UNIVERSITY OF STAVANGER

Investigations of Motifs with Positive Feedback

by

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Abstract

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Ironically, most of people know that the positive feedback is existent mechanism applied in everywhere as well as negative feedback, However, these are unknown that the positive feedback consists of eight basic two component loop structures as motifs and that these motifs have, specific, their own circuit and represent the positive feedback by activating or inhibiting each components.

Here I report that several property of each motif and how these motifs react variously with same constant rates. In addition, these 8 motifs could be combined with each other to create comprehensive and diverse strategies responding to variations of external and internal environments. All of motifs exist in different nature and play significant roles in each areas.

Most of data come from Fortran compiling the equations and then Gnuplot was used for visualization of whole results. Besides, Python, Matlab and Celldesigner program were used to explain the characteristic of motifs diversely.

Typical traits of motifs in positive feedback are steady state, linear increase. Above these, there are also logarithmic, exponential and hyperbolic increase. However when they integrate with the others, The property of components varies depending on constant rates and location of motifs as well as being saturated or unsaturated condition.

The understanding of motifs in positive feedback would provide researchers who study pathway or system in nature with keys for opening the door of undiscovered parts.

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Chapter 1

Introduction

1.1 Positive feedback

Positive feedback is literally system which make a feedback loop to exacerbates the effects of elements[11]. As well as negative feedback, this concept play a significant role everywhere. Feedback loops are therefore the process whereby a change to the system results in an alarm which will trigger a certain result[12]. This result will either increase the change to the system or reduce it to bring the system back to normal. A few question remain: where do we find these systems? what do they do? Many of examples of positive feedback can be found in biology such as onset of contractions in childbirth or process of blood clotting, lactation, estrogen, generation of nerve signals and so on[13, 14]. These plenty of biological examples represent that positive feedback is one of important axis for living organisms to maintain homeostasis[12]. In spite of this importance, some of people do not distingsh this system consists of eight different elementary loop structure as Figure 1.1. Hence, when scientists investigate biochemical pathway, they do not minutely subdivide in detail.

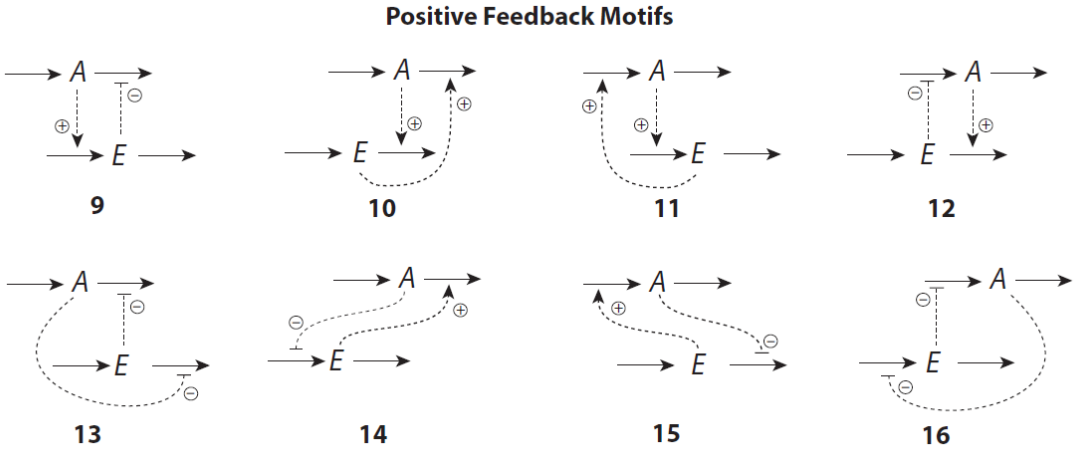


Figure 1.1: Basic set of eight positive feedback motifs.

So why should we know about motifs in detail? To be clear, all of motifs in positive feedback are somewhat distinguished with the others, because they have their own loops and react differently with even same constant rates. All of motifs are readily found when we inverstigate natural phenomena. Hence, If researchers overlook this importance of distinction, it is high potential to miss important elements in their thesis or studies. For these reasons, I will describe how I researched motifs and I am going to represent differences through results.

In the same context, the aim of this thesis is the following. To begin with, I will show how each motifs in positive feedback behave either similarly or differently with same constant rates. Besides, I represent that all of motifs exist in biological environment and that motifs react properly to suit each conditions. Third, a motif can have a different reaction when combined with the same or other motifs. Finally, motifs react differently due to being saturated or unsaturated.

In general, most of the motifs show steady states, linear increase. When degradation rates are zero, each motifs represent linear growth. If not, they become steady state. However, in special case, each motifs have their characteristic trait including exponential , hyperbolic and logarithmic growth.

Unlike linear growth, logarithmic growth is curved but does not become steady state. It increases constantly with curved state. In case of hyperbolic growth, It reaches infinity at specific times. On the other hands, exponential seems to similar hyperbolic but it grows as function of time. If log scale is applied to exponential growth, it is converted to linear growth as F in Figure 1.2. Conditions for making logarithmic, exponential and hyperbolic growth will be

described in Results.

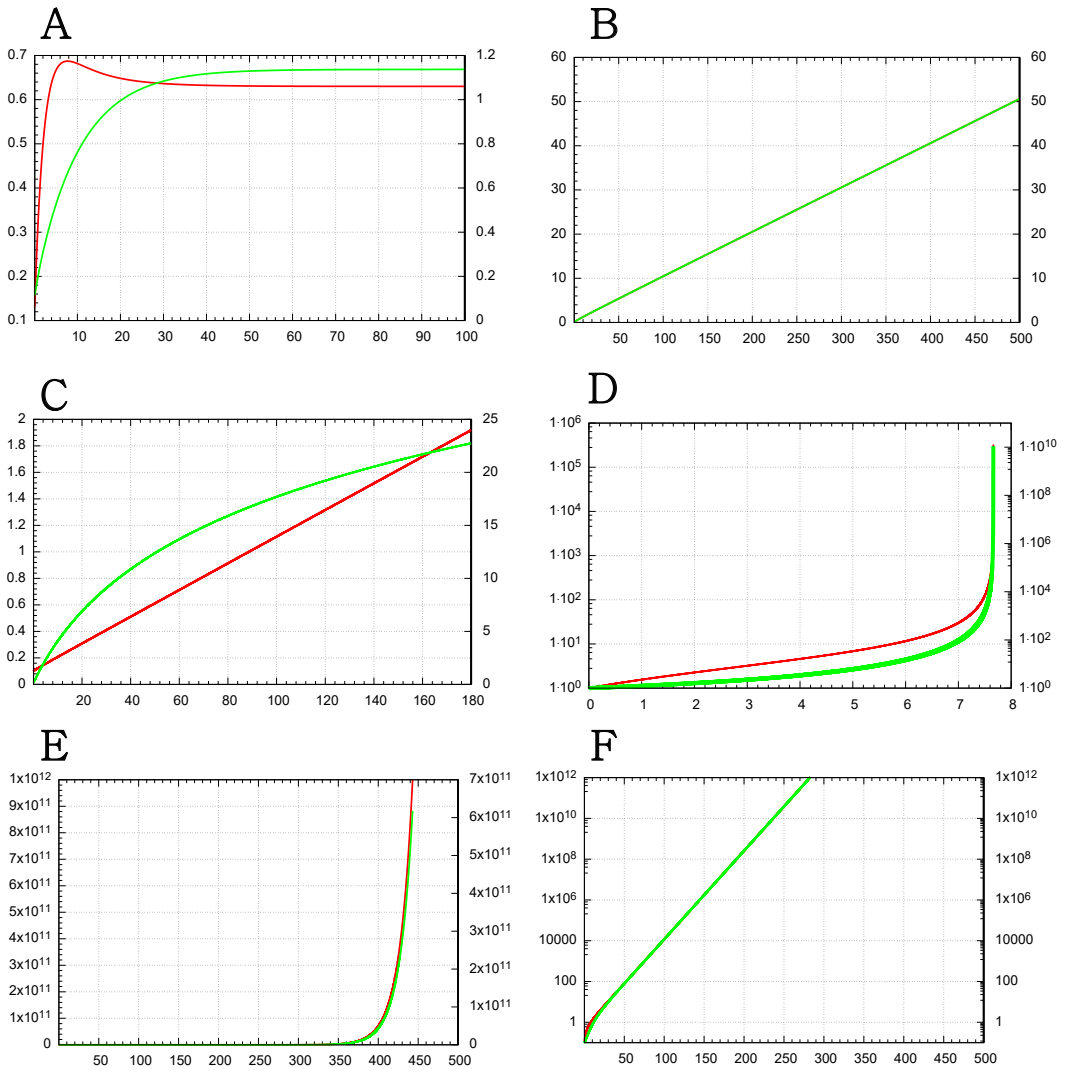


Figure 1.2: Various behaviours of motifs in positive feedback loops. (A) steady state (B) linear growth (C) logarithmic and linear growth (D) hyperbolic growth (E) exponential growth with linear scale (F) exponential growth with log scale.

Such research should not be limited in theory, but requires the comparison with real natural examples. In plants, there is positive feedback as motif 16, which two component inhibit each other. JAZ and DELLA are used when plant modulates growth and defense depending on external situations[15]. I will show how JAZ and DELLA change by applying various factors and then compare graphs between JAZ-DELLA and motif 16 to search common features and differences.

In terms of motif 15, I will describe the conversion from negative feedback to positive feedback in cell progression pathways. This transition occurs when cells are under stress

by adding just one element like miR-605 in negative feedback loops, p53 and MDM2[16]. Among various traits of motifs, steady state accounts for most parts. In motif 13, the conditions of steady state will be enumerated by calculation with Matlab.

In motif 12, I will not only define what hyperbolic growth and infinity time are but also reveal which factors influence these characteristics. In case of bacterial infection cycle, it proves that how motif 11 could trigger hyperbolic growth at specific condition, which make advantage for bacterial to survive and grow up in host[17]. Motif 11 also plays important role in blood clotting. Through comparison between presence and absence of motif 11 loops, the significance of positive feedback would be identified. Usually, motifs in positive feedback are linked with the other motifs including negative feedback. When it comes to mitotic entry cycle, Wee1-Cdk1-Cdc25 system made of motif 10 and motif 13 for bistable switch of mitosis[18].

In positive feedback, motifs can be divided by convertible and non-convertible motif.

Table 1.1: Convertible and Non-convertible motifs.

Table: convert and non-convert motifs				
convert	motif 9 <–>motif 15			
	motif 12 <–>motif 14			
non-convert	motif 10	motif 11	motif 13	motif 16

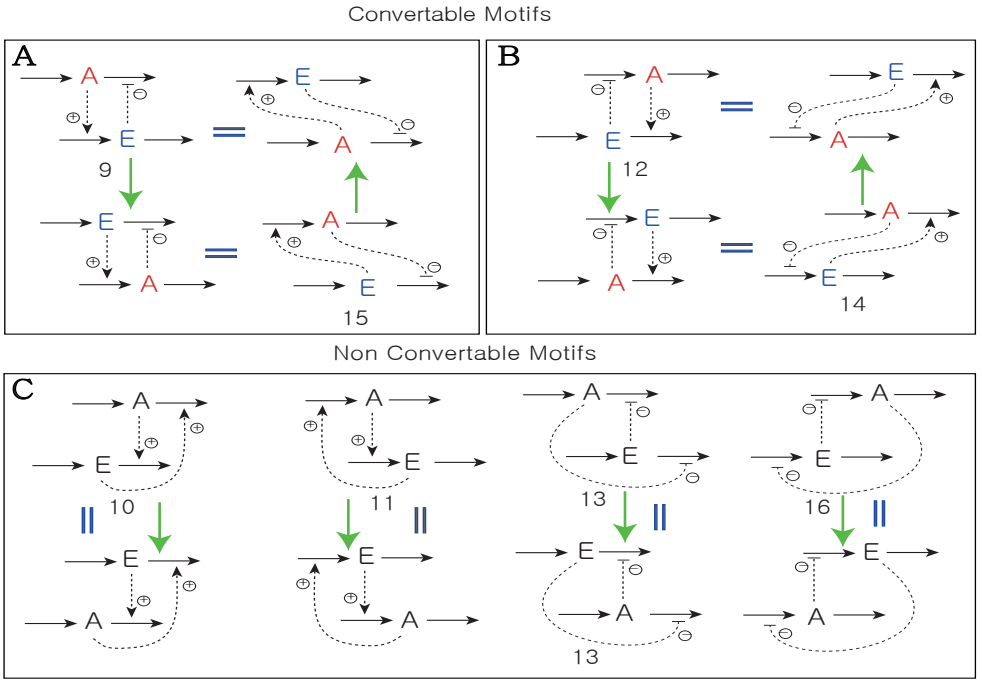


Figure 1.3: Converted and non converted motifs (A) Motif 9 and motif 15 can be transformed by substituting A and E. (B) Motif 12 and motif 14 also are able to switch eachother. (C) Motif 10, motif 11, motif 13, motif 16 are non convertable. Green arrow represents substitution of E and A and equal mark show the equal between two motifs.

By substituting components, A and E, motif 9 with motif 15, motif 12 with motif 14 can be transformed. In contrary to this, motif 10, motif 11, motif 13 and motif 16 are non-convertible as confirmed in the FIgure 1.3.

In addition to analyze individual motif, comparing each motif would be good method to find out indigenous trait of each motif. In doing so, similar logarithmic or linear growth and steady state pattern in each motif obviously could be discriminated.
In nature system, some of motifs are linked with the other motifs. They collaborate the other motifs or form double motifs to adapt the environmental changes[19–21]. By simulating these combined motifs in positive feedback, I can confirm that how nature develops strategies to survive and shed light on complexity of the life systems.
When components in motifs affect counterpart, they could be either saturated or unsaturated with activation rate. This small changes, sometime, trigger massive variation between them. This study will show difference not only single motifs but also combined motifs.
Since the expression in silico was used, there are many of in silico studies implemented in various fields of biology. The development of modeling and technique in silico help not only reducing cost and time but also understanding mechanism and system of nature more accurately. This research of motifs in positive feedback with computational approaches clearly will display traits and behavior of nature which experiments in vivo or in vitro could not prove. I used FORTRAN, Python, Matlab, Celldesigner programs to simulate and to predict consequence depending on several values. this research can be useful to understand positive feedback as well as homeostasis[22].

Chapter 2

Materials and Methods

Whole models for simulation were implemented in Fortran, Python, Matlab and Celldesigner. To enhance reliability of simulations, some models which had been not compiled by Fortran were implemented by Fortran and are shown in appendix.

2.1 Materials

Computations were performed by using the Fortran subroutine LSODE in conjunction with Absoft's Pro Fortran compiler (www.absoft.com). The graphs were generated with gnuplot (www.gnuplot.info) and Adobe Illustrator (www.adobe.com). To make notations simpler, concentrations of compounds are denoted by compound names without square brackets. Rate parameters are presented as k_i's (i=1,2,3) irrespective of their kinetic nature, i.e. whether they represent turnover numbers, Michaelis constants, or inhibition constants. I used the doubling time as an indicator of how fast a certain feedback arrangement could grow. In addition, python script which I scripted are used for seeking out relevant constant rate in short time (https://github.com/jaewoong-87/python). Simbiology in MATLAB and Celldesigner programs are used to visualize and simulate some pathways simultaneously.

2.2 Methods

2.2.1 Fortran

First of all, whole programs mentioned above, should be installed exactly. Some of them, specially Perl, require designation of environmental path. If not, proper compiling

could be impossible.

In Fortran subroutine LSODE, there are some important syntax that help operation[23]. In Figure 2.1, 1) FILE="A2B.INP" mean that open the A2B input file which include information about constant rates and time, initial concentration of components. READ(5, *) implements reading operation.

2) This syntax make files which have extension of text files and temporary file.

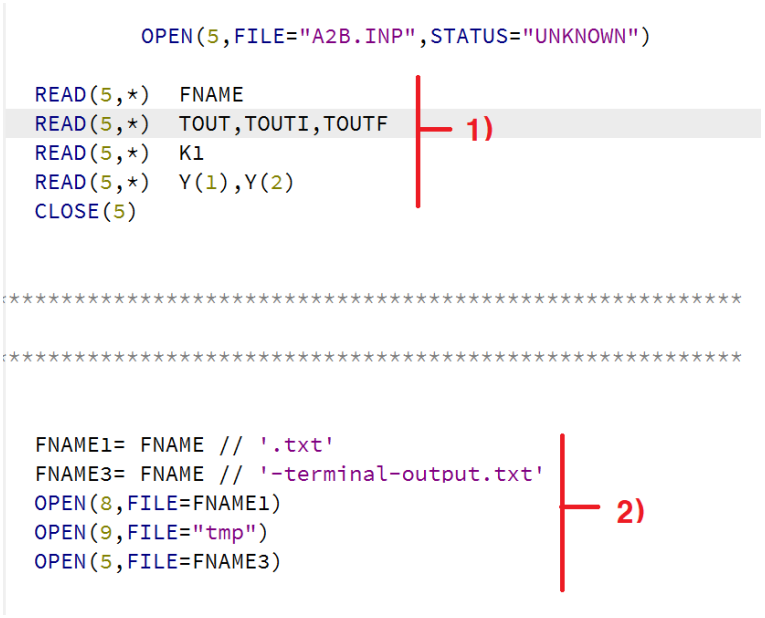


Figure 2.1: Open input file and additional files. 1)Read contents of Input file and 2)make file containing two text files and a tmp file.

In input file, first line is run-id which present name of representative experiment with different constant rates. As Figure 2.2, input file has information which Fortran file use for calculation.

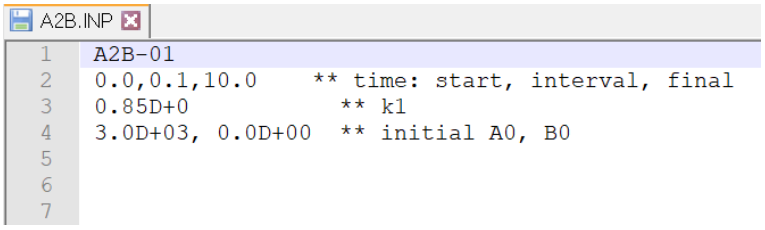


Figure 2.2: Input file It contains runtime name and time for start, interval, final and rate constant, initial concentration of components.

In Fortran file as Figure 2.3, 1) there are some syntax which modulate the equation Y(1), Y(2) refer to specific component concentration and these could calculation with parameters derived from input file.

```
SUBROUTINE FEX (NEQ, T, Y, YDOT)
    IMPLICIT NONE
DOUBLE PRECISION  T, Y, YDOT
DOUBLE PRECISION  K1

    INTEGER NEQ

DIMENSION Y(7),YDOT(7)
COMMON /RATCON/ K1

 YDOT(1)=-K1*Y(1)
 YDOT(2)=K1*Y(1)

  RETURN
  END
```

1)

Figure 2.3: Fortran equation syntax In fortran file, the equation for calculation could be modified.

As Figure 2.4, this diagram depicts how these process proceed. A2B.f create a2b.exe (executable file). But when a2b.exe is carried out, it just calculates equation in Fortran. Thus, to visualize results by graphs, we need to execute a2b.cmd file.

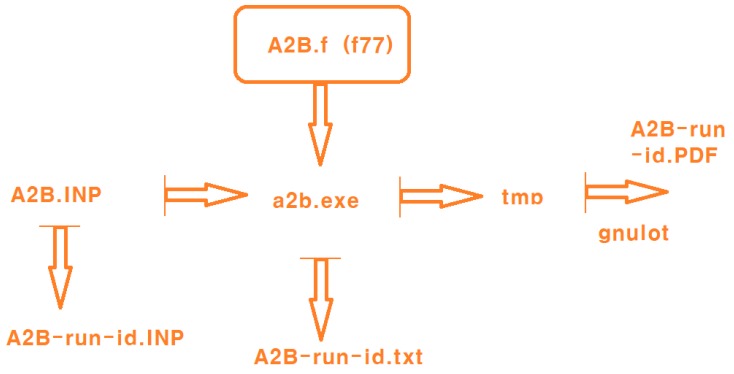


Figure 2.4: cmd:syntax This diagram presents how files are associated to plot graphs.

The cmd.file include

1) syntax for operating executable file made from compiling like Figure 2.5.

2) read input file by copy-input.perl file.

3) graph-AB.pl is used for making graphs.

4) syntax for implementing pdf file.

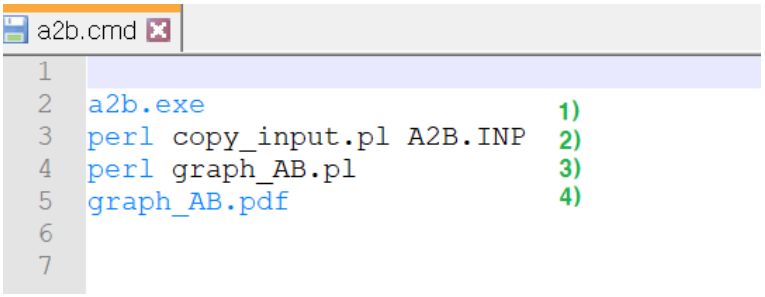


Figure 2.5: cmd file In command file, several commands run for each purpose. 1)executable file, 2)perl file for extracting information from input file, 3)plot graph, 4)make pdf file.

The copy-input Perl file can read A2B.INP and makes INP file which has run id title.

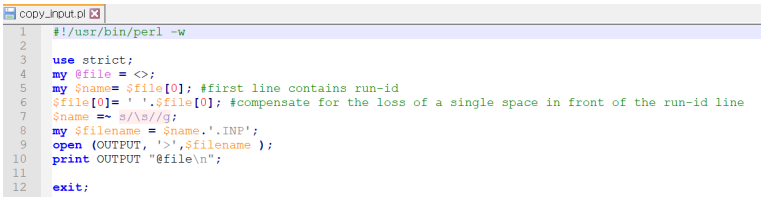


Figure 2.6: Copy input perl extract run-id and make new file.

The graphAP.pl is used for making plot by Gnuplot. It reads data file which have calculated values and call Gnuplot program. There are the commands that set font size and format of graph and that set output as pdf file with various shape of graph such as boxes, lines, dot and so on.

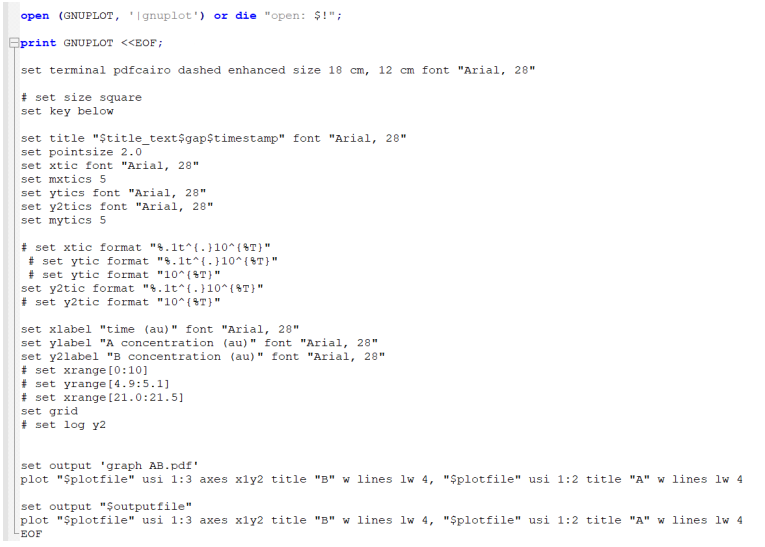


Figure 2.7: graphAP. This perl file reads data file and plots the graph.

To create executable file by Fortran, open the development command prompt (in my case, 64bit) and type in “f77 -o a2b -m64 -O2 A2B.f vms.lib” to make a2b.exe.

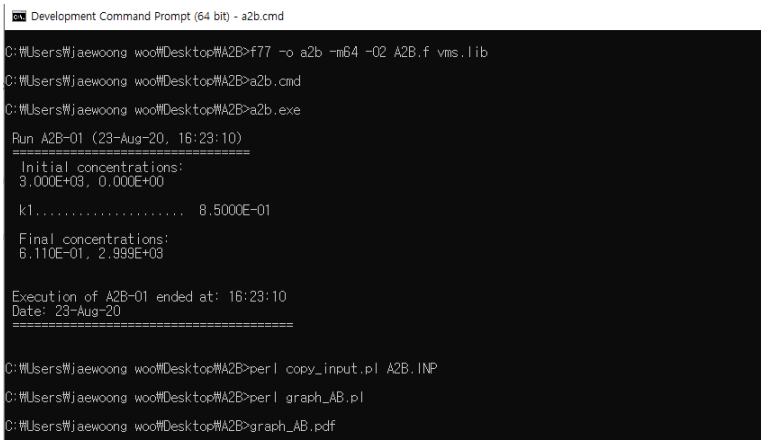


Figure 2.8: The command promt-cmd.

If all of process work without any problem, just type in a2b.cmd for indentify results.

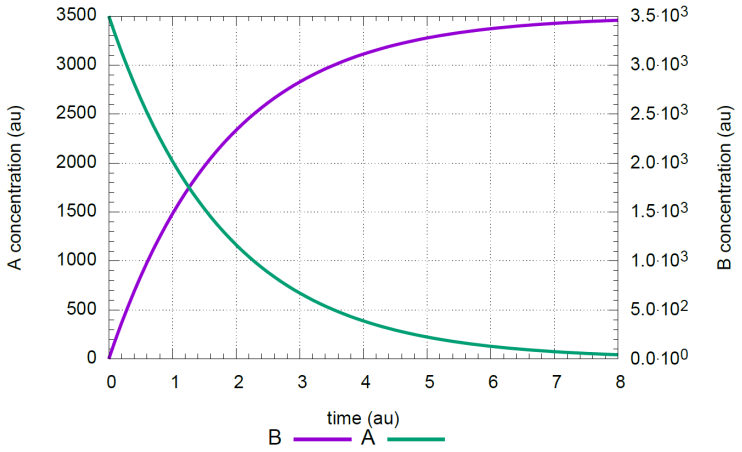
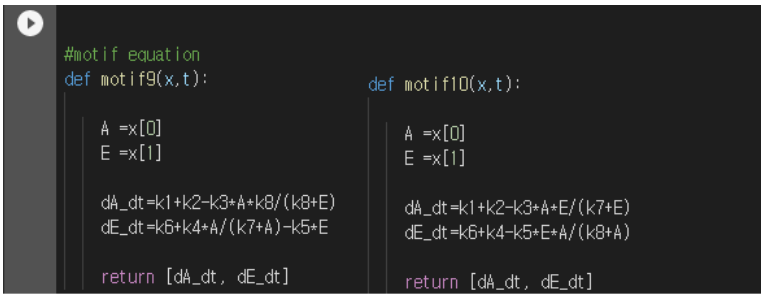


Figure 2.9: Graphic result by gnuplot.

The name, a2b, is arbitrary it mean you can change the name corresponding to purpose of projects. Sometimes, this method could be hard and recursive.

2.2.2 Python

For the sake of simplicity, I scripted Python code for ODE calculation and uploaded it in Git hub.

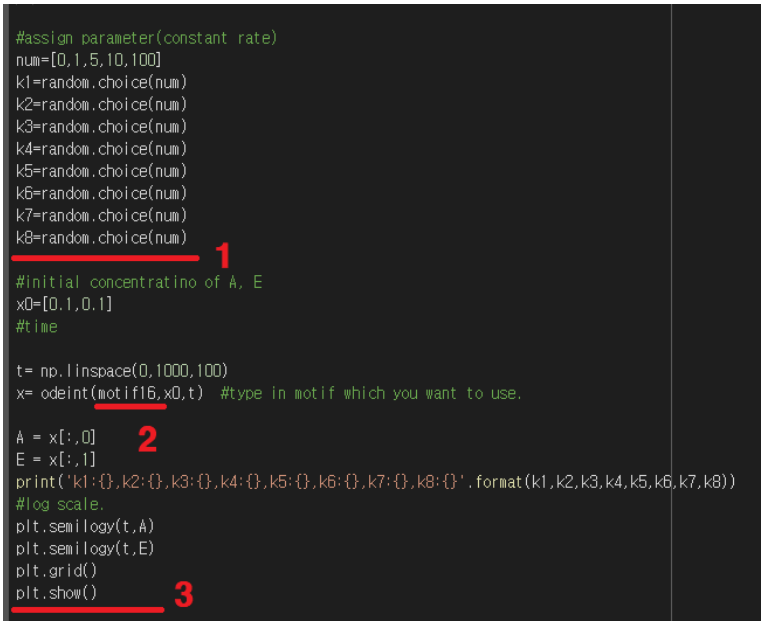


```
#motif equation
def motif9(x,t):

    A =x[0]
    E =x[1]

    dA_dt=k1+k2-k3*A*k8/(k8+E)
    dE_dt=k6+k4*A/(k7+A)-k5*E

    return [dA_dt, dE_dt]
```

```
def motif10(x,t):

    A =x[0]
    E =x[1]

    dA_dt=k1+k2-k3*A*E/(k7+E)
    dE_dt=k6+k4-k5*E*A/(k8+A)

    return [dA_dt, dE_dt]
```

Figure 2.10: Functions of motifs in positive feedback. Python script includes functions for calculation of each motifs.

These motifs functions could be used by selection.



```
#assign parameter(constant rate)
num=[0,1,5,10,100]
k1=random.choice(num)
k2=random.choice(num)
k3=random.choice(num)
k4=random.choice(num)
k5=random.choice(num)
k6=random.choice(num)
k7=random.choice(num)
k8=random.choice(num)
```

1

```
#initial concentratino of A, E
x0=[0.1,0.1]
#time

t= np.linspace(0,1000,100)
x= odeint(motif16,x0,t)  #type in motif which you want to use.

A = x[:,0]
E = x[:,1]
print('k1:{},k2:{},k3:{},k4:{},k5:{},k6:{},k7:{},k8:{}'.format(k1,k2,k3,k4,k5,k6,k7,k8))
#log scale.
plt.semilogy(t,A)
plt.semilogy(t,E)
plt.grid()
plt.show()
```

2

3

Figure 2.11: Script that include constant rate and time, module to calculate motifs. 1) It has rate constant which occur randomly among numbers in num list. 2) Any motif can be included by typing in. 3) The graph appear by this command.

To assign value of parameters, variable [num] is used, which include constant rates. Random.choice script choice one of value in num list. t is time and odeint script help to calculate with motif function and initial concentration and time. Plotting is made by plt.semilogy(t,A) and (t,E),which scale is log. But it can be changed depending on case.

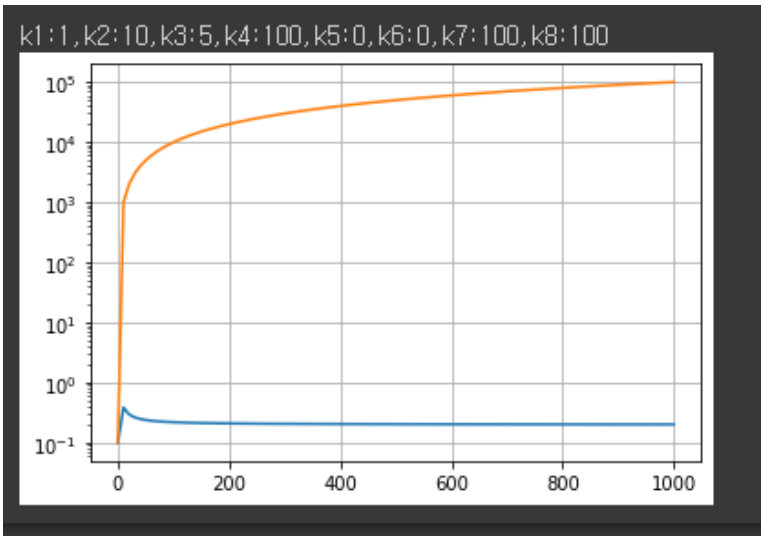


Figure 2.12: Graph that plotted by constant rates.

After clicking the compile, graph is shown with constant rates used for plotting. This script could help to figure out how motifs act at specific constant rates within ranges set by users with just one clicking.

2.2.3 Matlab

I used Simbiology application in MATLAB to simulate the motif 15 loops of p53 and MDM2.

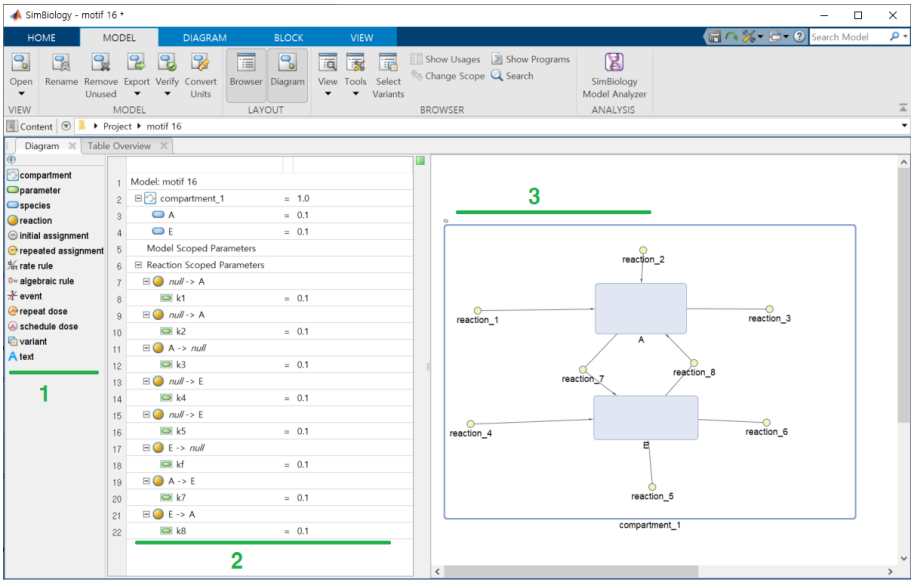


Figure 2.13: Simbiology application in MATLAB.

1) This function allows users to readily use Simbiology by drag and draw. 2) This table presents rate constant and initial concentration. 3) After drag and draw elements from 1), user can modify diagram by connecting each elements.

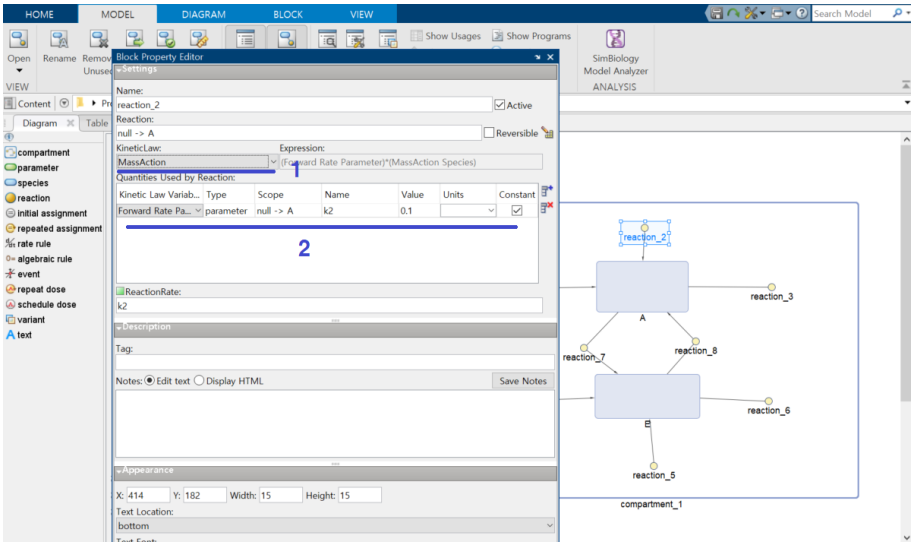


Figure 2.14: Modulation of rate constant and initial concentration and equation in Simbiology.

By double clicking, several values of each elements can be adjusted. 1) Basic kinetic laws are installed so that user should choose proper kinetic law. If there is no appropriate kinetic law, users could make their own kinetic laws. 2) After choosing kinetic laws, parameter should be typed in without blank.

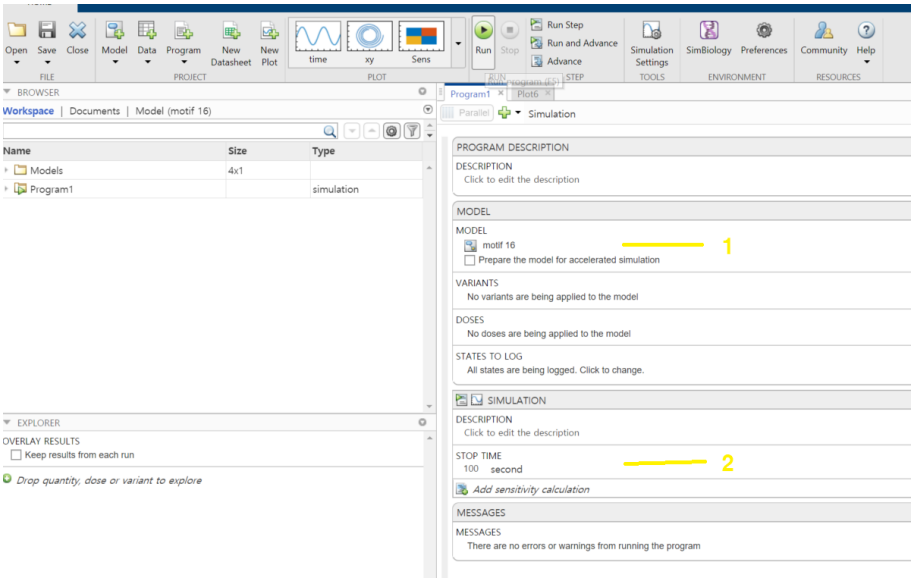


Figure 2.15: The selection of ODE and model

1)User should select one model which want to simulate. In this function, there is another option to choose ODE types. 2) Finish time could be modified.

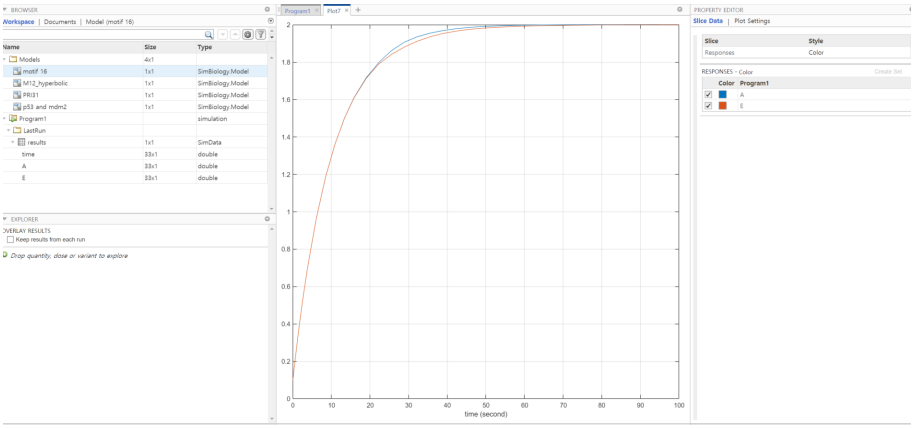


Figure 2.16: The plotting by simulated model in MATLAB.

If there are no errors in diagram and equations, plotting from chosen model appears with legends.

2.2.4 CellDesigner

CellDesigner allows users to draw a diagram which based on the process with graphical notation. this program also has ability to convert between SBGN(System Biology Graphical Notation) and SBML(Systems Biology Markup Language) so that user can readily not only identify pathway with visual diagram but also transfer or integration the information of model by SBML format[24].

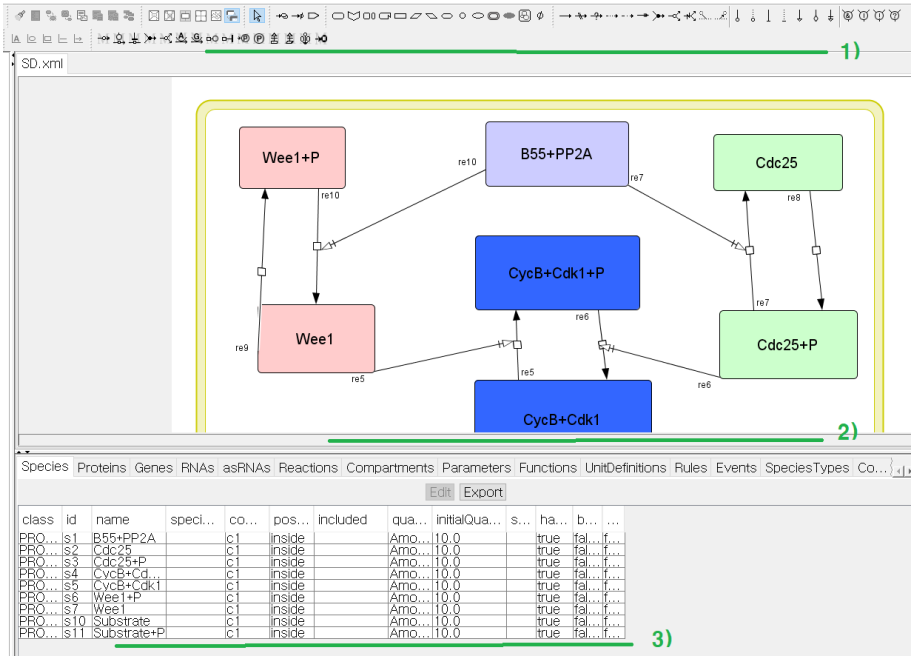


Figure 2.17: Diagram drawn by Celldesigner. (1) Cell designer has many tools on panel to draw diagrams. User can choose not only protein, gene ect but also various reactions like catalysis, trigger, activation or inhibition. (2) Each elements are placed and linked. (3) All elements and reactions are adjusted for exact experiment.

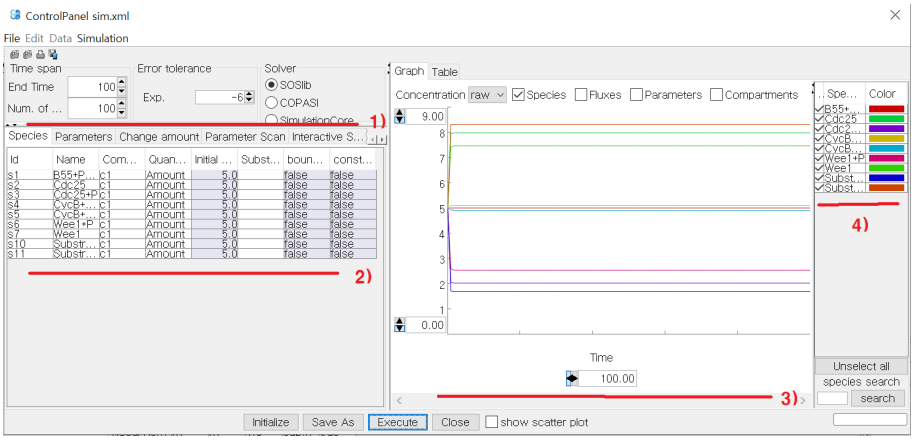


Figure 2.18: The results from simulation of program.(1)Time and error tolerance, solver for simulation could be chosen by user. (2) Parameters for simulation are shown and could be modified. (3) Graph of result appear. X axis as time and Y axis could be adjusted. (4) By color, user can identify several elements. Omitting specific lists is possible by not ticking.

Chapter 3

Results: Analysis of Motifs

3.1 Motif 16

In motif 16, two elements inhibit the flux of input of counterparts. E inhibits influx of A, which is k_2 and A inhibits influx of E as k_4. Due to this trait, some of people can not think that this show positive feedback, However, this motif presents exclusive increase.

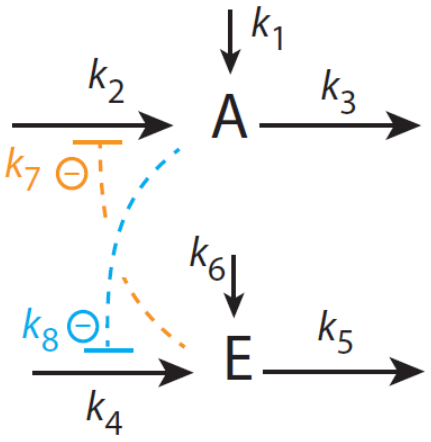


Figure 3.1: The circuit of motif 16

Through this circuit of motif 16, we can deduce the equation which respect to variation of both A and E as below.

$$\dot{A} = k_1 + k_2 \left(\frac{k_7}{k_7 + E} \right) - k_3 \cdot A \tag{3.1}$$

$$\dot{E} = k_6 + k_4 \left(\frac{k_8}{k_8 + A} \right) - k_5 \cdot E \tag{3.2}$$

There are some behaviors of motif 16 like steady state and linear growth. Among them, special trait of motif 16 is logarithmic growth. I will explain when logarithmic growth occurs after the introduction of doubling time.

3.1.1 Introduction of Doubling time

The concept of doubling time is simply that the identified time it takes for concentration to double. For the sake of understanding, there is the figure regarding to doubling time as below.

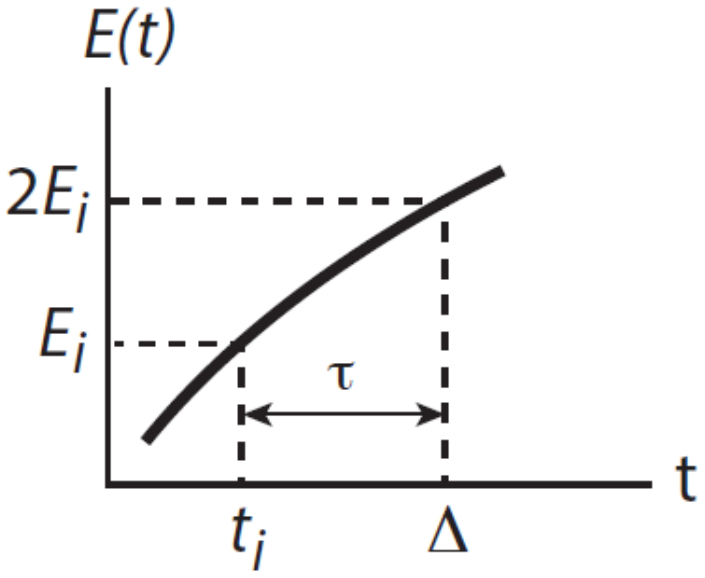


Figure 3.2: Diagram presenting the definition of doubling time. Doubling time τ is taken time when E_i double.

In a nutshell, doubling time refers to the time it takes for the initial concentration to double. Since, mathematical calcuation require a comprehensive explanation, I will explain them together with the Figure 3.3 below.

3.1.2 Various concentration changes and doubling time in motif 16

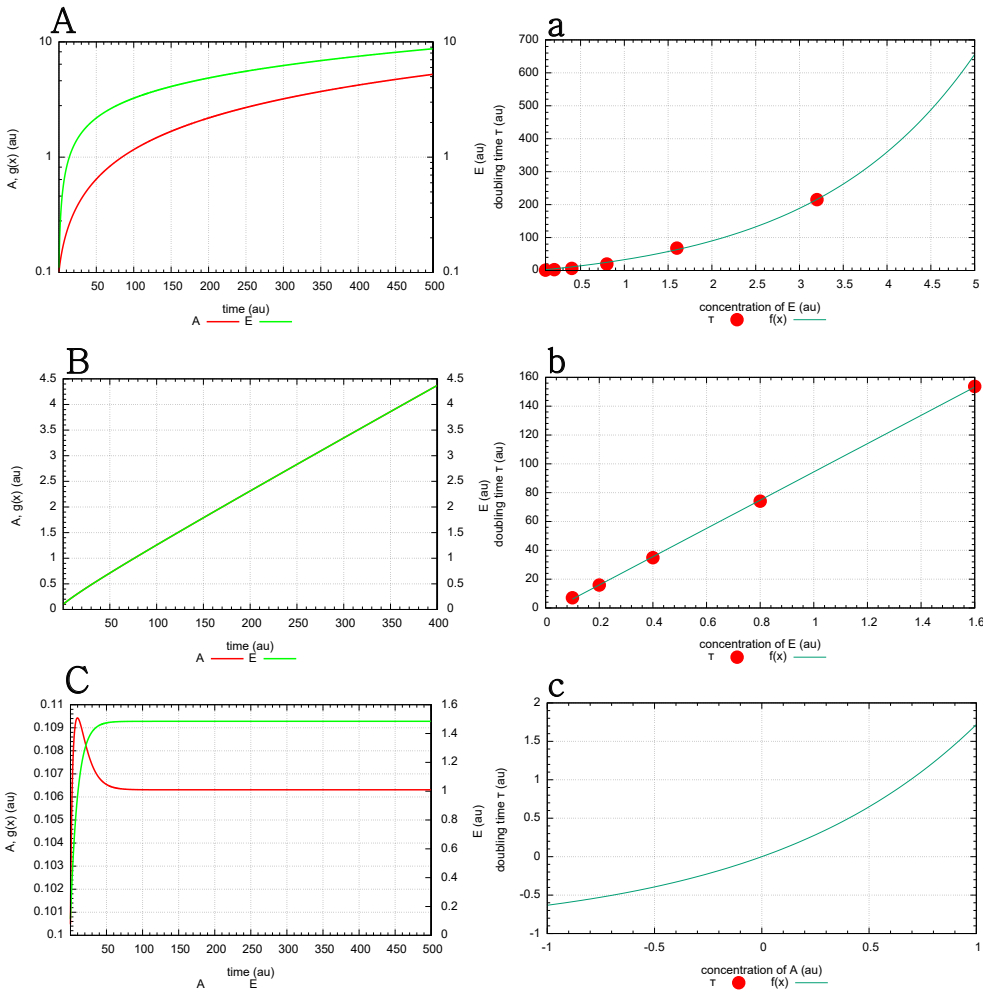


Figure 3.3: Various graphs of motif 16 and doubling time regarding to E. A) logarithmic growth occurs when k_1, k_2, k_6 are 0.01 and k_3, k_5 are zero while k_7, k_8 are 0.1 and k_4 is 1.0. The logarithmic scale is used only here to represent logarithmic growth. a) Doubling time of A increase exponentially. B) Linear growth when k_1, k_2 are 0.01 and k_3, k_5, k_6 are zero while, k_4 are 1.0. k_7, k_8 are 0.1. b)Doubling time of B increase linearly. C) Steady state when rate constants k_3, k_4, k_5, k_6, k_7, k_8 are 0.1 except k_1, k_2 are 0.01. c) Doubling time of C can not be measured due to transient increase. In doubling time graphs, f(x) is numerical E as fucntion of time, red dot T is analytical E with time. The initial concentration of A and E in all graph are 0.1.

In case of component E of graph (A) in Figure 3.3, when degradation rate is zero and k_4 is relatively higher than k_1 and k_2, logarithmic growth appears and doubling time of E represents exponential increase.

I describe doubling time calculation E In (a) of Figure 3.3. In this run, degradation rate, k_3,k_5 are zero, while k_1, k_2, k_6 are 0.01 and k_4 is 1.0, k_7,k_8 are 0.1. initial concentration

of A and E are zero. When degradation rate are zero, A and E increase as function of time but no steady state. Since k_4 is 2-orders of magnitude lager than k_1 and k_2, E grows initially more rapidly than A, so $k_2\left(\frac{k_7}{k_7+E}\right)$ is less than $k_4\left(\frac{k_8}{k_8+A}\right)$, thus A increases linearly.

$$A(t) = k_1 \cdot t \tag{3.3}$$

With k_5 is zero, the rate equation of E becomes as below.

$$\dot{E} = k_6 + k_4\left(\frac{k_8}{k_8 + A}\right) \tag{3.4}$$

After integral by Matlab, I got the E(t) as below.

$$E(t) = k_6 \cdot t + \frac{k_4 \cdot k_8 log(k_8 + k_1 \cdot t)}{k_1} \tag{3.5}$$

This equation will be used for curve-fit of the numerical E-t data. However, in case of analytical E-t data, it was difficult to calculate it because of complexity. if k_6 was zero, complexity of calculation somewhat get eased.

When initial concentration and rate constants used for graph (a) in 3.3, numerical function for this graph is as below.

$$f(x) = 44.883 \cdot (e^{(0.549607 \cdot x)} - 1) \tag{3.6}$$

In this case, f(x) stands for doubling time for E.

3.1.3 Regulation of growth and defence in plants with motif 16

In plants, the regulation of growth and defense is the one of important things in terms of energy efficiency. There are many proteins responsible for this regulation. Among them, JAZ and DELLA play significant role[1].

As Figure 3.4, JAZ regulates the defense of plant so that when the amount of JAZ increase, it inhibits MYC, which represses genes for defense. The other way, JAZ is regulated by JA(Jasmonic acid)[2]. In case of JA, it combines protein complex including JAZ, COI1, Fbox, ASK1 which lead the degradation of JAZ. JAZ-DELLA not only are repressed by JA and GA(Gibberellic acid) but also inhibit each other. That's why JA and GA affect on JAZ and DELLA oppositely. They represent positive feedback as motif 16[15]. Hence, when JA increases by various external stresses, amount of JAZ reduced, which allow not only MYC2 to transcript JA responsive genes but also DELLA represses GA responsive gene by combining with PIF for transcription. In case of DELLA, it inhibits PIF, which presses genes for growth[3]. When the amount of GA increase, GA

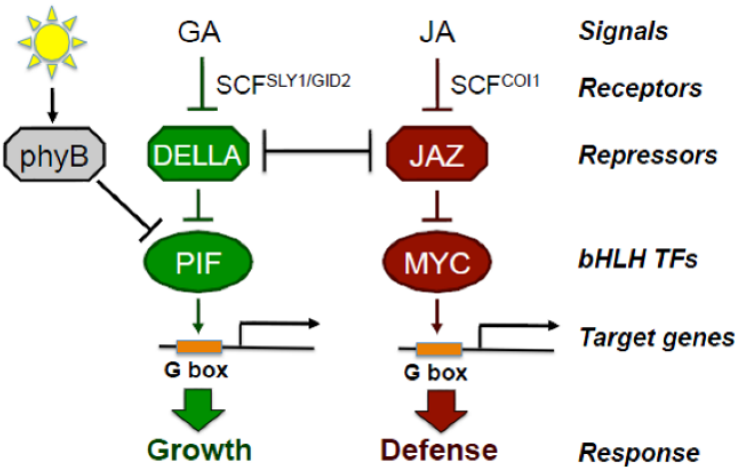


Figure 3.4: The network of JAZ and DELLA. GA and JA are signals which make DELLA and JAZ degraded. DELLA and JAZ not only inhibit each other but also repress PIF and MYC which are responsible for Growth and Defense in plant [1].

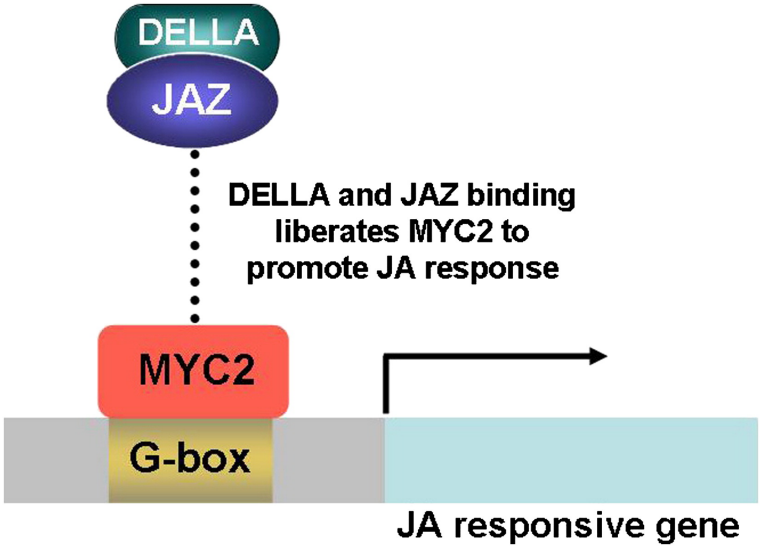


Figure 3.5: The role of JAZ. DELLA inhibit JAZ by binding together so that liberated MYC2 can succeed in promoting transcription of JA responsive gene[2].

also make protein complex and then DELLA becomes degraded. As a results, PIF can start transcription for growth. In addition, JAZ can hold role of MYC to inhibit JA responsive gene transcription.

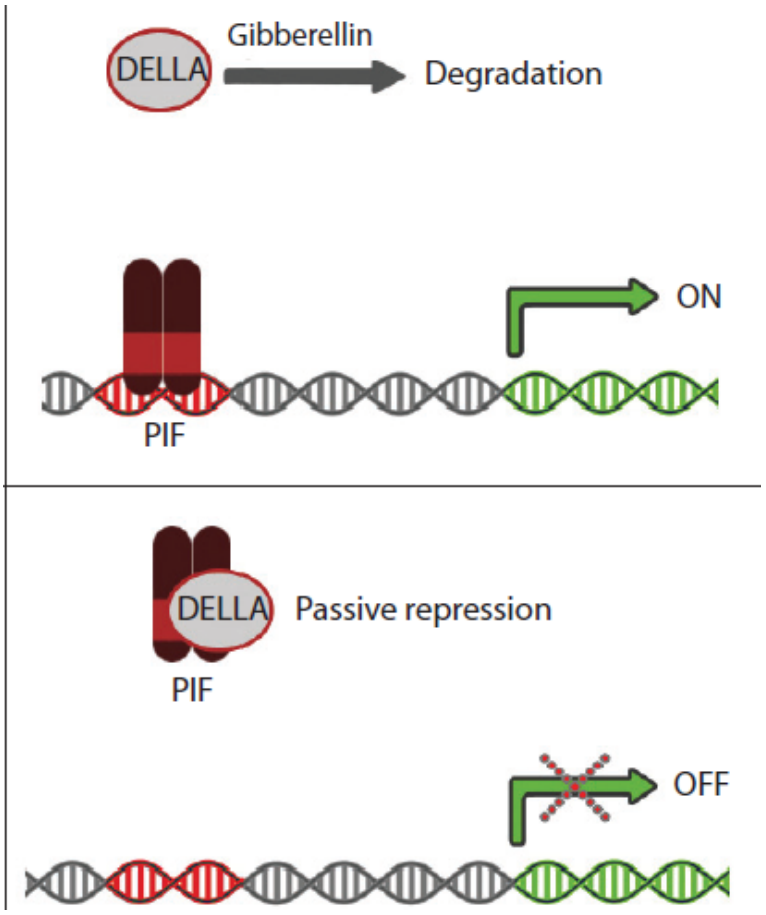


Figure 3.6: The role of DELLA. DELLA suppress PIF but when Ga or JAZ increase, DELLA separate with PIF thus, PIF can transcript of GA responsive genes[3].

By comparing with simulated both JAZ-DELLA and motif 16, We can identify that motif 16 works in biological environment not just in theory. The scheme of model JAZ1 which is for regulation of growth and defense in plants is as below.

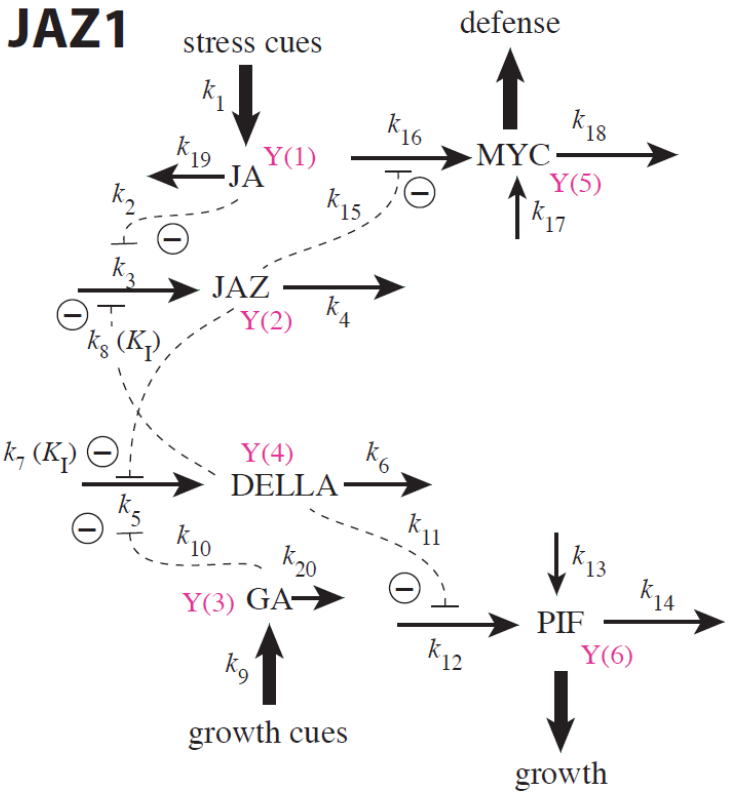


Figure 3.7: The circuit of JAZ1. Stress cues and growth cues are presented as k_1 and k_9 respectively. JA inhibits JAZ and JAZ suppresses not only MYC but also DELLA. GA inhibits DELLA and DELLA inhibit JAZ and PIF.

With this scheme, we can make formulas regarding to JAZ , DELLA, GA, JA, MYC and PIF as below.

$$\dot{JA} = k_1 - k_{19} \cdot JA \tag{3.7}$$

$$\dot{GA} = k_9 - k_{20} \cdot GA \tag{3.8}$$

$$\dot{JAZ} = \frac{k_2 \cdot k_8 \cdot k_3}{(K_2 + (JA))\,(k_8 + (DELLA))} - K_4 \cdot JAZ \tag{3.9}$$

$$\dot{DELLA} = \frac{k_5 \cdot k_7 \cdot k_{10}}{(K_7 + (JAZ))\,(k_{10} + (GA))} - K_6 \cdot DELLA \tag{3.10}$$

$$\dot{MYC} = k_{17} + k_{16} \cdot \frac{k_{15}}{k_{15} + (JAZ)} - k_{18} \cdot MYC \tag{3.11}$$

$$\dot{PIF} = K_{13} + K_{12} \cdot \frac{K_{11}}{K_{11} + (DELLA)} - K_{14} \cdot PIF \tag{3.12}$$

Under the various conditions, JAZ-DELLA shows some specific traits as motif 16.

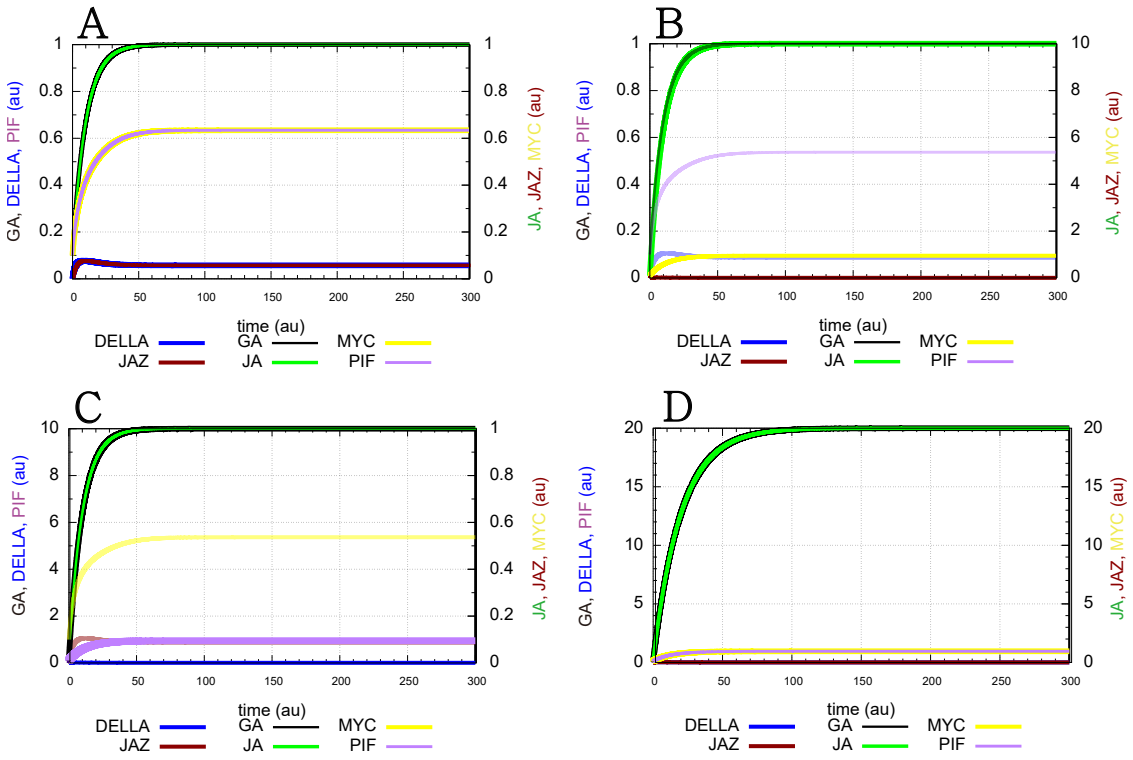


Figure 3.8: The outcome of JAZ-DELLA. (A)Control graph. (B)show result when stress cue increase. JA increase 10 time instead, JAZ extremely decrease so that MYC is liberated from JAZ thus, MYC increases too. (C)presents when growth cue increase. Oppositely, In this case, GA increase and DELLA decrease. As a result, PIF increase. (D)show When both growth and stress cue increase simultaneously. Both JA and GA increase so that JAZ and DELLA decrease. The initial concentration of all components are 0.1. Rate constant are given in Table 3.1.

Table 3.1: Rate constants for Figure 3.8. All of rate constants are 0.1. Red mark depicts 1.0 and yellow mark presents zero. k2∼k8 means that from k2 to k8, all of rate constants are same as 0.1.

Graph	k1	k2 ∼k8	k9	k10	k11	k12	k13	k14	k15	k16	k17	k18	k19	k20
A	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1
B	1.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1
C	0.1	0.1	1.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1
D	1.0	0.1	1.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1

In Figure 3.8, input rate constants for PIF and MYC, k_{13} and k_{17}, are set by zero because the values of k_{13} and k_{17} can be obstacle for observing the consequence of stress

and growth cues.
Graph A were used for control group to compare results with specific cues. When stress cue increase, JA increases more than 10 time thus, JAZ is repressed. As a result, MYC increase. On the other hand, if growth cue increase, GA increases hence, DELLA is inhibited. Therefore, PIF inhibited by DELLA can increase. When both stress and growth cue increase, JAZ and DELLA are suppressed and then MYC and PIF can increase. In this network, JAZ and DELLA are linked so that stress cue and growth cue affect not only their subunit but also that of counterparts such as PIF, MYC.
Through this experiment, we can identify that motif 16 exists in regulation system in arabidopsis plant and modulates for efficient usage of physiological energy depending on various stimuli like stress.

3.2 Motif 15

In motif 15, two elements behave differently. E activates flux of input of A, while A inhibits the degradation of E.

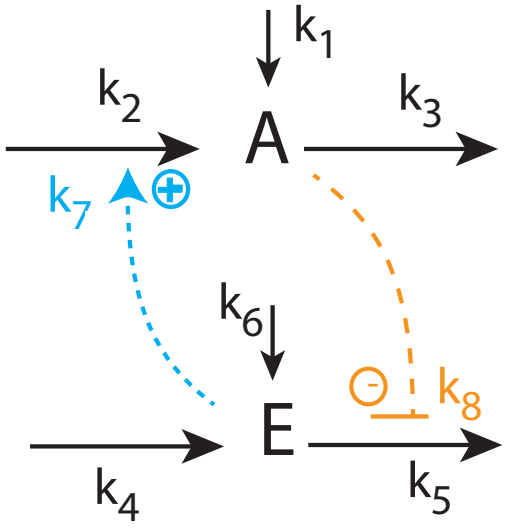


Figure 3.9: The circuit of motif 15.

By circuit of motif 15, we can deduce equations as below.

$$\dot{A} = k_1 + k_2 \left(\frac{E}{k_7 + E} \right) - k_3 \cdot A \tag{3.13}$$

$$\dot{E} = k_6 + k_4 - k_5 \cdot E \left(\frac{k_8}{k_8 + A} \right) \tag{3.14}$$

3.2.1 Linear growth

Most of motif show linear growth when degradation rates of A and E, k_3, k_5 are zero. In Motif 15, it is similar with the others but, there are some special case. Interestingly when k_3 are zero but k_5 is not zero, they also show linear growth, but not vice versa.

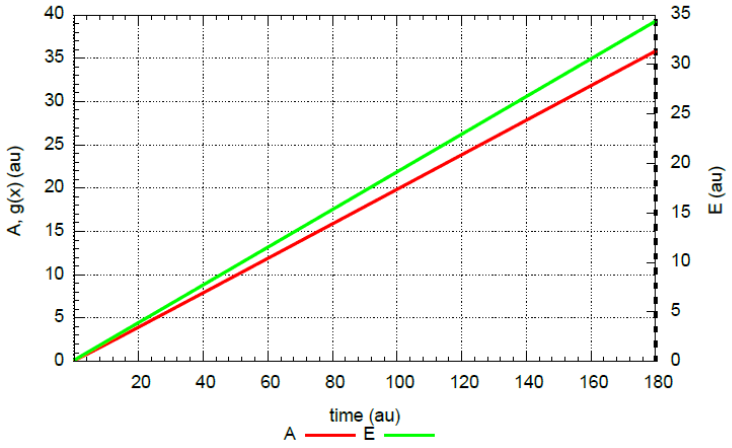


Figure 3.10: Linear growth when k_1,k_2,k_4,k_5,k_6,k_7 are 0.1, except k_3 is zero. Even though degradation rate of E, k_5 is not zero, E becomes linear growth unlike motif 16. Initial concentration of both A and E is 0.1.

3.2.2 Different time to reach steady state

Although all constant rate are same as 0.1, they show different times to become steady state. A is more sensitive to degradation rate as k_3 than k_5 of E. Because degradation of E is inhibited by $\left(\frac{k_8}{k_8+A}\right)$.

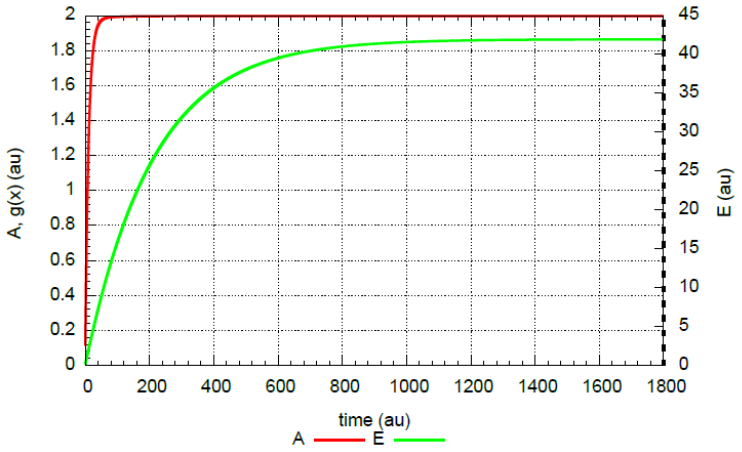


Figure 3.11: Steady state when k_1,k_2,k_3,k_4,k_5,k_6,k_7,k_8, all rate constants are 0.1. A increase rapidly and then become steady state. E relatively increase slowly but more increase than A. Initial concentration of both A and E is 0.1.

As I mentioned in the introduction, motif 15 is switchable with motif 9. Motif 12 and motif 14 also can switch each other. By substituting $A <-> E$, they can be transformed either from motif 15 to motif 9 or from motif 12 to motif 14 vice versa. More details will be explained in motif 9.

3.2.3 Conversion from negative to positive feedback

In special case, feedback loops can be convertible between negative and positive feedback. The balance of p53 and MDM2 is important for regulating the cell cycle progression. The p53 not only halts cell cycle progression but also acts as transcript factors which help transcription of MDM2[25]. This MDM2 protein combines p53 and qubiquitin for degradation of p53. This harmony can exist as negative feedback loop[4]. However, under stress case, interestingly, this negative feedback is converted to positive feedback by adding the other components, in this case mir-605 which is a microRNA[4]. Under cell stress, the p53 transcripts miR-605 which suppress MDM2 protein translations so that p53 can be accumulated in cells, which induce apoptosis[26].

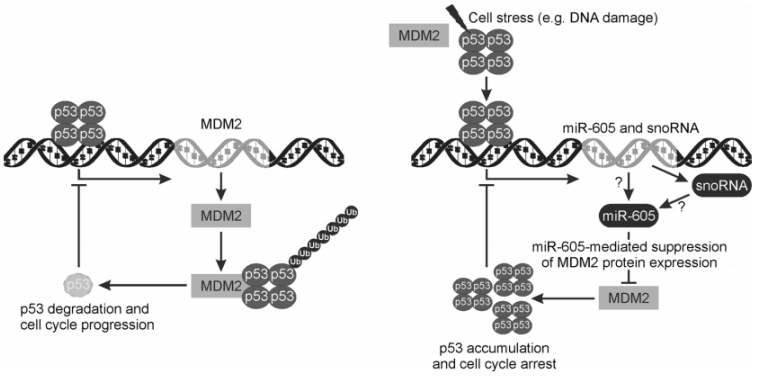


Figure 3.12: Schematic diagram of p53 and MDM2, miR605. p53 and MDM2 are regulated in negative feedback. As left Figure, p53 transcripts MDM2, which lead p53 to degradation. But in cells undergoing stress, this negative feedback becomes positive feedback. As right Figure, p53 starts to transcript mir-605 which inhibit MDM2 translation and this enhance the amount of p53. By doing so, increasing p53 represses cell cycle and induces the apoptosis[4].

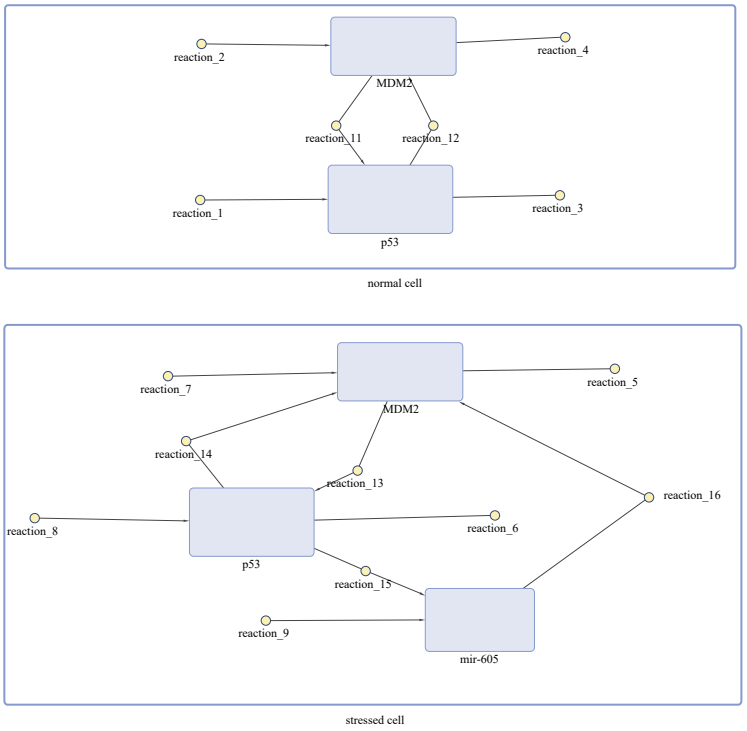


Figure 3.13: Schematic diagram of normal and stressed cell simbiology application in MATLAB. Each reactions present kinetic laws for input and degradation. Upper diagram shows negative feedback in normal cell. On the other hands, lower diagram presents positive feedback under stress cell.

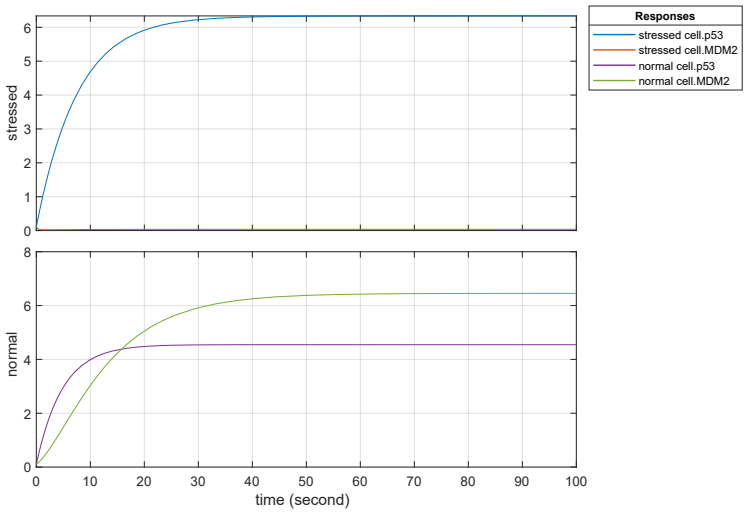


Figure 3.14: Different consequences in normal and stressed cell in Simbiology. Stress cell increase p53 and suppresses MDM2. Oppositively, p53 and MDM2 in normal cell show small gap between them.

In normal cell, p53 and MDM2 show homeostasis by negative feedback loop. Thus, gap of them is small. However when cell gets stressed or DNA damage, miR-605 is added to negative feedback and then convert it positive feedback. As a result, concentration of p53 increase by suppression of MDM2. The accumulative p53 starts to halt cell cycle arrest and lead apoptopsis. **In appendix A, same results which was calculated by Fortran can be observed.**

3.3 Motif 13

In motif 13, two elements inhibit the degradation of counterparts. E inhibits the degradation of A, k_3 and A inhibits the degradation of E, k_5. In this motif 13, there are linear growth, steady state and curved and linear growth.

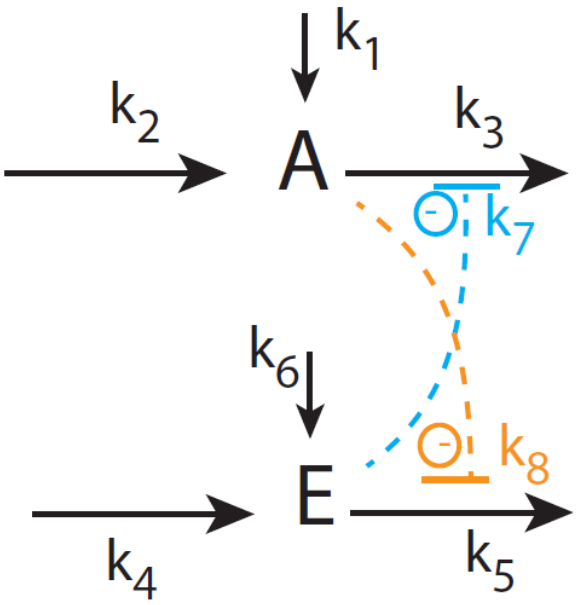


Figure 3.15: The circuit of motif 13

The rate equations of A and E in motif 13 are as below.

$$\dot{A} = k_1 + k_2 - k_3 \cdot A \left(\frac{k_7}{k_7 + E} \right) \tag{3.15}$$

$$\dot{E} = k_6 + k_4 - k_5 \cdot E \left(\frac{k_8}{k_8 + A} \right) \tag{3.16}$$

3.3.1 Curved and linear growth

This is specific trait of Motif 13. The concentration of element are usually affected by degradation. However, as time goes, magnitude of degradation diminished by increasing concentration of counterparts. That's why, at specific moment, shape of growth become linear growth.

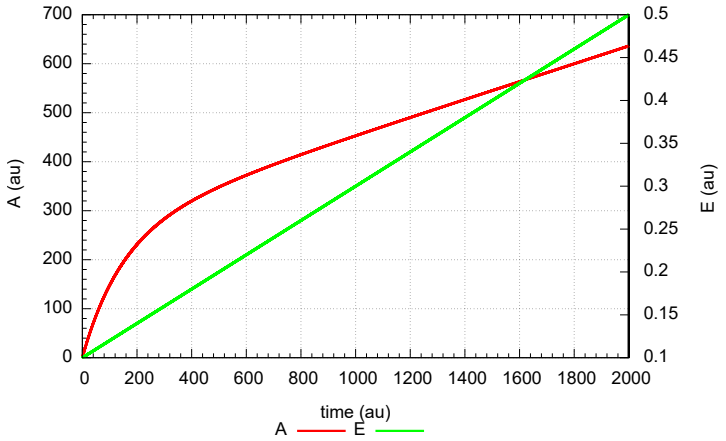


Figure 3.16: Curved and linear growth. when k_1, k_2 are 1.0 while k_3 is 0.01, k_4, k_8 are zero004, k_7 is 0.01, k_5, k_7 are 0.1. The initial concentration of A and E are 0.1.

3.3.2 Linear growth

It just looks like linear growth as motif 16 and motif 10. However, there is the difference which k_3, k_5 are not zero but this graph shows linear growth. $\left(\frac{k_7}{k_7+E}\right)$ and $\left(\frac{k_8}{k_8+A}\right)$ weaken the degradation rates as concentration of A and E increase so that linear growth occurs when k_3, k_5 are not zero.

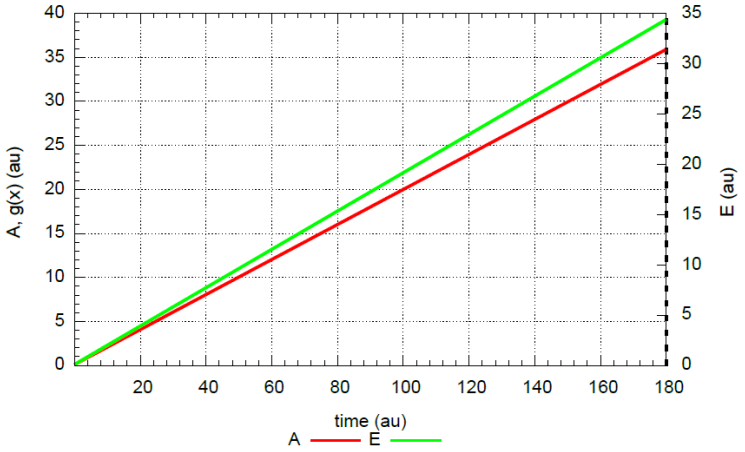


Figure 3.17: Steady state when k_1,k_2,k_3,k_4,k_5,k_6,k_8 are 0.1 except k_7 are 0.01. The initial concentration of A and E are 0.1.

3.3.3 Steady state

In this case, steady state occur when concentration of elements are 0.1 and inhibition rates, which are k_7, k_8 are same or bigger than 1.0. If they increase, the magnitude of degradation increase too so that the concentration of elements reach to steady state.

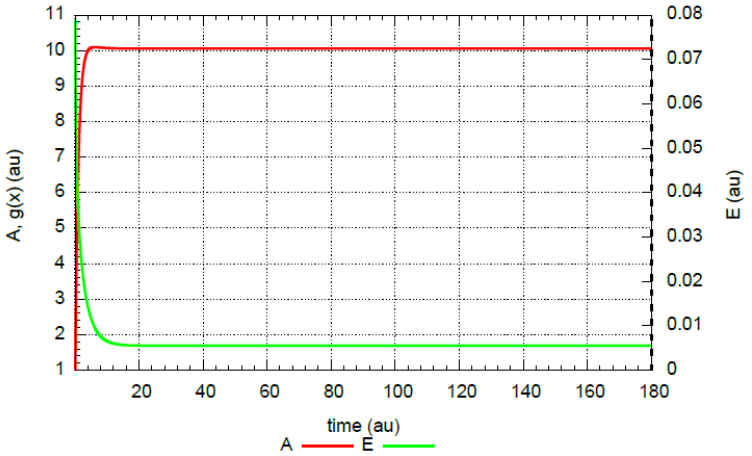


Figure 3.18: Steady state when k_1, k_2 are 5.0, while k_3, k_7, k_8 are 1.0, k_4, k_6 is zero01, k_5 is 4.0. The initial concentration of A and E are 0.1.

3.3.4 Conditions for steady state of motif 13

Most of motifs in positive feedback present steady state at specific conditions. To become steady state, $\dot{A}$ and $\dot{E}$ should be zero. With this assumption, we can modify the equation with former equation 3.15 and 3.16 as below.

$$A_{ss} = \left(\frac{k_1 + k_2}{k_3 \cdot \left(\frac{k_7}{k_7 + E_{ss}} \right)} \right) \tag{3.17}$$

$$E_{ss} = \left(\frac{k_6 + k_4}{k_5 \cdot \left(\frac{k_8}{k_8 + A_{ss}} \right)} \right) \tag{3.18}$$

```
g(E) =

k4 + (k6*(k8 + ((E + k7)*(k1 + k2))/(k3*k7)))/(k5*k8)

>> solve(g(E),E)

ans =

-(k3*k5*k7*k8*(k4 + (k6*(k8 + (k1 + k2)/k3))/(k5*k8)))/(k6*(k1 + k2))
```

Figure 3.19: The equation deduced by Matlab. This equation presents the condition of steady state in motif 13.

In other words, A_{ss} and E_{ss} should be as below.

$$A_{ss} = \left(\frac{E_{ss} \cdot k_5 \cdot k_8}{k_4 + k_6}\right) - k_8 \tag{3.19}$$

$$E_{ss} = \left(\frac{A_{ss} \cdot k_3 \cdot k_7}{k_1 + k_2}\right) - k_7 \tag{3.20}$$

Through equation 3.17, 3.18, we can know that $\left(\frac{k_1+k_2}{k_3}\right)$ and $\left(\frac{k_4+k_6}{k_5}\right)$ can be fixed depending on whether concentration of E and A continuously increase or not. If they increase, as result value of k_3 and k_5 are affected by decrease so that steady state can not be happened. if fixed, numerator and denominator maintain same values.
To make steady state, first of all, initial concentration of A and E need to be less than inhibition rates. If input constant rate are less than degradation rate, we can expect steady state readily. However, if not, we can increase inhibition rates more to maintain steady state. Lastly, k_3 and k_5 should not be zero.

3.4 Motif 12

Contrary to motif 15, In case of motif 12, it shows that A enhances degradation of E, while E inhibits input of A for positive feedback so that as time passes, they increase each other. In motif 12, hyperbolic growth can be found at specific case.

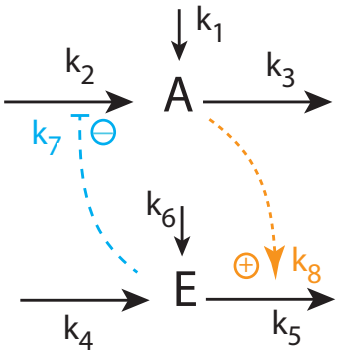


Figure 3.20: circuit of motif 12

By circuit of motif 12, we can deduce equations as below.

$$\dot{A} = k_1 + k_2 \left(\frac{k_7}{k_7 + E} \right) - k_3 \cdot A \tag{3.21}$$

$$\dot{E} = k_6 + k_4 - k_5 \cdot E \left(\frac{A}{k_8 + A} \right) \tag{3.22}$$

3.4.1 Steady state

As same as the other motifs, motif 12 presents steady state when all of constant rate are 0.1 but A increase more than E.

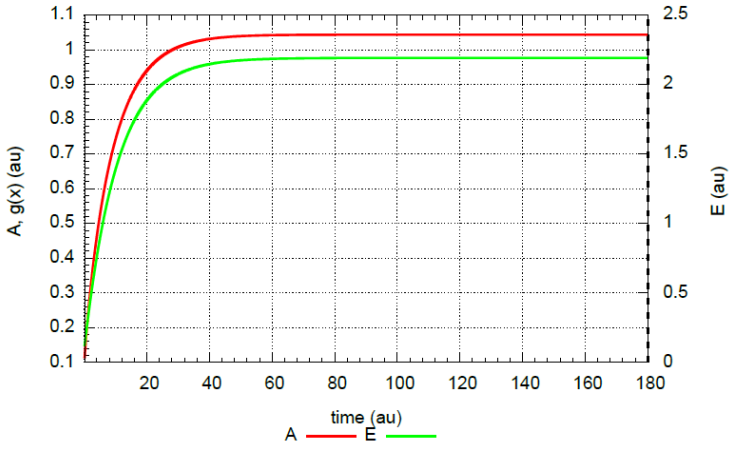


Figure 3.21: Steady state when k_1,k_2,k_3,k_4,k_5,k_6,k_7,k_8 all of rate constants are 0.1. The initial concentration of A and E are 0.1.

3.4.2 Linear growth

When degradation rates of all components become zero, this motif show linear increase.

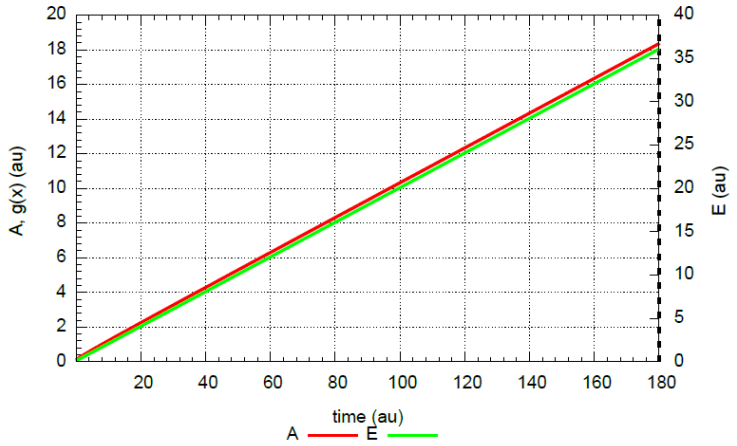


Figure 3.22: Linear growth when k_1,k_2,k_4,k_6,k_7,k_8 0.1 except k_3, k_5 are zero. The initial concentration of A and E are 0.1.

Unlike Motif 15, linear growth of A doesn't make E as linear.

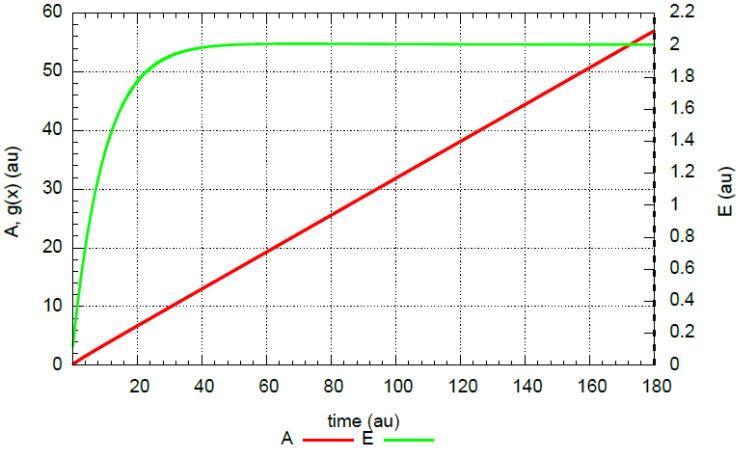


Figure 3.23: Linear growth and steady state when k_4,k_5,k_6,k_7,k_8 0.1 except k_1, k_2 are 0.3, while k_3 is zero. The initial concentration of A and E are 0.1.

3.4.3 Hyperbolic growth in Motif 12

With specific conditions, motif 12 can present hyperbolic growth. In this time, I will compile motif 12 with various rate constants and figure out which factors lead hyperbolic

growth.
Before that, I need to explain the definition of hyperbolic with higher-order autocatalysis example.

We consider the following autocatalytic reaction with reaction order p>1:

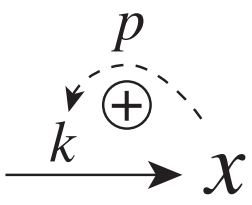


Figure 3.24: Scheme of an autocatalytic process in x with reaction order p>1.

The rate equation of x is given by

$$\frac{dx}{dt} = k \cdot x^p; \quad p > 1 \tag{3.23}$$

By separating the variables

$$\frac{dx}{x^p} = k \cdot dt \implies x^{-p} dx = k \cdot dt \tag{3.24}$$

and integration we can get

$$\int_{x_0}^{x(t)} \frac{dx}{x^p} = \int_0^t k \cdot dt = k \cdot t \tag{3.25}$$

$$\frac{x^{1-p}}{1-p} - \frac{{x_0}^{1-p}}{1-p} = k \cdot t \tag{3.26}$$

with the following expression for x

$$x(t) = \frac{x_0}{(1 - {x_0}^{p-1}(p-1)k \cdot t)^{\frac{1}{p-1}}} \tag{3.27}$$

Figure 3.25 shows x as a function of t when x_0=0.1, p=2, and k=1.0.

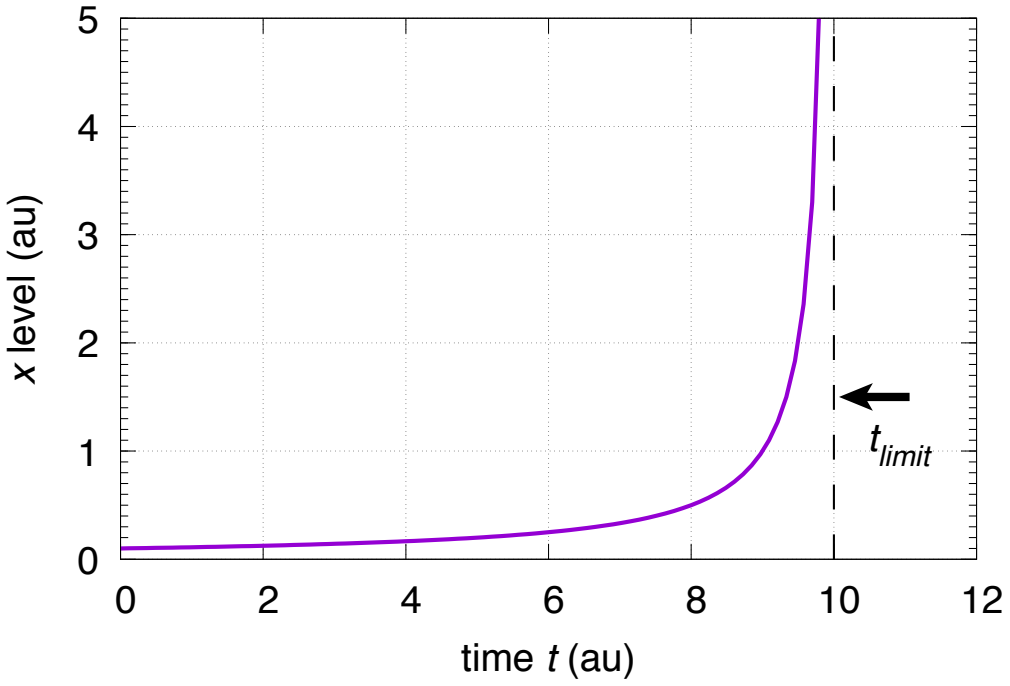


Figure 3.25: Hyperbolic growth of x when x_0=0.1, p=2, and k=1.0. t_{limit} is the infinity limit, i.e. the time when x reaches infinity.

As Figure 3.25, hyperbolic growth reaches infinity at point in time in a massive event as a singularity. This hyperboic growth can also appear temporarily and disappear. In addition, infinity time can be closer or farther away by various factors like rate constants and initial concentrations. I will explain it with Figure 3.26 as below.

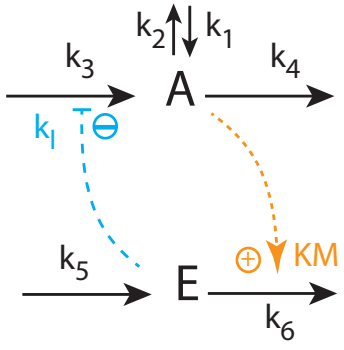


Figure 3.26: The circuit of motif 12.

With this scheme, we can separate equations as below(M12Y).

$$\dot{A} = k_1 - k_2 \cdot A + \left(\frac{k_3}{1.0 + \left(\frac{E}{KI}\right)} \right) - k_4 \cdot A \tag{3.28}$$

$$\dot{E} = k_5 - k_6 \cdot E \left(\frac{A}{KM + A} \right) \tag{3.29}$$

Or, depending on unsaturated formation of E, the equation of E can be presented as below(M12N).

$$\dot{A} = k_1 - k_2 \cdot A + \left(\frac{k_3}{1.0 + \left(\frac{E}{K_I} \right)} \right) - k_4 \cdot A \tag{3.30}$$

$$\dot{E} = k_5 - k_6 \cdot E \cdot A \tag{3.31}$$

As I marked, I compiled with these equations with M12Y and M12N.

M12Y: Component A activates the degradation of E by activation rate, KM

M12N: Component A activates the degradation of E without activation rate, KM

In Figure 3.27, first of all, log scale was used for component A to show hyperbolic pattern while linear scale was used for component E to prevent error of plotting. Becuase E decreases to the decimal point. If log scale is used, graph shows something that is not normal.

Compared to M12N, hyperbolic moments In M12Y not appear vividly so that difficult to identify results of changes among rate constants

In case of M12N, not only increasing k_3 but also increasing k_1 affects on early hyperbolic moment.

The K_I also acts as k_3. When K_I increase, hyperbolic moment get closer

On the other hand, when the initial concentration of E increase, hyperbolic moment become postponed.

In case of M12Y, it can present more clear hyperbolic moment when M12Y becomes unsaturated. If values of k_6 and KM increase very much like 1E+7, while the other rate constants are 0.1 or zero. I will prove it on next subsection, double motif 12.

Table 3.2: The rate constants for Figure 3.27. For the sake of simplicity, modified parts with control group are highlighted by green color.

Graph	k1	k2	k3	k4	k5	k6	K_I	KM	E0
A, a	0	0	1.E+2	0	0	0	1.E-1	1.E-6	1.E+3
B, b	0	0	1.E+4	0	0	0	1.E-1	1.E-6	1.E+3
C, c	1.E+2	0	1.E+2	0	0	0	1.E-1	1.E-6	1.E+3
D, d	0	0	1.E+2	0	0	0	1.E-2	1.E-6	1.E+3
E, e	0	0	1.E+2	0	0	0	1.E-1	1.E-6	1.E+6

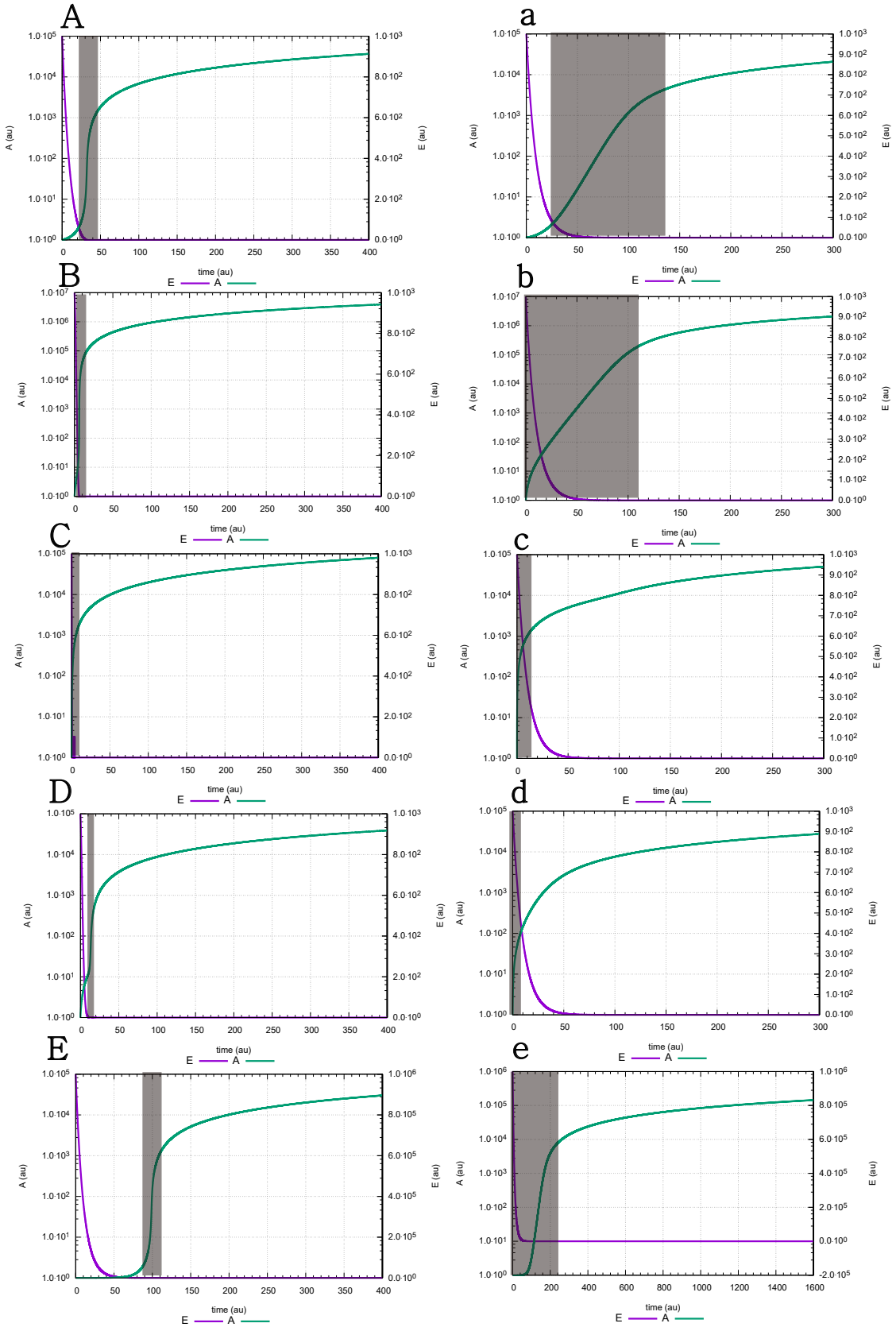


Figure 3.27: Comparison between M12N and M12Y. (A), (a) are control group of M12N and M12Y respectively. (B) and (b) show that hyperbolic moments get closer when $k_3$increase 1E+2 from 0 in control group. (C) and (c)present earlier hyperbolic moment when k_1 increase 1E+4 from 1E+2. (D) and (d) show earlier hyperbolic moment when k_I increase 1E+2 from 0.1. (E) and (e) represent postponed hyperbolic moment when initial concentration of E increase from 1E+3 to 1E+6. Gray area presents where hyperbolic growth occur. All rate constants are given in Table 3.2.

The reason why hyperbolic growth of A appear and disapper is following. First the concentration of E which represses the influx, k_3, of A is larger than A. Thus, A can not increase quickly, However, When concentration of E decreases close to 0, A sharply increase because k_3 is relatively high. After the influence of E decreases, A increases constanty so that the hyperbolic growth disappears.

3.4.4 Hyperbolic growth in combined identical motif 12

What if motif 12 combine identical motif 12, how will hyperbolic growth change?. To answer this question, I combined a pair motif 12 and named as **2 motif 12.** At specific constant rates, 2 motif 12 also can show hyperbolic growth. In this time, I will compile different scheme of 2 motif 12 which are models, 2M12 and 2M12K with various rates constant and figure out which factors lead hyperbolic growth.

2M12: both E_2 and E_1 inhibit input of A, k_3

2M12K: E_2 inhibits k_3 while E_1 inhibits, k_{13}

With this scheme, we can separate equations as below (2M12).

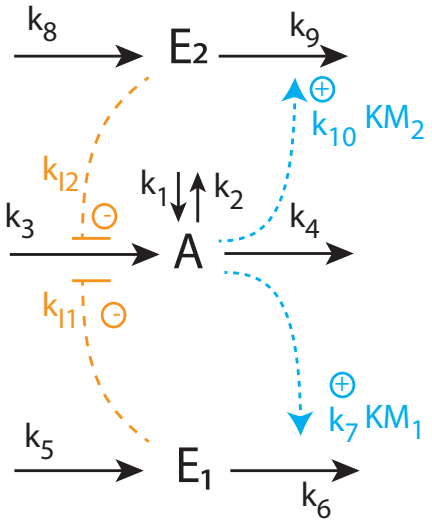


Figure 3.28: The circuit of 2M12.

$$\dot{A} = k_3 \cdot \left(\frac{K_{I1}}{K_{I1} + E_1}\right) \cdot \left(\frac{K_{I2}}{K_{I2} + E_2}\right) + k_1 - k_4 \cdot A - k_2 \cdot A \tag{3.32}$$

$$\dot{E_1} = k_5 - k_6 \cdot E_1 \cdot \left(\frac{A}{k_7 + A}\right) \tag{3.33}$$

$$\dot{E_1} = k_8 - k_9 \cdot E_2 \cdot \left(\frac{A}{k_{10} + A} \right) \tag{3.34}$$

Otherwise, the scheme of combined identical motif 12 and the equation can be presented as below (2M12K). All of constant rate are same with 2M12. However, k_{13} is inserted into additional influx of A.

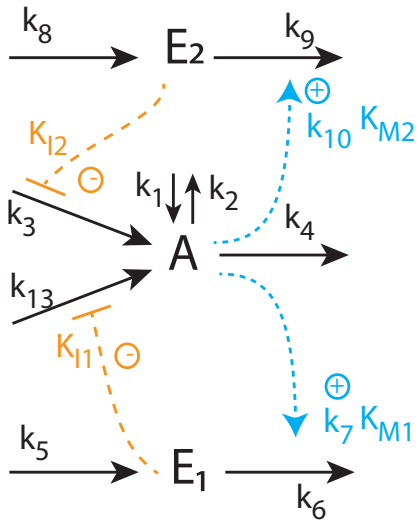


Figure 3.29: The circuit of 2M12k.

$$\dot{A} = k_{13} \left(\frac{K_{I1}}{K_{I1} + E_1} \right) + k_3 \left(\frac{K_{I2}}{K_{I2} + E_2} \right) + k_1 - k_4 \cdot A - k_2 \cdot A \tag{3.35}$$

$$\dot{E_1} = k_5 - k_6 \cdot E_1 \cdot \left(\frac{A}{k_7 + A} \right) \tag{3.36}$$

$$\dot{E_1} = k_8 - k_9 \cdot E_2 \cdot \left(\frac{A}{k_{10} + A} \right) \tag{3.37}$$

Table 3.3: The rate constants for Figure 3.30. A and a are rate constants for control. Yellow mark presents difference with control group. Orange mark shows another change of rate constants.

Graph	k1,k2	k3	k4,k5	k6	k7	k8	k9	k10	k11,k12	k13
A, a	0	1.E+2	0	1.E-1	1.E-6	0	1.E-1	1.E-6	1.E-1	1.E+2
B, b	0	1.E+2	0	1.E+9	1.E+8	0	1.E+9	1.E+8	1.E-1	1.E+2
C, c	0	1.E+2	0	1.E+9	1.E+8	0	1.E-1	1.E-6	1.E-1	1.E+2
D, d	0	1.E+2	0	1.E+8	1.E+8	0	1.E+8	1.E+8	1.E-1	1.E+2

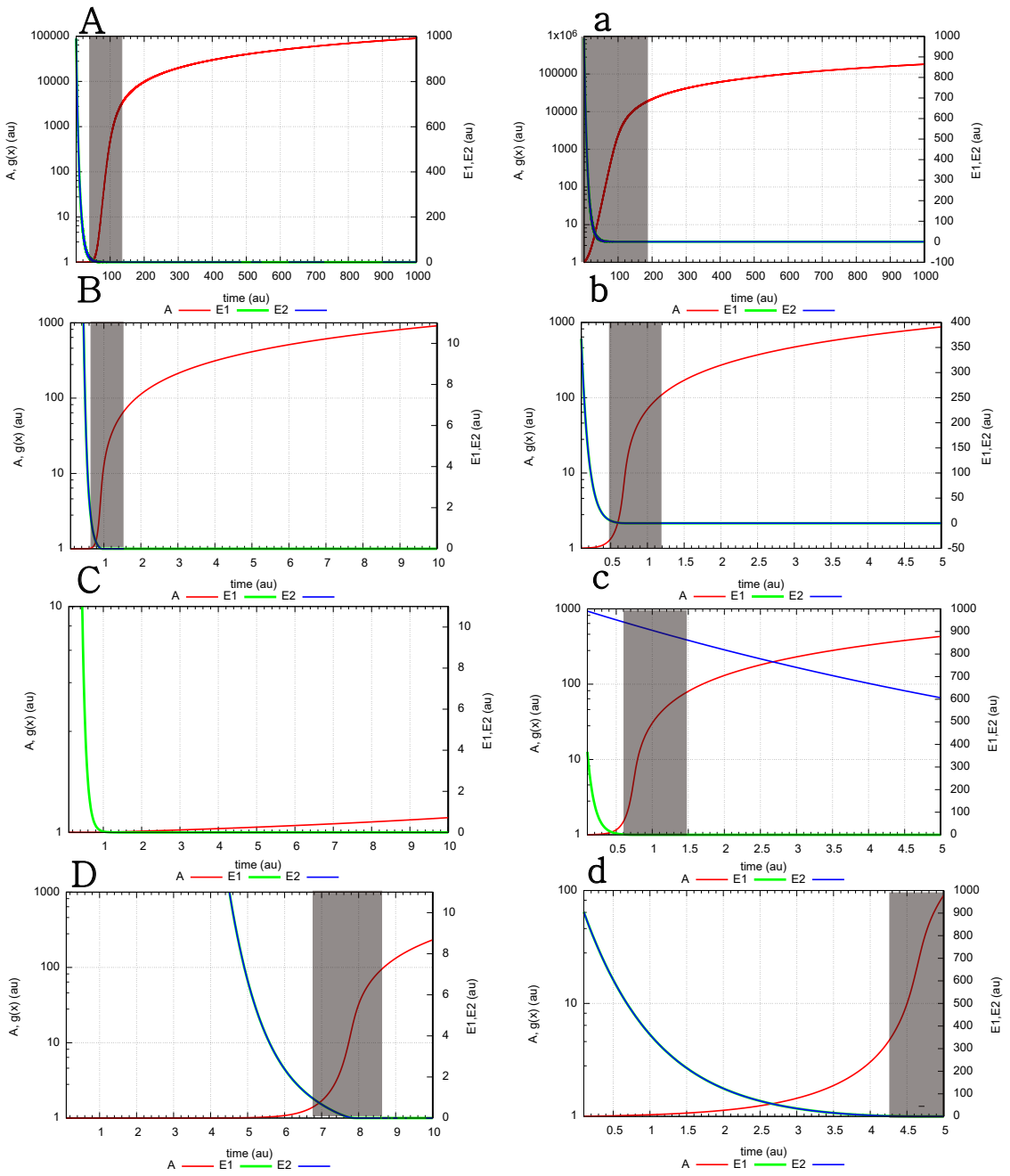


Figure 3.30: Outcome of saturated and unsaturated 2M12 and 2M12K. Upper letter:2M12, lower letter:2M12K. (A) and (a) are control group. (B) and (b) show hyperbolic graphs. In (C) and (c), k_9 and k_{10} One of unsaturated form is converted to saturated form. D) and (d) show distortion of hyperbolic moment when value of k_6,k_9 are less than those of k_7,k_{10}.

In this Figure 3.30, I took values of k_6,k_7,k_9,k_{10} to very large for making E_1, E_2 unsaturated in 2M12 and 2M12K. In A) and a), when value of k_6,k_9 and k_7mk_{10} increase 1E+9 and 1E+8 respectively, both 2M12 and 2M12K become unsaturated and then present clear hyperbolic graphs like B) and b). Both graph show similar hyperbolic

moments. However, when one of unsaturated E_1, E_2 become saturated, C) hyperbolic growth in 2M12 is diminished while c) that in 2M12K is maintained due to separation of k_3 and k_{13} in 2M12K. When value of k_6,k_9 are same as those of k_7mk_{10}, hyperbolic graph starts to distorted.

3.5 Motif 11

In the motif 11, both A and E enhance influx of conuterparts unlike, in motif 13, both components repress degradation of counterpart each other to form positive feedback.

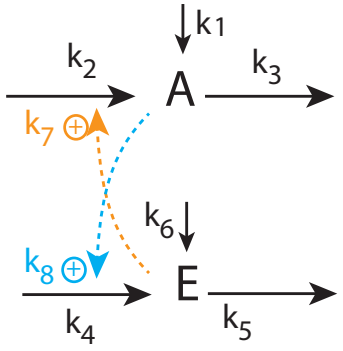


Figure 3.31: The circuit of motif 11.

$$\dot{A} = k_1 + k_2 \left(\frac{E}{k_7 + E} \right) - k_3 \cdot A \tag{3.38}$$

$$\dot{E} = k_6 + k_4 \left(\frac{A}{k_8 + A} \right) - k_5 \cdot E \tag{3.39}$$

3.5.1 Comparison between saturated and unsaturated motif 11

Sometime, In loops, there are unsaturated components affecting counterpart like Figure 3.32.

Saturated motif indicates that each components activate input of counterparts by activate rate constants. Otherwise, in unsaturated motif, each components activate influx of counterparts without rate constants.

In contrast with Figure 3.31, unsaturated motif 11 show direct influence of component toward input without activation rates. This difference make new dramatical reaction.

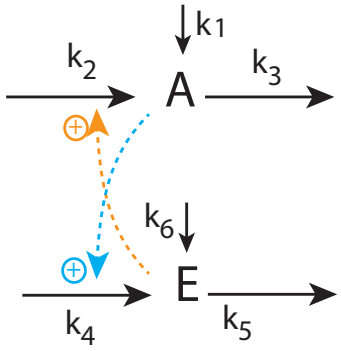


Figure 3.32: The circuit of unsaturated motif 11. Unlike saturated motif 11, there are no activation rates.

$$\dot{A} = k_1 + k_2 \cdot E - k_3 \cdot A \tag{3.40}$$

$$\dot{E} = k_6 + k_4 \cdot A - k_5 \cdot E \tag{3.41}$$

Same constant rates were applied in M11 and M112 respectively and then compared their patterns.

I will call saturated motif 11 as M11 and unsaturated motif11 as M112.

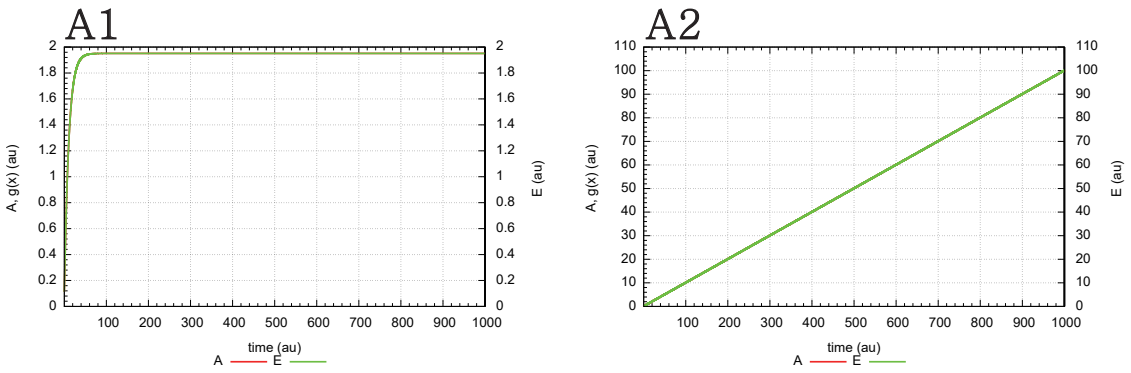


Figure 3.33: The steady state of A1:M11 and linear growth of A2:M112. k_1,k_2,k_3,k_4,k_5,k_6,k_7,k_8, all rate constant are 0.1. The initial concentration of A and E In both A1, A2 is 0.1.

In case of A1, it show steady state, whereas A2 presents linear growth in Figure 3.33.

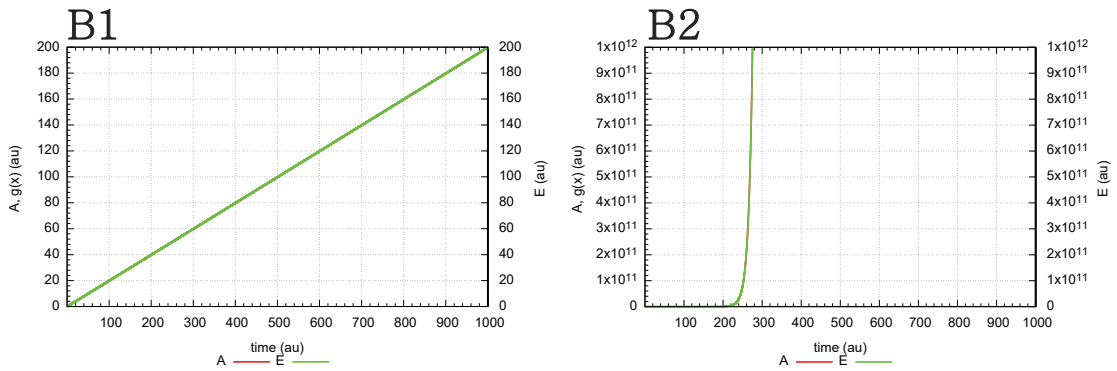


Figure 3.34: The linear growth of B1:M11 and exponential growth of B2:M112. k_1,k_2,k_4,k_6,k_7,k_8 are 0.1. only k_3,k_5 are zero. The initial concentration of A and E In both B1, B2 is 0.1.

When degradation of both A and E are zero, B1 shows linear growth, However B2 presents exponentially increase. The value of B1:M11 is 60 at 300 time(au), but that of B2:M112 reach over $1 \cdot 10^{12}$.

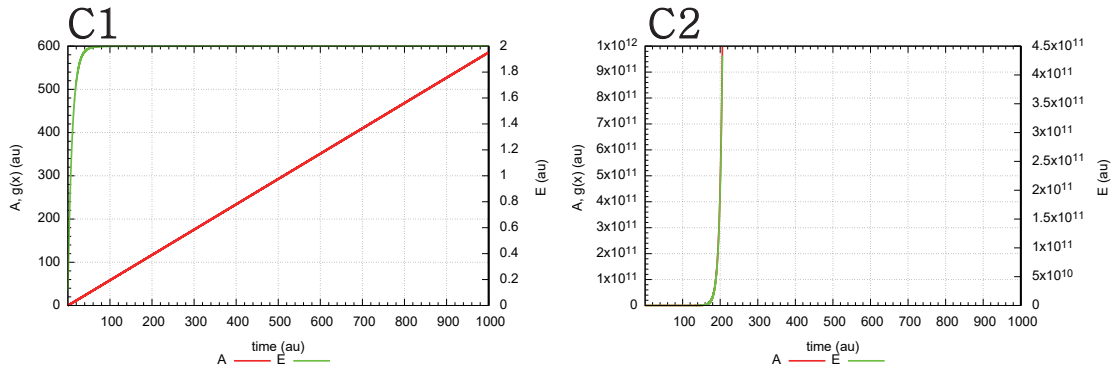


Figure 3.35: Steady state of E and linear growth of A In C1:M11 and both exponential growth in M112. k_1,k_2,k_4,k_5,k_6,k_7,k_8 are 0.1 except k_3 is zero. The initial concentration of A and E In both C1, C2 is 0.1.

C1 shows steady state of E and linear growth of A, but those in C2 presents exponentially the increase of A and E regardless of degradation which is zero.

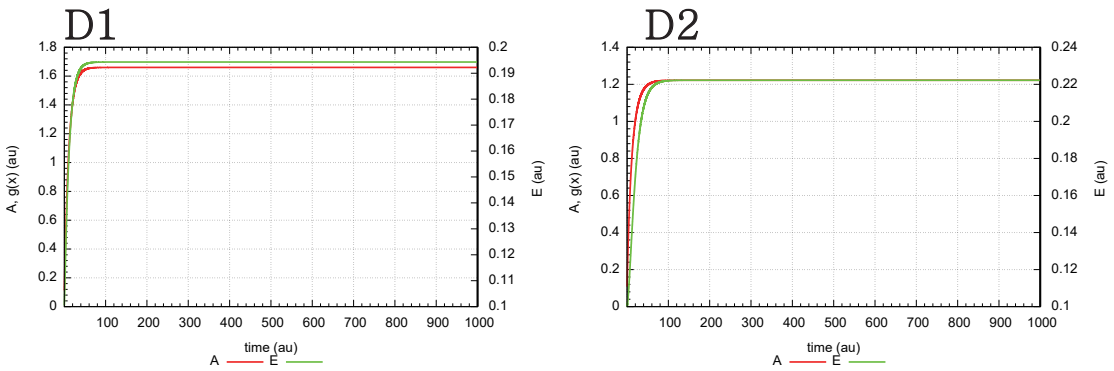


Figure 3.36: Both steady state in D1:M11 and D2:M112.
k_1,k_2,k_3,k_5,k_7,k_8:0.1 and k_4, k_6:0.01. The initial concentration of A and E In both D1, D2 is 0.1.

Interestingly, when input constant rate are less than the others, both graphs display steady state. In conclusion, compared to saturated motif 11, unsaturated one are affected regarding to the input rate in motif 11. On the other hands, saturated motif 11 are affected by activation rate. Without activation rate, motif 11 readily increases more than unsaturated motif 11, M112. In M112, When input rate, which are k_4 and $k6$ are less than the other constant rate, it becomes steady state, similar to graph of saturated motif 11, M11.

3.5.2 Combined unsaturated motif 11 with motif 9 present different patterns

If unsaturated motif 11 combines with motif 9, it denotes different behaviors.

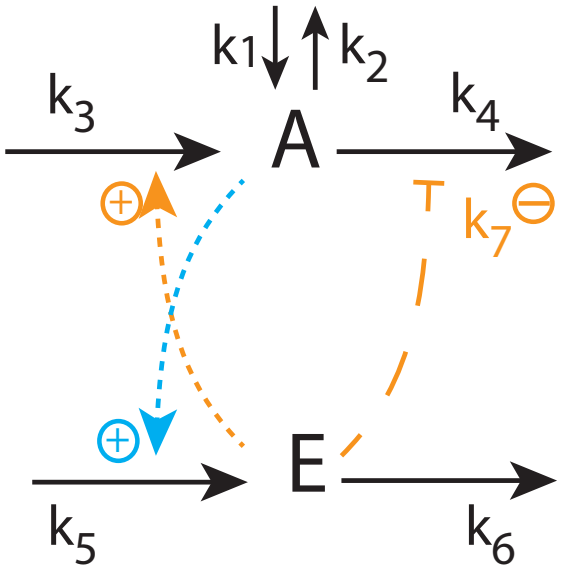


Figure 3.37: Diagram of combined motif 11 and motif 9.

$$\dot{A} = k_1 + k_3 \cdot E - k_4 \cdot A \cdot \left(\frac{k_7}{k_7 + E}\right) - k_2 \cdot A \tag{3.42}$$

$$\dot{E} = k_5 \cdot A - k_6 \cdot E \tag{3.43}$$

This motif depicts similarity and difference of motif 11. It shows steady state when all of rate constants are 0.1 while linear growth in A2 in Figure 3.33. When degradation rate of A and E becomes zero, it shows exponential growth. However inhibition rate, k_7, influences graph. It postpones exponential time and as (c) In Figure 3.38, exponential growth become linear growth.

Table 3.4: Table of rates constants for Figure 3.38

	k1	k2	k3	k4	k5	k6	k7
A	0.1	0.1	0.1	0.1	0.1	0.1	0.1
a	0.1	0.1	0.1	0.1	0.1	0.1	$1 \cdot 10^6$
B	0.1	**0.0**	0.1	0.1	0.1	**0.0**	0.1
b	0.1	**0.0**	0.1	0.1	0.1	**0.0**	$1 \cdot 10^6$
C	0.1	**0.0**	0.1	0.1	0.1	0.1	0.1
c	0.1	**0.0**	0.1	0.1	0.1	0.1	$1 \cdot 10^6$

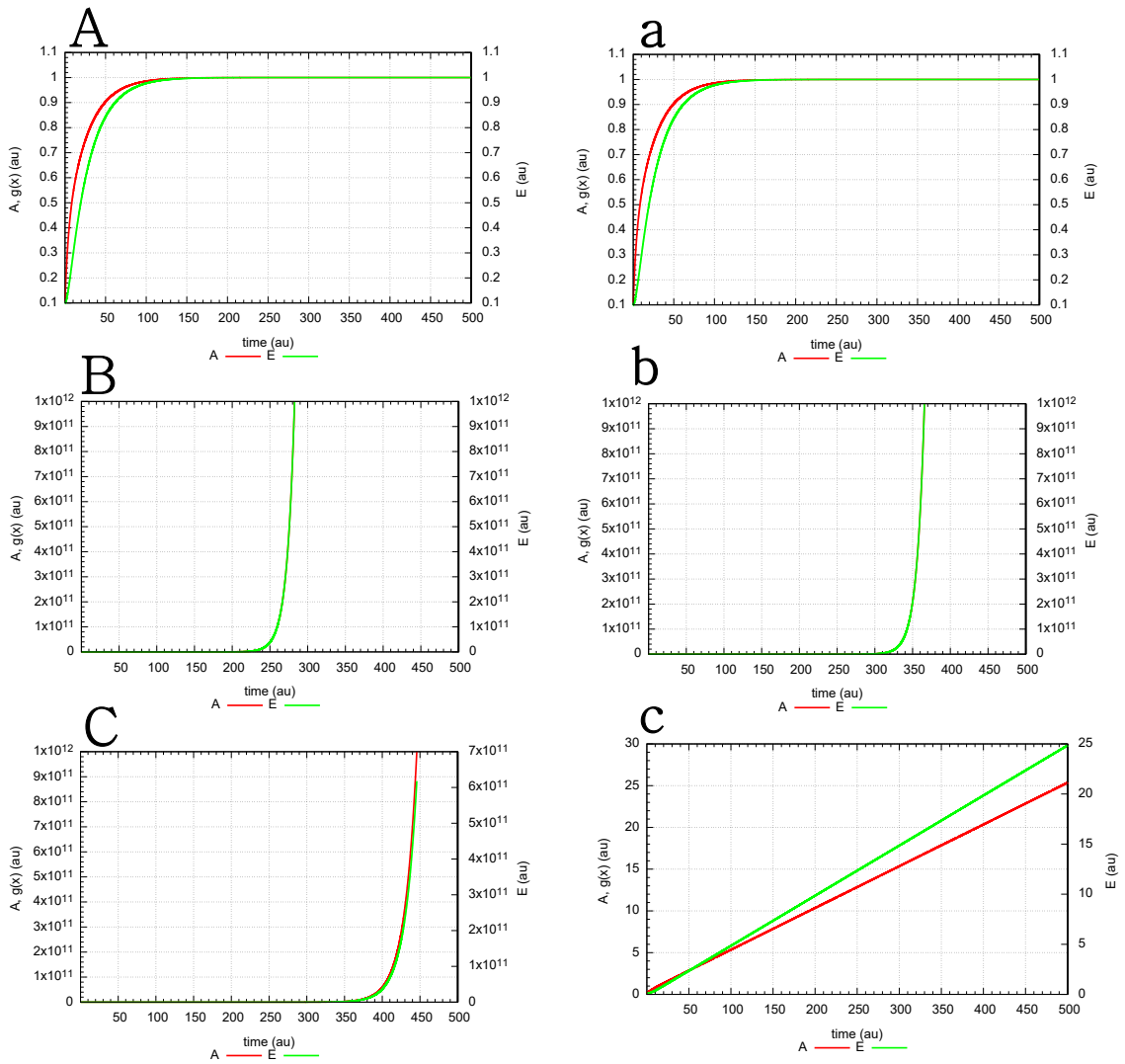


Figure 3.38: Various graphs of combined motif 11 and motif 9. All of rate constants are shown in Table 3.4. (A),(a) show no difference. (B),(b) presents exponential growth. With increasing k_7, exponential time get postponed. (C) denotes exponential growth whereas (c) show linear growth. The initial concentration of A and E in all graphs is 0.1.

3.5.3 Hyperbolic growth in bacterial infection cycles

A mechanism related with bacteria infection cycle has several phase[17].

1)initial phase where the invading RNA is used as RNA for the production of viral proteins with constant concentration of RNA and linear increase of the viral protein concentration.

2) a short phase where RNA and viral protein grow hyperbolically.

3) late phase where RNA and viral protein grow linearly and production rates of both are regulated at the maximum permissible levels[27].

Within these mechanisms, positive feedback exists and works for infection. I will analyze how the graph changes depending on the saturated and non-saturated states by classifying them into four models.

RPI31 shows cycle of bacteria for production with positive feedback when Enzyme E is unsaturated. In RPI31S, E is saturated with k_3 by activation rate k_6. In opposition, In RPI31S2, E is saturated with k_5. In RPI31S3, E is saturated with both k_3, k_5 by activation rate k_6, k_7 respectively. As Enzyme E get saturated, hyperbolic moment get postponed. Furthermore, when E totally saturated with both k_3, k_5, hyperbolic tendency is converted to exponential growth.

The scheme and the equation for this concept are as below.

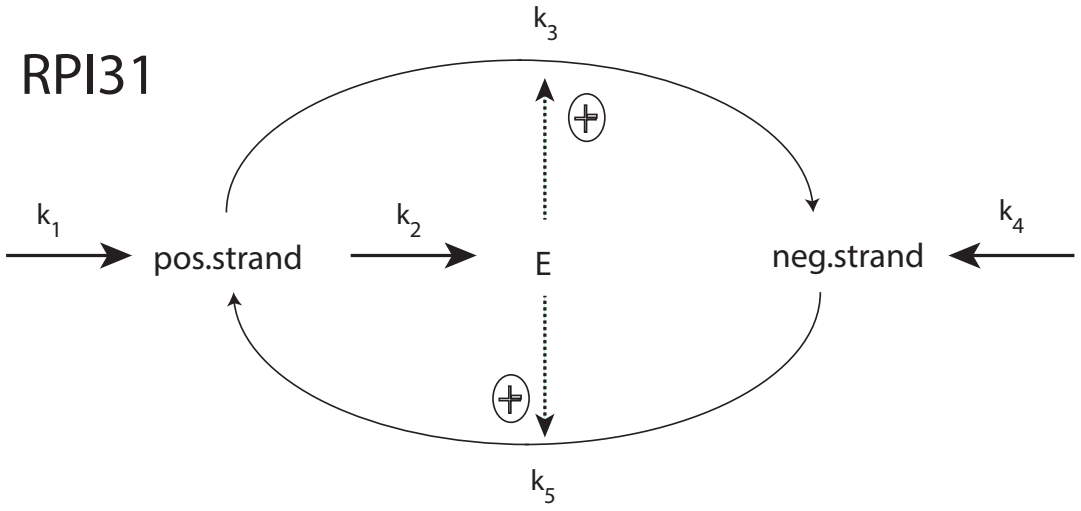


Figure 3.39: Scheme of RPI31 Enzyme E is not saturated

By circuit of RPI31, we can deduce equations as below.

$$\dot{E} = k_2 \cdot P_{str} \tag{3.44}$$

$$\dot{P_{str}} = k_1 + k_5 \cdot N_{str} \cdot E \tag{3.45}$$

$$\dot{N_{str}} = k_4 + k_3 \cdot P_{str} \cdot E \tag{3.46}$$

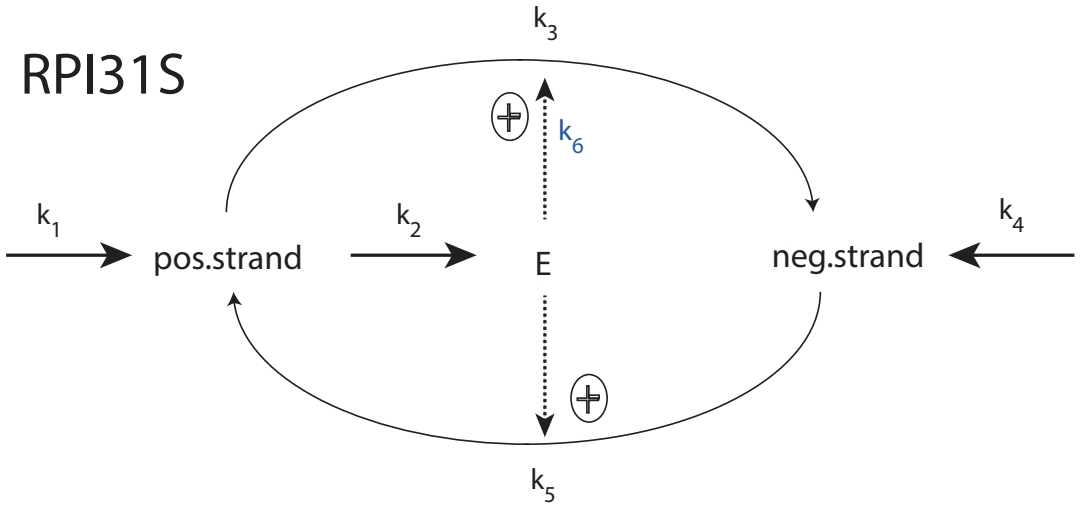


Figure 3.40: Scheme of RPI31S. Enzyme E is saturated with k_3 by k_6.

By circuit of RPI31S, we can deduce equations as below.

$$\dot{E} = k_2 \cdot P_{str} \tag{3.47}$$

$$\dot{P_{str}} = k_1 + k_5 \cdot N_{str} \cdot E_1 \tag{3.48}$$

$$\dot{N_{str}} = k_4 + k_3 \cdot P_{str} \cdot \left(\frac{E}{k_6 + E} \right) \tag{3.49}$$

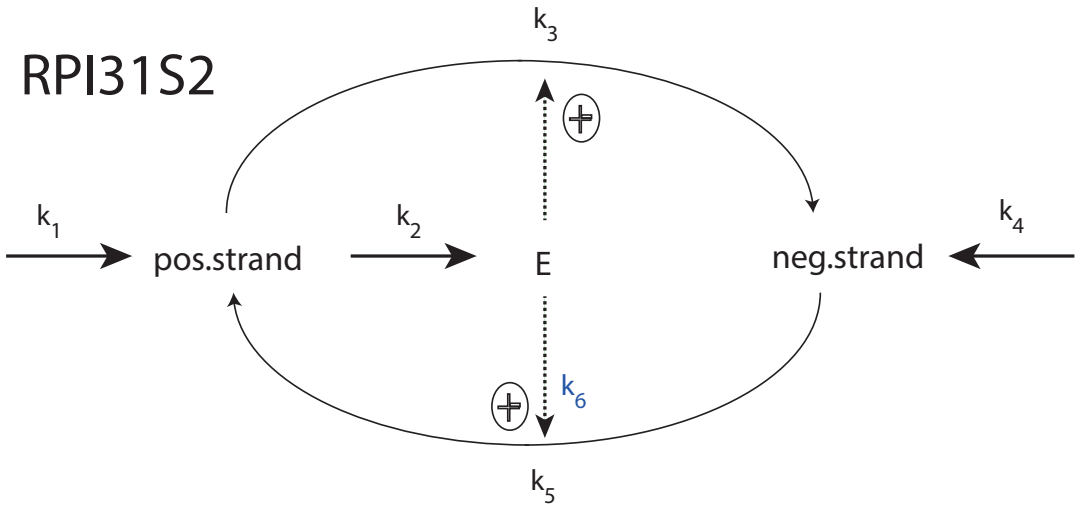


Figure 3.41: Scheme of RPI31S2. Enzyme E is saturated with k_5 by k_6.

By circuit of RPI31S2, we can deduce equations as below.

$$\dot{E} = k_2 \cdot P_{str} \tag{3.50}$$

$$\dot{P_{str}} = k_1 + k_5 \cdot N_{str} \cdot E_1 \cdot \left(\frac{E}{k_6 + E} \right) \tag{3.51}$$

$$\dot{N_{str}} = k_4 + k_3 \cdot P_{str} \tag{3.52}$$

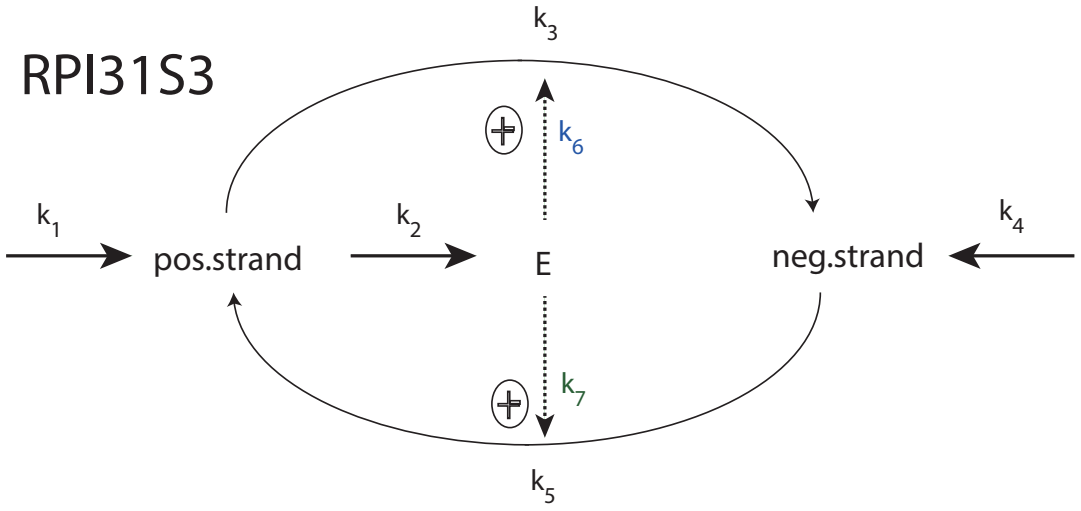


Figure 3.42: Scheme of RPI31S3. Enzyme E is saturated with both k_3, k_5 by k_6, k_7 respectively.

By circuit of RPI31S3, we can deduce equations as below.

$$\dot{E} = k_2 \cdot P_{str} \tag{3.53}$$

$$\dot{P_{str}} = k_1 + k_5 \cdot N_{str} \cdot E_1 \cdot \left(\frac{E}{k_6 + E} \right) \tag{3.54}$$

$$\dot{N_{str}} = k_4 + k_3 \cdot P_{str} \cdot E_1 \cdot \left(\frac{E}{k_7 + E} \right) \tag{3.55}$$

3.5.4 Saturation of E inhibits hyperbolic states

In Figure 3.43, all of constant rates are same as 0.1. All of graphs show hyperbolic growth except D:RPI31S3 presents exponential growth. As this motif get saturated, hyperbolic times are postponed. In (A), hyperbolic growth occurs at 14 time but in (B) and (C), they are 21 and 27. When two loops, k_3, k_5 are saturated with Enzyme E, hyperbolic state changes to exponential state.

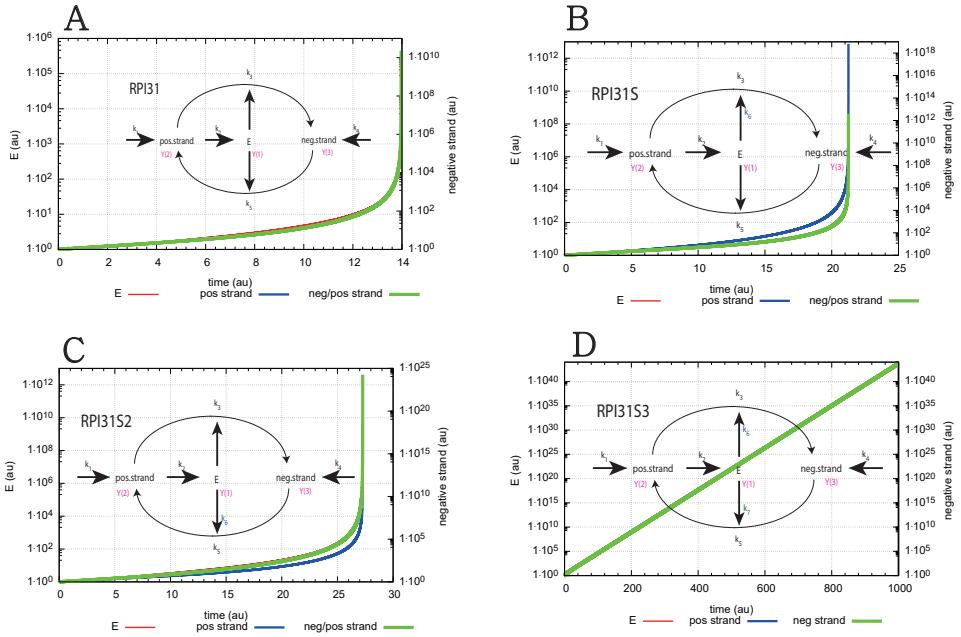


Figure 3.43: The outcome of A:RPI31, B:RPI31S, C:RPI31S2, D:RPI31S3. k_1,k_2,k_3,k_4,k_5,k_6,k_7:0.1. As loops between positive and negative strand get saturated by activation rates, hyperbolic graph become postpone. Finally it becomes exponential growth at (D). The initial concentration of E and positive, negative strand In all graph is 0.1.

3.5.5 Increase of k_3 and k_5 gets infinity time closer.

Compared to Figure 3.43, in Figure 3.44, as k_3 and k_5 increase, infinity time get shorter. On the other hand, when k_3 and k_5 decrease, hyperbolic moment become postponed.

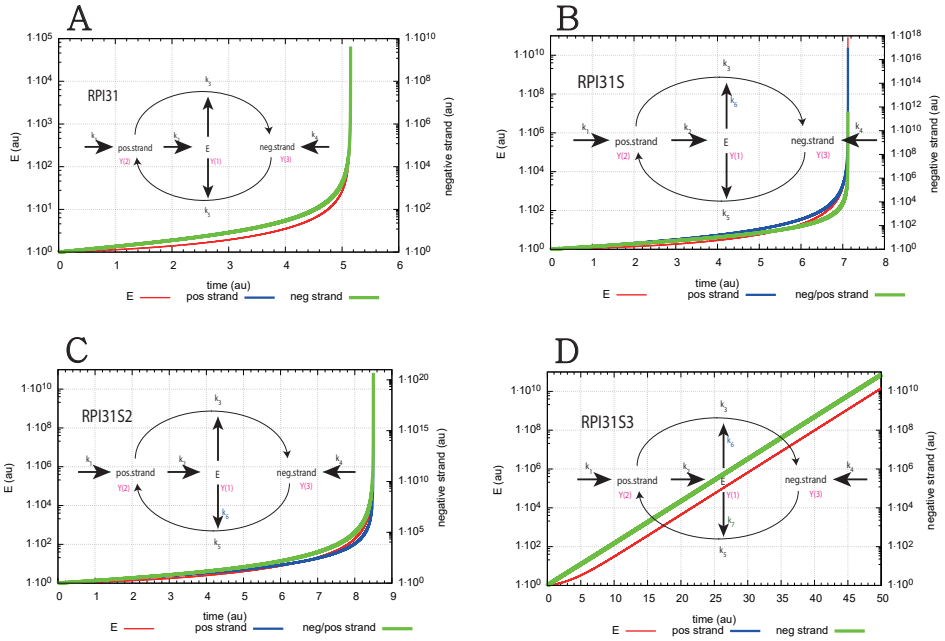


Figure 3.44: The outcome of A:RPI31, B:RPI31S, C:RPI31S2, D:RPI31S3. k_1,k_2,k_4,k_6,k_7:0.1 and k_3,k_5:0.5. increasing k_3, k_5 get hyperbolic moment closer. The initial concentration of E and positive, negative strand In all graph is 0.1.

3.5.6 Hyperbolic growth maintained by presence of k_3 and k_5.

If k_3 and k_5 are zero. hyperbolic growth disappear and then become linear growth.

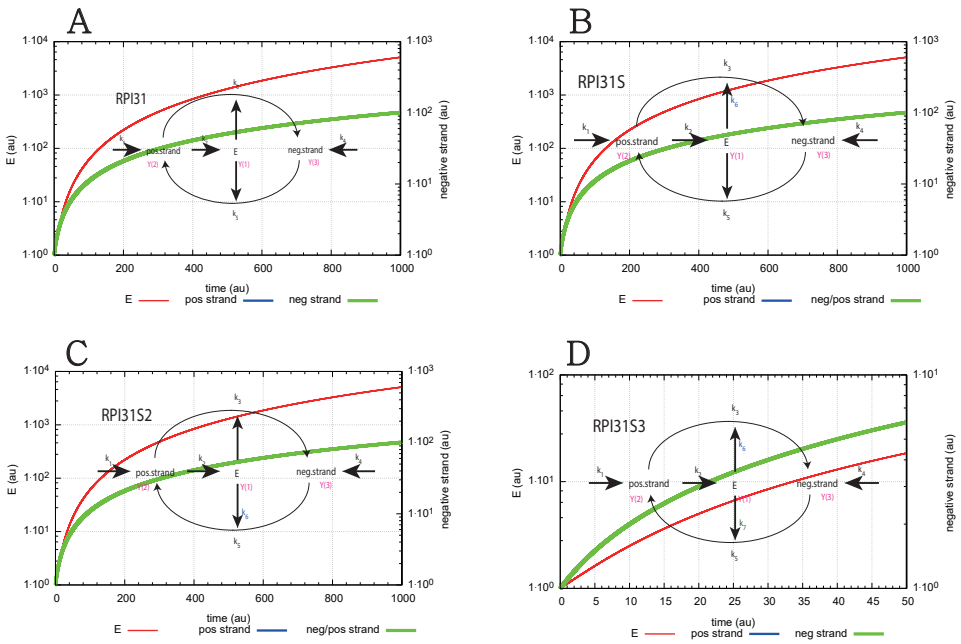


Figure 3.45: The outcome of A:RPI31, B:RPI31S, C:RPI31S2, D:RPI31S3. k_1,k_2,k_4,k_6,k_7:0.1 and k_3,k_5:zero. Disappearance of k_3, k_5 loops convert hyperbolic growth to linear growth. The initial concentration of E and positive, negative strand In all graph is 0.1.

3.5.7 Absence of influx of positive and negative strand has no effect for hyperbolic growth.

Even though influx of Pos.strand and Neg.strand, k_1, k_4, are removed, they can maintain hyperbolic growth on the basis of existence of k_3 and k_5 loops.

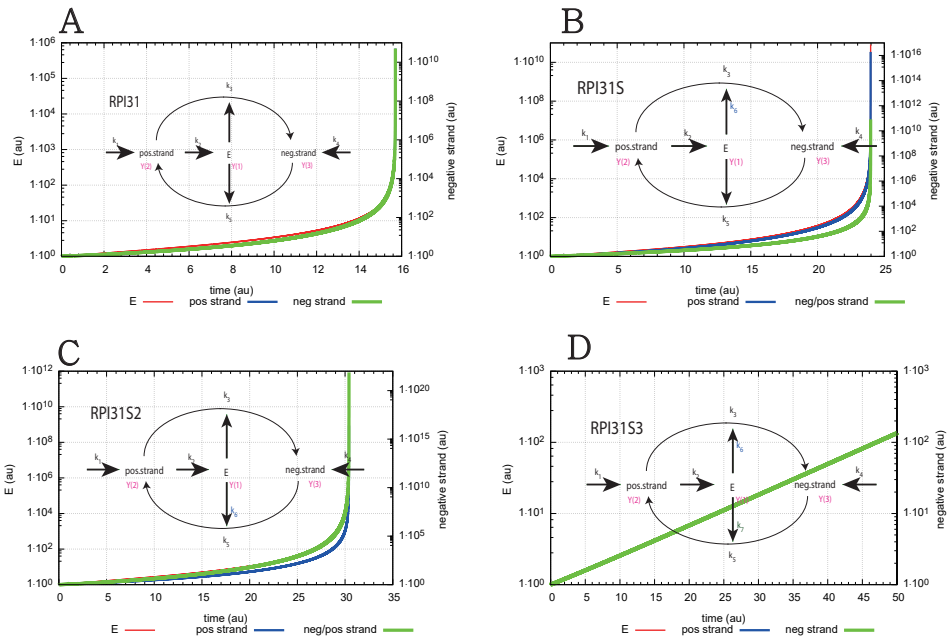


Figure 3.46: The outcome of A:RPI31, B:RPI31S, C:RPI31S2, D:RPI31S3. k_2,k_3,k_5,k_6,k_7:0.1 and k_1,k_4:zero. Even though there is no influx between positive and negative strand, hyperbolic growth is observed. The initial concentration of E and positive, negative strand In all graph is 0.1.

In conclusion, when all of constant rates are same, All of graphs show hyperbolic growth. But RPI31S3 represents exponential growth, which mean if k_3 and k_5 are saturated by k_6 and k_7, they are converted from hyperbolic to exponential growth.
Among k_3 and k_5, k_5 is more effective constant rate to get hyperbolic growth closer and when k_3 and k_5 both increase, hyperbolic time reached early.
When k_3, k_5 are zero, All of graphs have a tendency to become steady state.
Between k_6, k_7, the k_6 is more affect-able to modulate results of motifs. Finally, i confirmed that hyperbolic growth can be maintained even though influx of Pos. and Neg strand disappear, if loops,k_3 and k_5 exists.

3.5.8 The positive feedback of blood clotting cascade

Motif 11 can also be found in our body. When external damage occurs in vasculature, blood clotting cascade initiates with zymogen. Factor X which is first zymogen form positive feedback like motif 10 with Prothrombin II. When both components cleaved, they become activated and help to cleave their counterpart respectively[5].
This positive feedback triggers the formation of the fibrin clotting network.

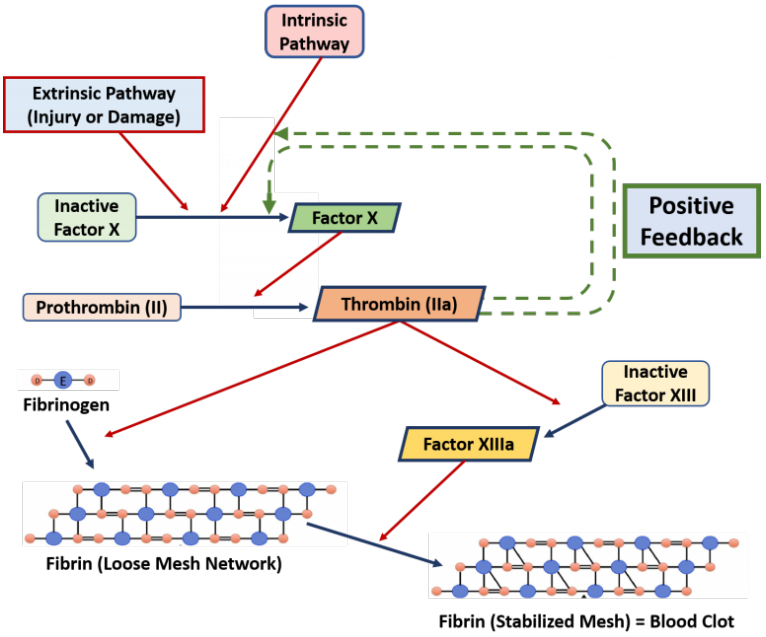


Figure 3.47: The diagram of motif 11 in blood clotting cascade[5].

To check importance of positive feedback in fibrin clotting network, I will presents the comparison between presence and absence of positive feedback loop in blood clotting regulation network.

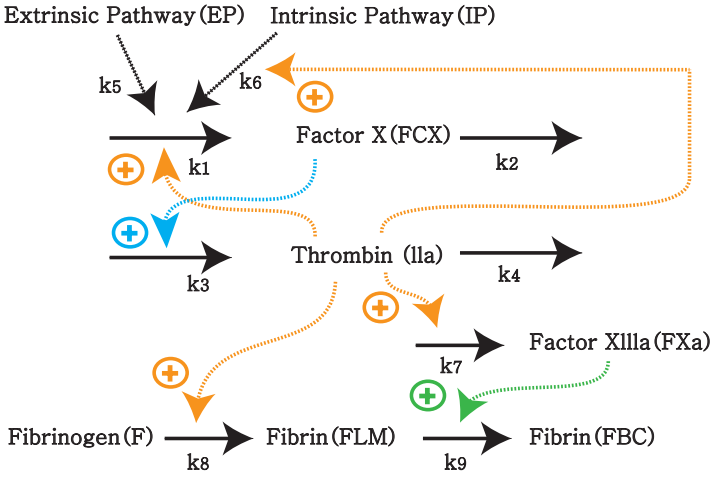


Figure 3.48: The diagram with positive feedback loop in blood clotting cascade.

With this diagram, we can deduce the equations as below. To prevent complexity of calculation, all of activate signals are presented as unsaturated forms.

$$\dot{FCX} = k_1 \cdot k_5 \cdot k_6 \cdot \ IIa^2 \ - FCX \cdot k_2 \tag{3.56}$$

$$\dot{lla} = k_3 \cdot FCX - k_4 \cdot lla \tag{3.57}$$

$$\dot{FXa} = k_7 \cdot lla \tag{3.58}$$

$$\dot{FLM} = k_8 \cdot lla - k_9 \cdot FLM \tag{3.59}$$

$$\dot{FBC} = k_9 \cdot FLM \cdot FXa \tag{3.60}$$

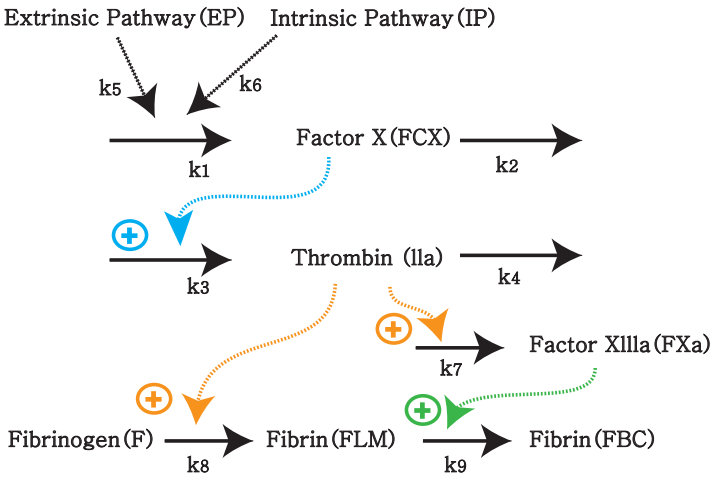


Figure 3.49: The diagram without positive feedback loop in blood clotting cascade.

In case of the absence of positive feedback only $\dot{FCX}$ has different equation as below.

$$\dot{FCX} = k_1 \cdot k_5 \cdot k_6 - FCX \cdot k_2 \tag{3.61}$$

However, this small difference cause significant difference as we can see in Figure 3.50. In presence of motif 11, most of components increase dramatically but in absence of motif 11, increase rate of each components is small or show decrease. With motif 11, stabilized fibrin increase so that blood clotting occurs rapidly. To sum it up, without motif 11, it would take long time or is difficult to prevent blooding. **Graphs and calculation with Fortran are in appendix B to compare the values beteewn Python and Fortran.**

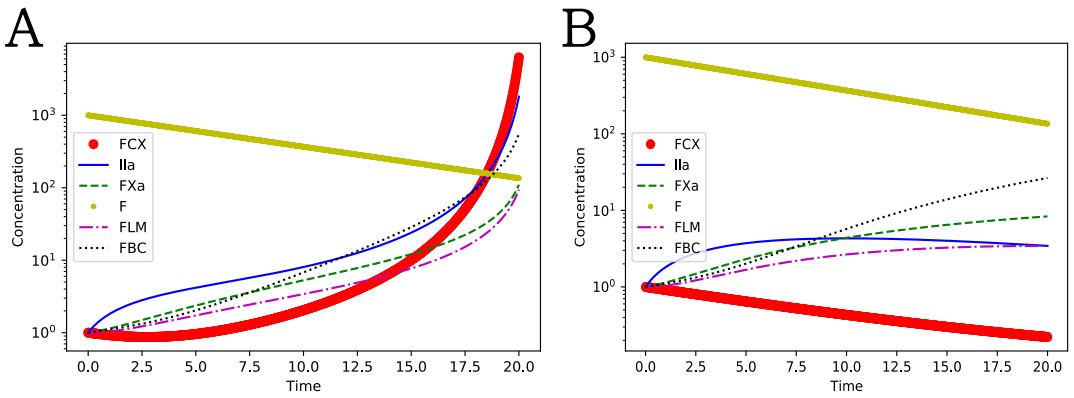


Figure 3.50: The comparison of graphs with and without positive feedback. (A) shows presence of motif 11. All of components except F increase hyperbolically (B) show absence of motif 11. Most of components increased a little. In case of FCX, it was rather decreased. In both graphs, rate constants and initial concentration are same. k_2,k_4,k_5,k_6,k_7,k_8,k_9 are 0.1, k_1,k_3 are 1.0. Initial concentratino of FCX, lla, FXa, F, FLM, FBC are 1,1,1,1000,1,1.

3.6 Motif 10

In motif 10, two elements accelerate the degradation of each counterparts. E enhances k_3 and A enhances k_5. In this motif, steady state and linear growth are typical patterns.

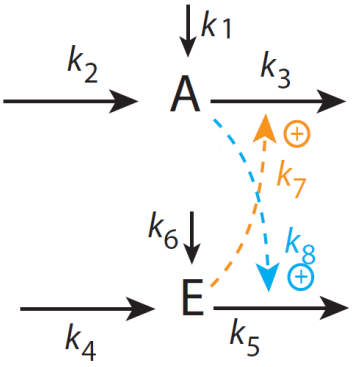


Figure 3.51: The circuit of motif 10.

By circuit of motif 10, we can deduce equation as below.

$$\dot{A} = k_1 + k_2 - k_3 \cdot A\left(\frac{E}{k_7 + E}\right) \tag{3.62}$$

$$\dot{E} = k_6 + k_4 - k_5 \cdot E\left(\frac{A}{k_8 + A}\right) \tag{3.63}$$

3.6.1 Linear growth

As Motif 16, Motif 10 also shows linear growth when k_3, k_5 are zero.

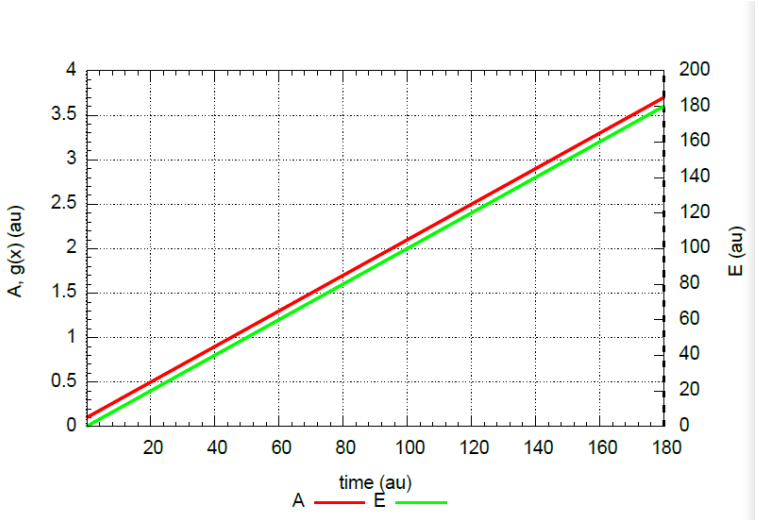


Figure 3.52: Linear growth when k_1, k_2 are 0.01 and k_3, k_5, k_6 are zero while, k_4 are zero. k_7, k_8 are 0.1. The initial concentration of A and E is 0.1.

3.6.2 Steady state

If k_3, k_5 are not zero, regardless of what the other constant rate have values, it become steady state.

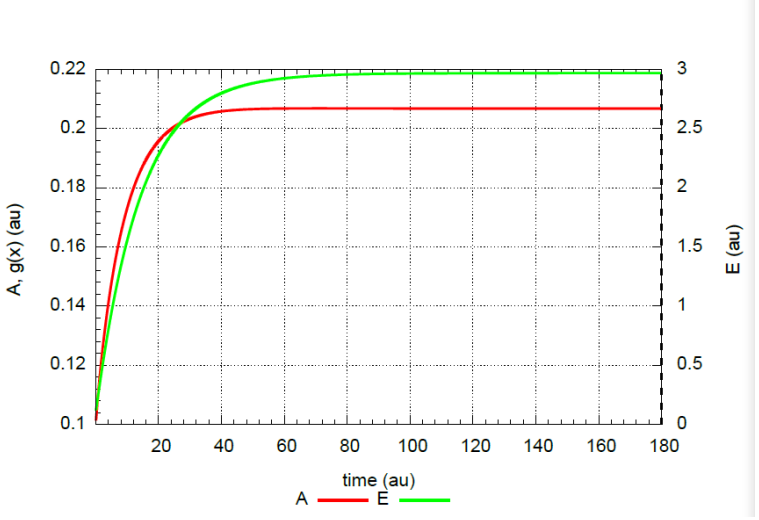


Figure 3.53: Steady state when k_3,k_4,k_5,k_6,k_7,k_8:0.1 and k_1, k_2: 0.01. The initial concentration of A and E is 0.1.

3.6.3 Combination of motif 10 and motif 13 in mitotic entry cycle

Motif 10 can be found in combination with motif 13 for cell cycling regulation. In the cell division cycle, circular sequence of four phases: G1 (un-replicated chromosomes) to S (DNA synthesis) to G2 (replicated chromosomes) to M (mitosis) and back to G1 [6]. Among them, G2 to M transition is irreversible due to bi-stability, which is actually governed by Wee1-Cdk1-Cdc25 system[28]. They are made of motif 10 and motif 13 loops.

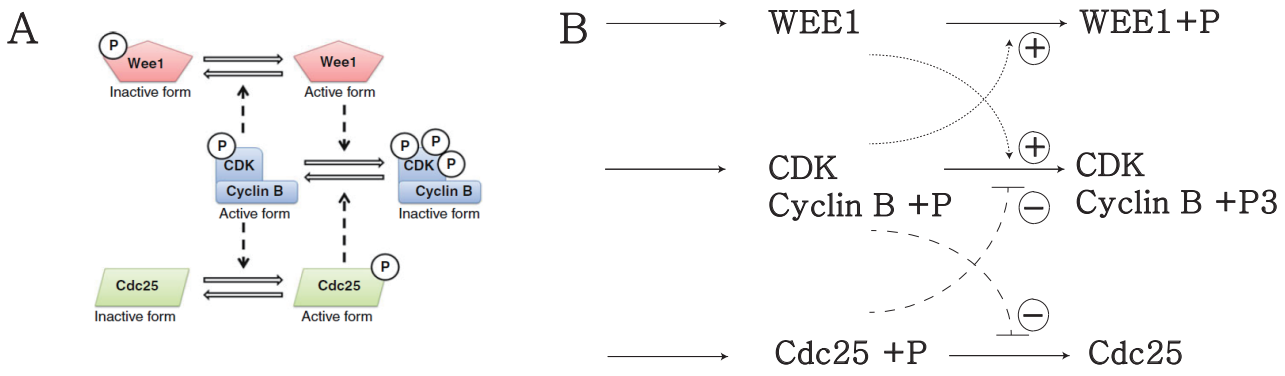


Figure 3.54: Diagram of Wee1-Cdk1-Cdc25 system. (A)Wee1-Cdk1-Cdc25 activated or inactivated by phosporyation [6][7] [8]. (B) In (A), active form Wee1 activate the conversion of active form CDK-Cyclin B to inactivate form. Activate form CDK-Cyclin B activates the conversion of inactivate form Cdc25 to active form. Activate form Cdc25 activates the conversion of inactivate form CDK-Cyclin B to activate. Through alignment, motif 10 and motif 13 are identified clearly.

WEE1 and CDK are active forms and they lead counterpart to become inactive like motif 10. In case of relationship between CDK and Cdc25, They make counterpart to become active form each other like motif 13.

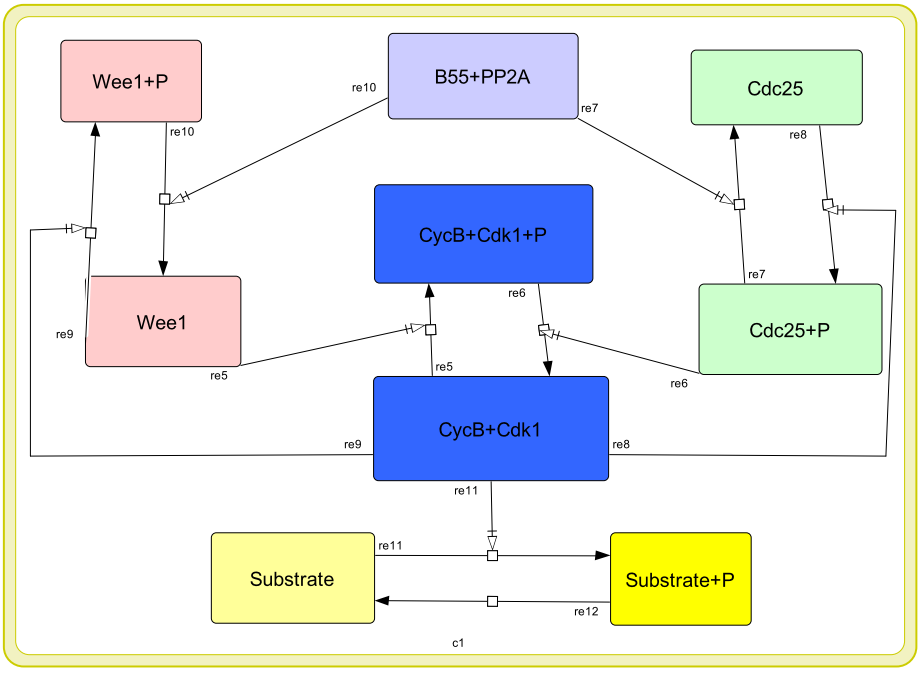


Figure 3.55: Diagram made by CellDesigner program. Mitosis of cell is regulated by phosphoryation by Cdk1-CycB and dephosphoryation of PP2A-B55 through mitotic substrate (Yellow)[9][10].

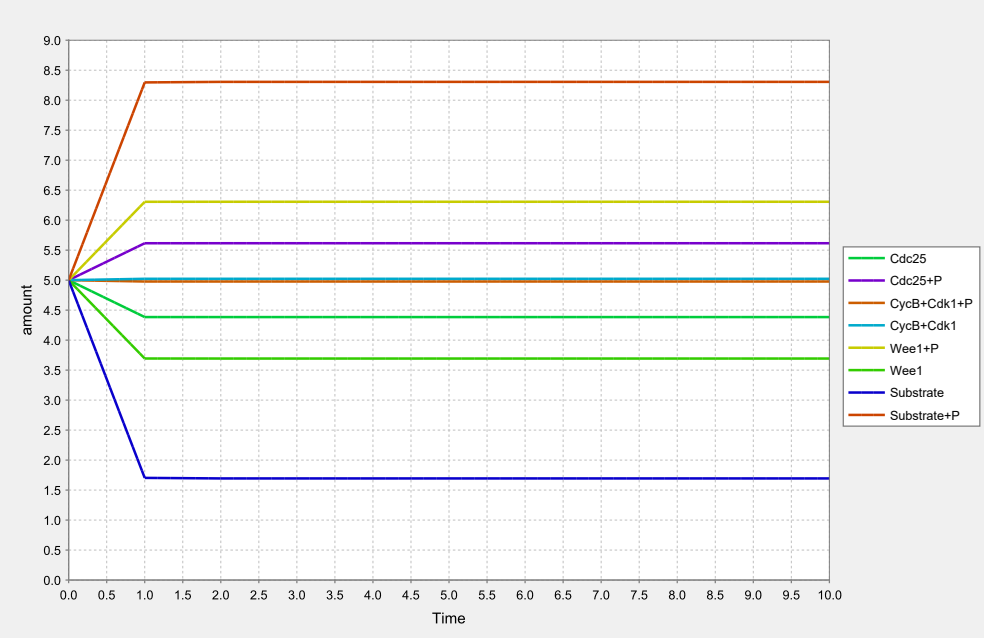


Figure 3.56: Mitosis stage when phosphoryated substrate increase. This graph shows result of increase when Wee1-Cdk1-Cdc25 loops activate mitotic substrate. The initial concentration of all components is 5.0. There is no degradation rate. Thus, both activate and inactivate form were switched by kinetic law in Celldesigner.

Through this combined motif, phosphoryated substrate increase, which lead mitotic entry. **In appendix C, calculation and graph for Wee1-Cdk1-Cdc25 bistable swtich were implemented by Fortran.**

3.7 Motif 9

In case of motif 9, it shows that A activate input of E, while E represses degradation of A for positive feedback so that as time passes, they increase each other.

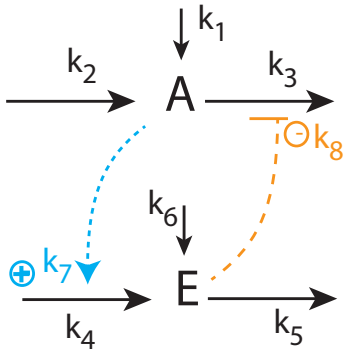


Figure 3.57: Circuit of motif 9.

By circuit of motif 9, we can deduce equations as below.

$$\dot{A} = k_1 + k_2 - k_3 \cdot A \left(\frac{k_8}{k_8 + E} \right) \tag{3.64}$$

$$\dot{E} = k_6 + k_4 \left(\frac{A}{k_8 + A} \right) - k_5 \cdot E \tag{3.65}$$

3.7.1 Switchable with Motif 15

As I mentioned in introduction, motif 9 can be transformed into motif 15, when A is substituted by E, and E by A. We can identify their similarity. This Figure 3.58 proves that two motifs act equally with same constant rate.

Table 3.5: The rate constants for Figure 3.51. The blue color of C1, C2 represents the increase of the input and the yellow and the green color represents the degradation rates.

Graph	Motif	k1	k2	k3	k4	k5	k6	k7	k8
A1	9	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
A2	15	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
B1	9	0.1	0.1	0	0.1	0	0.1	0.1	0.1
B2	15	0.1	0.1	0	0.1	0	0.1	0.1	0.1
C1	9	0.3	0.3	0.01	0.1	0.1	0.1	0.1	0.1
C2	15	0.1	0.1	0.1	0.3	0.01	0.3	0.1	0.1

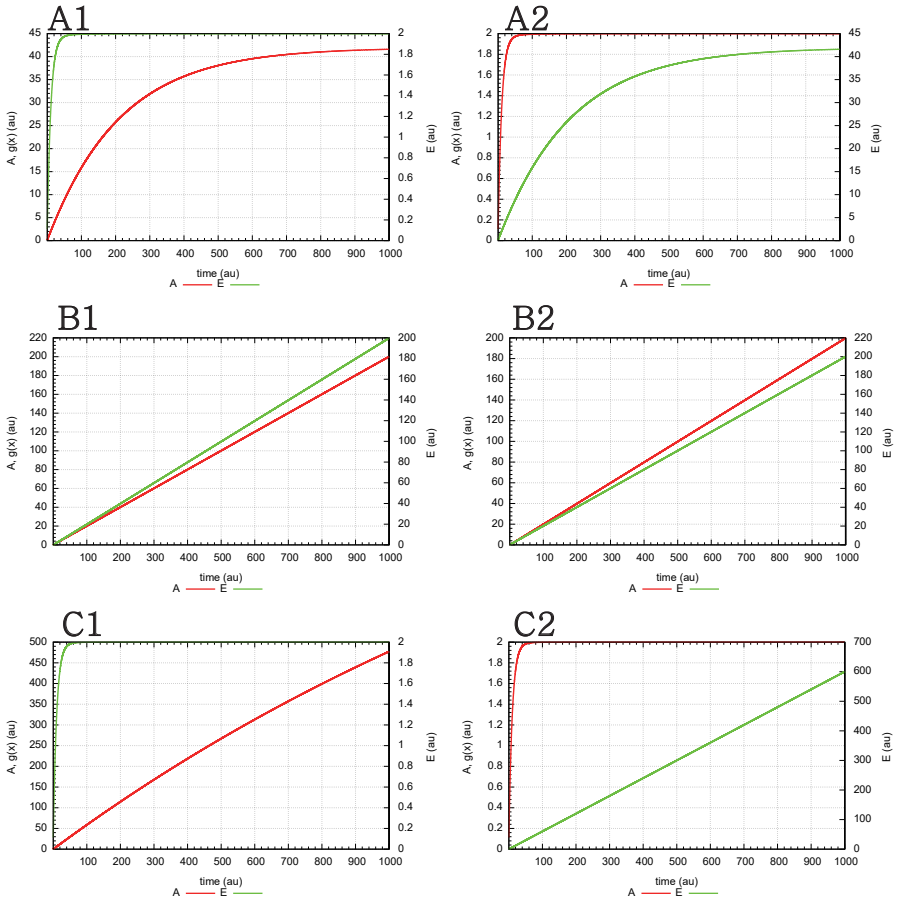


Figure 3.58: Graphs of motif 9 and motif 15. Both side graphs show complementary symmetery. Rate constants are given in Table 3.5. The initial concentration of A and E in all graphs is 0.1.

These graphs present that A and E in both motif 9 and motif 15 are oppositely same.

3.8 Comparison of single motifs

We have, so far, confirmed specific traits in each motifs respectively. Now I will compare these motifs to identify difference more in detail. With same constant rates which is 0.1, motif 16, 15, 11, 10, 9 show steady state, while motif 13 increase linearly. In case of motif 15, A become steady state faster than E.

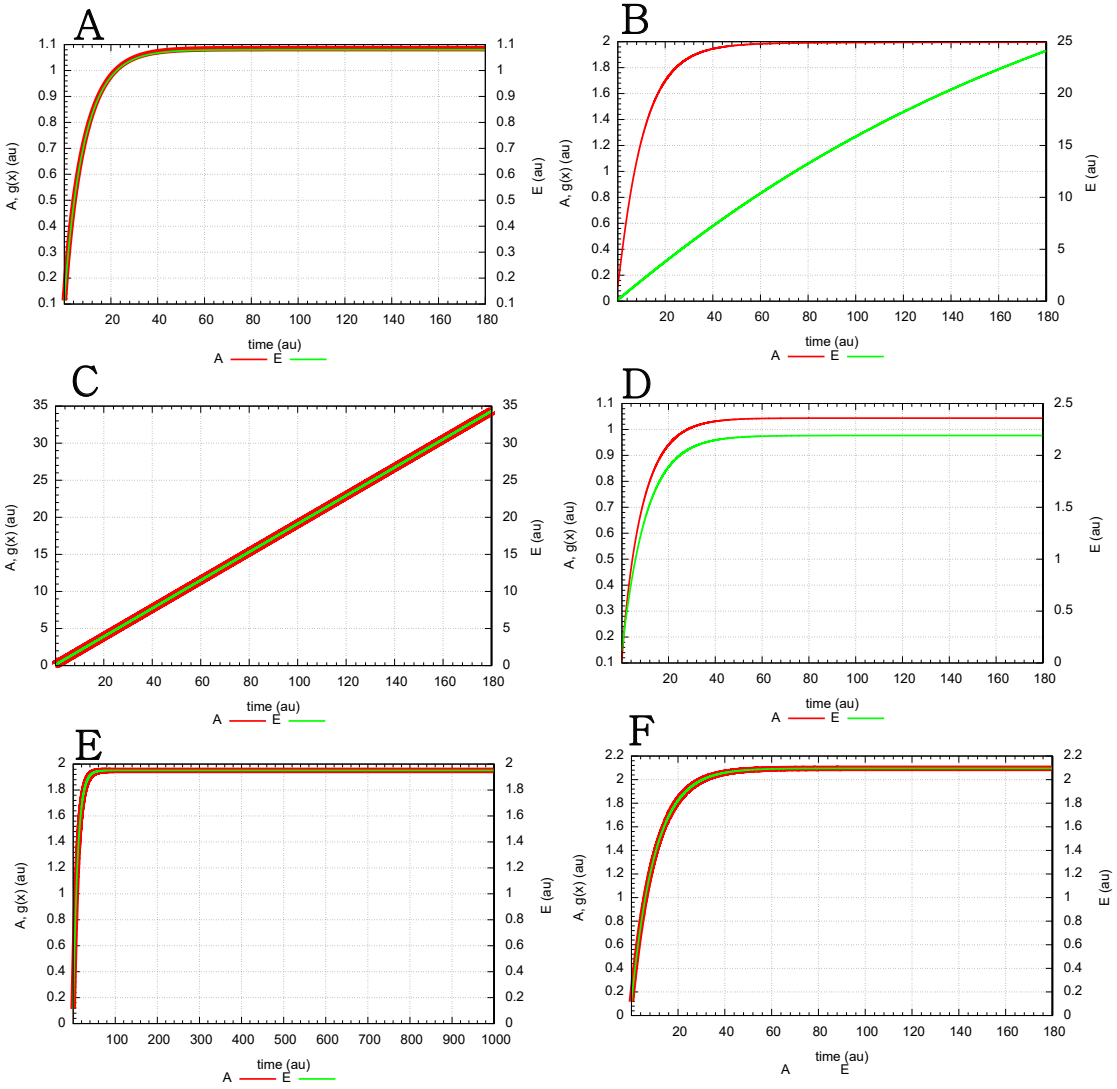


Figure 3.59: Different graphs with same rate constants in A:motif 16, B:motif 15, C:motif 13, D:motif 12, E:motif 11, F:motif 10. k_1,k_2,k_3,k_4,k_5,k_6,k_7,k_8 all of rate constants are 0.1. The initial concentration of A and E in all graphs is 0.1.

In FIgure 3.60, when k_4 are 1.0 and the other rate constants are less than k_4, while degradation rates, k_3, k_5, are zero, as we can see, all of motif present linear growth except E in motif 16 increase logarithmically.

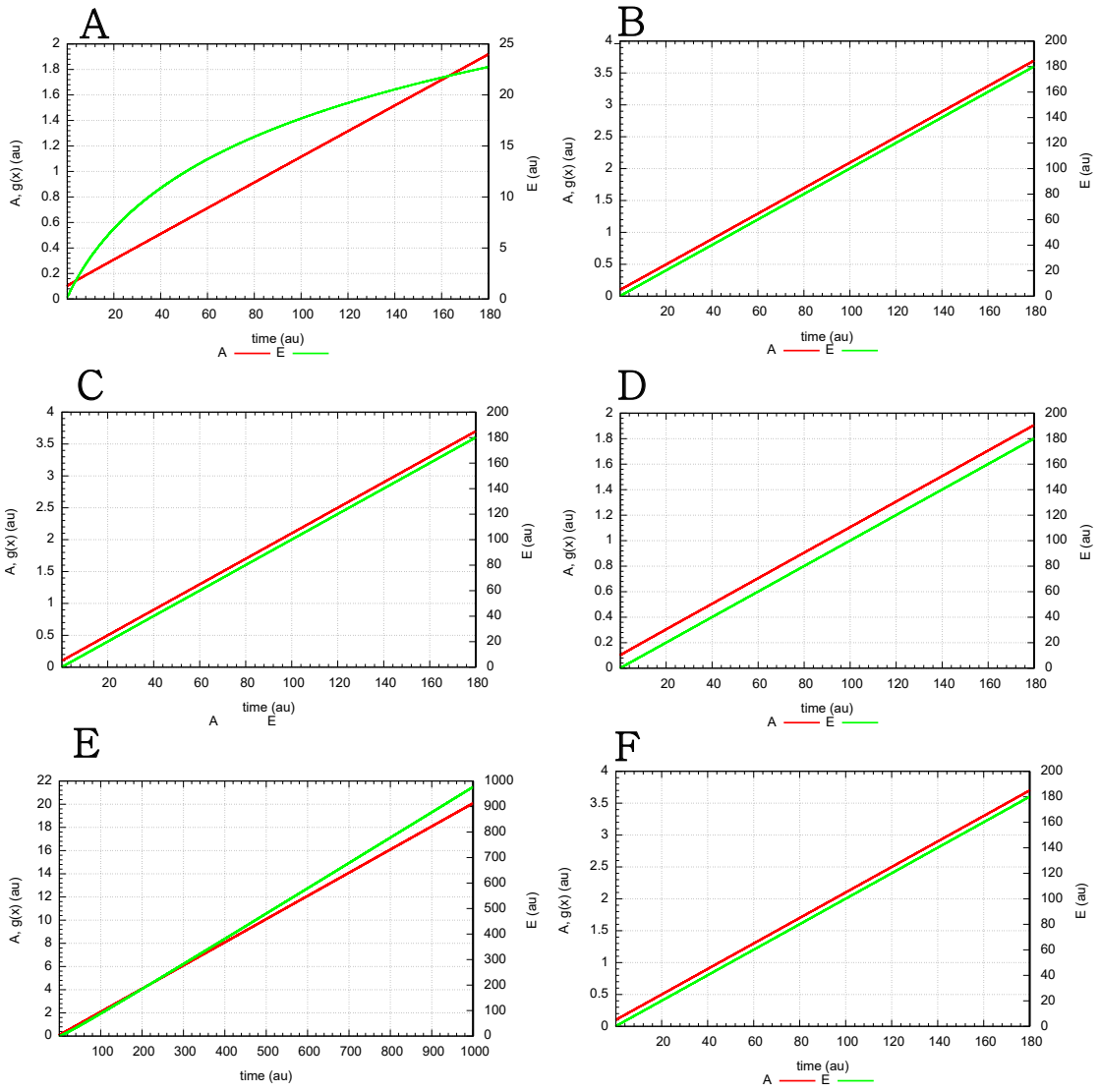


Figure 3.60: Different graphs with same rate constants in A:motif 16, B:motif 15, C:motif 13, D:motif 12, E:motif 11, F:motif 10. k_1,k_2,k_6,k_7,k_8 are 0.1 and k_4 is 1.0, degradation rate k_3,k_5 are zero. The initial concentration of A and E in all graphs is 0.1.

When only one of degradation, k_3 is zero and input rate constant of A is bigger than the other, motif 16, 11,10 and 9 show steady state of E and linear increase of A. Contrary to this, motif 13 and motif 15 present both linear growth in graphs.

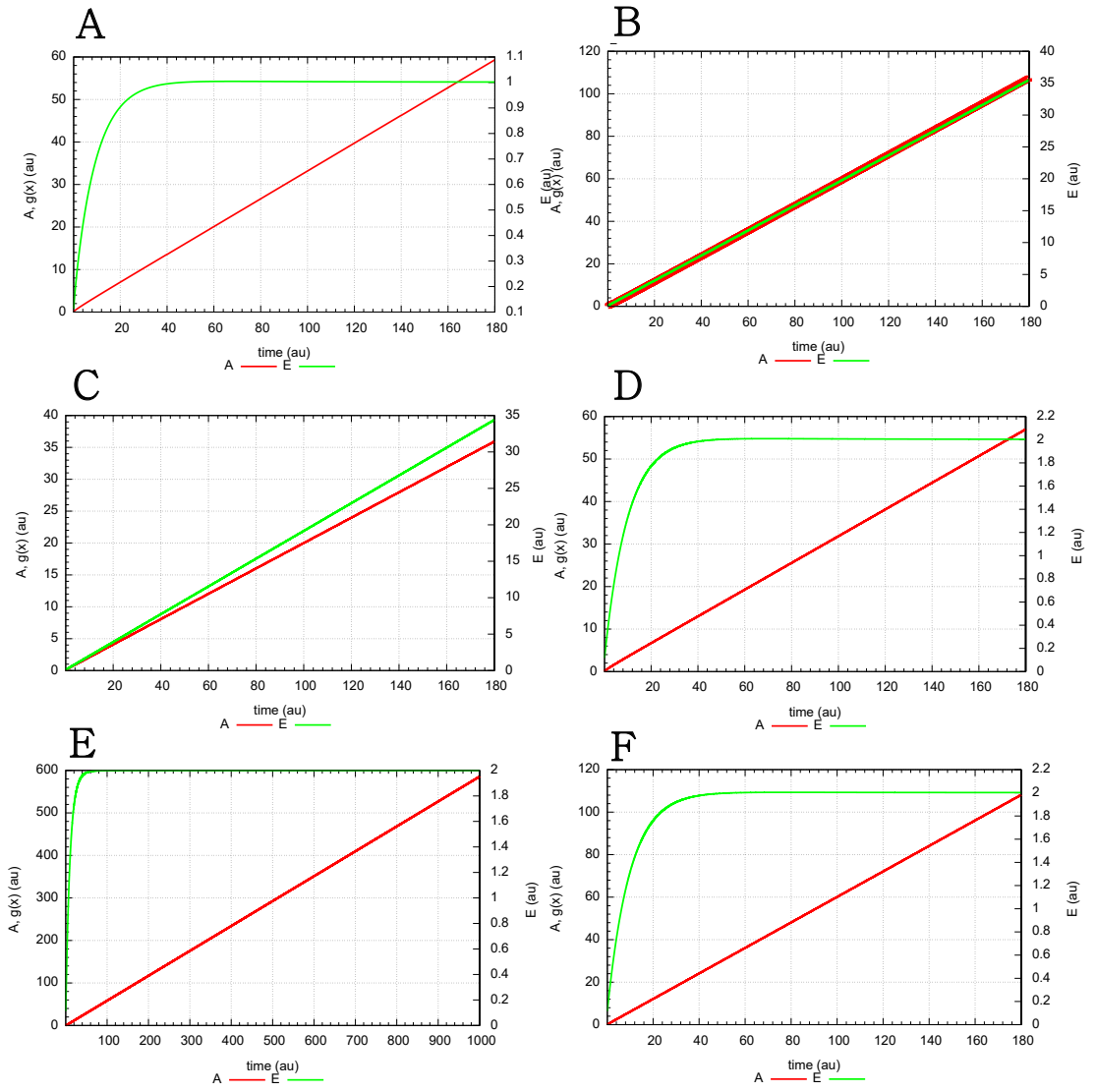


Figure 3.61: Different graphs with same rate constants in A:motif 16, B:motif 15, C:motif 13, D:motif 12, E:motif 11, F:motif 10. k_4,k_5,k_6,k_7,k_8 are 0.1 and k_1,k_2 are 0.3, k_3 is zero. The initial concentration of A and E in all graphs is 0.1.

With same constant rate, each motifs have their own values and tendency to correspond to specific condition. Motif 16 only presents logarithmic growth. In case of motif 13, most of graphs shows linear increase. In addition, even though tendency of graphs between motifs are same, each value are totally different. It is one of important reasons why we should consider positive feedback divided in detail.

3.9 Combined identical motifs

Let's think about if each motifs united with same motif. The combined identical motifs can either enhance or repress the reaction than single motifs. To be sure, they

display remarkably different shape of graphs, even though all of constant rate are same with single motifs. Schemes and equations for combined motifs are as below.

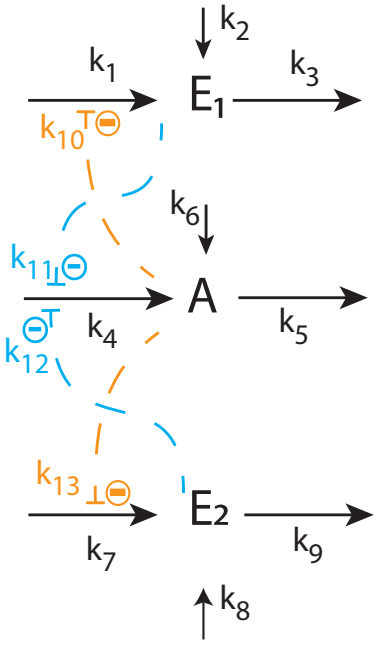


Figure 3.62: The scheme of coupled identical motif 16.

$$\dot{A} = k_4 \cdot \left(\frac{k_{11}}{k_{11} + E_1}\right) \cdot \left(\frac{k_{12}}{k_{12} + E_2}\right) + k_6 - k_5 \cdot A \tag{3.66}$$

$$\dot{E_1} = k_1 \cdot \left(\frac{k_{10}}{k_{10} + A}\right) + k_2 - k_3 \cdot E_1 \tag{3.67}$$

$$\dot{E_2} = k_7 \cdot \left(\frac{k_{13}}{k_{13} + A}\right) + k_8 - k_9 \cdot E_2 \tag{3.68}$$

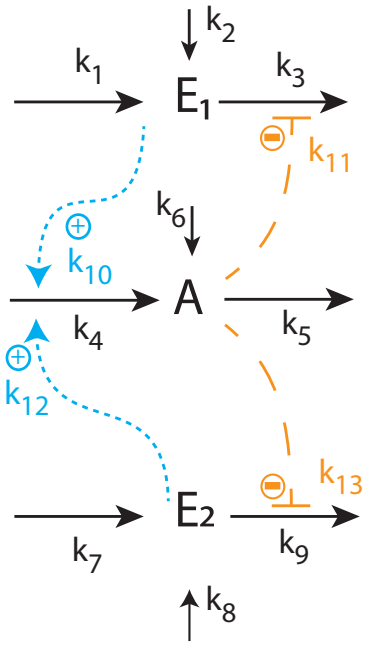


Figure 3.63: The scheme of coupled identical motif 15.

$$\dot{A} = k_4 \cdot \left(\frac{E_1}{k_{10} + E_1}\right) \cdot \left(\frac{E_2}{k_{12} + E_2}\right) + k_6 - k_5 \cdot A \tag{3.69}$$

$$\dot{E_1} = k_1 + k_2 - k_3 \cdot E_1 \left(\frac{k_{11}}{k_{11} + A}\right) \tag{3.70}$$

$$\dot{E_2} = k_7 + k_8 - k_9 \cdot E_2 \left(\frac{k_{13}}{k_{13} + A}\right) \tag{3.71}$$

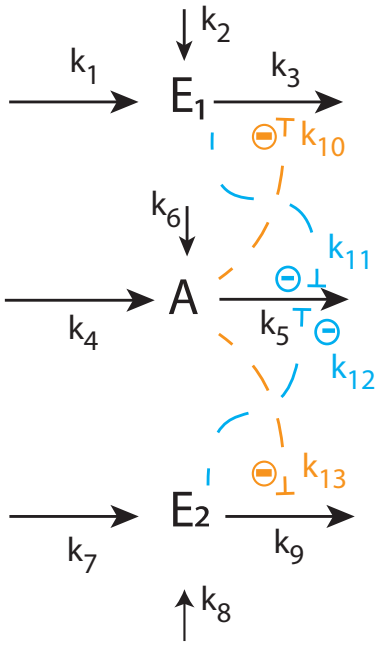


Figure 3.64: The scheme of coupled identical motif 13.

$$\dot{A} = k_4 + k_6 - k_5 \cdot A \cdot \left(\frac{k_{11}}{k_{11} + E_1}\right) \cdot \left(\frac{k_{12}}{k_{12} + E_2}\right) \tag{3.72}$$

$$\dot{E_1} = k_1 + k_2 - k_3 \cdot E_1 \left(\frac{k_{10}}{k_{10} + A}\right) \tag{3.73}$$

$$\dot{E_2} = k_7 + k_8 - k_9 \cdot E_2 \left(\frac{k_{13}}{k_{13} + A}\right) \tag{3.74}$$

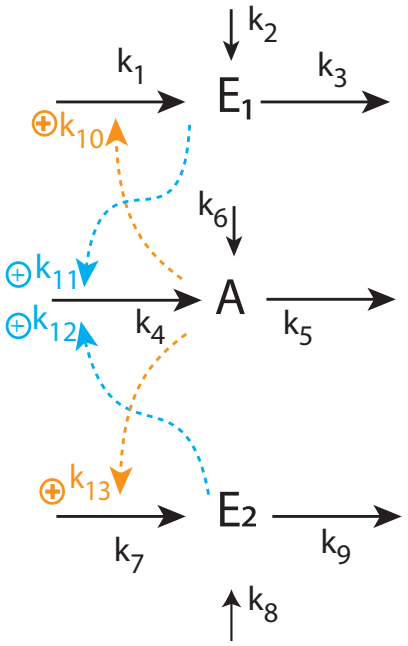


Figure 3.65: The scheme of coupled identical motif 11.

$$\dot{A} = k_4 \cdot \left(\frac{E_1}{k_{11} + E_1}\right) \cdot \left(\frac{E_2}{k_{12} + E_2}\right) + k_6 - k_5 \cdot A \tag{3.75}$$

$$\dot{E_1} = k_1 \left(\frac{A}{k_{10} + A}\right) + k_2 - k_3 \cdot E_1 \tag{3.76}$$

$$\dot{E_2} = k_7 \left(\frac{A}{k_{13} + A}\right) + k_8 - k_9 \cdot E_2 \tag{3.77}$$

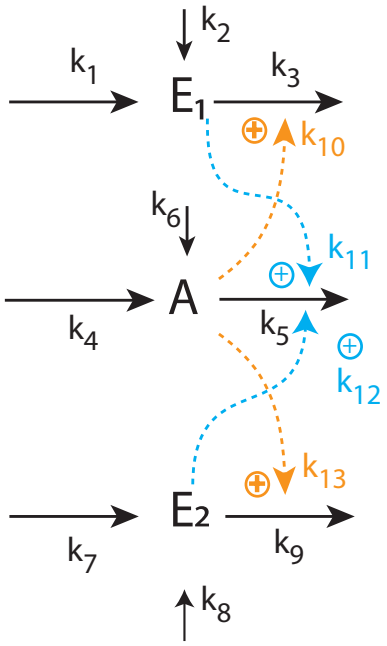


Figure 3.66: The scheme of coupled identical motif 10.

$$\dot{A} = k_4 + k_6 - k_5 \cdot A \cdot \left(\frac{E_1}{k_{11} + E_1}\right) \cdot \left(\frac{E_2}{k_{12} + E_2}\right) \tag{3.78}$$

$$\dot{E_1} = k_1 + k_2 - k_3 \cdot E_1 \left(\frac{A}{k_{10} + A}\right) \tag{3.79}$$

$$\dot{E_2} = k_7 + k_8 - k_9 \cdot E_2 \left(\frac{A}{k_{13} + A}\right) \tag{3.80}$$

From these equation, we can identify how components change with specific constants rate and then compare graphs of each motifs.

In case of combined identical motif 12, refer to Figure 3.28 and equations 3.32 to 3.34. **Before that, I put number 2 in front of each motif to present coupled identical motif.**

In Figure 3.67, states of combined motifs are similar to those of single motifs. For example, most of motifs show steady state except C in Figure 3.67 shows linear growth as motif 13. However, there is a difference when it comes to increased concentration. Unlike single motif, components of combined motifs can increase or decrease further depending on how the motifs are connected.

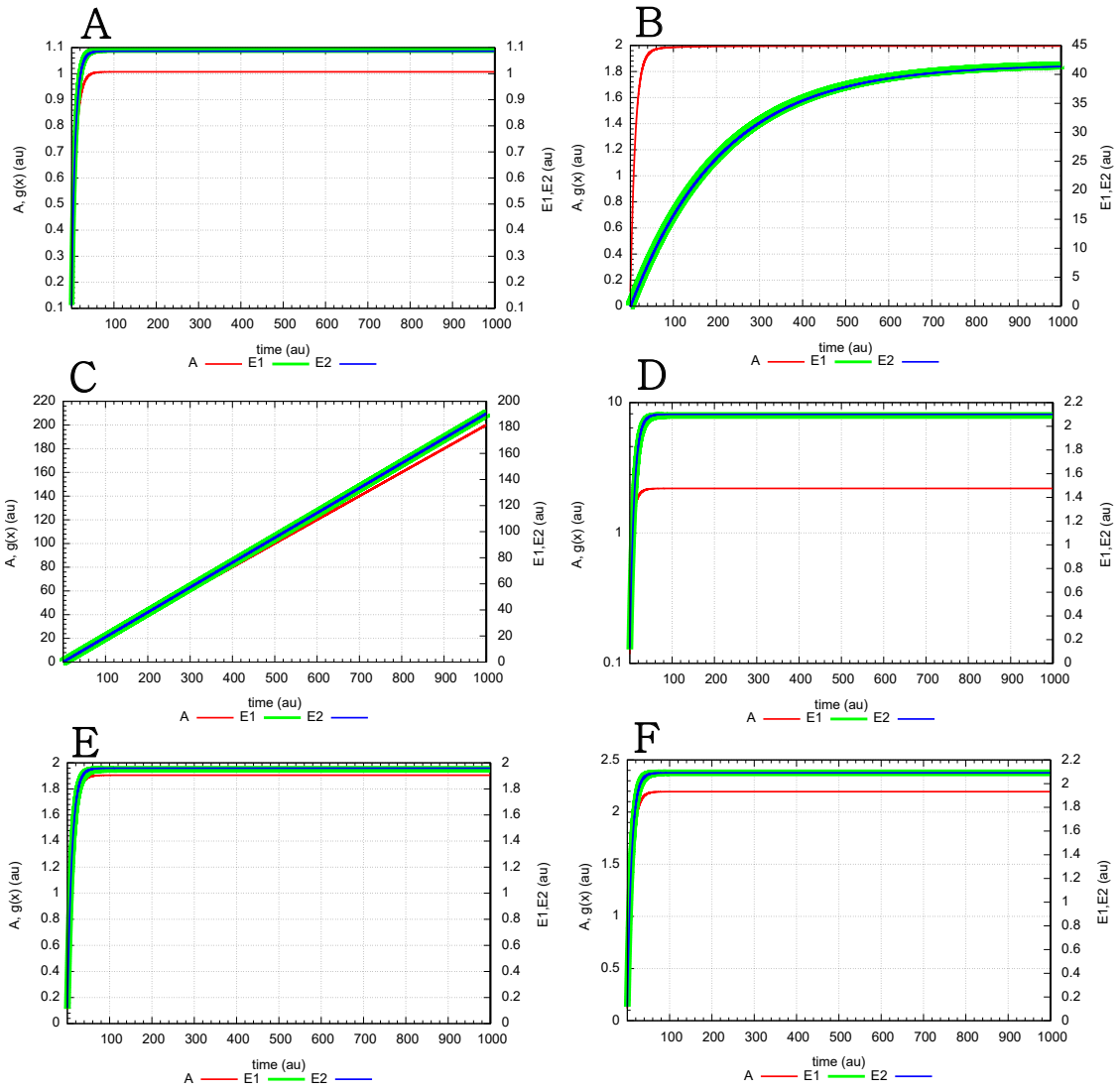


Figure 3.67: Different patterns with same rate constants In A:2motif 16, B:2motif 15, C:2motif 13, D:2motif 12, E:2motif 11, F:2motif 10. k_1,k_2,k_3,k_4,k_5,k_6,k_7,k_8,k_9,k_{10},k_{11},k_{12},k_{13} all of rate constants are 0.1. The initial concentration of A and E in all graphs is 0.1.

In Figure 3.68, various reactions occur when input rates, k_1 and k_2, of E_1 increase. Expectedly, E_1 records the highest values among components.

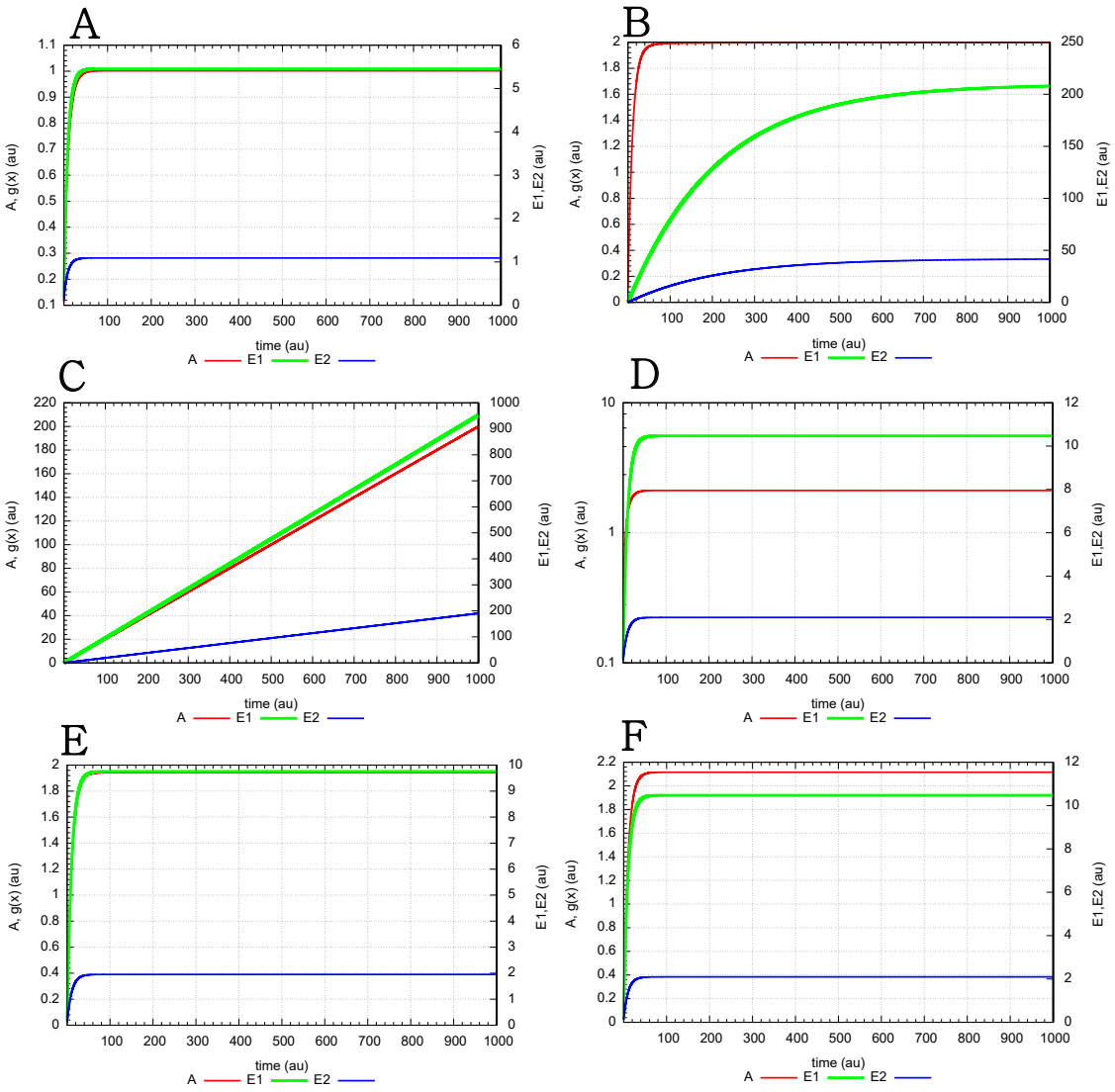


Figure 3.68: Different patterns with same rate constants In A:2motif 16, B:2motif 15, C:2motif 13, D:2motif 12, E:2motif 11, F:2motif 10. k_3,k_4,k_5,k_6,k_7,k_8, k_9,k_{10},k_{11},k_{12},k_{13} are 0.1 and k_1,k_2 are 0.5. The initial concentration of A and E in all graphs is 0.1.

In Figure 3.69, various reactions occur when inhibition or activation rate in each combined motifs increase.

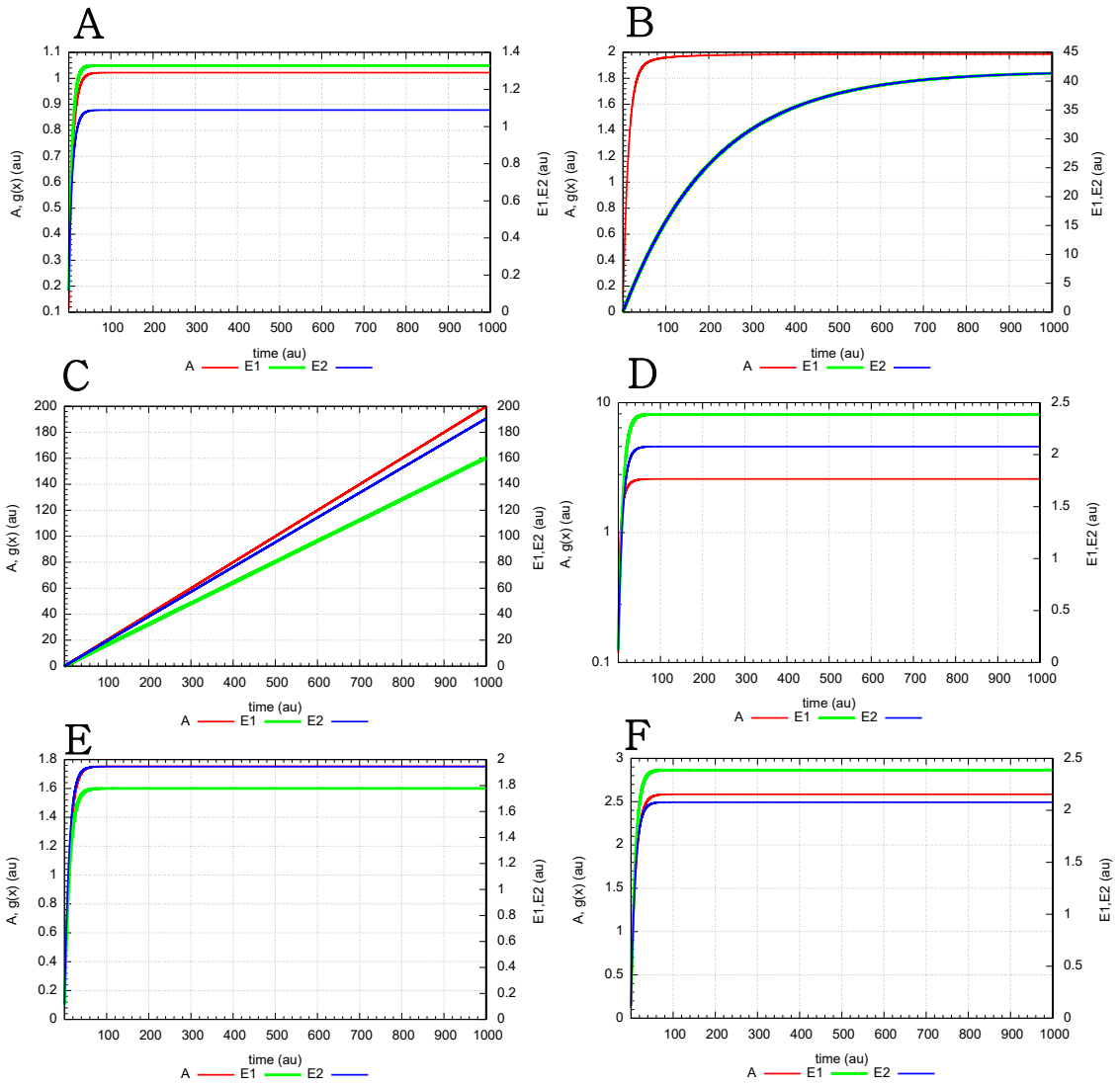


Figure 3.69: Different patterns with same rate constants In A:2motif 16, B:2motif 15, C:2motif 13, D:2motif 12, E:2motif 11, F:2motif 10 k_1,k_2,k_3,k_4,k_5,k_6,k_7,k_8,k_9,k_{11},k_{13} are 0.1 and k_{10},k_{12} are 0.5. The initial concentration of A and E in all graphs is 0.1.

The combined identical motifs show not only similarity with their own single motifs but also different patterns with them. Concentration of components in double motifs whether more increase or decrease depending on combination, indicating that motifs can enhance or represent their trait through interaction of same motifs.

3.10 Combined different motifs

The motifs can combine the other motifs so that they react and adjust for various stresses. In nature, as already shown former examples, some motifs do not exist alone,

instead they are linked with the other motifs. Among motifs I have studied, I combined motif 16 and the other motifs to identify traits of combined motifs.

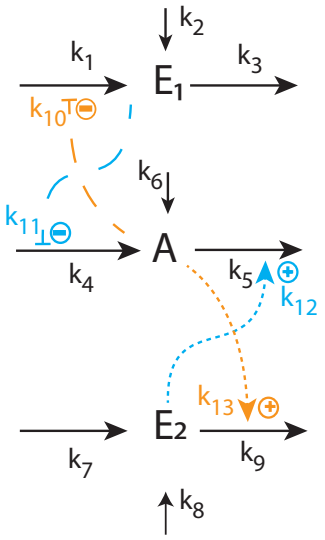


Figure 3.70: Circuit of mixed different motif 16-10.

By circuit of combined motif 16-10, we can deduce equations as below.

$$\dot{A} = k_4 \cdot \left(\frac{k_{11}}{k_{11} + E_1}\right) + k_6 - k_5 \cdot A \cdot \left(\frac{E_2}{k_{12} + E_2}\right) \tag{3.81}$$

$$\dot{E_1} = k_1 \cdot \left(\frac{k_{10}}{k_{10} + A}\right) + k_2 - k_3 \cdot E_1 \tag{3.82}$$

$$\dot{E_2} = k_7 + k_8 - k_9 \cdot E_2 \cdot \left(\frac{A}{k_{13} + A}\right) \tag{3.83}$$

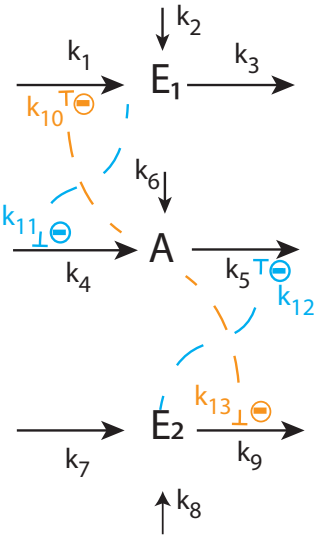


Figure 3.71: Circuit of mixed different motif 16-13.

By circuit of combined different motif 16-13, we can deduce equations as below.

$$\dot{A} = k_4 \cdot \left(\frac{k_{11}}{k_{11} + E_1}\right) + k_6 - k_5 \cdot A \cdot \left(\frac{k_{12}}{k_{12} + E_2}\right) \tag{3.84}$$

$$\dot{E_1} = k_1 \cdot \left(\frac{k_{10}}{k_{10} + A}\right) + k_2 - k_3 \cdot E_1 \tag{3.85}$$

$$\dot{E_2} = k_7 + k_8 - k_9 \cdot E_2 \cdot \left(\frac{k_{13}}{k_{13} + A}\right) \tag{3.86}$$

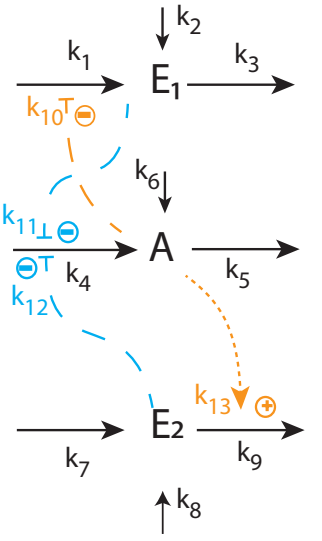


Figure 3.72: Circuit of mixed different motif 16-12.

By circuit of combined different motif 16-12, we can deduce equations as below.

$$\dot{A} = k_4 \cdot \left(\frac{k_{11}}{k_{11} + E_1}\right) \cdot \left(\frac{k_{12}}{k_{12} + E_2}\right) + k_6 - k_5 \cdot A \tag{3.87}$$

$$\dot{E_1} = k_1 \cdot \left(\frac{k_{10}}{k_{10} + A}\right) + k_2 - k_3 \cdot E_1 \tag{3.88}$$

$$\dot{E_2} = k_7 + k_8 - k_9 \cdot E_2 \cdot \left(\frac{A}{k_{13} + A}\right) \tag{3.89}$$

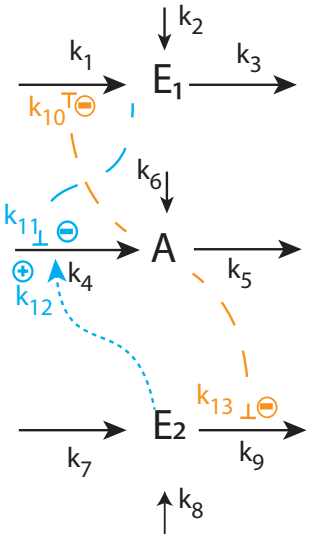


Figure 3.73: Circuit of mixed different motif 16-15.

By circuit of combined motif 16-15, we can deduce equations as below.

$$\dot{A} = k_4 \cdot \left(\frac{k_{11}}{k_{11} + E_1}\right) \cdot \left(\frac{E_2}{k_{12} + E_2}\right) + k_6 - k_5 \cdot A \tag{3.90}$$

$$\dot{E_1} = k_1 \cdot \left(\frac{k_{10}}{k_{10} + A}\right) + k_2 - k_3 \cdot E_1 \tag{3.91}$$

$$\dot{E_2} = k_7 + k_8 - k_9 \cdot E_2 \cdot \left(\frac{k_{13}}{k_{13} + A}\right) \tag{3.92}$$

Through this mixed different motifs, I compiled them with different constant rates. Among them, I will describe fraction of them, which present characteristic shapes in combined different motifs.

To represent mixed motif with motif 16, I put motif number behind of motif16 -. For instance, if motif 13 combined with motif 16, it is motif 16-13.

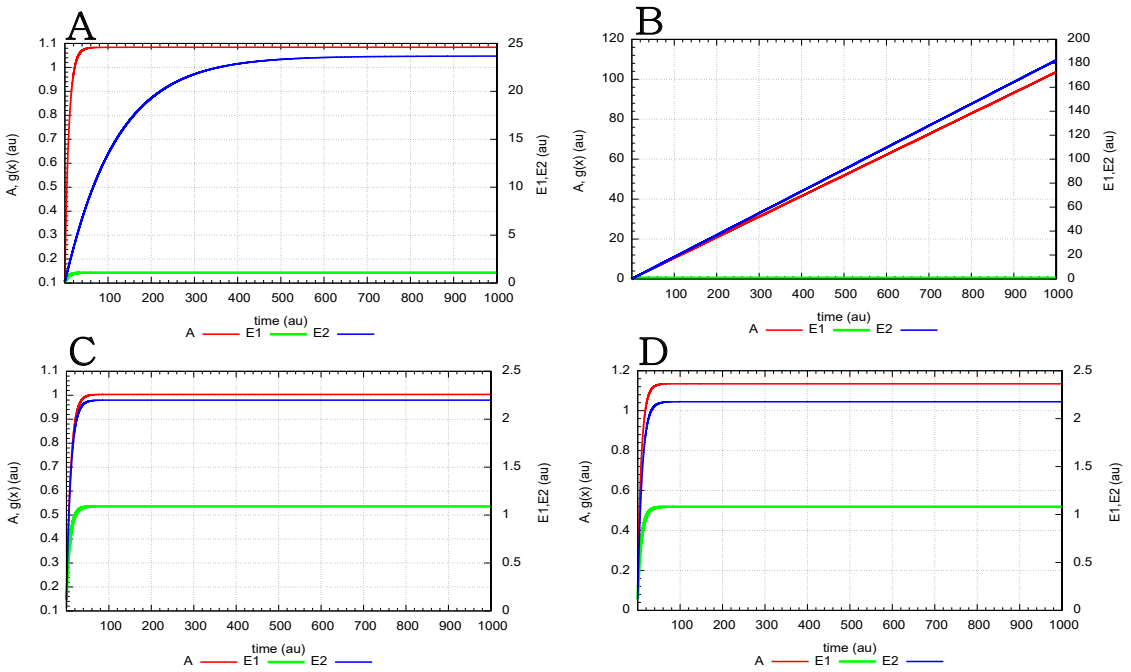


Figure 3.74: Different patterns with same rate constants in A:motif16-15, B:motif16-13, C:motif16-12, D:motif16-10.
k_1,k_2,k_3,k_4,k_5,k_6,k_7,k_8,k_9,k_{10},k_{11},k_{12},k_{13} all of rate constants are 0.1. The initial concentration of A and E in all graphs is 0.1.

In Figure 3.74, this mixed motif shows different traits which each single motifs have. All of E_1 record the lowest value because E_1 is directly impacted by motif 16. In case of E_2 and A, they are changed by which motif combine to motif 16.

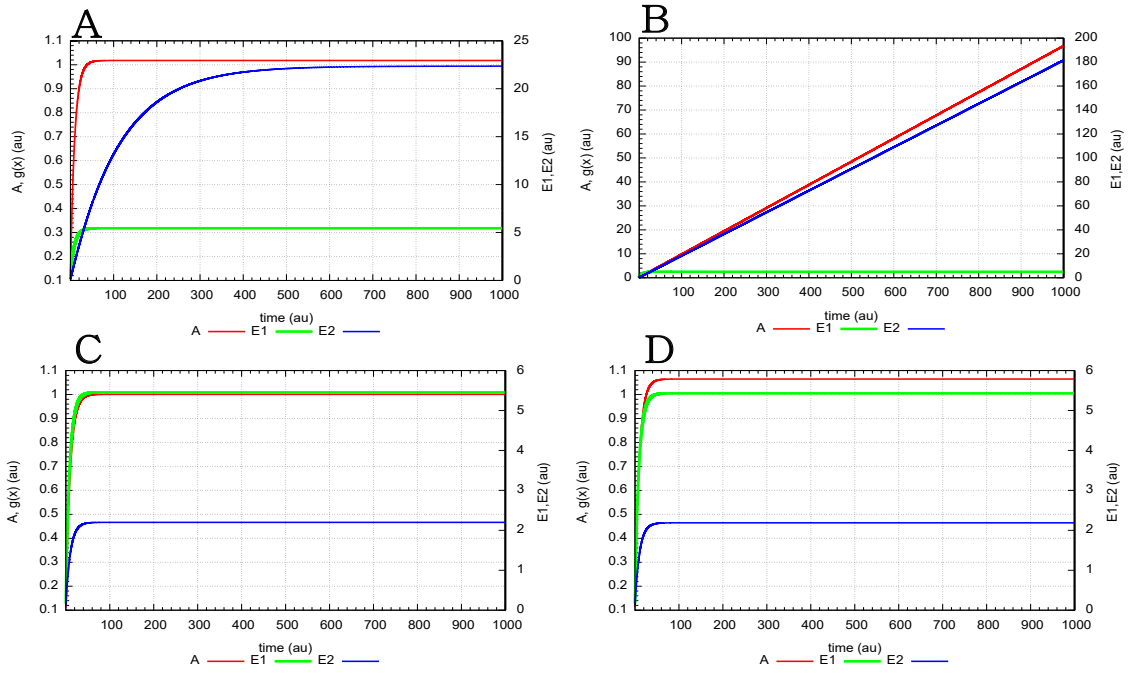


Figure 3.75: Different patterns with same rate constants in A:motif16-15, B:motif16-13, C:motif16-12, D:motif16-10.
k_3,k_4,k_5,k_6,k_7,k_8,k_9,k_{10},k_{11},k_{12},k_{13} are 0.1 and k_1,k_2 are 0.5. The initial concentration of A and E in all graphs is 0.1.

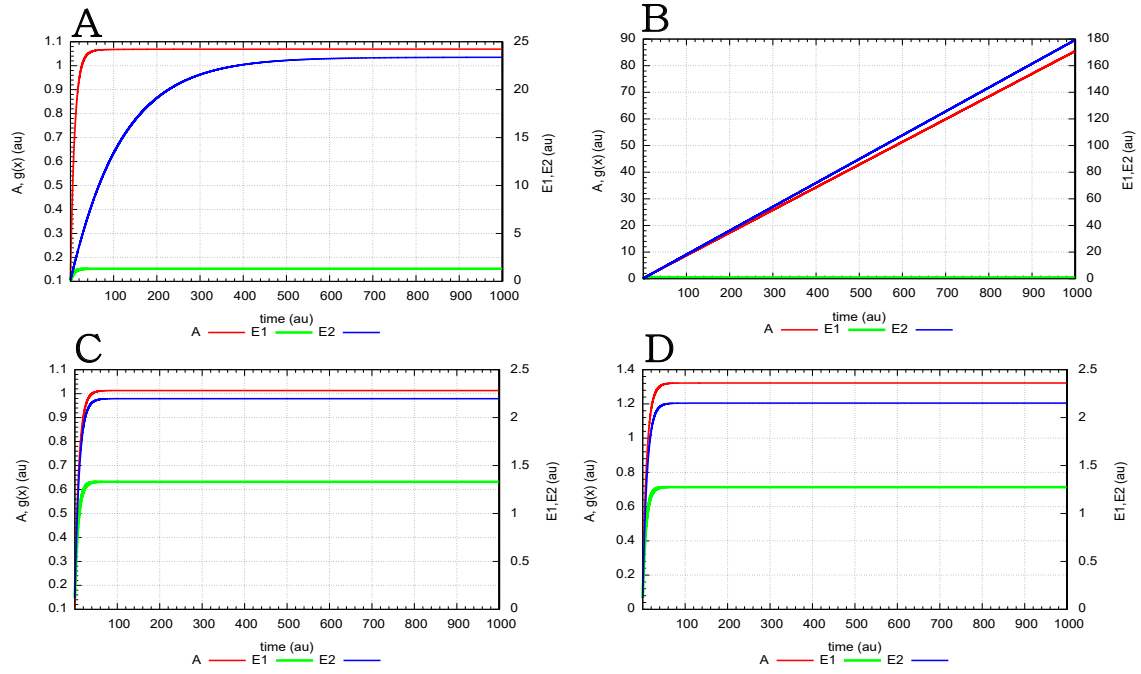


Figure 3.76: Different patterns with same rate constants in A:motif16-15, B:motif16-13, C:motif16-12, D:motif16-10. k_1,k_2, k_3,k_4,k_5,k_6,k_7,k_8,k_9,k_{11},k_{13} are 0.1 and k_{10},k_{12} are 0.5. The initial concentration of A and E in all graphs is 0.1.

The mixed motifs with motif 16 show different patterns with those of combined identical motifs else. Theses new combinations create inventive traits and react differently with same constant rates. In doing so, Positive feedback can retain diversity to cope with various environmental changes. Apart from motif 16, If the another motif collaborates the others, particular changes would occur.

3.11 Comparison of saturated and unsaturated mixed motifs

In the front, we observed changes of mixed motif. However, when these motifs get unsaturated, another behaviors are expected to appear. **As expained earlier, the same nomenclature was applied. Additionally, u and u2 are used. u means one of activation rate become unsaturated. Likewise, u2 means two of activation rate become unsaturated in combined motifs.**

Unlike saturated motif 16-10, this time I removed k_{12} to make E_2 unsaturated. The scheme and equation are as below.

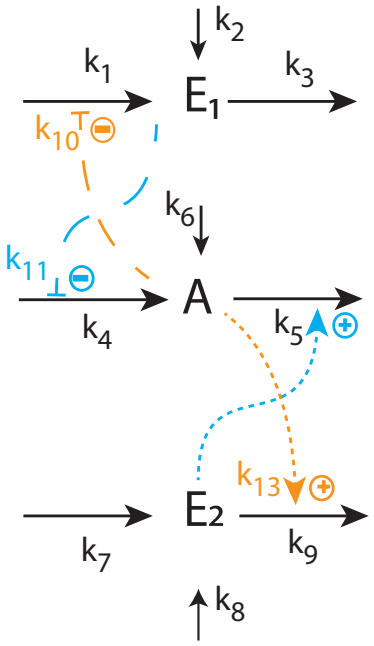


Figure 3.77: circuit of mixed motif 16-10u.

By circuit of combined motif 16-10u, we can deduce equations as below.

$$\dot{A} = k_4 \cdot \left(\frac{k_{11}}{k_{11} + E_1} \right) + k_6 - k_5 \cdot A \cdot E_2 \tag{3.93}$$

$$\dot{E_1} = k_1 \cdot \left(\frac{k_{10}}{k_{10} + A} \right) + k_2 - k_3 \cdot E_1 \tag{3.94}$$

$$\dot{E_2} = k_7 + k_8 - k_9 \cdot E_2 \cdot \left(\frac{A}{k_{13} + A} \right) \tag{3.95}$$

In addition, I removed k_{13} too. so now A and E_2 are unsaturated in $\dot{E}$ and $\dot{A}$ respectively.

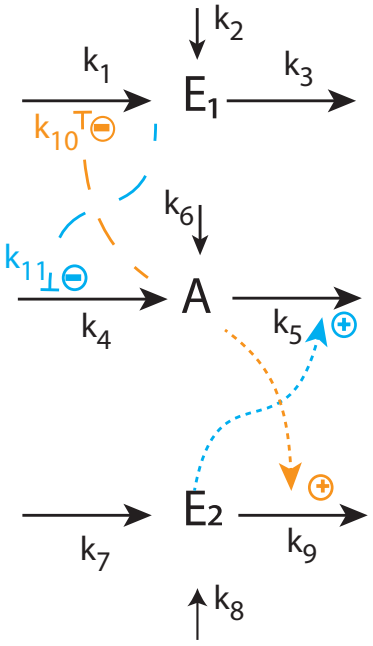


Figure 3.78: circuit of mixed motif 16-10u2.

By circuit of combined motif 16-10u2, we can deduce equations as below.

$$\dot{A} = k_4 \cdot \left(\frac{k_{11}}{k_{11} + E_1} \right) + k_6 - k_5 \cdot A \cdot E_2 \tag{3.96}$$

$$\dot{E_1} = k_1 \cdot \left(\frac{k_{10}}{k_{10} + A} \right) + k_2 - k_3 \cdot E_1 \tag{3.97}$$

$$\dot{E_2} = k_7 + k_8 - k_9 \cdot E_2 \cdot A \tag{3.98}$$

In this motif, k_{13} is removed to make unsaturated A in $\dot{E_2}$.

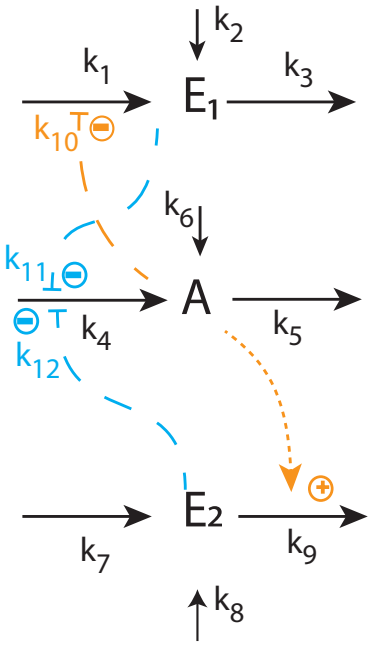


Figure 3.79: circuit of mixed motif 16-12u.

By circuit of combined motif 16-12u, we can deduce equations as below.

$$\dot{A} = k_4 \cdot \left(\frac{k_{11}}{k_{11} + E_1}\right) \cdot \left(\frac{k_{12}}{k_{12} + E_2}\right) + k_6 - k_5 \cdot A \tag{3.99}$$

$$\dot{E_1} = k_1 \cdot \left(\frac{k_{10}}{k_{10} + A}\right) + k_2 - k_3 \cdot E_1 \tag{3.100}$$

$$\dot{E_2} = k_7 + k_8 - k_9 \cdot E_2 \cdot A \tag{3.101}$$

This time, I removed k_{12} to make unsaturated A in $\dot{E_2}$.

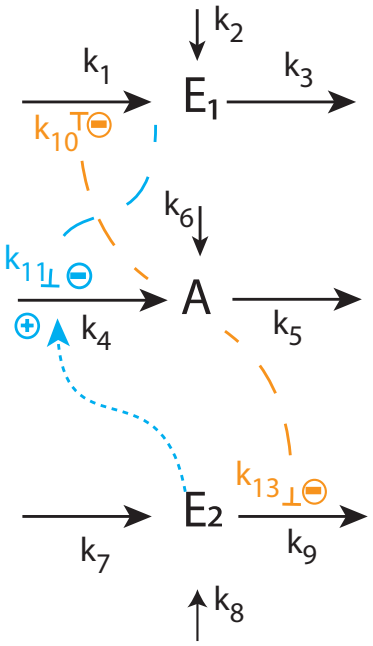


Figure 3.80: circuit of mixed motif 16-15u.

By circuit of combined motif 16-15u, we can deduce equations as below.

$$\dot{A} = k_4 \cdot \left(\frac{k_{11}}{k_{11} + E_1} \right) \cdot E_2 + k_6 - k_5 \cdot A \tag{3.102}$$

$$\dot{E_1} = k_1 \cdot \left(\frac{k_{10}}{k_{10} + A} \right) + k_2 - k_3 \cdot E_1 \tag{3.103}$$

$$\dot{E_2} = k_7 + k_8 - k_9 \cdot E_2 \cdot \left(\frac{k_{13}}{k_{13} + A} \right) \tag{3.104}$$

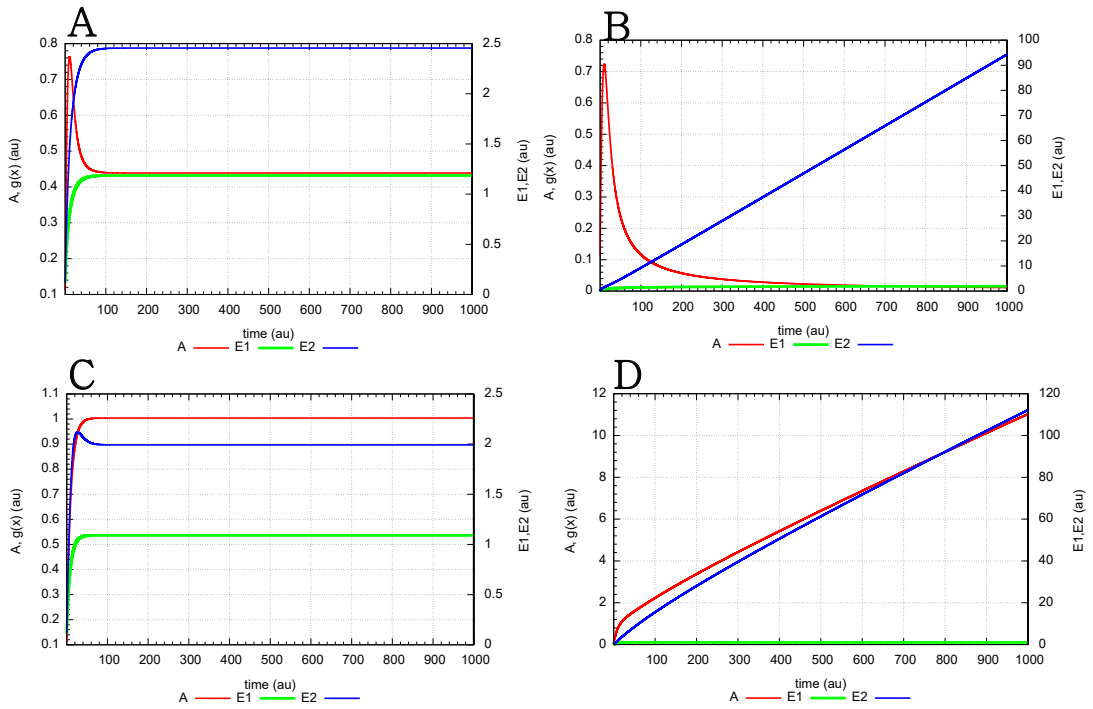


Figure 3.81: The outcome of unsaturated mixed motif with motif16 of A:motif 16-10u, B:motif 16-10u2, C:motif 16-12u, D:motif 16-15u. k_1,k_2,k_3,k_4,k_5,k_6,k_7,k_8,k_9,k_{10},k_{11},k_{12},k_{13} are 0.1. The initial concentration of A and E in all graphs is 0.1.

Through these unsaturated mixed motifs, I compiled them with same constant rates which used in Figure 3.74 to show the clear difference between saturated and unsaturated motifs.

Compared to Figure 3.74, shape of each graphs in Figure 3.81 changes when some component in motifs become unsaturated. We can verify the conversion to linear increase from steady state by graph A in Figure 3.74 with graph D in Figure 3.81. In case of graph C in Figure 3.81, there are overshooting in early period unlike graph C in Figure 3.74.

Chapter 4

Discussion

I have shown that motifs in positive feedback have their own special loops and traits. With same external or internal conditions, each motifs react in not only same like steady state and linear increase but also different ways, which could explain how natures adopt exclusive some motifs of positive feedback to cope with various stimuli. In each motifs, elements which cooperate or inhibit each other are affected by counterparts. In doing so, each motifs could present various traits.

The motif 16 presents not only steady state and linear increase but exclusively logarithmic increase which could not be found in the other motifs. This logarithmic pattern occur when rate constant of influx, which saturated with inhibition rate, is higher than the that of counterpart, while degradation rates of both components are zero. The doubling time graphs of motif 16 are different depending on rate of concentration change. In terms of logarithmic growth, doubling time of it show exponential increase. ln case of linear growth, doubling time increase linearly. When concentration graph show steady state, it could not be measured.
The motif 16 exists in one of loops of JAZ-DELLA, which regulate growth and defense in plants. that interaction influences JAZ-DELLA as well as MYC and PIF too. This connection allow plants to modulate their physiological reactions when stress or growth cues occur.

The motif 15 also show similar patterns like steady state and linear growth. However, There are some differences. First of all, in motif 15, components could be linear growth even though the degradation rate of one of components is not zero. In addition, on steady state, E increase more than A when all rate constants are same.
Through p53 and MDM2 relationship, I found that this negative feedback could be

converted to positive feedback like motif 15 by intervening of miR-605. Normally, In cell, the amount of p53 is regulated by MDM2 because p53 involve in cell apoptosis. But when cells are under stress, they choose amplication of p53 instead of homeostasis for halting cell progression.

The motif 14 was not explained in this thesis. As I mentioned in introduction and motif 9 section, some of motifs can be exchanged each other like motif 9 with motif 15 and motif 14 with motif 12.

The motif 13 represents that curved linear growth as well as steady state and linear growth. The condition of steady state in each motif can be identified by calculation with the equation deduced from schemes. One of important factors is values of degradation rate. Low Initial concentrations of two components and influx are followed. The importance of activation or repress rates is depended on where they are designated, whether influx or degradation.

The motif 12 shows not only steady state and linear growth but also hyperbolic increase and it is clearly shown when counterpart is unsaturated. To see clear hyperbolic graph with saturated counterpart, degradation rate and activation rate should be higher than initial concentration. In case of combined motif 12, difference of loops determine behavior according to the change of rate constants. In both single and combined motif 12, as influx and inhibition rate increase, hyperbolic growth period get closer, otherwise, increasing initial concentration of counterpart postpones the hyperbolic growth period.

The motif 11 depicts that the comparison between saturated and unsaturated loops. The unsaturated loops result in strong growth compared to saturated loops. The transition from steady state to linear growth and from linear growth to exponential increase supports previous arguments.
The hyperbolic growth in bacteria infection cycles prove that saturation of motif 11 hinder the hyperbolic tendency. When loops are totally saturated by activation rates, hyperbolic growth is converted to exponential growth.
When unsaturated motif 11 is combined with motif 9, exponential trait is repressed. Unlike unsaturated motif 11, it shows steady state if degradation rate is not zero. Instead, if either one or all of degradation are zero, exponential growth occurs. This reaction could be changed when inhibition rate become massively high. When inhibition rate is high and one of degradation rate which directly inhibited is not zero, exponential growth change to linear growth. In the bacterial infection cycle, loops which linked with positive strand and negative strand plays important role for hyperbolic effect. Unsaturated loop allow bacterial cycle to grow hyperbolically, even though one of them is

saturated, hyperbolic trait are maintained. But, both of them become saturated, they show exponential growth. If one of them get disconnected, hyperbolic growth becomes linear growth. Contrary to this, If that loop exist, while influx of both positive and negative removed, still hyperbolic tendency is maintained.
The comparison between presence and absence of motif 11 prove that how positive feedback is important for blood clotting quickly. With positive feedback loop, Fibrin becomes stabilized immediately, on the other hand, without it, there was no immediate response.

The motif 10 is involved in regulation of cell cycle. This loop is combined with motif 13 and constitute bistable switch for entering or evading mitosis stage. Within this mixed loops, motif 10 switch off otherwise, motif 13 switch on the mitosis.

The motif 9 and motif 15 display the ability to switch when two components swap each others. This ability is proved In Figure 3.58.

Comparison of each single motifs in positive feedback indicates the reason explicitly why motifs in positive feedback should be distinguish. All of results are different even though initial concentration and rate constants are same. Each motifs have not only common but also its own traits.

Combined motifs have tendency that whether repress or activate the concentration of components.

Mixed motifs also show different patterns compared with those of single motif. Through these results, we can assume that in nature, positive feedback could cope with various stimuli by combining several motifs. If positive and negative feedback are joined, It is clear that more and more various patterns will emerge.

The combination of saturated and unsaturated component contribute to complexity of various patterns in positive feedback. Through modification of one component, entire patterns of motifs change.

When it comes to examples in each motifs, to emphasize the effect of motifs, I omitted some pathways which associated with motifs. Besides, I just describe positive feedback not negative feedback hence, it could be somewhat not enough for explanation of entire mechanism of pathway.

To research several motifs in positive feedback, various computer programs were used including Fortran, Perl, Python, Matlab and Celldesigner. All of programs denoted same results when exact kinetic laws were applied. However, each programs have different error tolerances so that they sometime have different values when whether value is extremely small or high. For complement this problem, scale of y axis was adjusted or x axis, which is time was limited.

Finally, this is obvious that my research gives insight to those who research the mechanism of nature including positive feedback. Beyond simply positive feedback, understanding and analysis of accurate motifs will become a major framework for studying nature characteristics.

Chapter 5

Conclusion

The positive feedback is not subject to overlook because it has various motifs which consist of specific traits. The each motifs have their own properties such as steady state, linear increase, logarithmic, exponential or hyperbolic growth and they are implemented respectively with same external and internal stimuli. They could exist in either saturated or unsaturated form thus, the resulting manifestation can vary depending on the situation. Besides, when these motifs combined or mixed, they represent immeasurable capabilities to deal with varied macro environment. While researching the property of nature, this detail analysis regarding to these motifs would help to clarify what human being have not found before.

Through this research, it is clear that all of motifs exist in natures with different patterns with the other motifs. In addition, one of most important find is that by adding or omitting one element, negative feedback and positive feedback could be switched. Through this observation, we can know that motifs in nature are not fixed but flexible so that they can react with proper situations. Another impressive finding is that one of rate constant in loop could influence entire patterns of motif. It could be inhibition or activation rate constants and these variations cause significant transitions.

This study would be impossible without the support of computational methodologies. However there is still limitation of error tolerance so that computer can not calculate extremely small or high values in graphs. As computational biology gets developed fast, the more complicated issues could be solved out. Hence, we can pinpoint the local minimum or local maximum more exactly.

If overlook this cornerstone of positive feedback, it is definitely impossible to predict the exact principle and mechanisms of nature.

Nowadays, researchers have investigated organisms, tissue, or cells as system biology. My research would play significant role to complete study of system biology. The lack of explanation of entire pathway would be supplemented when additional research of motifs in negative feedback added. One day, research for combination of positive and negative feedback would provide us with informations which complete biology maps.

Appendix A

Calculation of p53 and MDM2 with Fortran

In advance, conversion between negative feedback and positive feedback in p53 and MDM2 network implemented by simbology application in Matlab. For accuracy, I also implement calculation with Fortran.

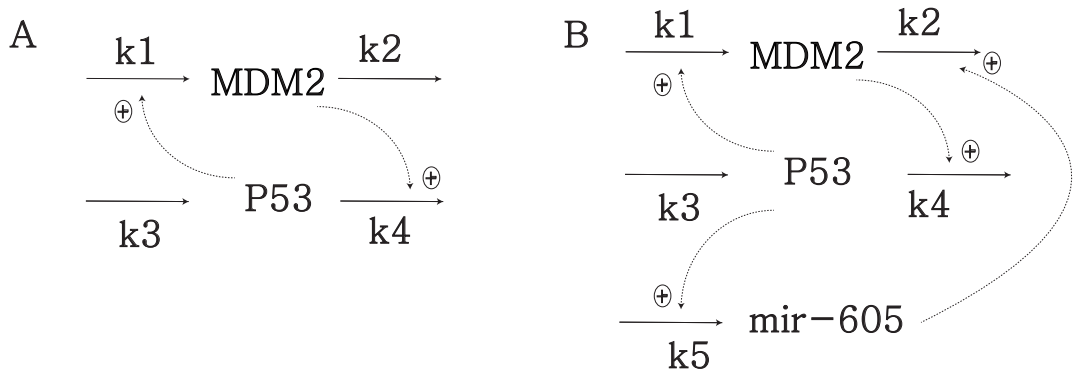


Figure A.1: Diagram of p53 and MDM2. (A) presents negative feedback in p53 and MDM2 network (B) shows positive feedback in p53 and MDM2 network when mir-605 inserted.

In negative feedback, the equations of p53 and MDM2 could be shown as below.

$$\dot{MDM2} = k_1 \cdot P53 - k_2 \cdot MDM2 \tag{A.1}$$

$$\dot{P53} = k_3 - k_4 \cdot P53 \cdot MDM2 \tag{A.2}$$

With mir-605, above equations change as below. Additionally, equation of mir-605 is added.

$$\dot{MDM2} = k_1 \cdot P53 - k_2 \cdot MDM2 \cdot mir - 605 \tag{A.3}$$

$$\dot{P53} = k_3 - k_4 \cdot P53 \cdot MDM2 \tag{A.4}$$

$$\dot{mir - 605} = k_5 \cdot P53 \tag{A.5}$$

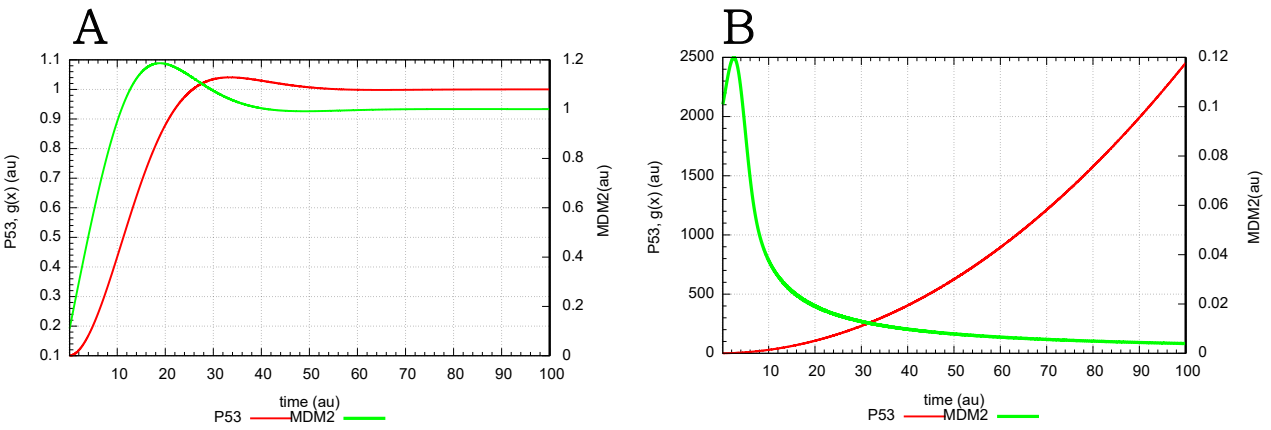


Figure A.2: Graph of p53 and MDM2. (A) Both p53 and MDM2 show steady state (B) MDM2 starts to decrease while p53 continuously increase.

In Figure A.2, both graphs show similarity with those in Figure 3.14. In negative feedback, p53 and MDM2 have similar concentration. However, when mir-605 starts to be transcript-ed under cell stress, network becomes positive feedback and p53 increase while MDM2 get inhibited by mir-605.

Appendix B

Blood clotting calculation with Fortran

In motif 11 section, I implemented calculations with Python to compare the difference between presence and absence of motif 11 in blood clotting cascade. In this Appendix B, I will calculate it with Fortran to compare the results. Diagrams and equations of this experiment refer to Figure 3.48], Figure 3.49 and equation from 3.51 to 3.56.

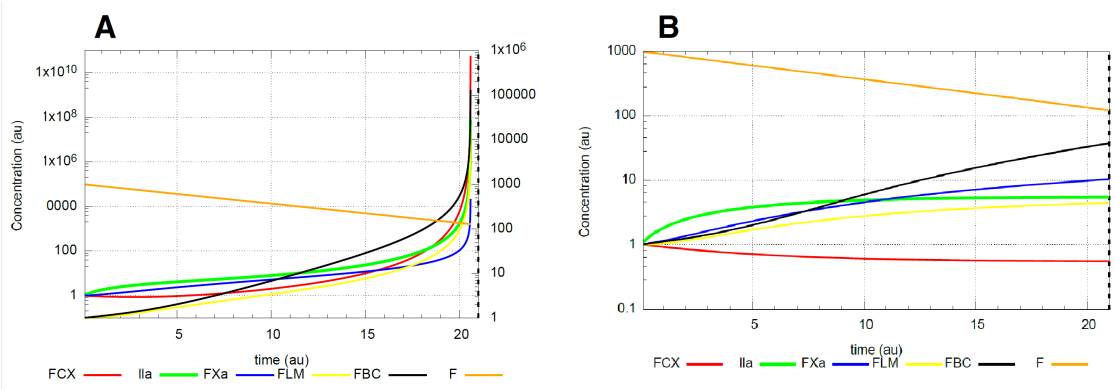


Figure B.1: Comparison of graphs with and without positive feedback. (A) shows presence of motif 11 (B) show absence of motif 11 shows.

With positive feedback, blood clotting elements increase hyperbolically.

Appendix C

Calculation of cell cycle progression

In motif 10, cell cycle progression with WEE1-CDK-CDC network was calculated by Cell-designer. In this appendix, I will calculate with Fortran to show how graph look like.

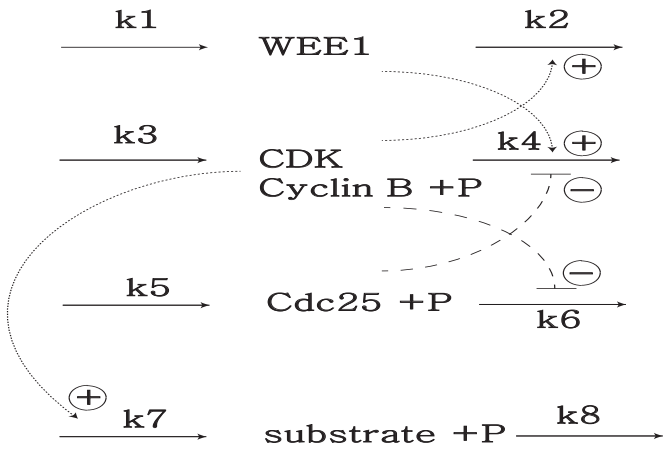


Figure C.1: Diagram of WEE1-CDK-CDC network.

Through Figure C.1, several equations could be deduced as below.

$$\dot{WEE1} = k_1 - k_2 \cdot CDK \tag{C.1}$$

$$\dot{CDK} = k_3 - k_4 \cdot WEE1 \cdot CDK \cdot \left(\frac{k_9}{k_9 + Cdc}\right) \tag{C.2}$$

$$\dot{Cdc} = k_5 - k_6 \cdot Cdc \cdot \left(\frac{k_{10}}{k_{10} + CDK} \right) \tag{C.3}$$

$$\dot{Substrate + P} = k_7 - k_8 \cdot Substrate + P \tag{C.4}$$

With these equations, Fortran can calculate and plot graphs as results.

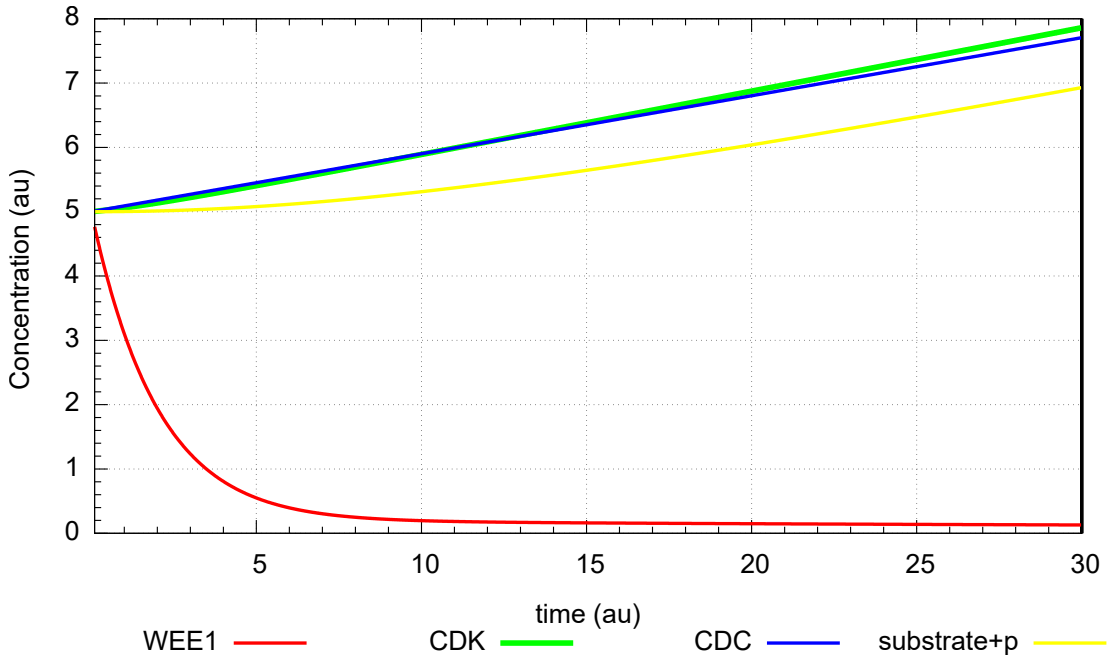


Figure C.2: Graph of WEE1-CDK-CDC network. While WEE1 decrease, CDK and CDC gradually increase, thus concentration of substrate +P also increase.

As Figure 3.56, In Figure C.2, WEE1 that inhibits CDK decrease and then CDK and Cdc increase, making phosphoryated substrate increase.

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