Modelling the Spatio-Temporal Dynamics of Nuclear Proteins

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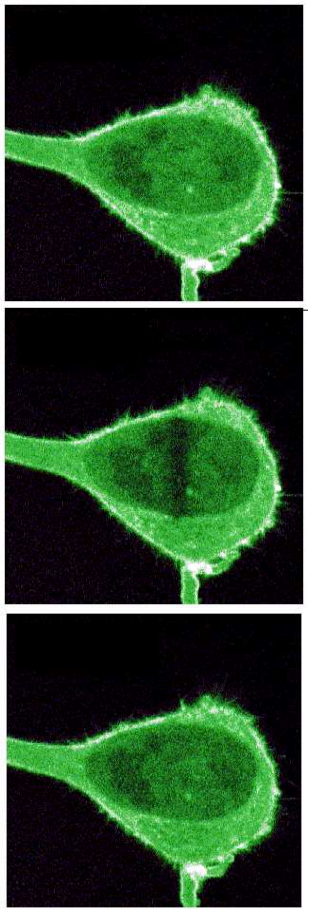
Overview of Presentation

- Modelling problem 1: Mobility of nuclear proteins

- Modelling problem 2: Spatial organization of nuclear proteins

FRAP:
Fluorescence Recovery After Photobleaching

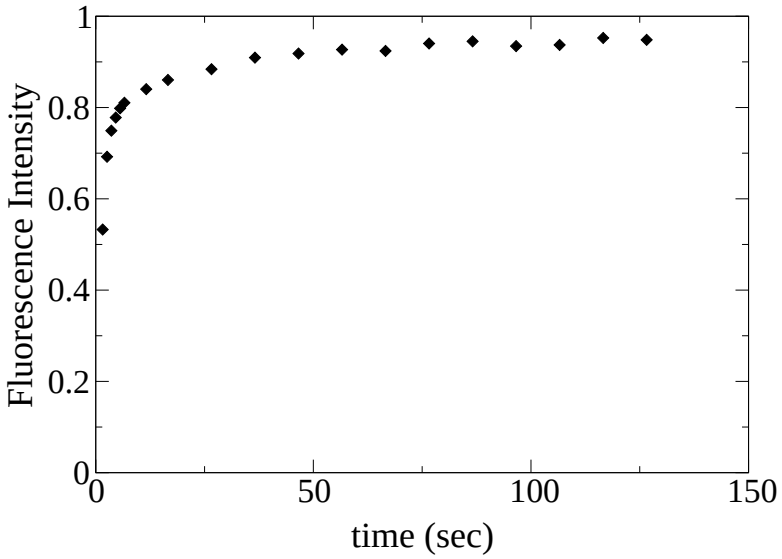


Proteins of interest are tagged with Green Fluorescence Protein (GFP).

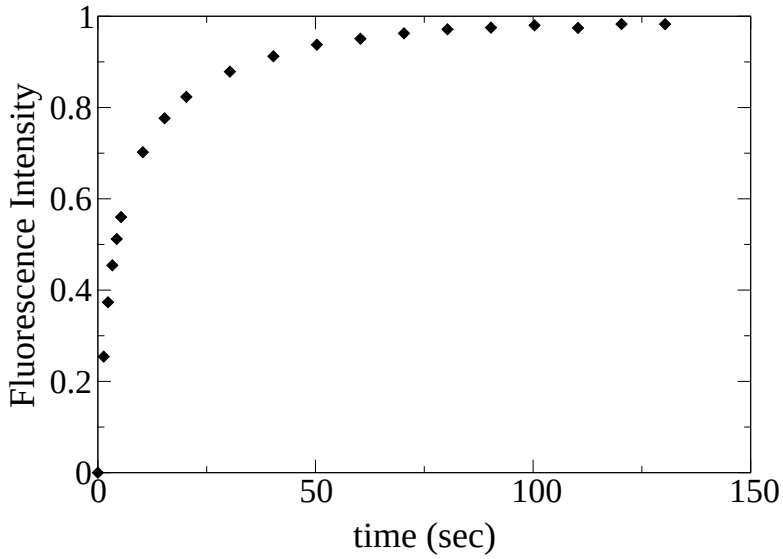
The fluorescent proteins are photobleached within a small region of the nucleus.

Due to diffusional exchange between bleached and unbleached proteins, fluorescence in the targeted area recovers.

Fluorescence Recovery Data



GFP-Actin data



GFP-Histone data

Modelling Problem 1 (Mobility)

From the fluorescence recovery data, how does one deduce information about the mobility of the proteins being studied?

Outline

- Historical approach
- New models and analysis
- Concluding remarks for the mobility problem

Historical Approach: Determining an Effective Diffusion Coefficient

(D. Axelrod et al., 1976)

- Solve the diffusion equation:

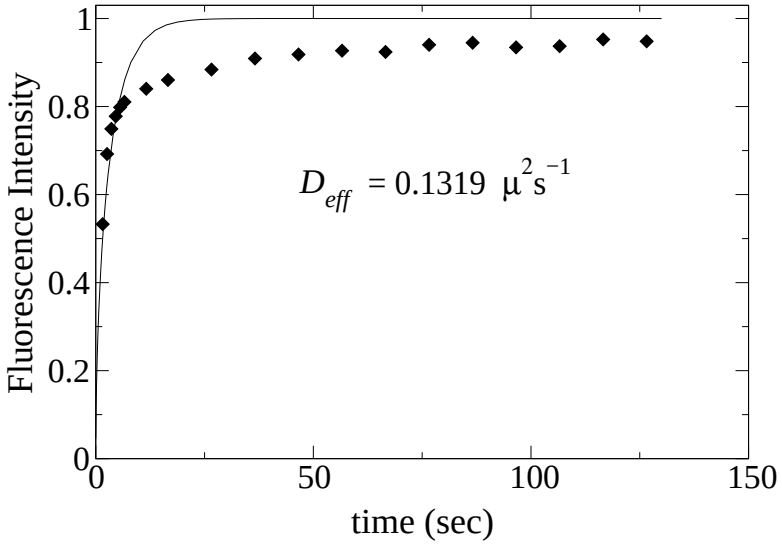
$$\frac{\partial}{\partial t}u(x,t) = D_{eff}\frac{\partial^2 u(x,t)}{\partial x^2}.$$

- Obtain the theoretical fluorescence recovery curve:

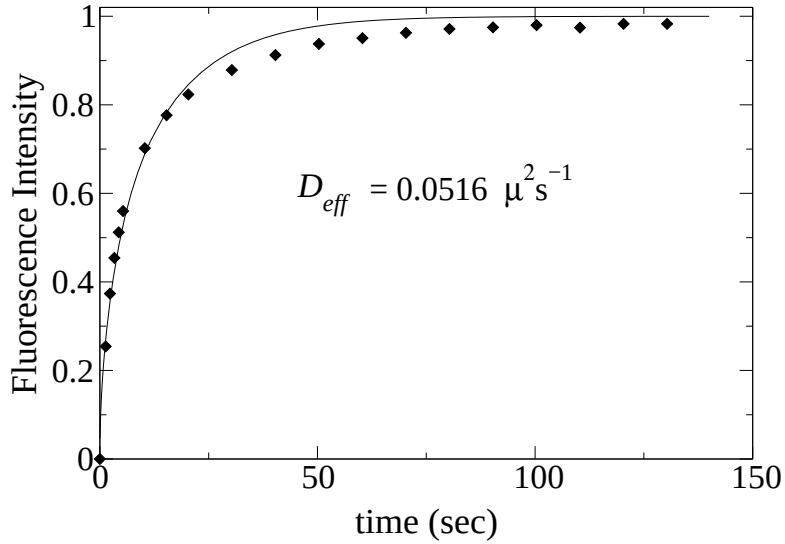
$$R(t) = \int_{\Lambda} u(x,t)dx.$$

- Fit the theoretical fluorescence recovery curve to the experimental data to find D_{eff}.

Fitting the Diffusion model to Experimental Data



GFP-Actin data



GFP-Histone data

The diffusion model does not provide satisfactory fits because the mobility of nuclear proteins is governed not only by diffusion, but also by interactions with other structures in the nucleus.

Assumptions for New Models

- Proteins undergo a reversible binding process with a structure that is assumed to be immobile on the time scale of the experiment.

- The structure is assumed to be spatially homogeneously distributed.

- Unbound proteins are free to diffuse.

- The profile of the photobleaching is given by a narrow band so that we can reduce the problem to one spatial dimension.

Model 1: A Simple Reaction-Diffusion Model

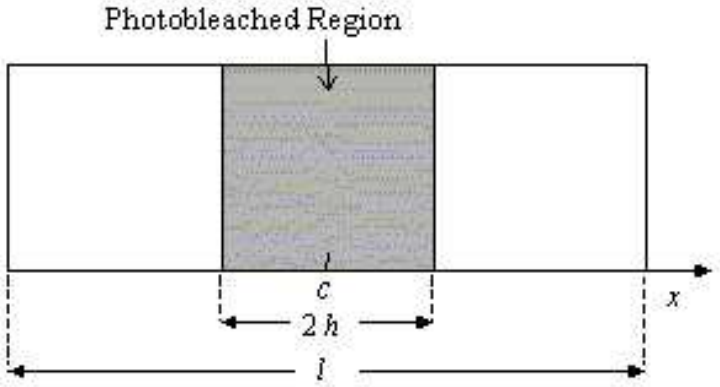
(Y. Tardy et al., 1995)

$u(x,t)$ is the density of fluorescent biomolecules free to diffuse
$v(x,t)$ is the density of bound fluorescent biomolecules

$$\begin{aligned}\frac{\partial}{\partial t}u(x,t) &= D\frac{\partial^2}{\partial x^2}u(x,t) - k_b u(x,t) + k_u v(x,t)\\ \frac{\partial}{\partial t}v(x,t) &= k_b u(x,t) - k_u v(x,t)\end{aligned}$$

Boundary conditions: no flux
Initial conditions: determined by photobleaching profile

Solution for the Reaction-Diffusion Model

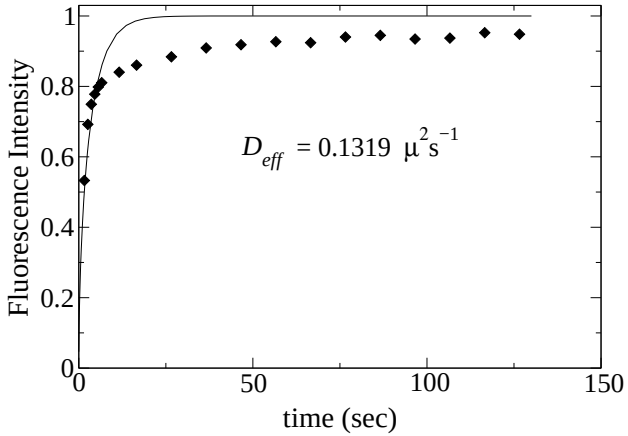


The theoretical fluorescence recovery curve is given by

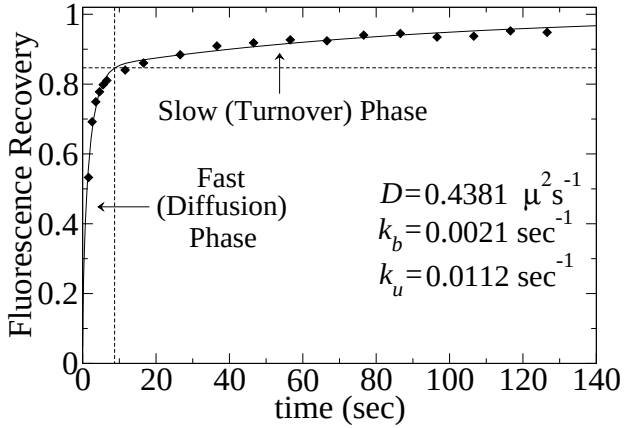
$$R(t) = \int_{c-h}^{c+h} [u(x,t) + v(x,t)]\, dx.$$

$R(t)$ can be solved for exactly - one obtains a messy expression in terms of the model parameters k_b, k_u, and D.

Fitting the Reaction-Diffusion Model to Data



Previous fit to GFP-actin data with the diffusion equation.



Improved fit to GFP-actin data with the reaction-diffusion model.

Quantitative Information Obtained

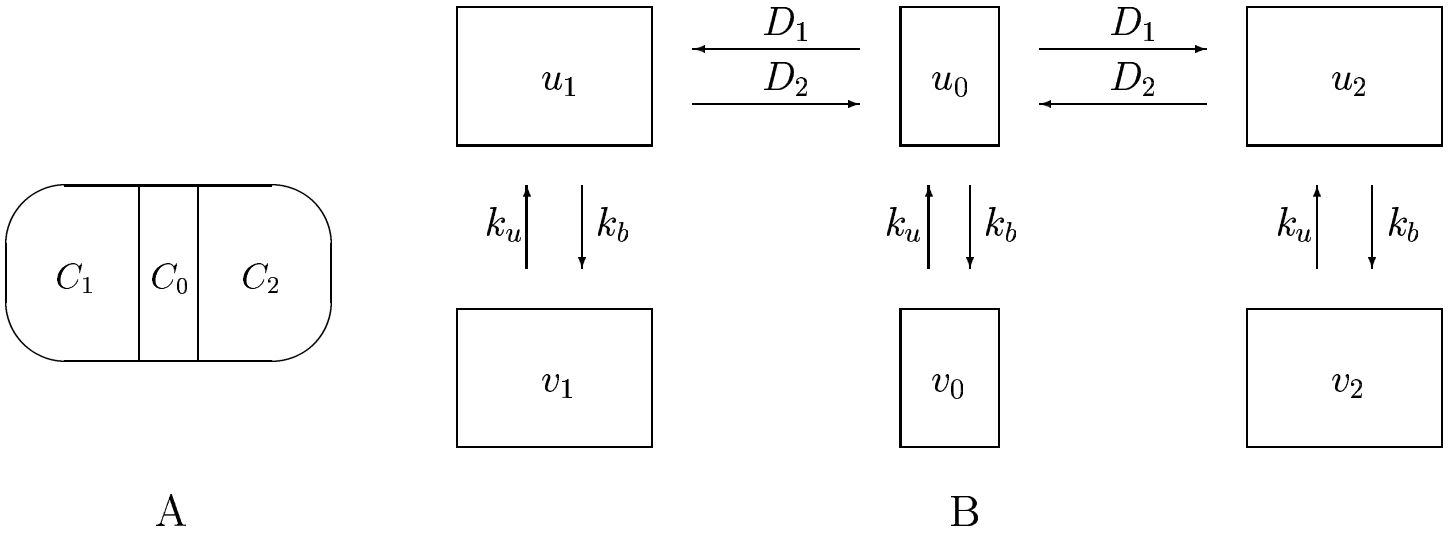
$$
\begin{aligned}
D &= 0.4381 \mu \text{m}^2/\text{s} \\
k_b &= 0.0021 \text{s}^{-1} \\
k_u &= 0.0112 \text{s}^{-1}
\end{aligned}
$$

Interpretation:

Proportion of GFP-actin in bound form: $k_b/(k_b + k_u) = 0.16$
Proportion of GFP-actin free to diffuse: $k_u/(k_b + k_u) = 0.84$
Average residency time of bound GFP-actin: $1/k_u = 89$ seconds
Average wandering time of free GFP-actin: $1/k_b = 476$ seconds

Model 2: A Compartmental Model

(G. Carrero et al., 2003)



The compartmental model can be written as a system of 6 ordinary differential equations in terms of 3 model parameters, $D_t = D_1 + D_2$, k_b, and k_u. Since the system is linear, its solution can be obtained easily.

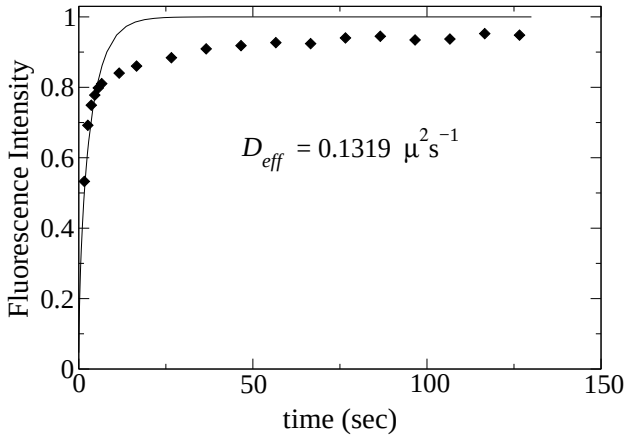
Solution for the Compartmental Model

The theoretical fluorescence recovery curve is given by

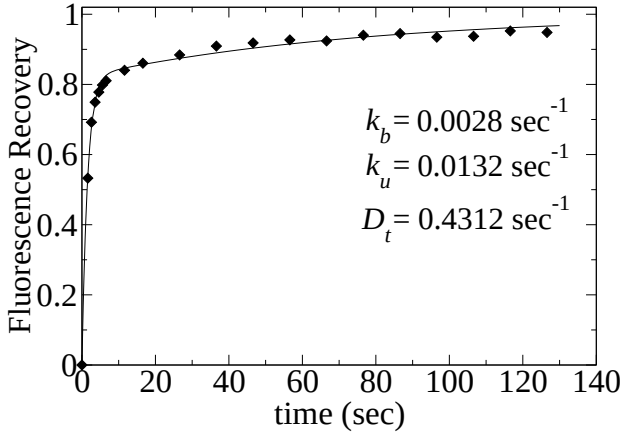
$$\begin{aligned} R(t) &= u_0 + v_0 \\ &= 1 - \gamma \exp(\alpha t) - (1 - \gamma) \exp(\beta t), \end{aligned}$$

where α, β, and γ are known nonlinear functions of the three model parameters k_b, k_u, and D_t.

Fitting the Compartmental Model to Data



Previous fit to GFP-actin data with the diffusion equation.



Improved fit to GFP-actin data with the compartmental model. Quantitative results are comparable to those from the reaction-diffusion model.

Qualitative Behaviour of Fluorescence Recovery Curves

The theoretical fluorescence recovery curves can exhibit different qualitative types of behaviour depending on the relative magnitude of the binding and unbinding rate parameters k_b and k_u.

A perturbation analysis of the models leads to an elegant explanation of two important limiting types of behaviour exhibited by fluorescence recovery data, namely

1. Reduced diffusive behaviour

2. Biphasic behaviour

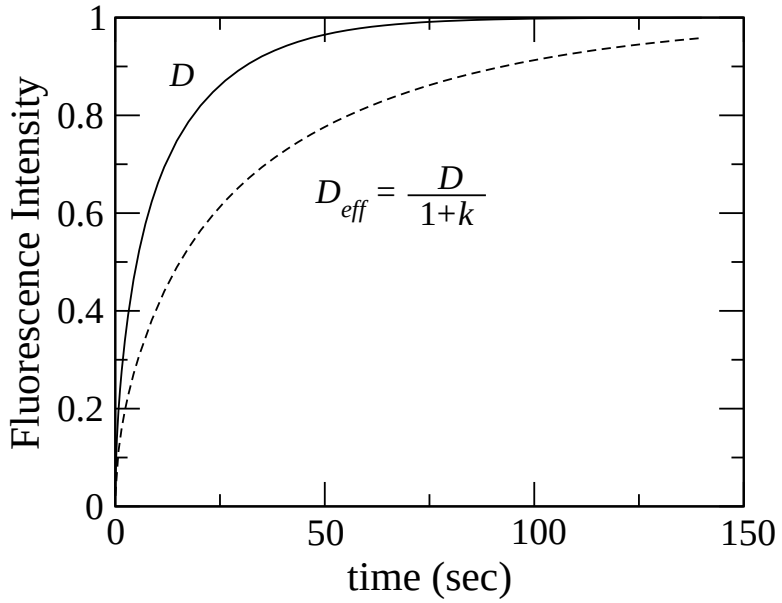
Type 1: Reduced Diffusive Behaviour

$$\boxed{k_b = \gamma_b/\varepsilon \text{ and } k_u = \gamma_u/\varepsilon} \quad \text{(rapid turnover)}$$

In this case, the reaction-diffusion and compartmental models reduce to simple diffusion models, *but with reduced effective diffusion coefficients:*

$$\boxed{D_{eff} = \frac{D}{1+k}}$$

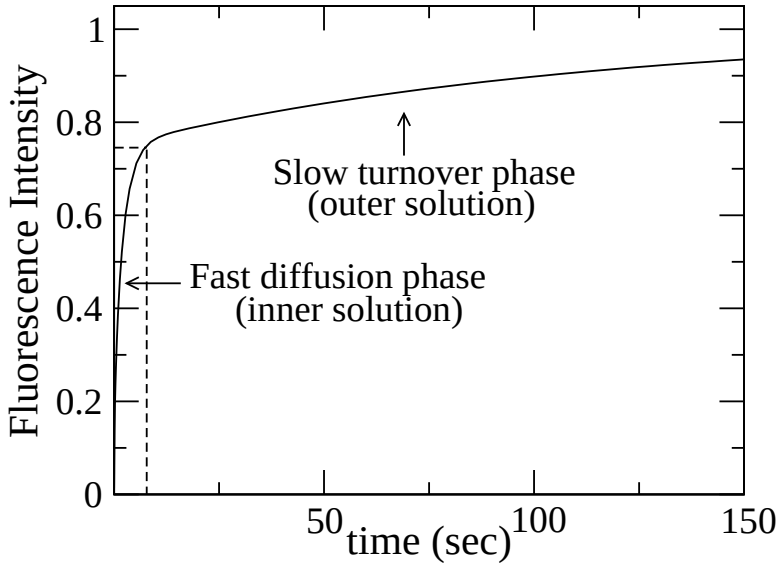
where $k = k_b/k_u$



Type 2: Biphasic Behaviour

$$\boxed{k_b = \varepsilon\gamma_b \text{ and } k_u = \varepsilon\gamma_u} \quad \text{(slow turnover)}$$

In this case, the fluorescence recovery curves exhibits two phases:
1) Fast diffusion phase (inner solution)
2) Slow turnover phase (outer solution)



Concluding Remarks for Modelling Problem 1 (Mobility)

- A simple diffusion model does not provide a good fit for all experimental fluorescence recovery data curves.

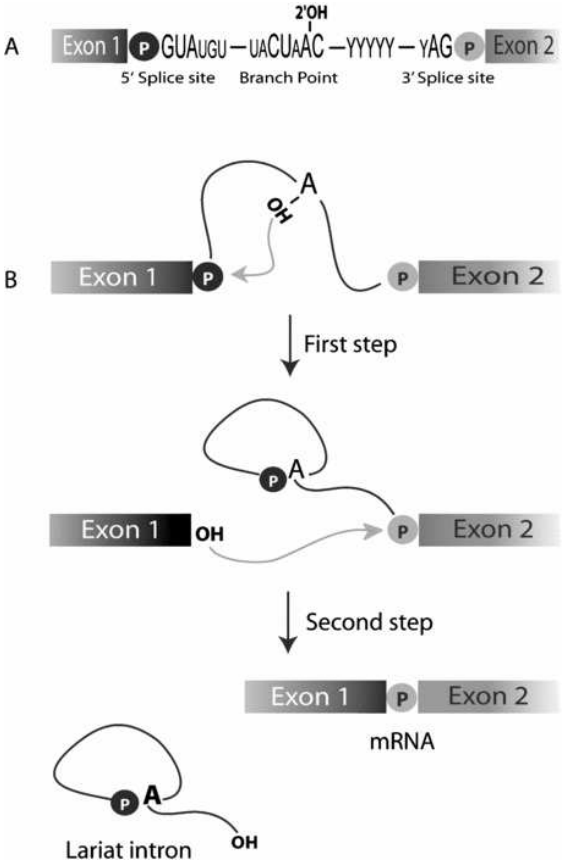
- A model taking into account diffusion plus a reversible binding process can give a much better fit to experimental fluorescence recovery data.

- Two limiting types of behaviour can be characterized:
  1. Reduced diffusive behaviour (caused by a rapid turnover)
  2. Biphasic behaviour (caused by a slow turnover)

Overview of Presentation

- Modelling problem 1: Mobility of nuclear proteins
- Modelling problem 2: Spatial organization of nuclear proteins

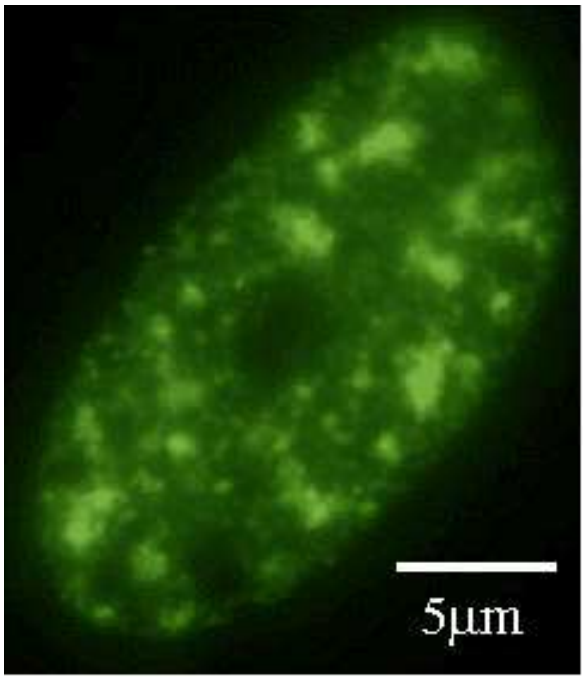
Splicing Factors



- Splicing factors are proteins found in the nucleus of a eukaryotic cell.
- Splicing factors move randomly throughout the nucleus.
- Splicing factors remove introns (noncoding sequences) from mRNA before it is transported to the cytoplasm of the cell.

Image: www.biochemsoctrans.org/bst/032/0928/bst0320928f01.gif

Splicing Factor Compartments (SFCs/speckles)



- SFCs are clusters within the cell nucleus enriched with splicing factors.
- During the interphase of the cell cycle, splicing factors are concentrated in approximately 25–50 SFCs.
- During mitosis, the SFCs disassemble.

Image: courtesy of Hendzel's lab

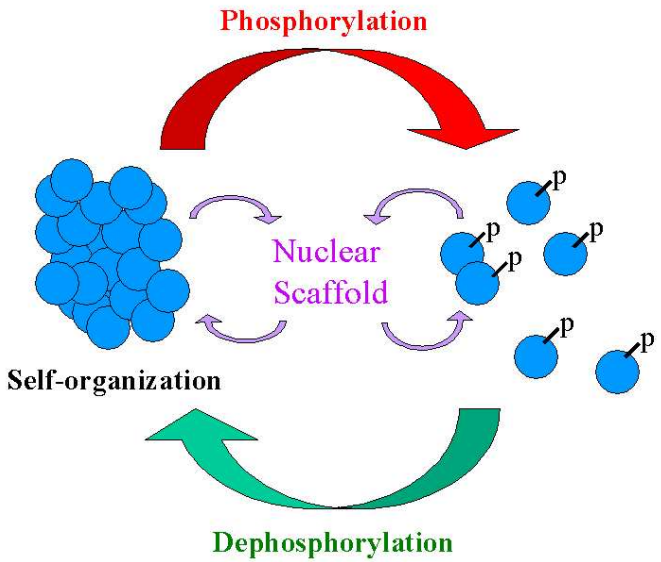
Modelling Problem 2 (Spatial Organization)

What is the mechanism responsible for the formation, maintenance, and disassembly of Splicing Factor Compartments?

Outline

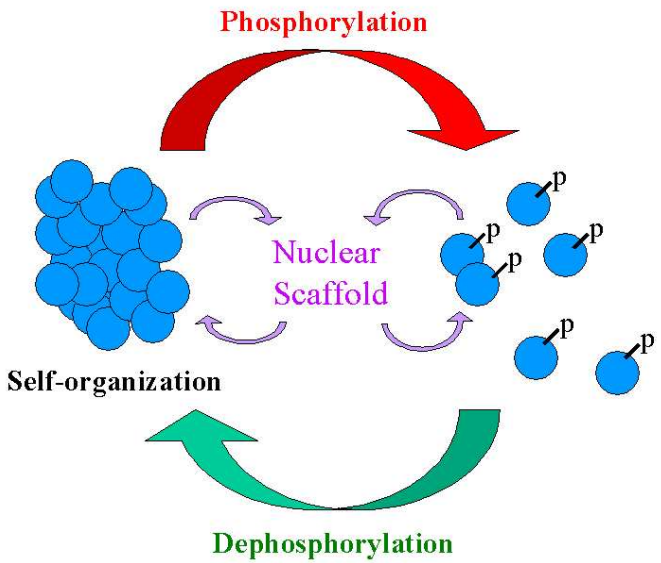
- Biological hypotheses and translation to mathematical model
- Model analysis
- Concluding remarks for the spatial organization problem

Hypothesis 1: Self-Organization



- Splicing factors are in continuous flux between the SFC's and the nucleoplasm.
- Splicing factors exist in phosphorylated and dephosphorylated forms.
- Increased phosphorylation promotes the release of splicing factors from SFC's and disassembly of SFC's.
- Increased dephosphorylation is required for the formation of SFC's.
- Dephosphorylated splicing factors have a tendency to self-interact; phosphorylated splicing factors do not.

Hypothesis 2: Underlying Nuclear Scaffold



- Splicing factors move randomly within the nucleus two orders of magnitude slower than expected.
- The low mobility of the splicing factors is attributed to rapid transient binding to an underlying nuclear scaffold (matrix).
- The existence of an underlying scaffold is thought to be a major determinant in the organization of splicing factors.

Model Skeleton

$$\frac{\partial v}{\partial t} = D\frac{\partial^2 v}{\partial x^2} - \delta v + \rho u$$

$$\frac{\partial u}{\partial t} = \text{“motion and self-interaction term”} + \delta v - \rho u$$

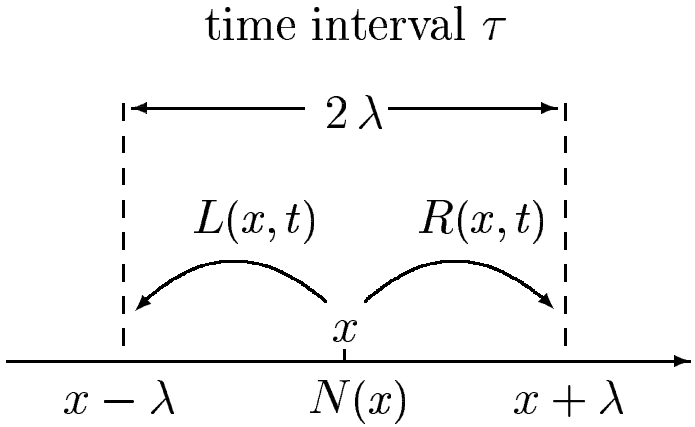
$v(x,t)$	=	density of phosphorylated splicing factors
$u(x,t)$	=	density of unphosphorylated splicing factors
δ	=	dephosphorylation rate
ρ	=	phosphorylation rate
D	=	effective diffusion coefficient of splicing factors

Question:

What does the “motion and self-interaction term” look like?

Deriving the "Motion and Self-Interaction Term"

Consider the following random walk:



τ	$=$	size of the time step
λ	$=$	size of the space step
$L(x,t), R(x,t)$	$=$	probability that molecule located at position x and time t moves one unit to the left, right
$N(x,t)$	$=$	$1 - L(x,t) - R(x,t)$

Deriving the "Motion and Self-Interaction Term"

The diffusion approximation leads to the following general Fokker-Planck equation for the unphosphorylated splicing factors:

$$\frac{\partial u}{\partial t} = \frac{\partial^2(\mu u)}{\partial x^2} - \frac{\partial(\beta u)}{\partial x}$$

where

$$\mu = \frac{\lambda^2}{2\tau}(R+L) = D(1-N) \qquad \text{"motility"}$$

$$\beta = \frac{\lambda}{\tau}(R-L) \qquad \text{"bias"}$$

Assumption: $R = L$, so that the bias term disappears
Question: How do we model the probability $N(x,t)$?

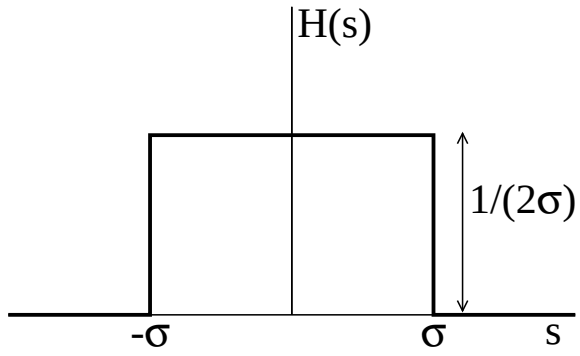
Modelling the Probability $N(x,t)$

Assume that $N(x,t)$ depends on the number of bound unphosphorylated splicing factors within a neighbourhood of size σ:

$$N(x,t) = \frac{\kappa}{\omega} \int_{-\infty}^{\infty} H(s) u(x+s,t) ds$$

with kernel function

$$H(s) = \begin{cases} \frac{1}{2\sigma} & \text{for } |s| \leq \sigma \\ 0 & \text{for } |s| \geq \sigma \end{cases}$$



κ = aggregative sensitivity
ω = critical density dictated by space limitations
σ = range of detection

Deriving the "Motion and Self-Interaction Term"

The general Fokker-Planck equation was

$$\frac{\partial u}{\partial t} = \frac{\partial^2(\mu u)}{\partial x^2} \quad \text{with} \quad \mu = D(1-N).$$

Using the Taylor expansion for $u(x+s,t)$ about x in

$$N(x,t) = \frac{\kappa}{\omega}\int_{-\infty}^{\infty} H(s)u(x+s,t)ds$$

and neglecting $\mathcal{O}(s^4)$ terms yields the following PDE describing the motion and self-interaction of unphosphorylated splicing factors:

$$\frac{\partial u}{\partial t} = \frac{\partial}{\partial x}\left[(D - 2D\kappa\frac{u}{\omega})\frac{\partial u}{\partial x}\right] - \frac{\partial^2}{\partial x^2}\left[\left(\frac{D\kappa\sigma^2}{6}\frac{u}{\omega}\right)\frac{\partial^2 u}{\partial x^2}\right]$$

Summary of the Model

- The model skeleton from before:

$$\frac{\partial v}{\partial t} = D\frac{\partial^2 v}{\partial x^2} - \delta v + \rho u$$

$$\frac{\partial u}{\partial t} = \text{“motion and self-interaction term”} + \delta v - \rho u$$

- With the details filled in:

$$\frac{\partial v}{\partial t} = D\frac{\partial^2 v}{\partial x^2} - \delta v + \rho u$$

$$\frac{\partial u}{\partial t} = \frac{\partial}{\partial x}\left[(D - 2D\kappa\frac{u}{\omega})\frac{\partial u}{\partial x}\right] - \frac{\partial^2}{\partial x^2}\left[\left(\frac{D\kappa\sigma^2}{6}\frac{u}{\omega}\right)\frac{\partial^2 u}{\partial x^2}\right] + \delta v - \rho u$$

Model Analysis Part I: The Aggregation-Diffusion Equation

In non-dimensionalized form, we have

$$\frac{\partial u}{\partial t} = \frac{\partial}{\partial x}\left[(1-u)\frac{\partial u}{\partial x}\right] - \frac{\partial^2}{\partial x^2}\left[\left(\frac{\sigma^2}{12}u\right)\frac{\partial^2 u}{\partial x^2}\right]$$

subject to no-flux boundary conditions.

Observation: Any constant density $u(x,t) = u_{eq}$ is a uniform steady state of this equation.

Question: Under what conditions does this equation exhibit pattern formation (under what conditions is the uniform steady state unstable)?

Stability Analysis

- Linearize the PDE about the uniform steady state u_{eq} to get

$$\frac{\partial U}{\partial t} = (1 - u_{eq})\frac{\partial^2 U}{\partial x^2} - \frac{\sigma^2}{12} u_{eq} \frac{\partial^4 U}{\partial x^4},$$

  where $U(x,t)$ is the deviation from u_{eq}.

- Study the normal mode solutions of the form

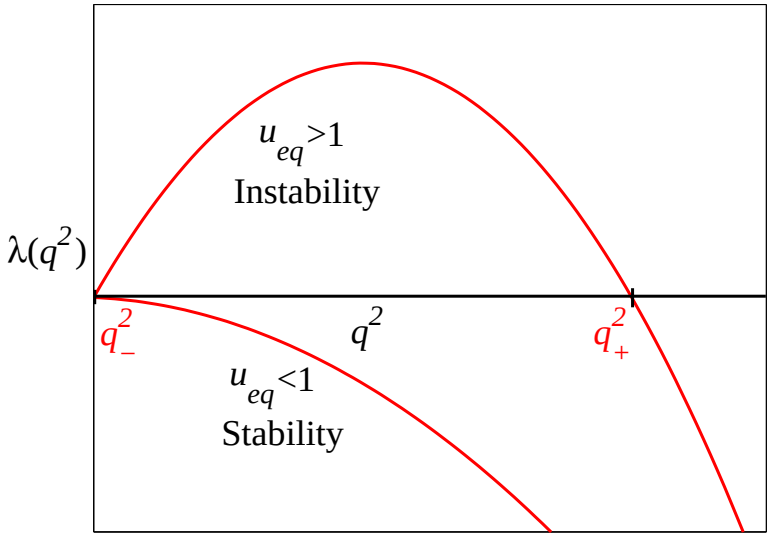
$$U(x,t) = \exp(\lambda t + iqx),$$

  where λ is the growth rate corresponding to the wave number q.

- $\lambda < 0$ indicates that u_{eq} is stable
  $\lambda > 0$ indicates that u_{eq} is unstable

The Dispersion Relation

The dispersion relation summarizes the relationship between the growth rate λ and the wavenumber q.

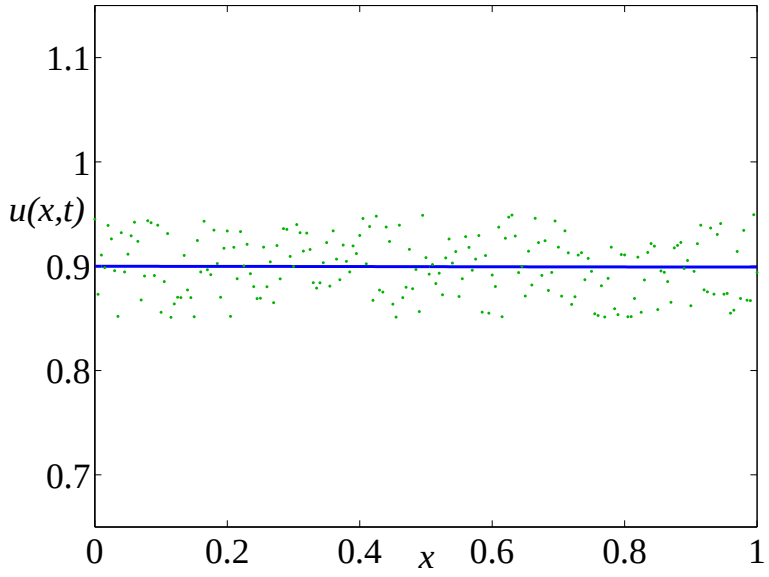


- When $u_{eq} < 1$, it is stable. When $u_{eq} > 1$, it is unstable.
- The condition $u_{eq} > 1$ for instability means that the population of unphosphorylated splicing factors must be sufficiently large for pattern formation to occur.
- The fastest growing mode determines the spatial pattern.

Sample Solutions

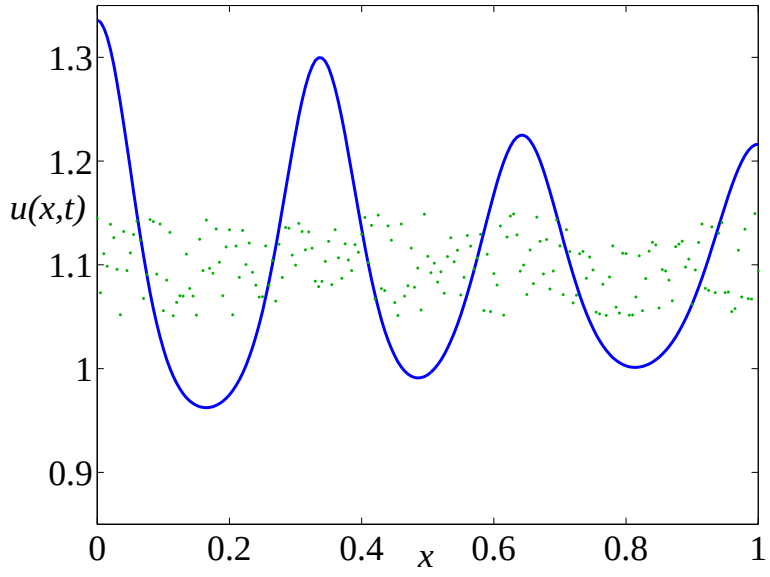
$u_{eq} = 0.9$:
steady state is **stable**

(solution shown for $t = 3$)



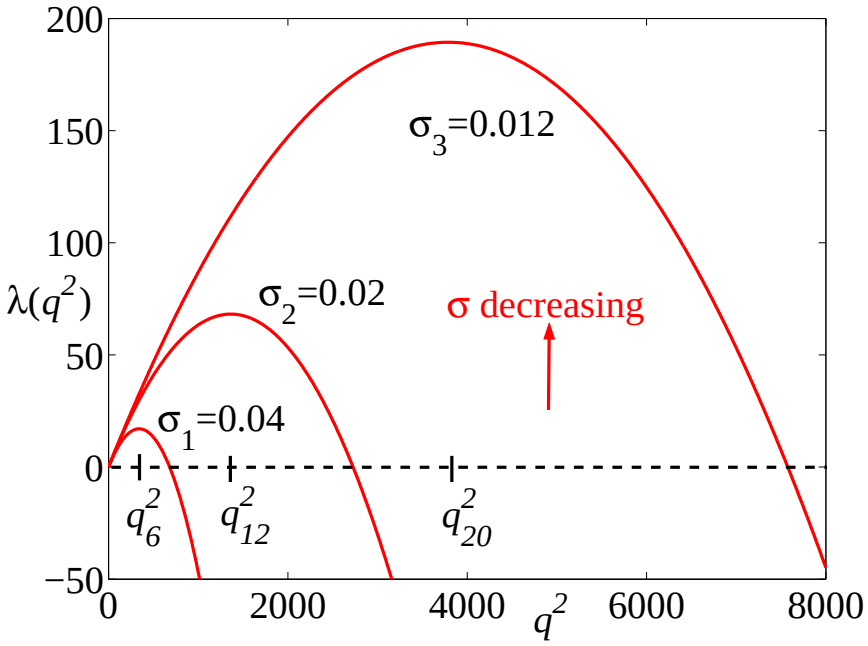
$u_{eq} = 1.1$:
steady state is **unstable**

(solution shown for $t = 0.25$)

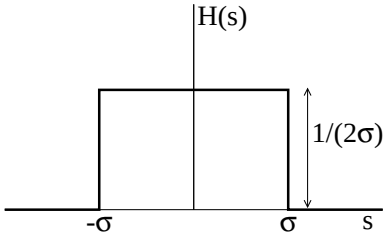


Question: What is the effect of changing the value of σ?

Dispersion Relation: Influence of σ

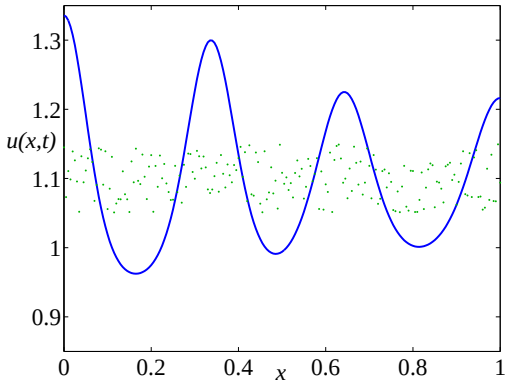


The smaller the range of detection σ, the larger the dominating wave number and the smaller the wavelength.

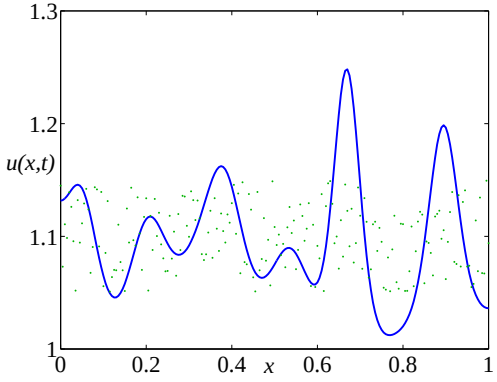


Sample Solutions: Influence of σ

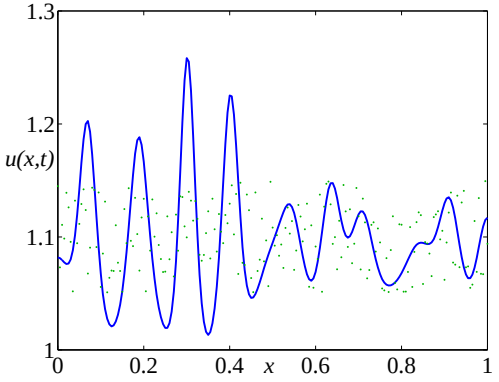
$\sigma_1 = 0.04$ (solution at $t = 0.25$)



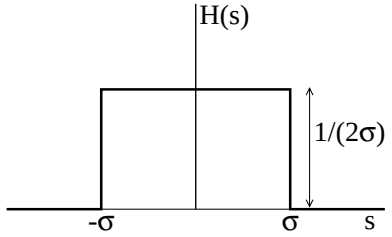
$\sigma_2 = 0.02$ (solution at $t = 0.05$)



$\sigma_3 = 0.012$ (solution at $t = 0.01$)



The smaller the range of detection σ, the more SFC's are formed.



Model Analysis Part II:
The Aggregation-Reaction-Diffusion Equations

(in non-dimensionalized form)

$$\begin{aligned}
\frac{\partial v}{\partial t} &= \frac{\partial^2 v}{\partial x^2} - \delta v + \rho u \\
\frac{\partial u}{\partial t} &= \frac{\partial}{\partial x}\left[(1-u)\frac{\partial u}{\partial x}\right] - \frac{\partial^2}{\partial x^2}\left[\left(\frac{\sigma^2}{12}u\right)\frac{\partial^2 u}{\partial x^2}\right] + \delta v - \rho u
\end{aligned}$$

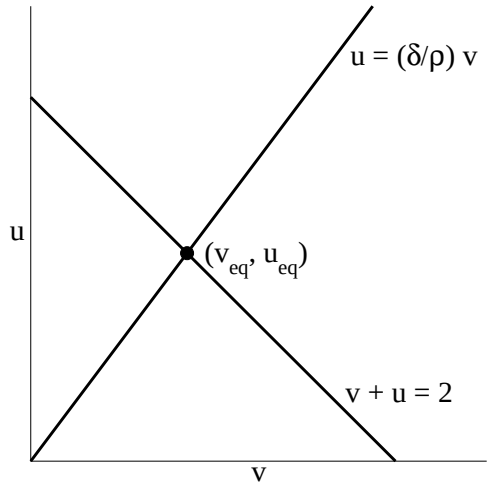
(subject to no-flux boundary conditions)

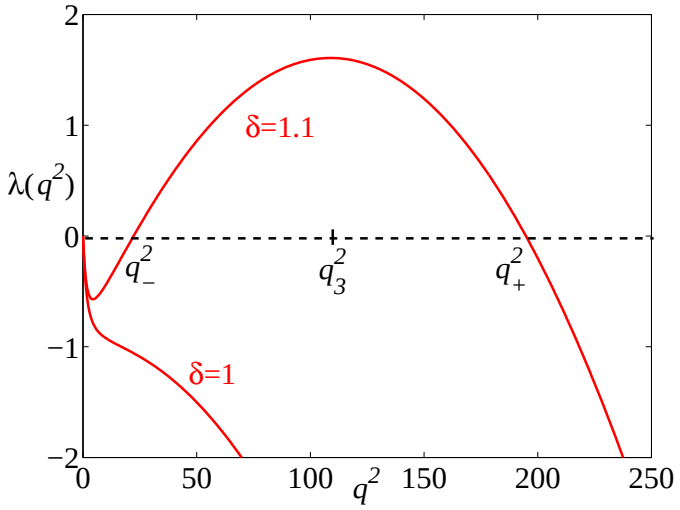
These equations also exhibit pattern formation (compartmentalization), provided the population of splicing factors is sufficiently large.

Question of interest: How does the dephosphorylation parameter δ influence the compartmentalization?

Steady States and the Dispersion Relation

$$\begin{aligned} \sigma &= 0.05 \\ \rho &= 1 \\ u + v &= 2 \\ \delta &= \text{free} \end{aligned}$$





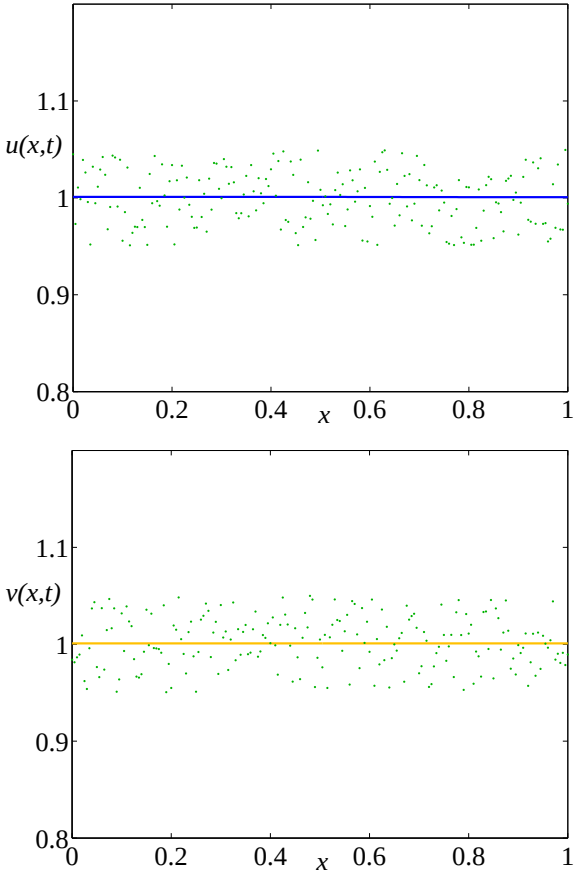
For small values of δ, there is no compartmentalization.

For large values of δ, compartmentalization occurs.

Sample Solutions

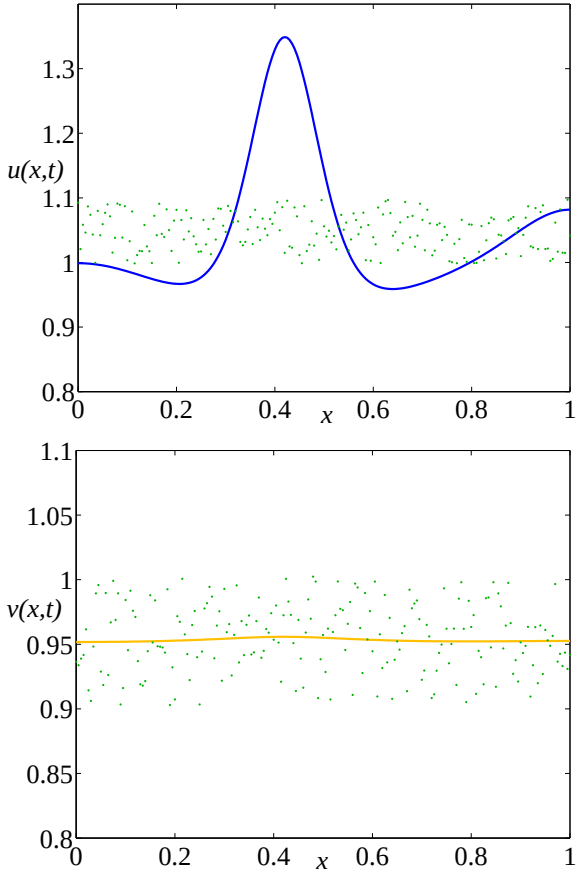
$\delta = 1$

$(v_{eq}, u_{eq}) = (1, 1)$



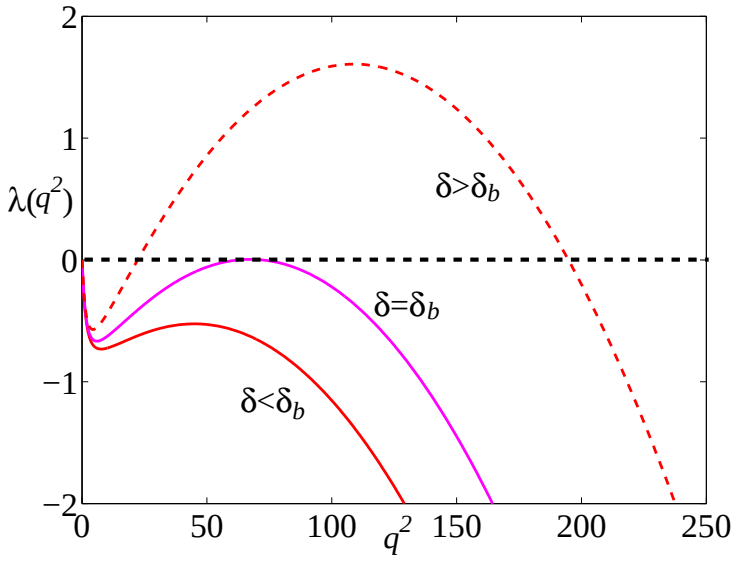
$\delta = 1.1$

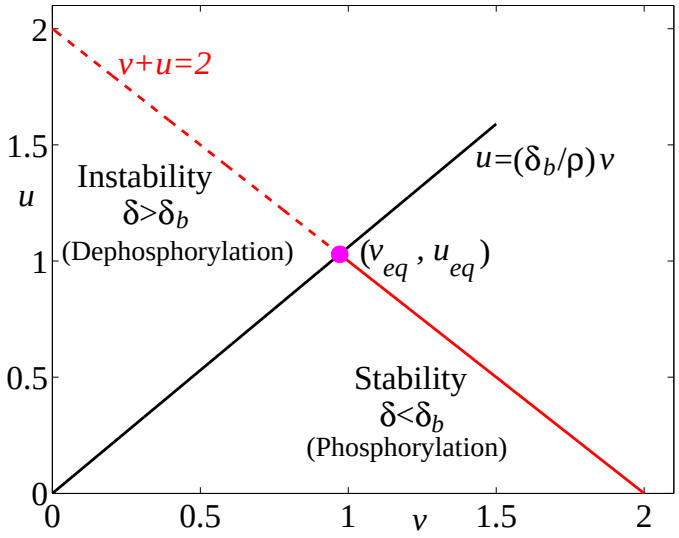
$(v_{eq}, u_{eq}) = (0.95, 1.05)$



Real Bifurcation

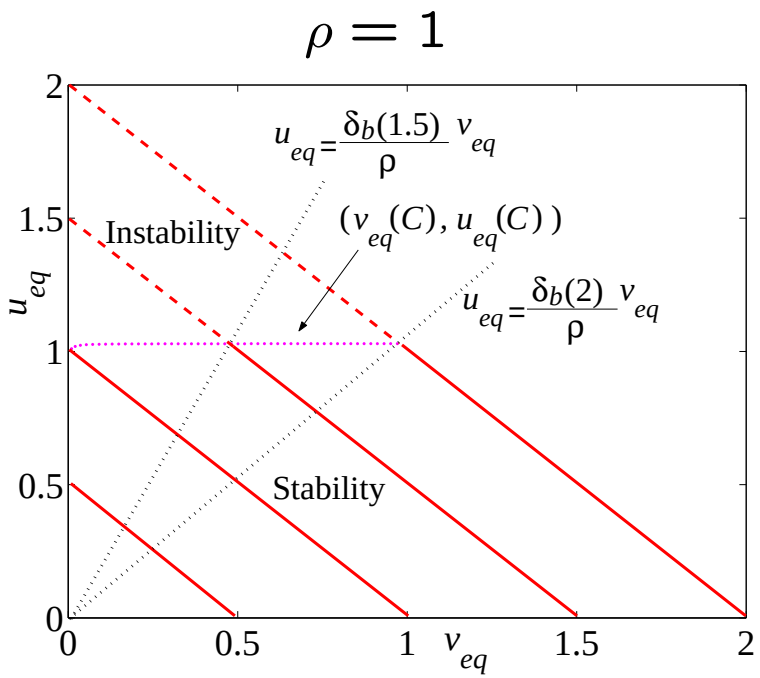
As δ increases, the uniform steady state (v_{eq}, u_{eq}) becomes unstable at a real bifurcation when $\delta = \delta_b$.

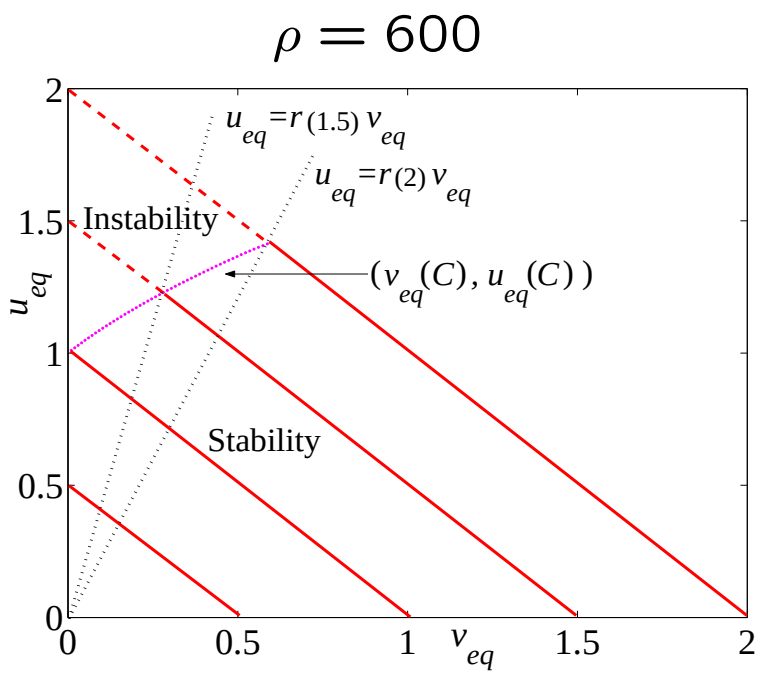




Question: How does the location of the real bifurcation depend on the total amount of biomolecules present in the system?

Regions of Stability and Instability

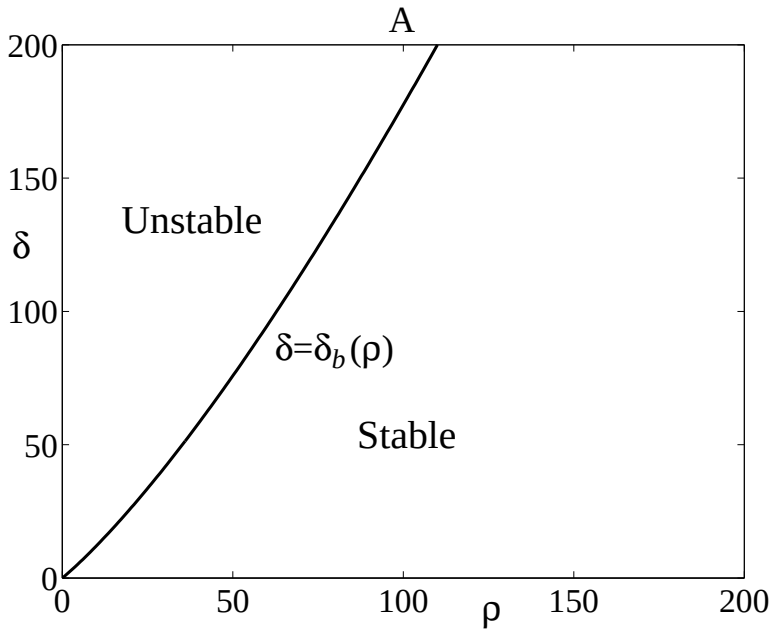




Regions of Stability and Instability in the (ρ, δ)-plane

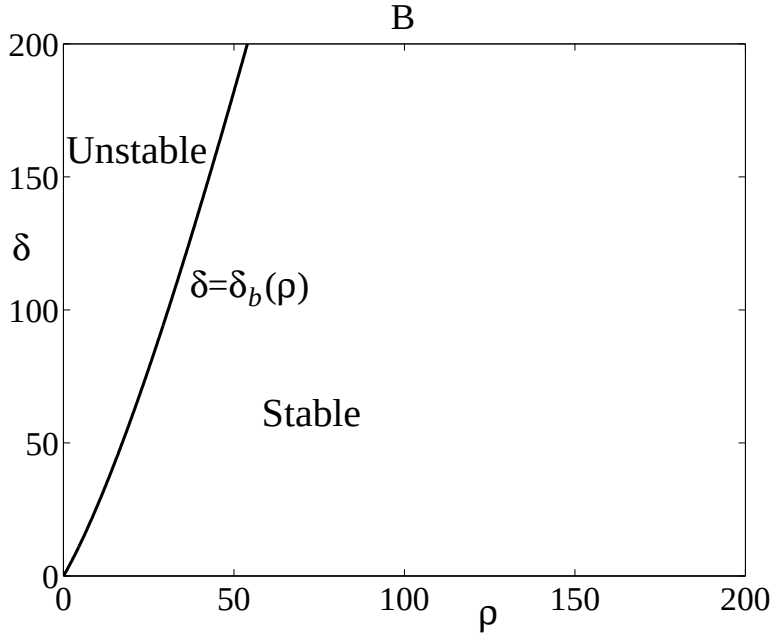
$\sigma = 0.05$

$v + u = 2$



$\sigma = 0.05$

$v + u = 1.5$



Predictions

- The formation of SFC's is enhanced by increased dephosphorylation and/or an increased number of splicing factors.

- The number of SFC's increases as the range of detection σ decreases.

- No SFC's are formed when the underlying nuclear scaffold is removed/destroyed.

Concluding Remarks for Modelling Problem 2 (Spatial Organization)

- We have developed a model that supports the hypothesis that SFC's are formed by a process of self-organization.

- The dynamic behaviour of the model is consistent with the biological observation that a cycle of phosphorylation and dephosphorylation modulates the aggregation of splicing factors.

- Future work includes the design of experiments to determine parameter values and test the predictions of the model.