Engineering Genetic Circuits

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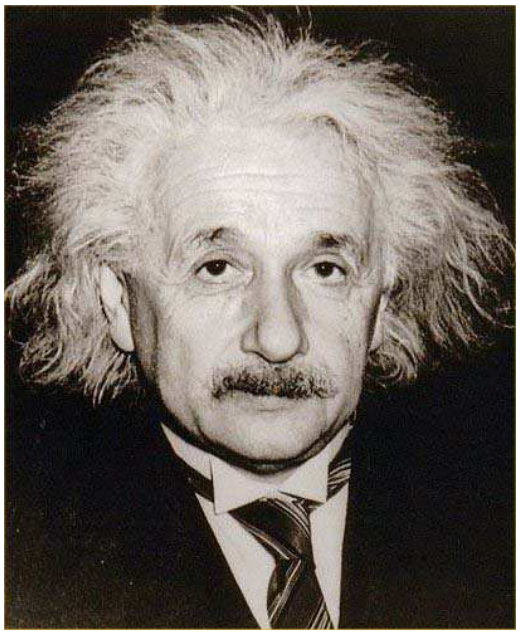
Lecture 6: Differential Equation Analysis

Albert Einstein



Yes, we have to divide up our time like that, between our politics and our equations. But to me our equations are far more important, for politics are only a matter of present concern. A mathematical equation stands forever.

Introduction

- Next step of the engineering approach is analysis.
- Goal of analysis is to be able to both reproduce experimental results and make predictions *in silico*.
- Simulation provides unlimited controllability and observability.
- Traditional *classical chemical kinetics* (CCK) utilizes *ordinary differential equations* (ODE) to represent system dynamics.
- Law of mass action can translate a chemical reaction model into ODEs known as *reaction rate equations*.
- ODEs typically analyzed using numerical simulation.
- Qualitative analysis utilized to understand behavior as initial conditions and parameter values vary.
- *Partial differential equations* (PDE) utilized when spatial considerations are important.

Overview

- Classical chemical kinetics
- Differential equation simulation
- Qualitative ODE analysis
- Spatial methods

A Classical Chemical Kinetic Model

- A CCK model tracks concentrations of each chemical species (i.e., number of molecules divided by volume, Ω, of cell or compartment).
- Assumes reactions occur in a *well-stirred* volume (i.e., molecules are equally distributed within the cell) and *spatial effects* can be neglected.
- Assumes reactions occur continuously and deterministically.
- Requires that the number of molecules of each species are large.

A Classical Chemical Kinetic Model (cont)

- A CCK model is composed of n chemical species $\{S_1, \ldots, S_n\}$ and m chemical reaction channels $\{R_1, \ldots, R_m\}$.
- Each reaction R_j has the following form:

$$v^r_{1j} S_1 + \ldots + v^r_{nj} S_n \quad \underset{k_r}{\overset{k_f}{\rightleftarrows}} \quad v^p_{1j} S_1 + \ldots + v^p_{nj} S_n$$

  where v^r_{ij} is the stoichiometry for species S_i as a reactant in reaction R_j and v^p_{ij} is the stoichiometry for species S_i as a product in reaction R_j.
- The values of v^r_{ij} and/or v^p_{ij} are 0 when species S_i does not participate as a reactant and/or product in reaction R_j.
- Parameter k_f is *forward rate constant* while k_r is *reverse rate constant*.
- If the reaction is *irreversible*, then k_r is 0.

A Classical Chemical Kinetic Model (cont)

- Law of mass action states that the rate of an irreversible reaction is proportional to the product of concentrations of reactant molecules.
- The rate of a reversible reaction is also reduced by a value proportional to the product of the concentrations of product molecules.
- Formally, the reaction rate V_j for reaction R_j is defined as follows:

$$V_j = k_f \prod_{i=1}^{n} [S_i]^{v_{ij}^r} - k_r \prod_{i=1}^{n} [S_i]^{v_{ij}^p}$$

  where $[S_i]$ is the concentration of species S_i.
- An ODE model can be constructed as follows:

$$\frac{d[S_i]}{dt} = \sum_{j=1}^{m} v_{ij} V_j, \quad 1 \leq i \leq n$$

  where $v_{ij} = v_{ij}^p - v_{ij}^r$ (i.e., the net change in species S_i due to reaction R_j).
- CCK model consists of one ODE for each species which is sum of the rates of change of species due to each reaction that affects the species.

ODE Model Example

$$
\begin{aligned}
\mathrm{CI} &\xrightarrow{k_d} () \\
\mathrm{CII} &\xrightarrow{k_d} () \\
P_{RE} + \mathrm{RNAP} &\xleftrightarrow{K_{o1}} S1 \\
P_R + \mathrm{RNAP} &\xleftrightarrow{K_{o2}} S2 \\
S1 &\xrightarrow{k_b} S1 + np\ \mathrm{CI} \\
S2 &\xrightarrow{k_o} S2 + np\ \mathrm{CII} \\
2\mathrm{CI} &\xleftrightarrow{K_d} \mathrm{CI}_2 \\
P_R + nc\ \mathrm{CI}_2 &\xleftrightarrow{K_r} S3 \\
P_{RE} + na\ \mathrm{CII} + \mathrm{RNAP} &\xleftrightarrow{K_a} S4 \\
S4 &\xrightarrow{k_a} S4 + np\ \mathrm{CI}
\end{aligned}
$$

Constant	*Value*
RNAP_0	30 nM
$K_d = k_{df}/k_{dr}$	$0.1 M^{-1}$
$K_{o1} = k_{o1f}/k_{o1r}$	0.01 M^{-1}
$K_{o2} = k_{o2f}/k_{o2r}$	0.69422 M^{-1}
$K_r = k_{rf}/k_{rr}$	0.2165 M^{-nc}
$K_a = k_{af}/k_{ar}$	0.00161 $M^{-(na+1)}$
k_o	0.014 sec^{-1}
k_b	0.00004 sec^{-1}
k_a	0.015 sec^{-1}
k_d	0.0075 sec^{-1}
nc	1
na	1
np	10

ODE Model Example

$$\frac{d[CI]}{dt} = np\,k_b[S1] + np\,k_a[S4] - 2(k_{df}[CI]^2 - k_{dr}[CI_2]) - k_d[CI]$$

$$\frac{d[CI_2]}{dt} = k_{df}[CI]^2 - k_{dr}[CI_2] - nc(k_{rf}[P_R][CI_2]^{nc} - k_{rr}[S3])$$

$$\frac{d[CII]}{dt} = np\,k_o[S2] - na(k_{af}[P_{RE}][RNAP][CII]^{na} - k_{ar}[S4]) - k_d[CII]$$

$$\frac{d[P_R]}{dt} = k_{o2r}[S2] - k_{o2f}[P_R][RNAP] + k_{rr}[S3] - k_{rf}[P_R][CI_2]^{nc}$$

$$\frac{d[P_{RE}]}{dt} = k_{o1r}[S1] - k_{o1f}[P_{RE}][RNAP] + k_{ar}[S4] - k_{af}[P_{RE}][RNAP][CII]^{na}$$

$$\frac{d[RNAP]}{dt} = k_{o1r}[S1] - k_{o1f}[P_{RE}][RNAP] + k_{o2r}[S2] - k_{o2f}[P_R][RNAP] + k_{ar}[S4] - k_{af}[P_{RE}][RNAP][CII]^{na}$$

$$\frac{d[S1]}{dt} = k_{o1f}[P_{RE}][RNAP] - k_{o1r}[S1]$$

$$\frac{d[S2]}{dt} = k_{o2f}[P_R][RNAP] - k_{o2r}[S2]$$

$$\frac{d[S3]}{dt} = k_{rf}[P_R][CI_2]^{nc} - k_{rr}[S3]$$

$$\frac{d[S4]}{dt} = k_{af}[P_{RE}][RNAP][CII]^{na} - k_{ar}[S4]$$

Differential Equation Simulation

- The differential equations for a set of reaction rate equations are:

$$\frac{dx_i}{dt} = f_i(\mathbf{x}), \text{ where } 1 \leq i \leq n$$

  where $\mathbf{x} = [x_1, \ldots, x_n] \geq \mathbf{0}$ is vector of species concentrations.
- Solving this ODE model analytically is very difficult, if not impossible.
- Numerical simulation can approximate time evolution of $\mathbf{X}(t)$ assuming $\mathbf{X}(t_0) = \mathbf{x}_0$ (*initial value problem*).

Euler's Method

- Simplest approach to solve the initial value problem is *Euler's method*.
- Initial instantaneous rate of change for each S_i at time t_0:

$$\frac{dX_i(t_0)}{dt} = f_i(\mathbf{x}_0), \text{ where } 1 \leq i \leq n.$$

- If the rate of change, $f_i(\mathbf{X}(t))$, remains constant for all $t \geq t_0$, then $X_i(t) = \mathbf{x}_{0i} + f_i(\mathbf{x}_0)(t - t_0)$.
- Not true in general, but may be reasonable to assume value remains close to $f_i(\mathbf{x}_0)$ for some small time step Δt (*step size*).

Forward Euler Method

- With this assumption,

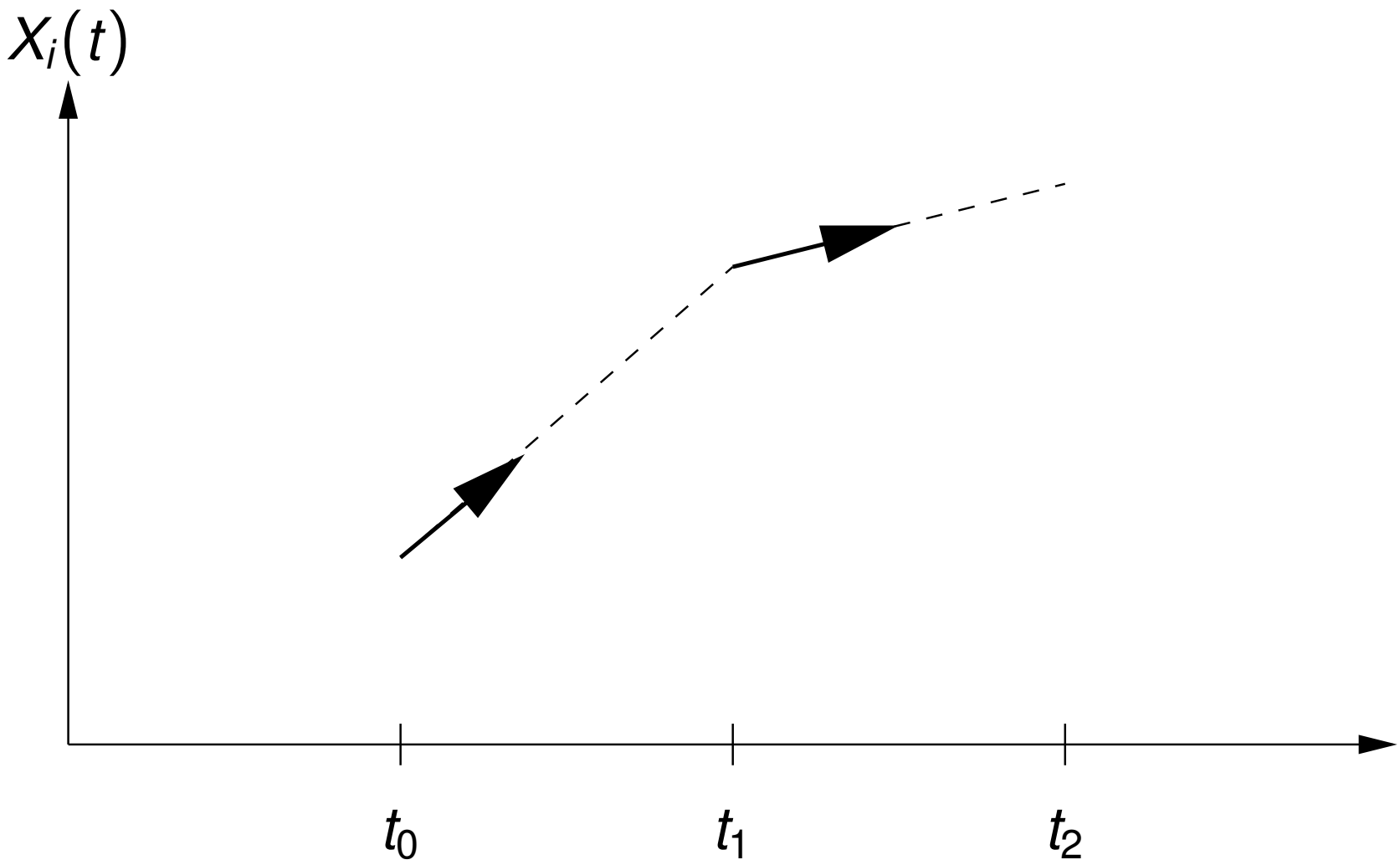
$$X_i(t_1) \approx X_i(t_0) + f_i(\mathbf{X}(t_0))\Delta t$$

- In general, for any $t_j = t_0 + n\Delta t$ where $j = 0, 1, 2, 3, \ldots$:

$$X_i(t_{j+1}) \approx X_i(t_j) + f_i(\mathbf{X}(t_j))\Delta t$$

- This algorithm is known as the *forward Euler Method*, and it is an example of an *explicit* ODE simulation method.

Forward Euler Method



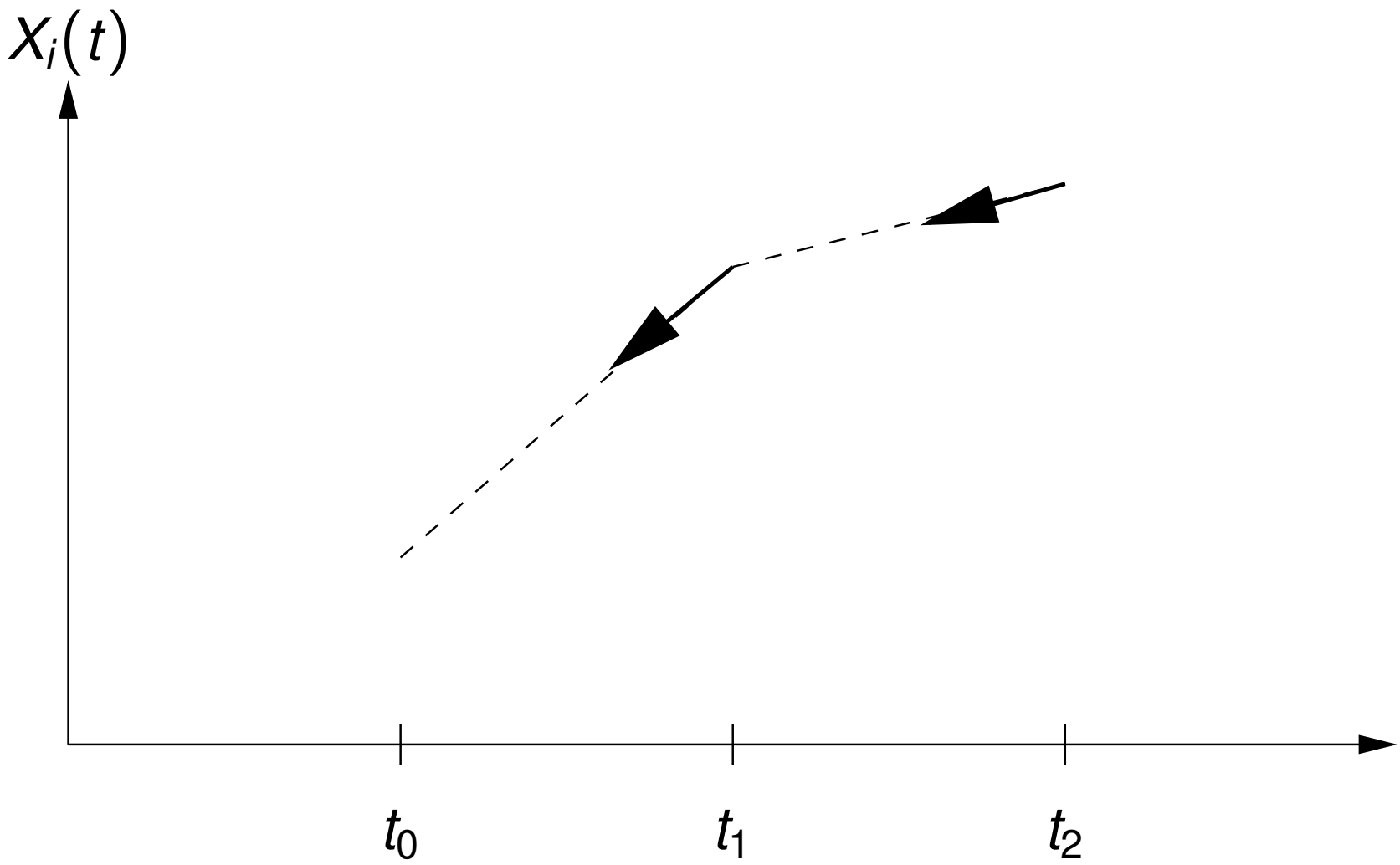
Backward Euler Method

- *Backward Euler method* is an *implicit* ODE simulation method.
- New rate of change cannot be determined directly from current state, but it must be found implicitly by an equation that must be solved.
- Backward Euler method is defined by follows:

$$X_i(t_{j+1}) \approx X_i(t_j) + f_i(\mathbf{X}(t_{j+1}))\Delta t.$$

- New value of $\mathbf{X}(t_{j+1})$ is determined as the rate at the point that would have taken you there in a Δt step.
- Since $\mathbf{X}(t_{j+1})$ is not yet known, this equation must be solved using a root finding technique such as the Newton-Raphson method.
- Implicit methods are more complicated, but often more stable for *stiff equations* (i.e., those that require a very small time step).

Backward Euler Method



Drawback of Euler's Method

- Using the fundamental theorem of calculus, exact solution for each species, S_i, must satisfy:

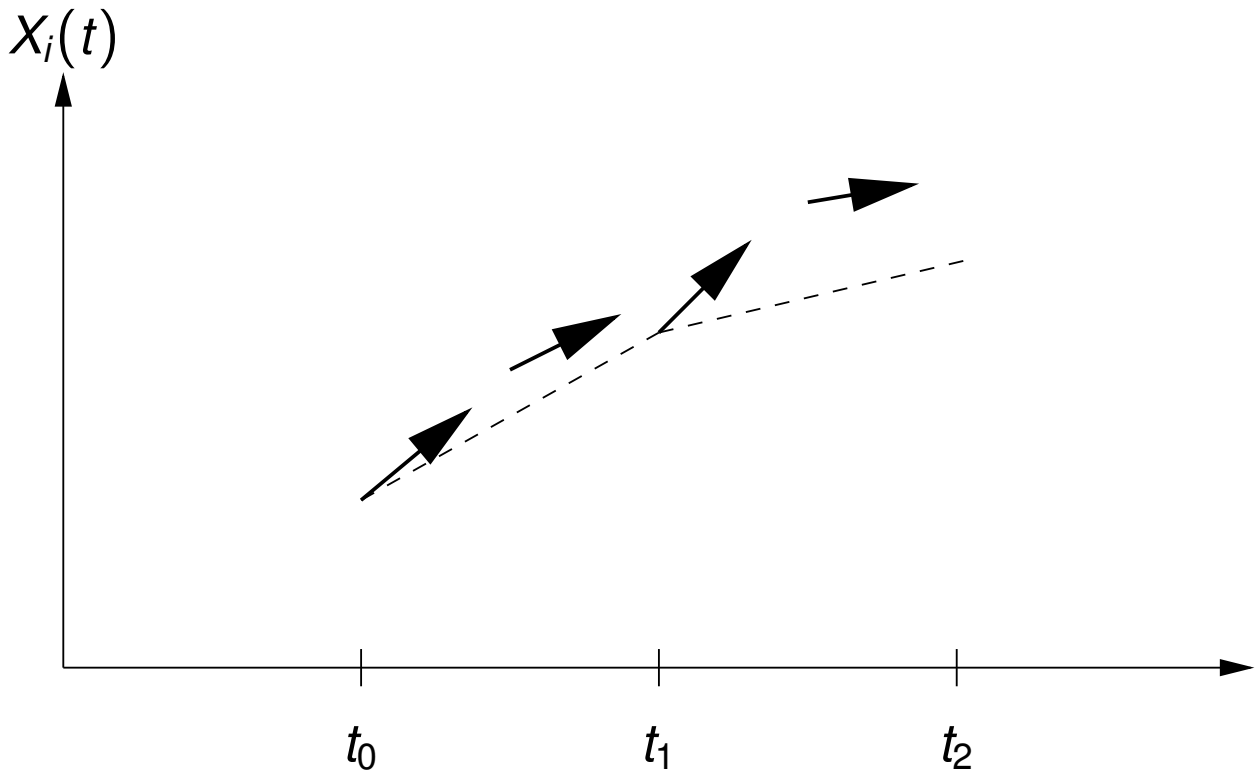
$$X_i(t_{j+1}) = X_i(t_j) + \int_{t_j}^{t_{j+1}} f_i(\mathbf{X}(t))dt$$

- Drawback of Euler methods is they approximate this integral by assuming that $f_i(\mathbf{X}(t))$ is constant throughout the entire Δt interval from t_j to t_{j+1}.

Midpoint Method (Second-Order Runge-Kutta)

- Approximates rate of change in Δt interval using rate at the midpoint:

$$X_i(t_{j+1}) \approx X_i(t_j) + \left[f_i(\mathbf{X}(t_j) + \frac{1}{2} f(\mathbf{X}(t_j))\Delta t) \right] \Delta t$$



Fourth-Order Runge-Kutta

- More points can be combined to further improve accuracy:

$$
\begin{aligned}
\alpha_1 &= f(\mathbf{X}(t_j)) \\
\alpha_2 &= f\left(\mathbf{X}(t_j) + \frac{\Delta t}{2}\alpha_1\right) \\
\alpha_3 &= f\left(\mathbf{X}(t_j) + \frac{\Delta t}{2}\alpha_2\right) \\
\alpha_4 &= f\left(\mathbf{X}(t_j) + \Delta t\alpha_3\right) \\
\mathbf{X}(t_{j+1}) &= \mathbf{X}(t_j) + \frac{\Delta t}{6}\left[\alpha_1 + 2\alpha_2 + 2\alpha_3 + \alpha_4\right]
\end{aligned}
$$

- Implicit Runge-Kutta methods can also be used for stiff equations.

Adaptive Stepsize Control

- To obtain good results, should modify Δt during the simulation.
- Simulation should slow down when rates are changing rapidly and speed up when the rates are changing slowly.
- With the *step doubling* approach, $\mathbf{X}(t_{j+1})$, is found in one Δt step and $\mathbf{X}'(t_{j+1})$ is found by taking two $\Delta t/2$ steps.
- Estimate of the error for each species:

$$E_i = |X_i'(t_{j+1}) - X_i(t_{j+1})|$$

- Goal of adaptive stepsize control is to achieve a desired error level:

$$D_i = abs + rel \cdot |X_i(t_{j+1})|$$

where *abs* is *absolute error level* and *rel* is *relative error level*.

Adaptive Stepsize Control (cont)

- If for any species, E_i, exceeds D_i by more than 10%, simulation step should be performed again using a new stepsize:

$$\Delta t \quad = \quad 0.9 \cdot \Delta t \cdot \left(\frac{D}{E} \right)^q$$

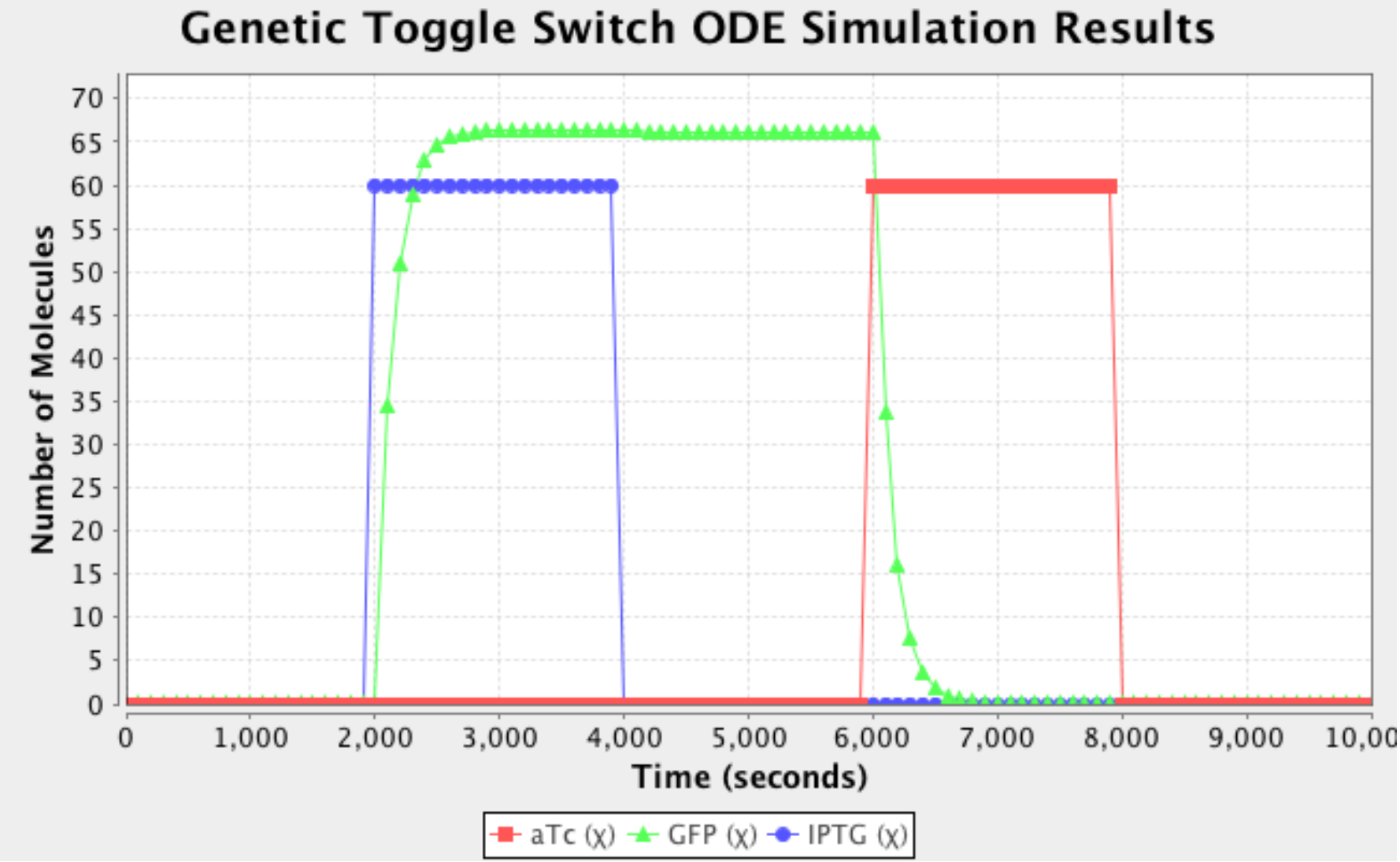
  where D/E is the minimum of the ratios D_i/E_i and q is the order of the method (i.e., 4 for a fourth-order Runge-Kutta method).

- If for all species, E_i is less than 50% of D_i, stepsize can be increased:

$$\Delta t \quad = \quad 0.9 \cdot \Delta t \cdot \left(\frac{D}{E} \right)^{(q+1)}$$

- Previous simulation step can be accepted and the state found by taking half steps, $\mathbf{X}'(t_{j+1})$, can be used.

ODE Simulation Results



Assignment #5

- Perform ODE simulation on your genetic toggle switch model.
  - Set the initial value of LacI (in all models) to 60 molecules.
  - Add events that set IPTG to 60 at 2000s, IPTG to 0 at 4000s, aTc to 60 at 6000s, and aTc to 0 at 8000s.
  - Perform ODE simulation for 10000s and compare with the expected toggle simulation results. If it does not match, correct your model until they do.
  - Upload an archive of your project to `https://synbiohub.utah.edu`, and provide a share link.
- Perform ODE simulation on your paper's genetic circuit model
  - Set initial conditions/parameters and add events to test your genetic circuit model and update your model as needed to get the results you expect.
  - Upload an archive of your project to `https://synbiohub.utah.edu`, and provide a share link.

Assignment #5 (cont)

- Use the law of mass action to create an ODE model for the reactions given below.
- Simulate this ODE model using the Forward Euler method and Fourth Order Runge-kutta for 1 second with a 0.2 second time step.
- Comment on any differences in results using these two ODE simulation methods.
- You may either do this simulation by hand, using a spreadsheet, or writing a simple program.
- Submit all your work.

$$\begin{aligned}
\text{LacI} &\xrightarrow{k_d} () \\
\text{TetR} &\xrightarrow{k_d} () \\
pLac &\xrightarrow{k_o} np\ \text{TetR} \\
pTet &\xrightarrow{k_o} np\ \text{LacI} \\
pLac + nc\ \text{LacI} &\xleftrightarrow{K_r} pLacLacI \\
pTet + nc\ \text{TetR} &\xleftrightarrow{K_r} pTetTetR
\end{aligned}$$

Constant	*Value*
$K_r = k_{rf}/k_{rr}$	$(0.1/1.0)\ M^{-nc}$
k_o	$0.1\ \text{sec}^{-1}$
k_d	$0.1\ \text{sec}^{-1}$
nc	2
np	10
$pLac$	1
$pTet$	1

Qualitative ODE Analysis

- Goal of ODE analysis is to determine properties of the *phase space*.
- Phase space is the set of all possible *states* of the system.
- States are values and current rates of change of each variable.
- Numerical simulation only shows one trajectory in the phase space starting in a given initial condition with specific parameter values.
- Goal of *qualitative ODE analysis* is to determine the complete phase space based upon any initial condition.
- Can also discover how parameter variation affects the phase space.

One-Dimensional ODE Models

- A *one-dimensional ODE model* with a single parameter, r:

$$\frac{dx}{dt} = f(x, r)$$

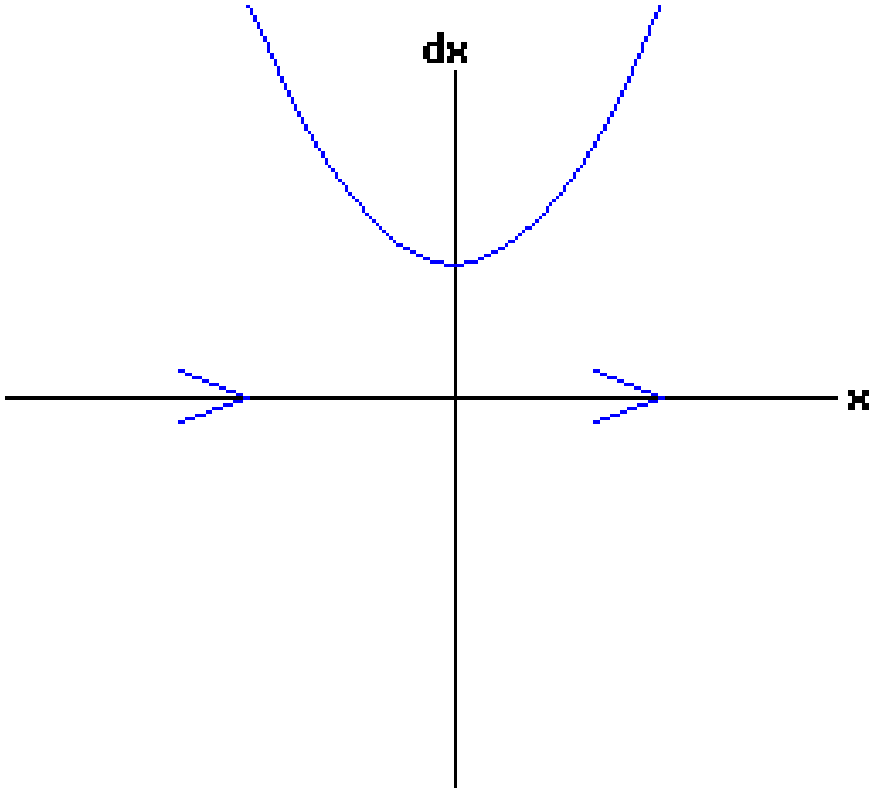
- A state for such a model includes only the value of x and its current rate of change (or *flow*) of x, $\frac{dx}{dt}$.
- Can visualize phase space with a graph of x versus $\frac{dx}{dt}$.

Saddle-Node Example

$$\frac{dx}{dt} = -r + x^2$$

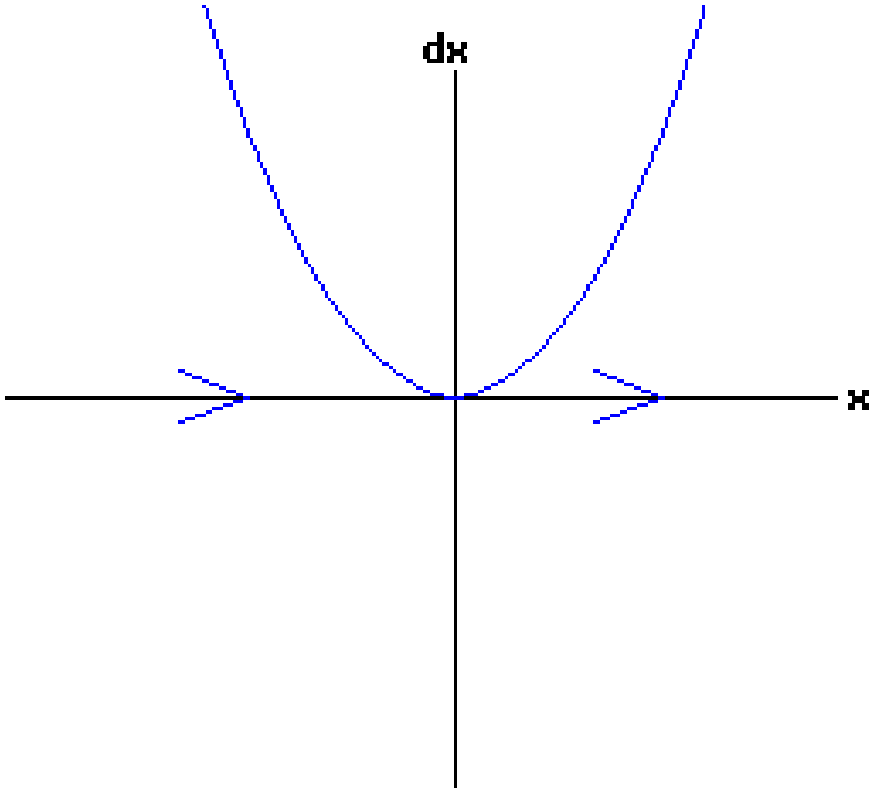
Saddle-Node Example ($r < 0$)

$$\frac{dx}{dt} = -r + x^2$$



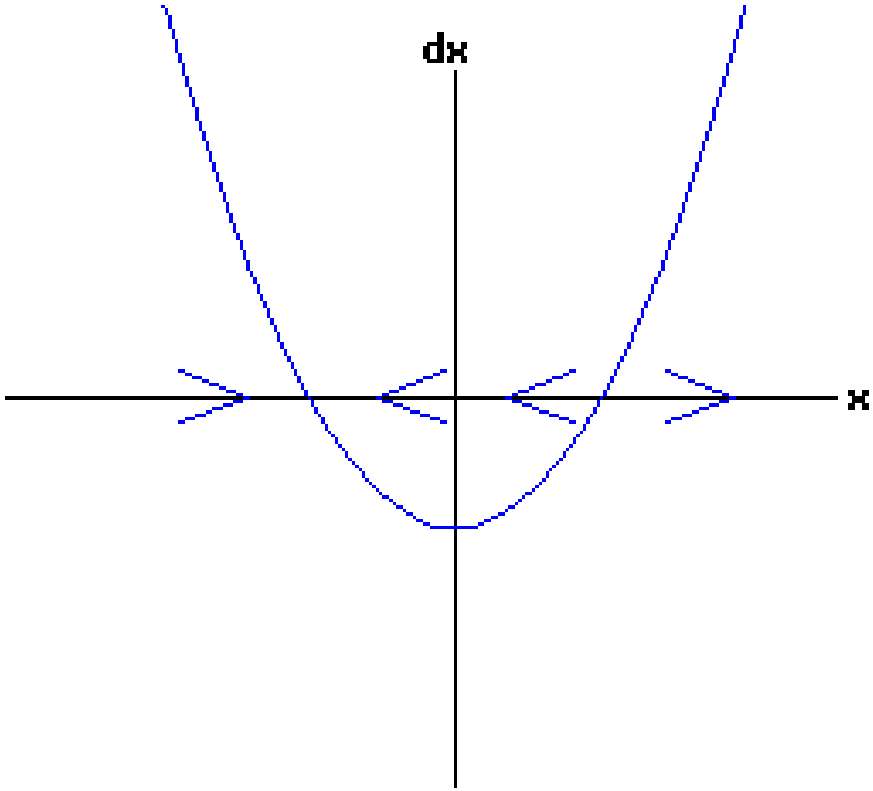
Saddle-Node Example ($r = 0$)

$$\frac{dx}{dt} = -r + x^2$$



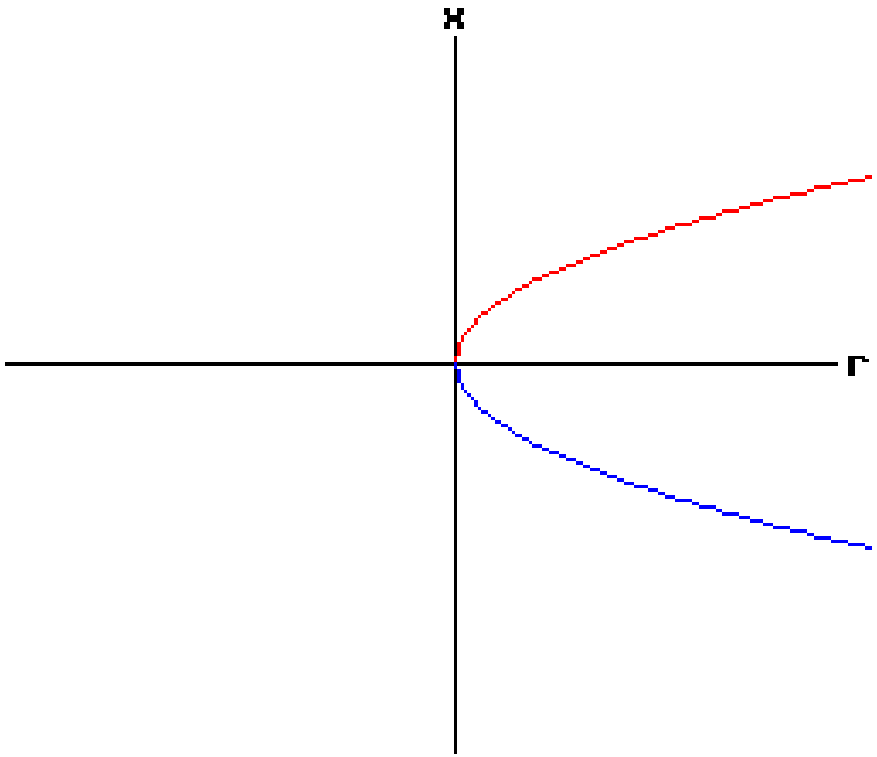
Saddle-Node Example ($r > 0$)

$$\frac{dx}{dt} = -r + x^2$$



Saddle-Node Example (Bifurcation Diagram)

$$\frac{dx}{dt} = -r + x^2$$

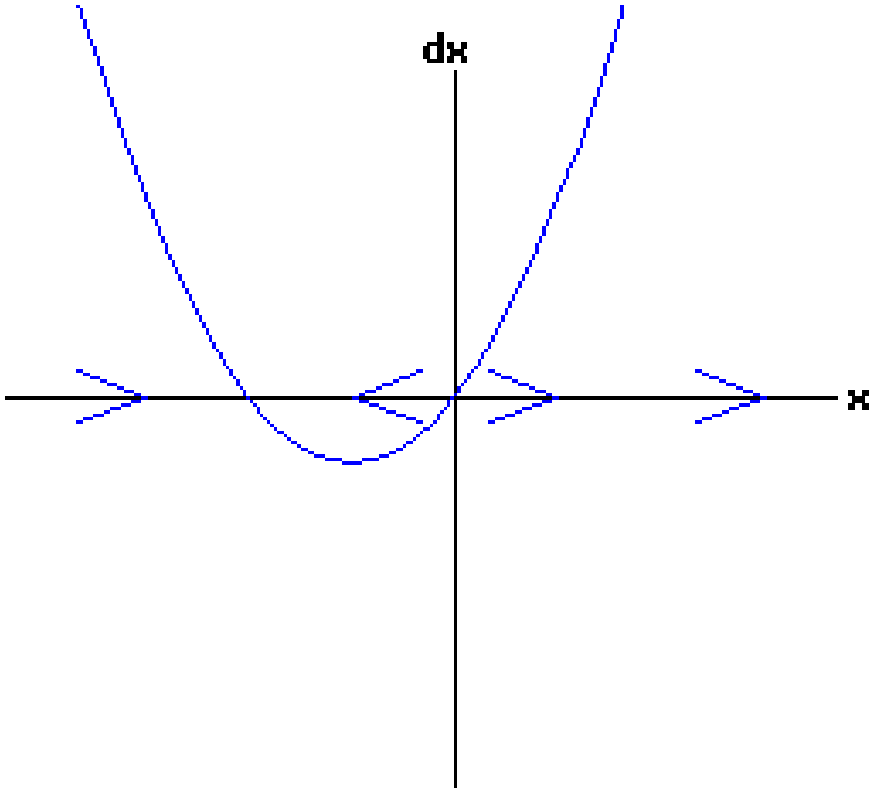


Transcritical Bifurcation Example

$$\frac{dx}{dt} = -rx + x^2$$

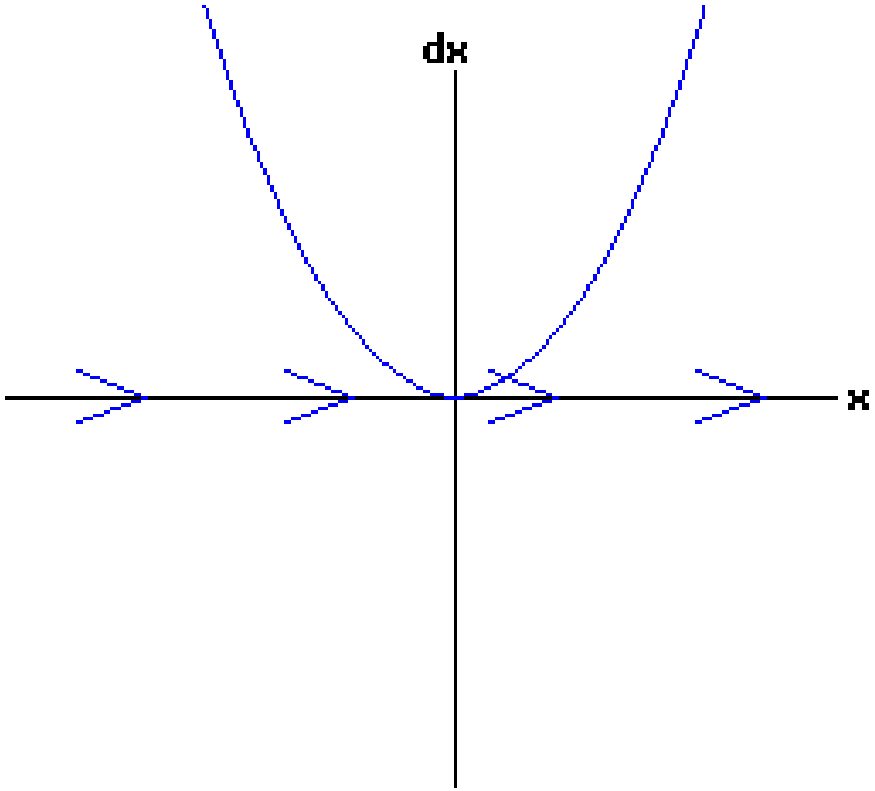
Transcritical Bifurcation Example ($r < 0$)

$$\frac{dx}{dt} = -rx + x^2$$



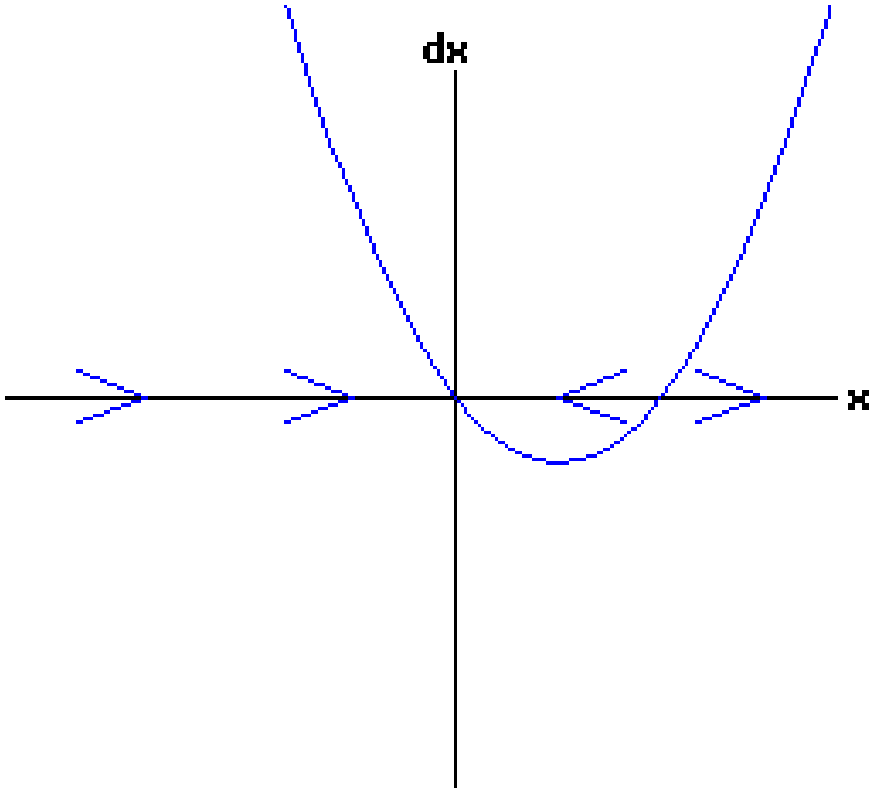
Transcritical Bifurcation Example ($r = 0$)

$$\frac{dx}{dt} = -rx + x^2$$



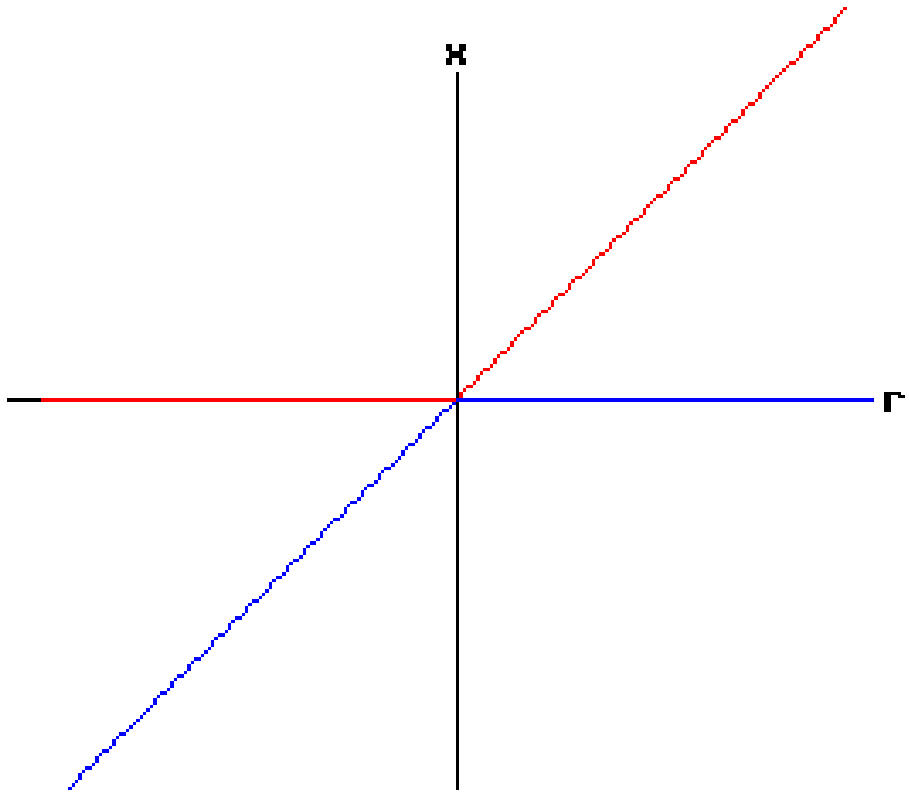
Transcritical Bifurcation Example ($r > 0$)

$$\frac{dx}{dt} = -rx + x^2$$



Transcritical Bifurcation Example (Bifurcation Diagram)

$$\frac{dx}{dt} = -rx + x^2$$

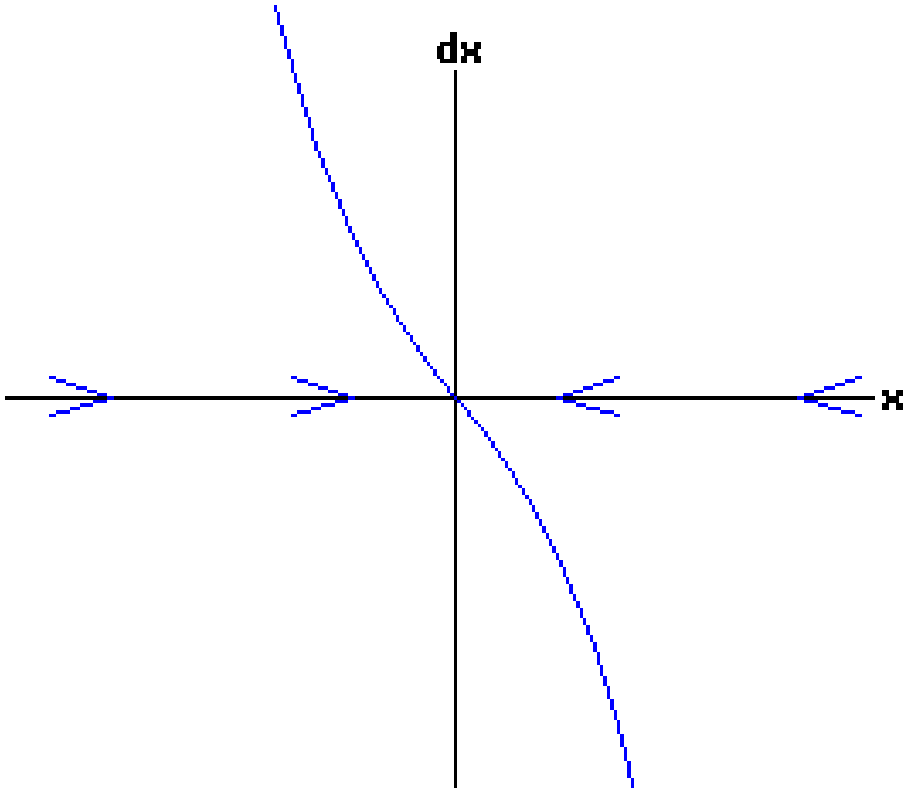


Pitchfork Bifurcation Example

$$\frac{dx}{dt} = rx - x^3$$

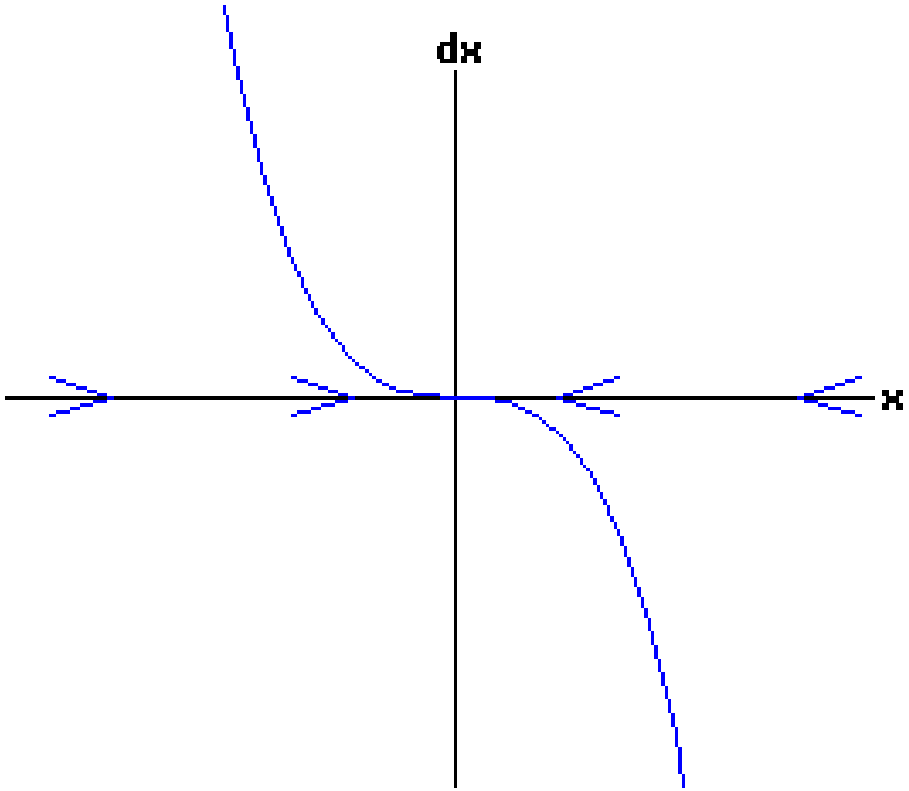
Pitchfork Bifurcation Example ($r < 0$)

$$\frac{dx}{dt} = rx - x^3$$



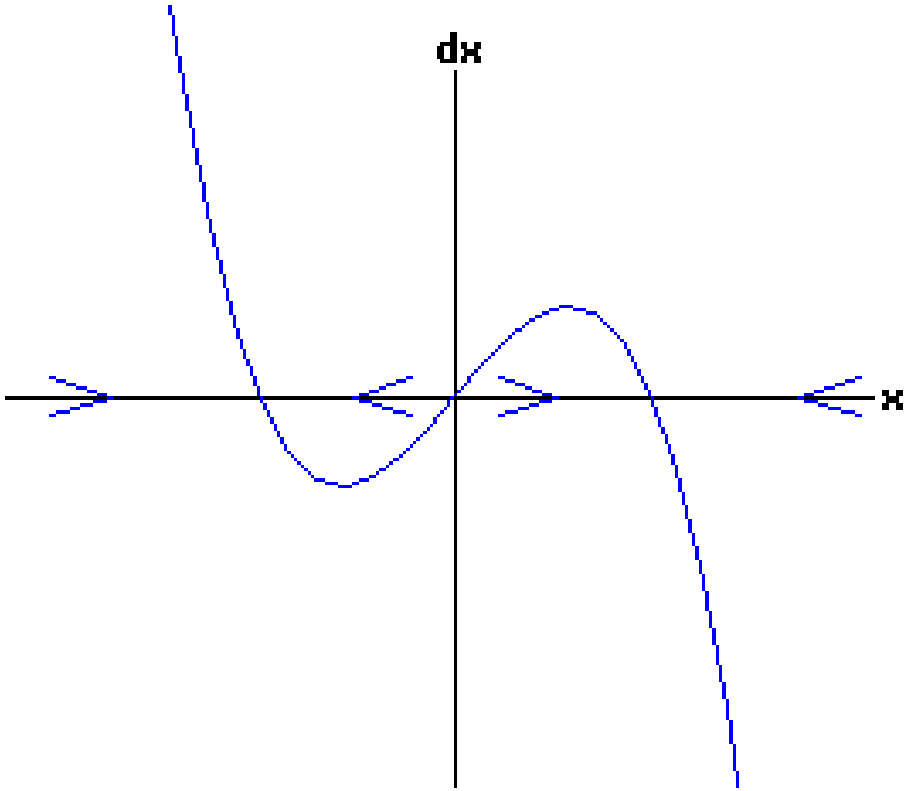
Pitchfork Bifurcation Example ($r = 0$)

$$\frac{dx}{dt} = rx - x^3$$



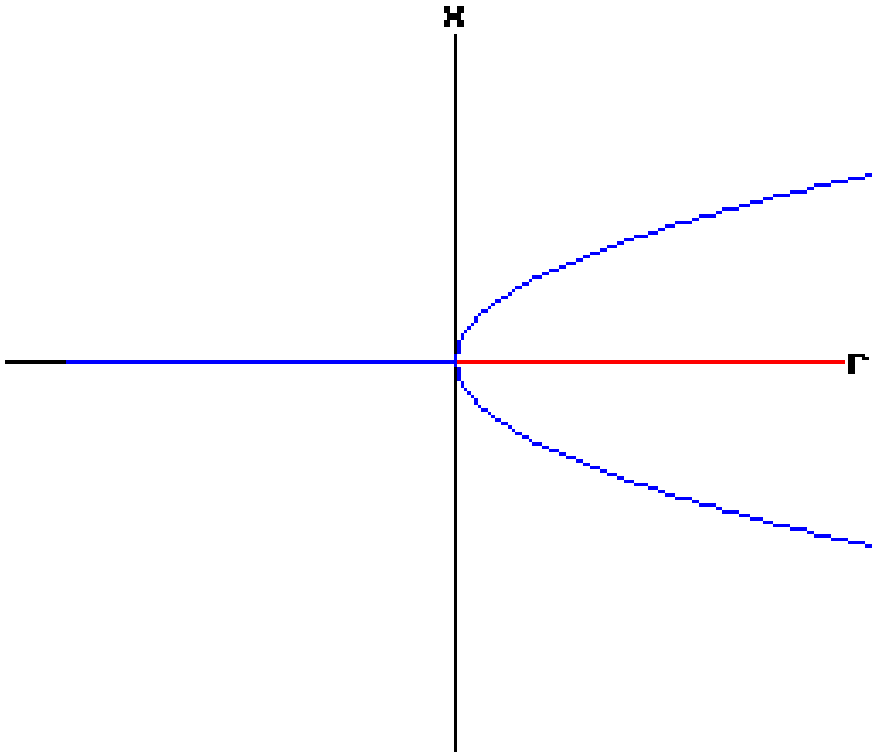
Pitchfork Bifurcation Example ($r > 0$)

$$\frac{dx}{dt} = rx - x^3$$



Pitchfork Bifurcation Example (Bifurcation Diagram)

$$\frac{dx}{dt} = rx - x^3$$



Two-Dimensional ODE Model

- A *two-dimensional ODE model* has the following form:

$$\begin{aligned} \frac{dx}{dt} &= f(x,y) \\ \frac{dy}{dt} &= g(x,y) \end{aligned}$$

- Now four state variables (i.e., values of x, y, and their flows).
- Graphical technique uses *vector fields* in which values of x and y are the axes and a vector assigned to each point to indicate direction of flow.
- Graph is constructed by first determining the *nullclines* (i.e., lines in which the flow of a variable is zero):

$$\begin{aligned} 0 &= f(x,y) \\ 0 &= g(x,y) \end{aligned}$$

- Vectors drawn at various points to indicate the direction of flow.

Two-Dimensional ODE Model (cont)

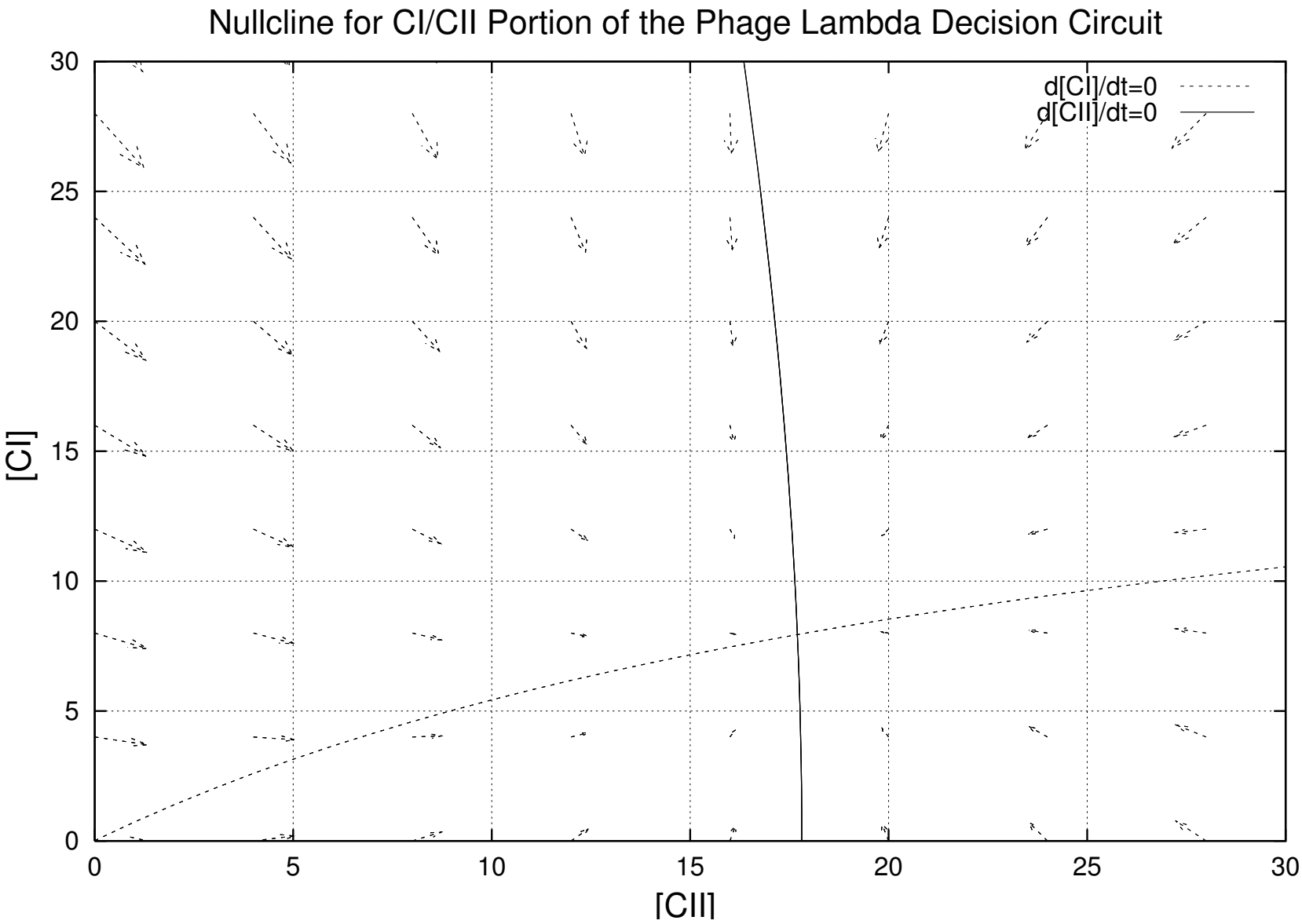
- Points in which two nullclines intersect are equilibrium points.
- Stability can be determined by looking at the flows in the vicinity of the equilibrium point.
- Qualitative ODE analysis for systems of more than 2 dimensions is difficult, so one often reduces dimensionality to 2.

Two Dimensional Model for CI/CII Portion of Phage λ

$$\frac{d[CI]}{dt} = \frac{np\ P_{RE} RNAP(k_b K_{o1} + k_a K_a [CII])}{1 + K_{o1} RNAP + K_a RNAP[CII]} - k_d[CI]$$

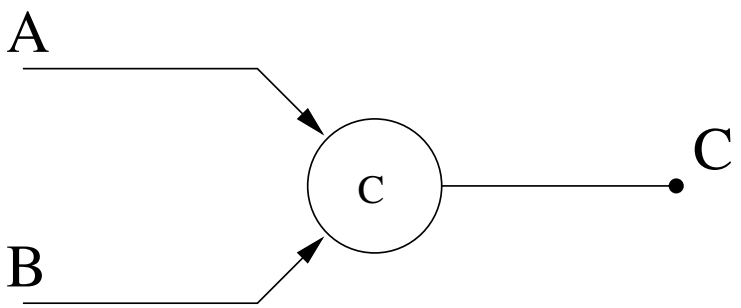
$$\frac{d[CII]}{dt} = \frac{np\ k_o P_R K_{o2} RNAP}{1 + K_{o2} RNAP + K_r K_d [CI]^2} - k_d[CII]$$

Nullcline for the CI/CII Portion of Phage λ



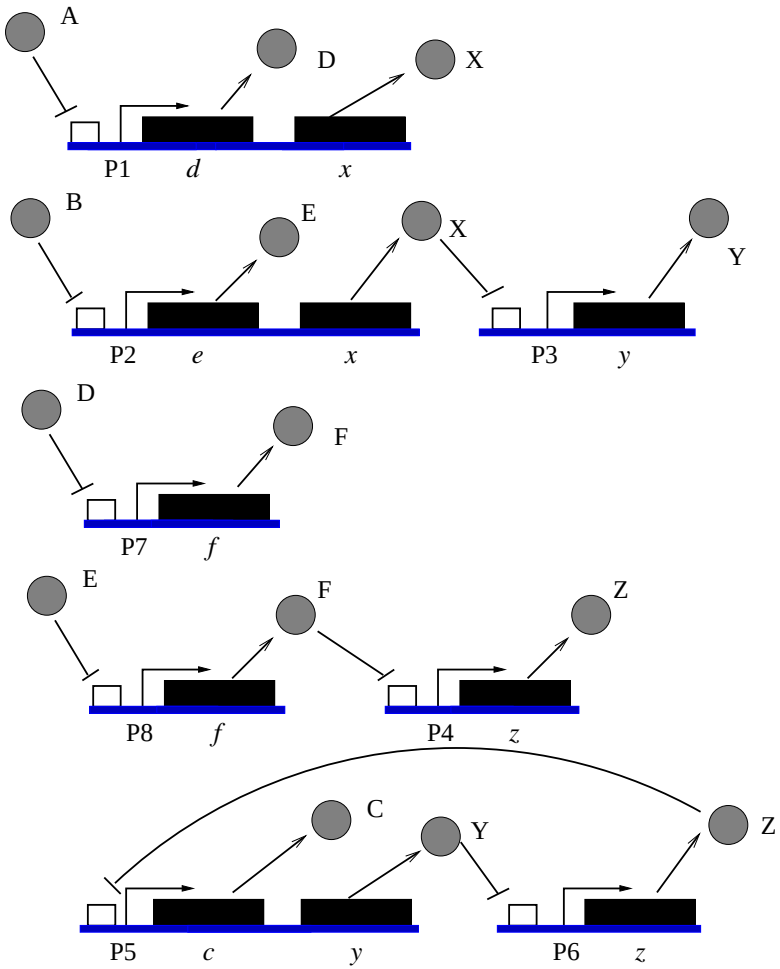
Genetic Muller C-Element

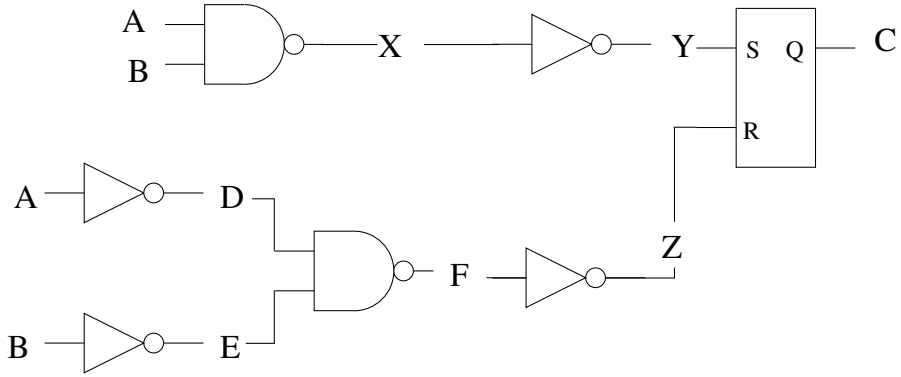
- A Muller C-element is a state holding gate common in many asynchronous design methods that is used to synchronize multiple independent processes.
- A genetic Muller C-element would allow for the design of any asynchronous FSM.

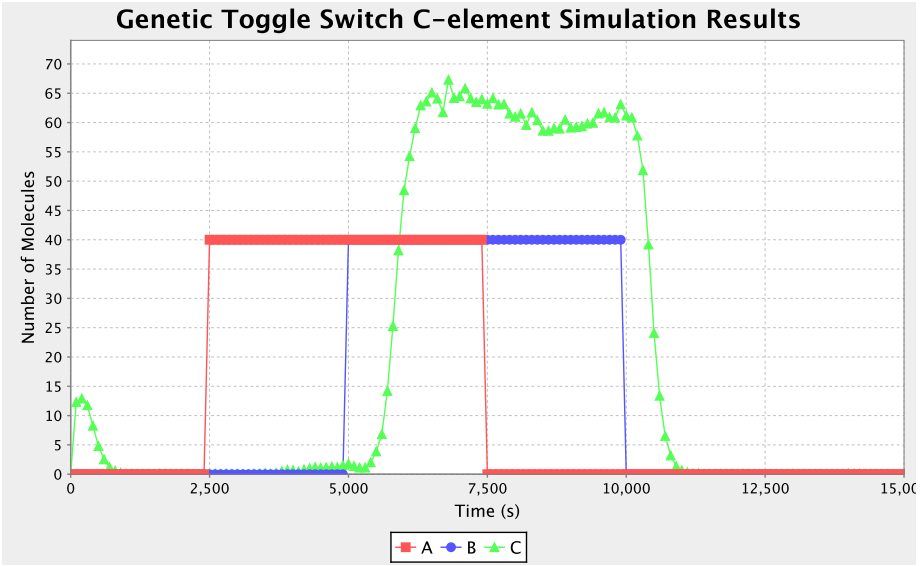


A	B	C
0	0	0
0	1	C
1	0	C
1	1	1

Genetic Toggle Switch Muller C-Element



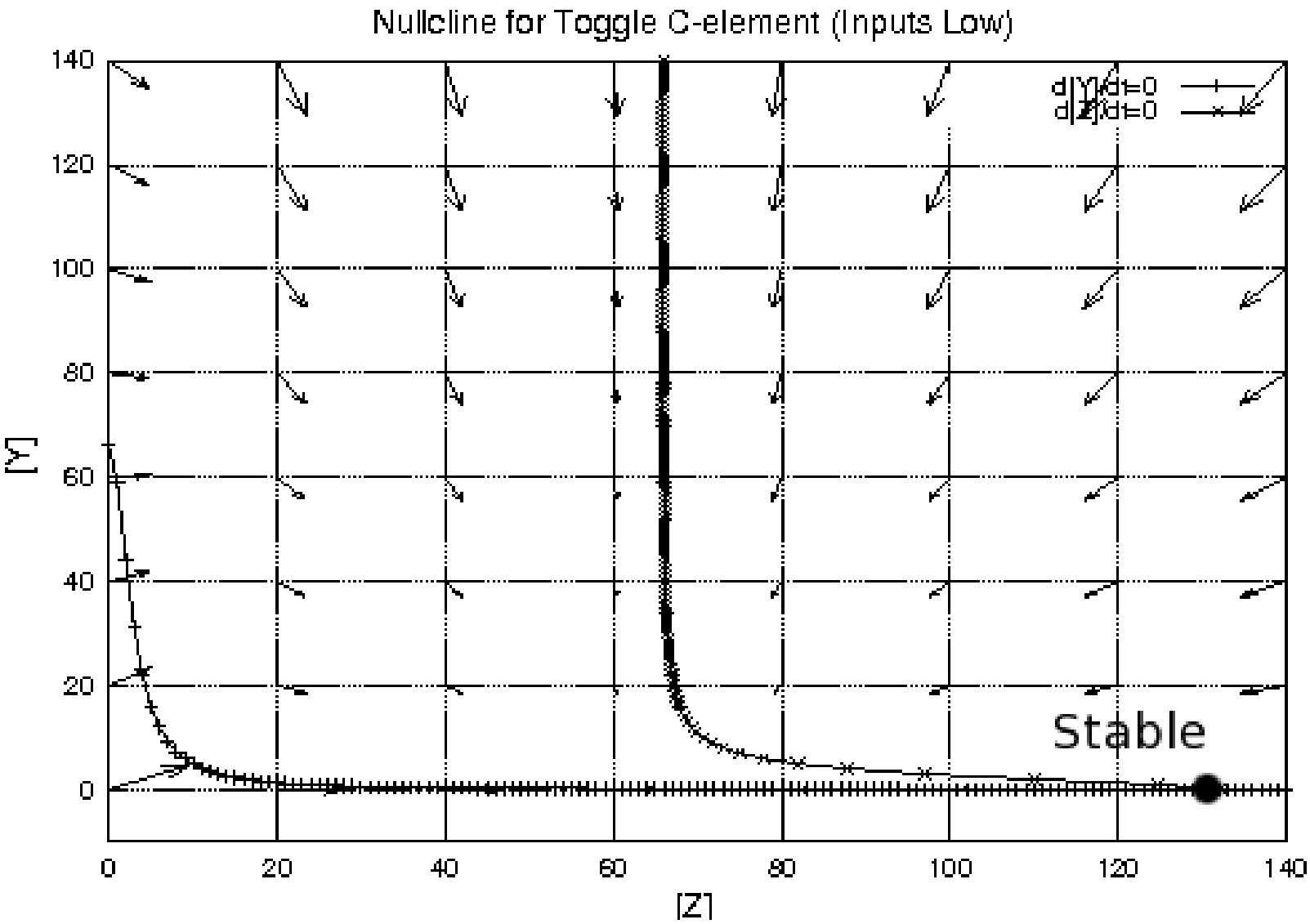




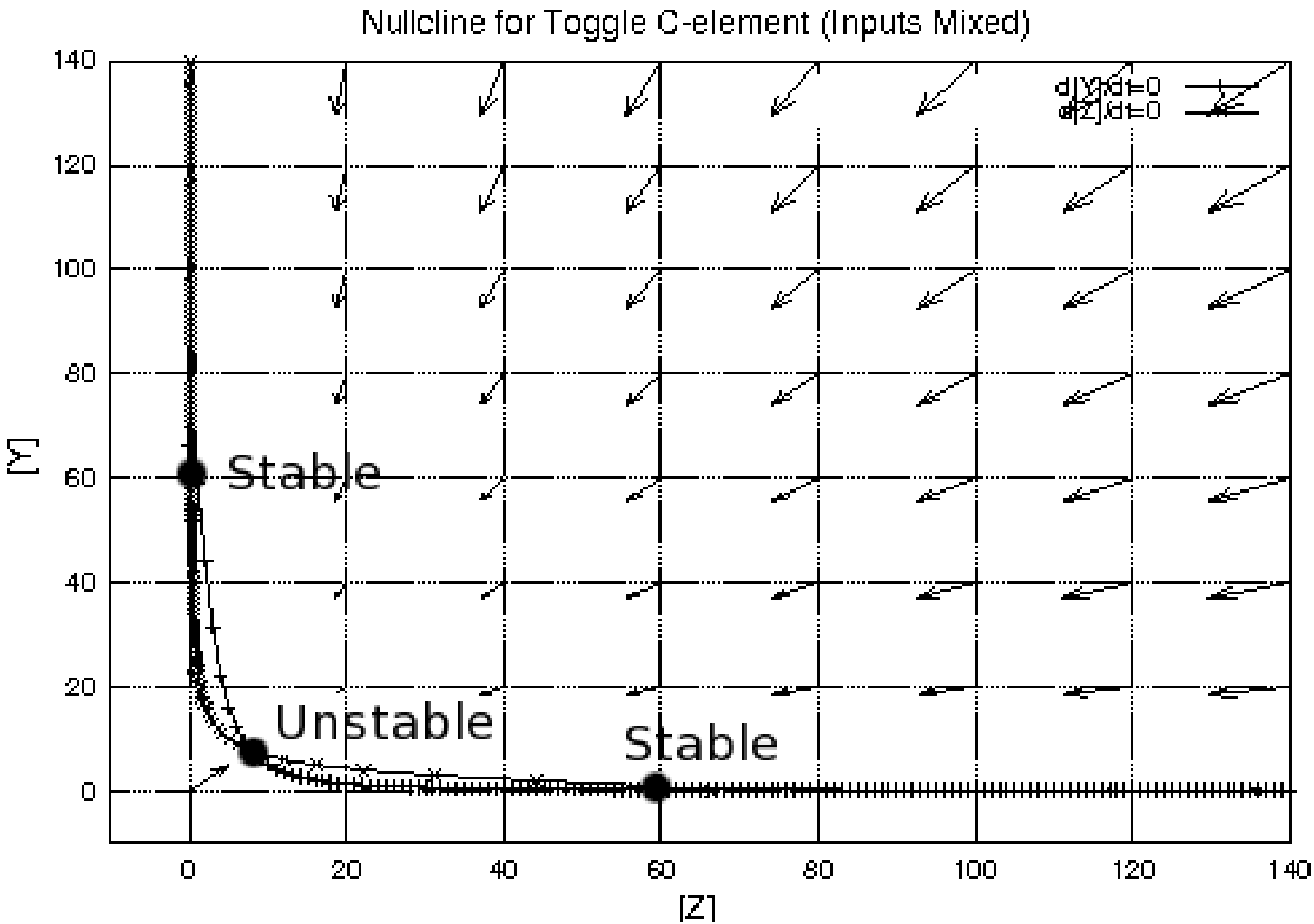
Nguyen et al., 13th Symposium on Async. Ckts. & Sys., 2007 (**best paper**)

Nguyen et al., Journal of Theoretical Biology, 2010.

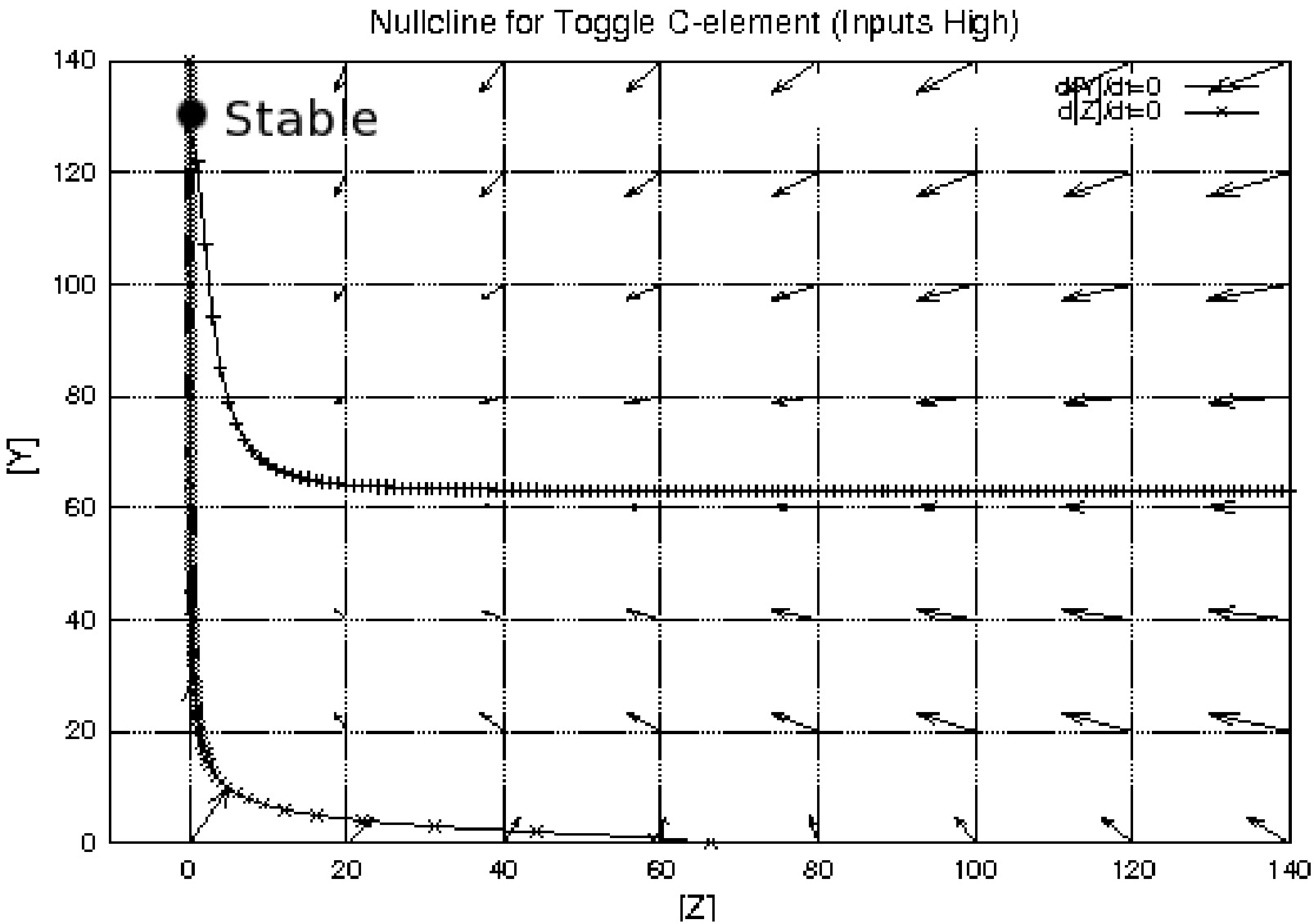
Nullcline Analysis for the Genetic Toggle C-element



Nullcline Analysis for the Genetic Toggle C-element



Nullcline Analysis for the Genetic Toggle C-element



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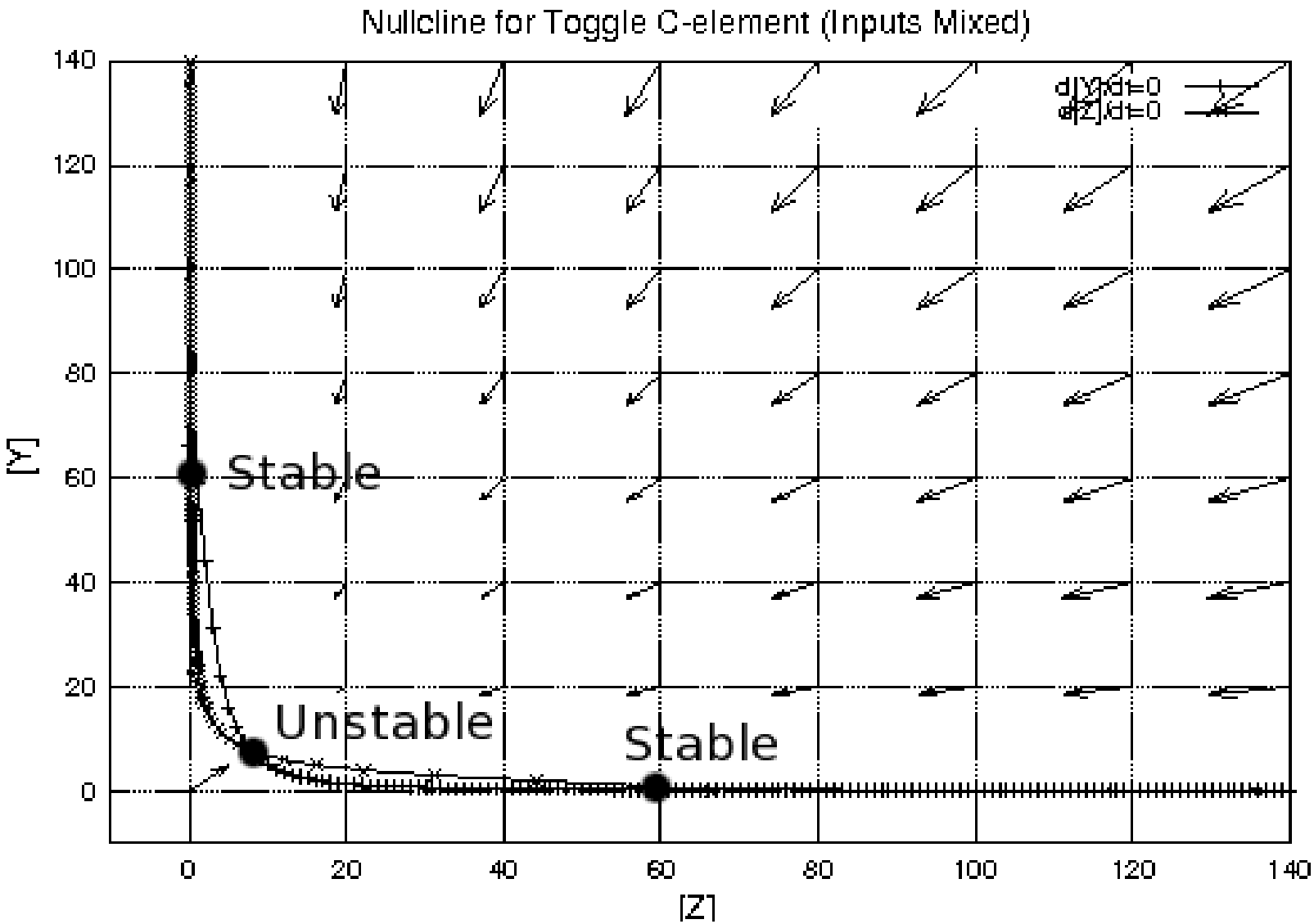

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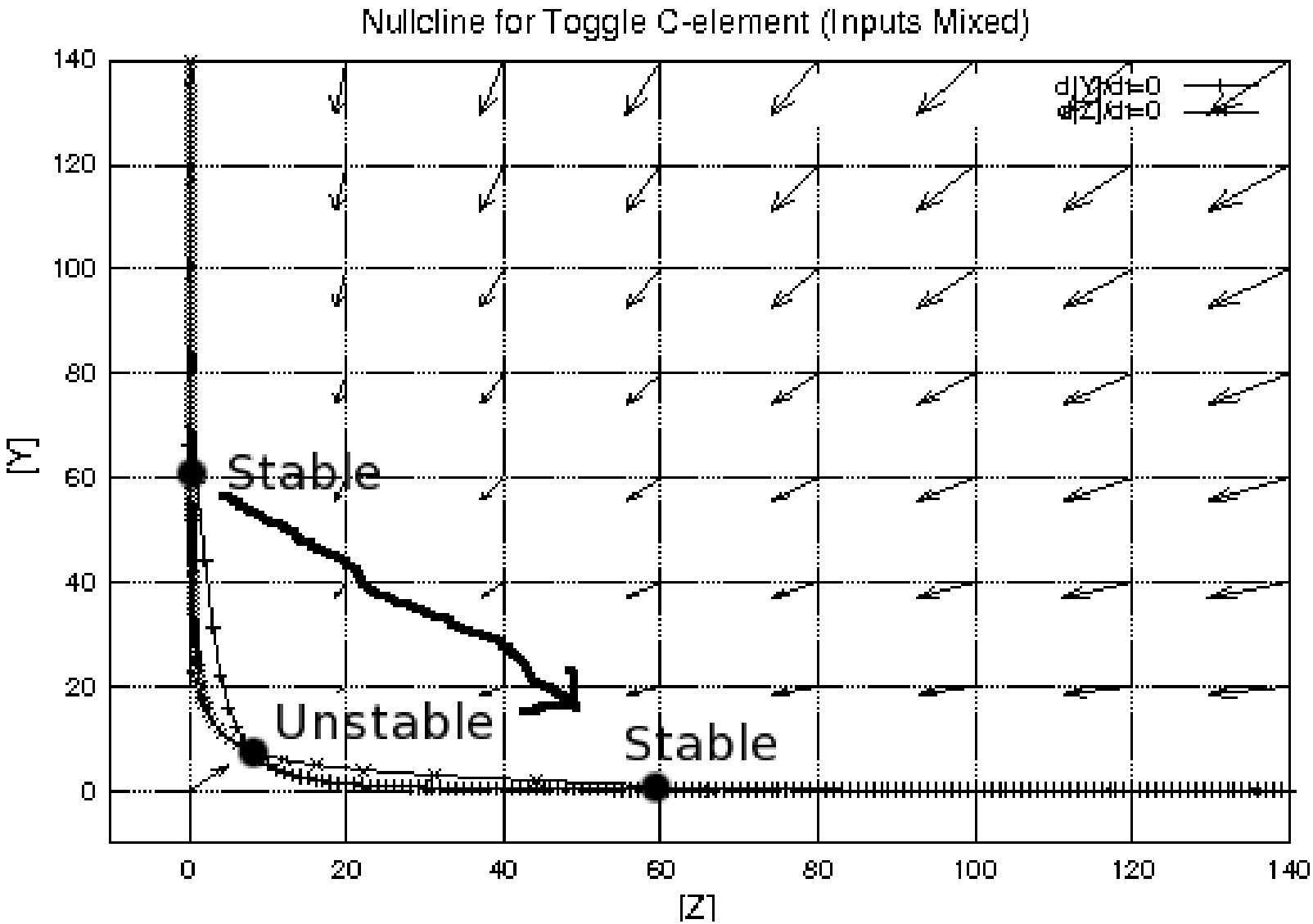
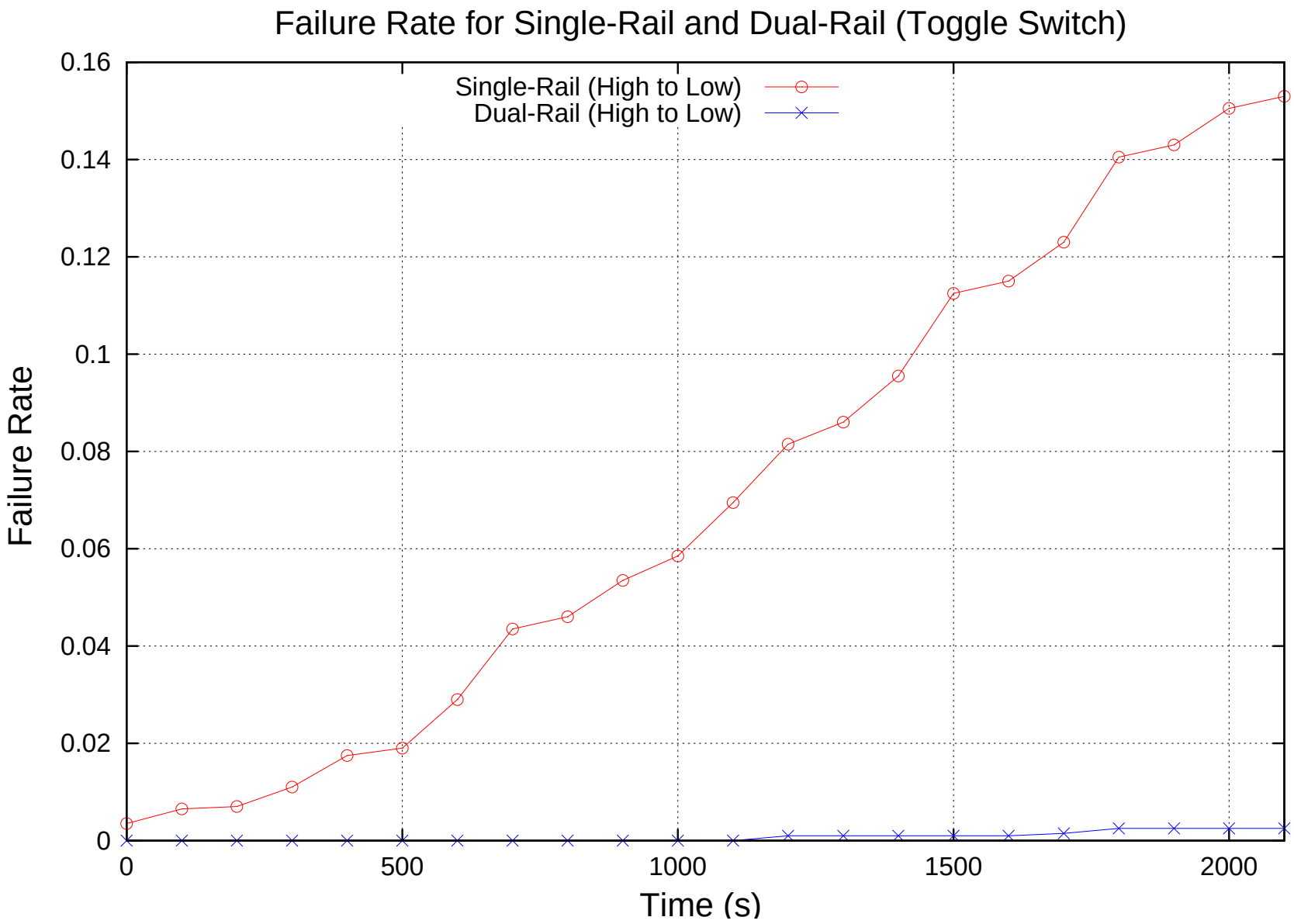


Failure Rate for Single-Rail and Dual-Rail



Spatial Methods

- ODE models assume spatial homogeneity.
- Delays due to diffusion may be important.
- Examples:
  - Diffusion between the nucleus and cytoplasm or other compartments.
  - Cell differentiation and embryonic development in multicellular organisms appears to be controlled by gradients of protein concentrations.

One-Dimensional Spatial Configuration

- Consider p cells (or regions of a single cell) are arranged in a row in which each cell l has an amount of each species denoted by the vector $\mathbf{X}^l(t)$.

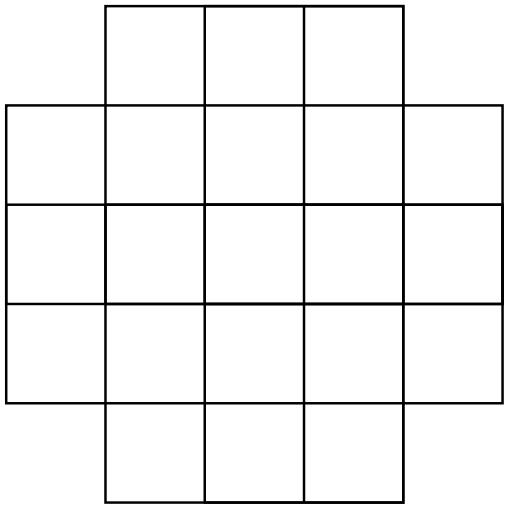
- Assuming that diffusion between cells is at a rate proportional to their differences in concentration (i.e., $x_i^{(l+1)} - x_i^{(l)}$ and $x_i^{(l-1)} - x_i^{(l)}$), the following *reaction-diffusion equations* can be obtained:

$$\frac{dx_i^{(l)}}{dt} = f_i(\mathbf{x}^{(l)}) + \delta_i(x_i^{(l+1)} - 2x_i^{(l)} + x_i^{(l-1)}), 1 \leq i \leq n, 1 < l < p$$

  where δ_i is a diffusion constant.

- These equations are still ODEs, so they can be numerically solved using the simulation methods described earlier.

Two-Dimensional Spatial Configuration



Partial Differential Equations

- As the number of cells becomes large, l can be taken to be a continuous variable resulting in a partial differential equation (PDE) model:

$$\frac{dx_i}{dt} = f_i(\mathbf{x}) + \delta_i \frac{\partial^2 x_i}{\partial l^2}, 1 \leq i \leq n, 0 \leq l \leq \gamma$$

  where the system size is assumed to be γ and diffusion does not occur beyond the boundaries at $l = 0$ and $l = \gamma$.
- Analysis and numerical solutions for PDE models is more involved.

More on Spatial Modeling

- Reaction-diffusion equations for modeling cell differentiation were originally proposed by Turing (1951).
- Goal of his work is a mathematical model of a growing embryo.
- Turing suggested that systems consist of masses of tissues within which certain substances called *morphogens* react chemically and diffuse.
- Diffusing into a tissue persuades the tissue to develop differently.
- The embryo in the spherical blastula stage has spherical symmetry.
- Systems with spherical symmetry whose state is changed by chemical reactions and diffusion remains spherically symmetric.
- This cannot result in a non-spherically symmetric organism like a horse.
- There are *some* asymmetries which cause instability and lead to a new and stable equilibrium without symmetry.
- This behavior is very similar to how electrical oscillators get started.
- Successfully applied to modeling pattern formation in the *Drosophila* embryo (see Kauffman et al. (1978), Bunow et al. (1980), Goodwin and Kauffman (1990), Lacalli (1990), and Myasnikova et al. (2001)).

Sources

- Law of mass action - Waage and Guldberg (1864).
- Classical chemical kinetics - Wright (2004).
- Models of genetic circuits - Goodwin (1963, 1965).
- Numerical simulation - Press et al. (1992).
- Qualitative ODE analysis - Strogatz (1994).
- Chapter 3 of Engineering Genetic Circuits - Myers (2009).