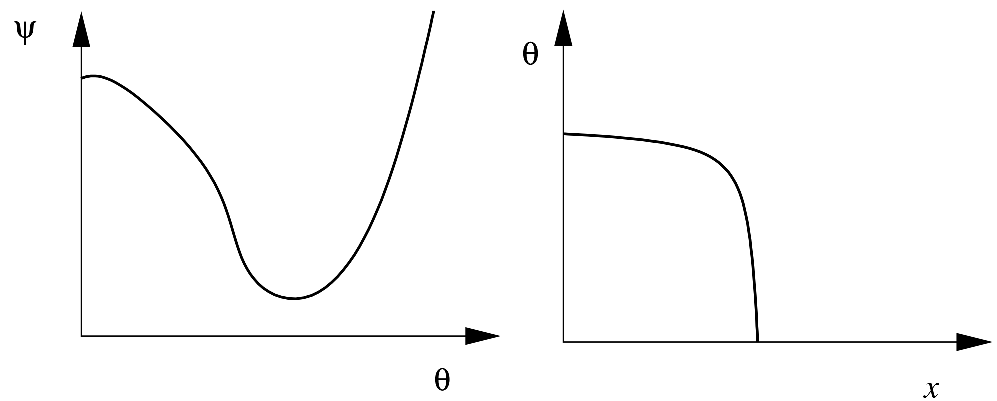
What is the meaning of the term $-\nabla\psi(\theta)$?



$\psi'(\theta) > 0$ gives expansion (swelling)
$\psi'(\theta) < 0$ gives contraction (deswelling)

Osmotic Pressure

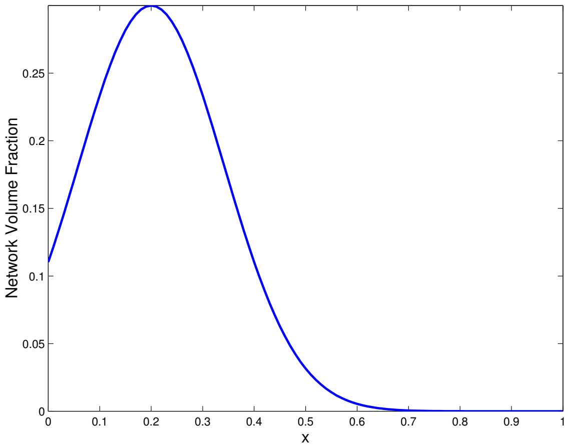
What is the meaning of the term $-\nabla\psi(\theta)$?



$\psi'(\theta) > 0$ gives expansion (swelling)
$\psi'(\theta) < 0$ gives contraction (deswelling)

To maintain an edge, $\psi(\theta)$ must be of the form $\psi(\theta) = \theta^2 F(\theta)$

Movement by Swelling

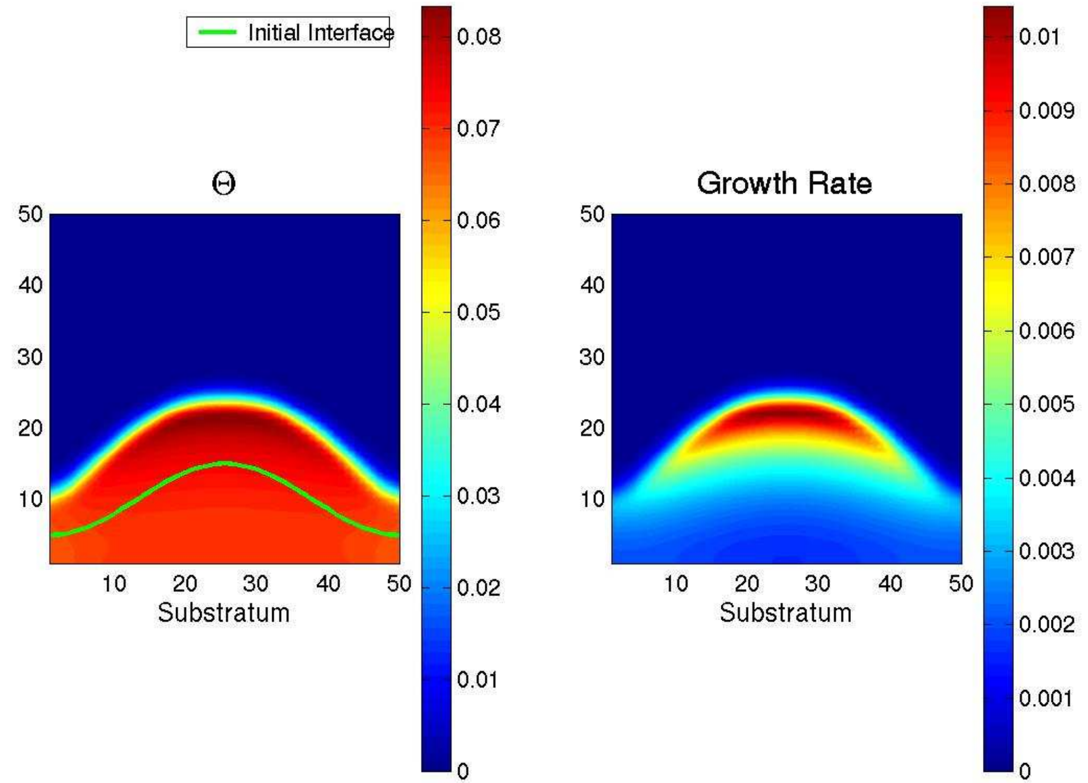


Movement by Swelling

WT Biofilm Growth

Mutant Cell Growth

Fingering Instability



"Nutrient Poor" Fingering Instability

Channeling

Modified Network Model: Include elastic strains, $\sigma_n = \gamma\epsilon$

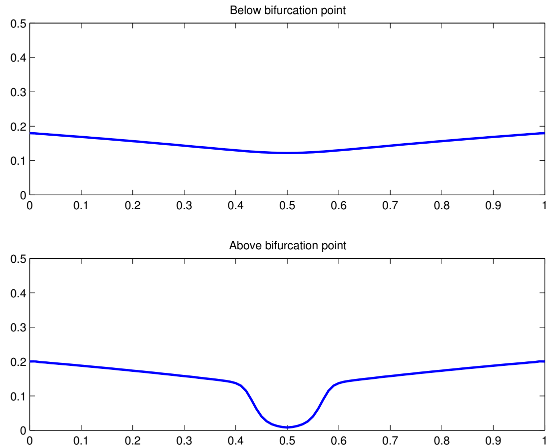
$$\eta\nabla(\theta(\nabla V_n + \nabla V_n^T)) + \boxed{\nabla \cdot (\gamma\theta\epsilon)} - h_f\theta\theta_s(V_n - V_s) - \nabla\psi(\theta) - \theta\nabla p = 0$$

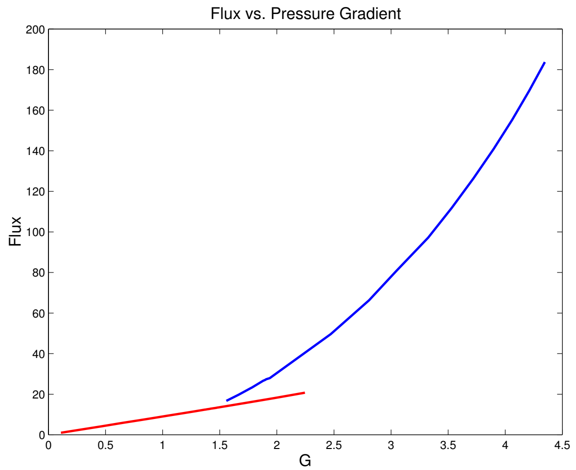
and displacements D

$$\frac{\partial D}{\partial t} + \nabla \cdot (V_n D) = V_n$$

where ϵ is the Cauchy-Green strain tensor.

Channel Formation





The "Moses Bifurcation"

Remark: The existence of this channeling "Moses Bifurcation" can be established using singular perturbation arguments.

Summary

- Quorum sensing is via a hysteretic switch involving diffusible autoinducer
- Fingering and mushrooming may be driven by a substrate deficiency-fingering instability.
- Channeling may be driven by a gel-osmosis "Moses Bifurcation".

Acknowledgments

Collaborators

- Jack Dockery, Montana State University
- Nick Cogan, Tulane University

Notes

- Funding provided by a grant from the NSF.
- This talk can be viewed at http://www.math.utah.edu/~keener/lectures/biofilmdynamics
- No Microsoft products were used or harmed during the production of this talk.

The End

Structure of the "Moses Bifurcation"

The steady state equation is

$$\epsilon\theta\frac{d}{dy}\left(\frac{\frac{d\theta}{dy}}{\theta}\right)+\frac{1}{\theta}H(\theta,y)=k$$

subject to

$$\int_0^1 \theta dy=\hat{\theta}$$

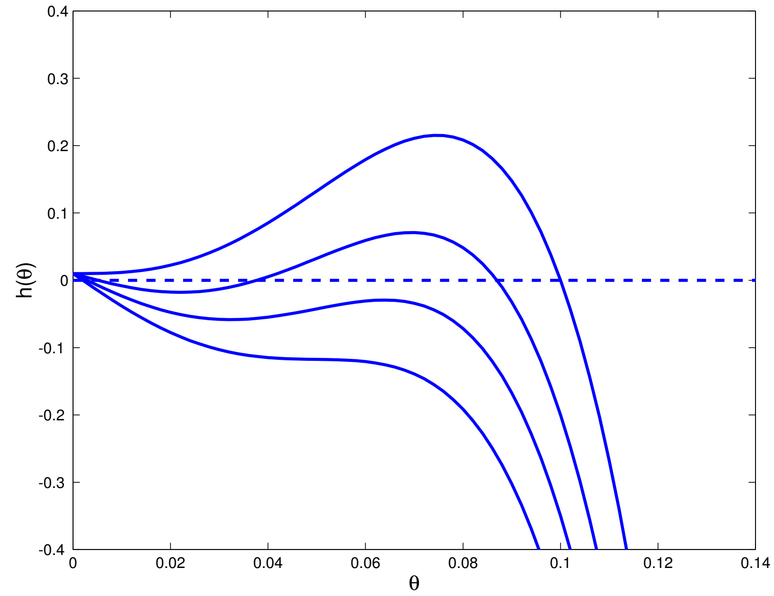
where

$$H(y,\theta)=G^2(y-\frac{1}{2})^2-\theta\Psi(\theta)+\hat{\theta}^2-\theta^2, \Psi(\theta)=\kappa\theta^2(\theta-\theta_{ref})$$

Singular Perturbation Analysis

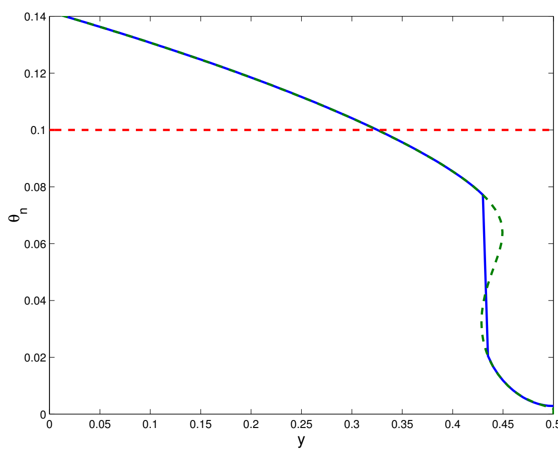
This is a singular perturbation problem.

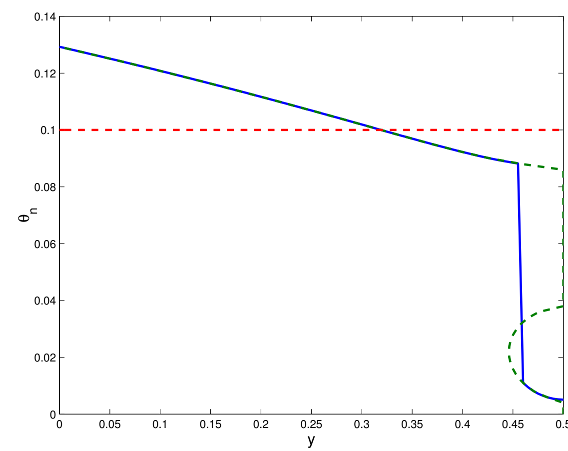
For $\epsilon = 0$ (the "outer solution"), we must solve an algebraic equation for θ as a function of y. However, the equation $H(\theta, y) = k\theta$ has (possibly) multiple solutions.



Boundary Layer Analysis

The governing equation is a "bistable equation", so transition layers can be inserted at certain locations.





It is possible that boundary layer solutions coexist with non-boundary solutions, as is seen in the bifurcation diagram.

(Go back)