UNIVERSITY OF STAVANGER

Dynamics of Hypercycles in Viral Replication and Investigations of positive feedback loops

by

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Master Thesis in Biological Chemistry

submitted to the
Faculty of Science and Technology
Department of Biology, Chemistry and Environmental Engineering

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"Growing old is mandatory, growing up is optional."

Chili Davis

UNIVERSITY OF STAVANGER

Abstract

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Positive feedbacks are of importance in biochemical processes, including also replication mechanisms. Positive feedback is a process that takes place in a feedback loop that amplifies small changes. It means that the impact of perturbation on the system requires an increase in perturbation. Hypercycle is a certain kind of positive feedback. The notion of hypercycle was developed in order to explain how organisms could survive in the natural selection process. Replication of nucleic acids is an essential process in the reproduction process, which means that replication plays a significant role in natural selection. The replication stage of viral infection leads to a hyperbolic growth. In this thesis, positive feedback motifs and hypercyclic models were investigated in order to see the increased type of these biochemical processes and explore under what conditions this type of development achieved.

Acknowledgements

I would like to express my gratitude to my supervisor, Peter Ruoff, for his irreplaceable support and guidance during this process. Peter Ruoff continuously encouraged me to realize my new ideas and was always willing and enthusiastic to assist in any way he could throughout the research project. I would also like to thank my daughter, my husband and my family for supporting me during writing my thesis.. . .

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Dedicated to my wonderful and lovely daughter Sara and my beloved husband Sabuhi...

Chapter 1

Introduction

Most molecular and physiological processes in nature are controlled by feedback mechanisms. Two types of feedback mechanisms are present in nature. Positive feedback is a process that takes place in a feedback loop that amplifies small changes. It means that the impact of perturbation on the system requires an increase in perturbation [7]. To simplify the term of positive feedback, the process that starts to increase the product and this product leads to an increase in the synthesis of other products. It can be described as a chain effect. The process will increase the synthesis of one product, which will then go ahead and stimulate the production of another product, and it continues. In comparison, negative feedback occurs when effects arise to reduce or counteract it. [7]. If the rate of the reaction or process prevents the accumulation of the product, negative feedback occurs. So it inhibits the process by decreasing the synthesis and accumulation of the product. Negative feedback has the capability to play a destabilizing role in organisms. In contrast, positive feedback may be required for a system to maintain its stability [8].

1.1 The aim of thesis

In this study, I have investigated two basic positive feedback motifs and three models developed to investigate positive feedbacks and hypercycles. Positive feedback occurs in nature when the reaction product increases reaction rate and, as a result, its own concentration. For example, homeostasis, osmoregulation, blood pressure regulation, temperature regulation, replication of viruses etc. are examples of positive feedback in nature. Because of their small size, acellular form, and host-dependent metabolism, viruses are the best examples in order to investigate positive feedback loop and hypercycle. This thesis aims to find under which conditions basic positive feedback motifs and

hyperbolic models can show different types of increase, steady-state, or degradation. I focused more on the viral replication cycle and investigated certain circumstances that can lead to linear, exponential, and hyperbolic growth.

1.2 Positive feedback schemes

Figure 1.1 describes eight fundamental positive feedback motifs. How processes sustain a positive feedback loop can be mathematically described by generating positive feedback motifs. These two-component motifs act as building blocks for the most fundamental positive feedback motif, which is made up of two substances A and E. The positive feedback loop variable is A, and the loop species is E. A and E may affect each other in a variety of ways to create a positive feedback loop. A and E can create eight different positive feedback motifs by activating or inhibiting each other's synthesis or degradation, as shown in Figure 1.1.

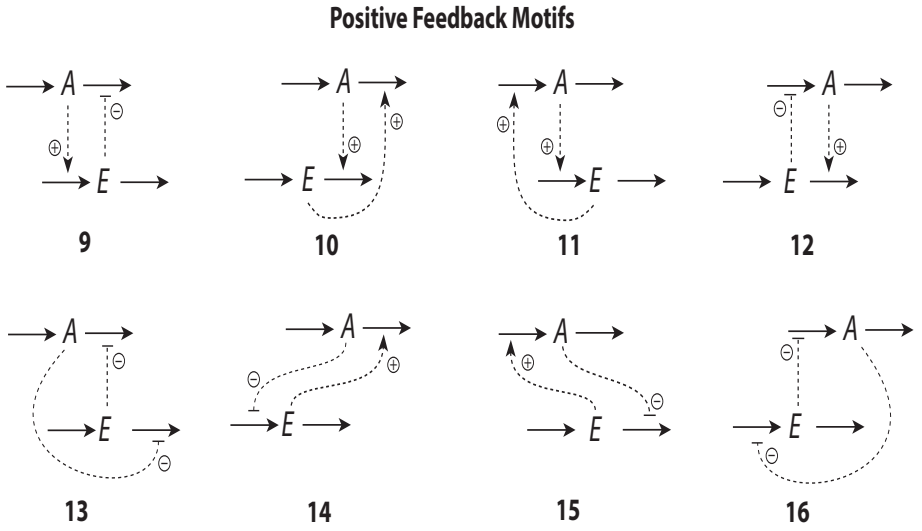

FIGURE 1.1: Basic positive feedback motifs

Motif 9 in Figure 1.1 indicates that both inhibition and activation are involved in the process: A activates E, and E inhibits A's synthesis. There are two activation processes present in Motif 10. But the effect of E on A is not significant because it can not activate A. Motif 11 is also called double activation motif. Motif 11 is a model of positive feedback loop known as double activation. This motif is the best example of autocatalysis and the DNA replication. The feedback loop's two substances interact and cause rapid activation. That's why it's known as double activation. There may also be very rapid degradation depending on the rate parameter values. It means that sometimes "positive feedback" is "not positive feedback". One of the motifs associated with positive feedback is Motif 12. This motif has both activation and inhibition parts,

and this motif can lead to hyperbolic increase for a certain time depending on the rate parameters values. Motif 13 and 16 have double inhibition in their schemes. In motif 13, A inhibits E. In contrast in motif, 16 E inhibits A. In Motif 14, E activates A and leads to an increased process. Motif 15 shows that E activates A's formation and leads to the increase or decrease depending on rate parameters values.

Chapter 2

Materials and Methods

The LSODE Fortran Subroutine [9] with Absoft's Pro Fortran compiler were used to perform the calculations.(www.absoft.com). Gnuplot (www.gnuplot.info) with a Perl interface (www.perl.org) was used to generate the plots of calculations . Adobe Illustrator used in order to generate and edit graphs (www.adobe.com). Variables in each calculation equation are given as A and E in Motif 11 and Motif 12; P, E, and M in hyperbolic model 1; Ps, Ns, and E in Manfred Eigen's viral hypercyclic replication model and Manfred Eigen's viral hypercyclic replication model with saturation. Concentrations of compounds are usually denoted by compound names without square brackets to simplify notations. Rate parameters are represented as k_n (n=1,2,3...). KM is the representative of the Michaelis Menten constant, and K_I is the inhibition constant.
Matlab Programming (www.mathworks.com) was used in order to confirm the results of calculations.

Chapter 3

Results

3.1 Autocatalysis

Autocatalysis refers to a mechanism in which the substance itself activates its synthesis.[10]. It means that in the autocatalytic process chemical compound can increase the rate of its own formation. The German chemist Wilhelm Ostwald invented the term "autocatalysis" to describe reactions that show time-dependent rate acceleration [10]. Hydrolysis of ester is the best example for this process. In this reaction same acid catalyzes and produces another organic acid:

$$CH_3COOCH_2CH_3 + H_2O \longrightarrow CH_3COOH + CH_3CH_2OH$$

Kelly Kruger discovered ribosomes in 1982. He stated that some RNA molecules could act as catalysts. Under certain conditions, these RNA sequences, called ribosomes, can act as catalysts for their own replication [1][11]. On the other hand, autocatalytic reactions have never been widely used in traditional or industrial chemistry for two purposes: 1)Autocatalysis is a form of positive feedback, which is hard to observe and control in large reactors and 2) Multiple dynamical behaviors can be produced by autocatalytic systems, which are very difficult to handle [10]. These problems were solved with modern chemistry improvements, and autocatalysis got particular importance. There were identified first-order and higher-order autocatalysis. The most general example for autocatalysis :

$$A + B \longrightarrow 2\,B \tag{3.1}$$

[1]Please see page 215, [11]

[12] A single autocatalytic molecule B and A molecule synthesize together 2 B molecules.

$$\frac{dx}{dt} = kx^p; p = 1 \tag{3.2}$$

Equation (3.2) describes the first-order autocatalytic reaction. When p=1, this refers to a typical autocatalytic reaction and results in an exponential increase in the growth curve. An exponential type of increase is the result of autocatalysis. According to Darwinian theory, autocatalysis can lead to natural selection [13].

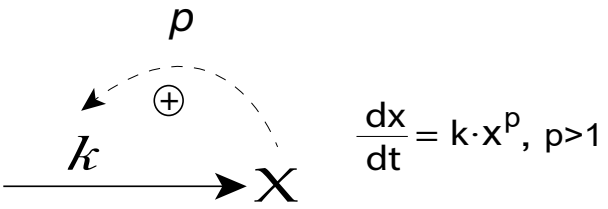

FIGURE 3.1: Higher-order autocatalytic reaction.

When there is higher-order autocatalytic reaction (Figure 3.1) an equation for the reaction is provided in Figure 3.1. The most general example of investigating the autocatalytic process is the nucleic acids replication process. DNA and RNA are self-replicative molecules. We know that reproduction stands at the core of life. Reproduction itself occurs by the replication process of self-replicating nucleic acids. I want to start by looking at viral DNA replication.

3.1.1 DNA virus replication

Since positive feedback is observed in viral replication and viral replication gives good examples for positive feedback systems, I will provide a brief overview of what we know about DNA virus replication. Viruses are infectious agents which can "live" only inside the cell. Viruses are distinguished from other organisms because of their small size, acellular form, fast adaptability to all conditions, and their dependent metabolism. Their replication process depends on the host cell. Viruses can not replicate until they are inside a host cell. According to Manfred Eigen [14] the virus carries genetic information, and this information consists of only one message: "Reproduce me". Viruses may be categorized by what they have as genetic material: RNA or DNA. DNA and RNA both can be double-stranded or single-stranded ones. The Baltimore classification is also an option to classify viruses by their way of mRNA synthesis. The classification was based on whether the genome has DNA or RNA, is

nucleic acid double-stranded or single-stranded and is RNA or DNA has positive or negative sense. There are seven virus groups identified [15].

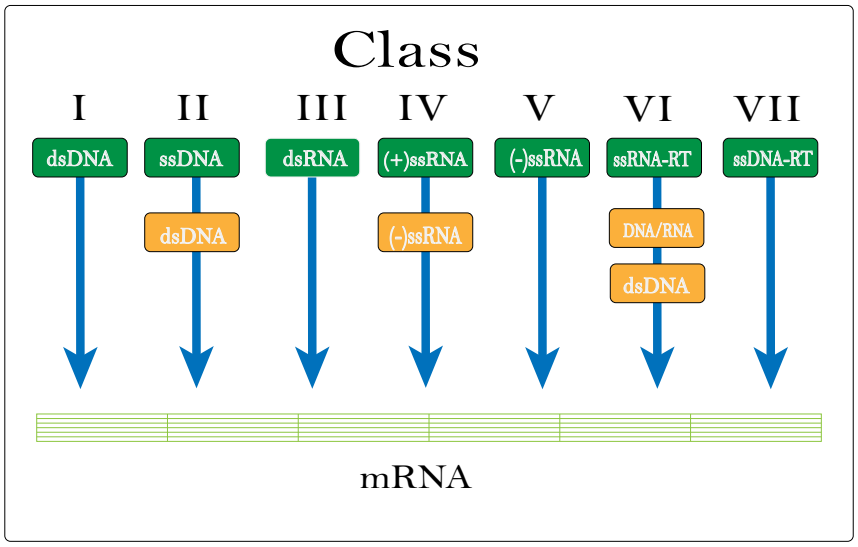

FIGURE 3.2: Baltimore classification of viruses. Baltimore classification is a system for classifying viruses according to their way of mRNA synthesis.Redrawn from [1].

Group I viruses possess double-stranded DNA viruses. Group II viruses have single-stranded DNA. Double-stranded RNA viruses classified in group III. Positive sense single-stranded RNA viruses summarized in Group IV and negative single-stranded RNAs in group V. Group VI represents single-stranded viruses with DNA in their life cycle. It means that these viruses replicate through DNA. Group VII viruses possess double-stranded DNA with RNA intermediate in their life. The replication process takes part by RNA [15].

Viruses do not have essential organelles and systems for independent metabolism and multiplication. They need the host cell's machine to multiply their genetic information. Outside the host cell viruses can not replicate their nucleic acids. DNA replication occurs when a double-stranded DNA molecule is replicated to create two similar DNA molecules. Replication is an essential step of the division process because, after division, there are two daughter cells, and both need their own genetic information. Class I and class II viruses (dsDNA and ssDNA) (Figure 3.2) replicate the genome via dsDNA. In order to convert the genome into the dsDNA, class II viruses need to synthesize a complementary strand [3]. Per viral DNA has minimum one spesific sequence where replication starts. This sequence calls replication origin. Most DNA viruses' genomes replicate in the nucleus, but some viruses from class I (dsDNA) replicate in the cytoplasm [3]. There are eight steps in virus replication cycle.

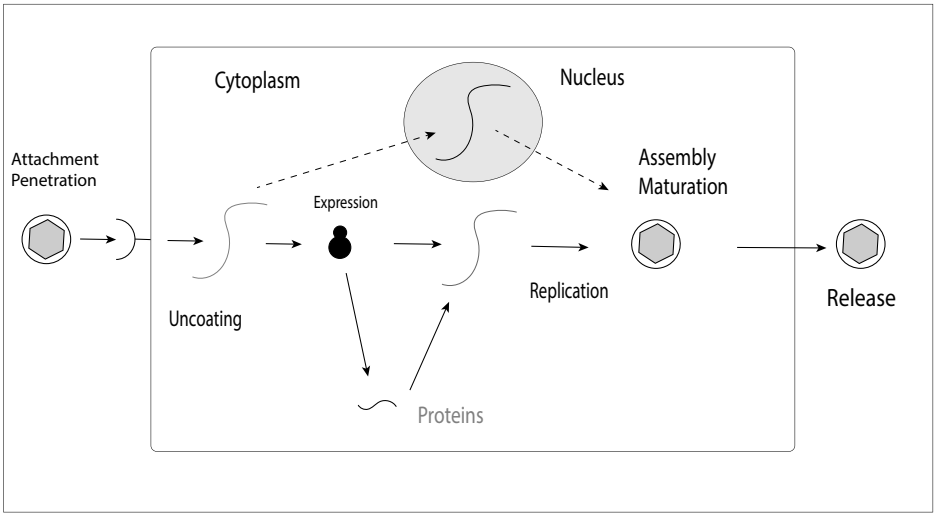

FIGURE 3.3: Virus replication scheme. The viral replication process is consists of eight essential steps: attachment, penetration, uncoating, gene expression, replication, assembly, maturation, and release. Redrawn from [2]

1) Attachment occurs when the viral particle binds to the host cell membrane with the help of particular proteins called virus attachment proteins.
2) In the penetration process viral particle injects its genetic material into the host cell.
3) Uncoating process occurs right after penetration process, virus removes its capsid, and viral genome becomes exposed.
4-5) Gene expression and replication: In double-stranded DNA viruses (except Poxviruses), the replication process is nuclear, which is why fully dependent on host cell factors. In Poxviruses, replication occurs in the cytoplasm, where viruses have acquired all of the essential factors for genome transcription and replication and are thus independent of cellular machinery[2]. In ssDNA viruses replication process take part in the nucleus. The replication of the viral genome is an essential part of virus replication. The synthesis of viral mRNA from genes is also a part of replication.
6) The assembly step requires collecting all of the components needed to form the mature virion at a specific location within the cell. The basic structure of the virus particle establishes during assembly [2].
7) In the maturation part, essential structural changes occur in virus particles. This step is a crucial step for viruses because, in this step, virion completes its formation and becomes an infectious agent.
8) In the last part, newly formed viruses releases from the host cell in three ways: i) by causing it to break, waiting for the cell to die, or budding off through the host membrane. Enveloped viruses mostly use budding. They "make" an envelope for themselves from the host cell membrane. This process is very damaging for the host cell.

The viral DNA replication process starts with a specific nucleotide sequence. The proteins recognize this particular sequence, and the replication process begins. Most viral DNA molecules use RNA molecules as a primer. Priming means a reaction of a nucleotide with a hydroxy (-OH) group at the replication origin (Figure 3.4) [2] [3].

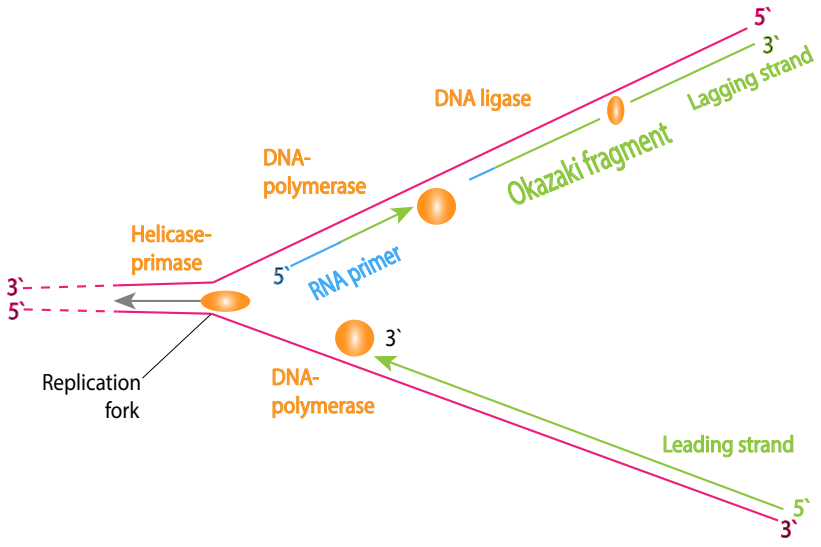

FIGURE 3.4: DNA replication. Parental DNA as shown by pink line, green lines represent new DNA, and arrow indicates synthesis in 5‘ to 3‘ direction. The dsDNA unwinds by helicase-primase. So helicase-primase synthesizes RNA primers, which uses by DNA polymerase in order to start DNA synthesis. The leading strand is continually generated, whereas the lagging strand is generated as Okazaki fragments that are linked together via a DNA ligase. Redrawn from [3].

RNA molecules act as primers, as shown in Figure 3.4. One for the leading strand and one for the lagging strand are needed. For the Okazaki fragments of the lagging strand, multiple primers must be synthesized. Proteins that bind to the replication origin site and start the process are: a helicase, ssDNA binding protein, and a DNA polymerase. DNA molecules are synthesized near a replication fork. The leading strand is one of the newly synthesized daughter strands. The other daughter strand synthesized as Okazaki fragments joins to the DNA ligase and becomes a lagging strand.[3].

3.1.2 Poxviruses replication

Viruses in the Poxviridae family are giant, complex DNA viruses that can infect vertebrates and invertebrates. Chardopoxviruses and entomopoxviruses are the best examples of this family. Unlike other DNA viruses, poxviruses replicate in the cytoplasm of the invaded cell's nucleus. The virions in this family are highly complex,

[2] Please see page 86 [3]

with 75 different proteins and an internal core that protects the viral genome and transcriptional machinery [4].

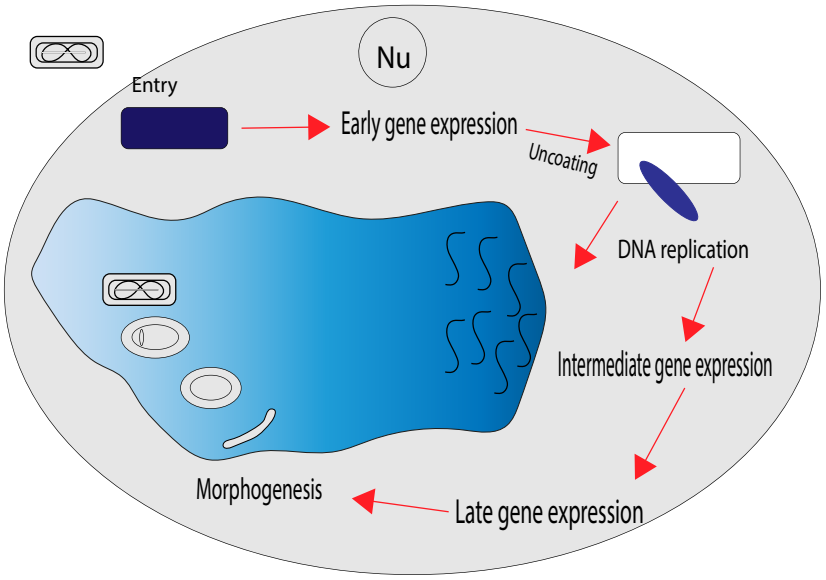

FIGURE 3.5: Life cycle of poxviruses. The first virion enters the cell, and then the viral core releases to the cytoplasm. Early gene expression takes part in the cytoplasm, which follows by the replication process. Intermediate and late gene expression gives place to morphogenesis, where mature virions produce. Redrawn from [4]

Figure 3.5 depicts the life cycle of the poxvirus. The viral core releases into the cytoplasm after virion entry into the host cell. Inside the core, early gene expression occurs. As a result, proteins involved in DNA replication produces. After the uncoating process, the viral genome releases into the cytoplasm, and replication initiates. Intermediate and late gene expressions take part, and the process ends up with morphogenesis. In the last phase (morphogenesis), mature infectious virions produce [4]. I want to investigate the replication process of poxviruses more. For that purpose I redraw replication mechanism of these viruses from [5].

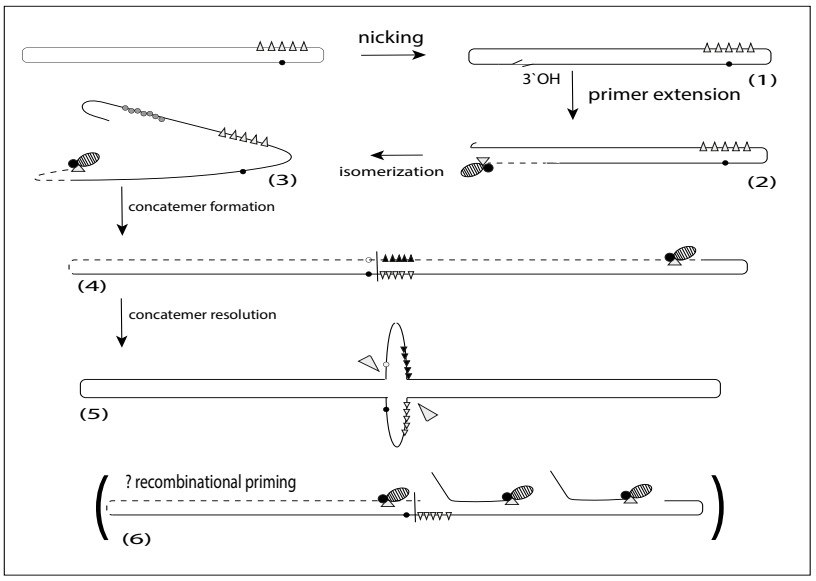

FIGURE 3.6: Replication cycle of poxviruses. The process starts with the insertion of nick and follows by primer extension, isomerization, concatemer formation, concatemer resolution, and recombinational priming. Redrawn from [5]

The following (Figure 3.6) is a figure of a linear genome with covalently closed hairpin termini: triangles and circles are described for the right telomere [5]. The replication process seems to be started with the insertion of a nick. This nick exposes the 3‘OH group, which acts as a primer for DNA polymerase III holoenzyme. Dashed lines and light gray circles represent the nascent and displaced strands, respectively. They can each form self-complementary hairpins, enabling leading strand synthesis to replicate the molecule entirely. The process continues with concatemer formation and at least with the generation of a tail. Recombination priming events may occur during the final step of replication.

3.2 Motif 11: the model of autocatalysis and replication

In the following section, I will describe Motif 11. Motif 11 is a positive feedback loop model, which is called double activation. This motif is the best example of autocatalysis and DNA replication model. Both substances act together and lead to rapid growth or rapid degradation. That is why we call it double activation. Depending on the rate parameters‘ values, there can also be very rapid degradation. It means that positive feedback sometimes is ”not positive feedback”. Motif 11 consists of 6 rate constants. I have changed rate parameters values and the concentrations of A and E during the first and second phases. I mainly used one or two phases to precisely see the system’s behavior.

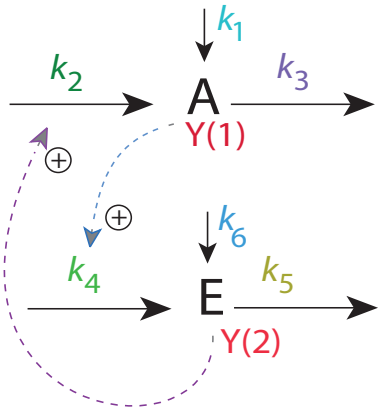

FIGURE 3.7: Motif 11 scheme shows that there is two positive feedback loop.

$$\dot{A} = k_1 - k_3 A + k_2 E \tag{3.3}$$

and

$$\dot{E} = k_6 - k_4 A + k_5 E \tag{3.4}$$

From the scheme (Figure 3.7) and equations 3.3 and 3.4, it has been shown that rate parameters work together with two and two. k_1 is a "couple" with k_6, k_2 with k_4 and k_3 with k_5. That is why this motif is called "double activation." The interaction between two rate constants can lead the system not only to development but also to degradation. The results and discussion section will clarify how I obtained linear growth, exponential growth, steady-state, and degradation in the motif 11 program under various conditions.

3.3 Dynamic behaviours of Motif 11

In motif 11, I have experimented that all rate parameters have different effects on the positive feedback loop system. In this motif, all rate parameters "work" together and lead the system to different types of states. Every "couple" of rate parameters has a unique effect on the system. As a result of the interaction between rate constants, I got four positive feedback loop states: steady-state, linear increase, exponential increase, and degradation. I want to start with a steady-state.

3.3.1 Steady-State

In this calculation, I wanted to investigate under what conditions I can obtain a steady state. For that purpose I gave following values to rate parameters in the first phase: k_2, k_3, k_4 and k_5 are 0.05; k_1 and k_6 are 0 (Figure 3.8, part c).

In these circumstances, the system goes to a steady state (Figure 3.8). I also changed A and E values to see the difference and their effect on the system. I kept A as 0.1 and E value was 0.6.
A steady state is a state or condition of one system or process that does not change in a given time. It means that the positive feedback loop system is a constant during the first phase. In other words, if we have k_1 and k_6 zero, then we have no change in A and E values. In this case, the positive feedback process is active, but actually, it is nonfunctional.

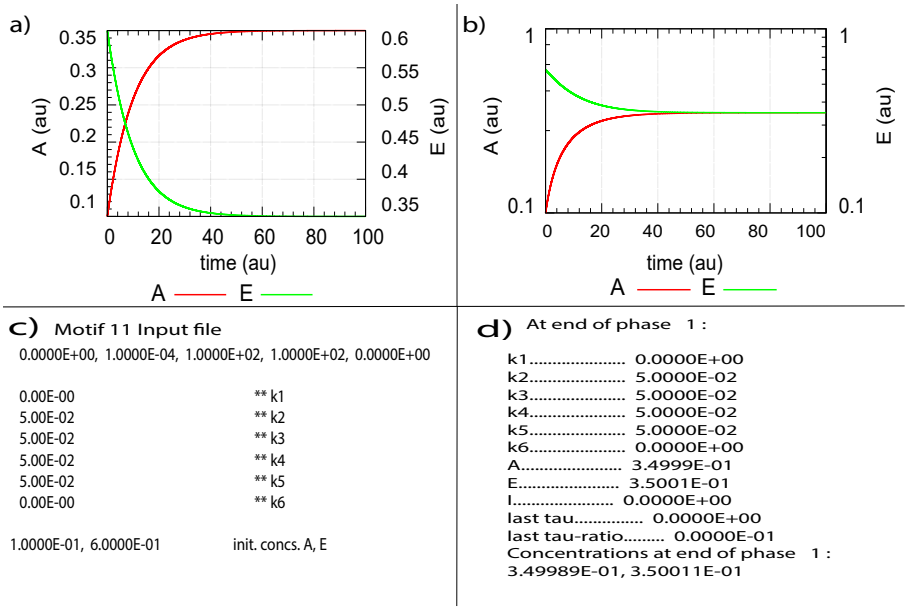

FIGURE 3.8: Steady state observed in Motif 11 under following conditions: k_1=k_6=0; k_2=k_3=k_4=k_5=0.05. Graph a) represents linear scale, while graph b) demonstrates logarithmic plotting. Input and output files are represented in part c) and d).

Since the added expressions of $\dot{A}$ and $\dot{E}$ (Equation (3.3), (3.4)) give:

$$\dot{A} = k_1 - k_3A + k_2E \text{ and } \dot{E} = k_6 - k_4A + k_5E$$

$$\dot{A}+\dot{E} = k_1+k_6-k_3\cdot A+k_2\cdot E-k_4\cdot A+k_5\cdot E\cdot k_1+k_6+(k_4-k_3)\cdot A+(k_2-k_5)\cdot E = 0 \quad (3.5)$$

this condition leads to constant A and E values during the first step.

The positive feedback loop system manages to keep a constant state because of the balance between all four rate parameters. Four rate parameters (k_2,k_3,k_4 and k_5) which can have an impact to the process were constant. In this case, only k_1 and k_6 can alter the process, but since they are 0, the process goes to a steady state.

In Figure 3.8, I have summarized all results from this calculation. Part a) in Figure 3.8 demonstrates a plotting of the concentration of A and E against time. It shows a steady state in the concentration of A and E.

Part b) shows the logarithmic plotting of this calculation. I used logarithmic plotting of concentration against time as an indicator in this program. In part b) we can see a steady state in the first phase.

Part c) is the Input file of the program. It shows the values of all rate parameters, initial concentrations of A and E, and length of the phases.

Part d) is the output file of the program. It shows the final concentration values of A and E.

As an indicator of the Fortran program, I used the Matlab program to see if I get the same results. Firstly, I inserted same equations (3.3 and 3.4) of this motif to the Matlab‘s ODE file (Figure 3.9). Values of rate parameters were the same as in Fortran program:
k_1=k_6=0; k_2=k_3=k_4=k_5=0.05.
Results are shown in Figure 3.10.

HC3.m

```
clear all
close all

% Initial values (should be equal to the number of ODEs):
y0=[0.1, 0.6];
% Integration time:

tspan=[0, 100.0];

% Rate constants k1,    k2,  k3,   k4,  k5,   k6
              k=[0.00,  0.05, 0.05, 0.05, 0.05, 0.00];

%Solving ODE
options = odeset('AbsTol',1.e-11,'RelTol',1.e-7,'MaxStep',0.01);
[t,y]=ode15s(@ODE_for_HC3,tspan,y0,options,k);

figure % make a figure


plot(t,y(:,1),t,y(:,2),'LineWidth',2.0);
title('HC3');
ylabel('A (au)');
xlabel('time (au)');
legend('E')
grid on;
```

ODE_for_HC3.m

```
function dy = ODE_for_HC3(t,y,k);

dy=zeros(2,1); % number of ODEs (rate equations)


dy(1) = k(1)-k(3)*y(1)+k(2)*y(2);
dy(2) = k(6)+k(4)*y(1)-k(5)*y(2);


end
```

FIGURE 3.9: The Matlab program's input file, with both equations 3.3 and 3.4 inserted into the ODE.m file and all rate constant values added into the HC3.m file.

I got the same result-steady state, same final calculation value from the Matlab program (Figure 3.10).

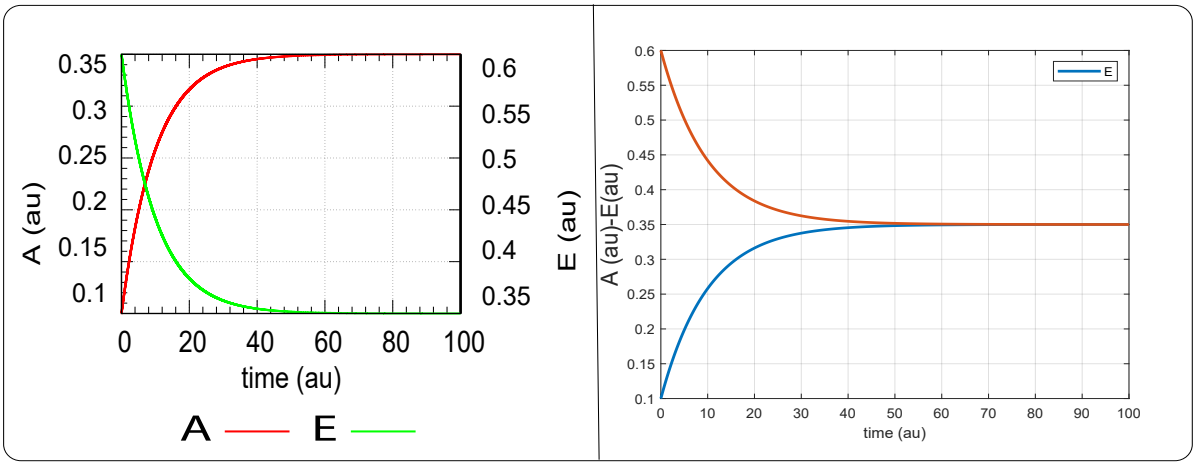

FIGURE 3.10: The right graph represents an output from the Fortran program; the left graph is obtained from Matlab Programming and indicates a steady-state in both A and E concentration.

3.3.2 Linear progression

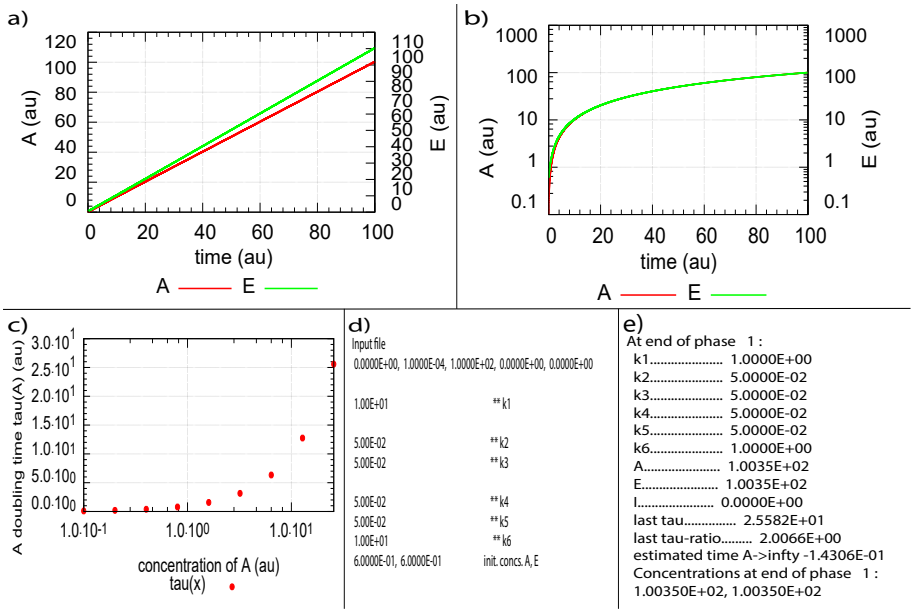

FIGURE 3.11: Linear growth that observed from Motif 11. Graph a) represents linear scale, while graph b) demonstrates logarithmic plotting. Doubling time graph is shown in graph c). Doubling time shows how fast concentration are growing. Input and output files are represented in part d) and e).

In the next calculation, I want to see the impact of the k_1 and k_6 on the process. For that reason I kept all other rate constants (k_2, k_3, k_4 and k_5)= 0.05. In this case k_1 and k_6 are 1.0 : k_1=k_6=1.0 , much more than other parameters. I make changes also in the A and E values. I kept the E value at A=6.000E-01 and decreased the A value to A=1.000E-01. It has not been observed such a certain change where A and E are at the same or different value. Differences in the concentrations of A and E do not affect the process. I used only one phase in this calculation. The second and third phases are omitted. We can see that from the graph that I got linear progression (Figure 3.11, part a)). Linear progression means an increase by the same amount at the same time unit.

The summary of the second calculation has been described in figure 3.11 Part a) of Figure 3.11 depicts a time-dependent plot of the concentrations of A and E. It demonstrates a linear progression of A and E concentrations in the first step. Both concentrations increase by the same amount at the same time.

Part b) illustrates this calculation's logarithmic plot. I used logarithmic plotting of concentration versus time as an indicator in this program. In section b), we see a curve that represents the sign of linear development.

Part c) is the doubling time graph. Doubling time is the opposite of half-life. When something is growing, we have doubling time. Doubling time is constant when we have an exponential increase. When we have hyperbolic growth, doubling time decreases exponentially. In this graph We can see an apparent linear increase in this graph.

Part d) is the program's input file. It provides all rate parameters' values, the A and E concentrations in the beginning, and the phase lengths.

Part e) is the program's output register. It demonstrates the final concentrations of A and E.

As a result of the current calculation, I can state that k_1 and k_6 play a significant role in the process. With the increase of k_1 and k_6, the positive feedback loop will kick off and increase. This increase will start and continue linearly. I noticed the same rate of increase over the same period.

I used the Matlab program to check whether the calculation results are equal and correct. Equations 3.3 and 3.4 inserted I set all rate parameters values (k_1=k_6=1. and k_2=k_3=k_4=k_5=0.05) and initial concentrations (A=0.6 and E=0.6) in the HC3.m input file and run the program. I got the following results (Figure 3.12):

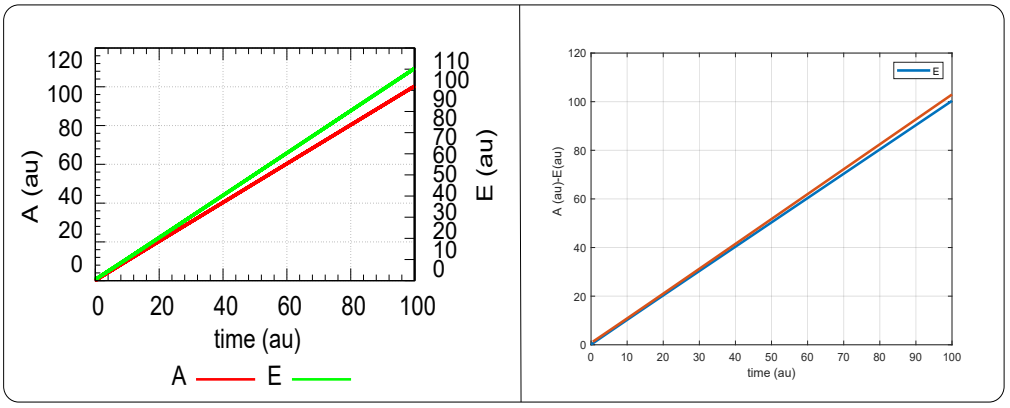

FIGURE 3.12: Output file of the Matlab program that shows a linear growth in both variables. The right graph represents an output from the Fortran program, the left graph is from Matlab Programming and confirms results achieved from Fortran.

Figure 3.12 indicates that results from the Matlab program are precisely equal to the Fortran program. It shows a linear growth in both A and E concentrations.

3.3.3 Degradation

In the following calculation I want to see the effect of the k_3 and k_5 to the process. I used two phases. In the first phase I gave following values to rate parameters: k_1=k_6=0.1 and k_2=k_3=k_4=k_5=0.5. In the second phase I have reduced values of all rate constants. k_3=k_5=0.1 and k_1=k_2=k_4=k_6=0.01. There is a significant difference between k_3, k_5, and other rate parameters in the second phase.

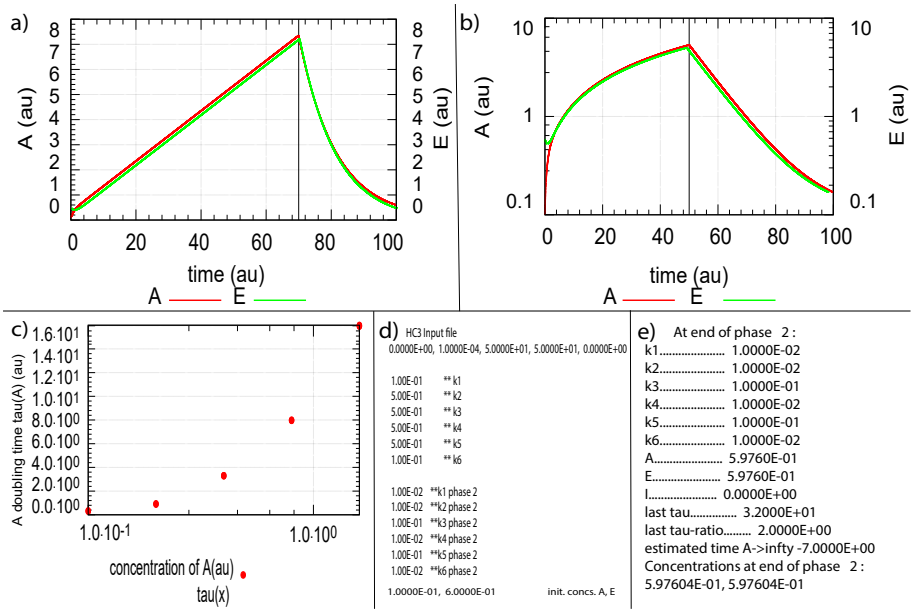

FIGURE 3.13: Degradation is achieved from Motif 11 when k_3 and k_5 are dominant in the process. Both graph a) and graph b) shows that degradation on A and E values are present in the system.

In the first phase, the program provides linear progression. As I mentioned earlier, the increase in k_1 and k_6 triggers the process to rise linearly. The high value of k_3 and k_5 causes positive feedback to go down and degrades. It can be precisely seen also in the logarithmic plot. It starts from a high value of A and E respectively and rapidly goes down.

I have listed all of the results from this calculation in Figure 3.13. Part a) of Figure 3.13 represents a time-dependent plot of the concentrations of A and E.In the first step, the concentrations of A and E rise linearly. The second phase involves the onset of degradation. Degradation starts and progresses quickly.

Part b) illustrates this calculation's logarithmic plot. The logarithmic plot of concentration versus time provides an indication. In part b), we see a curve in the initial phase that reveals linearity. The second phase demonstrates decay, which is a sign of degradation.

Part c) represents doubling time graph. When there is degradation we can see slight increase in graph which indicates degradation.

Part d) is the program's input file. It contains the values for all rate parameters, the initial A and E concentrations, and the duration of the first and second phases.

Part e) is the program's output file. It reveals the final concentrations of A and E.

I used Matlab software to verify that the calculation results were accurate. Equations 3.3 and 3.4 were the same in Matlab Programming too. I defined all rate parameters (k_3=k_5=0.1 and k_1=k_2=k_4=k_6=0.01) and initial concentrations (A=7.0 and E=6.0) in the HC3.m input file and ran the program. I achieved the following results (Figure 3.14):

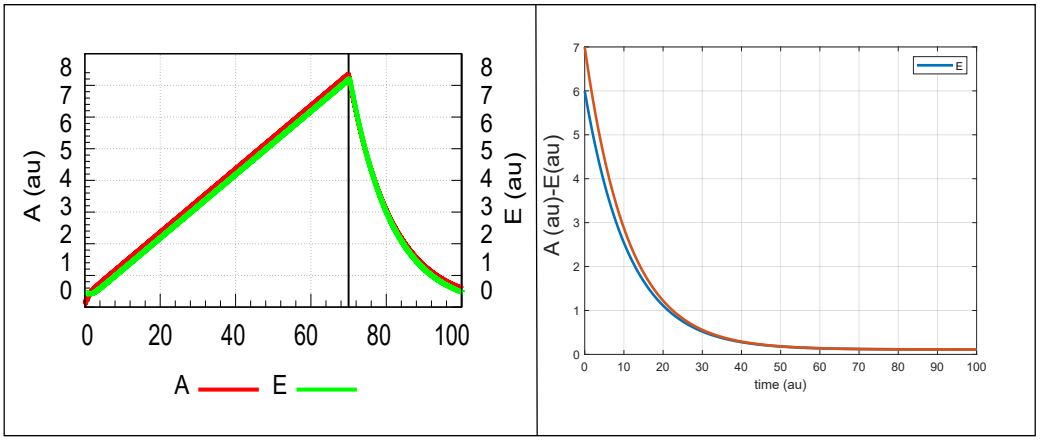

FIGURE 3.14: Output file of the Matlab program (left graph) indicates that there is degradation in both concentrations under following conditions: k_3=k_5=0.1; k_1=k_2=k_4=k_6=0.01.

In conclusion, k_3 and k_5 plays a particular role in the positive feedback pool. Their overactivity leads the process to slow down and breaks off at the end.

3.3.4 Exponential growth

In this calculation, I observed the effect of the k_2 and k_4 on the process. For that reason, I keep only one phase and start to investigate the process. I give 0.1 to k_2 and k_4: k_2=k_4=0.1. Other rate parameters have following values: k_3=k_5=0.05 and k_1=k_6=0.01.

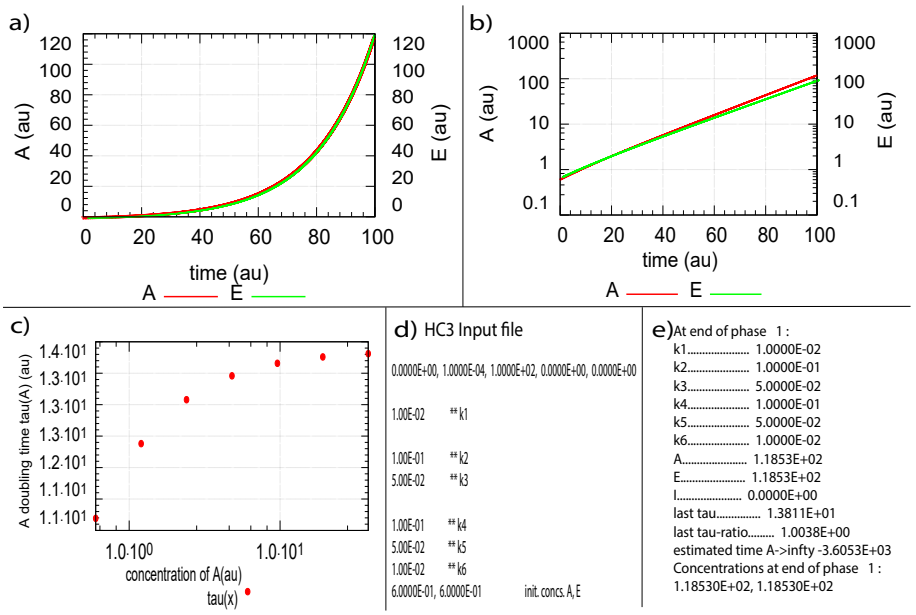

FIGURE 3.15: Exponential growth was obtained from Motif 11. Graph a) represents the linear scale, and it shows that there is exponential growth. Graph b) demonstrates a linear increase in logarithmic plotting, which is an indicator of exponential increase. Constant doubling time in graph c) indicates an exponential growth. Input and output files are represented in parts c) and d).

I give more value to k_3 and k_5 than k_1 and k_6 to see how it affects the positive feedback process. In Figure 3.15, we see that the velocity of the reaction is proportional to a particular increase value. It means that the concentration of A and E and the entire positive feedback process is in exponential growth. In Figure 3.15, I have summarized all of the results from this calculation.

Part a) is a graph of the concentrations of A and E over time. From the graph, we can see an apparent exponential increase. Exponential growth is a mechanism in which quantity increases over time, when a quantity's rapid rate of change with time is proportional to the quantity itself [16].

Part b) provides a logarithmic plot of this section. There is a linear progression which indicates the exponential type of growth.

Part c) gives information about doubling time increase of concentration A by time. As I mentioned before, doubling time is constant when we have an exponential increase. We can see a constant doubling time graph which is the sign of exponential growth.

Part d) is this section's input file and yields information about initial concentrations, rate parameters values, and phases.

Part e) contains results of this calculation. It gives information about final concentrations of both A and E and shows that their final concentrations are precisely same.

The Matlab software has been used again to confirm the exponential growth.I set into HC3.m input file values of all rate parameters (k_2=k_4=0.1 and k_3=k_5=0.05 and k_1=k_6=0.01) and equal initial concentrations (A=0.6 and E=0.6). After running the program, I achieved the following results (Figure 3.16):

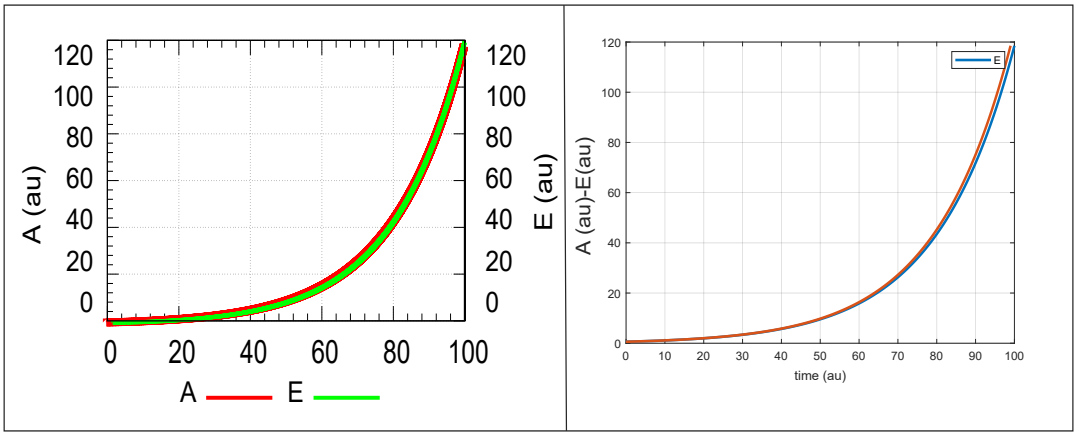

FIGURE 3.16: Output file of the Matlab program indicates an exponential growth in this implementation.

3.4 Summary of Motif 11 Calculations

In conclusion, I can say about motif 11 that, it is typical autocatalytic motif.

$$\frac{dx}{dt} = kx^p, p = 1 \tag{3.6}$$

When p=1, this indicates that the mechanism is autocatalytic. By autocatalytic mechanism, I mean that exponential growth arises when there is full positive feedback. It means that plotting A as a function of time on a logarithmic scale yields a linear relationship.

i) When k_1 and k_6 are more larger than other parameters process goes to linear progression.

ii) When k_2 and k_4 are more greater than other rate constants, it forces the system to increase exponentially.

iii) When k_3 and k_5 are larger than other variables, the process degrades.

iv) When k_1 and k_6 are 0, and other rate parameters are equal and constant, it causes a steady-state.

3.5 The Hypercycle

The adaptation and alteration processes of populations of living organisms to the environmental perturbations is called natural selection. [17]. Individuals in a group are variables by nature, which means they may vary in specific ways. Because of this variety, some organisms have characteristics that are better suited to their environments than other organisms. All organisms have different phenotypes, so individuals survive and reproduce differently. Reproduction is necessary for natural selection, and replication of nucleic acids is required for reproduction[6]. Let's look at the sequence of reactions where the products experience further transformation at each step, either with or without the participation of additional reagents. If the product produced in this sequence is identical to the reagent from the previous step, the system appears to be a reaction cycle, and the whole cycle is the catalyst. A catalyst is a single molecule, such as an enzyme, that converts a substrate to a product [6]:

$$S \xrightarrow{\mathrm{E}} P \tag{3.7}$$

This formal scheme's mechanism requires at least a three-term interval. (Fig. 3.17).

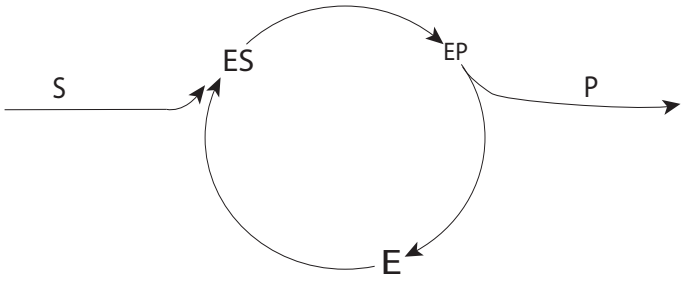

FIGURE 3.17: The common catalytic mechanism of an enzyme according to Michaelis and Menten.

The following is the general reaction scheme for an enzyme-catalyzed reaction:

$$E + S \underset{\mathrm{k}_{-1}}{\overset{\mathrm{k}_1}{\rightleftarrows}} [ES] \xrightarrow{\mathrm{k}_2} E + P$$

According to Michaelis and Menten, an enzyme's common catalytic mechanism consists of at least three components: free enzyme (E), enzyme-substrate (ES), and enzyme-product complex (EP) (Figure 3.17). The graph in Figure 3.17 shows the equivalence of the enzyme's catalytic activity and the cyclic restoration of substances during the transfer of the substrate (S) to the product (P) [18]. Thus, a catalytic hypercycle is a mechanism that maintains a cyclic linkage between autocatalytic or self-replicating units. Such a system is described in Figure 3.18 [6].

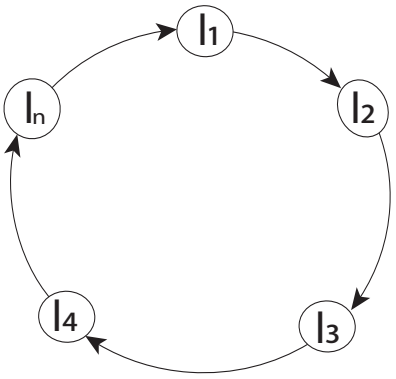

FIGURE 3.18: A catalytic cycle consists of self-replicative units, which indicates a cyclic hierarchy. This type of cycle is observed a lot in biochemical reactions, such as Krebs-cycle, citric acid cycle, etc. But when every self-replicative unit: I_1, I_2.. are autocatalytic, then this calls a hypercycle. For instance viral replication cycle can be good example for hypercycles. Redrawn from [6]

I_1-I_n are intermediates of this autocatalytic cycle. They are self-replicative units that generate a hierarchic process. Generally, all autocatalytic systems are hypercycles that symbolize a circular arrangement of catalysts, each of which is a chemical cycle [19]. As an example for catalytic cycle, single-stranded RNA viruses are given in the system.

3.6 Viral RNA replication

In this section, I want to describe the best example of hypercycles in biological systems. Single-stranded RNA virus replication is an excellent example of it.

I want to focus on the genome replication part of RNA viruses. The infected virus's genome is replicated in order for viral transcription to be amplified and provide copies of the genome for progeny virus particles. RNA viruses, in general, copy their genomes straight to RNA. Certain RNA viruses replicate their genomes via DNA via reverse transcription. Viruses in prokaryotes lack such reverse transcription[3].

Single-stranded viruses are classified as plus or minus in relationship to the virus mRNA. The plus-strand RNA genomes have the same sequence as the messenger RNA, but the minus-strand genomes complement the messenger RNA. The genomes of most RNA viruses replicate in the cytoplasm, although there are rare exceptions [3].

Retroviruses and pararetroviruses are minus-strand RNA viruses that replicate in the nucleus. Viruses that replicate the genome in the cytoplasm synthesize nucleic acids within the cytoplasm structures [2]. Viruses avoid replicating their nucleic acids in the cytoplasm in order to avoid being targeted by the host cell's defensive mechanisms.

For RNA viruses, genome replication does not require a primer; replication begins when the new strand's first ribonucleotide base pairs with a ribonucleotide in the template RNA. When its 3-OH group is connected to the second ribonucleotide, this initial ribonucleotide works as a primer [3]. RNA polymerases are the primary enzymes involved in virus genome replication. While many viruses encode their own polymerase, others utilize an enzyme from the host cell. As an example of RNA virus replication I want to describe Coronaviruses replication process.

3.7 Coronavirus replication

Coronaviridae viruses are unique among RNA viruses in terms of their genome size, which is estimated to have approximately 30 000 bases. In compared to Coronaviruses, the genomes of hepadnaviruses (Hep. B) are 3000 bases, retroviruses (HIV) are 10000 bases, and papillomaviruses (HPVs) are 8000 bases. Coronaviruses are distinctive in that they have a helical nucleocapsid, which is uncommon for plus-stranded RNA viruses but not for minus-stranded RNA viruses. Coronaviruses are extremely successful, infecting a wide variety of animal species, including bats, birds, and mammals such as humans [5]. Coronavirus species are categorized into three groups based on antibody cross-reactivity and, more recently, nucleotide sequence similarity. Coronavirus genomes range in length from 27.5 to 31 kb.

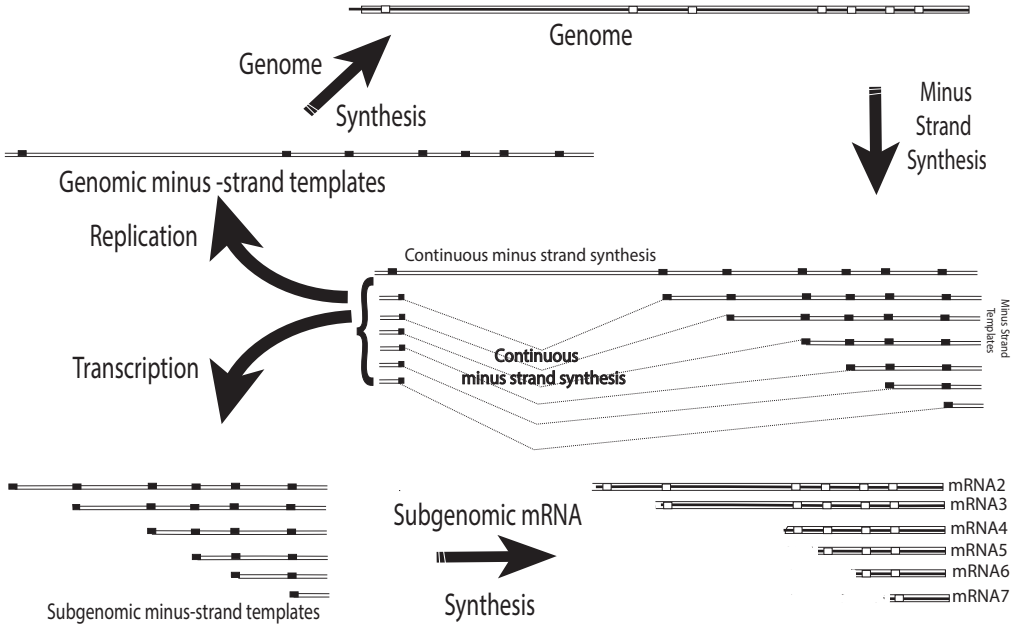

FIGURE 3.19: Coronavirus genome replication. Redrawn from [5]

The coronavirus genome replication mechanism, first suggested in 1996 [5], is represented in Figure 3.19. The ORF1 (Open Reading Frame in the genome) sequence in the genome is translated by the replicase, which then copies the genome constantly from one end to the other to create a complementary copy of the genome, the genomic minus-strand template, which can subsequently be reproduced into more genomes, a process known as genome replication [5]. Replicase has another function in this process besides making minus-strand templates. Sites that surround internal TRS (Transcriptional Regulatory Sequence) recognizes by replicase, and after the copying process, it moves continuously to the 5‘ end of the genome [5]. It elongates after duplicating the leader sequence. Subgenomic minus-strand templates are created, including a sequence complementary to the leader sequence, at both the 3’ genomic and subgenomic end of minus-strand templates [5]. The viral transcriptase would recognize the subgenomic minus-strand templates and the genomic minus-strand templates, then it will copy them into subgenomic mRNAs or genomes accordingly [5]. Due to the fact that only the genome serves as a template for the generation of subgenomic minus-strand templates, a replication signal would be present on the genome but absent from the subgenomic mRNAs. By comparison, both the genomic and subgenomic minus strands seem to include a transcription signal that regulates their potential as templates for plus-strand synthesis [5]. Coronaviruses are the most common type of plus-stranded RNA virus. They do not encapsulate an RNA-dependent RNA polymerase in their virions or deliver it to the infected cell. As a

result, they must create a polymerase from scratch by translating its essential components from the Genome. To replicate their genomes, coronaviruses must develop two distinct types of RNA synthesizers. One uses the genome as a template and synthesizes minus strands utilizing continuous and discontinuous synthesis to create genome synthesis and subgenomic mRNA synthesis templates. The other macromolecular machine utilizes minus-strand templates and ongoing transcription to generate viral genomes and subgenomic mRNA.

In order to investigate hypercycles, RNA replication, and coronavirus replication, Hypercyclic model 1 was created.

3.8 About the hypercyclic model 1

The hypercyclic model 1 is created in order to give a good description of hypercycles and RNA virus replication. This program is a clear-cut example of the viral replication process and its consequences. In the beginning, I want to give a brief description of the hypercyclic model 1. The model 1 aims to get hyperbolic growth and have a clear explanation of under what conditions we can obtain the hyperbolic growth. The system investigated by me requires two positive feedback loops to get hyperbolic increase. It can be described as phenotype and genotype dichotomy [19]. A genotype is a complete collection of genetic material for an organism. Phenotype itself determines by genotype, and for that purpose, one positive feedback loop is required. In order to synthesize desirable phenotype, we need one more feedback loop that commands genotype. In the hypercyclic model 1 (Figure 3.20), I will investigate how these two loops act together and lead the system to infinite growth. Model 1 consists of eleven rate constants and three P, E, and M variables. I have assigned only one phase to see the more clear result of each calculation. I could change all rate parameters k_1-k_{11} during this phase.

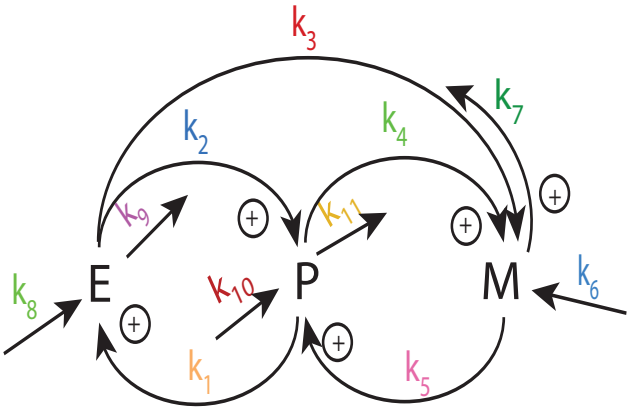

FIGURE 3.20: Hypercyclic model 1 scheme indicates that there are three variables: P,E,M and eleven rate parameters: k_1-k_{11} on the system.

Here are equations of hypercyclic model 1:

$$\dot{P} = k_2 \cdot E + k_{10} - k_{11} \cdot P + k_5 \cdot M; \tag{3.8}$$

$$\dot{E} = k_1 \cdot P \cdot E + k_8 - k_9 \cdot E \tag{3.9}$$

$$\dot{M} = k_3 \cdot E + k_4 \cdot P + k_6 - k_7 \cdot M \tag{3.10}$$

P is positive strand virus parts.
E is enzyme.
M is negative strand viruses.
P, E, and M have stable initial concentration values during all calculations:
P=E=M=0.1.
We have made some changes and have seen that it has no particular effect on the system, so we kept them constant. Duration of the first phase is 50 time units. The First 5 rate constants play a more significant role in the positive feedback loop, but $k_6 - k_{11}$ also has specific importance for the system. During some calculations, I kept $k_6 - k_{11}$ constant $k_6 - k_{11}$=0 with a focus on seeing the behavior of the first five rate constants with each other. All rate parameters have their particular effect on the system. They lead a positive feedback loop to progression or steady-state. The critical task in this program is to determine what type of increase is that. As a result, we got three states in this program: hyperbolic growth, exponential growth, and steady-state. I would like to start with hyperbolic growth.

3.8.1 Hyperbolic growth

First of all, I would like to start with the following mathematical background of hyperbolic growth. The rate equation of x is given below:

$$\frac{dx}{dt} = k \cdot x^p; p > 1 \tag{3.11}$$

Lets separate the variables:

$$\frac{dx}{x^p} = k \cdot dt \Rightarrow x^{-p} dx = k \cdot dt \tag{3.12}$$

Now we get the integration:

$$\int_{x_0} Cx(t) \frac{dx}{x^p} = \int_0^t k \cdot dt = k \cdot t \tag{3.13}$$

$$\frac{x^{1-p}}{1-p} - \frac{x_0^{1-p}}{1-p} = k \cdot t \tag{3.14}$$

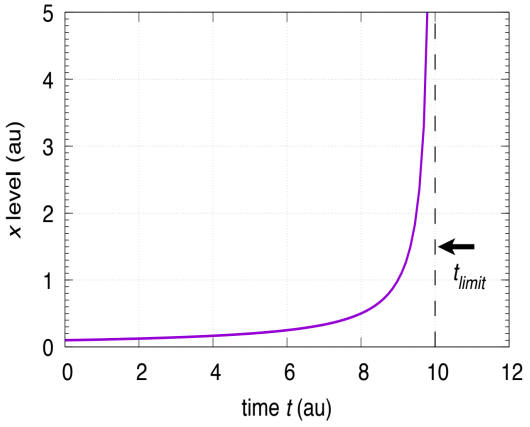

FIGURE 3.21: Hyperbolic growth of x when x_0=0.1, p=2 and k=1.0. t_{limit} is the infinity limit, in other words, the time when x reaches infinity.

with the following expression of x:

$$x(t) = \frac{x_0}{(1 - X_0^{p-1}(p-1)k \cdot t)^{\frac{1}{p-1}}} \tag{3.15}$$

Figure 3.21 shows x as a function of t when x_0=0.1, p=2 and k=1.0 ([20]

In our first calculation of this hypercyclic model, we wondered how the system reaches hyperbolic growth. For that reason, we have increased or decreased almost all rate constants' values. We got hyperbolic growth in two conditions. I would like to start with first condition. In the first calculation, I gave following values to rate parameters: k_1=1.0, k_2=k_3=k_4=k_5=0.01. Other rate constants have following values: k_6=0.1; k_7=k_8=1.0; k_9=0.1 and k_{10}=k_{11}=0.0. As a result, the system goes to a very rapid and hyperbolic growth. This implies that since k_1 has a higher value than k_2-k_5 and k_7-k_8 are equivalent to k_1, feedback loop approaches the maximum increase value. It means that positive strands activates the synthesis of enzyme and increases its concentration. As a result of increased concentration of the enzyme, positive and negative strands become more increased. Results of this implementation are presented in Figure 3.22:

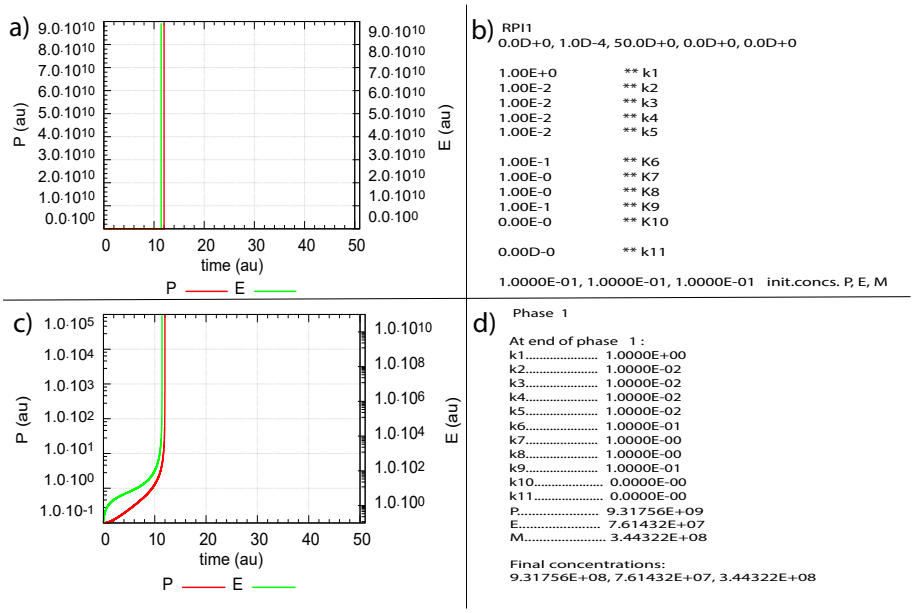

FIGURE 3.22: Figure shows a hyperbolic grows in Hyperbolic model 1 and consist of four parts. Part a) is linear plotting, while part c) represents logarithmic plotting. Part b) is an input file of the calculation and part d) represents final results of this calculation. Hyperbolic growth obtained from model 1 program while k_1=1.0; k_2=k_3=k_4=k_5=0.01; k_6=0.1; k_7=k_8=1.0; k_9=0.1 and k_{10}=k_{11}=0.0.

In Figure 3.22, I summed up all data related to this calculation.

Part a) shows plotting of positive virus strand particles and enzyme concentration versus time. It can be seen from the graph that there is a very rapid, hyperbolic increase in a short time, which was termed a hyperbolic type of growth. Negative strand virus particles also increase hyperbolically by the increase of enzymes.

The logarithmic plotting of enzyme-positive strand viruses against time as seen in part b). We got the same result from this plotting as well - hyperbolic growth. The logarithmic plot indicates that the progression is precisely hyperbolic.

The input file is part c). It provides a description of the values of all rate parameters, the duration of the first phase, and the initial P, E, and M concentration values: P=E=M=0.1 and t=50.

Part d) is the calculation's output file. The final concentrations of positive-negative strand viruses and enzymes are seen in this section. Positive strand virus concentration differs from enzyme and negative-strand concentration and is the highest one. Enzyme concentration is slightly lower than others. For verification of the results, we decided to use Matlab software. We needed to insert hyperbolic Model 1's equations (3.8, 3.9 and 3.10) and rate parameter values to the ODE and RPI1.m file (Figure 3.23).

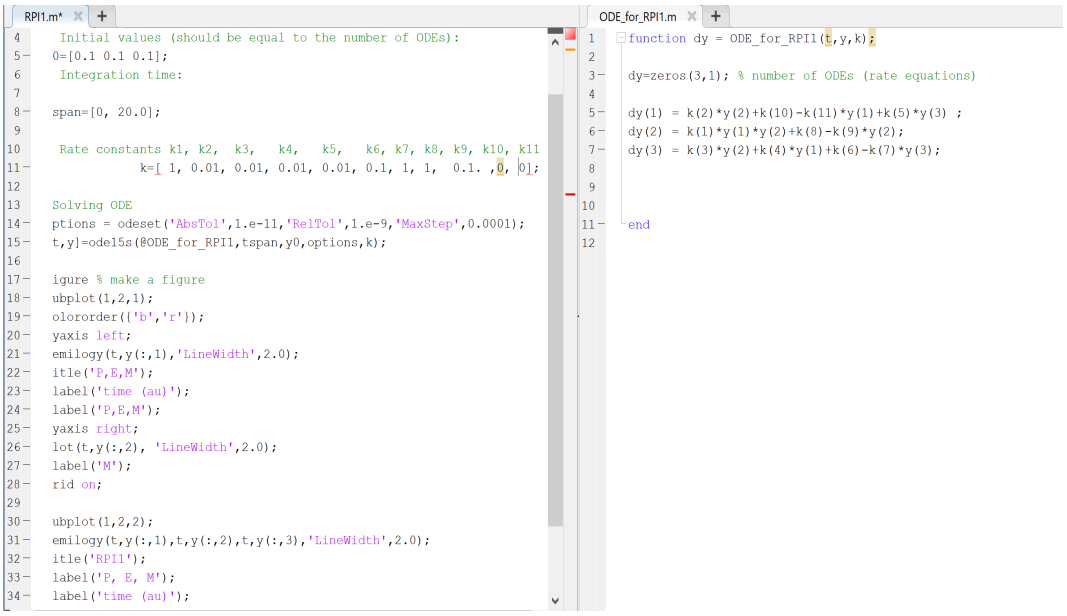

FIGURE 3.23: Matlab input file. k_1=1.0; k_2=k_3=k_4=k_5=0.01; k_6=0.1; k_7=k_8=1.0; k_9=0.1 and k_{10}=k_{11}=0.0.

Matlab results were precisely the same as those obtained by Fortran software. The increase in all variables is a hyperbolic increase (Figure 3.24).

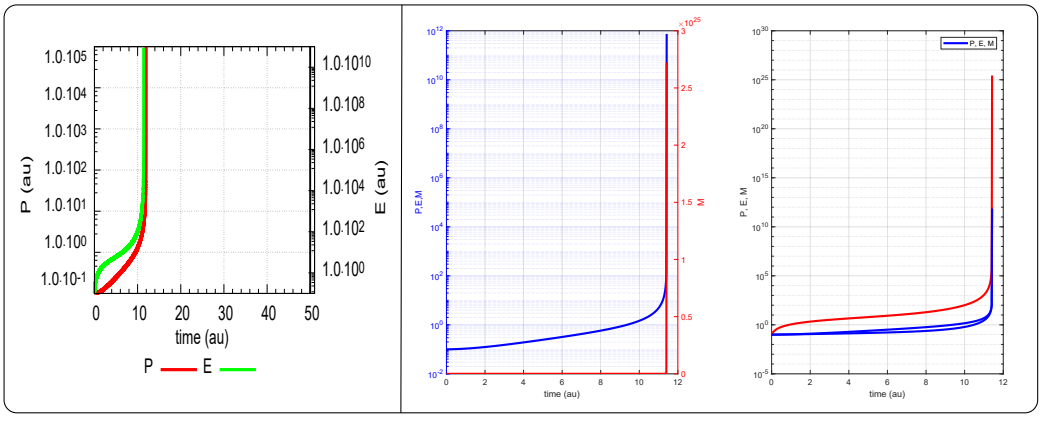

FIGURE 3.24: Matlab output file shows a hyperbolic increase in all three variables: positive-negative virus strands and enzyme

3.8.2 Hyperbolic growth in Model 1, condition 2

I have investigated more conditions that leads the system to hyperbolic growth. In the previous implementation, we have seen that (Figure 3.22) k_1 has a remarkable effect on the system. It is observed that the positive feedback pool reaches its highest value by k_1's and k_7-k_8's impact. In the following calculation, we wanted to see if there is another rate constant that cooperates with k_1 and guides the system achieve hyperbolic progression. For this, I utilized only first five rate constants and kept k_6-k_{11} as zero: k_6-k_{11}=0.0. k_3-k_5 remained 0.01 as in the prevoius implementation: k_3-k_4=k_3=0.01. I kept the value of k_1 as 1.0: k_1=1.0, but I have changed k_2 from 0.01 to 0.1: k_2=0.1. Starting concentrations of P, E, and M and length of the first phase remained the same: 1.0000E-01, t=50. Figure 3.25 represents the data of this implementation.

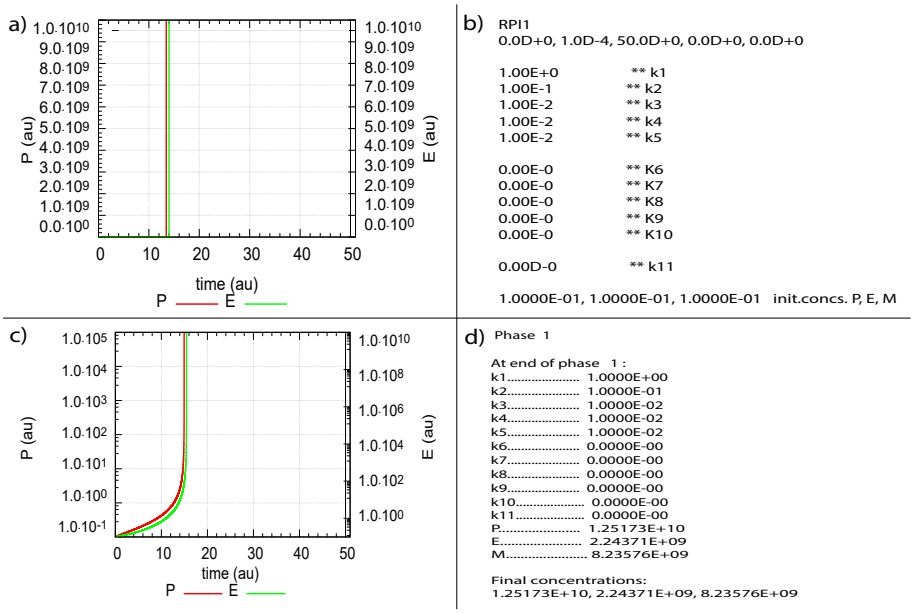

FIGURE 3.25: Figure shows a hyperbolic grows in Hyperbolic model 1 and consist of four parts. Part a) is linear plotting, while part c) represents logarithmic plotting. Part b) is an input file of the calculation and part d) represents final results of this calculation. Hyperbolic growth obtained from Model 1 while k_1=1.0; k_2=0.1 k_3=k_4=k_5=0.01; k_6=0.1; k_7=k_8=1.0; k_9=0.1 and k_{10}=k_{11}=0.0.

We observe that in these circumstances, the same hyperbolic growth is obtained in the system. It implies that k_2 collaborates with k_1 in order to get the highest and hyperbolic type of increase. The growth rate in both enzyme and positive virus strands is the same (Figure 3.25). We have reviewed the results of this implementation below: Part a) depicts the plotting of concentrations of positive-strand virus particles and enzymes over time. Both variables exhibit an apparent hyperbolic increase. Increased enzyme concentration results in an increase in positive-strand virus particle concentration and vice versa.

Logarithmic plotting is represented in part b). As an indicator of part a), it proves hyperbolic increase. There is same level of increase in this plotting too.
Part c) demonstrates the input file for this calculation. The initial concentrations of variables, the rate parameters values, and the length of the first phase are revealed. Section d) includes the detailed final concentrations of variables. We can figure out from this section that positive-strand virus concentration is the highest and slightly higher than negative-strand virus concentration. The lowest enzyme concentration indicates that the enzyme is responsible for the rise in positive-negative strands. As a result, it grows more slowly than others.
As usual, we used the Matlab program as a confirmation of our results. All three equations (3.8, 3.9 and 3.10) added to the ODE.m file in the Matlab programming. We used logarithmic plotting in Matlab because linear scale can not indicate whether growth is hyperbolic or not. Rate parameter values were the same as in the Fortran program: k_1=1.0; k_2=0.1 k_3=k_4=k_5=0.01; k_6=0.1; k_7=k_8=1.0; k_9=0.1 and k_{10}=k_{11}=0.0. Results from Matlab (Figure 3.26) confirm that there is a hyperbolic growth in all three variables in the case of k_1 and k_2 dominance. Even if k_6-k_{11} are out of the functionality and have no impact on the system, it results in a hyperbolic increase.

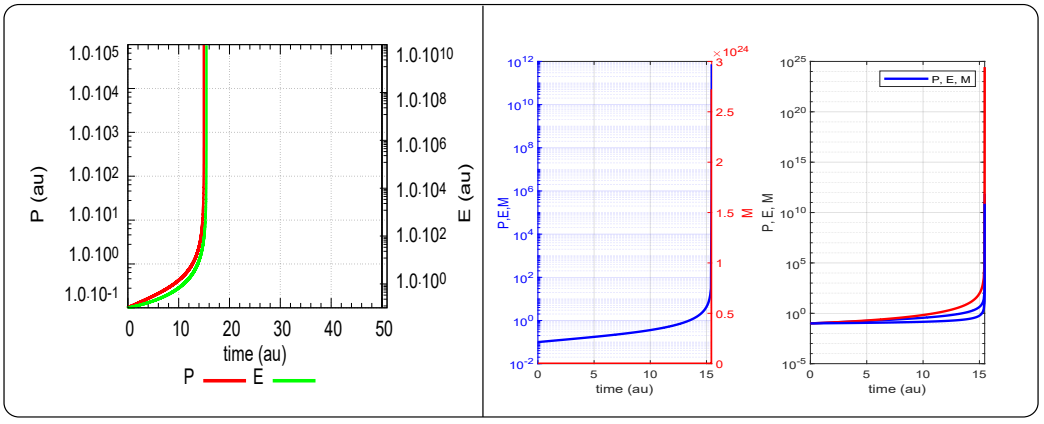

FIGURE 3.26: Matlab output file shows a hyperbolic increase in all three variables: positive-negative virus strands and enzyme in case of k_1 and k_2 dominance.

3.8.3 Exponential increase

We wanted to investigate under what conditions we can get exponential growth in this motif in the following calculation. For that reason, we have increased set following values to the rate parameters:

k_1=k_3=k_4=0.1 k_2=k_5=0.01 k_6-k_{11}=0.0

As it can be seen above k_6-k_{11} are out of the system and ineffective in this implementation. k_1, k_3 and k_4 have highest value in the process. Initial concentrations of P,E and M are 1.0000E-01 and t=50. We can see that from the graph (Figure 3.27), we got exponential growth. The term "exponential growth" refers to a mechanism in which quantity grows over time [21]. A proportional rate of change exists when the instantaneous rate of change (that is, the derivative) of a quantity concerning time is equal to the quantity itself. A quantity that grows exponentially is described as an exponential function of time, with the exponent as the time variable (in contrast to other types of growth, such as quadratic growth) [21].

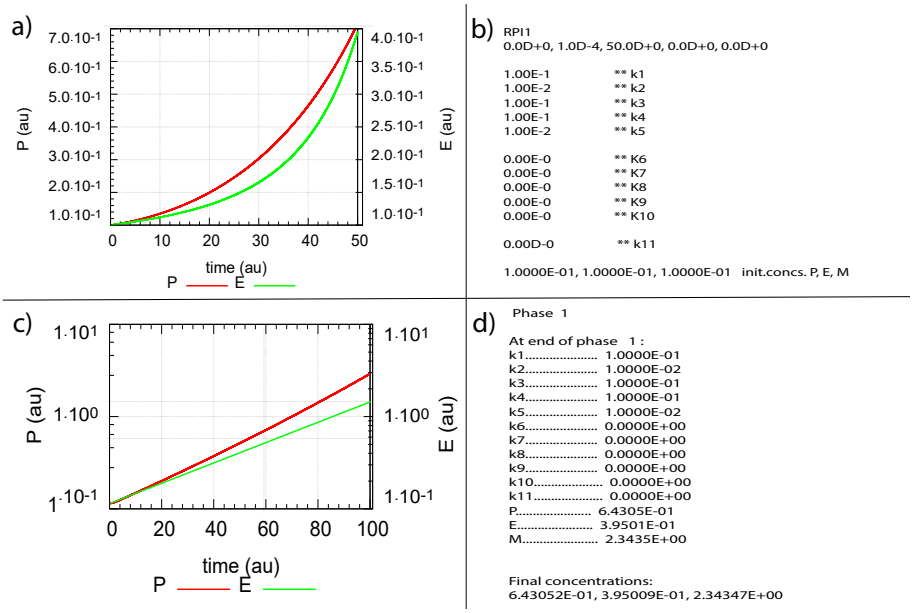

FIGURE 3.27: Exponential growth obtained from the hypercyclic model 1 in following circumstances: k_1=k_3=k_4=0.1; k_2=k_5=0.01 and k_6-k_{11}=0.0

All results from this implementation are summed up in Figure 3.27.
Part a) shows the plotting graph of P and E versus time. There is an apparent exponential increase in positive-strand virus concentration and enzyme concentration. Negative strand viruses are not present in the graph, but they have also increased exponentially.
The graph has been shown in part b) is an indicator of the previous graph. It demonstrates logarithmic plotting of strand viruses and enzymes against time. The result is linear, which verifies the exponential type of growth.
Part c) is an input file for this calculation that contains the initial concentrations, phases' time units, and values for the rate parameters.
Part d) is the output file obtained from the program. It shows the final concentration of all three P, E, and M variables at the end of the first phase. We can say that there is a minor difference between negative and positive virus strand concentration.

Negative virus strand particles‘ concentration is higher than the other two participants. The enzyme has the lowest value of final concentration.

As always, the Matlab program was used to validate the calculation. Equations (3.8, 3.9 and 3.10) and values for rate constants (k_1=k_3=k_4=0.1; k_2=k_5=0.01 and k_6-k_{11}=0.0), initial concentrations of P, E, and M, and the duration of the first phase were introduced into the Matlab project.

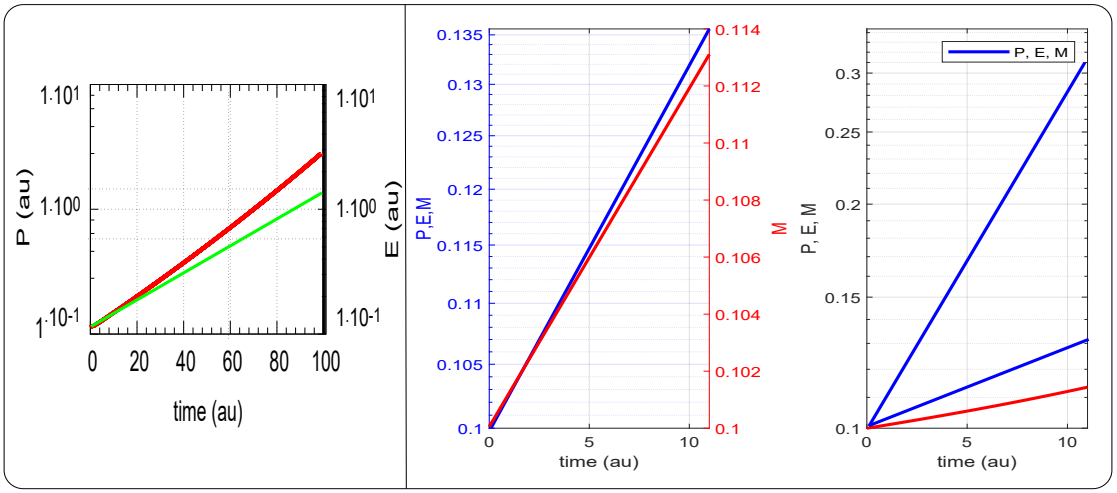

FIGURE 3.28: Matlab output file shows an exponential increase in all three variables: positive-negative virus strands and enzyme in case of k_1,k_3, and k_4 are higher than other parameters. Logarithmic plotting from Matlab shows a linear growth which is an indicator of exponential increase.

Results acquired from Matlab are precisely the same as the results achieved from the Fortran program. As shown in Figure 3.28, there is an exponential increase (linear growth in logarithmic plotting indicates the exponential type of growth) in virus particles and enzyme concentration. While interpreting results from the graph, I mentioned that negative strand viruses had shown more increase in their concentration than others (Figure 3.27). It is confirmed in Figure 3.28. The graph indicates that negative strand viruses have more increase than enzyme and positive virus strand particles. The enzyme has the lowest increase among them. In conclusion, k_1, k_3 and k_4 has a major effect in obtaining exponential increase in hypercyclic model 1.

3.8.4 Steady-state

We used different values for all rate constants in the following implementation than in our previous calculations. First five rate constants k_1-k_5 are 0.01. Other rate parameters k_6-k_9 have a value of 1. The last two parameters, which are k_10 and k_11,

have a value of 0.01 :k_1-k_5=0.01; k_6-k_9=1.0 and k_10=k_11=0.01. Three variables :P,E and M have the same value of 1.0000E-01 in this calculation too. Both graphs in figure 3.29 represent a steady state. A steady-state can be described as a system in which all or certain parts of this system are not dependent on time. Concisely, the whole system is constant during this calculation.

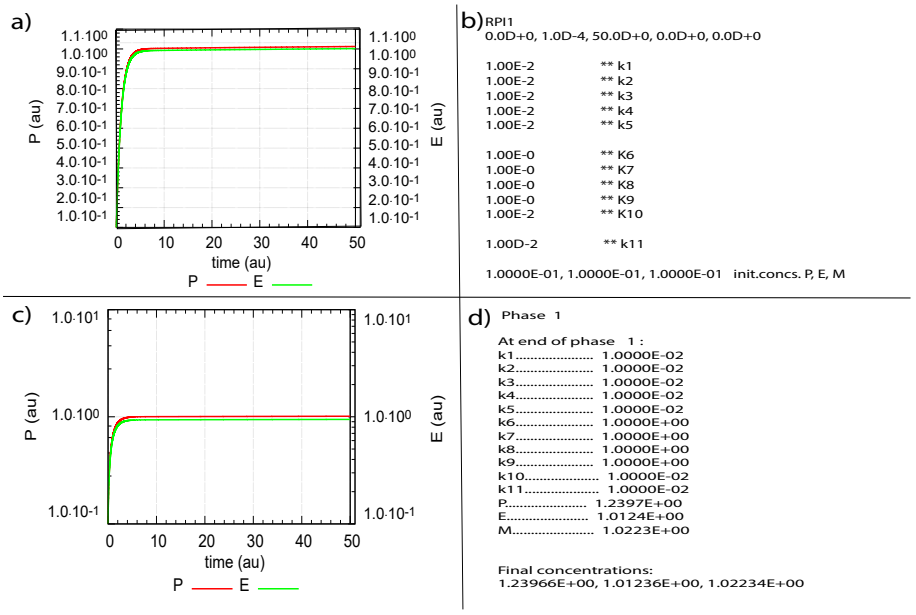

FIGURE 3.29: Steady state obtained from hyperbolic model 1. Figure consist of four parts. Graph a) is linear plotting of P, E and M concentration against time. Graph c) is the logarithmic plotting which indicates steady state. Part b) and d) are input and output files.

All results are outlined in Figure 3.29.

Part a) demonstrates that plotting of positive virus particles and enzyme versus time reveals a steady state. Due to the low values of the first five rate parameters, the steady-state could be managed. k_1-k_{11} constants have a remarkable impact on the system, and their constancy during the process leads the feedback loop to the constant state. From the plotting graph, we can see a slight increase first, but it gives a place to the steady-state after a concise growth.

Part b) shows logarithmic plotting of virus strands and enzymes over time. Result is the same steady-state in both positive strand viruses and enzyme concentration.

Part c) is the implementation's input file, which contains all of the calculation's initial data.

Section d) contains the final concentrations of virus particles and enzymes. Negative virus strands and enzyme concentrations are almost the same; positive-strand viruses concentration is slightly more than others.

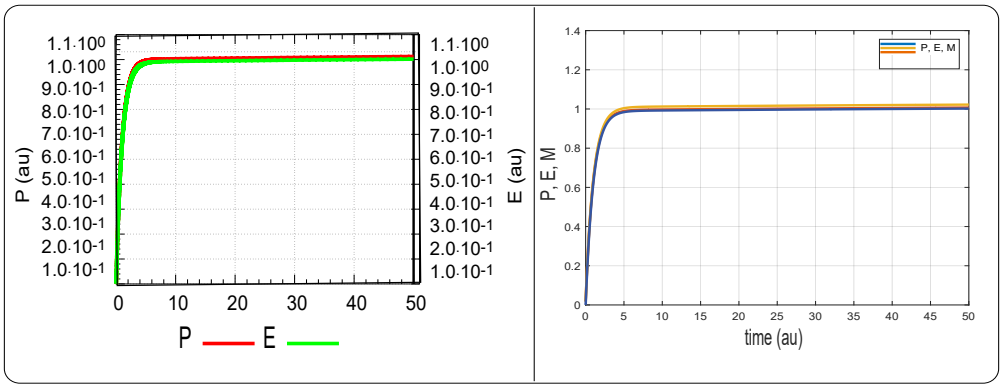

FIGURE 3.30: Matlab output file shows a steady-state condition when k_6-k_9 are higher than other parameters: k_1-k_5=0.01; k_6-k_9=1.0 and k_10=k_11=0.01

Figure 3.30 is a confirmation implementation done by the Matlab program. Graph shows that there is a steady-state in both: positive-negative virus strand and enzyme.

3.8.5 Summary of dynamics of Hypercyclic model 1

I want to stress essential points of the implementation of hypercyclic model 1:
i) k_1 plays an essential role in achieving hyperbolic growth. Without the increase of this parameter, the system can not gain an hyperbolic increase.
ii) k_2 with k_1 leads the system to hyperbolic growth Without the assistance of any other rate constant.
iii) k_1, k_3 and k_4 get the system to exponential increase.
IV) When first five rate parameters k_1-k_5 are constant and have a very low values, increasing k_6-k_{11} cause a steady-state.

3.9 Manfred Eigens‘ model of viral hyperbolic growth

The best prototype of the hypercycles is the viral replication process. When the viral RNA enters the host cell, it acts as a messenger for the host translation apparatus, producing the replication factor that catalyzes the amplification of the viral RNA and complementary minus-strand RNA in the cyclic reaction [6]. A hypercycle is generated by the interaction of translation and replication cycles. The hypercycle notion was initially suggested as a self-organization theory relevant to early evolution [19]. Its significance in early evolution was identical to its role in viral evolution nowadays: hypercyclic coupling allows for functional evaluation and evolutionary improvement of

the phenotype by encouraging replicating its own genotype. The host's role in virus infection had to be played by the primordial reaction medium during precellular evolution. Viruses are almost certainly entirely postbiotic in origin, meaning they are derived from host-cell components [6]. However, analyzing their infection cycles enables us to have a good sense of how the hypercycle principle functions in nature, which may help us understand how prebiotic hypercycles were established.

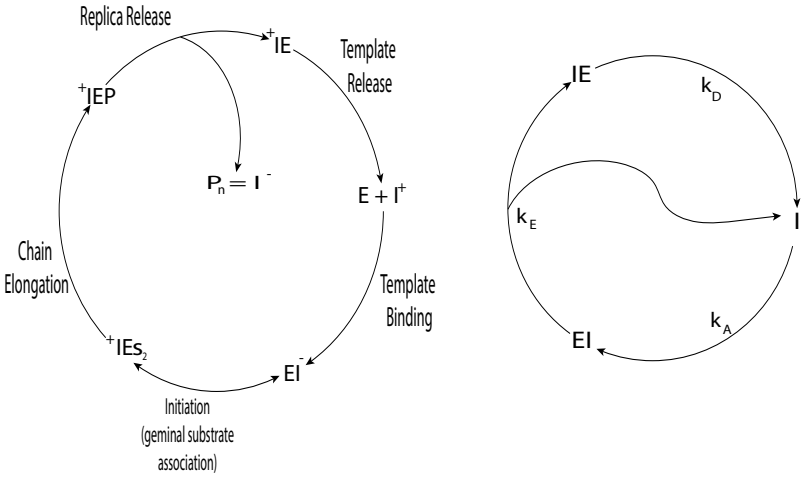

FIGURE 3.31: Manfred Eigen's simplified five-step and three-step replication cycle processes. E is free replicase, I symbolizes free RNA, EI defines the active enzyme-template complex, and IE denotes the inactive enzyme-template complex [6]

Manfred Eigen's simplified five-step and three-step replication cycle processes are depicted in Figure 3.31.
E represents free replicase, I symbolizes free RNA, EI defines the active enzyme-template complex, and IE denotes the inactive enzyme-template complex [6]. I complexes with E at a rate of $k_A[E]$ to create the EI, EI then produces I at a rate of k_E.
Rate equations of this processes are:

$$\frac{d[EI]}{dt} = k_A \cdot [E] \cdot [I] - k_E \cdot [EI] \tag{3.16}$$

$$\frac{d[IE]}{dt} = k_E \cdot [EI] - k_D \cdot [IE] \tag{3.17}$$

$$\frac{d[I]}{dt} = k_E \cdot [EI] + k_D \cdot [IE] - k_A \cdot [E] \cdot [I] \tag{3.18}$$

Figure 3.31 and equations 3.16, 3.17 and 3.18 indicates that I complexes with E at a rate of $k_A[E]$ to create the EI, EI then produces I at a rate of k_E [6].

After infecting the host cells viral RNA is mainly responsible for protein production. In this stage replicase and viral coats begin to accumulate. Synthesis of coat protein is relatively continuous, although production of replicase is nearly terminated once coat protein inhibits translation. The infection cycle begins when the first phase has occurred. Between 6-12 minutes of the infection cycle hyperbolic step comes up. As replicase concentration increases, RNA replicates. Replicase and RNA creation is accelerated at this point due to hyperbolic growth. Asymmetry in binding of the coat protein to viral RNA is the reason for deviations from the analytical descriptions. Plus-strand RNA is coupled to ribosomes, replicase, and coat protein, while minus-strand RNA is fully saturated with replicase. During the hyperbolic phase, the plus- and minus-strand synthesis rates of RNA are balanced by their strong synthesis coupling [6].

3.9.1 Eigens' model of hypercyclic viral growth

To begin, I would like to include a brief overview of the Eigens' model of hypercyclic viral growth. This model was developed to provide an accurate explanation of hyperbolic growth using Manfred Eigen's model. The system under investigation contains two positive feedback loops. Together, these two loops allow the positive feedback loop process to extend hyperbolically. Five rate constants and three variables represent in Eigens' model of hypercyclic viral growth: E enzyme, positive-strand pStr, and negative-strand nStr. All rate parameters k_1-k_5 can be modified during all three phases. Figure 3.32 illustrates Eigens' model of hypercyclic viral growth scheme.

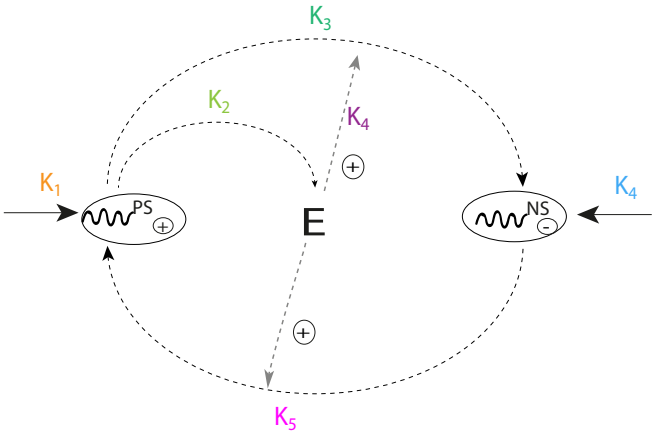

FIGURE 3.32: Eigens' model of hypercyclic viral growth scheme which consists of three variables and five rate parameters. Scheme indicates that there are two positive feedback loop which can lead to hyperbolic and exponential type of growth.

Ps or pStr is positive strand virus parts. E is enzyme. Ns or nStr is negative strand viruses. In some calculations, I used one phase, but in most calculations, all three phases were involved to the process. Five rate parameters together lead the system to an increase. The critical task in this program is to establish the nature of the increase. The rate equations for the method are as follows, based on the Eigens' model of hypercyclic viral growth scheme, which represents hyperbolic growth:

$$\dot{E} = k_2 \cdot Ps \tag{3.19}$$

$$\dot{P}s = k_1 + k_5 \cdot E \cdot Ns \tag{3.20}$$

$$\dot{N}s = k_4 + k_3 \cdot E \cdot Ps \tag{3.21}$$

Additionally, I had three graphs. The first is graph-EN, representing the plotting of the E -enzyme and Ns or nStr -negative virus strands concentrations versus time. The second graph is graph-EP, which illustrates the relative increase in E and Ps or pStr -positive strand virus populations over time. The final one, graph-PN, demonstrates the plotting of positive and negative strands against time. This model provides me with hyperbolic growth, exponential growth and linear growth. All of these states are determined by the values of the rate constants. I want to start with hyperbolic increase.

3.9.2 Hyperbolic growth in Eigens' viral hypercyclic model

I discovered that each rate parameter has a unique impact on the Eigens' viral hypercyclic model's positive feedback loop mechanism. Some of them have an effect on the system that causes it to grow at an infinite rate, a mechanism referred to an as hyperbolic growth. In this implementation, I assigned the lowest value to k_5 ,and the highest value to k_3 and k_4. The following are the values of the rate constants: k_1=k_2=0.1, ; k_3=k_4=0.2, and k_5=0.05.

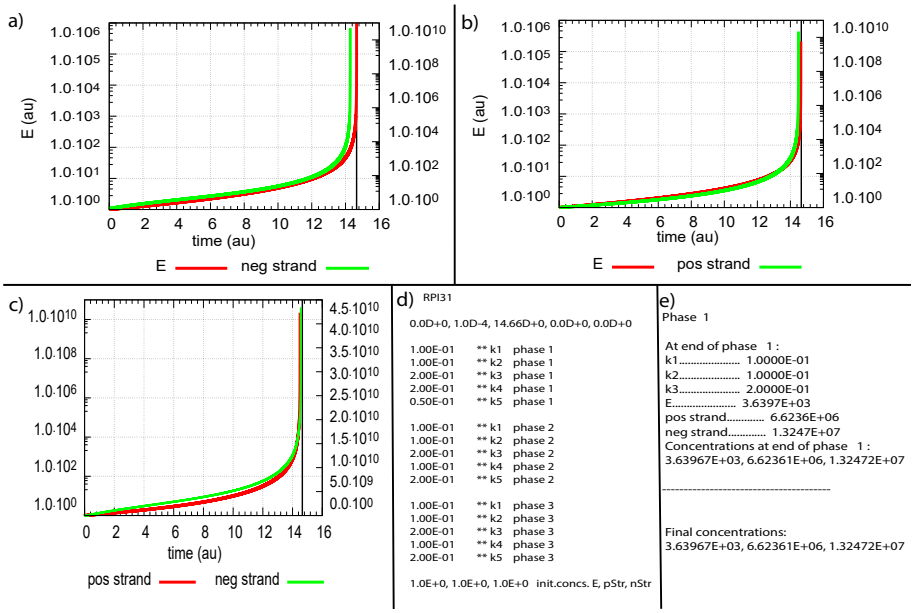

FIGURE 3.33: Hyperbolic growth under following conditions: k_1=k_2=0.1, ; k_3=k_4=0.2, and k_5=0.05

Initial concentrations of E, nStr, and pStr are 1.0E+0, and we have only one phase in this implementation. Figure 3.33 demonstrates the results of this calculation. I used only logarithmic plotting in both Fortran and Matlab programming because the linear scale can not prove whether growth is exponential or hyperbolic. Figure 3.33 shows the results of the logarithmic plotting. This plotting is an indicator that shows how the concentration of all particles, including enzymes, positive and negative strand viruses, is increasing hyperbolically. Increased positive strand concentration boosts enzyme synthesis, which in turn boosts negative strands' rate of synthesis. As a result, the concentration of negative strand viruses is slightly higher than that of positive strand viruses. The positive-negative strand graph indicates this (graph-PN). In the graph, the negative strand viruses concentration (green curve) is higher than the positive strand viruses concentration (red curve).

Part a) depicts the plotting of enzyme and negative-strand virus concentrations versus time. It shows that both enzyme and negative virus strands grow hyperbolically in a short period.

Part b) contains a graph depicting the plotting of enzyme and positive virus particles against time. This graph demonstrates that both E and positive virus strands increase hyperbolically over a brief time.

We got the same result in the third graph - part c). The hyperbolic increase is observed in both positive and negative virus strands concentration as time progressed. It means that positive strands cause enzyme synthesis to be enabled, resulting in a rise in concentration. If the concentration of enzymes rises, the increase of negative strands

rises as well.

Part d) is an input file of the implementation. Initial concentrations of E, nStr, and pStr, duration of the phases, and rate parameters values are described in this part.

Part e) is the output file for the implementation. It demonstrates the final concentrations of an enzyme and pStr, nStr. Increased positive-strand concentration boosts enzyme synthesis, which in turn boosts negative strands' rate of synthesis. As a result, the concentration of negative strand viruses is slightly higher than that of positive-strand viruses. The enzyme concentration was found to be the lowest.

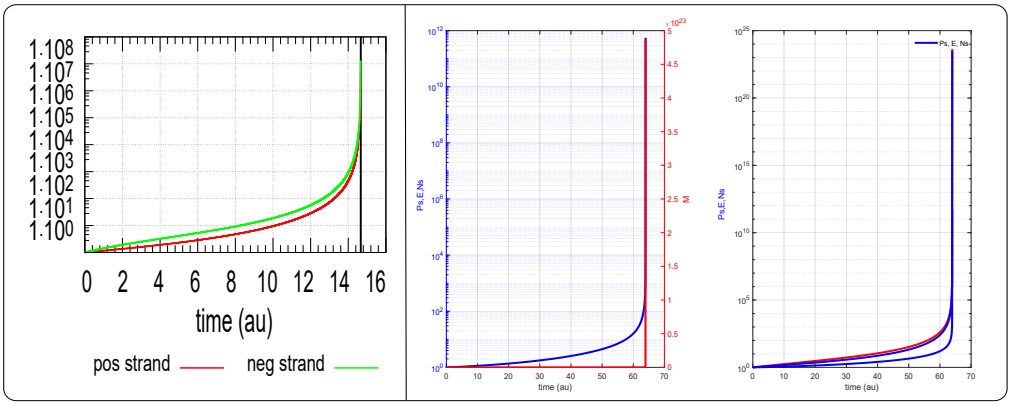

FIGURE 3.34: Matlab output file shows an exponential increase in all three variables: positive-negative virus strands and enzyme in case of k_1,k_3 and k_4 are higher than other parameters.

As a confirmation of our results, we decided to use the Matlab program. For that purpose, the Eigens' viral hypercyclic model 2.m and ODE file for this model was created. Equations 3.19, 3.20 and 3.21 added to the ODE.m file. Initial concentrations of variables (E, nStr, and pStr are 1.0), values of rate parameters (k_1=k_2=0.1; k_3=k_4=0.2, and k_5=0.05) and duration of the first phase (70) are inserted into the program. Results from this calculation are demonstrated in Figure 3.34. The graph indicates that there are a hyperbolic growth in enzyme, positive and negative virus particles.

3.9.3 Exponential growth in Eigens' viral hypercyclic model

In the following implementation, we wanted to figure out which rate constant causes the process to grow exponentially. In order to get exponential growth I decreased k_1 and k_2 from 1.00E-01 to 1.00E-02. Meanwhile I decreased k_3 and k_4 from 2.00E-01 to 2.00E-02. k_5 is the same as in the previous calculation. In this case k_5 value is much more than other parameters. k_1=k_2=1.00E-02, k_3=k_4=2.00E-02, k_5=0.50E-01.

E, pStr and nStr values remain unchanged: E=pStr=nStr=1.0E+0. In this case k_5 value is much more than other parameters. In this calculation, I used only logarithmic plotting, since as I mentioned before, we can not decide whether growth is exponential or hyperbolic from the linear scale. We can see from the graph that (Figure 3.35) system shows an exponential progression. Since we have linear growth in logarithmic plots, it indicates that there is an exponential increase. The term "exponential growth" refers to a phase of increasing quantity over time. It occurs if a quantity's instantaneous rate of change with respect to time is proportional to the amount itself.

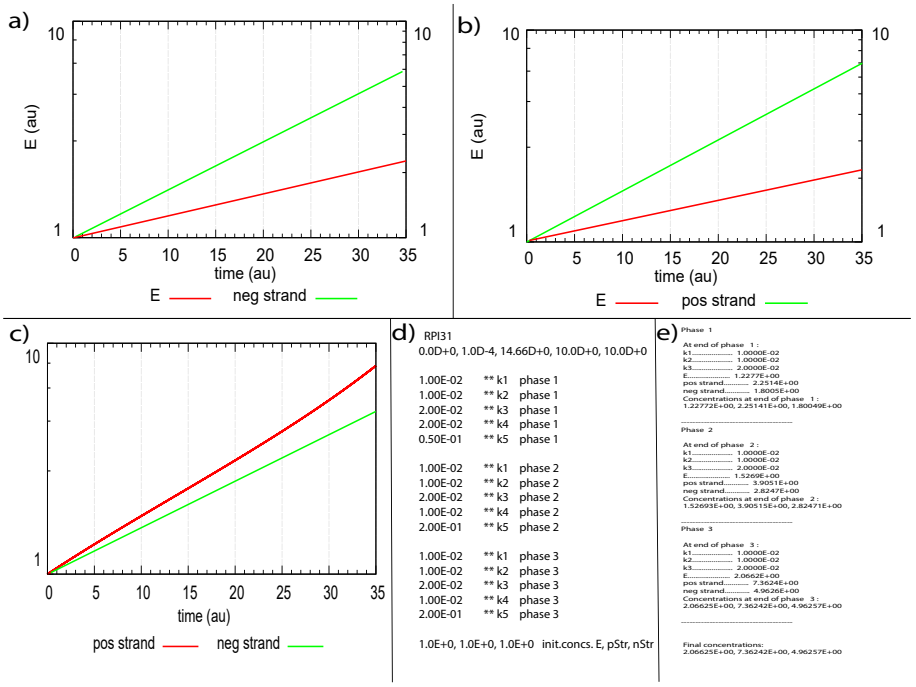

FIGURE 3.35: Exponential growth achieved from this model under following conditions: k_1=k_2=0.01, k_3=k_4=0.02, k_5=0.05. Graph a) represents a logaritmic plotting of an enzyme and negative virus particles. Graph b) demonstrates plotting of enzyme and positive virus strands against time. Graph c) is the plotting og positive and negative virus particles versus time. Part d) and e) are input and output files respectively

Figure 3.35 shows that there is an exponential increase in all three graphs. The linear increase in logarithmic plotting is an indicator of exponential growth.

Graph a) demonstrates an exponential growth in both enzyme and negative strands increasing with respect to time.

Graph b) indicates that there is an increasing quantity of enzyme and positive strands over time.

The graph c) represents the exponential increase in the concentration of both negative and positive virus strands through time.

Part d) is an input file containing initial concentrations of pStr, nStr, and enzyme, rate parameters values, and length of phases.

Part e) is the output file that contains final concentrations of enzyme, positive and negative virus particles in every phase. According to part e), positive virus strands have the highest concentration value, and the lowest value belongs to the enzyme. In comparing graph-PN to graph-EN, I found that positive-strand viruses reached higher concentration levels simultaneously than negative strand viruses. In graph-PN, the concentrations of positive strands increase more quickly.
I used logarithmic plotting on Matlab to verify exponential increase in this calculation. Confirmation of this implementation has been done with the Matlab program as usual. By using equations 3.19, 3.20 and 3.21 program runned in Matlab. The Matlab illustration shows there is an exponential growth in all particles and enzyme (Figure 3.36).

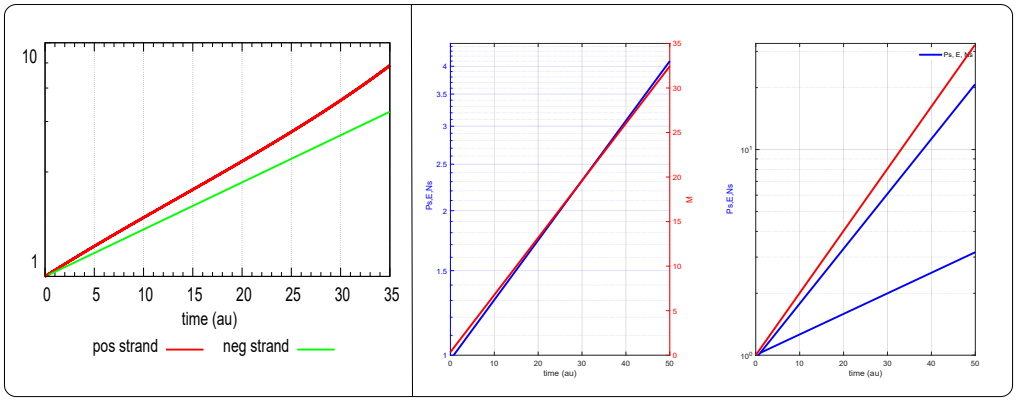

FIGURE 3.36: Matlab output file shows an exponential increase in all three variables: positive-negative virus strands and enzyme in case of k_5 is higher than other parameters under following conditions: k_1=k_2=0.01, k_3=k_4=0.02, k_5=0.05

Figure 3.36 confirms all results that have been achieved from Fortran software. Positive virus strands‘ concentration is highest than other particles—the lowest exponential growth rate acquired from enzymes.

3.9.4 Linear growth

In the following implementation, I'd like to see under what conditions the process switches to linear growth and increases by the same amount over time.We decided to make a significant difference in the value of k_5 in the first phase by decreasing it from 0.05 to 0.0005. All other rate parameters remained unchanged. k_1=k_2=0.01, k_3=k_4=0.02, k_5=0.0005. Three phases are involved in this implementation. Except k_4 and k_5, I kept all rate parameters constant over the three phases. I changed k_4 in the second phase and decreased it from 0.02 to 0.01. It had the exact value of 0.001 in the

third phase too. I changed k_5 in both phases. In the second phase, we have increased k_5 from 0.0005 to 0.002, and in the third phase, we expanded this value ten times and made it 0.02. I made no changes to the values of E, pStr, or nStr : 1.0.

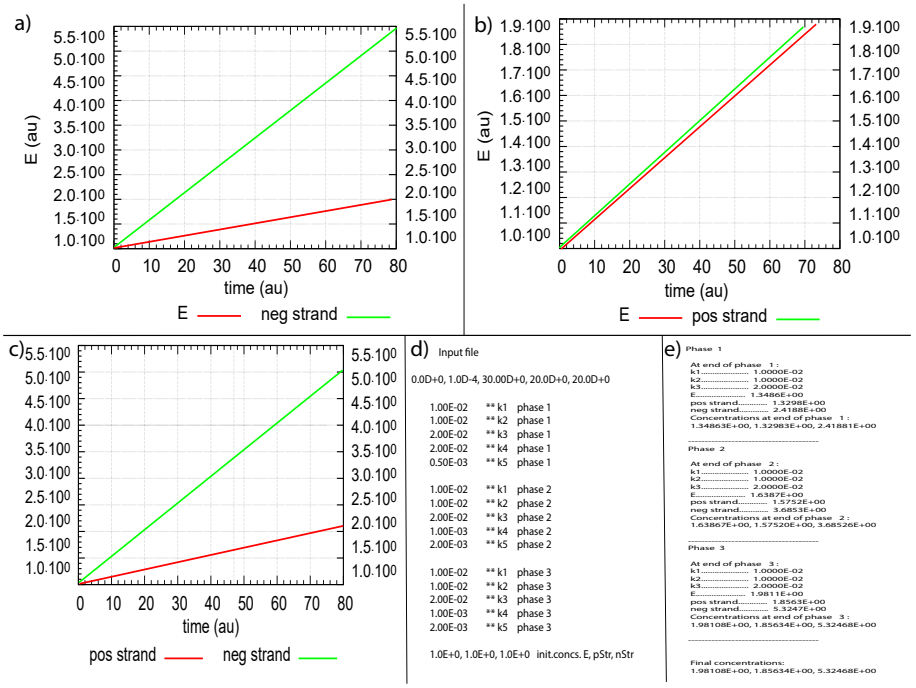

FIGURE 3.37: Linear growth observed in Eigens' viral replication model. Figure consists of five parts, graph a), b) and c) represents plotting between enzyme-negative virus strands, enzyme-positive virus particles, and positive-negative virus particles respectively. Part d) and e) provides information about initial values of variables and rate parameters and final calculations.

Figure 3.37 presents a linear growth in Eigens' viral replication model. Linear growth means that it grows by the same amount at each time level. All three graphs in figure 3.37 have a linear progression.

Part a) shows a plotting of enzyme and negative virus particles concentration over time. This graph demonstrates same amount of the increase in both enzyme and negative virus strand concentration at the each time step.

Part b) demonstrates the linear increase in the concentration of both enzyme and the negative virus strands.

Part c) depicts the same rise in the concentration of the negative and the positive strands over time.

Part d) is the implementations' input file, which contains the values of the rate constants, beginning concentrations, and duration of all phases.

Part e) is the output file of this calculation containing final concentrations of pStr, nStr and enzyme. Negative strand virus concentration shows the highest level among them. Implementations results were confirmed using the Matlab program. Results

from the Matlab program demonstrate a linear increase in all variables (Figure 3.38). Equations 3.19, 3.20 and 3.21 inserted to the ODE file. Rate parameters values used in order to observe the dynamics of this calculation. Only first phase rate parameters values used in the Matlab programming: k_1=k_2=0.01, k_3=k_4=0.02, k_5=0.0005.

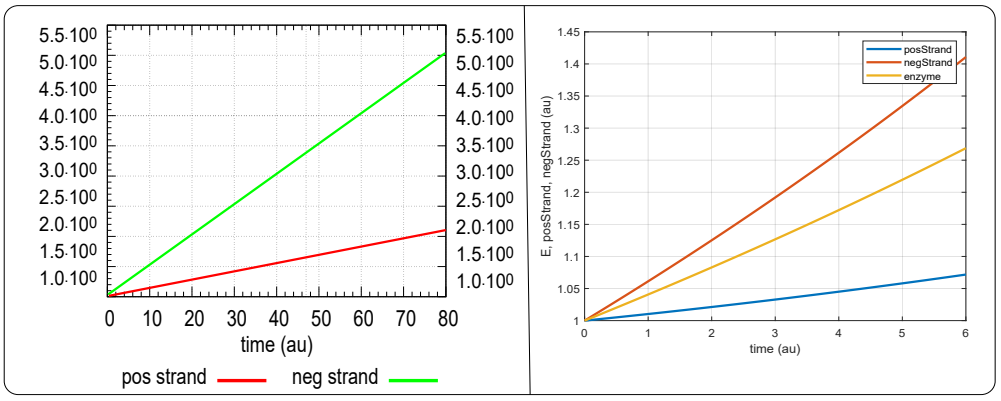

FIGURE 3.38: Matlab output file shows a linear increase in all three variables: positive-negative virus strands and enzyme in case of k_1,k_3 and k_4 are higher than other parameters.

Figure 3.38 validates that there is linear growth in positive-negative virus particles and enzymes. The lowest concentration level belongs to aa positive virus particles (blue line) and the highest to negative-strand viruses (orange line).

3.9.5 Summary of calculations of Manfred Eigens' viral hypercyclic model

In conclusion, I want to summarize all of the findings:
i) If k_3 and k_4 have a higher value than the other rate parameters, the system grows hyperbolically.
ii) When k_5 has a greater value than the rest of the rate constants, the feedback loop mechanism develops exponential growth.
iii) The linear increase was discovered when k_5 has the lowest value, and high-value belongs to k_3.

3.10 Saturation in Eigens' viral hypercyclic model

According to Manfred Eigen's experimentation, after a short hyperbolic step entire system goes to an equilibrium point. Regardless of whether the rate constant values

are chosen to have an exponential or a hyperbolic relationship, the hyperbolic phase will always return to the equivalence point we mentioned earlier. After only a little period, the metabolism of viral growth is radically transformed as the RNA becomes completely saturated with replicase and ribosomes. Meanwhile, the majority of the plus strand is attached to ribosomes; the minus strands are currently being used as a supply of replicase[6].

$$\frac{d[I_0]}{dt} = \frac{d[I]}{dt} = k_A \cdot [I] \cdot [E] = k_E \cdot [EI] = k_D \cdot [IE] = constant \tag{3.22}$$

In order to investigate saturation level in the replication process Eigens' viral hypercyclic model with saturation is generated.

3.10.1 Eigens' viral hypercyclic model with saturation

Eigens' viral hypercyclic model with saturation developed in order to give a clear overview of saturation in Manfred Eigens' hypercyclic replication. After a very rapid hyperbolic increase saturation process takes place. In other words, the system gets to a constant level for a while. This period calls the saturation phase in the process. This model investigates how this period occurs, under what conditions we can achieve this level, and how it affects variables' final concentrations. This program consists of 6 rate parameters: k_1-k_6, three variables- E (enzyme), pStr (positive virus strands), nStr (negative virus strands). The scheme of the Eigens' viral hypercyclic model with saturation is described in Figure 3.39.

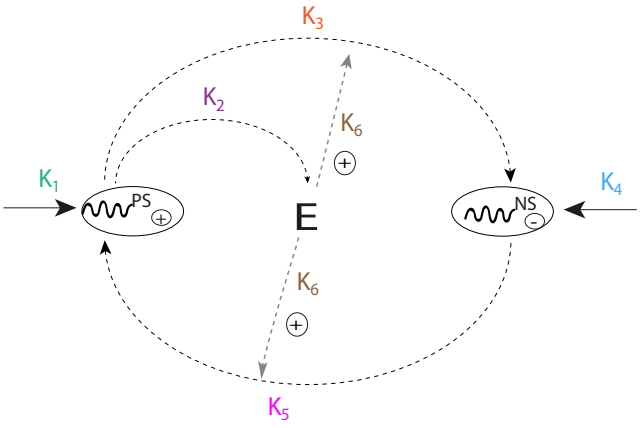

FIGURE 3.39: Eigens' viral hypercyclic model with saturation scheme consist of three variables and six rate parameters

Rate equations of Eigens' viral hypercyclic model with saturation scheme are following:

$$\dot{E} = k_2 \cdot P \tag{3.23}$$

$$\dot{P} = k_1 + k_5 \cdot E \cdot N/(k_6 + E); \tag{3.24}$$

$$\dot{N} = k_4 + k_3 \cdot E \cdot P/(k_6 + E) \tag{3.25}$$

The difference between Eigens' viral hypercyclic model and Eigens' viral hypercyclic model with saturation is the inner k_4 rate parameter was replaced with k_6 in this scheme, and there is two k_6 parameter in the system. We decided to use only one phase in this program, and all rate parameters‘ values could be changed and adjusted in this phase. Three states have been achieved from this program: hyperbolic increase, exponential increase, and linear increase. Saturation has been observed in all these states. We chose to start with hyperbolic growth.

3.10.2 Hyperbolic growth in Eigens' viral model with saturation

In order to achieve a hyperbolic type of increase in this program, I assigned the following values to rate parameters: k_1=k_2=0.1; k_3=2.00E+02; k_4=0.2; k_5=0.50E+02; k_6=1.00E+02. The initial concentration of Enzyme, negative virus strands (nStr), and positive virus particles (pStr) are equal and 1.0E+0. The duration of the first phase is 3.85D+0 time units. Figure 3.40 demonstrates under these circumstances system reaches to hyperbolic increase.

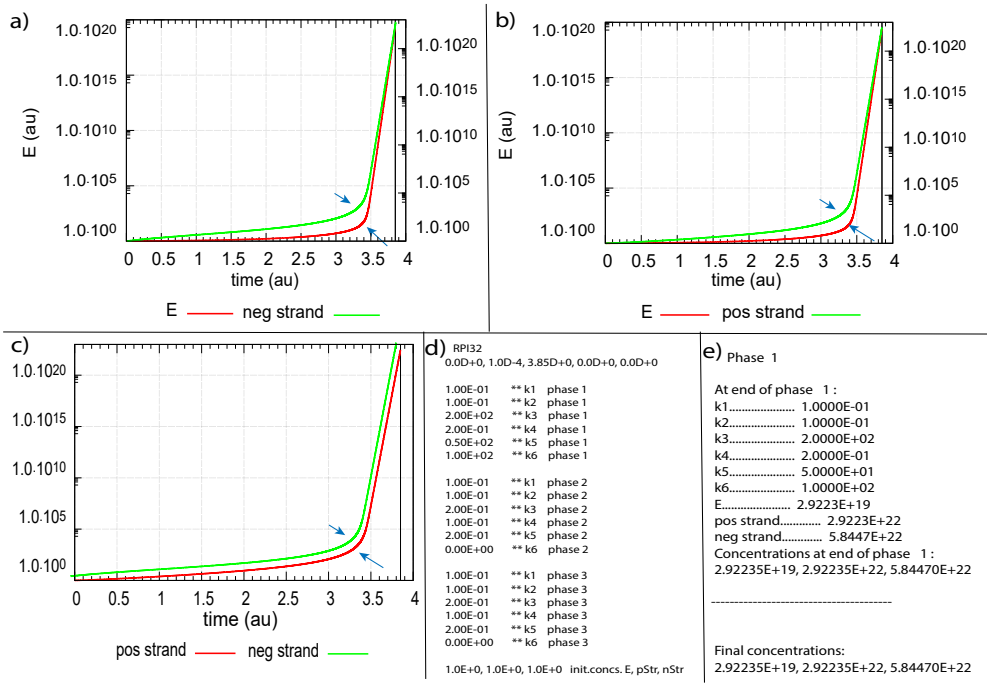

FIGURE 3.40: Hyperbolic increase that achieved under following conditions: k_1=k_2=0.1; k_3=200; k_4=0.2; k_5=50; k_6=100

Figure 3.40 depicts hyperbolic increase with logarithmic plotting. I used logarithmic plotting in order to confirm whether growth is hyperbolic or not.
Part a) is a graph EN logarithmic plotting of the concentration of enzyme and negative-strand RNA virus over time. Graph EN describes hyperbolic growth, with a saturation period before the start of hyperbolic growth.
Part b) logarithmically plots the enzyme and positive-strand RNA virus concentrations against time. They seem to be a growing hyperbolically. After a brief period of time, the saturation phase is replaced by a hyperbolic increase.
Part c) is a logarithmic plotting graph of the positive and negative virus strands against time. Additionally, there is a hyperbolic increase following a brief saturation stage.
Part d) is the implementation's input file; it contains all of the implementation's data, including initial concentration values, rate parameters' values, and step durations.
Part e) is the output file for the measurement, containing information about the final concentrations of pStr, nStr, and enzyme. Positive-strand RNA viruses and negative-strand RNA viruses have almost identical final concentrations. It has been discovered that certain enzymes have a lower concentration than others.

We used Matlab programming for confirmation of our results. Logarithmic plotting used in Matlab Programming too. All rate equations 3.23, 3.24 and 3.25 inserted to the matlab file and values of rate parameters, variables concentrations, and phase duration inserted into the program. Results have been shown in Figure 3.41.

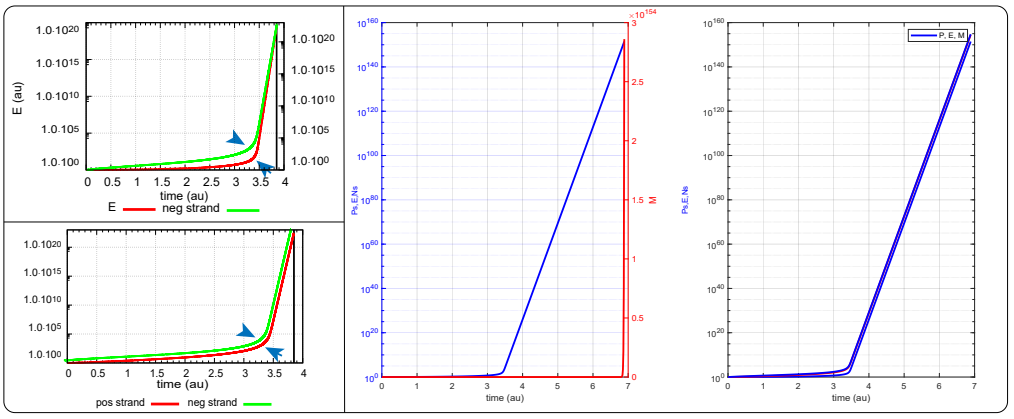

FIGURE 3.41: Matlab output file shows a hyperbolic increase in all three variables: positive-negative virus strands and enzyme in case of k_1=k_2=0.1; k_3=200; k_4=0.2; k_5=50; k_6=100

Figure 3.41 confirms that there is a hyperbolic growth after s short saturation in this calculation.

3.10.3 Exponential increase in Eigens' viral model with saturation

As written in Manfred Eigen's model [6], usually after the saturation process in RNA replication, an exponential increase comes up. As a result, we wanted to determine the conditions under which we could increase our implementation exponentially. We chose the following values for rate parameters in order to evaluate the saturation phase of exponential growth: k_1=0.1; k_2=10; k_3=20; k_4=0.2; k_5=0.5; k_6=1.0.

Initial concentrations of E, pStr and nStr are equal: 1.0. The duration of first phase is 50. Results from implementation is described in Figure 3.42.

Figure 3.42 summarizes all results and is divided into five parts. Following the saturation point, as shown in Figure 3.42, there is an exponential rise.
Part a) plots the concentrations of enzyme and negative RNA virus strands against time. It demonstrates that the mechanism experiences an exponential increase following a brief saturation period. There is a brief period of stability in concentrations during this constant period. This phase is referred to as saturation. The final concentrations of enzyme and nStr are approximately equal.
Part b) depicts the concentrations of enzyme and positive RNA virus strands as a function of time. After maintaining constant enzyme and pStr concentrations, an apparent exponential increase occurs. It confirms that the enzyme concentration is greater than the concentration of the positive virus strand.

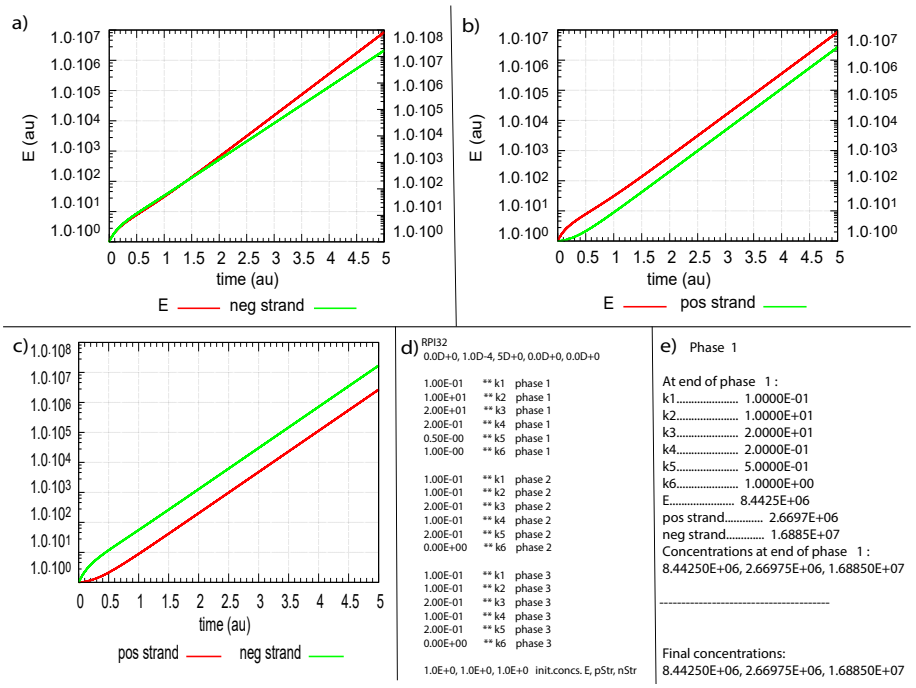

FIGURE 3.42: Exponential growth in logarithmic plotting in Eigens' viral hypercyclic model with saturation

The graph in Part c) plots the concentration of positive and negative RNA virus strands against time. There is a saturation phase in the graph during which both concentrations are constant. Negative virus strands are more dominant than positive virus strands.
Part d) includes data on initial concentrations and rate parameter values for the implementation. This section also specifies the length of the stages.
Part e) contains the calculation's final results. According to yielding data, negative strand viruses have the highest concentration. The lowest observed concentration of positive RNA viruses.

Findings from the Matlab program are the same as the results in Fortran program (figure 3.43). There are an exponential increase in enzyme, positive and negative virus strands. The highest concentration level has been found in negative strands and the lowest level in positive RNA virus particles.

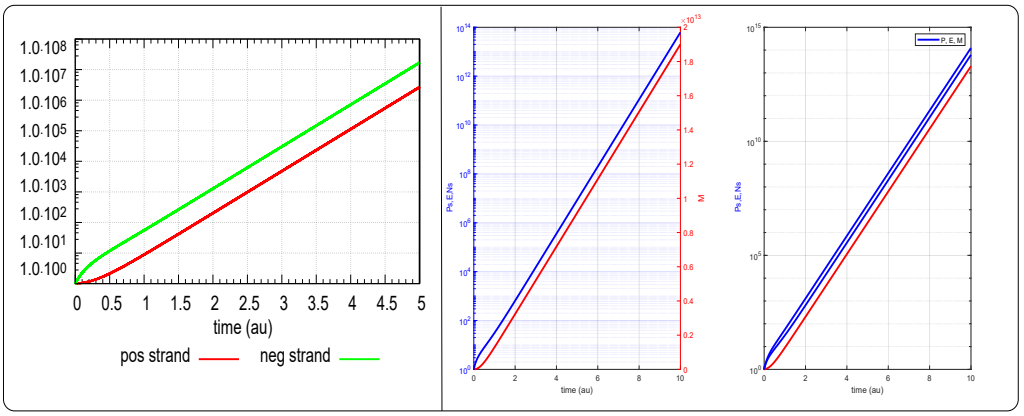

FIGURE 3.43: Matlab output file shows a exponential increase (linearity in logarithmic plottin is an indicator of exponential increase) in all three variables: positive-negative virus strands and enzyme in case of k_2,k_5 and k_6 are higher than other parameters: k_1=0.1; k_2=10; k_3=20; k_4=0.2; k_5=0.5; k_6=1.0.

3.10.4 Linear growth

To achieve a linear increase in this model, I set the following rate parameters to the following values: k_1=0.01; k_2=1.0; k_3=k_4=2.0; k_5=0.005; k_6=0.01. Enzyme, negative virus strands (nStr), and positive virus particles (pStr) have an initial concentration of 1.0E+0. The first phase lasts 50 time units. As shown in Figure 3.44, the system achieves linear growth under these conditions.

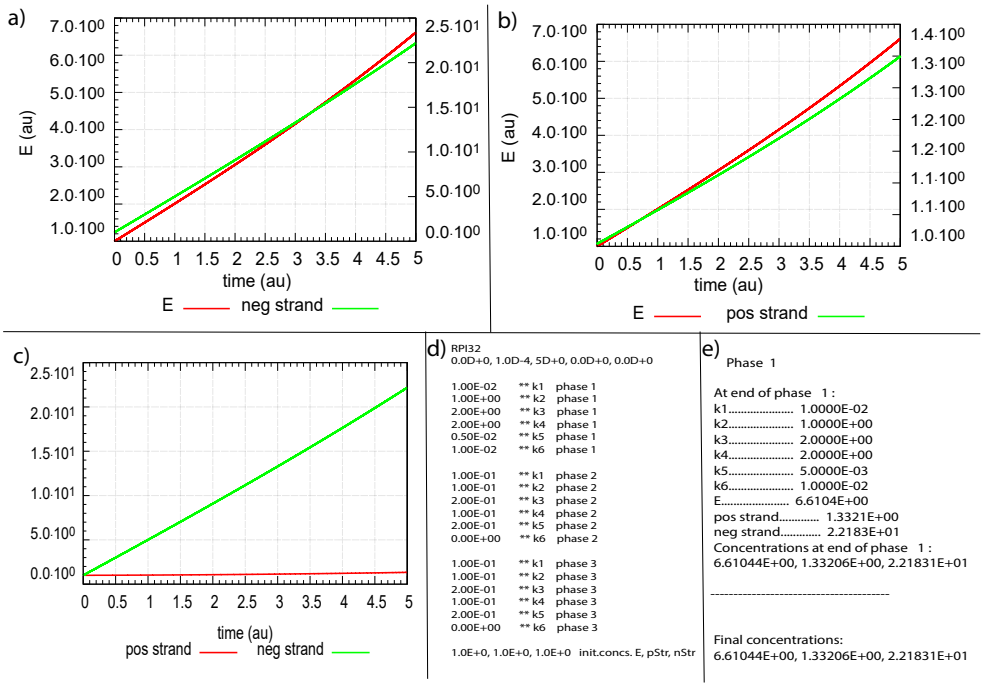

FIGURE 3.44: Linear increase that achieved under following conditions: k_1=0.01; k_2=1.0; k_3=k_4=2.0; k_5=0.005; k_6=0.01

Part a) demonstrates time-dependent plotting of enzyme and negative strand viruses. The graph shows that both negative RNA viruses and enzyme concentrations increase linearly.

Part b) is the plotting graph of positive virus strands and enzymes against time. The graph confirms that there is a linear progression in both parts, but the enzyme growth level is higher than positive viruses.

Part c) illustrates the time dependent plotting of positive and negative strand viruses. Surprisingly, while positive virus particles maintain a steady state, negative virus particles appear to grow linearly.

Part d) is an input file of the program showing the initial data of the implementation.

There has been revealed all final data in part e). It shows a high concentration level in negative RNA viruses, while the negative virus strand has the lowest concentration.

As is common, the Matlab program was used to verify the implementation. The figure shown in figure 3.45 summarizes the results from Matlab.

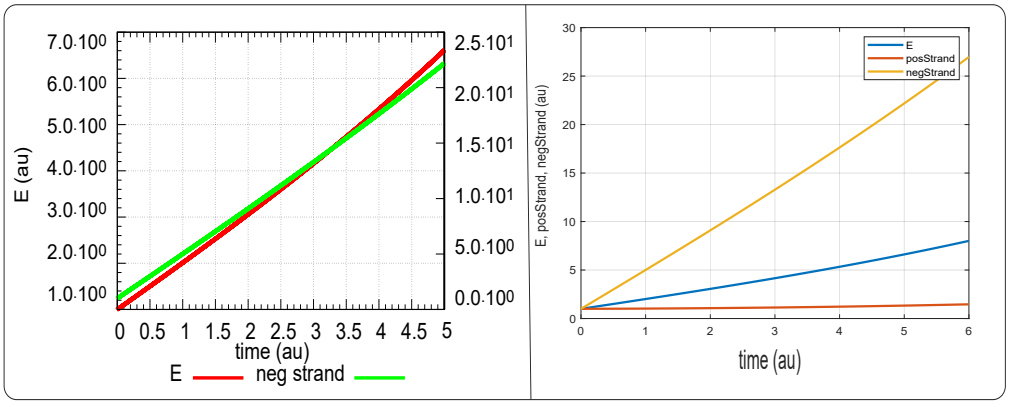

FIGURE 3.45: Matlab output file shows a linear increase in all three variables: positive-negative virus strands and enzyme in case of $k_3$and k_4 are higher than other parameters: k_1=0.01; k_2=1.0; k_3=k_4=2.0; k_5=0.005; k_6=0.01

Figure 3.45 verifies the precision of previous Fortran results. There is the highest linear growth in negative virus strand concentration. Enzyme concentration also increases linearly, but positive RNA viruses are in a steady state in comparison with other particles.

3.10.5 Summary of calculations in viral hypercyclic model with saturation

In conclusion to this section, I may state the following:
i) When k_3, k_5 and k_6 are dominant in the process, the system goes to an unlimited hyperbolic increase.
ii) If k_2 and k_3 are more significant than other rate parameters system grows exponentially.
iii) The high level of k_3 and k_4 lead the system to linear growth.

3.11 Motif 12

In the following section, I want to include a brief overview of Motif 12. Motif 12 is one of the motifs associated with positive feedback. Motif 12 is interesting since, in this motif, we see a negative feedback motif that can oppose perturbations that are hyperbolic, but only as long as there is enough E. E acts as an inhibitor in the process, whereas A acts as an activator. The Motif 12 scheme is depicted in Figure 3.46.

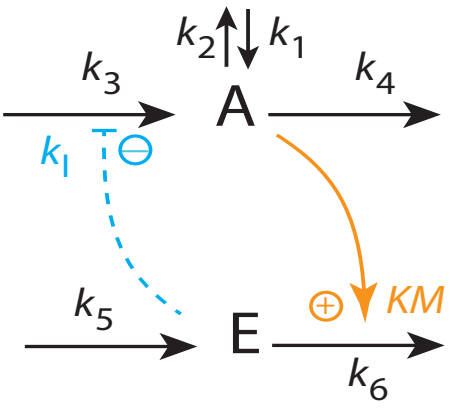

FIGURE 3.46: Motif 12 scheme

According to Motif 12, the following are the scheme equations for this calculation:

$$\dot{A} = k_1 - k_2 \cdot A + k_3/((1.0 + (E/K_I)) \cdot 1.0) \tag{3.26}$$

$$\dot{E} = k_5 - k_6 \cdot E \cdot A/(KM + E) \tag{3.27}$$

Motif 12 is composed of eight rate parameters and two variables, A and E. I used only one phase in all calculations. k_1-k_6 are rate parameters and each has a particular role in the system. Especially k_3 plays an inhibitive role and this helps the system to obtain hyperbolic growth. K_I is an inhibitive constant, where KM represents Michaelis Menten constant. As a result, I got three states in Motif 12 program: hyperbolic growth, linear growth and steady state.

3.11.1 Hyperbolic growth in Motif 12

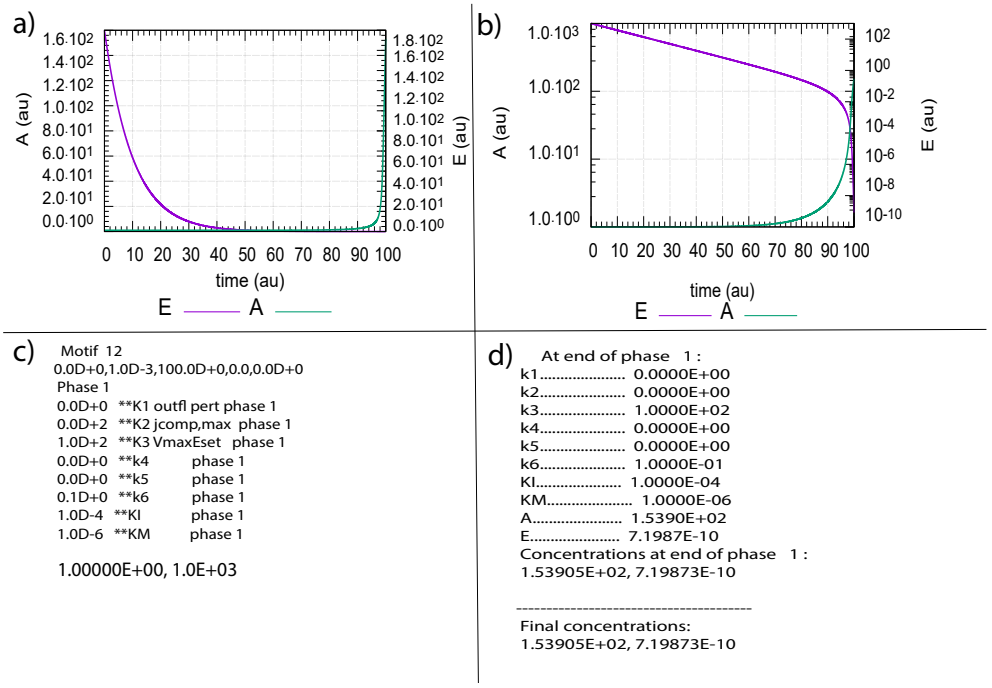

FIGURE 3.47: The hyperbolic increase was observed in Motif 12. A increases hyperbolically while E decreases. The logarithmic scale confirms hyperbolic growth.

In the following calculation I used only one phase and assigned following values to the rate parameters: k_1=k_2=k_4=k_5=0.0; k_3=1.0D+02; k_6=0.1D+0; K_I=1.0D-4 and KM=1.0D-6. Initial concentrations are highly different: A=1.000E+00 and E=1.000E+03. Results are shown in Figure 3.47. There is a hyperbolic growth in both A and E values. All findings from this calculation are summed up in different parts in Figure 3.47.

Part a) is the plotting graph of A and E against time. It shows that there is a hyperbolic increase in A value. Despite that, the concentration of E decreases.

Part b) is an indicator of the calculation. It demonstrates logarithmic plotting of A and E as a function of time. Graph b) indicates a hyperbolic growth in concentrations of A and a decrease in E concentration.

Part c) is the calculation's input file. It reveals the initial concentrations of A and E,

the values of the rate constants, and the length of the first phase.
Part d) contains the calculation's output file. This section contains the final concentrations of both A and E. It confirms that concentration of A is higher than E.

3.11.2 Linear growth in Motif 12

In order to observe a linear increase in Motif 12, I set the following values to rate constants: k_1, k_2, k_4 and k_5 are zero and unfunctional in this calculation. k_3=1.0D+2 and k_6=0.1D+0. I have made changes in the value of K_I: K_I=1.0D-1 and KM=1.0D-6. Initial concentrations are: A=1.000E+00; E=1.000E+03.

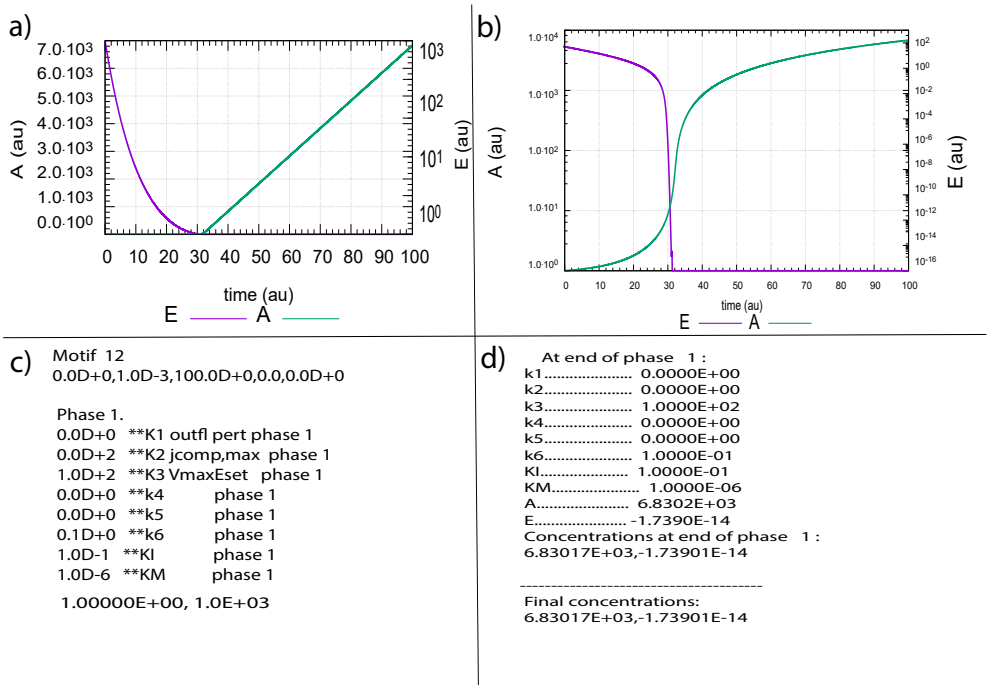

FIGURE 3.48: Hyperbolic growth followed by linear growth in A. It can be seen better n graph b) that there is a hyperbolic growth until the 30-time unit, which gives place to linear growth.

All results have been shown in Figure 3.48.
In part a), there is a linear growth in the concentration level of A and a decrease in the concentration of E. But before a linear growth, a very short hyperbolic increase occurs in A. We can see that clearly in logarithmic scale.
Part b) is the indicator graph of this implementation. It shows that shows the concentrations of A and E plotted as a logarithmic function of time. Results are the same: linear progression in the concentration of A and decrease in the level of E. Linear progression is shown as a curve in the graph. But before linear progression, we can see hyperbolic growth, which takes part in a very short time. E is inhibiting the

process; in contrast, A is activating. In the beginning, there are enough (1.000E+03) in the process and very low A concentration. k_3 is relatively high than other rate parameters. Then, in the beginning, we have the inhibition process. But because A is activating by KM, there will increase. We will get a hyperbolic increase for a relatively short time. Then there will be no inflow in E, and it will go to zero. When E goes to zero, then there is no inhibition anymore, and A increases linearly.
Initial concentration values, rate parameters values, and length of the phase are given in part c).
Part d) demonstrates that the final concentration level of A is higher than E.

3.11.3 Steady state in Motif 12

In the last part of this calculation, I want to investigate conditions under which we can get a steady-state in this calculation. For that reason I assigned following values to rate parameters: k_1=0; k_2=1.0D-1; k_3=1.0D+2; k_4=1.0D-1; k_5=0; k_6=1.0D-0. K_I and KM values are the same as in previus calculation: K_I=1.0D-1; KM=1.0D-6.
Concentrations of E and A are: 1.000E+0 and 1.000E+1 respectively.

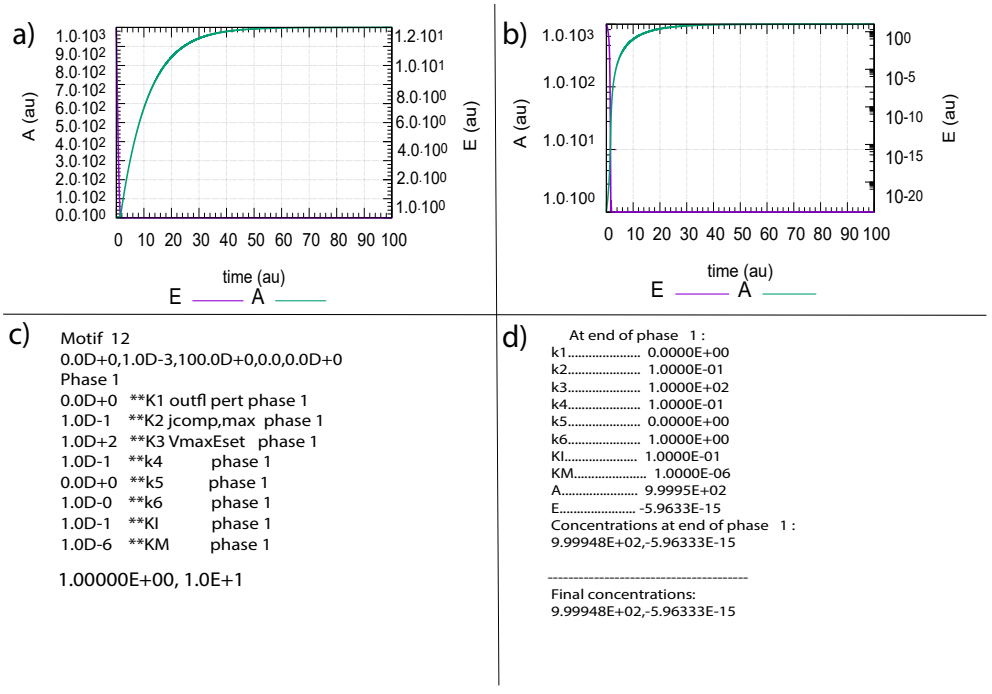

FIGURE 3.49: Steady state observed in Motif 12 program under following conditions: k_1=0; k_2=1.0D-1; k_3=1.0D+2; k_4=1.0D-1; k_5=0; k_6=1.0D-0; KI=1.0D-1; KM=1.0D-6

Results is given in Figure 3.49 and divided into four parts: Part a) shows that concentrations of A and E are plotted versus time. Both A and E have constant concentration levels. After a rapid increase, A gets into a steady-state, despite that E

achieved steady-state after decreasing process. Part b) is the logarithmic plotting of both concentrations over time and indicates a steady state. Part c) is the input file of this implementation containing all initial data. Part d) is the output file and reveals the final details of the calculation.

In conclusion, I want to summarize the results of Motif 12:
i) k_3 and KM have a unique role in getting the system to a hyperbolic growth.
ii) K_I has significant importance in leading the system to linear increase.
iii) k_2 and k_4 gets the system to a steady state.

Chapter 4

Discussion

All motifs and viral hypercyclic models in this thesis show a different type of behavior. Furthermore, their behavior varied depending on their variables' initial concentrations, rate parameter values, and phase lengths. The dynamics of all these motifs and models have been classified as hyperbolic growth, exponential growth, linear growth, and steady-state.

Motif 11 is an excellent example of exponential growth. This motif contains two activating feedback loops; when both loops are activated, the motif grows exponentially. This type of growth is a characteristic of autocatalytic reactions. Motif 11 also resulted in a linear increase in which both variable's concentrations increases by the same amount in each period. A steady-state has also been observed in this motif in constant values of rate parameters. Degradation has been seen in Motif 11 in the case of the increased value of inhibitive rate constants.

In Motif 12, hyperbolic growth is observed, and it is unique to this motif. This motif is very special because a negative feedback motif inhibits the process, but this inhibition activates the system to go to a hyperbolic growth. Hyperbolic increase in Motif 12 has be observed only when there is enough inhibitor concentration. If there is no more inhibitor that can inhibit the process:

$$i_3 = \frac{k_3 \cdot K_I}{K_I + E} \tag{4.1}$$

When the inhibitor (E) has no inflow, A grows linearly. In this motif, hyperbolic growth, linear growth, and steady-state were observed. All these states are observed only in activators' (A) concentration. Inhibitor (E) decreases to zero in all calculations.

While investigating hypercyclic viral replication models, we have found that viral replication might result in not only exponential but also hyperbolic growth. Hyperbolic growth in viral replication lasts for a very short period of time and gives place to a

saturation process. The saturation process also lasts for a while and results mostly in an exponential increase. While this saturation process occurs concentration of all virus particles and enzyme are constant for a very short time, and it calls steady state.
A steady-state is observed in hypercyclic model 1. When rate parameters that play an inhibitive role took part in the process, they lead the system to a steady-state. It means that the concentrations of negative and positive virus strands and enzyme were constant.
Linear growth was observed in Manfred Eigens' viral replication model.
Concentrations of all particles, minus, and plus-strand viruses and enzymes are in linear growth. The interesting feature of this calculation was that in linear growth, negative virus particles' concentration was the highest among other virus strands and enzymes. In contrast, in an exponential type of growth, positive virus particle concentration is higher than negative ones.

Bibliography

[1] Wikipedia contributors. Baltimore classification — Wikipedia, the free encyclopedia, 2021. URL `https://en.wikipedia.org/w/index.php?title=Baltimore_classification&oldid=1020800164`. [Online; accessed 6-May-2021].

[2] Alan J Cann. *Principles of Molecular Virology (Standard Edition)*. Academic Press, 2001.

[3] John Carter, Venetia Saunders, and Venetia A Saunders. *Virology: Principles and Applications*. John Wiley & Sons, 2007.

[4] Kathleen Boyle and Paula Traktman. Poxviruses. In *Viral Genome Replication*, pages 225–247. Springer, 2009.

[5] Craig Eugene Cameron, Matthias Götte, and Kevin Douglas Raney. *Viral Genome Replication*. Springer, 2009.

[6] Manfred Eigen, Christof K Biebricher, Michael Gebinoga, and William C Gardiner. The hypercycle. coupling of rna and protein biosynthesis in the infection cycle of an rna bacteriophage. *Biochemistry*, 30(46):11005–11018, 1991.

[7] Wikipedia contributors. Positive feedback — Wikipedia, the free encyclopedia, 2021. URL `https://en.wikipedia.org/w/index.php?title=Positive_feedback&oldid=1024012404`. [Online; accessed 25-May-2021].

[8] Olivier Cinquin and Jacques Demongeot. Roles of positive and negative feedback in biological systems. *Comptes Rendus Biologies*, 325(11):1085–1095, 2002.

[9] Krishnan Radhakrishnan and Alan C Hindmarsh. Description and use of lsode, the livermore solver for ordinary differential equations. 1993.

[10] Raphael Plasson, Axel Brandenburg, Ludovic Jullien, and Hugues Bersini. Autocatalyses. *The Journal of Physical Chemistry A*, 115(28):8073–8085, 2011.

[11] Ricard V Solé and Brian C Goodwin. *Signs of Life: How Complexity Pervades Biology*. Basic Books New York, 2000.

[12] Raphaël Plasson, Axel Brandenburg, Ludovic Jullien, and Hugues Bersini. Autocatalysis: At the root of self-replication. *Artificial life*, 17(3):219–236, 2011.

[13] Manfred Eigen. *From Strange Simplicity to Complex Familiarity: a Treatise on Matter, Information, Life and Thought*. Oxford University Press, Oxford, 2013.

[14] Manfred Eigen. Viruses: evolution, propagation, and defense. *Nutrition Reviews, Oxford University Press, UK*, 58(2):S5, 2000.

[15] Charlie Bowman. *Plant Virology*. Scientific e-Resources, 2019.

[16] Wikipedia contributors. Exponential growth — Wikipedia, the free encyclopedia, 2021. URL `https://en.wikipedia.org/w/index.php?title=Exponential_growth&oldid=1021351393`. [Online; accessed 6-May-2021].

[17] Charles Darwin. *The origin of species*. PF Collier & son New York, 1909.

[18] Manfred Eigen and Peter Schuster. A principle of natural self-organization. *Naturwissenschaften*, 64(11):541–565, 1977.

[19] Manfred Eigen and Peter Schuster. *The hypercycle: a Principle of Natural Self-Organization*. Springer Science & Business Media, 2012.

[20] Gorana Drobac, Qaiser Waheed, Behzad Heidari, and Peter Ruoff. An amplified derepression controller with multisite inhibition and positive feedback. *PloS one*, 16(3):e0241654, 2021.

[21] Wikipedia contributors. Exponential growth — Wikipedia, the free encyclopedia, 2021. URL `https://en.wikipedia.org/w/index.php?title=Exponential_growth&oldid=1021351393`. [Online; accessed 14-May-2021].