Gene Network Models Including mRNA and Protein Dynamics

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1. Motivation for qualitative models
2. Protein-only models
3. An mRNA-protein model framework

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2. Protein-only models
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4. Non-uniqueness again
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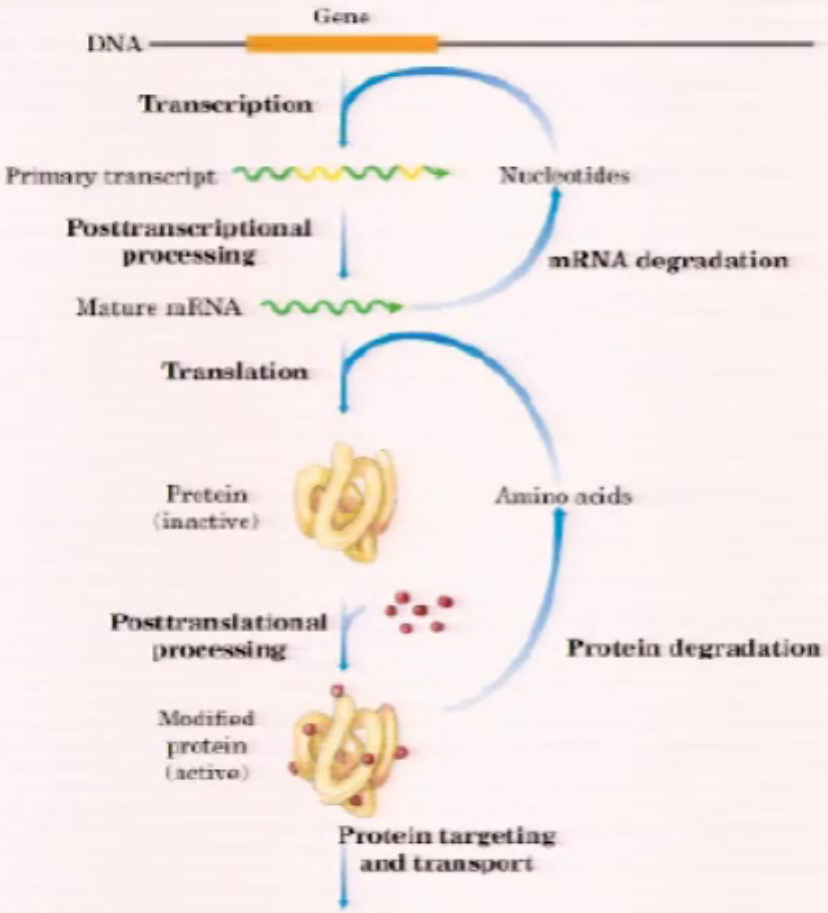
Transcription and translation



Gene Regulatory Networks

Why do we need to qualitative mathematical analysis of these networks?

Current Model Framework

- Since the qualitative analysis of such networks began in the 1970*s*, the network has been viewed as a single product system.
- Protein products are taken to directly promote or inhibit the growth of other products within the network.
- The interaction between products is given the "switch on" and "switch off" characteristic present within each gene.

Current Model Framework

A system of n genes then takes the form

$$\begin{aligned}
\dot{x_1} &= F_1(x_1, x_2, ..., x_n) - \gamma_1 x_1 \\
\dot{x_2} &= F_2(x_1, x_2, ..., x_n) - \gamma_2 x_2 \\
&\vdots \\
\dot{x_n} &= F_n(x_1, x_2, ..., x_n) - \gamma_n x_n,
\end{aligned} \tag{1}$$

where $x_i(t)$ is the concentration of the product i at time t.

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where $x_i(t)$ is the concentration of the product i at time t.

- The functions F_i are production rates corresponding to the protein concentration x_i which, in general, are taken to satisfy $F_i \geq 0$.
- The F_i's capture how x_i changes given the current concentrations of proteins within the network which promote or inhibit x_i's production.

Current Model Framework

Since protein regulation within a gene network is often well approximated by steep sigmoids, we take

$$F_i(x_1, x_2, ..., x_n) = F_i(Z_1(x_1), Z_2(x_2), ..., Z_n(x_n)),$$

where each $Z_i(x_i)$ is a Hill function

$$Z_i(x_i) = H(x_i, \theta_i, q) = \frac{x_i^{\frac{1}{q}}}{\theta_i^{\frac{1}{q}} + x_i^{\frac{1}{q}}}, \quad q \in (0, 1].$$

Current Model Framework

This results in the model framework

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\end{aligned}$$

This is a smooth system of differential equations but highly-nonlinear. Qualitative analysis is facilitated by taking the limit as $q \to 0$, giving a non-smooth system where each $Z_i = 0$ or 1 depending on the value of x_i.

But now there are new analytic difficulties when flows are constrained to switching domains for non-zero time intervals.

Analysis of flows in switching domains

There are two main ways of analyzing the flow in switching domains.

1. Singular Perturbation Theory
2. Filippov Theory

Both of these methods have problems in some situations.

- Singular Perturbation Theory requires restrictive conditions to apply; generalizations deal with some other cases, but then non-uniqueness can arise.

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- Singular Perturbation Theory requires restrictive conditions to apply; generalizations deal with some other cases, but then non-uniqueness can arise.
- Filippov Theory ignores information about how a trajectory arrived in a particular domain, and has significant trouble with non-uniqueness and spurious solutions, only partially solved by using an alternative definition of Filippov solution.

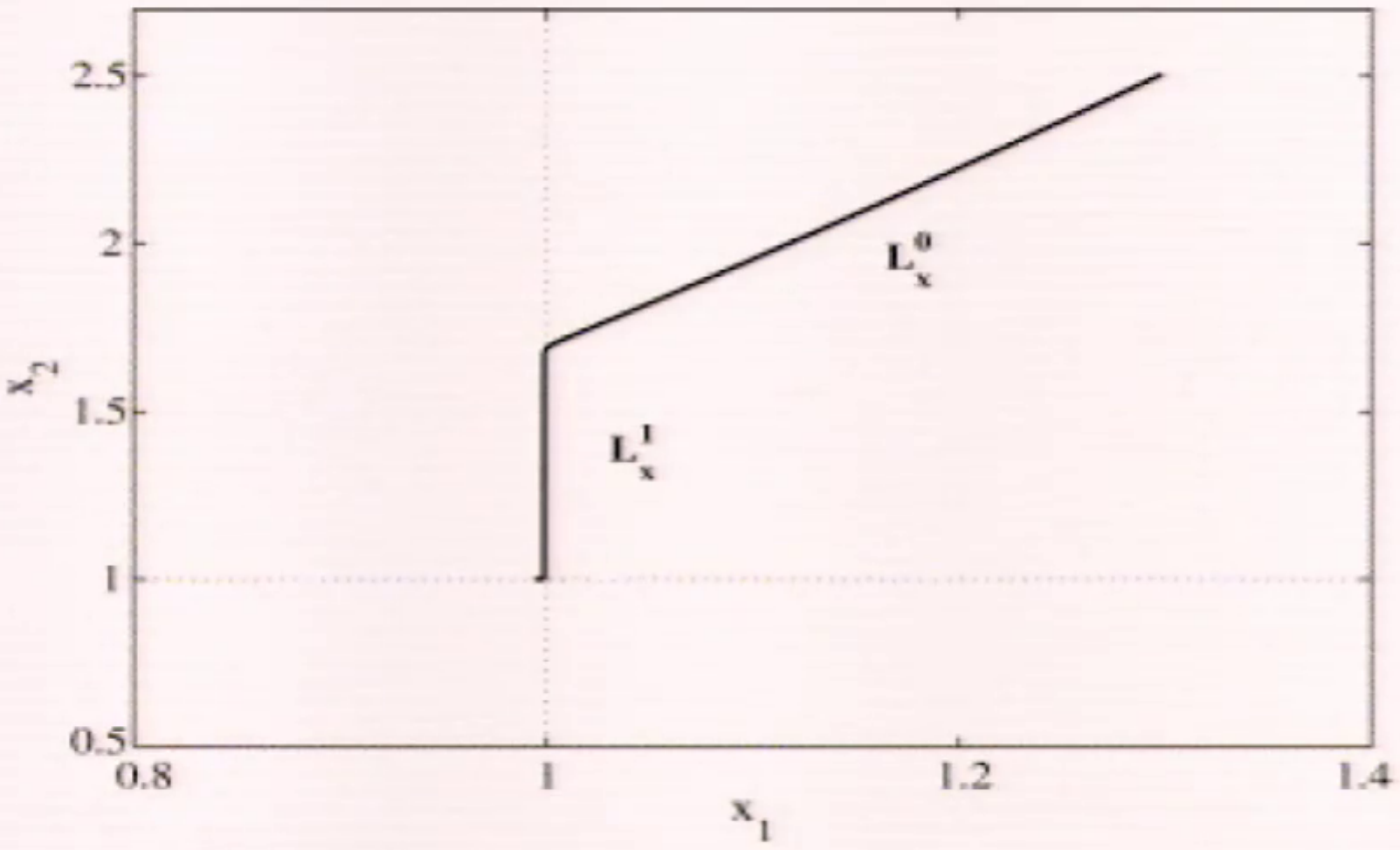
Singular Perturbation Analysis

Consider the following 2-gene system (Plahte & Kjøglum)

$$\dot{x_1} = Z_1 + Z_2 - 2Z_1Z_2 - \gamma_1 x_1$$
$$\dot{x_2} = 1 - Z_1Z_2 - \gamma_2 x_2,$$

where $\gamma_1 = 0.6$, $\gamma_2 = 0.9$, $\theta_1 = \theta_2 = 1$ with initial conditions $x_1(0) = 1.4$ and $x_2(0) = 1.9$.

Singular Perturbation Analysis



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where $\gamma_1 = 0.6$, $\gamma_2 = 0.9$, $\theta_1 = \theta_2 = 1$ with initial conditions $x_1(0) = 1.4$ and $x_2(0) = 1.9$.
Since $x_1(0) > \theta_1$ and $x_2(0) > \theta_2$, we have $Z_1 = Z_2 = 1$. Thus,

$$\dot{x_1} = -0.6x_1$$
$$\dot{x_2} = -0.9x_2,$$

$x_1 = \theta_1$ at $t = 0.56$
What happens once x_1 hits its theshold?
Look at behaviour of Z_1 instead of x_1 as it reaches θ_1!

Singular Perturbation Analysis

Letting $'$ denote the derivative with respect to fast time, $\tau = \frac{t}{q}$, we arrive at the system

$$\frac{Z_1'}{q} = \frac{Z_1(1-Z_1)}{x_1 q}(1 - Z_1 - 0.6x_1) \tag{2}$$
$$\frac{x_2'}{q} = 1 - Z_1 - 0.9x_2,$$

since $x_2 > 1$.
Muliplying by q and analyzing the system as $q \to 0$, we have

$$Z_1' = \frac{Z_1(1-Z_1)}{\theta_1}(1 - Z_1 - 0.6\theta_1) \tag{3}$$
$$x_2' = 0.$$

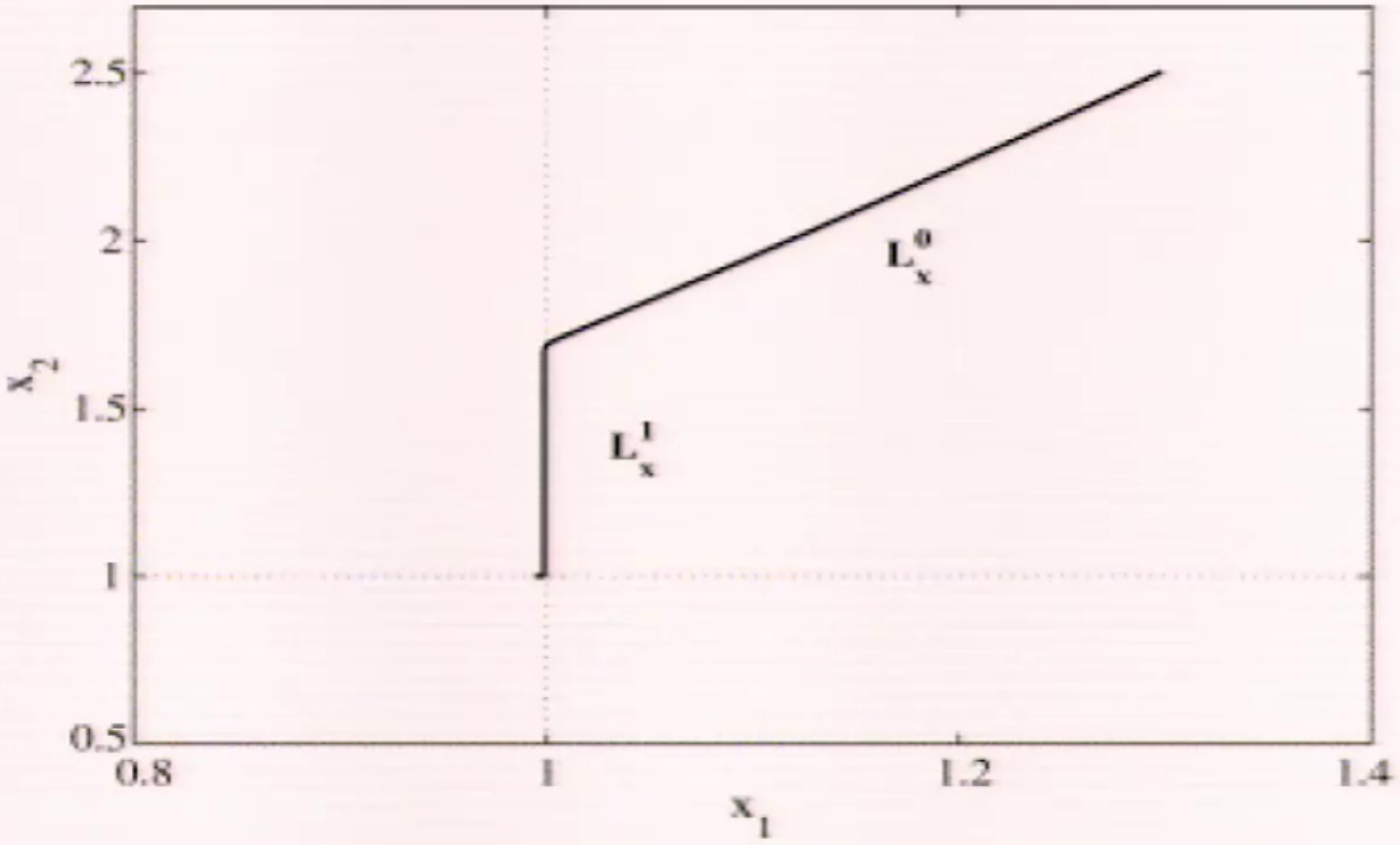
Singular Perturbation Analysis

The result is a 1-dimensional system with an asymptotically stable fixed point $Z_1^* = 0.4$.
This implies that x_1 stays fixed at θ_1, while x_2 resumes regular flow with $Z_1 = 0.4$.
Plugging this into the x_2 equation we get

$$\dot{x_2} = 1 - 0.4 - 0.9x_2.$$

This means the x_2 variable decreases towards the value $\frac{0.6}{0.9}$ until it hits its threshold θ_2. This sliding motion is known as 'sliding' or singular flow.

Singular Perturbation Analysis



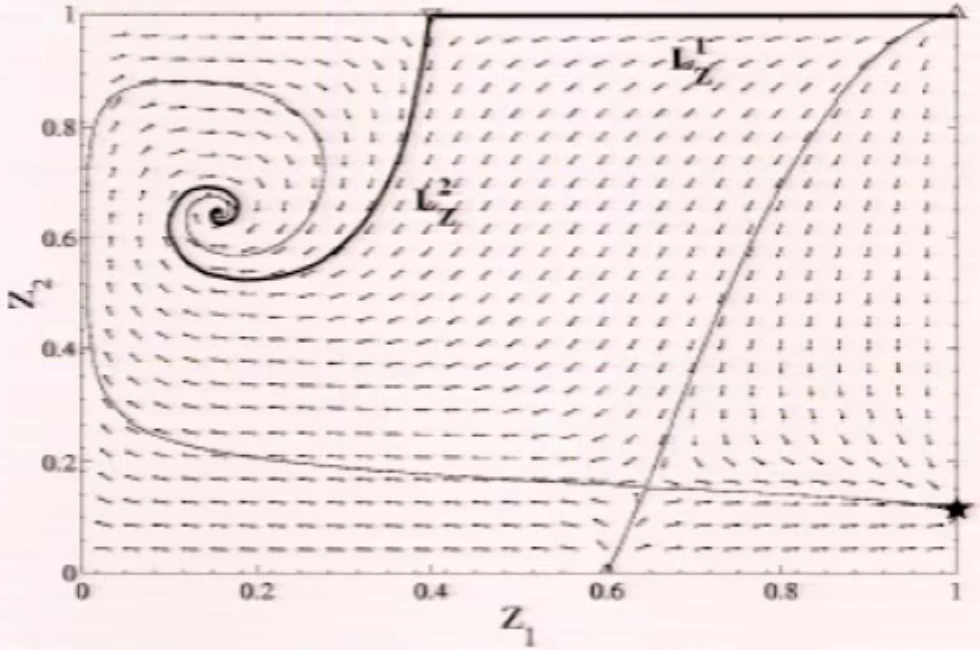
Singular Perturbation Analysis

Although we omit the details, a similar process can be used to analyze the 2-dimensional switching domain

$$Z_1' = \frac{Z_1(1-Z_1)}{\theta_1}\left(Z_1 + Z_2 - 2Z_1Z_2 - 0.6\theta_1\right) \tag{4}$$

$$Z_2' = \frac{Z_2(1-Z_2)}{\theta_2}\left(1 - Z_1Z_2 - 0.9\theta_2\right)$$

which has Z-dynamics shown here



Singular Perturbation Analysis

- There are 2 fixed points in the Z representation of this switching domain, a stable spiral and a saddle.

Singular Perturbation Analysis

- There are 2 fixed points in the Z representation of this switching domain, a stable spiral and a saddle.
- Singular perturbation theory says that when trajectories enter this 2-dimensional switching domain, where they end up depends on where they entered. This information is not taken into account in Filippov Theory.
- Our sample trajectory would enter from the top and spiral into the stable fixed point, and thus the solution remains at the threshold intersection.
- If instead our trajectory entered the bottom, it would exit along the line $x_1 > \theta_1$ and $x_2 = \theta_2$.

An alternative approach

- In gene network models, singular flow happens when a gene actively regulates its own production, i.e., autoregulates.
- In current models, autoregulation can cause variables to become "stuck" at a threshold, but **this is a consequence of only tracking protein concentrations**.

Consider the following single gene equation:

$$\dot{x_1} = 1 - Z_1 - 0.5x_1,$$

with $\theta_1 = 1$, $x(0) = 2$ and Z_1 a step function.

An alternative approach

Singular perturbation theory shows that the concentration reduces until it reaches its threshold and then remains there with $Z_1 = 0.5$.
Is this realistic?

- In the true biological system represented by this equation, when $x_1(0)$ starts above its threshold, the gene is off and therefore not transcribing any mRNA, so x_1 drops as a result of degradation.

An alternative approach

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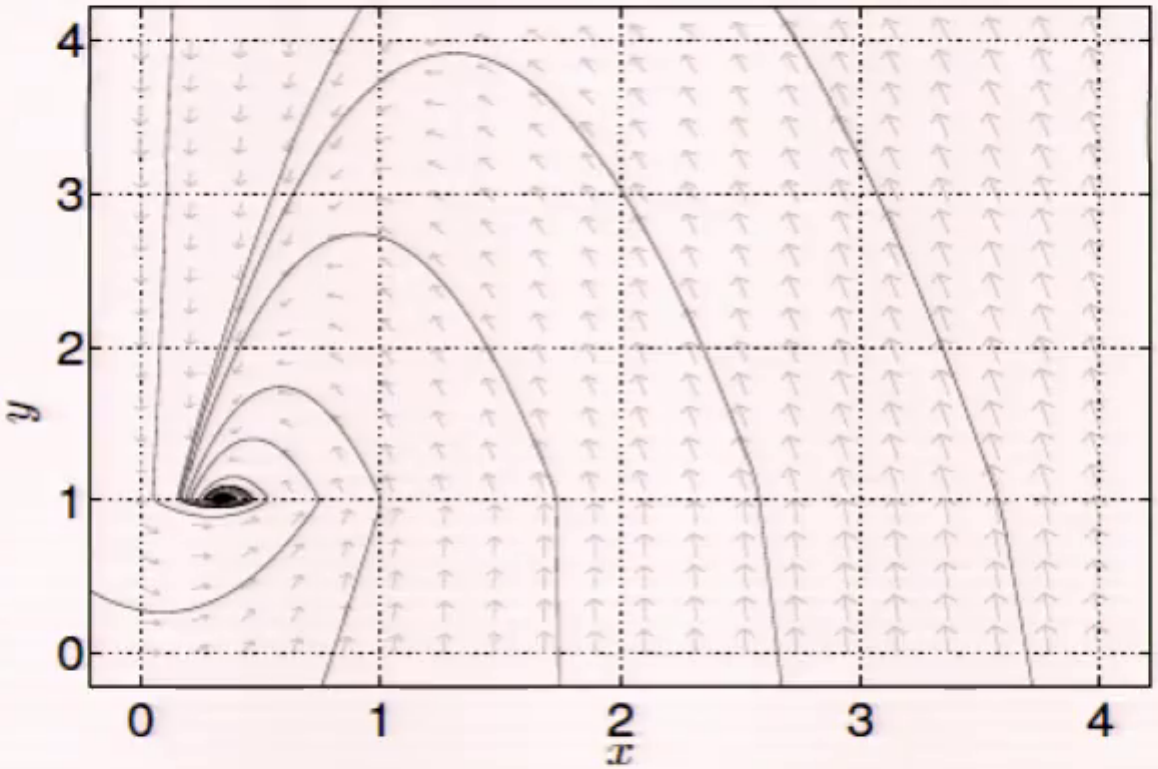
Is this realistic?

- In the true biological system represented by this equation, when $x_1(0)$ starts above its threshold, the gene is off and therefore not transcribing any mRNA, so x_1 drops as a result of degradation.
- Once x_1 crosses its threshold, the gene begins producing mRNA, but not enough at first for translation of new protein to exceed protein degradation, so x_1 continues to drop.
- After a non-zero interval, there is enough mRNA for protein concentration x_1 to increase again.
- This behaviour, under normal circumstances, results in damped oscillations until the protein concentration converges to its threshold, the same asymptotic result as predicted by the protein-only model.

An alternative approach



- This behaviour, under normal circumstances, results in damped oscillations until the protein concentration converges to its threshold, the same asymptotic result as predicted by the protein-only model.

An alternative approach

- More drastic differences can arise at theshold intersections, as we will see.
- The mRNA-protein model framework we believe better captures behaviour of gene networks around thresholds when autoregulation is present in the system.

The mRNA-protein model framework

The new approach includes an additional variable for each gene in the system:

$$
\begin{aligned}
\dot{x_1} &= F_1(Z_1, Z_2, ..., Z_n) - \gamma_1 x_1 \\
\dot{y_1} &= \kappa_1 x_1 - \beta_1 y_1 \\
\dot{x_2} &= F_2(Z_1, Z_2, ..., Z_n) - \gamma_2 x_2 \\
\dot{y_2} &= \kappa_2 x_2 - \beta_2 y_2 \\
&\vdots \\
\dot{x_n} &= F_n(Z_1, Z_2, ..., Z_n) - \gamma_n x_n. \\
\dot{y_2} &= \kappa_n x_n - \beta_n y_n
\end{aligned}
\tag{5}
$$

where $Z_i = H(y_i, \theta_i, q)$, a function of y_i instead of x_i.

The mRNA-protein model framework

- System (5) is an mRNA-Protein model (or transcription-translation model), where x_i is the concentration of mRNA transcribed by the i^{th} gene and y_i is the concentration of the protein product corresponding the i^{th} gene.
- The mRNA equations contain the switching functions to reflect the switching on and off of transcription.

Basic properties of the new model

- The first notable property of the expanded system is that the i^{th} production term, F_i, does not contain x_i.
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- The first notable property of the expanded system is that the i^{th} production term, F_i, does not contain x_i.
- This means that this system behaves more like a protein only model that does not have autoregulation.
- Autoregulation may still be present in the network, but it shows up as damped oscillations instead of hitting a wall and sliding.
- This results in each threshold being "transparent", meaning that trajectories cannot hit a threshold and stay there.
- They may, however, spiral into an asymptotically stable fixed point on a threshold or at an intersection of thresholds.
- Thus, this expansion largely avoids singular flow.

Limiting behaviour of the new model (fast mRNA)

Theorem

Let $q > 0$ and an initial condition $(x_i(0), y_i(0))$ for $i = 1, ...n$ of (5) be fixed. Let y_i^ε, for $i = 1, ..., n$ be a solution for the protein concentrations of the system

$$\dot{x}_i = \frac{1}{\varepsilon}(F_i(Z_1, Z_2, ..., Z_n) - \gamma_i x_i) \tag{6}$$
$$\dot{y}_i = \kappa_i x_i - \beta_i y_i \qquad \text{for } i = 1, ..., n.$$

Then in the limit as $\varepsilon \to 0$, the solution y_i^ε converges uniformly to the solution of the system

$$\dot{y}_i = \frac{\kappa_i}{\gamma_i} F_i(Z_1, Z_2, ..., Z_n) - \beta_i y_i, \quad \text{for } i = 1, .., n,$$

on any finite time interval.

Limiting behaviour of the new model (fast mRNA)

- If our expanded system's new parameters, β_i and κ_i, happen to have the right values, as we take the mRNA rates arbitrarily fast, we recover the dynamics of the unexpanded system on ANY finite time interval.
- **But**, this theorem does **not** give us information regarding the asymptotics of the system for any $\varepsilon > 0$.

Analysis of the expanded Plahte-Kjøglum system

Recall the system of equations studied by Plahte and Kjøglum

$$\dot{x_1} = Z_1 + Z_2 - 2Z_1Z_2 - \gamma_1 x_1$$
$$\dot{x_2} = 1 - Z_1Z_2 - \gamma_2 x_2.$$

Using our new framework, we expand this system to include both protein and mRNA concentrations

$$\begin{aligned} \dot{x_1} &= Z_1 + Z_2 - 2Z_1Z_2 - \gamma_1 x_1 \\ \dot{y_1} &= \kappa_1 x_1 - \beta_1 y_1 \\ \dot{x_2} &= 1 - Z_1Z_2 - \gamma_2 x_2 \\ \dot{y_2} &= \kappa_2 x_2 - \beta_2 y_2 \end{aligned} \tag{7}$$

Analysis of the expanded Plahte-Kjøglum system

Setting the RHS of (7) to zero,

$$\begin{aligned}
\dot{x_1} &= Z_1 + Z_2 - 2Z_1Z_2 - \gamma_1 x_1 = 0 \\
\dot{y_1} &= \kappa_1 x_1 - \beta_1 y_1 = 0 \\
\dot{x_2} &= 1 - Z_1Z_2 - \gamma_2 x_2 = 0 \\
\dot{y_2} &= \kappa_2 x_2 - \beta_2 y_2 = 0,
\end{aligned}$$

then solving for x_i in the $\dot{x_i}$ equation and plugging it into $\dot{y_i}$, we get

$$\begin{aligned}
\dot{y_1} &= \frac{\kappa_1}{\gamma_1}(Z_1 + Z_2 - 2Z_1Z_2) - \beta_1 y_1 = 0 \\
\dot{y_2} &= \frac{\kappa_2}{\gamma_2}(1 - Z_1Z_2) - \beta_2 y_2 = 0.
\end{aligned}$$

Analysis of the expanded Plahte-Kjøglum system

Therefore, fixed points in the fast dynamics at the threshold intersection $y_1 = \theta_1$, $y_2 = \theta_2$ occur when Z_1 and Z_2 satisfy

$$Z_1 + Z_2 - 2Z_1Z_2 - \frac{\beta_1\gamma_1}{\kappa_1}\theta_1 = 0$$

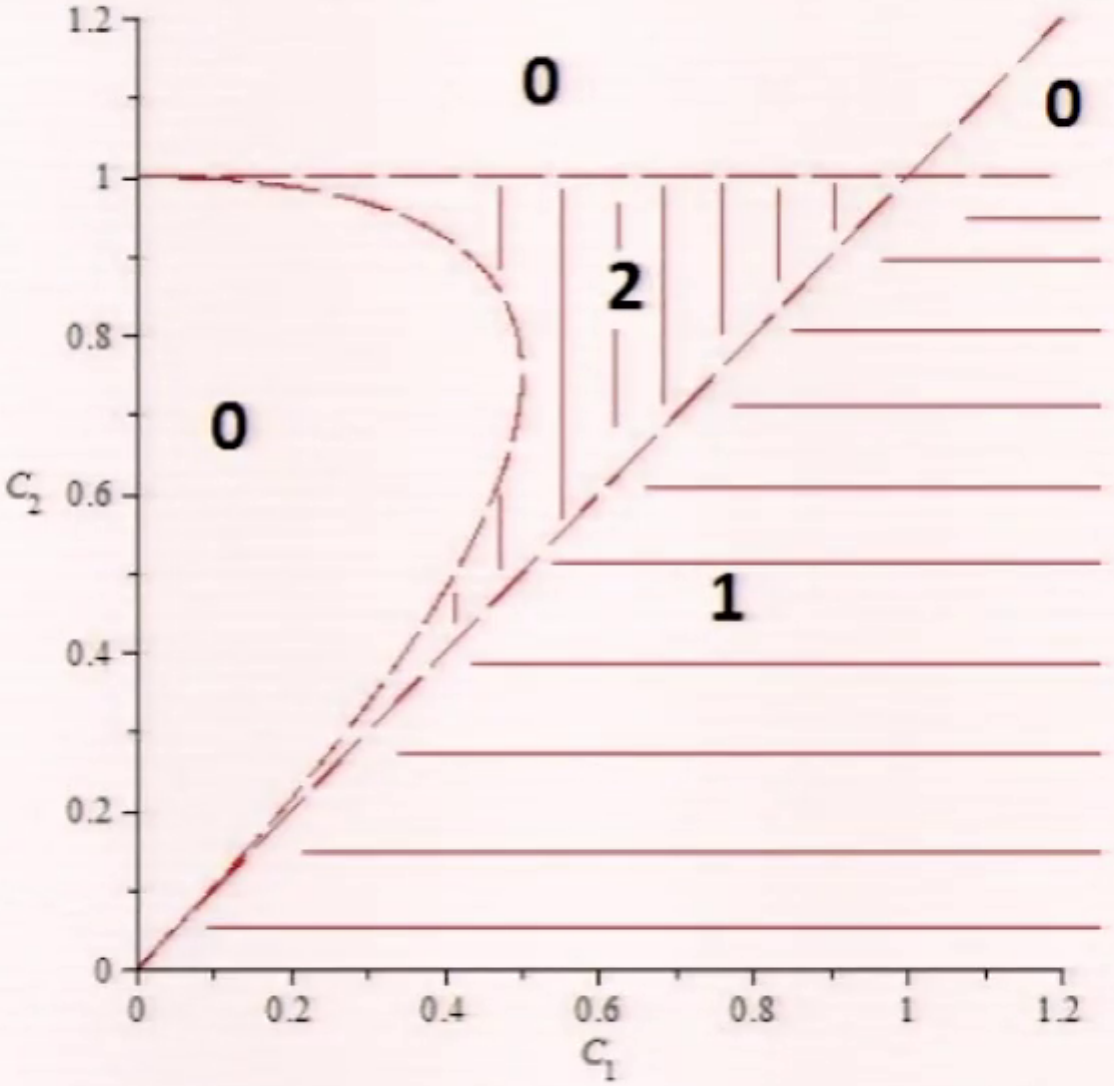
$$1 - Z_1Z_2 - \frac{\beta_2\gamma_2}{\kappa_2}\theta_2 = 0.$$

This is has the same form as the requirement for a fixed point in the original system, except that the parameters in front of the y_i's (θ_i's) are different.

In fact, there are values of the κ_i and β_i for which no fixed point of the fast dynamics exists.

Setting $c_i = \frac{\beta_i\gamma_i}{\kappa_i}\theta_i$ we compute the parameter regions for which there are $0, 1$ or 2 fixed points.

Analysis of the expanded Plahte-Kjøglum system



Analysis of the expanded Plahte-Kjøglum system

- This is the first major difference between the protein and mRNA-protein models.
- Leaving the protein degredation rate the same, the expanded model allows for 0, 1 or 2 fixed points in the threshold intersection.
- Thus for some values of the new parameters, there is no fixed point at the threshold intersection and the macroscopic behaviour must be different.
- These possible differences in structure are suppressed when the gene network is only modeled by a single protein concentration.

Analysis of the expanded Plahte-Kjøglum system

- Taking $\kappa_i = \gamma_i$ with $\beta_1 = 0.6$ and $\beta_2 = 0.9$ we recover the same two protein equations as in the Plahte system with 2 fixed points in the threshold intersection.
- In the protein only model, stability analysis in the threshold intersection is done in the fast time, $\tau = \frac{t}{q}$
- Here however, this approach does not help because there are always slow variables

We therefore need to analyze the system as a whole without taking $q \to 0$.

Analysis of the expanded Plahte-Kjøglum system

The Jacobian at $\mathcal{P} = (x_1^*, x_2^*, Z_1^*, Z_2^*)$ with $q > 0$ is given by,

$$J(\mathcal{P}) = \begin{bmatrix} -\beta_1 & 0 & \frac{\partial \dot{x}_1}{\partial Z_1} & \frac{\partial \dot{x}_1}{\partial Z_2} \\ 0 & -\beta_2 & \frac{\partial \dot{x}_2}{\partial Z_1} & \frac{\partial \dot{x}_2}{\partial Z_2} \\ \frac{A_1}{q} & 0 & \frac{-\alpha_1}{\varepsilon} & 0 \\ 0 & \frac{A_2}{q} & 0 & \frac{-\alpha_2}{\varepsilon} \end{bmatrix}$$

Denoting λ as an eigenvalue of this matrix, we can compute the characteristic polynomial in terms of $\mu = \lambda\sqrt{q}$, where $A_i = \frac{\kappa_i Z_i^*(1-Z_i^*)}{\theta_i\left(\frac{Z_i^*}{1-Z_i^*}\right)^q}$.

Multiplying through by q^2, then taking the limit as $q \to 0$ we obtain the polynomial

$$\mu^4 - \left(\frac{\partial \dot{x}_2}{\partial Z_2}A_2 + \frac{\partial \dot{x}_1}{\partial Z_1}A_1\right)\mu^2 - A_1A_2\left(\frac{\partial \dot{x}_2}{\partial Z_1}\frac{\partial \dot{x}_1}{\partial Z_2} - \frac{\partial \dot{x}_2}{\partial Z_2}\frac{\partial \dot{x}_1}{\partial Z_1}\right) = 0.$$

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- We note that the roots of (8) come in $\pm$ pairs
- Since roots of polynomials depend continuously on their coefficents, we can make the roots of the characteristic polynomial in μ as close to the roots of (8) as we wish by taking q small enough.
- Therefore, if (8) has a root with a non-zero real part, then we can find a $\delta > 0$ such that the characteristic polynomial in μ has a root with non-zero real part for all $0 < q < \delta$.
- Since the roots of (8) come in $\pm$ pairs, μ has a root with positive real part for all $0 < q < \delta$, and λ is a positive scalar multiple of μ.
- Therefore, if we have a root of (8) with a non-zero real part, then we can find a $\delta > 0$ such that the fixed point $\mathcal{P} = (x_1^*, x_2^*, Z_1^*, Z_2^*)$ is unstable for all $0 < q < \delta$.

Analysis of the expanded Plahte-Kjøglum system

- This analysis does not tell us when a fixed point is stable, but it gives us an easy way to test if it is unstable.
- If the roots of (8) are all pure imaginary, then we are unable to say for sure whether the fixed point is stable or unstable.
- Asymptotic spiralling approach to a threshold intersection is possible; this may correspond to the above case.
- Apart from such special cases, expanding the model of gene regulatory networks in this way generally has a destabilizing effect on fixed points in co-dimension 2 or higher switching domains.

Numerics for the expanded Plahte-Kjøglum example

If we compute the polynomial (8) in the expanded Plahte-Kjøglum example, the first fixed point gives the (rounded) roots

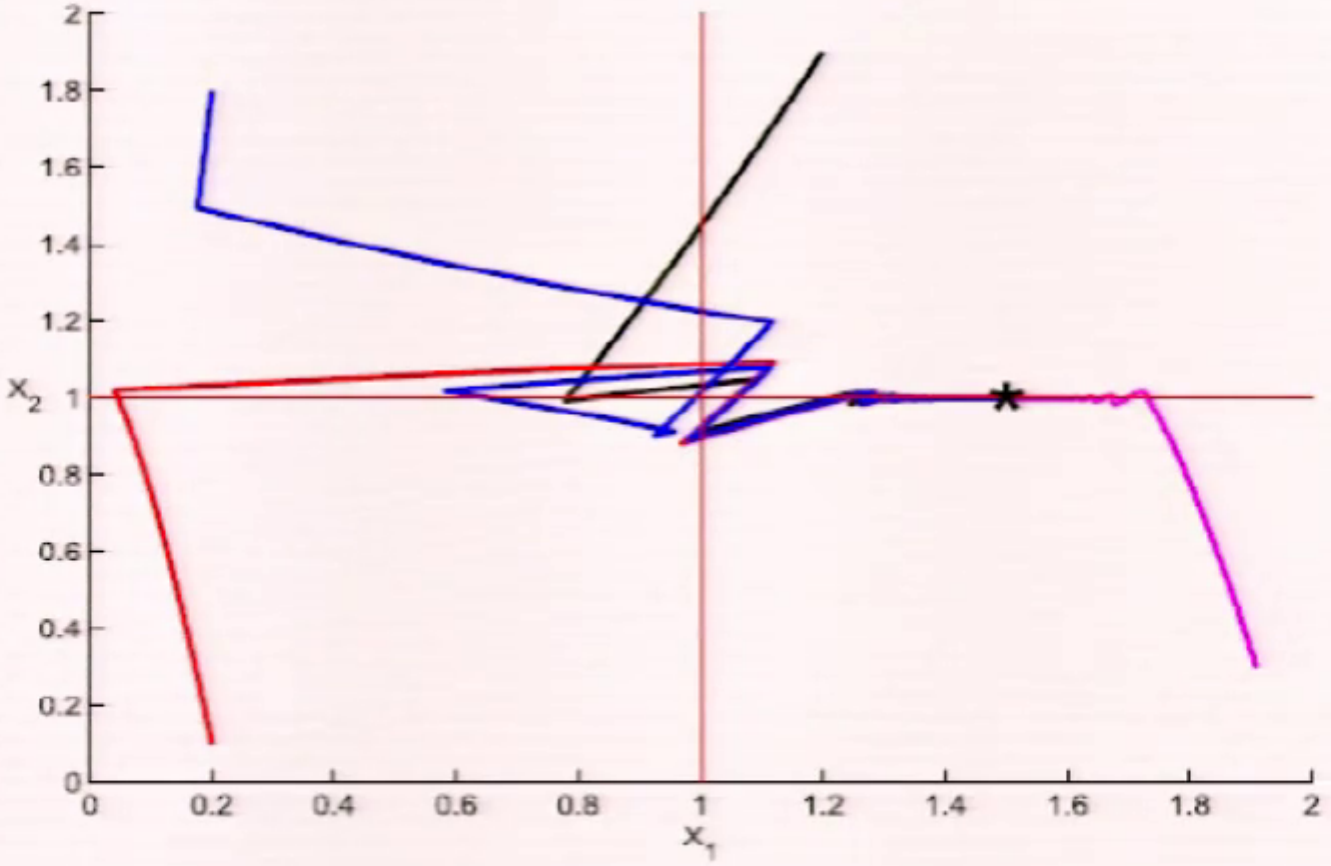
$$\mu_1 = 0.28321i$$
$$\mu_2 = -0.28321i$$
$$\mu_3 = 0.31456$$
$$\mu_4 = -0.31456,$$

and the second gives

$$\mu_1 = 0.17325 - 2.4305i$$
$$\mu_2 = -0.17325 + 2.4305i$$
$$\mu_3 = 0.17325 + 2.4305i$$
$$\mu_4 = -0.17325 - 2.4305i.$$

Numerics for the expanded Plahte-Kjøglum example

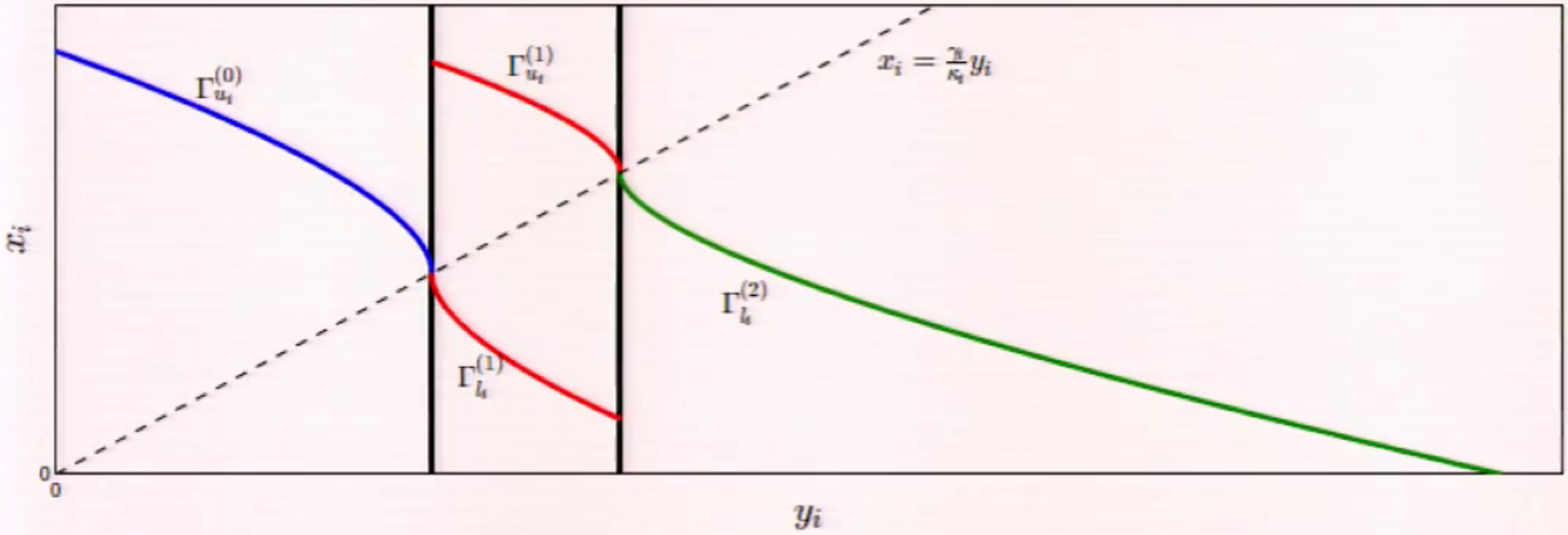
Thus, even with parameters that give us the same protein equations as in the original system, both fixed points in the threshold intersection of the expanded system are unstable. Further analysis shows that the fixed point on the right wall is asymptotically stable.



Non-uniqueness again

The mRNA-protein model allows new behaviours:

- Flow crosses walls in both directions, but each wall can be partitioned into smaller domains to preserve unique flow direction.
- There are solutions that graze the wall from both sides at the same point, leading to non-uniqueness again!



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- The problem of singular flow results from modelling choices.
- Including mRNA and protein avoids singular flow.
- However, sensitive behaviour near threshold intersections can cause drastic changes to macroscopic behaviour of solutions.
- Even if mRNA dynamics is much faster than protein dynamics, suppressing it can lead to misleading conclusions.
- The mRNA-protein model can be analyzed, but still allows non-uniqueness in the step-function limit.
- Expanding other non-smooth systems in this way might be a useful mathematical tool for analyzing dynamics.