

Systems Biology

Simulation of Dynamic Network States



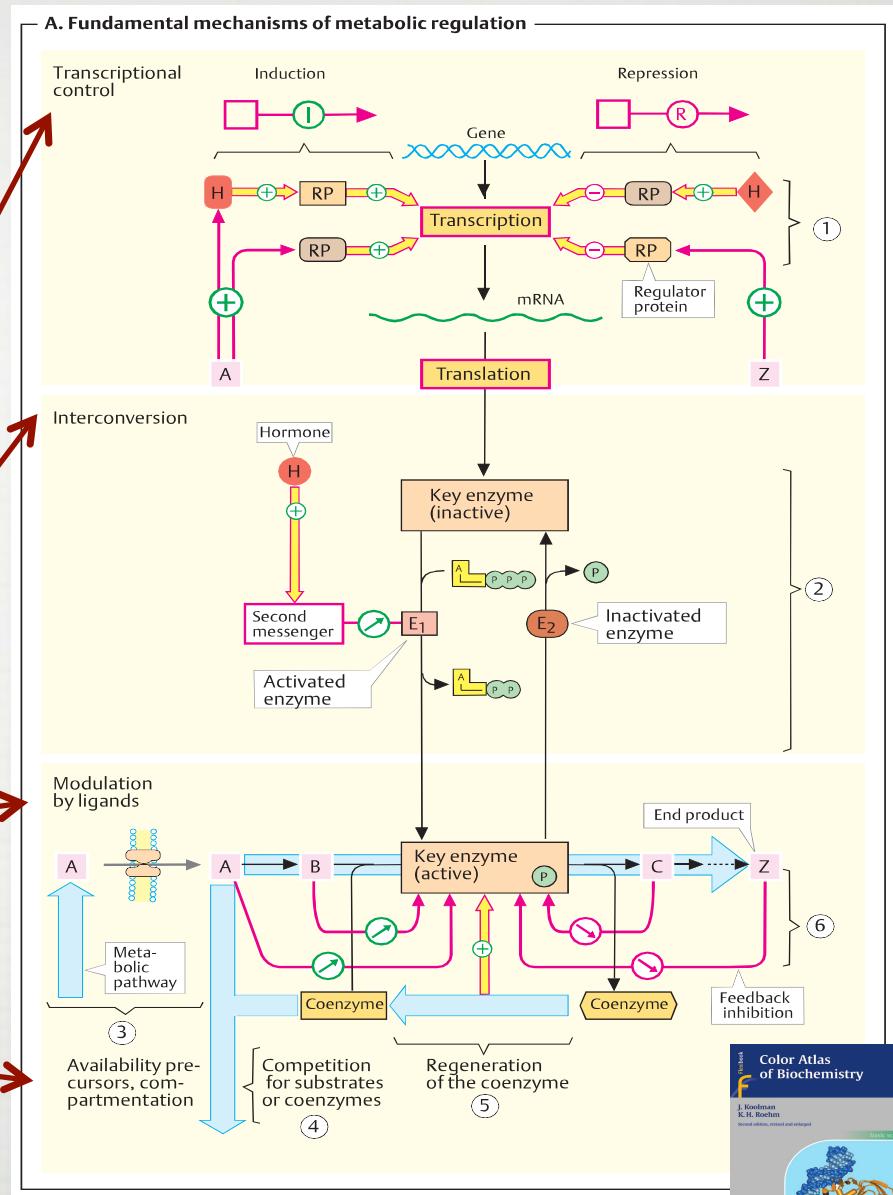
Lecture #9

Regulation

Bernhard Ø. Palsson

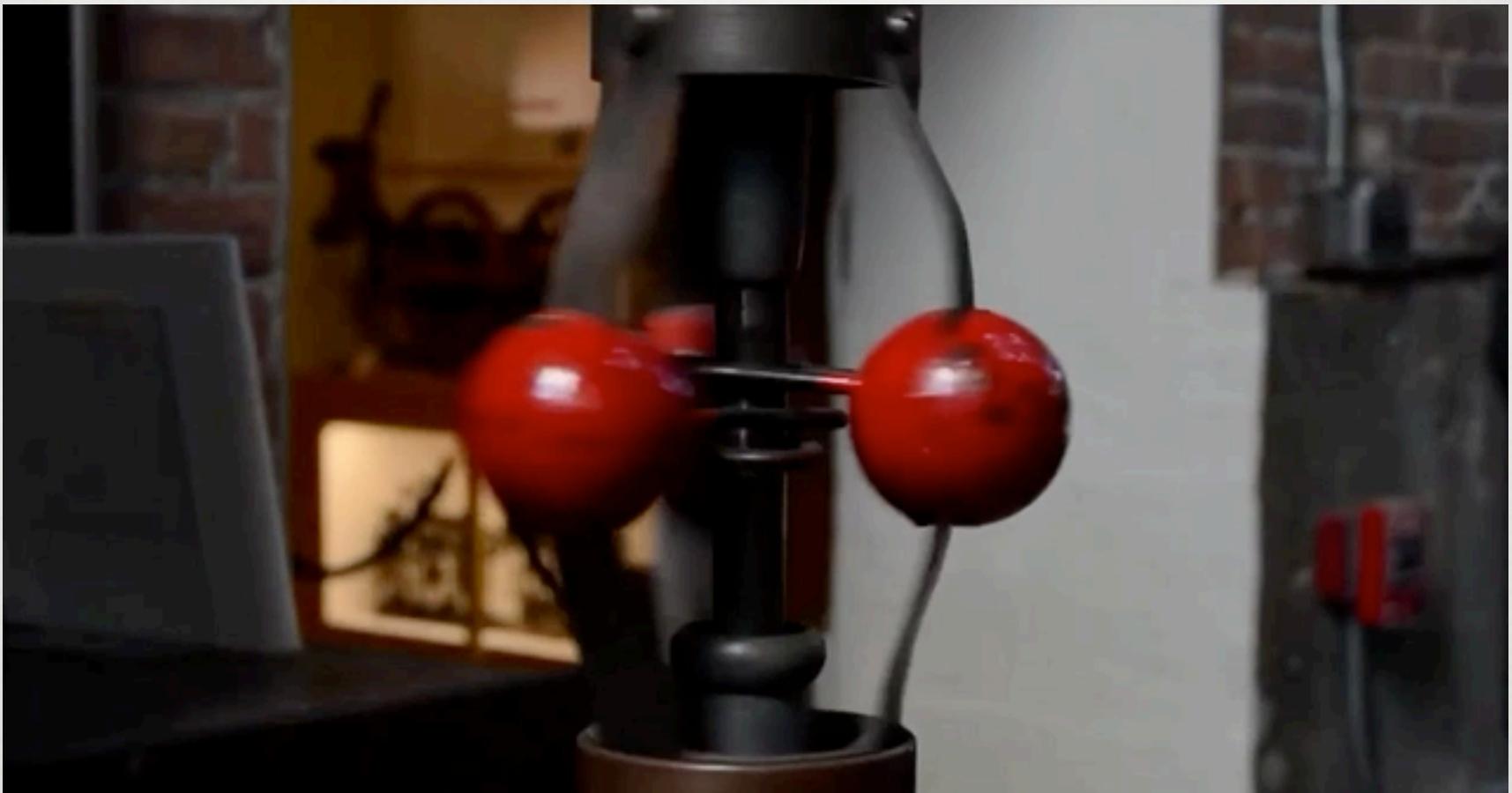
Multiple levels of enzyme regulation:

- 1) gene expression,
- 2) interconversion,
- 3) ligand binding,
- 4) cofactor availability



Outline

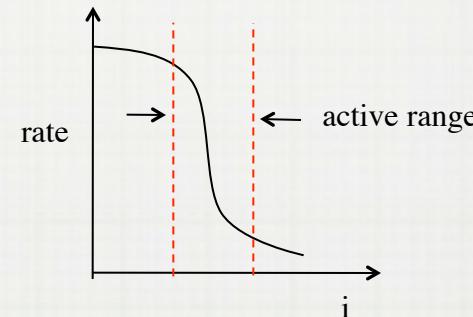
- Phenomenology of regulation and signaling
- Analyzing regulation (the only exception):
 - The mathematics of regulatory coupling
- Simulating regulation:
 - Enzymes as molecules in simulation
 - Fractional states of macromolecular pools
 - Monomers, dimers, tetramers, ...



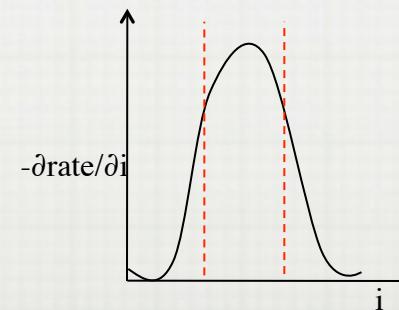
Phenomenology

1. Built-in bias; '+' or '-'
activation *inhibition*

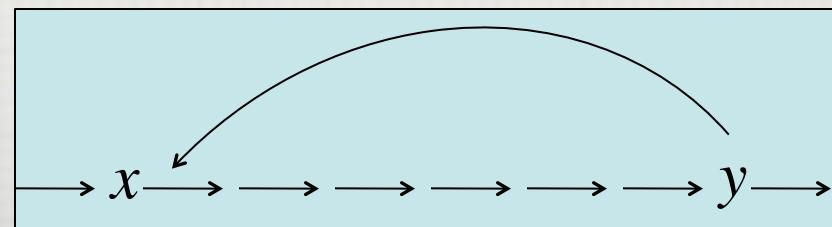
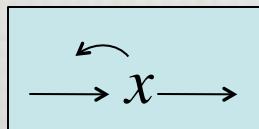
2. Active concentration range



3. Gain

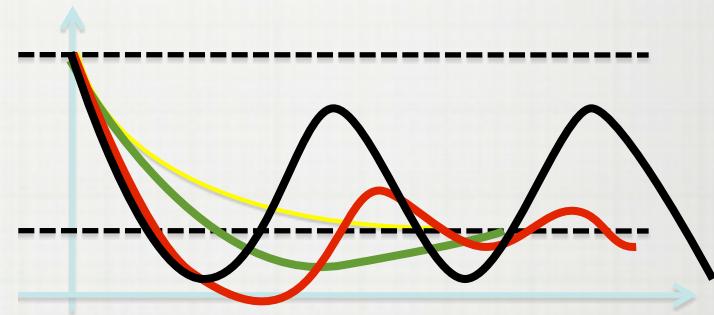
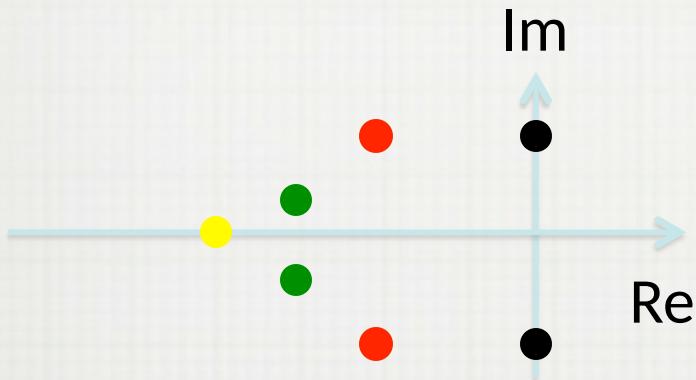


4. Local vs. distant



Eigenvalues and their location in the complex plane (IMPORTANT)

With n roots: L1, l2, ...Ln



Linear Algebra flashback:

$$\det(J - \lambda I) = 0$$

$a_0 + a_1\lambda^1 + \dots + a_{n-1}\lambda^{(n-1)} + a_n\lambda^n$
with n roots : $\lambda^1, \lambda^2, \dots, \lambda^n$
coefficients a_i : $(0 \leq i \leq n)$

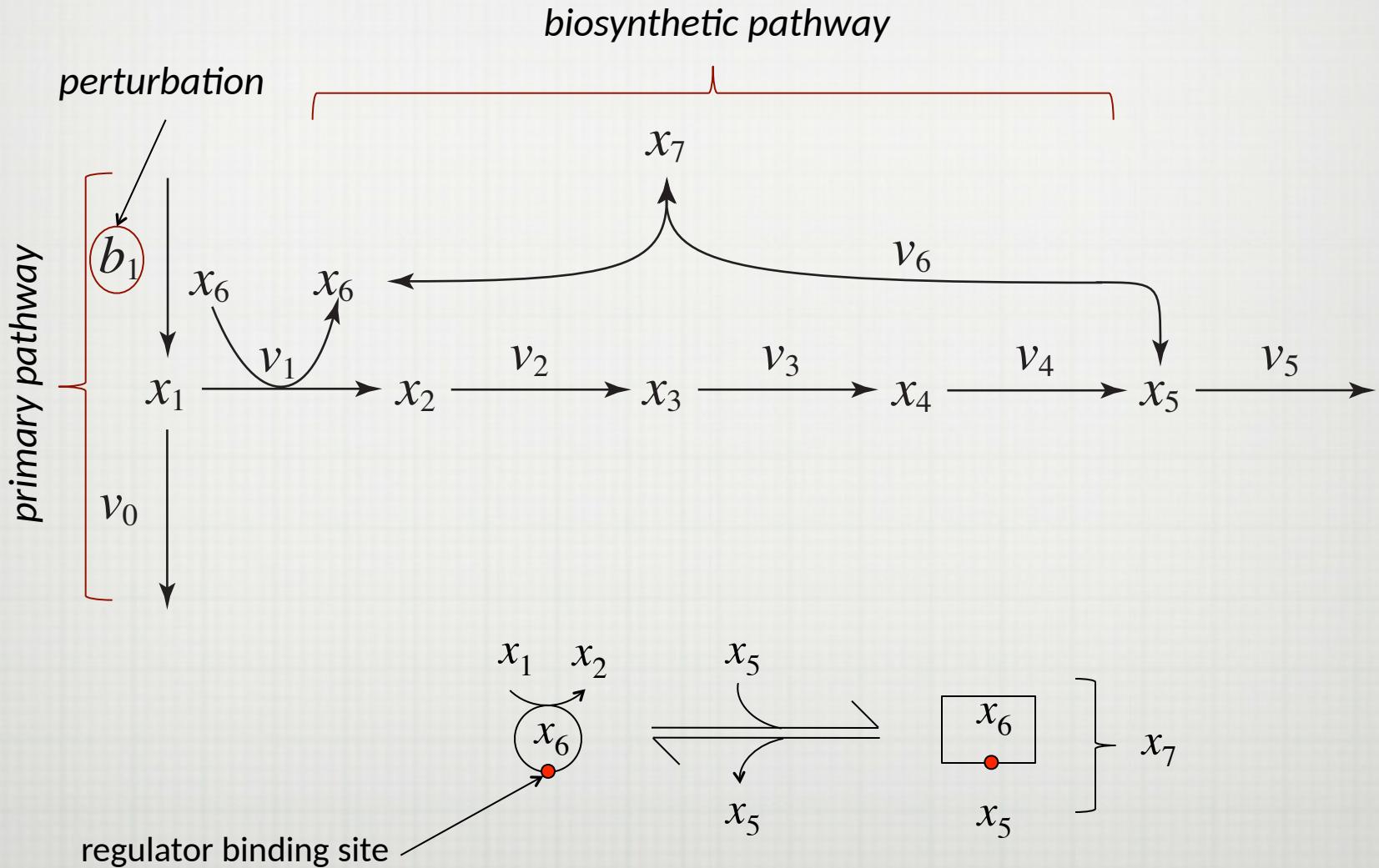
Transient response:

1. “smooth” landing
2. overshoot
3. damped oscillation
4. sustained oscillation
5. chaos

Simulating regulation

ENZYMES AS MOLECULES

Regulation at a “Distance”



Ways to find the Steady State

1. Analytically from the steady state equations — possible in this case
2. Numerically from the steady state equations
3. Numerically by integrating the dynamic solution to a very long time — longer than the longest time scale in the system

The Dynamic Equations

<i>Time derivative</i>	<i>Fluxes</i>	<i>Kinetic expressions</i>
$\frac{dx_1}{dt} =$	$b_1 - v_0 - v_1$	$= b_1 - k_0x_1 - k_1x_6x_1$
$\frac{dx_2}{dt} =$	$v_1 - v_2$	$= k_1x_6x_1 - k_2x_2$
$\frac{dx_3}{dt} =$	$v_2 - v_3$	$= k_2x_2 - k_3x_3$
$\frac{dx_4}{dt} =$	$v_3 - v_4$	$= k_3x_3 - k_4x_4$
$\frac{dx_5}{dt} =$	$v_4 - v_5 - v_6$	$= k_4x_4 - k_5x_5 - (k_6x_5x_6 - k_{-6}x_7)$
$\frac{d(x_6 + x_7)}{dt} = 0$	$\frac{dx_6}{dt} = -v_6$ $\frac{dx_7}{dt} = v_6$	$= -k_6x_5x_6 + k_{-6}x_7$ $= k_6x_5x_6 - k_{-6}x_7$

$\Rightarrow = 0;$
at equilibrium in the stst

The sum of the last two differential equations gives us the mass balance in the enzyme

→ $x_6 + x_7 = e_t$
where e_t is the total amount of enzyme. $0 \leq \frac{x_6}{e_t} \leq 1$

The Steady-State Equations

$$\frac{dx_i}{dt} = 0 \quad \left\{ \begin{array}{l} 0 = b_1 - (k_0 + k_1 x_6) x_1 \\ 0 = k_1 x_6 x_1 - k_2 x_2 \\ 0 = k_2 x_2 - k_3 x_3 \\ 0 = k_3 x_3 - k_4 x_4 \\ 0 = k_4 x_4 - k_5 x_5 - (k_6 x_5 x_6 - k_{-6} x_7) \\ 0 = k_6 x_5 x_6 - k_{-6} x_7 \\ 0 = x_6 + x_7 - e_t \end{array} \right. = 0;$$

at equilibrium in the stst

Option #1 (of three) to get the st.st.

These equations can be combined to give a quadratic equation

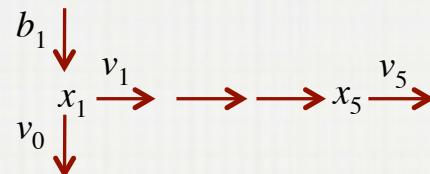
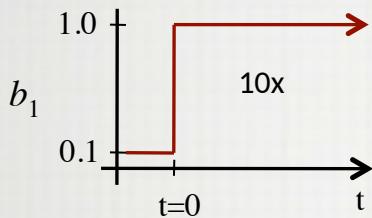
$$y^2 + ay - b = 0$$

where

$$y = k_2 x_2, \quad a = k_5 \left(\frac{k_{-6}}{k_6} \right) \left(1 + \frac{k_1 e_t}{k_0} \right), \quad b = k_5 \left(\frac{k_{-6}}{k_6} \right) \left(\frac{k_1 e_t}{k_0} \right) b_1$$

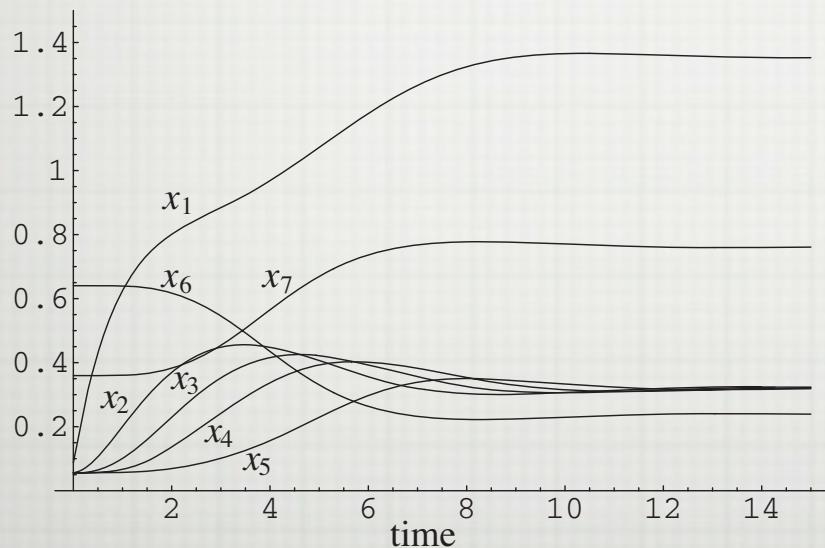
that has one positive root.

Simulation Results

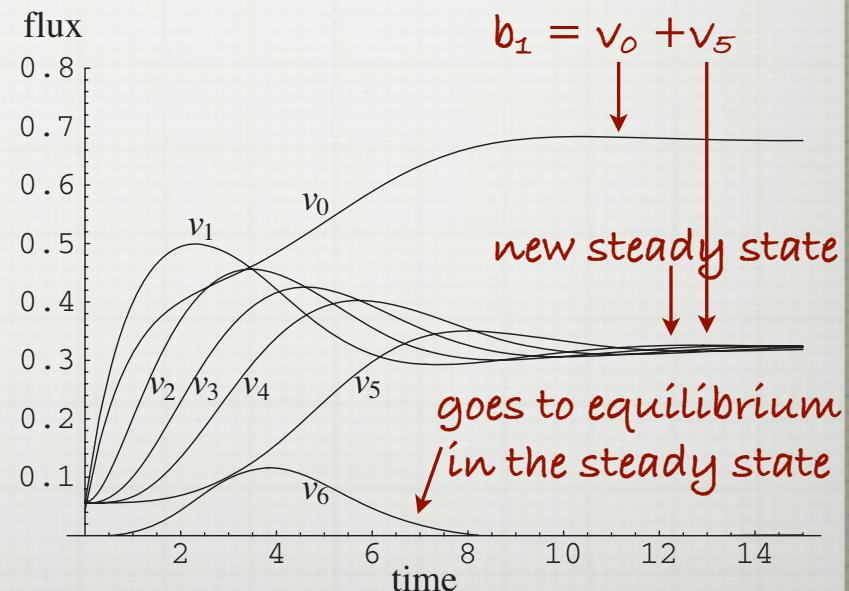


$k_0 = .5$	$k_1 = 1$
$k_2 = 1$	$k_3 = 1$
$k_4 = 1$	$k_5 = 1$
$k_6 = 10$	$k_{-6} = 1$
$e_t = 1.0$	

(a)
concentration

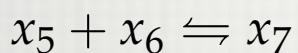
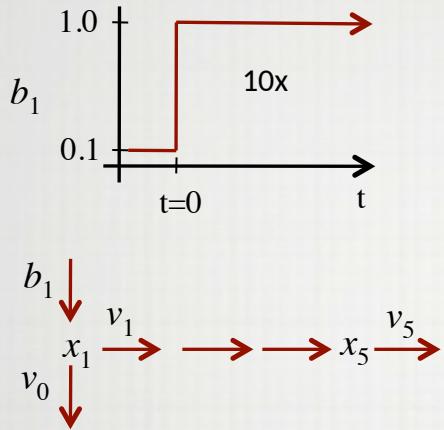


(b)

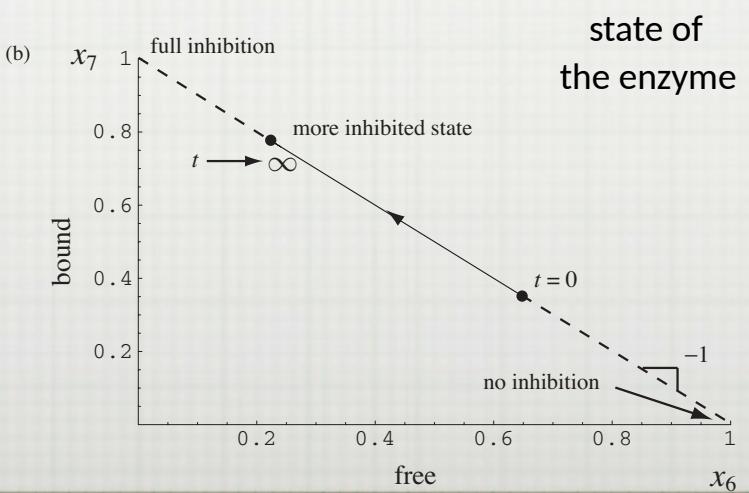
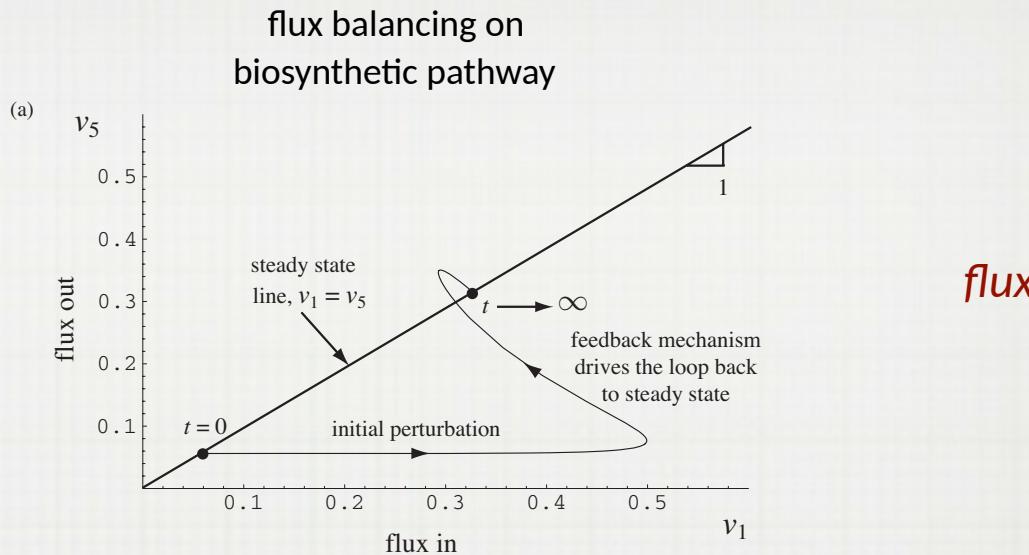


Complicated to interpret the time responses: what is going on?

Phase Portrait and Pool Interpretation

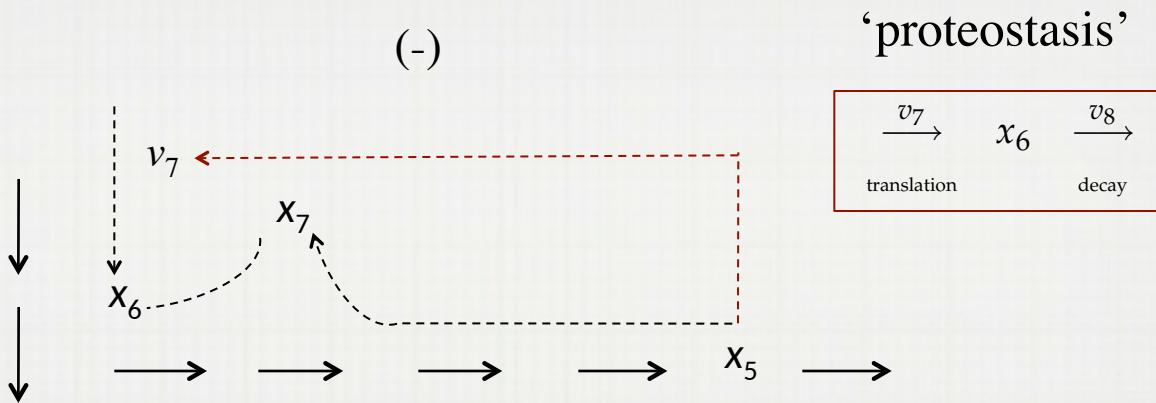


$$\begin{aligned} \text{frac} &= \frac{x_7}{x_6+x_7} \\ &= \frac{\text{inhibited}}{\text{total}} \end{aligned}$$



concentration

Regulation of Gene Expression



Additions to the dynamic equations This regulation of protein synthesis can be simulated by adding a synthesis and degradation rate in the dynamic equation for the enzyme, x_6 :

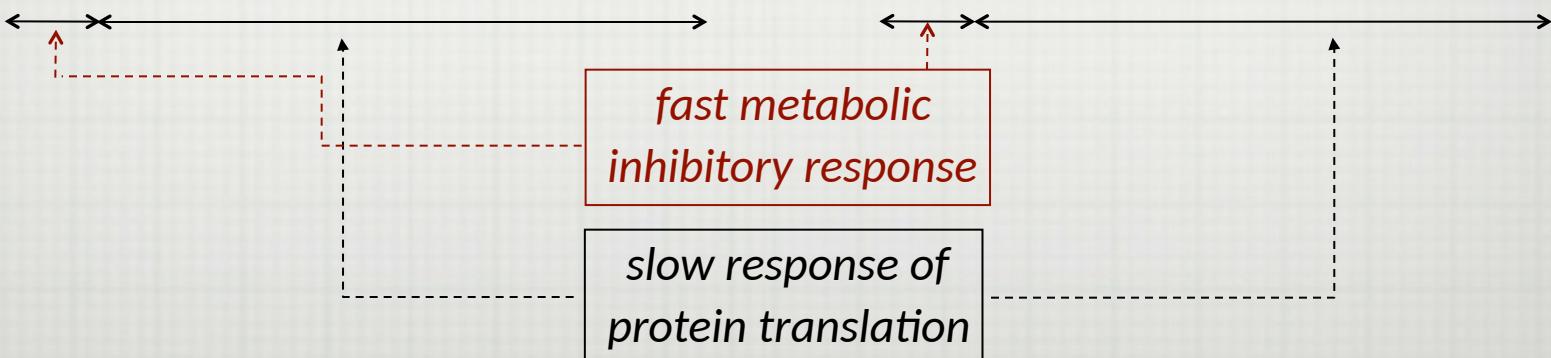
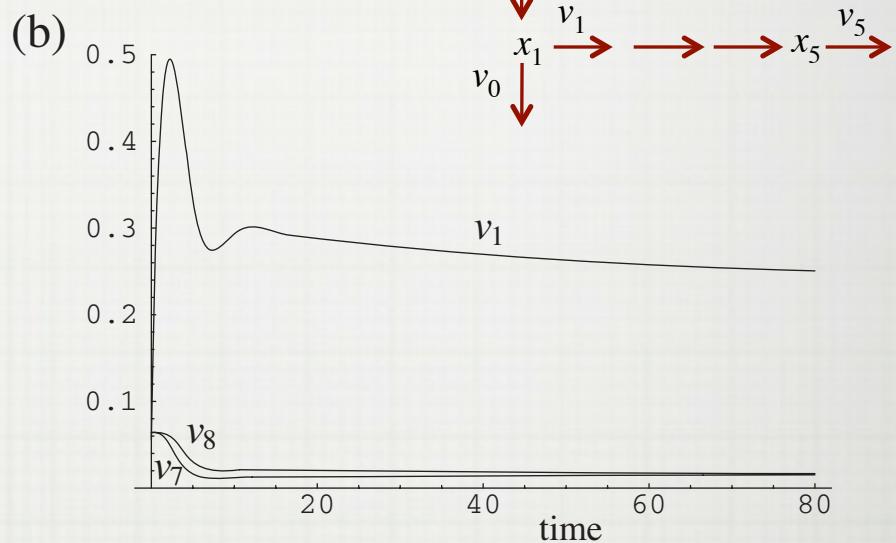
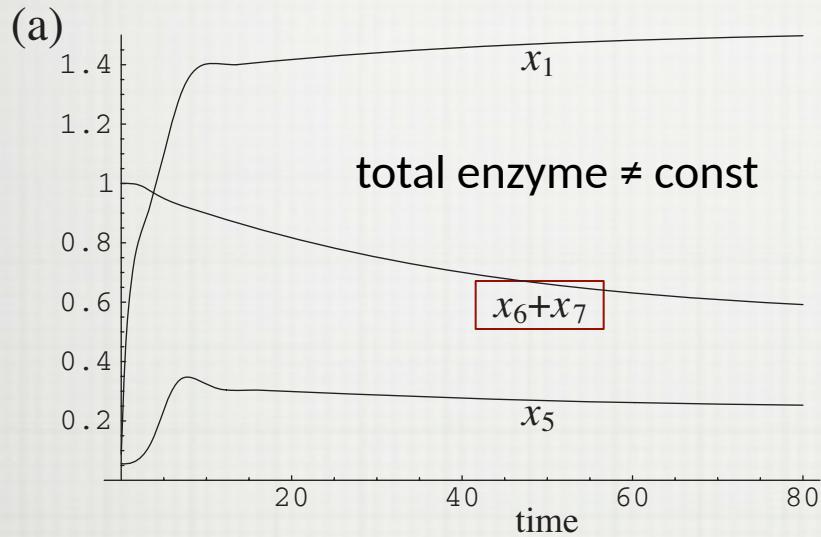
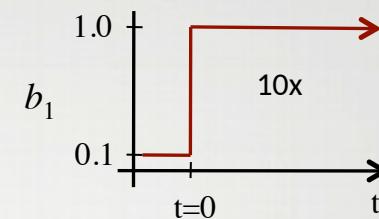
$$\frac{dx_6}{dt} = -k_6 x_5 x_6 + k_{-6} x_7 + [v_7 - v_8] \quad (9.47)$$

where we can use an inhibition rate of the Hill form with $\nu = 1$:

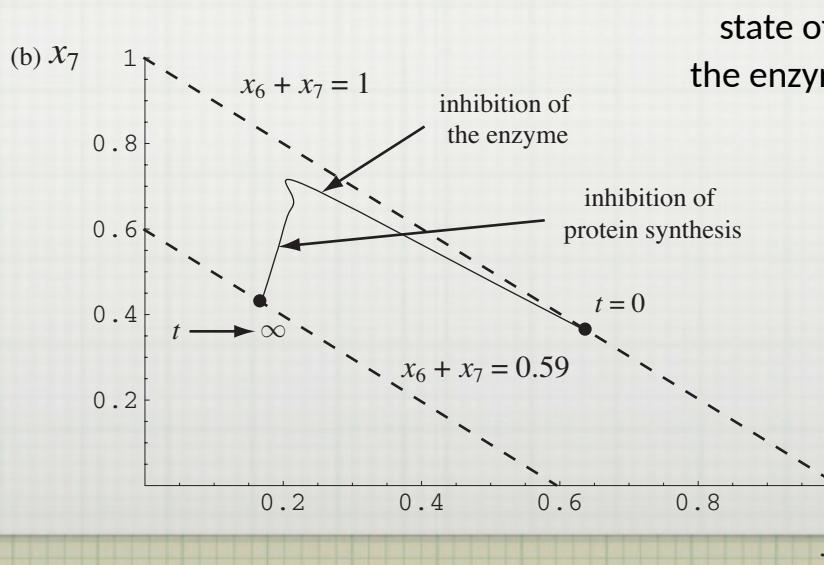
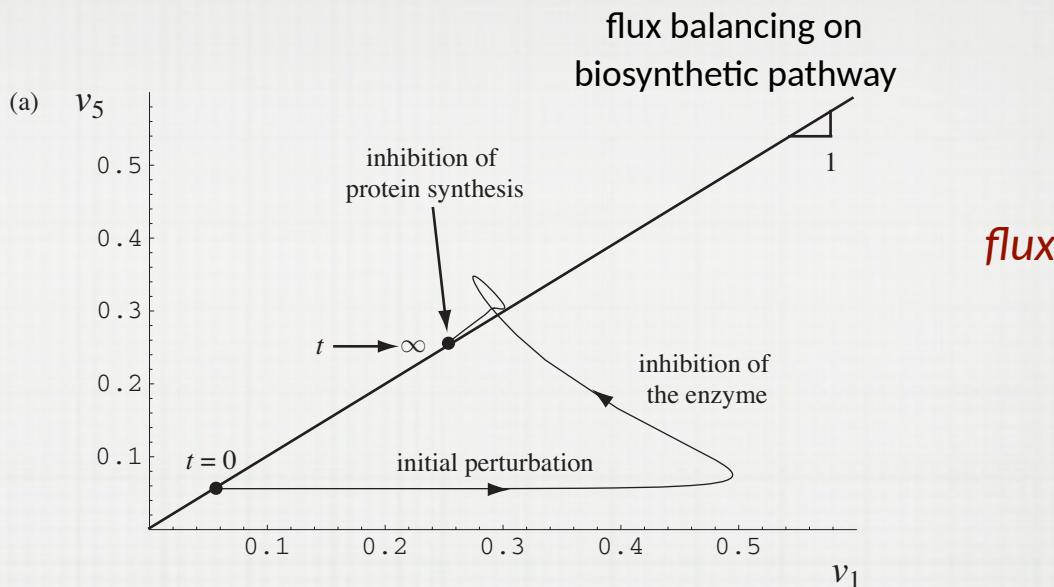
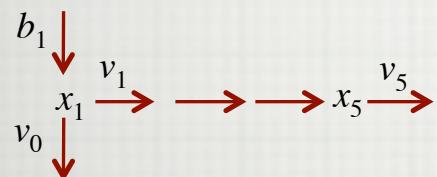
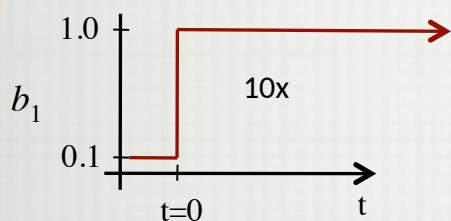
$$v_7 = \frac{k_7}{1 + K_7 x_5} \quad (9.48)$$

inhibition of translation

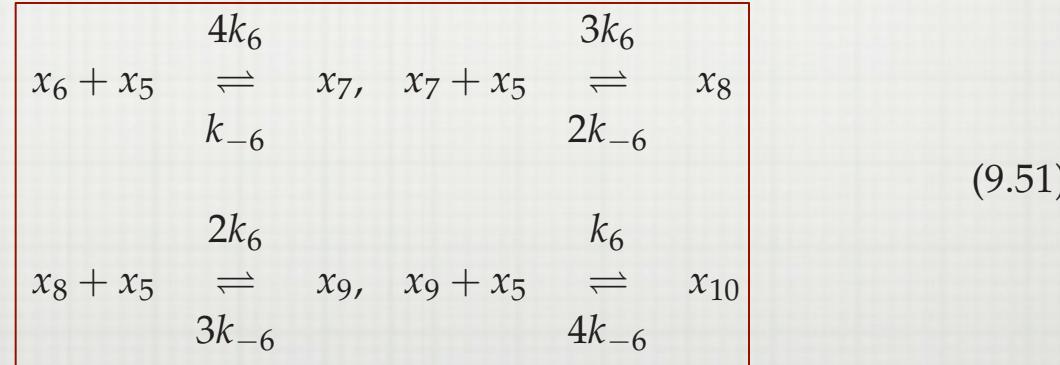
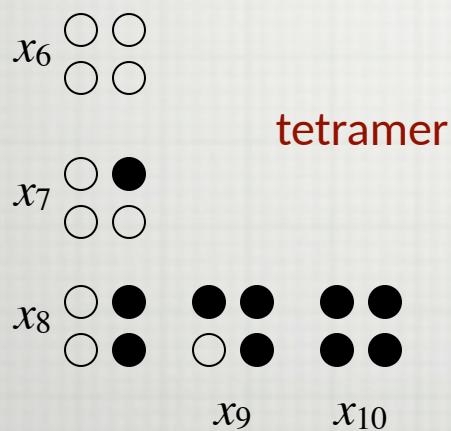
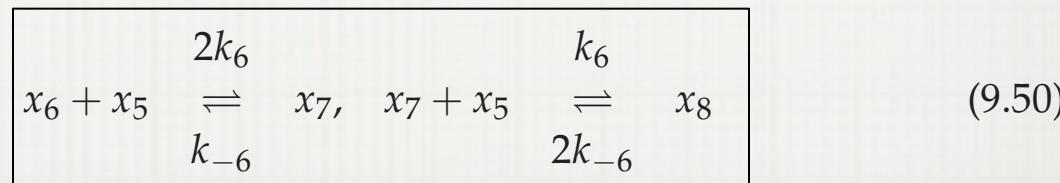
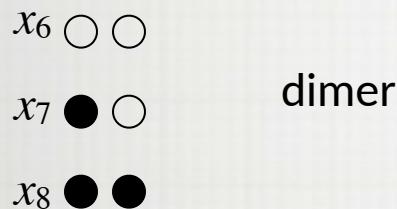
Simulation Results



Flux Phase Portrait and Pool Interpretation

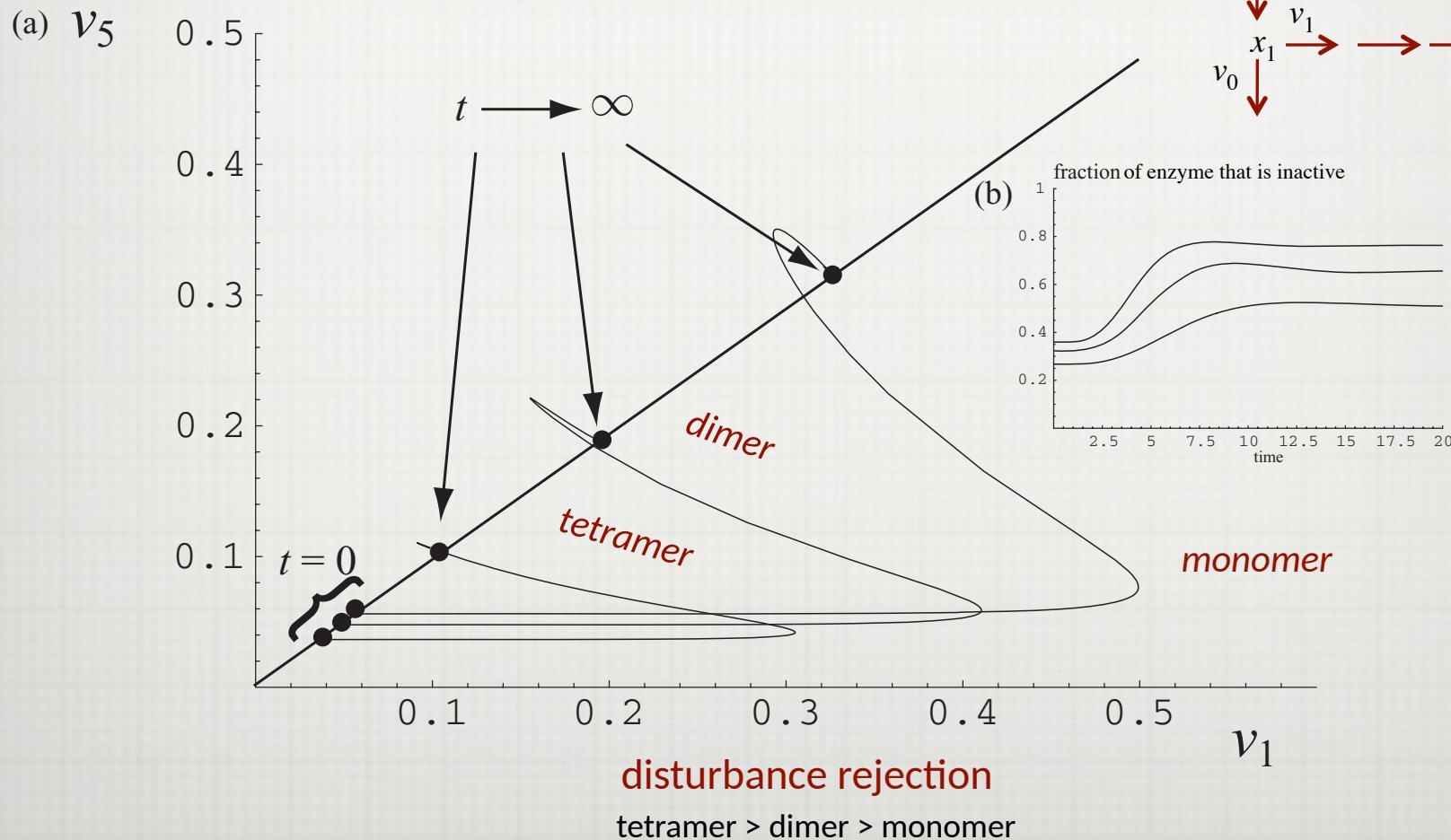


Allosteric Regulation of Enzyme Activity



Allosteric regulation is like ‘augmented’ mass action

Simulation Results: monomer, dimer, tetramer



Some observations

- Enzymes can be added as molecules into simulation models (more in part 4)
- Enzymes will have multiple functional states
- The fractional state is important
- Tetramers are more effective than dimers that are more effective than monomers when it comes to regulation

Summary

- The activities of gene products are often directly regulated.
- Regulation can be described by:
 - i) its bias,
 - ii) the concentration range over which the regulatory molecule is active and
 - iii) its strength, that is how sensitive the flux is to changes in the concentration of the regulator.

Summary (con't)

- Regulation of enzyme activity comes down to:
 - i) the functional state of the gene product (typically fast),
 - ii) regulating the amount of the gene product present (typically slow)
 - examining the functional state of the pool formed by the amount of the active gene product and then the total amount itself.
- Regulatory mechanisms:
 - can be built on top of the basic stoichiometric structure of a network being analyzed and its description by elementary mass action kinetics
 - are described by additional reactions that transform the regulated gene product from one state to the next with elementary reaction kinetics
 - fundamentally they work through mass action kinetics

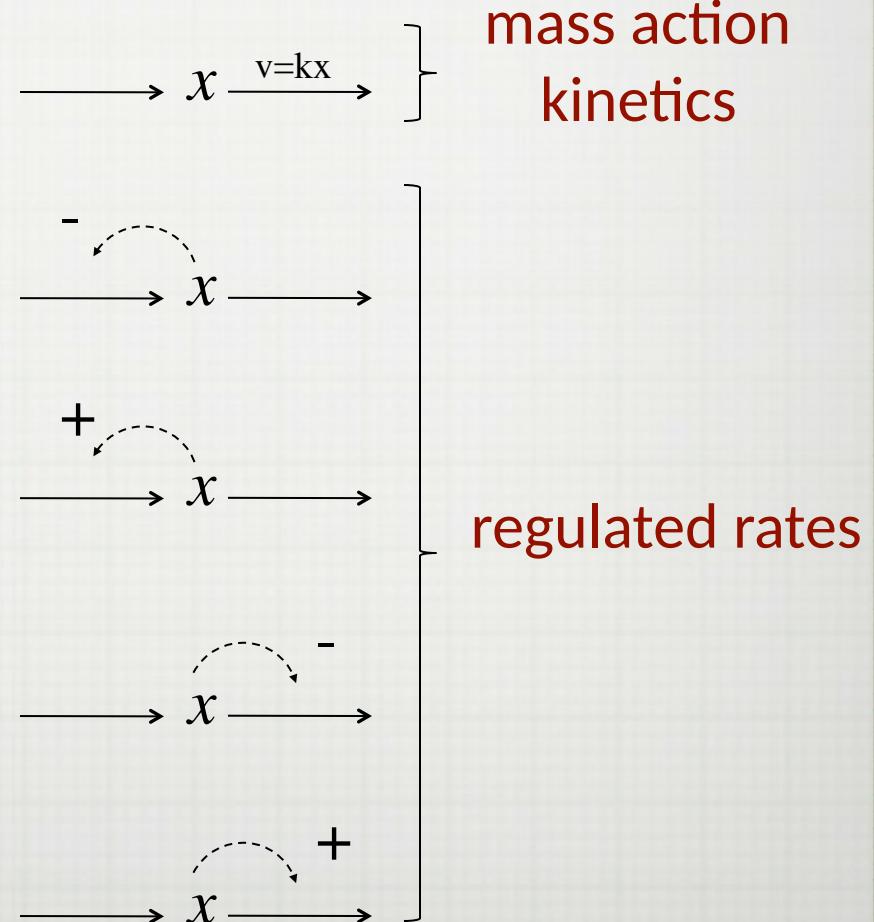
The end of main slides:
Appendix on finding eigenvalues and the
effect of regulation

ADVANCED TOPIC: Mathematically analyzing regulation

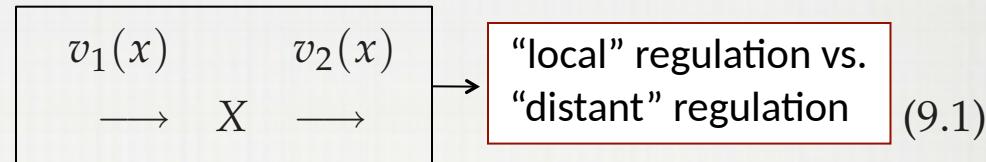
THE MATHEMATICS OF REGULATION OF ENZYME ACTIVITY

Local Regulation: The five basic cases

- No regulation
- Feedback inhibition
- Feedback activation
- Feedforward inhibition
- Feedforward activation



Basic mathematical features: To examine the qualitative effects of signals on network dynamics, let us examine the simple scheme:



where the concentration of metabolite X , x , directly influences the rates of its own formation, $v_1(x)$, and degradation, $v_2(x)$. The following discussion is graphically illustrated in Figure 9.3.

The dynamic mass balance on X is

$$\frac{dx}{dt} = v_1(x) - v_2(x) \quad (9.2)$$

that in a linearized form is

$$\frac{dx'}{dt} = \left(\frac{\partial v_1}{\partial x} - \frac{\partial v_2}{\partial x} \right) x' = \lambda x'$$

gain → magnitude

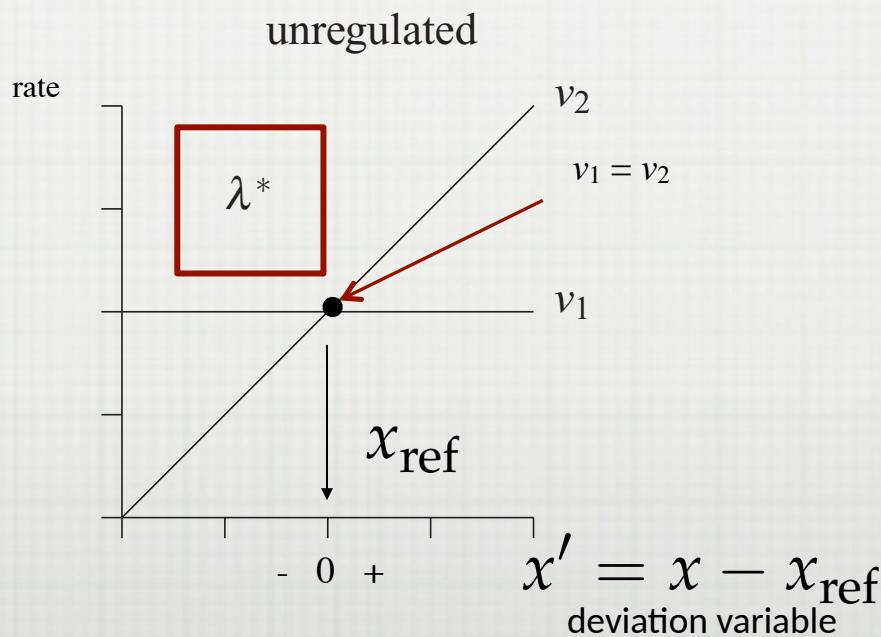
$x' = x - x_{\text{ref}}$
deviation variable
(9.3)

where λ is the ‘net’ rate constant. The value of λ determines the rate of response of this system to changes in the concentration of X .

The Deviation Variable:

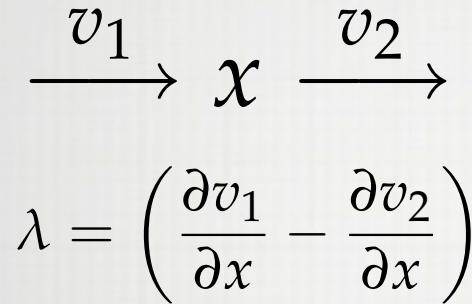
measures the distance from a reference point

$$\xrightarrow{v_1} x \xrightarrow{v_2}$$

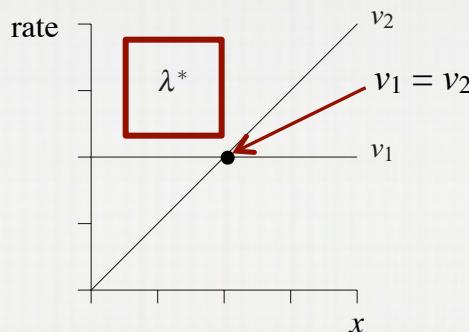


The ‘Net’ Rate Constant:

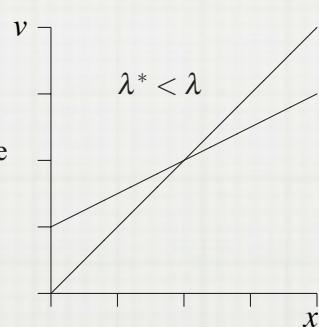
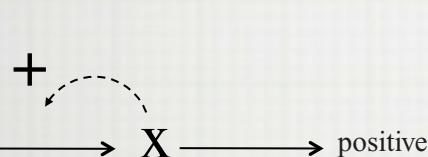
an eigenvalue or a systems time constant



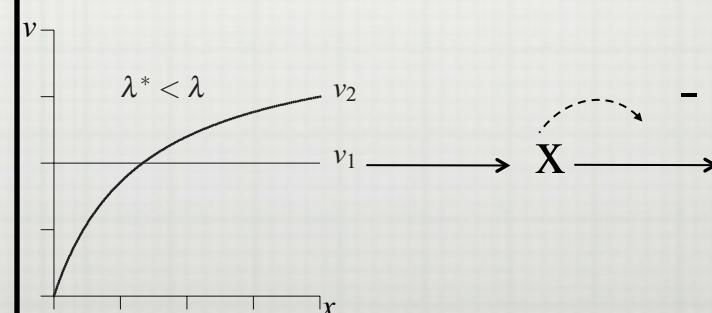
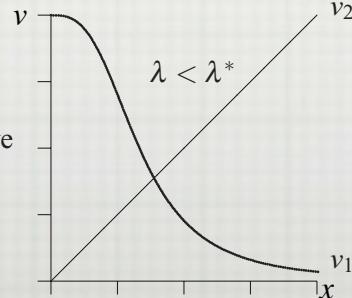
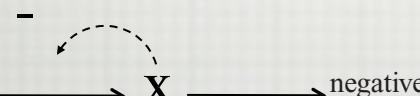
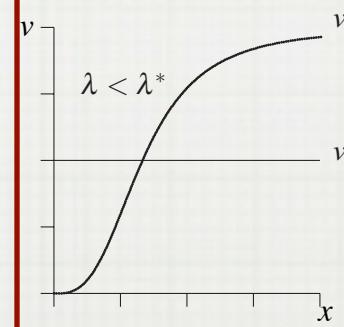
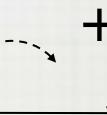
unregulated



feedback regulation



feedforward regulation



Principle for ‘Local’ Regulation

Regulatory principles: Regulatory signals can either support or antagonize the mass action trend in a network. Thus, we arrive at the following principles:

1. Local negative feedback and local positive feedforward controls support the mass action trend and are stabilizing in the sense that they try to maintain the intrinsic dynamic properties of the stoichiometric structure.
2. Local positive feedback and local negative feedforward controls counteract the mass action trend and can create instabilities. Many of the creative functions associated with metabolism can be attributed to these control modes. These signals allow the cell to behave in apparent defiance to the laws of mass action and stoichiometric trends.

Dynamic Effects of Regulation (advanced)

Measuring the dynamic effects of regulation The effect of regulation can be evaluated in the following way. Equation 9.4 may be rewritten as

$$\lambda = -\frac{\partial v_2}{\partial x} \left(1 - \frac{\partial v_1 / \partial x}{\partial v_2 / \partial x} \right) \quad (9.5)$$

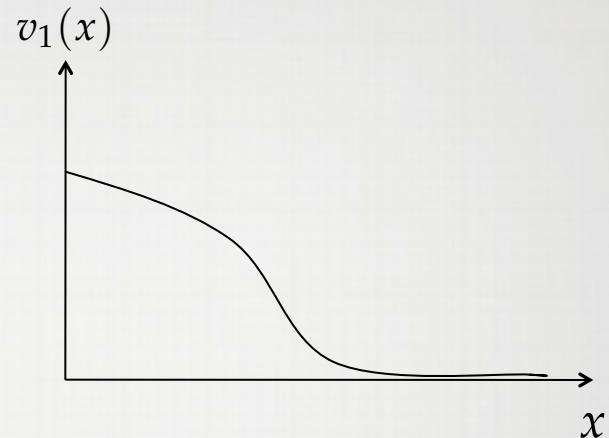
$$\approx -\frac{\partial v_2}{\partial x} \left(1 \pm \frac{t_{turnover}}{t_{regulation}} \right) \quad (9.6)$$

if the time constants indicated are good estimates of the corresponding partial derivatives. The dimensionless ratio:

$$a = \frac{t_{turnover}}{t_{regulation}} \quad (9.7)$$

thus becomes an important dimensionless group in characterizing local regulatory signals. If a is less than unity or on the order of unity, the regulation is dynamically about as important as the natural turnover time. However, if it significantly exceeds unity, one would expect that dynamics then would become dominated by the regulatory action.

Inhibition



9.4.1 Inhibition

We can look quantitatively at the effects of local feedback inhibition. We can consider specific functional forms for v_1 and v_2 in equation 9.2:

$$v_1(x) = \frac{v_m}{1 + (x/K)^2} \quad \text{and} \quad v_2(x) = kx \quad (9.8)$$

where the production rate is a Hill-type equation with $\nu = 2$ and the removal is an elementary first-order equation. The dynamic mass balance is

$$\frac{dx}{dt} = \frac{v_m}{1 + (x/K)^2} - kx \quad (9.9)$$

The dynamics of this simple system can be analyzed to determine the dynamic effects of the feedback inhibition.

The Steady State

The steady state The steady state equation, $v_1 = v_2$, for this network is a cubic equation

$$\left(\frac{x}{K}\right)^3 + \frac{x}{K} - \frac{v_m}{kK} = 0 \quad (9.10)$$

Introducing a dimensionless concentration $\chi = x/K$ we have that

$$\chi^3 + \chi - a = 0 \quad (9.11)$$

where $a = v_m/kK$. This equation has one real root

$$\chi_{ss} = \frac{\sqrt[3]{9a + \sqrt{3\sqrt{27a^2 + 4}}}}{3\sqrt[3]{\frac{2}{3}}} - \frac{\sqrt[3]{\frac{2}{3}}}{\sqrt[3]{9a + \sqrt{3\sqrt{27a^2 + 4}}}}$$

from mathematica
(try it)

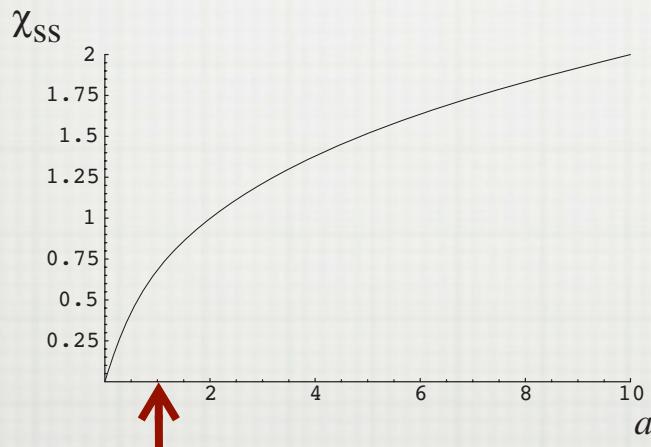
(9.12)

The steady state level of x , relative to K , is dependent on a single parameter, a , as shown in Figure 9.4. We see that

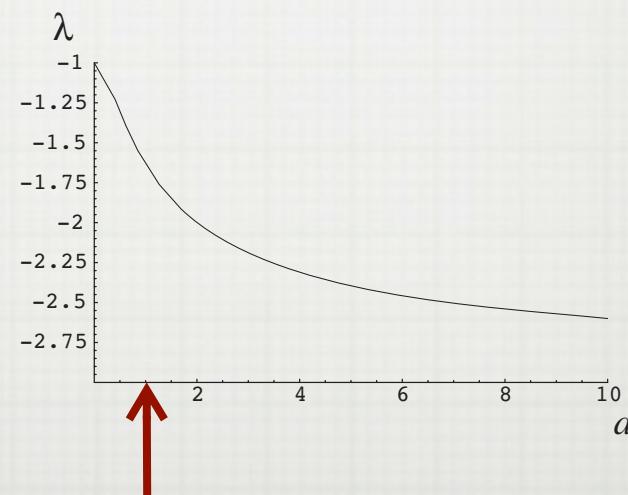
$$a = \frac{1/k}{K/v_m} = \frac{t_{turnover}}{t_{regulation}} \quad (9.13)$$

Parametric Sensitivity

$$a = \frac{1/k}{K/v_m} = \frac{t_{turnover}}{t_{regulation}}$$



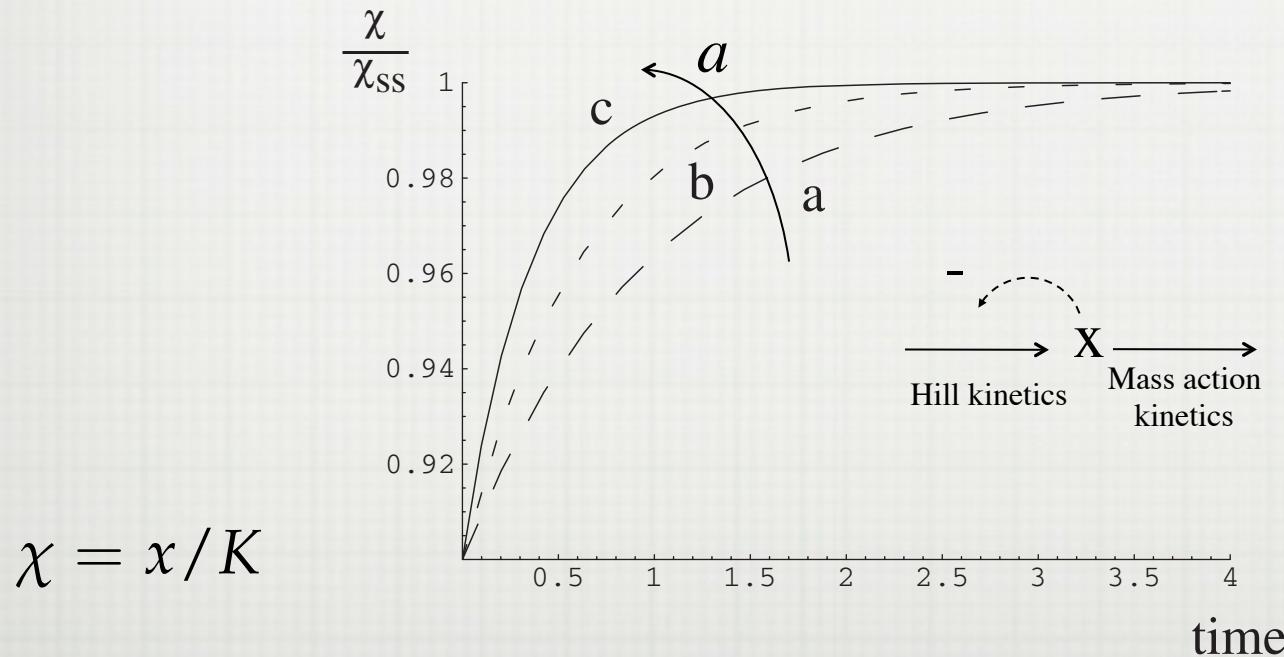
steady state
concentration increases

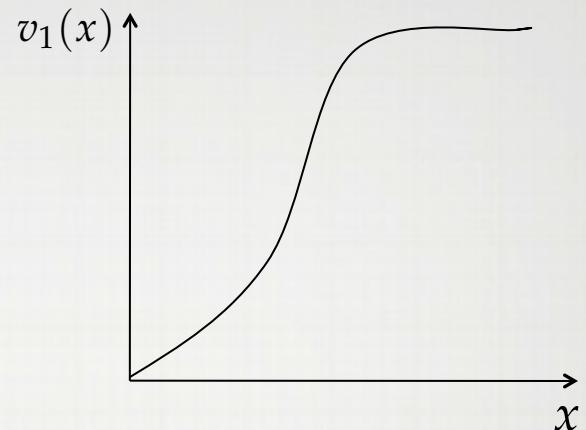


response is faster

Dynamic Response

return to steady state





Activation

We can look quantitatively at the effects of local feedback activation. We can consider specific functional forms for v_1 and v_2 in equation 9.2:

$$v_1(x) = v_m \frac{1 + \alpha(x/K)^\nu}{1 + (x/K)^\nu} \quad \text{and} \quad v_2(x) = kx \quad (9.16)$$

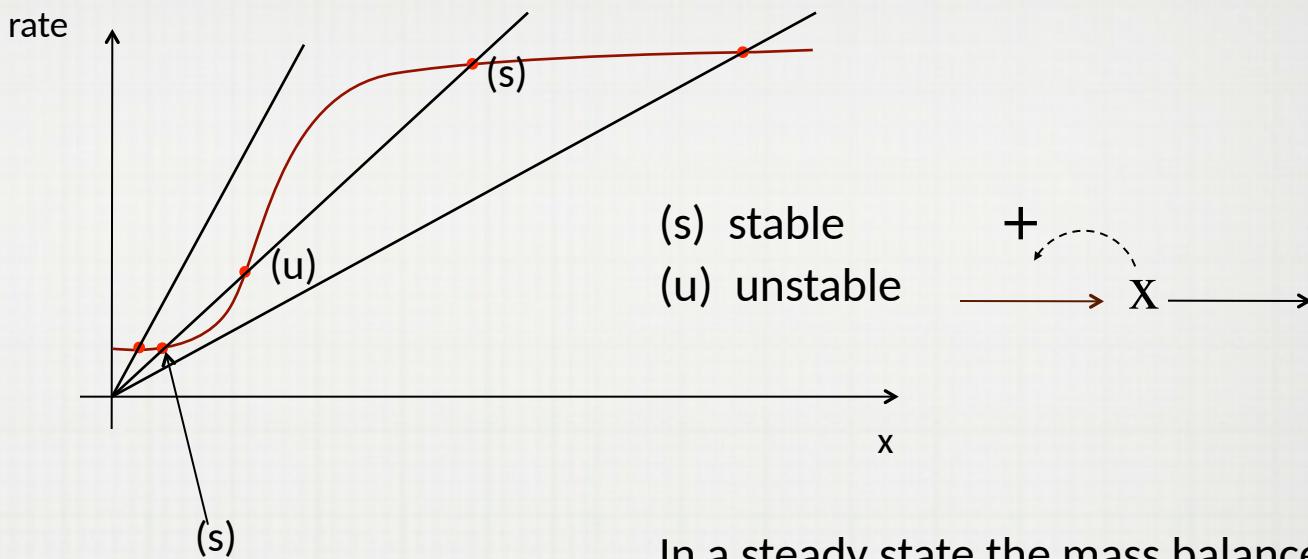
with a dynamic mass balance

$$\frac{dx}{dt} = v_m \frac{1 + \alpha(x/K)^\nu}{1 + (x/K)^\nu} - kx \quad (9.17)$$

We can make this equation dimensionless using the same dimensionless variables as above

$$\frac{d\chi}{d\tau} = a \frac{1 + \alpha\chi^\nu}{1 + \chi^\nu} - \chi \quad (9.18)$$

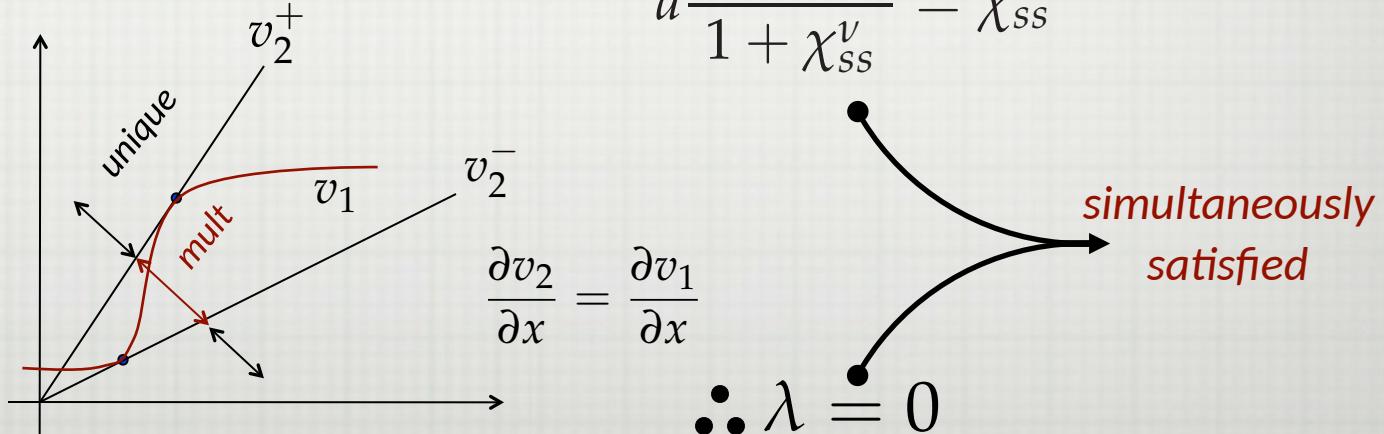
Activation



In a steady state the mass balance is:

$$a \frac{1 + \alpha \chi_{ss}^\nu}{1 + \chi_{ss}^\nu} = \chi_{ss}$$

Find 'critical' conditions



Key Quantities

Determining the eigenvalue The eigenvalue can be determined by the linearization of equation 9.18

$$\lambda = \frac{\nu a(\alpha - 1)\chi_{ss}^{\nu-1}}{(1 + \chi_{ss}^\nu)^2} - 1 \quad (9.20)$$

since $\alpha > 1$, the first term in equation 9.20 is positive. This leads to the possibility that the eigenvalue is zero. This condition in turn leads to a situation where one can have multiple steady states as will now be demonstrated.

Existence of multiple steady states We are looking for conditions where equations 9.19 and 9.20 are simultaneously zero, that is, the steady state condition and a zero eigenvalue. The two equations can be combined by multiplying equation 9.20 by χ and adding the equations together. After rearrangement, the equations become

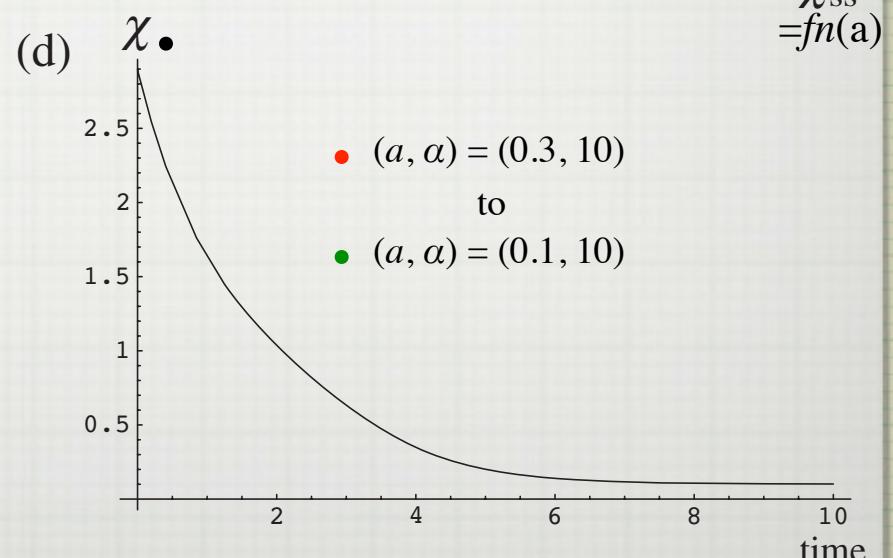
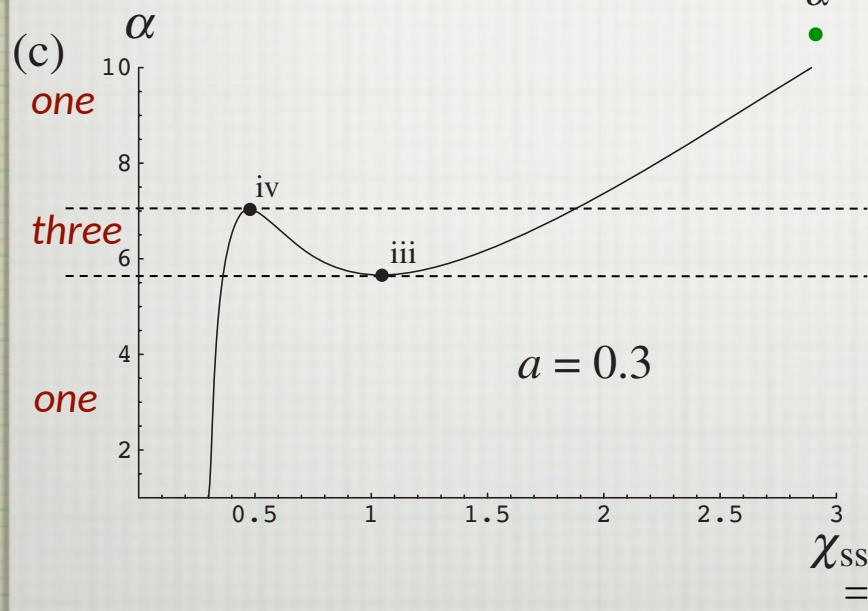
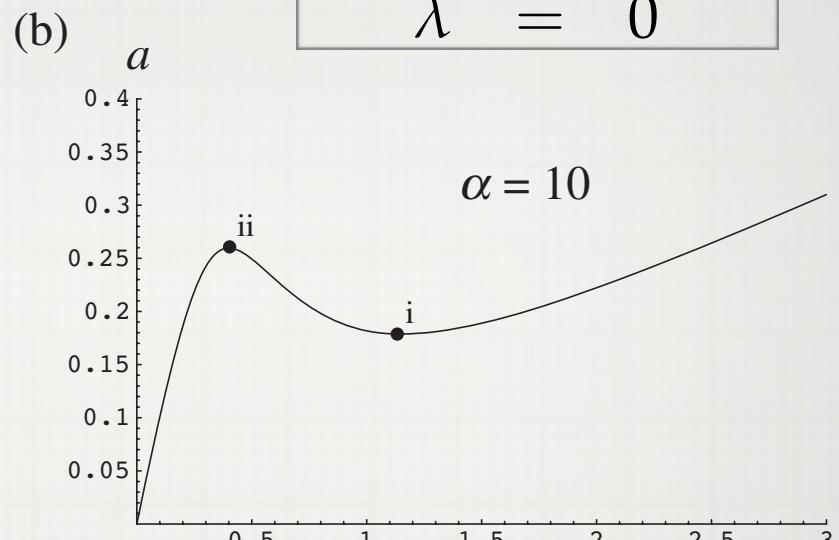
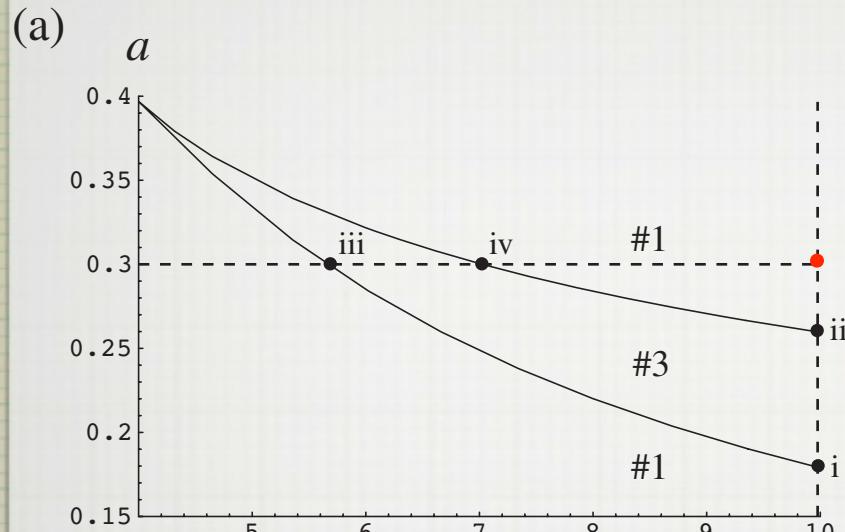
$$z^2 + [(1 - \nu) + (1 + \nu)/\alpha]z + 1/\alpha = 0, \quad z = \chi^\nu \quad (9.21)$$

This equation can only have a real positive solution if

$$\alpha > \alpha_{min} = \left(\frac{1 + \nu}{1 - \nu} \right)^2 \quad (9.22)$$

If α exceeds this minimum value, the steady state equation will have multiple solutions for χ for a range of values for a .

Multiple Steady States



$$\begin{array}{lll} v_1(x) & = & v_2(x) \\ \lambda & = & 0 \end{array}$$

$$\chi_{ss} = fn(a)$$

time

Some observations

- Regulation moves the eigenvalues in the complex plane
- Eigenvalues are systemic time constants
- The mathematics needed to analyze regulation is complex
- Local feedback inhibition/feedforward activation is stabilizing
 - $\text{Re}(\lambda) \rightarrow$ more negative
- Local feedback activation/feedforward inhibition is destabilizing

Summary

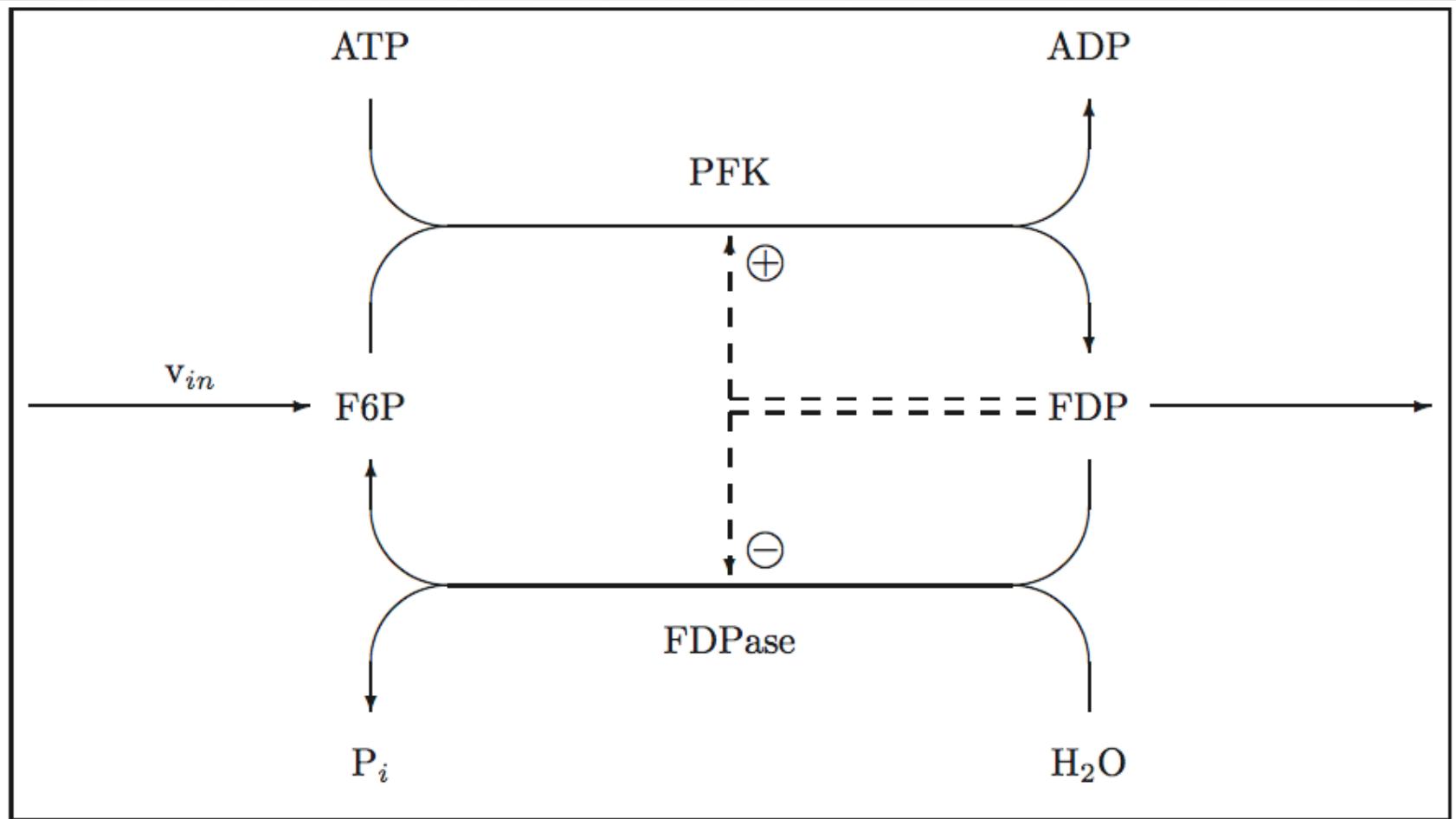
- The activities of gene products are often directly regulated.
- Regulation can be described by:
 - i) its bias,
 - ii) the concentration range over which the regulatory molecule is active and
 - iii) its strength, that is how sensitive the flux is to changes in the concentration of the regulator.
- In addition the 'distance' in the network between the site of regulation and the formation of the regulator is an important consideration.
- In general, *local signals* that:
 - support the natural mass action trend in a network are 'stabilizing'
 - counter the mass action trend may destabilize the steady state and create multiple steady states.

Summary (con't)

- Regulation of enzyme activity comes down to:
 - i) the functional state of the gene product (typically fast),
 - ii) regulating the amount of the gene product present (typically slow)
 - examining the functional state of the pool formed by the amount of the active gene product and then the total amount itself.
- Regulatory mechanisms:
 - can be built on top of the basic stoichiometric structure of a network being analyzed and its description by elementary mass action kinetics
 - are described by additional reactions that transform the regulated gene product from one state to the next with elementary reaction kinetics
 - fundamentally they work like mass action

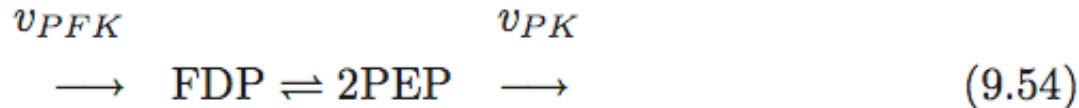
End of appendix

Key Regulatory Step in Glycolysis (Advanced)



Regulatory Signals (Advanced)

Regulatory signals PFK is a key regulatory enzyme in glycolysis (see Chapter 10). The net glycolytic pathway downstream from PFK is:

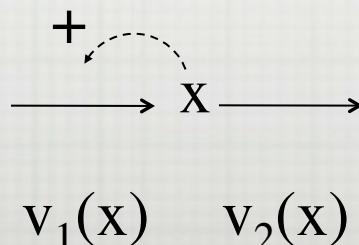


Since the two kinases are slow relative to the reactions that take place in the pathway between them we may assume an equilibrium between PEP and FDP as

$$K = \frac{\text{PEP}}{\text{FDP}^{1/2}} \quad (9.55)$$

Feedback activation of PFK by FDP in glycolysis has been postulated to lead to multiple steady states [8, 76]. NMR studies support that PFK responds primarily to F6P and FDP *in vivo* but is relatively insensitive to other effectors, i.e., [67].

Effective schema:



Kinetic Description (Advanced)

Kinetic description A simple model [76] that accounts for feedback activation of PFK by FDP has two rate laws:

$$v_1 = \frac{v_{m,1}x}{K_1 + x}, \quad v_2 = \frac{v_{m,2}x}{K_2 + x}y = \frac{v_{m,2}Kx^{3/2}}{K_2 + x} \quad (9.56)$$

where x is the concentration of FDP, y is the concentration of PEP, and the subscripts 1 and 2 refer to PFK and PK respectively. The center portion of glycolysis is assumed to be in equilibrium so that $K = \text{PEP}/\text{FDP}^{1/2} = y/x^{1/2}$.

Scaling the Equations (Advanced)

Scaling the equations Before scaling the equations we estimate the turnover time of FDP due to PK by:

$$\frac{\partial v_2}{\partial x} = \frac{v_{m,2}K}{2\sqrt{K_2}} \left(\frac{x}{K_2}\right)^{1/2} \frac{3+x/K_2}{(1+x/K_2)^2} \approx \frac{v_{m,2}K}{2\sqrt{K_2}} \quad \text{for } 0.1 < \frac{x}{K_2} < 2$$

We thus scale the model by using

$$\tau = \frac{t}{2\sqrt{K_2}/v_{m,2}K} \quad \chi = \frac{x}{K_2} \tag{9.57}$$

The equations in scaled form are:

$$\frac{d\chi}{d\tau} = \frac{a\kappa\chi}{\kappa + \chi} - \frac{2\chi^{3/2}}{1 + \chi} \tag{9.58}$$

where the two dimensionless parameters are:

$$a = \frac{2\sqrt{K_2}/v_{m,2}K}{K_1/v_{m,1}} = \frac{t_{turnover}}{t_{activation}}, \quad \kappa = \frac{K_1}{K_2} \tag{9.59}$$

a ratio of time constants and a ratio of concentration action scales, see Section 9.1.

Criteria for Existence of Multiple Steady States (Advanced)

Criteria for existence of multiple steady states To evaluate the onset of multiple steady states we evaluate:

$$\lambda = \frac{a\kappa^2}{(\kappa + \chi)^2} - \frac{\chi^{1/2}(3 + \chi)}{(1 + \chi)^2} = 0 \quad (9.60)$$

and, when combined with the steady state equation this yields:

$$\chi^2 - (\kappa - 3)\chi + \kappa = 0 \quad (9.61)$$

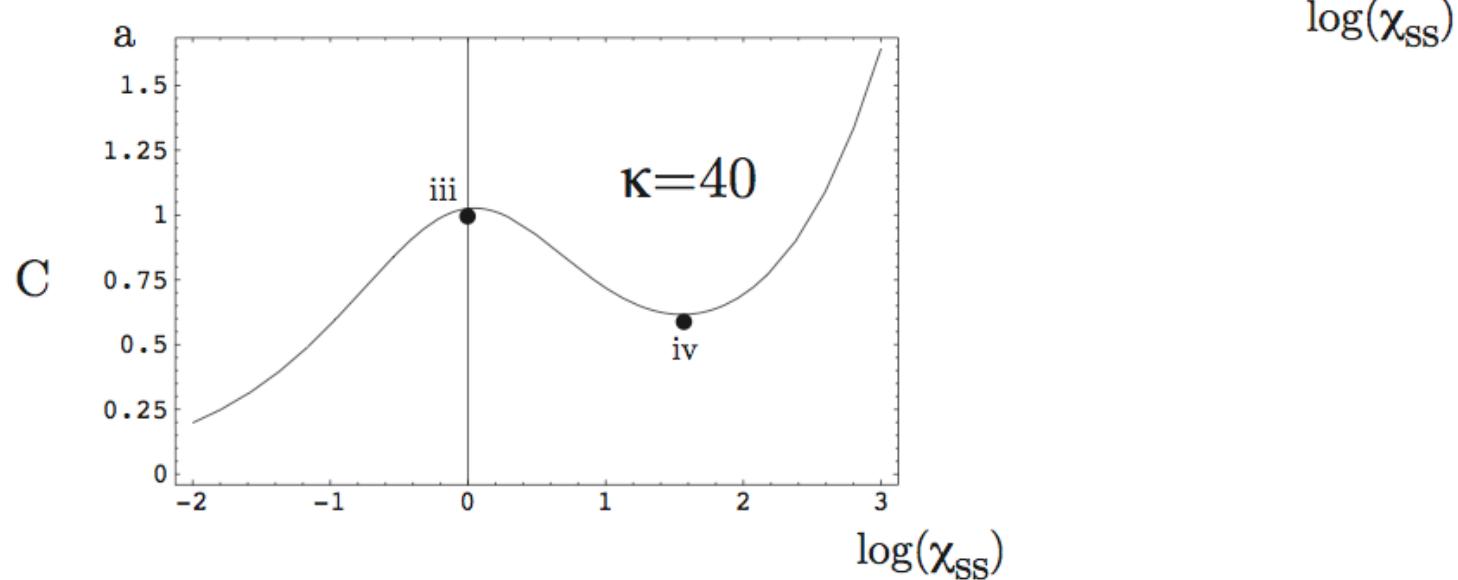
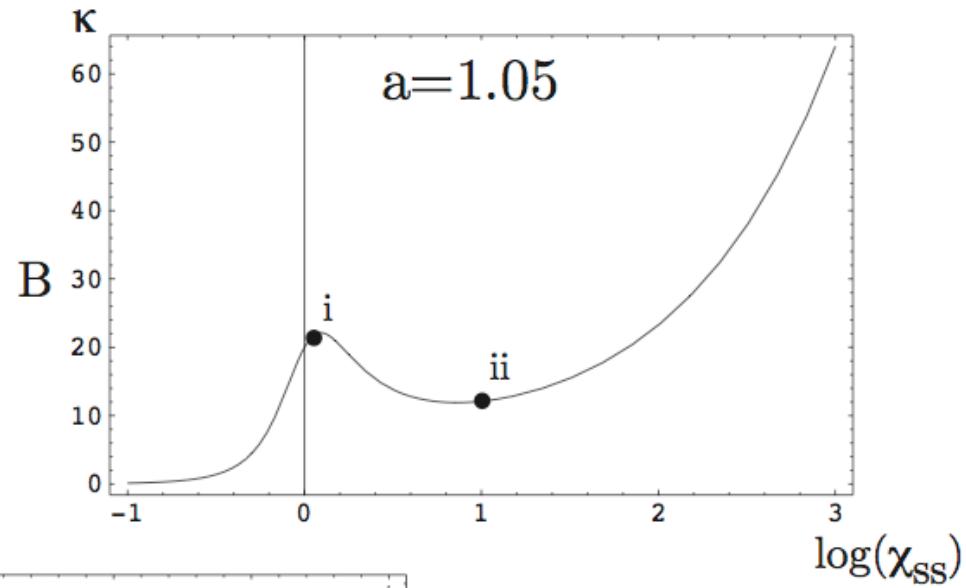
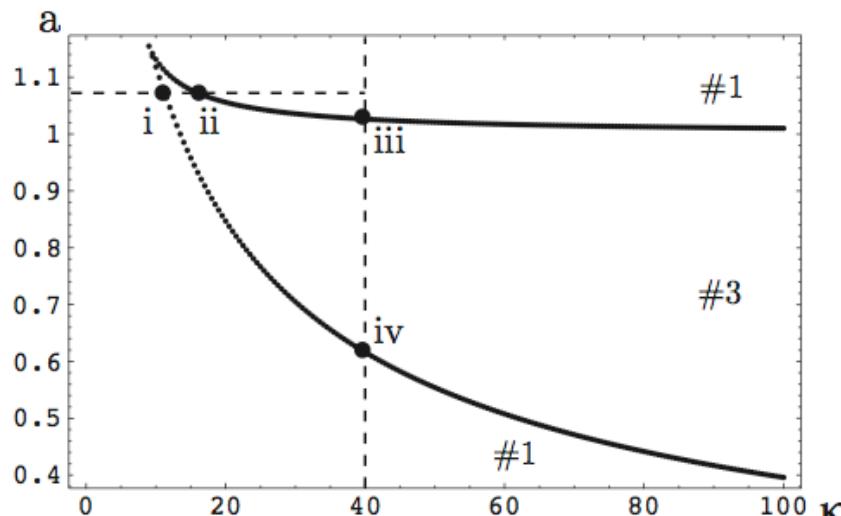
and therefore

$$\kappa \geq \kappa_{min} = 9 \quad (9.62)$$

A similar procedure (see homework 9.8) leads to:

$$a \leq a_{max} = \frac{2}{\sqrt{3}} = 1.155, \quad (9.63)$$

Computation of Multiple Steady States (Advanced)



Additions

- Compute the fluxes across the multiple steady state region