Stability of Enzymatic Reaction Chains

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1 Introduction

A human cell has a lot of internal processes. One of these processes is the production of one or more product metabolites from one or multiple substrate metabolites. Enzymes are usually involved in this process, as a catalyst. We can make a mathematical model of this process. One of the simplest models is a linear chain

$$X_0 \xleftrightarrow{e_1} X_1 \xleftrightarrow{e_2} X_2$$

where X_0 is the substrate, X_2 the product, X_1 an internal metabolite and e_i are the enzymes.

We write x_i for the concentration of metabolite X_i and v_i, v_{i+1} for the reaction rate of the reaction which produces X_i and the reaction rate of the reaction which consumes X_i respectively. Now we can look at the dynamics of

$$\dot{x_i} = v_i - v_{i+1}.$$

To look at the dynamics, we first need to make some assumptions. We want to work in a steady state. This means that the input and output flow need to be equal. This gives us that

$$v_1 = \cdots = v_{n+1}.$$

We also assume that the concentration in the in- and output reaction rates are given and are constant. In the simplest example this means that $\dot{x_0} = 0 = \dot{x_2}$. In the next section, we will look at some more advanced models for the enzymatic reactions.

2 Michael-Menten Kinetics in Simple Enzymatic Reaction Chains

We can look at larger linear chains such as

$$X_0 \overset{e_1}{\longleftrightarrow} X_1 \overset{e_2}{\longleftrightarrow} \cdots \overset{e_{N+1}}{\longleftrightarrow} X_{N+1} \tag{2.1}$$

Here we have X_0 and X_{N+1} external and $X_1, ..., X_N$ internal metabolites, and $e_j, j \in J = \{1, ..., N+1\}$ the enzymes. The reaction functions f_j may depend only on the substrates and products of the corresponding reaction, so

$$f_j(\mathbf{x}) = f_j(x_{j-1}, x_j)$$

in case of (2.1). Or the reaction functions f_j can also depend on other metabolic concentrations, for instance if metabolites can form complexes with enzymes catalysing reactions in which they do not appear as substrate or product. These functions $f_j(\mathbf{x})$ are often referred to as the saturation levels of the corresponding enzyme.

Using Michaelis-Menten kinetics these levels can be derived from mass action kinetics involving different time scales. In the simplest case we have the form

$$S + E \rightleftarrows ES \rightleftarrows EP \rightleftarrows P + E \tag{2.2}$$

This can be simplified as the reversible substrate and product reaction

$$S \overset{e}{\longleftrightarrow} P \tag{2.3}$$

For computing the reaction functions we first introduce some notation for denoting concentrations

$$s = [S], p = [P], c_0 = [C_0] = [E], c_1 = [C_1] = [ES], c_2 = [C_2] = [EP]$$

This gives us a system of differential equations

$$\begin{cases} \dot{s} = -k_1 s c_0 + k_2 c_1 \\ \dot{c_0} = -k_1 s c_0 + k_2 c_1 + k_5 c_2 - k_6 p c_0 \\ \dot{c_1} = k_1 s c_0 - k_2 c_1 - k_3 c_1 + k_4 c_2 \\ \dot{c_2} = k_3 c_1 - k_4 c_2 - k_5 c_2 + k_6 p c_0 \\ \dot{p} = k_5 c_2 - k_6 p c_0 \end{cases}$$

We say that the model (2.2) is in steady state if $\dot{s} = \dot{p} = \dot{c_1} = \dot{c_2} = 0$.
In a steady state, the concentrations of C_1 and C_2 are constant even if the

concentrations of E, S and the product P are changing. This can occur when the binding and unbinding of the ES and EP complexes have an equal rate.

A steady state is rapidly established such that the concentrations of E and ES are maintained at a constant concentration until the equilibrium position is approached. Thus, the concentrations of E and ES do not change with time.

We use the term quasi-steady state when we neglect the short period of time to reach equilibrium for the fast reaction. This means that we assume that the reactions almost instantaneously will go into steady state. The reason for using this approach is that this allows us to break up our model into two smaller systems of equations, which significantly increases the solubility of the model. Of course, by making this assumption, our results are at best an estimation of the actual steady state. Theoretically a lot can happen in the the short period of time to reach equilibrium. Nevertheless such an analysis does prove useful because it deepens our understanding of said enzymatic reaction chains.

We can also write the system of differential equations in matrix form

$$\begin{bmatrix} \dot{s} \\ \dot{c_0} \\ \dot{c_1} \\ \dot{c_2} \\ \dot{p} \end{bmatrix} = \begin{bmatrix} -k_1 s & k_2 & 0 \\ -k_1 s - k_6 p & k_2 & k_5 \\ k_1 s & -k_2 - k_3 & k_4 \\ k_6 p & k_3 & -k_4 - k_5 \\ -k_6 p & 0 & k_5 \end{bmatrix} \begin{bmatrix} c_0 \\ c_1 \\ c_2 \end{bmatrix}$$

We see that in these differential equations we get constants k_i, which correspond to the rate of a specific binding. For example, k_1 corresponds to the rate of S and E binding, k_2 to the rate of ES unbinding, and so forth.

Using this notation is possible, however, we will use a slightly different notation, namely, we put the system in a graph theoretic framework, which will help us to derive similar ODE's for even more complicated reactions. In this notation, k_{ij} stands for an arrow from node C_j to C_i. The matrix form of the differential equations will now look like

$$\begin{bmatrix} \dot{s} \\ \dot{c_0} \\ \dot{c_1} \\ \dot{c_2} \\ \dot{p} \end{bmatrix} = \begin{bmatrix} -k_{10} s & k_{01} & 0 \\ -k_{10} s - k_{20} p & k_{01} & k_{02} \\ k_{10} s & -k_{01} - k_{21} & k_{12} \\ k_{20} p & k_{21} & -k_{02} - k_{12} \\ -k_{20} p & 0 & k_{02} \end{bmatrix} \begin{bmatrix} c_0 \\ c_1 \\ c_2 \end{bmatrix}$$

We can now write this differential system as a graph with nodes C_0, C_1 and C_2 and with edges k_{ij}. These constants k_{ij} correspond to the reactions

$$C_i \leftarrow C_j.$$

The corresponding directed graph in this case will be

$$C_0 \rightleftarrows C_1 \rightleftarrows C_2 \rightleftarrows C_0. \tag{2.4}$$

When we drop s and p, this gives the square matrix

$$K_2 = \begin{bmatrix} -k_{10} - k_{20} & k_{01} & k_{02} \\ k_{10} & -k_{01} - k_{21} & k_{12} \\ k_{20} & k_{21} & -k_{02} - k_{12} \end{bmatrix}$$

for the graph denoted in (2.4). With this matrix K_2 we can derive the reaction function for the linear case. We do this by first determining a vector which spans the nullspace.

$$v_0 = \begin{bmatrix} k_{01}k_{02} + k_{01}k_{12} + k_{02}k_{21} \\ k_{02}k_{10} + k_{10}k_{12} + k_{12}k_{20} \\ k_{01}k_{20} + k_{10}k_{21} + k_{20}k_{21} \end{bmatrix}$$

After obtaining this nullvector v_0, we replace the s and the p which we have dropped before. So in this case we replace k_{10} with $k_{10}s$ and k_{20} with $k_{20}p$.

$$v_0 = \begin{bmatrix} k_{01}k_{02} + k_{01}k_{12} + k_{02}k_{21} \\ k_{02}k_{10}s + k_{10}k_{12}s + k_{12}k_{20}p \\ k_{01}k_{20}p + k_{10}k_{21}s + k_{20}k_{21}p \end{bmatrix}$$

Now we can take the first entry of v_0 to be C_0 and the second entry of v_0 as C_1 and the last entry as C_2.

$$\begin{aligned} C_0 &= k_{01}k_{02} + k_{01}k_{12} + k_{02}k_{21} \\ C_1 &= k_{02}k_{10}s + k_{10}k_{12}s + k_{12}k_{20}p \\ C_2 &= k_{01}k_{20}p + k_{10}k_{21}s + k_{20}k_{21}p \end{aligned}$$

Subsequently we can take

$$\begin{aligned} \dot{p} &= \epsilon \frac{k_{02}C_2 - pk_{20}C_0}{C_0 + C_1 + C_2} \\ &= \epsilon \frac{k_{02}k_{01}k_{20}p + k_{10}k_{21}s + k_{20}k_{21}p - pk_{20}k_{01}k_{02} + k_{01}k_{12} + k_{02}k_{21}}{k_{01}k_{02} + k_{01}k_{12} + k_{02}k_{21} + k_{02}k_{10}s + k_{10}k_{12}s + k_{12}k_{20}p + k_{01}k_{20}p + k_{10}k_{21}s + k_{20}k_{21}p} \end{aligned}$$

As we can see this becomes a very large fraction. To make this more compact and more useful, we can take $K_{eq1} = \frac{k_{10}}{k_{01}}$ and $K_{eq2} = \frac{k_{20}}{k_{02}}$ to be constant. Then by replacing k_{10} with $K_{eq1}k_{01}$ and k_{20} with $K_{eq2}k_{02}$ and taking the limits of k_{01} and k_{02} to infinity we get a much smaller fraction. By dropping the ϵ this leaves us with the much more simpler reaction function

$$\frac{-k_{12}K_{eq2}p + k_{21}K_{eq1}s}{1 + K_{eq2}p + K_{eq1}s}$$

for the linear model in (2.3).

Of course this can be done with even more complicated reactions with more complexes, for example

$$A + B \overset{e}{\longleftrightarrow} P \tag{2.5}$$

This model has the corresponding K_3-matrix

$$K_3 = \begin{bmatrix} -k_{10} - k_{20} - k_{30} & k_{01} & k_{02} & k_{03} \\ k_{10} & -k_{01} - k_{31} & 0 & k_{13} \\ k_{20} & 0 & -k_{02} - k_{32} & k_{21} \\ k_{30} & k_{31} & k_{32} & -k_{03} - k_{13} - k_{23} \end{bmatrix}.$$

We will once again determine the vector which spans the nullspace and replace the a, b and p back in the vector. Remark that we now have five places where we need to do this replacement.

$$\begin{aligned} k_{10} &\longrightarrow k_{10}a \\ k_{20} &\longrightarrow k_{20}b \\ k_{30} &\longrightarrow k_{30}p \\ k_{32} &\longrightarrow k_{32}a \\ k_{31} &\longrightarrow k_{31}b \end{aligned}$$

We will once again name the entries of this vector with C_0, C_1, C_2, C_3. Next we will look at the fraction

$$\frac{k_{03}C_3 - pk_{30}C_0}{C_0 + C_1 + C_2 + C_3} \tag{2.6}$$

which represents the reaction function. In this model there are some special loops. In this case we have the loops

$$C_0 \to C_1 \to C_3 \to C_2 \to C_0 \quad \text{and} \quad C_0 \to C_2 \to C_3 \to C_1 \to C_0.$$

Since both of these loops start and end in C_0, the flow in total through these loops has to be zero. This is because there can't be a difference in the concentration C_0 on either side, in both loops. Compare the loop with walking around a lake. When walking clockwise around the lake, the distance has to be the same as the distance when walking counterclockwise around the lake. This gives us a restraint on the k_{ij} representing these loops, namely

$$k_{10}k_{31}k_{23}k_{02} = k_{01}k_{13}k_{32}k_{20}.$$

Now we can replace k_{32} with $\frac{k_{10}k_{31}k_{23}k_{02}}{k_{01}k_{13}k_{20}}$ in (2.6). We will again take $K_{eq1} = \frac{k_{10}}{k_{01}}$, $K_{eq2} = \frac{k_{20}}{k_{02}}$ and $K_{eq3} = \frac{k_{30}}{k_{03}}$ to be constant. Then by replacing k_{10} with $K_{eq1}k_{01}$, k_{20} with $K_{eq2}k_{02}$ and k_{30} with $K_{eq3}k_{03}$ and taking the limits of k_{01}, k_{02} and k_{03} to infinity. We finally get our final reaction function

$$\frac{(k_{13} + k_{23})(k_{31}K_{eq1}ab - k_{13}K_{eq3}p)}{k_{13}(1 + K_{eq1}a + K_{eq2}b + K_{eq3}p}$$

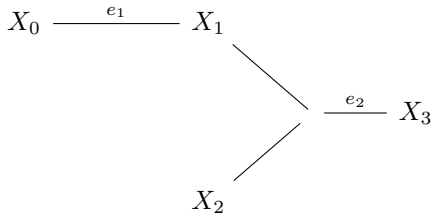
for the model in (2.5).

The Mathematica codes for these processes can be found in appendix A.

These were two of the simplest examples of models, however, there are also much more difficult chains possible. In this project, we will take a look at some more difficult chains and how they behave.

3 More Complex Enzymatic Reaction Chains

We will first look at a very simple non-linear chain.



In this chain X_0 and X_2 are substrates and X_3 is the product. The only dynamical variable in this case is X_1. This means we can only look at the dynamics of $\dot{x_1}$. We want to know if there is a point where $\dot{x_1} = 0$, since that is where the equilibrium point of this chain lies. Now we know from chapter 2 that we can write

$$\dot{x_1} = e_1 f_1(x_0, x_1) - e_2 f_2(x_1, x_2, x_3),$$

where the reaction functions are of the form

$$f_1(x_0, x_1) = \frac{x_0 - K_1 x_1}{L_1 + M_1 x_0 + N_1 x_1}$$
$$f_2(x_1, x_2, x_3) = \frac{x_1 x_2 - K_2 x_3}{L_2 + M_2 x_1 + N_2 x_2 + O_2 x_3}.$$

As we have seen before, all the constants K_i, L_i, M_i, N_i, O_i are combinations of the positive k_{ij}. Now we can make a graph where we draw $\dot{x_1}$ against x_1. This gives us the following figure:

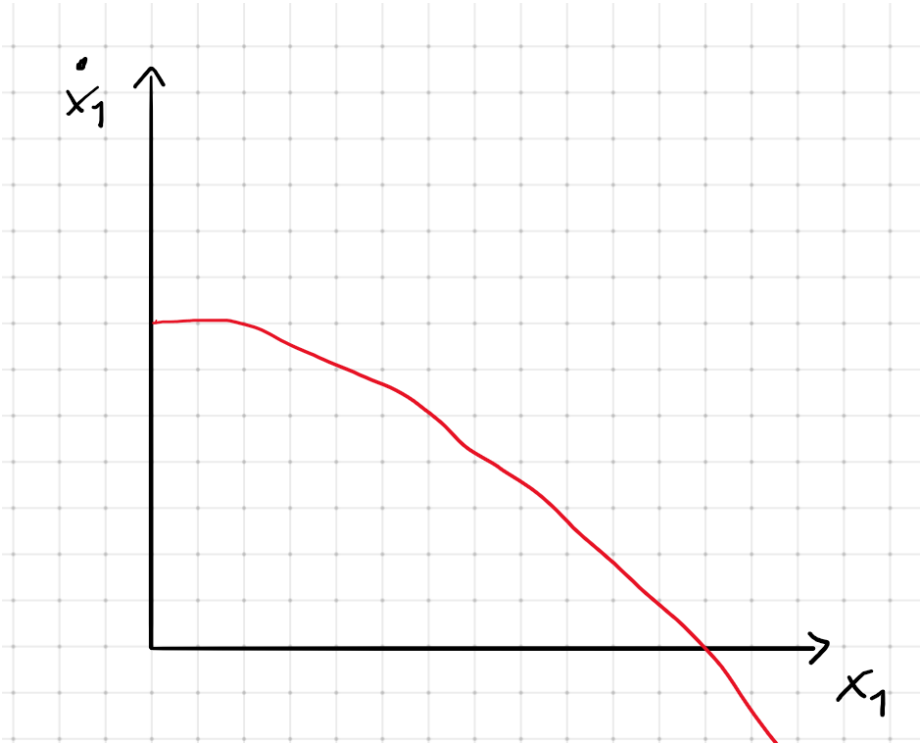
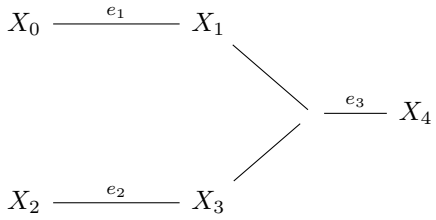


Figure 1: Sketch of $\dot{x}$ against x

We know it has this form, because the denominators are positive and when $x_1 = 0$, f_1 becomes positive and f_2 becomes negative. So we get that $\dot{x_1}$ equals a positive term minus a negative term equals a positive outcome. Now if we let x_1 become very large, f_1 will become negative and f_2 will become positive, so $\dot{x_1}$ equals a negative term minus a positive term equals a negative outcome. Since $\dot{x_1}$ is a polynomial and continuous, it follows that there is a point $x_1 = \bar{x_1}$ for which $\dot{x_1} = 0$. So we can conclude that this chain has a unique equilibrium point at $x_1 = \bar{x_1}$

We can expand this simple example to the following non-linear chain.



In this chain X_0 and X_2 are substrates and X_4 is the product. We can once

again look at the dynamics by looking at $\dot{x_1}$ and $\dot{x_3}$. From chapter 2 we know again that we can write

$$\dot{x_1} = e_1 f_1(x_0, x_1) - e_3 f_3(x_1, x_3, x_4)$$
$$\dot{x_3} = e_2 f_2(x_2, x_3) - e_3 f_3(x_1, x_3, x_4)$$

where the reaction functions are of the form

$$f_1(x_0, x_1) = \frac{x_0 - K_1 x_1}{L_1 + M_1 x_0 + N_1 x_1}$$
$$f_2(x_2, x_3) = \frac{x_2 - K_2 x_3}{L_2 + M_2 x_2 + N_2 x_3}$$
$$f_3(x_1, x_3, x_4) = \frac{x_1 x_3 - K_3 x_4}{L_3 + M_3 x_1 + N_3 x_3 + O_3 x_4}.$$

When drawing this system with Mathematica (Figure 2 and 3) and looking at the phase portrait in the first quadrant (because x_1 and x_3 have to be positive), it seems like there is a unique stable equilibrium. We want to prove these assumptions.

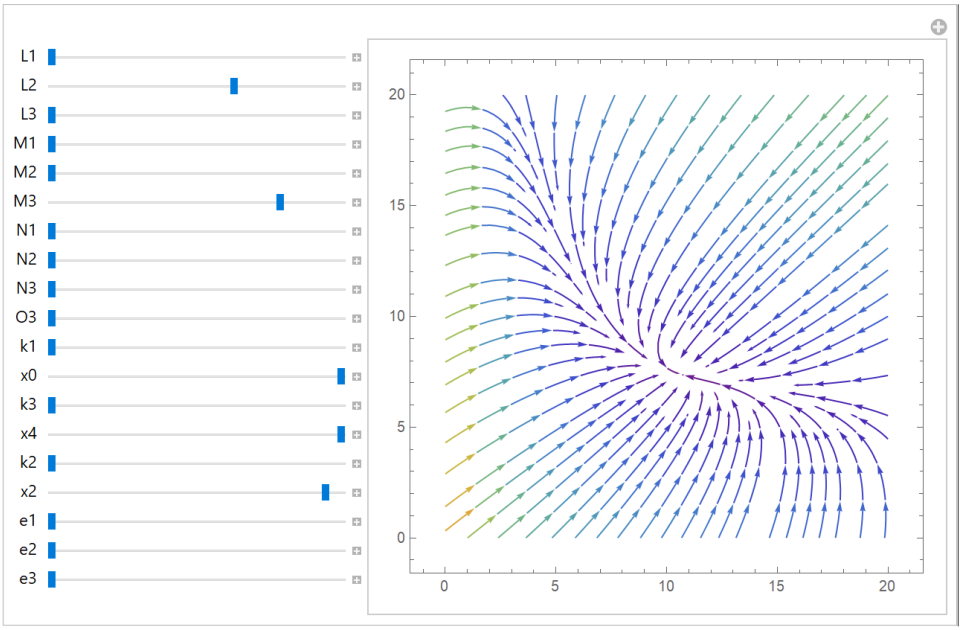


Figure 2: Phase portrait 1

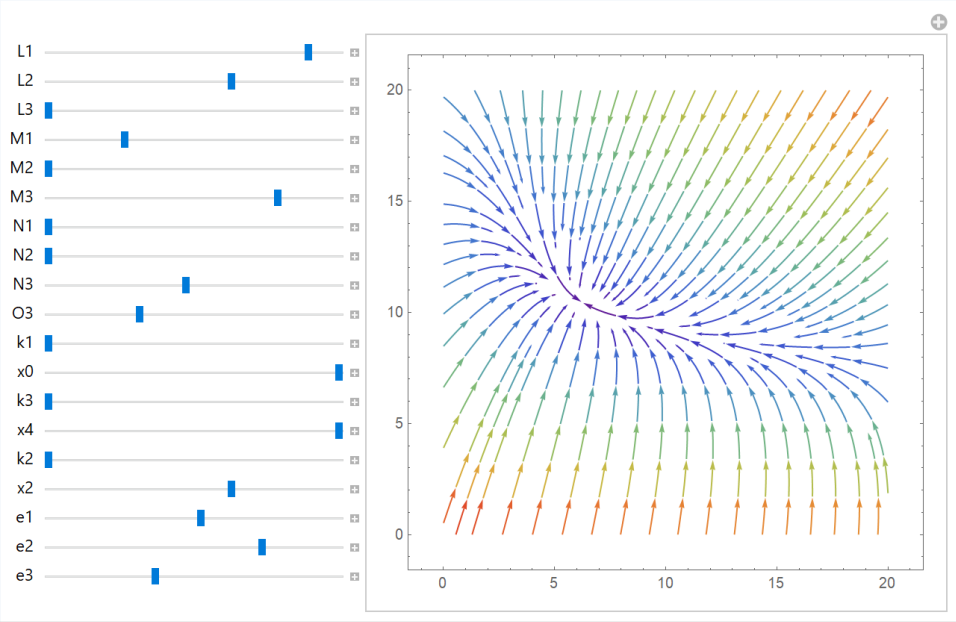


Figure 3: Phase portrait 2

We will first prove that there is an equilibrium. To do this we want to construct an invisible 'rectangle' from which the flow cannot leave. We know that we must stay in the first quadrant since we cannot have a negative concentration. So the rectangle already has the two boundaries $x_1 = 0$ and $x_3 = 0$. It is clear from the function that if $x_1 = 0$, then $\dot{x_1} > 0$ and if $x_3 = 0$, then $\dot{x_3} > 0$. So the flow moves towards the first quadrant from the x_1 and x_3-axis.

We take a look at what happens with $\dot{x_1}$ and $\dot{x_3}$ when we take x_1 and x_3 as a constant. For this we first look at the partial derivatives.

$$\frac{\partial f_1}{\partial x_1} = \frac{-K_1(L_1 + M_1x_0 + N_1x_1) - N1(x0 - K_1x_1)}{(L_1 + M_1x_0 + N_1x_1)^2} < 0 \tag{3.1}$$

$$\frac{\partial f_3}{\partial x_1} = \frac{x_3(L_3 + M_3x_1 + N_3x_3 + O_3x_4) - M_3(x_1x_3 - K_3x_4)}{(L_3 + M_3x_1 + N_3x_3 + O_3x_4)^2} > 0 \tag{3.2}$$

$$\frac{\partial f_3}{\partial x_3} = \frac{x_1(L_3 + M_3x_1 + N_3x_3 + O_3x_4) - N_3(x_1x_3 - K_3x_4)}{(L_3 + M_3x_1 + N_3x_3 + O_3x_4)^2} > 0 \tag{3.3}$$

We now see that when we let x_1 be a great constant, then f_1 will become a negative term since f_1 is a descending function in x_1 and it is clear to see from f_1 that it becomes negative for x_1 large enough. Since f_3 is increasing in x_1 we see that for large x_1, f_3 will be positive. Note that setting x_1 as a constant will not change f_2. This means we have

$$\dot{x_1} < 0$$

In the same way, if we now take x_3 as a great constant we see that f_1 will not change, f_2 will be negative and f_3 will be positive. Thus again we get:

$$\dot{x_3} < 0$$

This proves that the vectors in the phase portrait for x_1 and x_3 large will always point to the interior of the 'rectangle'.
Now we can use a useful theorem of Brouwer which says

Theorem 3.1 (Brouwer's Fixed Point)**.** *Every continuous function from a convex compact subset K of an Euclidean space to K itself has a fixed point.*

In our case we work in the first quadrant in $\mathbb{R}^2$, which we know is an Euclidean space. Now we can use the next theorem:

Theorem 3.2 (Heine-Borel)**.** *A subset $K \subset \mathbb{R}^k$ is compact if and only if K is closed and bounded.*

This tells us that the rectangle constructed above is a compact subset of an Euclidean space (since it is closed and bounded) and thus the continuous functions $\dot{x_1}$ and $\dot{x_3}$ must have a fixed point in the rectangle itself. This means that this point will be sent to itself, so the horizontal and vertical movement in this point will be equal to zero, which translates to $\dot{x_1} = 0 = \dot{x_3}$. Now we have proven that there exists an equilibrium point in the first quadrant.

Next we show that this equilibrium must be unique. We know that if a dynamical system is at an equilibrium, all of it's derivatives must be 0, so

$$\dot{x_1} = 0 = \dot{x_3},$$

which implies

$$e_1 f_1(x_0, x_1) = e_3 f_3(x_1, x_3, x_4) \tag{3.4}$$
$$e_2 f_2(x_2, x_3) = e_3 f_3(x_1, x_3, x_4), \tag{3.5}$$

which combined also give us

$$e_1 f_1(x_0, x_1) = e_2 f_2(x_2, x_3). \tag{3.6}$$

Recall from the first chapter that this falls in line with our assumption that in order to work in a steady state, all reaction rates must be equal. Our argument unfolds as follows. First we look at equation (3.4). Note that x_1 and x_3 are the only variables in our chain, so we can see f_1 as a function of only x_1 and f_3 as a function of only x_1 and x_3. Remember the partial derivatives we've determined in (3.1-3.3)

Now consider equation (3.4) for fixed x_1. From the derivations we've seen we know that $e_3 f_3$ is an increasing function (regardless of the variable we choose to look at). We also find that

$$\lim_{x_3 \to \infty} f_3 = \frac{x_1}{N_3}.$$

Furthermore

$$f_3(x_1, x_3) = 0 \implies x_3 = \frac{x_4 K_3}{x_1}.$$

Note that this is well-defined since $x_1 \neq 0$. So we know for fixed x_1 that $e_3 f_3$ is an increasing function that starts at $x_3 = \frac{x_4 K_3}{x1}$ with range $[0, \frac{x_1}{N_3}]$. Assume that for this fixed x_1 , there is an $\bar{x_3}$ such that

$$e_1 f_1(x_1) = e_3 f_3(x_1, \bar{x_3})$$

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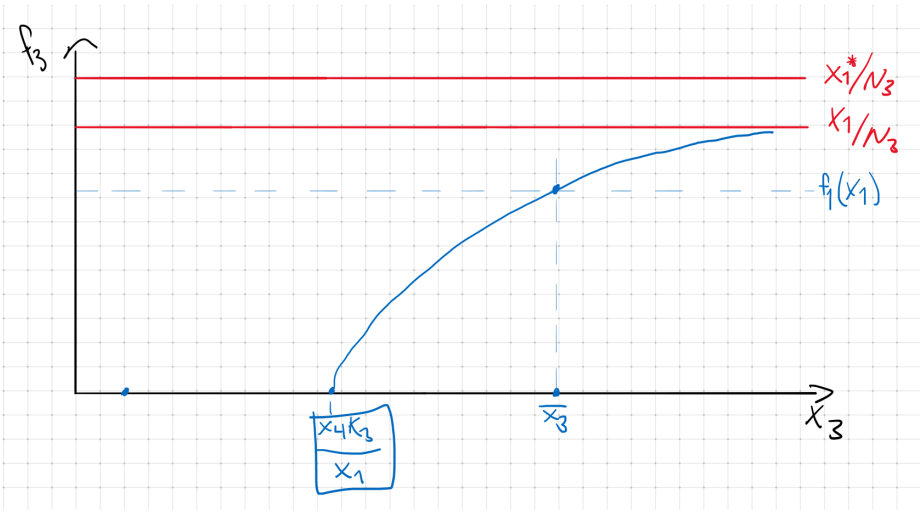


Figure 4: Sketch of $y = f_3(x_1, x_3)$ for fixed x_1

Now take a larger x_1^* such that $x_1^* > x_1$. Then the start of f_3 decreases because $\frac{x_4 K_3}{x_1^*} < \frac{x_4 K_3}{x_1}$. However, f_3 remains an increasing function, now with limit Since $f_1(x_1)$ is a decreasing function in x_1, we find $f_1(x_1^*) < f_1(x_1)$. Which tells us that we must have $\bar{x_3^*} < \bar{x_3}$ in order to maintain the equality $e_1 f_1(x_1^*) = e_3 f_3(x_1^*, \bar{x_3^*})$.

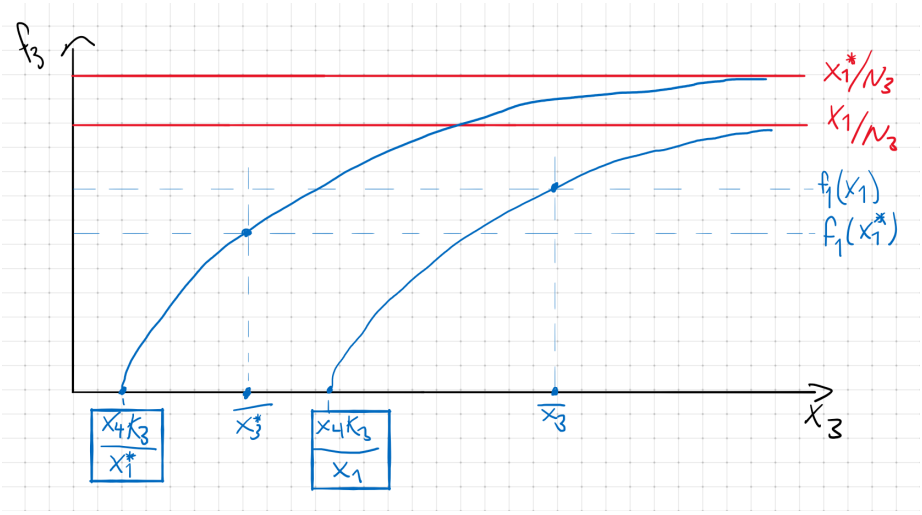


Figure 5: Sketch of $y = f_3(x_1^*, x_3)$ for fixed x_1^* with $x_1^* > x_1$

So when x_1 increases, x_3 decreases, or in other words

$$\frac{\partial x_3}{\partial x_1} < 0,$$

which describes a decreasing curve in the $(x_1, x_3)-$plane, that satisfies equation (3.4).

Next, let's look at equation (3.6) for fixed x_3. We have seen that $f_1(x_1)$ is a decreasing function. Furthermore

$$f_1(x_1) = 0 \implies x_1 = \frac{x_0}{K}.$$

Since f_1 doesn't depend on x_3 we can conclude that f_1 is always a decreasing function. So we can assume that for any x_3 there is a $\bar{x_1}$ such that

$$e_2 f_2(x_3) = e_1 f_1(\bar{x_1}).$$

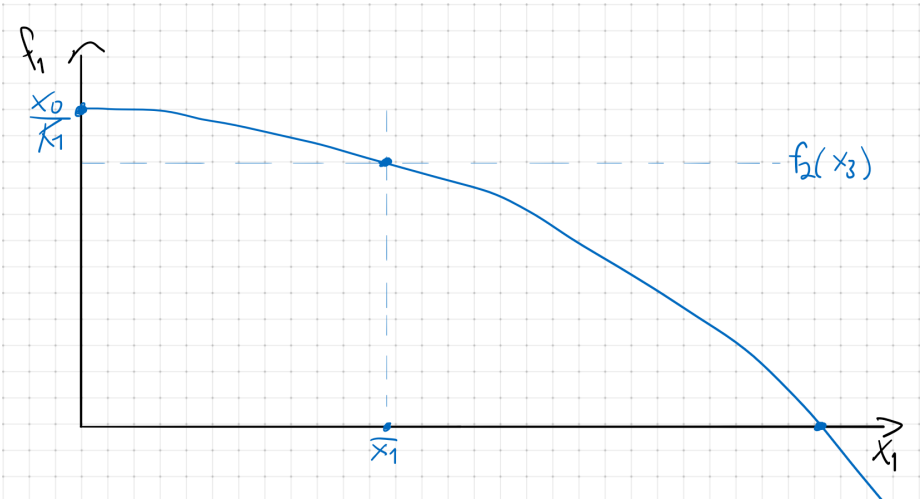


Figure 6: Sketch of $y = f_1(x_1)$ for fixed x_3

Similar to f_1, f_2 also is a decreasing function. Now take a larger x_3^* such that $x_3^* > x_3$. This merely lowers the (fixed) level of f_2, giving us $f_2(x_3^*) < f_2(x_3)$, but doesn't affect f_1. This gives us that there must be a $x_1^* > x_1$ such that $f_2(x_3^*) = f_1(x_1^*)$.

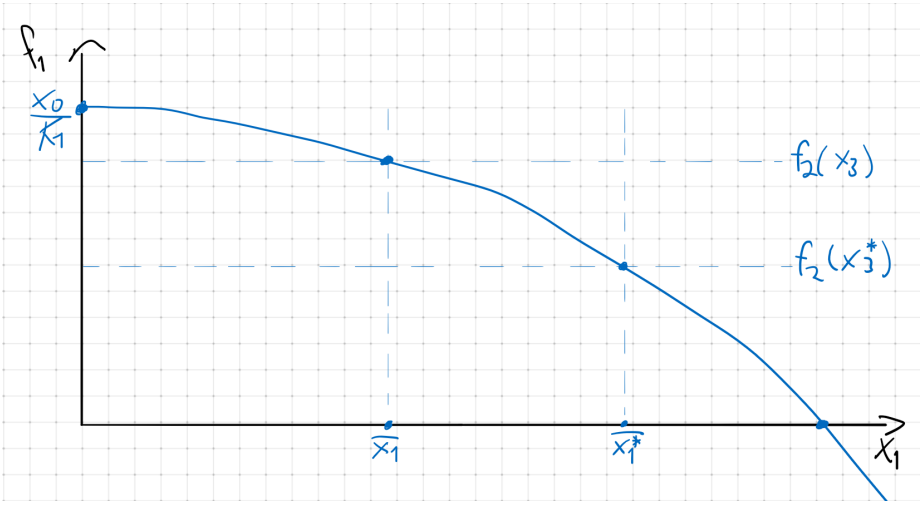


Figure 7: Sketch of $y = f_1(x_1^*)$ for fixed x_3^* with $x_3^* > x_3$

This makes us conclude that

$$\frac{\partial x_1}{\partial x_3} > 0,$$

which describes an increasing curve in the $(x_1, x_3)-$plane, that satisfies equation (3.6)

So we have found two curves in the $(x_1, x_3)-$plane that each respectively satisfy equations (3.4) and (3.6). Their single intersection must satisfy both conditions, and as such must be a unique point of equilibrium for this chain.

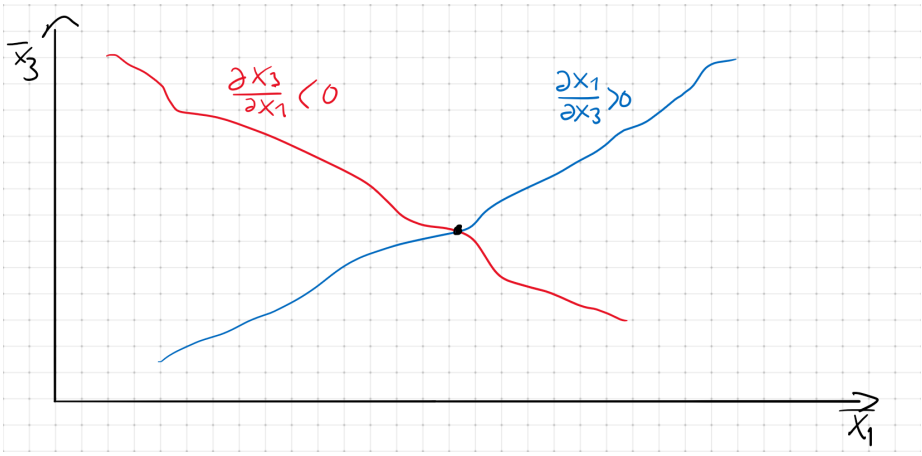


Figure 8: Sketch of the two curves in the $(x_1, x_3)-$plane

Now to prove this equilibrium is stable (in the first quadrant), we will look at the Jacobian. We know that the system is asymptotically stable if all of the real parts of the eigenvalues of the Jacobian are negative. The Jacobian J is of the form

$$J = \begin{bmatrix} \frac{d\dot{x_1}}{dx_1} & \frac{d\dot{x_1}}{dx_3} \\ \frac{d\dot{x_3}}{dx_1} & \frac{d\dot{x_3}}{dx_3} \end{bmatrix}.$$

We will first write out the entries of the Jacobian.

$$\begin{aligned}
\frac{\partial \dot{x_1}}{\partial x_1} &= e_1 \frac{-K_1(L_1 + M_1 x_0) - N_1 x_0}{(L_1 + M_1 x_0 + N_1 x_1)^2} - e_3 \frac{x_3(L_3 + N_3 x_3 + O_3 x_4) + M_3 K_3 x_4}{(L_3 + M_3 x_1 + N_3 x_3 + O_3 x_4)^2} \\
&= -a - b \\
\frac{\partial \dot{x_1}}{\partial x_3} &= -e_3 \frac{x_1(L_3 + M_3 x_1 + O_3 x_4) + N_3 K_3 x_4}{(L_3 + M_3 x_1 + N_3 x_3 + O_3 x_4)^2} \\
&= -c \\
\frac{\partial \dot{x_3}}{\partial x_1} &= -e_3 \frac{x_3(L_3 + N_3 x_3 + O_3 x_4) + M_3 K_3 x_4}{(L_3 + M_3 x_1 + N_3 x_3 + O_3 x_4)^2} \\
&= -b \\
\frac{\partial \dot{x_3}}{\partial x_3} &= e_2 \frac{-K_2(L_2 + M_2 x_2) - N_2 x_2}{(L_2 + M_2 x_2 + N_2 x_3)^2} - e_3 \frac{x_1(L_3 + M_3 x_1 + O_3 x_4) + N_3 K_3 x_4}{(L_3 + M_3 x_1 + N_3 x_3 + O_3 x_4)^2} \\
&= -d - c
\end{aligned}$$

Now since all the constants (the capitol letters, the enzymes e_i and x_0, x_2, x_4) are positive, we can see that $a, b, c, d > 0$. So all the entries of J are negative. So we get

$$J = \begin{bmatrix} -a - b & -c \\ -b & -d - c \end{bmatrix}.$$

The determinant of J $\det(J) = (-a - b)(-d - c) - bc = ad + bd + ac > 0$ and the trace of J $\mathrm{Tr}(J) = -a - b - d - c < 0$. So we know that the sum of the eigenvalues is negative and the product of the eigenvalues is positive. This leaves us with two possibilities

- Either both eigenvalues λ_1, λ_2 are real, in which case it is now clear that they are both negative.
- Or both eigenvalues λ_1, λ_2 are imaginary, in which case they are each others conjugate. This means they are of the form $\lambda_1 = a + bi, \lambda_2 = a - bi$. So because $0 > \lambda_1 + \lambda_2 = a + bi + a - bi = 2a$, we get that the real part of the eigenvalues λ_1, λ_2 are negative.

So the equilibrium is asymptotically stable in the first quadrant. This means we now proven have local stability of the equilibrium.

To prove global stability we will use the following theorem

Theorem 3.3. *Let the equilibrium point be locally asymptotically stable. If there are no periodic orbits, then this point is globally asymptotically stable if and only if there are no saddles at infinity.*

We have just shown that the equilibrium point is locally stable and we've already shown that for large enough x_1 and x_3 we get an invariant rectangle. So we've shown no trajectories are leaving the first quadrant or going to infinity and by the existence of the rectangle we have precluded the existence of saddles at infinity.

The only thing left to show now is that there are no closed trajectories, then we can use theorem 3.3. We can show this easily with the Bendixson criterion.

Theorem 3.4 (Bendixson's criterion)**.** *If*

$$\frac{\partial \dot{x_1}}{\partial x_1} + \frac{\partial \dot{x_3}}{\partial x_3}$$

doesn't change sign on the first quadrant, there are no closed trajectories in the first quadrant.

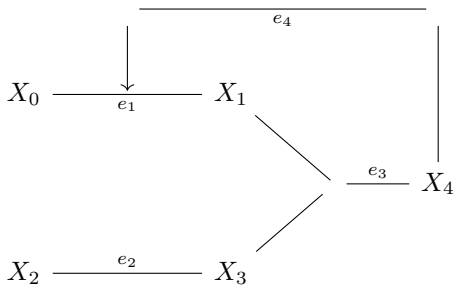
As we've shown above, we have

$$\frac{\partial \dot{x_1}}{\partial x_1} + \frac{\partial \dot{x_3}}{\partial x_3} = -a - b - d - c < 0.$$

Thus we have no periodic orbits, a locally asymptotically stable equilibrium and no saddles at infinity. Theorem 3.3 tells us now that the point is globally asymptotically stable.

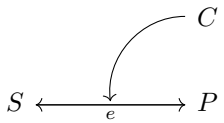
4 Feedback in an Enzymatic Reaction Chain

Another possibility for enzymatic reactions is for there to be a feedback or a feedforward within the reaction chain. This means that enzymes can influence earlier or later reactions in the reaction chain. In case of an inhibiting feedback, this gives us the following reaction chain scheme



From the scheme it is easy to see that the system changes in $\dot{x_1}$, because $f_1(x_0, x_1)$ will also depend on x_4. We will look at how this changes with the

simplified chain



with model

$$\begin{aligned} S + E &\rightleftarrows SE \\ SE &\rightleftarrows PE \\ PE &\rightleftarrows P + E \\ C + E &\rightleftarrows CE. \end{aligned}$$

We will write

$$s = [S], p = [P], c = [C], c_0 = [E] = [C_0], c_1 = [SE] = [C_1], c_2 = [PE] = [C_2], c_3 = [CE] = [C_3]$$

for the concentrations. We will follow the same process as we did in the case of

$$S \overset{e}{\longleftrightarrow} P.$$

First, we write

$$K_{3.1} = \begin{bmatrix} -k_{10} - k_{20} - k_{30} & k_{01} & k_{02} & k_{03} \\ k_{10} & -k_{01} - k_{21} & k_{12} & 0 \\ k_{20} & k_{21} & -k_{02} - k_{12} & 0 \\ k_{30} & 0 & 0 & -k_{03} \end{bmatrix}.$$

Now we compute the vector which spans the nullspace and replacing the s, p, c concentrations in the vector. Then we compute the reaction function by taking

$$\frac{k_{02}C_2 - pk_{20}C_0}{C_0 + C_1 + C_2 + C_3}$$

and substituting k_{i0} with $K_{eqi}k_{0i}$ for $i = 1, 2, 3$ and letting the limit of k_{0i} go to infinity. This leaves us with a reaction function of the form

$$\frac{s - K_1 p}{L_1 + M_1 s + N_1 p + O_1 c}.$$

For the Mathematica code look at appendix A.

So we can see that an inhibiting feedback gives an extra term in the denominator of the reaction function. The same holds for a feedforward. So were we to build in a feedforward, for example from X_0 to e_3, we would also get an extra term in the denominator of the reaction function of f_3. We can now use this to see if the model with a feedback loop still has a unique equilibrium in the first quadrant and if this is still locally and globally stable.

We now have

$$\dot{x_1} = e_1 f_1(x_0, x_1, x_4) - e_3 f_3(x_1, x_3, x_4)$$
$$\dot{x_3} = e_2 f_2(x_2, x_3) - e_3 f_3(x_1, x_3, x_4)$$

where the reaction functions are of the form

$$f_1(x_0, x_1, x_4) = \frac{x_0 - K_1 x_1}{L_1 + M_1 x_0 + N_1 x_1 + O_1 x_4}$$
$$f_2(x_2, x_3) = \frac{x_2 - K_2 x_3}{L_2 + M_2 x_2 + N_2 x_3}$$
$$f_3(x_1, x_3, x_4) = \frac{x_1 x_3 - K_3 x_4}{L_3 + M_3 x_1 + N_3 x_3 + O_3 x_4}.$$

When it comes to the existence of the equilibrium point we again can construct a compact rectangle such as in chapter 3 in which the flow cannot leave. We are going to give a little recap about this. The only function that changes is f_1 such that it has an extra term in its denominator. This term, however, is a positive constant so effectively, the behaviour of f_1 doesn't change. Take again x_1 large, since the behaviour of f_1 does not change by the feedback, we still see that this function will be negative. Function f_3 will still be positive since it is not changed. Thus again $\dot{x_1} < 0$. Same for when we take x_3 large again, we see that $\dot{x_3} < 0$, since we know that f_2 and f_3 do not change by the feedback. This means that all the vectors point to the interior of the rectangle. Now again according to Theorem 3.1 and Theorem 3.2, we have a fixed point for $\dot{x_1}$ and $\dot{x_3}$.

When it comes to the uniqueness of the equilibrium point it still suffices to analyse when

$$\dot{x_1} = 0 = \dot{x_2}.$$

For the most part this analyses is identical to the one we did in chapter 3. As described above the behaviour of f_1 doesn't change. Which means we can still conclude that there are two curves with $\frac{\partial x_3}{\partial x_1} < 0$ and $\frac{\partial x_1}{\partial x_3} > 0$ in the $(x_1, x_3)-$plane such that they satisfy

$$e_1 f_1(x_0, x_1, x_4) = e_3 f_3(x_1, x_3, x_4)$$
$$e_1 f_1(x_0, x_1, x_4) = e_2 f_2(x_2, x_3),$$

respectively. The intersection of these two curves give us the unique equilibrium point.

After proving that there is a unique equilibrium we can once again look at the Jacobian to see if it is locally stable. Remark that $\frac{\dot{x_3}}{x_1}, \frac{\dot{x_1}}{x_3}$ and $\frac{\dot{x_3}}{x_3}$ do not change, since only f_1 is changed. Since the partial derivative of the new f_1 is

$$\frac{\partial \dot{x_1}}{\partial x_1} = e_1 \frac{-K_1(L_1 + M_1 x_0 + O_1 x_4) - N_1 x_0}{(L_1 + M_1 x_0 + N_1 x_1 + O_1 x_4)^2}$$

all entries of the Jacobian are still negative. So we still have that $\det(J) > 0$ and $\mathrm{Tr}(J) < 0$, so the real parts of the eigenvalues are still negative. We can conclude that the feedback in the system doesn't change the local stability.

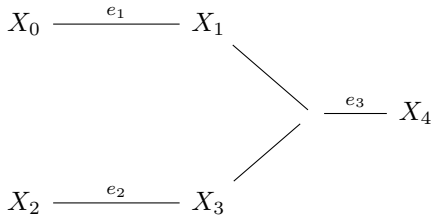
Since the feedback doesn't change the fact that the partial derivatives are negative, we still have that

$$\frac{\partial \dot{x_1}}{\partial x_1} + \frac{\partial \dot{x_3}}{\partial x_3} < 0$$

in the first quadrant. So with Bendixson's criterion we still have that there are no closed trajectories. With the rectangle where all vectors point inwards, we get global stability.

5 Further Research

We can conclude that enzymatic reaction chains of the form



with or without inhibiting feedback loops are very stable. After looking at these very stable chains, one might start to wonder if there is a possibility to make this reaction chain unstable. We have tried to simulate this in various ways in Mathematica to see if the system will start to oscillate and become unstable. We tried adding a delayed feedback, by adding a long linear chain to X_4 with a feedback from the end of this chain. This did not seem to make the system unstable. Furthermore, we tried to put in a sort of feedback from X_1 to X_3 or vice versa. However, this also did not make the system oscillate. See appendix A for an example we tried. For further research, it can be interesting to look at other possibilities to make this system unstable.

A Mathematica Code

```
In[217]:= K2 = {{-k10 - k20, k01, k02}, {k10, -k01 - k21, k12}, {k20, k21, -k02 - k12}}
Out[217]= {{-k10 - k20, k01, k02}, {k10, -k01 - k21, k12}, {k20, k21, -k02 - k12}}

In[218]:= K2 // MatrixForm
Out[218]//MatrixForm=
( -k10 - k20     k01          k02
    k10      -k01 - k21      k12
    k20          k21      -k02 - k12 )

In[235]:= nulvector3 = NullSpace[K2] * (k01 k20 + k10 k21 + k20 k21)
Out[235]= {{k01 k02 + k01 k12 + k02 k21, k02 k10 + k10 k12 + k12 k20, k01 k20 + k10 k21 + k20 k21}}

In[236]:= nulvector3 = nulvector3 /. k10 → k10 s;
          nulvector3 = nulvector3 /. k20 → k20 p
Out[237]= {{k01 k02 + k01 k12 + k02 k21, k12 k20 p + k02 k10 s + k10 k12 s, k01 k20 p + k20 k21 p + k10 k21 s}}

In[262]:= c000 = nulvector3[[1]][[1]];
          c111 = nulvector3[[1]][[2]];
          c222 = nulvector3[[1]][[3]];

In[248]:= vpp = (k02 c222 - k20 p c000) / (c000 + c111 + c222)
                  -k20 (k01 k02 + k01 k12 + k02 k21) p + k02 (k01 k20 p + k20 k21 p + k10 k21 s)
Out[248]= ------------------------------------------------------------------------------------------
          k01 k02 + k01 k12 + k02 k21 + k01 k20 p + k12 k20 p + k20 k21 p + k02 k10 s + k10 k12 s + k10 k21 s

In[254]:= vpp = vpp /. k10 → Keq01 * k01;
          vpp = vpp /. k20 → Keq02 * k02;
          vpp = Limit[vpp, k01 → ∞];
          vpp = Limit[vpp, k02 → ∞];
          vpp = Simplify[vpp]
Out[258]= -k12 Keq02 p + k21 Keq01 s
          ---------------------------
          1 + Keq02 p + Keq01 s
```

```
In[117]:= K3 = {{-k10 - k20 - k30, k01, k02, k03}, {k10, -k01 - k31, 0, k13}, {k20, 0, -k02 - k32, k23},
  {k30, k31, k32, -k03 - k13 - k23}}

Out[117]= {{-k10 - k20 - k30, k01, k02, k03}, {k10, -k01 - k31, 0, k13},
  {k20, 0, -k02 - k32, k23}, {k30, k31, k32, -k03 - k13 - k23}}
```

```
In[91]:= K3 // MatrixForm

Out[91]//MatrixForm=
⎛ -k10 - k20 - k30     k01          k02            k03          ⎞
⎜      k10         -k01 - k31       0              k13          ⎟
⎜      k20             0       -k02 - k32         k23          ⎟
⎝      k30            k31          k32      -k03 - k13 - k23   ⎠
```

```
In[118]:= nulvector =
  NullSpace[K3] *
   (k01 k02 k30 + k02 k10 k31 + k02 k30 k31 + k01 k20 k32 + k01 k30 k32 + k10 k31 k32 +
     k20 k31 k32 + k30 k31 k32)

Out[118]= {{k01 k02 k03 + k01 k02 k13 + k01 k02 k23 + k02 k03 k31 +
    k02 k23 k31 + k01 k03 k32 + k01 k13 k32 + k03 k31 k32, k02 k03 k10 + k02 k10 k13 +
    k02 k10 k23 + k02 k13 k30 + k03 k10 k32 + k10 k13 k32 + k13 k20 k32 + k13 k30 k32,
   k01 k03 k20 + k01 k13 k20 + k01 k20 k23 + k01 k23 k30 + k03 k20 k31 + k10 k23 k31 +
    k20 k23 k31 + k23 k30 k31, k01 k02 k30 + k02 k10 k31 + k02 k30 k31 +
    k01 k20 k32 + k01 k30 k32 + k10 k31 k32 + k20 k31 k32 + k30 k31 k32}}
```

```
In[119]:= nulvector2 = nulvector /. k10 → a k10 ;
  nulvector2 = nulvector2 /. k20 → b k20;
  nulvector2 = nulvector2 /. k30 → p k30;
  nulvector2 = nulvector2 /. k32 → a k32;
  nulvector2 = nulvector2 /. k31 → b k31

Out[123]= {{k01 k02 k03 + k01 k02 k13 + k01 k02 k23 + b k02 k03 k31 + b k02 k23 k31 +
    a k01 k03 k32 + a k01 k13 k32 + a b k03 k31 k32, a k02 k03 k10 + a k02 k10 k13 + a k02 k10 k23 +
    a^2 k03 k10 k32 + a^2 k10 k13 k32 + a b k13 k20 k32 + k02 k13 k30 p + a k13 k30 k32 p,
   b k01 k03 k20 + b k01 k13 k20 + b k01 k20 k23 + b^2 k03 k20 k31 + a b k10 k23 k31 + b^2 k20 k23 k31 +
    k01 k23 k30 p + b k23 k30 k31 p, a b k02 k10 k31 + a b k01 k20 k32 + a^2 b k10 k31 k32 +
    a b^2 k20 k31 k32 + k01 k02 k30 p + b k02 k30 k31 p + a k01 k30 k32 p + a b k30 k31 k32 p}}
```

In[244]:=
```
c0 = nulvector2[[1]][[1]];
c1 = nulvector2[[1]][[2]];
c2 = nulvector2[[1]][[3]];
c3 = nulvector2[[1]][[4]];
```

In[208]:=
```
vp = (k03 c3 - k30 p c0) / (c0 + c1 + c2 + c3)
```

Out[208]=
$$\Big(-k30\,(k01\,k02\,k03 + k01\,k02\,k13 + k01\,k02\,k23 + b\,k02\,k03\,k31 + b\,k02\,k23\,k31 + a\,k01\,k03\,k32 + a\,k01\,k13\,k32 + a\,b\,k03\,k31\,k32)\,p + k03\,\big(a\,b\,k02\,k10\,k31 + a\,b\,k01\,k20\,k32 + a^2\,b\,k10\,k31\,k32 + a\,b^2\,k20\,k31\,k32 + k01\,k02\,k30\,p + b\,k02\,k30\,k31\,p + a\,k01\,k30\,k32\,p + a\,b\,k30\,k31\,k32\,p\big)\Big) \Big/ \big(k01\,k02\,k03 + a\,k02\,k03\,k10 + k01\,k02\,k13 + a\,k02\,k10\,k13 + b\,k01\,k03\,k20 + b\,k01\,k13\,k20 + k01\,k02\,k23 + a\,k02\,k10\,k23 + b\,k01\,k20\,k23 + b\,k02\,k03\,k31 + a\,b\,k02\,k10\,k31 + b^2\,k03\,k20\,k31 + b\,k02\,k23\,k31 + a\,b\,k10\,k23\,k31 + b^2\,k20\,k23\,k31 + a\,k01\,k03\,k32 + a^2\,k03\,k10\,k32 + a\,k01\,k13\,k32 + a^2\,k10\,k13\,k32 + a\,b\,k01\,k20\,k32 + a\,b\,k13\,k20\,k32 + a\,b\,k03\,k31\,k32 + a^2\,b\,k10\,k31\,k32 + a\,b^2\,k20\,k31\,k32 + k01\,k02\,k30\,p + k02\,k13\,k30\,p + k01\,k23\,k30\,p + b\,k02\,k30\,k31\,p + b\,k23\,k30\,k31\,p + a\,k01\,k30\,k32\,p + a\,k13\,k30\,k32\,p + a\,b\,k30\,k31\,k32\,p\big)$$

In[209]:=
```
vp = vp /. k32 → (k02 k10 k23 k31) / (k01 k13 k20);
vp = vp /. k10 → Keq01 * k01;
vp = vp /. k20 → Keq02 * k02;
vp = vp /. k30 → Keq03 * k03;
vp = Limit[vp, k01 → ∞];
vp = Limit[vp, k02 → ∞];
vp = Limit[vp, k03 → ∞];
vp = Simplify[vp]
```

Out[216]=
$$\frac{(k13 + k23)\,(a\,b\,k31\,Keq01 - k13\,Keq03\,p)}{k13\,(1 + a\,Keq01 + b\,Keq02 + Keq03\,p)}$$

```
In[479]:= K31 = {{-k10 - k20 - k30, k01, k02, k03}, {k10, -k01 - k21, k12, 0}, {k20, k21, -k02 - k12, 0}, {k30, 0, 0, -k03}}
Out[479]= {{-k10 - k20 - k30, k01, k02, k03}, {k10, -k01 - k21, k12, 0}, {k20, k21, -k02 - k12, 0}, {k30, 0, 0, -k03}}

In[266]:= K31 // MatrixForm
Out[266]//MatrixForm=
⎛ -k10 - k20 - k30     k01          k02      k03 ⎞
⎜      k10         -k01 - k21      k12       0  ⎟
⎜      k20            k21      -k02 - k12   0  ⎟
⎝      k30             0           0       -k03 ⎠

In[480]:= nulvector1 = NullSpace[K31] * k30 (k01 k02 + k01 k12 + k02 k21)
Out[480]= {{k03 (k01 k02 + k01 k12 + k02 k21), k02 k03 k10 + k03 k10 k12 + k03 k12 k20,
  k01 k03 k20 + k03 k10 k21 + k03 k20 k21, (k01 k02 + k01 k12 + k02 k21) k30}}

In[481]:= nulvector1 = nulvector1 /. k10 → s k10;
          nulvector1 = nulvector1 /. k20 → p k20;
          nulvector1 = nulvector1 /. k30 → c k30

Out[483]= {{k03 (k01 k02 + k01 k12 + k02 k21), k03 k12 k20 p + k02 k03 k10 s + k03 k10 k12 s,
  k01 k03 k20 p + k03 k20 k21 p + k03 k10 k21 s, c (k01 k02 + k01 k12 + k02 k21) k30}}

In[484]:= c00 = nulvector1[[1]][[1]];
          c11 = nulvector1[[1]][[2]];
          c22 = nulvector1[[1]][[3]];
          c33 = nulvector1[[1]][[4]];

In[488]:= vb = (k02 c22 - k20 p c00) / (c00 + c11 + c22 + c33)
Out[488]= (-k03 k20 (k01 k02 + k01 k12 + k02 k21) p + k02 (k01 k03 k20 p + k03 k20 k21 p + k03 k10 k21 s)) /
  (k03 (k01 k02 + k01 k12 + k02 k21) + c (k01 k02 + k01 k12 + k02 k21) k30 +
    k01 k03 k20 p + k03 k12 k20 p + k03 k20 k21 p + k02 k03 k10 s + k03 k10 k12 s + k03 k10 k21 s)

In[489]:= vb = vb /. k10 → Keq01 * k01;
          vb = vb /. k20 → Keq02 * k02;
          vb = vb /. k30 → Keq03 * k03;
          vb = Limit[vb, k03 → ∞];
          vb = Limit[vb, k01 → ∞];
          vb = Limit[vb, k02 → ∞];
          vb = Simplify[vb]

Out[495]=  -k12 Keq02 p + k21 Keq01 s
          ------------------------------------
          1 + c Keq03 + Keq02 p + Keq01 s
```

In[175]:=
```
ode2 =
 {x1'[t] == (20 - 6 x1[t]) / (1 + 6 x1[t] + 20) -
    (3 x1[t] 2 x3[t] - x4[t]) / (1 + 3 x1[t] + 2 x3[t] + x4[t] + x9[t]),
  x3'[t] == (10 - x3[t]) / (1 + 10 + x3[t]) -
    (3 x1[t] 2 x3[t] - x4[t]) / (1 + 3 x1[t] + 2 x3[t] + x4[t] + x9[t]),
  x4'[t] == (3 x1[t] 2 x3[t] - x4[t]) / (1 + 3 x1[t] + 2 x3[t] + x9[t]) -
    (x4[t] - 7 x5[t]) / (1 + x4[t] + 7 x5[t]),
  x5'[t] == (x4[t] - 7 x5[t]) / (1 + x4[t] + 7 x5[t]) -
    (4 x5[t] - 8 x6[t]) / (1 + 4 x5[t] + 8 x6[t]),
  x6'[t] == (4 x5[t] - 8 x6[t]) / (1 + 4 x5[t] + 8 x6[t]) -
    (3 x6[t] - 2 x7[t]) / (1 + 3 x6[t] + 2 x7[t]),
  x7'[t] == (3 x6[t] - 2 x7[t]) / (1 + 3 x6[t] + 2 x7[t]) -
    (x7[t] - 2 x8[t]) / (1 + x7[t] + 2 x8[t]),
  x8'[t] == (x7[t] - 2 x8[t]) / (1 + x7[t] + 2 x8[t]) -
    (7 x8[t] - 6 x9[t]) / (1 + 7 x8[t] + 6 x9[t]),
  x9'[t] == (7 x8[t] - 6 x9[t]) / (1 + 7 x8[t] + 6 x9[t]) - (x9[t] - 2) / (1 + x9[t] + 2),
  x1[0] == x3[0] == 5, x4[0] == 3, x5[0] == 8, x6[0] == 2, x7[0] == 1, x8[0] == 10, x9[0] == 20}
```

In[176]:=
```
sol = NDSolve[ode2, {x1, x3, x4, x5, x6, x7, x8, x9}, {t, 1000}]
```

In[177]:=
```
Plot[{x1[t], x3[t], x4[t], x5[t], x6[t], x7[t], x8[t], x9[t]} /. sol, {t, 0, 100}]
```

Out[177]=