Assignment:

MODELLING OF BIOLOGICAL SYSTEMS

Negatively auto-regulated feedback system Question No.04

Contents:

INTRODUCTION:

In natural systems, different types of arrangements of feedback are present to generate a variety of dynamics. This interaction between different regulatory processes can lead to overall functional dynamics. Complex regulation of biochemical pathways in a cell is brought about by the interaction of simpler regulatory structures.

Both theoretical and experimental approaches are used. Theoretical approach: To develop mathematical models of simple reaction pathways and carry out mathematical analysis & numerical simulations in-order to study their dynamics under various conditions. Experimental approach: To construct simple regulatory networks (gene) which mimic the mathematical model as closely as possible and also study the behaviors of these constructs and see if they satisfy the predictions of the mathematical model.

Genome is the entirety of an organism's hereditary information. It is made up of a stretch of DNA (or RNA) sequence comprising of 4 bases (A, T, G and C). The central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information.

Genetic regulatory network consists of set of genes, proteins, small molecules, and their mutual regulatory interactions. Development and functioning of organisms cell emerges from interactions in genetic regulatory networks.

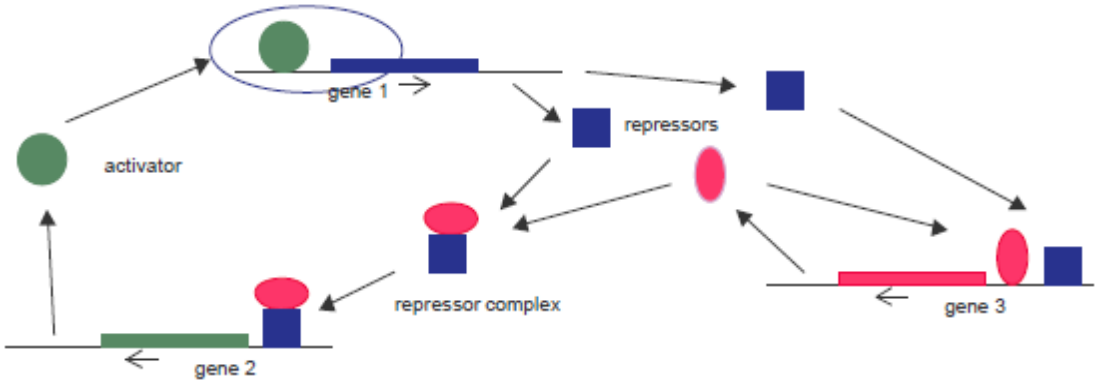

Fig: *A representation of gene regulation networks.*

Choice between alternative developmental pathways is controlled by network of genes, proteins, and mutual regulatory interactions. Most genetic regulatory networks are large and complex. Dynamics of large and complex genetic regulatory processes are hard to understand by intuitive approaches alone. Therefore Mathematical methods for modeling and simulation are required for precise and unambiguous description of network of interactions and systematical derivation of behavioral predictions.

Model of negative feedback system:

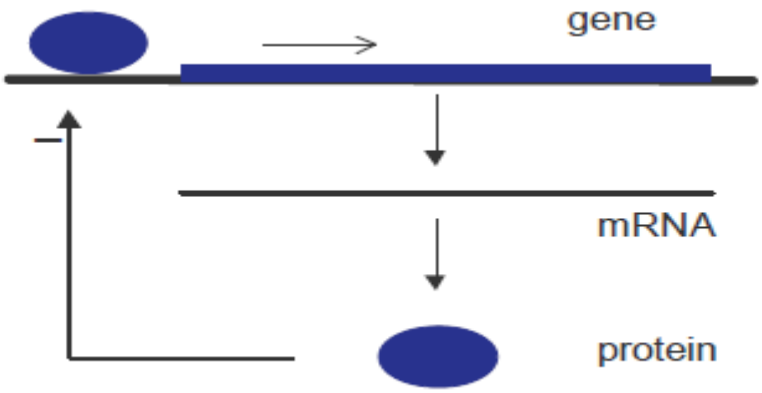

Fig: *Model of a simple negative feedback system in a biological system.*

Gene encodes a protein inhibiting its own expression. A gene regulatory network, also called a genetic circuit, is a group of genes whose protein products regulate one another's expression. The simplest genetic circuit consists of a single gene that regulates its own activity. If the gene's protein product enhances expression, the gene is called as auto-activator and if the product inhibits expression, the gene is an auto-inhibitor.

Many genes are auto-inhibitory. One advantage of this regulatory scheme is reduction of sensitivity to certain perturbations. Autoinhibition decreases the sensitivity to variation in the maximal expression rate. Because rate depends on a host of background processes, this increased robustness can provide a significant advantage over unregulated expression. Another advantage of autoinhibition is a fast response to changes in demand for protein product. Consider an unregulated gene whose product attains a particular concentration.

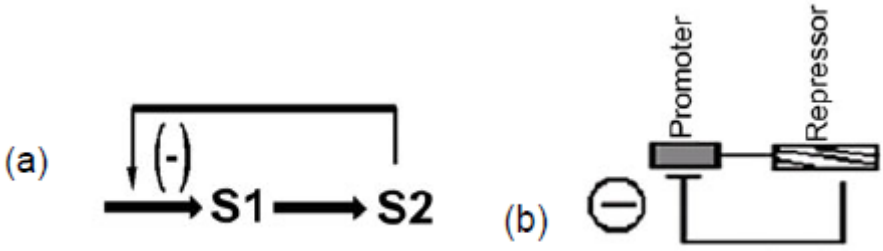

Fig: (a) *A simple negatively auto-regulated pathway; (b) The corresponding gene circuit*

Methodology:

Given:

The transcription of the repressor gene (**G**) yields mRNA molecules (**M**), which are translated to produce the repressor protein (**P**). The repressor protein acts as a negative regulator of its own transcription by binding to the operator-promoter complex (shown as "promoter" here). $k1$ and $k2$ represent the rates of these forward and backward reactions, respectively. The amount of mRNA produced at any given instant is a function of the number of free promoters available for transcription. The parameters $\beta 1$ and $\beta 2$ represent the rates of transcription and translation, and $\alpha 1$ and $\alpha 2$ are the degradation rates of M and P that follow first order kinetics.

A mathematical model (Ordinary differential equation) of the circuit is given by:

$$\frac{dM}{dt} = \beta_1 G - \alpha_1 M$$

$$\frac{dP}{dt} = \beta_2 M - \alpha_2 P$$

$$\frac{dG}{dt} = k_2(G_t - G) - k_1 G P$$

Parameters	Deterministic value
β_2	$4.3 \times 10^{-2}\,\text{sec}^{-1}$
$\boldsymbol{\beta_1}$	$0.6 \times 10^{-9}\,\text{M sec}^{-1}$
α_1	$5.7 \times 10^{-3}\,\text{sec}^{-1}$
α_2	$1.15 \times 10^{-3}\,\text{sec}^{-1}$
$\mathbf{k_1}$	$1.2 \times 10^{+7}\text{M}^{-1}\,\text{sec}^{-1}$
k_2	$0.9\,\text{sec}^{-1}$
G_t	50

Using MATLAB program we simulated the model using the basal parameter values that were given.

Script:

```
function ddt=negatively_autoregulated_pathway(t,x)
A1=5.7*10^-3;
A2=1.15*10^-3;
B1=0.6*10^-9;
B2=4.3*10^-2;

K1=1.2*10^7;
K2=0.9;
Gt=50;

G=x(1);
M=x(2);
P=x(3);
ddt=zeros(3,1);

% dM/dt=B1*G - A1*M
% dP/dt=B2*M-A2*P
% dG/dt=K2*(Gt-G) - K1*G*P

ddt(1)=(G*B1)-(M*A1);
ddt(2)=(M*B2)-(A2*P);
ddt(3)= K2*(Gt-G)-K1*G*P;
```

Using MATLAB command window commands we initialized the G, M & P as zero. By varying different parameters one at a time the model is analyzed and its stability is determined.

Report of test of analysis:

Fig 1: Time (0-20) Seconds

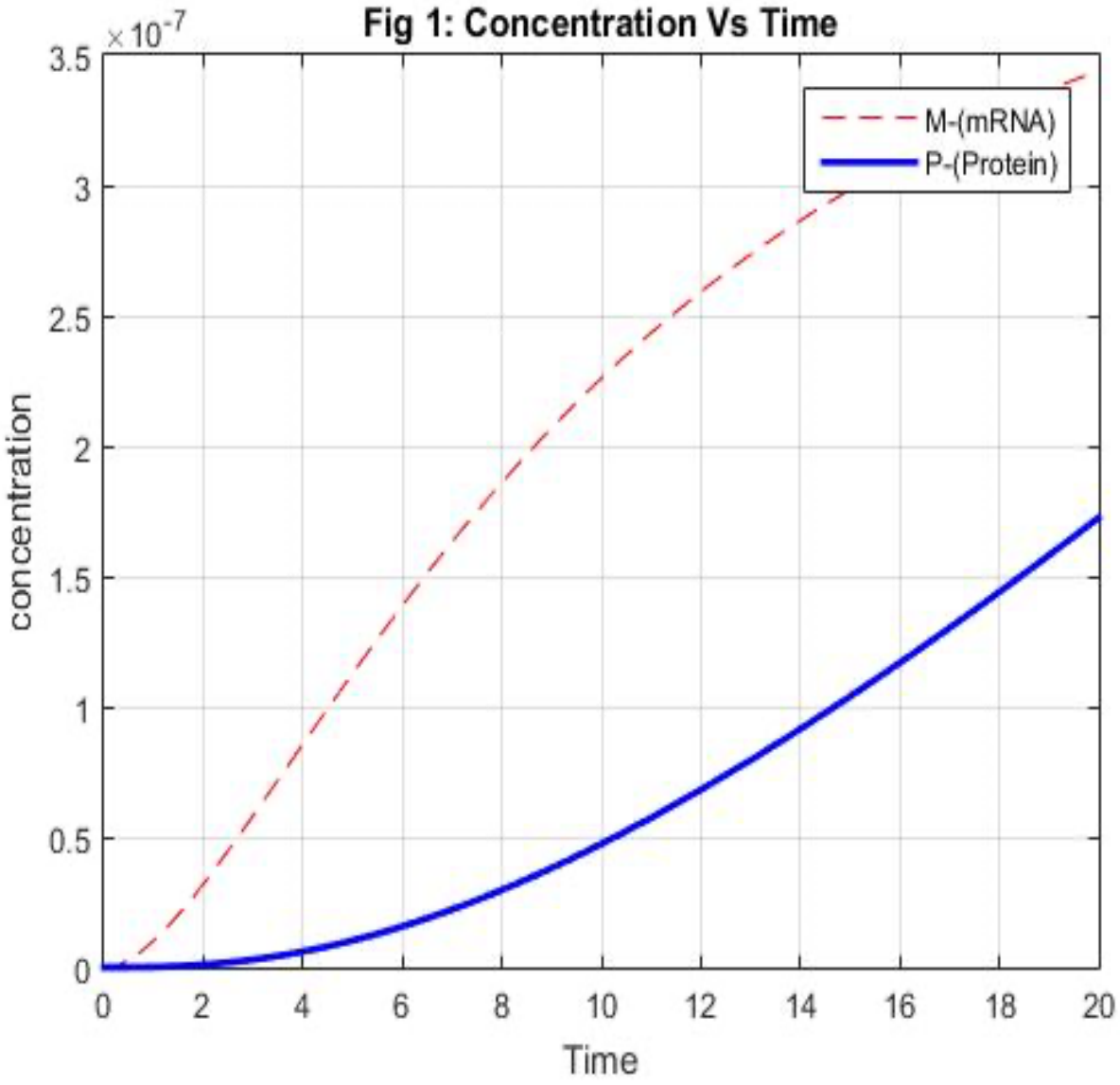

Fig 1: For time 0 to 20 simulation is run, where **'M'** is mRNA and **'P'** is Protein. A series of steps in the *mRNA (M)* concentration lead to changes in the production of the *Protein ('P')* reflecting in the abundance of *mRNA ('M')*. There is a negligible rise in the production of *Protein ('P')* at time 0 to 4 seconds and later increases as *mRNA ('M')* increases rapidly.

Fig 1.1: Time (0-100) Seconds

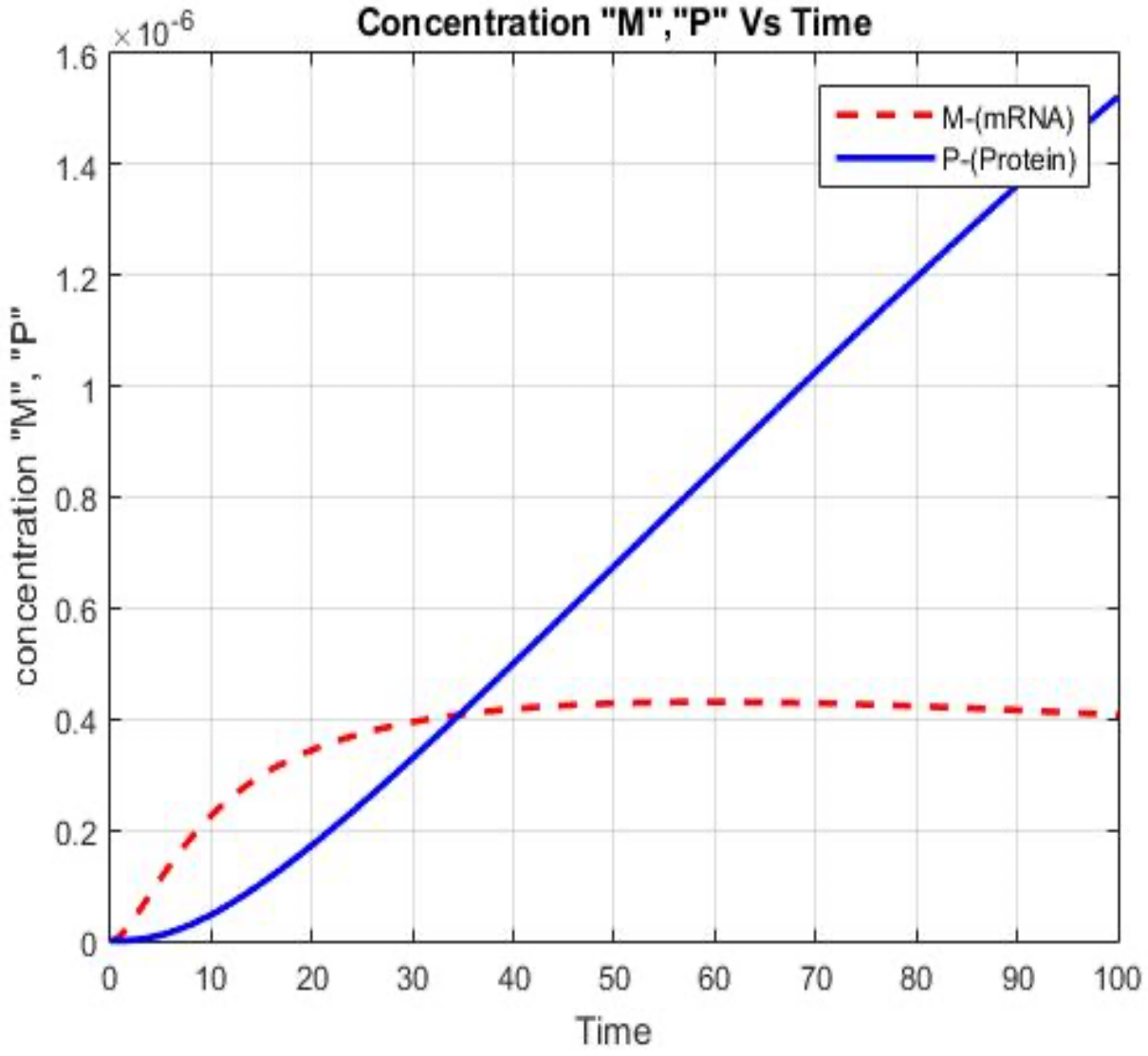

Fig 1.a: For time 0 to 100 simulation is run, where **'M'** is mRNA and **'P'** is Protein. There is a rapid rise in the production of *mRNA ('M')* at time 0 to 50 seconds and later it slowly reduces the production of *mRNA ('M')*. Whereas in case of *Protein ('P')* at time 0 to 20 seconds there is transient increase and later it increases rapidly.

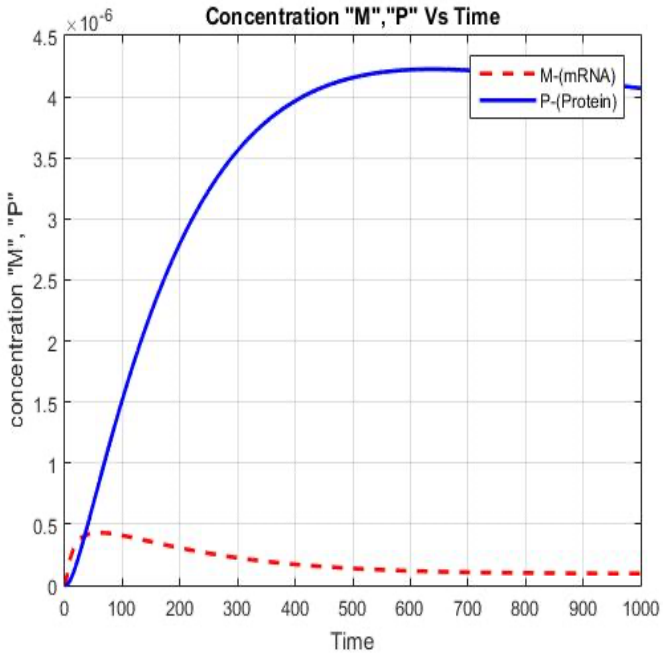

Fig 1.2: Time (0-1000) Seconds

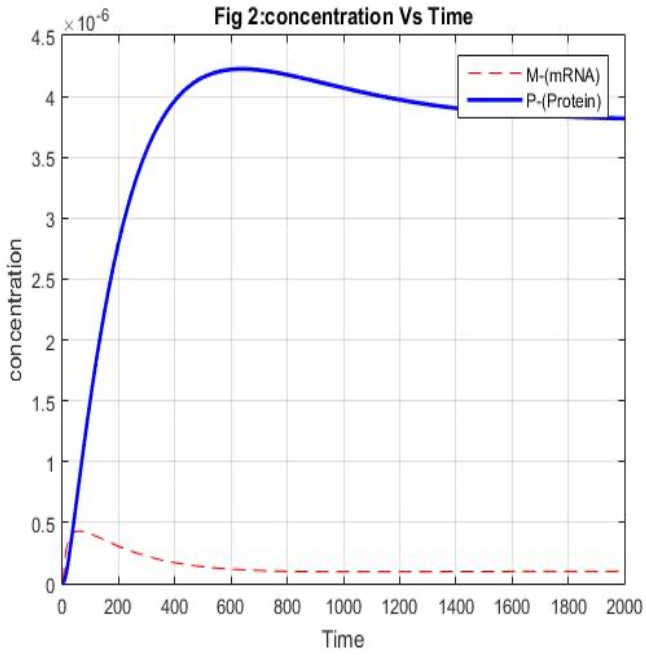

Fig 2: Time (0-2000) Seconds

From the above figure we can see that there is an increase of *mRNA ('M')* at time t=0-50 and decreases its production from t=50-500, at t=600 steady state is achieved. Whereas in case of *Protein ('P')* at time t=0-600 there is an increase in production of protein and decreases its production from t=600-1400, at t=1400 steady state is achieved.

From the above graph as mRNA ('M') production increases Protein ('P') production also increases. At t=50-600 as protein production increases rapidly the mRNA started to decay its production, because protein inhibits the production of mRNA.

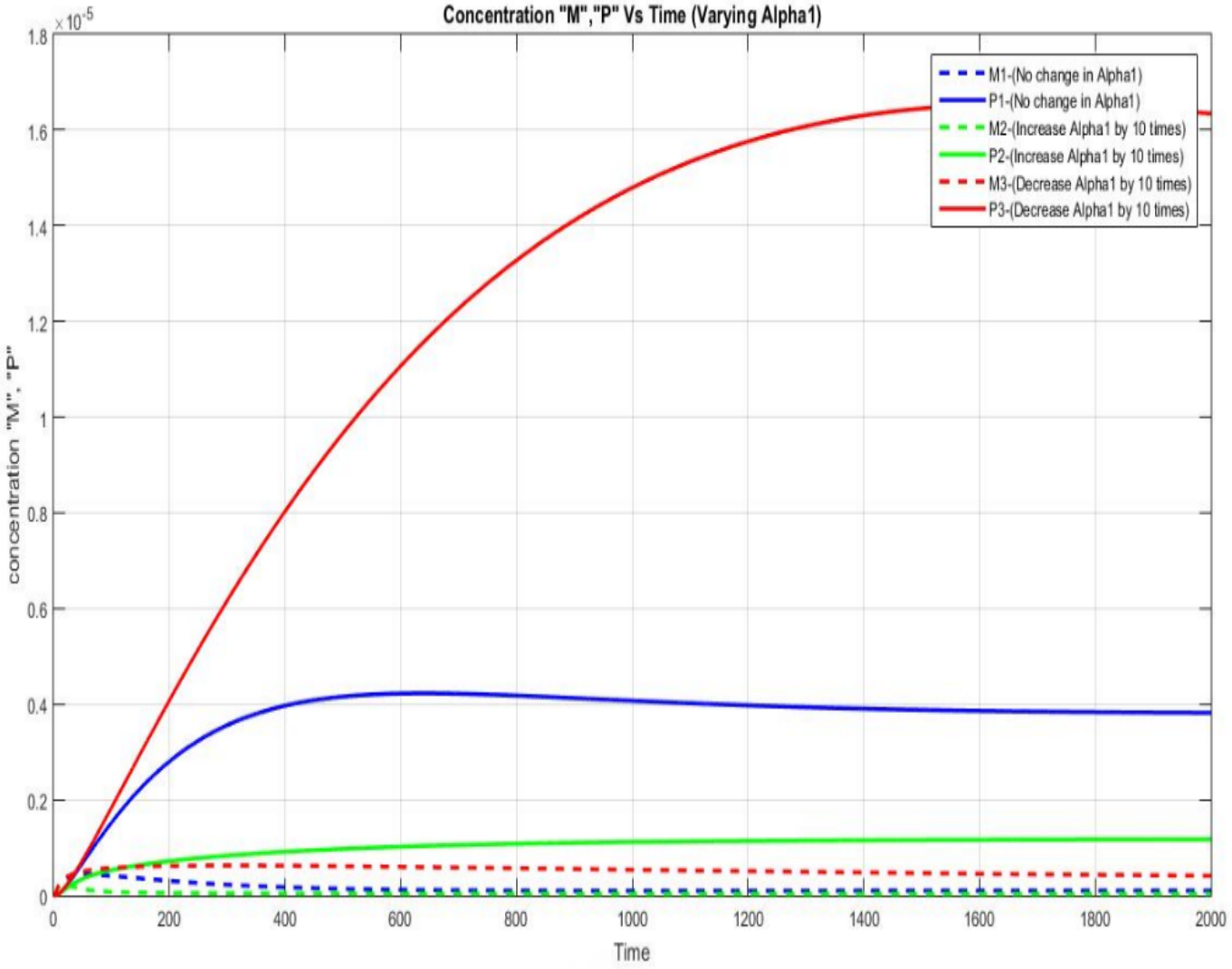

Fig 2.1; Time (0-2000) Seconds.

1. *M1=mRNA, P1=Protein:* Parameter values: Alpha1= $5.7X10^{-3}$, Alpha2= $1.15X10^{-3}$, Beta1= $0.6X10^{-9}$, Beta2= $4.3X10^{-2}$, K1= $1.2*10^{7}$, k2= 0.9.
2. *M2=mRNA, P2=Protein:* Where Alpha1 is increase by 10 times, the rest are same.
3. *M3=mRNA, P3=Protein:* Where Alpha1 is decrease by 10 times, the rest are same.

Varying Alpha1 (α1):

In autoregulatory system the concentrations is induced by reducing **α1** and delayed by increasing the **α1** (as shown in fig. 2.1).

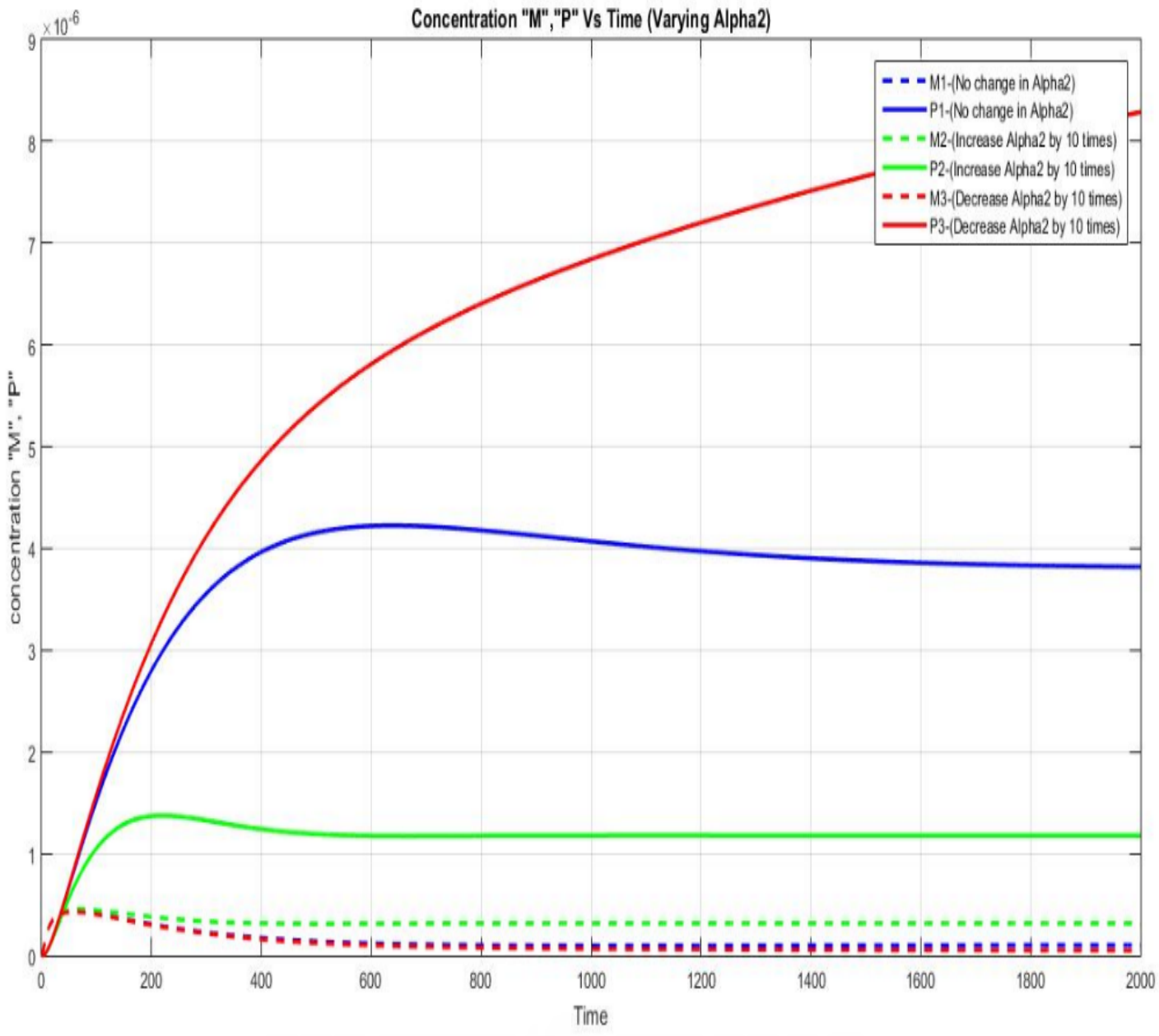

Fig 2.2; Time (0-2000) Seconds.

1. *M1=mRNA, P1=Protein:* Parameter values: Alpha1= $5.7X10^{-3}$, Alpha2= $1.15X10^{-3}$, Beta1= $0.6X10^{-9}$, Beta2= $4.3X10^{-2}$, K1= $1.2*10^{7}$, k2= 0.9.
2. *M2=mRNA, P2=Protein:* Where Alpha2 is increase by 10 times, the rest are same.
3. *M3=mRNA, P3=Protein:* Where Alpha2 is decrease by 10 times, the rest are same.

Varying Alpha2 (α2):

In autoregulatory system the concentrations is induced by reducing **α2** and delayed by increasing the **α2** (as shown in fig. 2.2).

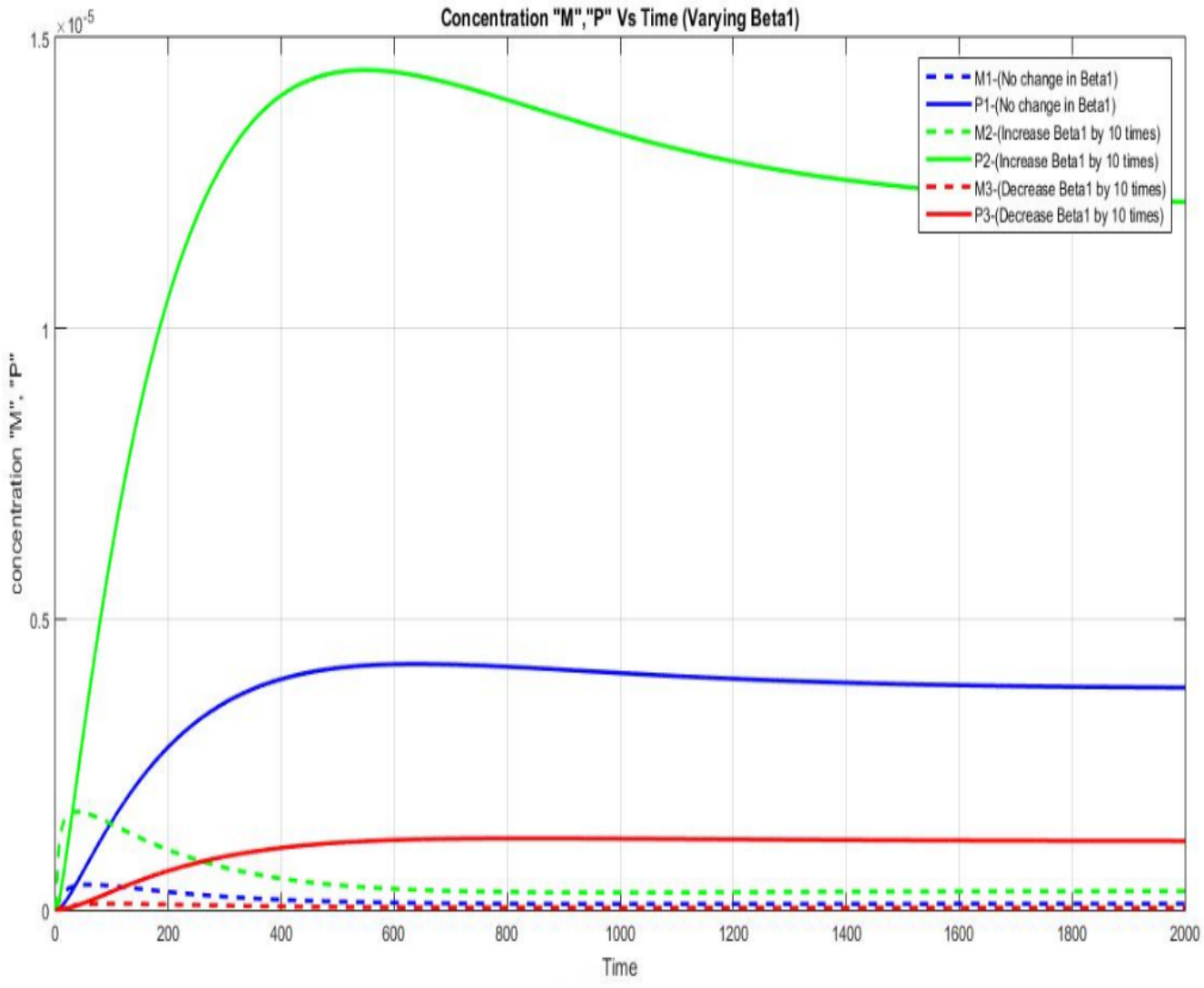

Fig 2.3; Time (0-2000) Seconds.

1. *M1=mRNA, P1=Protein:* Parameter values: Alpha1= $5.7X10^{-3}$, Alpha2= $1.15X10^{-3}$, Beta1= $0.6X10^{-9}$, Beta2= $4.3X10^{-2}$, K1= $1.2*10^{7}$, k2= 0.9.
2. *M2=mRNA, P2=Protein:* Where Beta1 is increase by 10 times, the rest are same.
3. *M3=mRNA, P3=Protein:* Where Beta1 is decrease by 10 times, the rest are same.

Varying Beta1 (β1):

In autoregulatory system the concentrations is induced by increasing **β1** and delayed by reducing the **β1** (as shown in fig. 2.3).

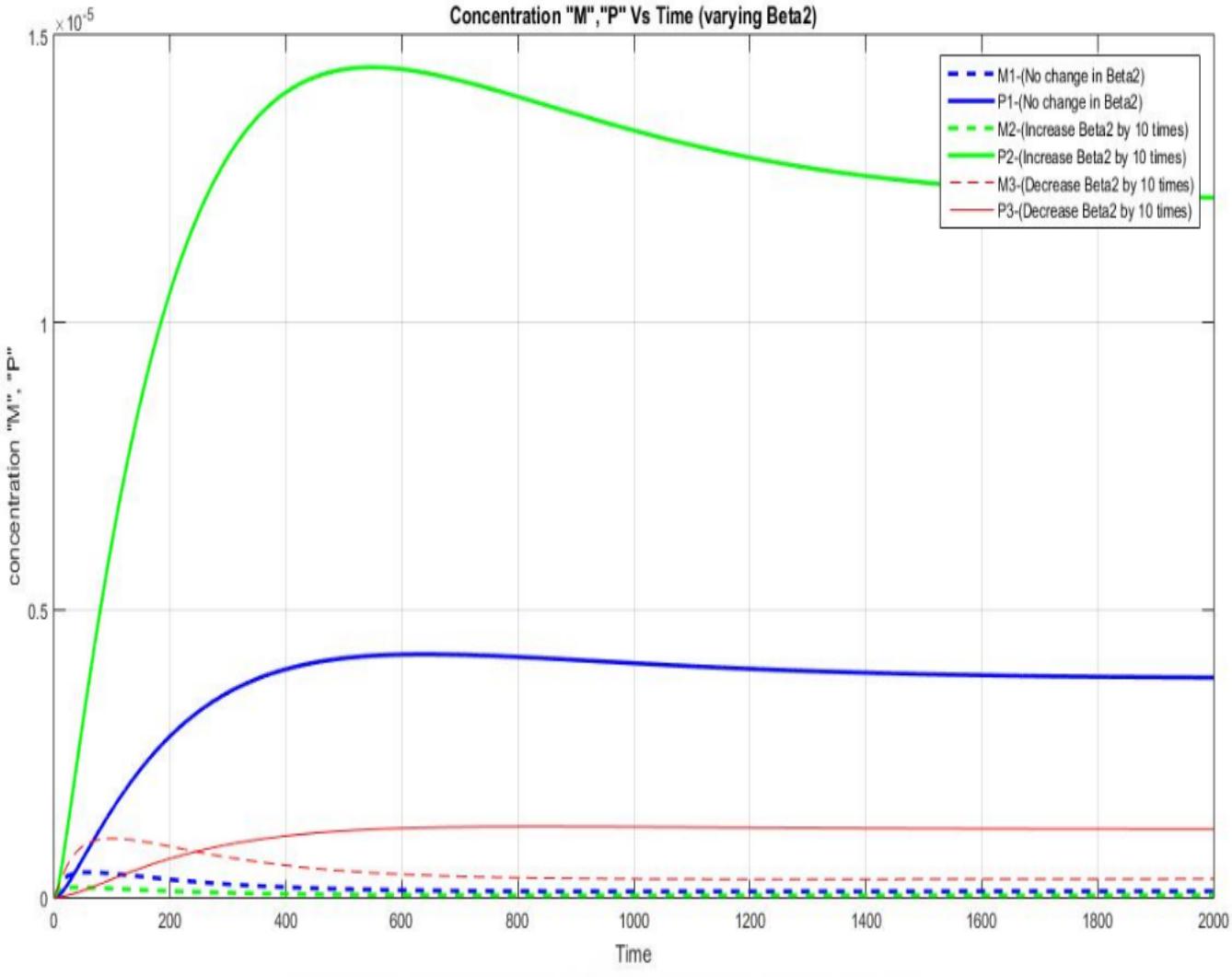

Fig 2.4; Time (0-2000) Seconds.

1. *M1=mRNA, P1=Protein:* Parameter values: Alpha1= $5.7X10^{-3}$, Alpha2= $1.15X10^{-3}$, Beta1= $0.6X10^{-9}$, Beta2= $4.3X10^{-2}$, K1= $1.2*10^{7}$, k2= 0.9.
2. *M2=mRNA, P2=Protein:* Where Beta2 is increase by 10 times, the rest are same.
3. *M3=mRNA, P3=Protein:* Where Beta2 is decrease by 10 times, the rest are same.

Varying Beta1 (β1):

In autoregulatory system the concentrations is induced by increasing **β2** in-case of Protein but delayed in-case of mRNA and reducing the **β2** induces mRNA and delays protein (as shown in fig. 2.4).

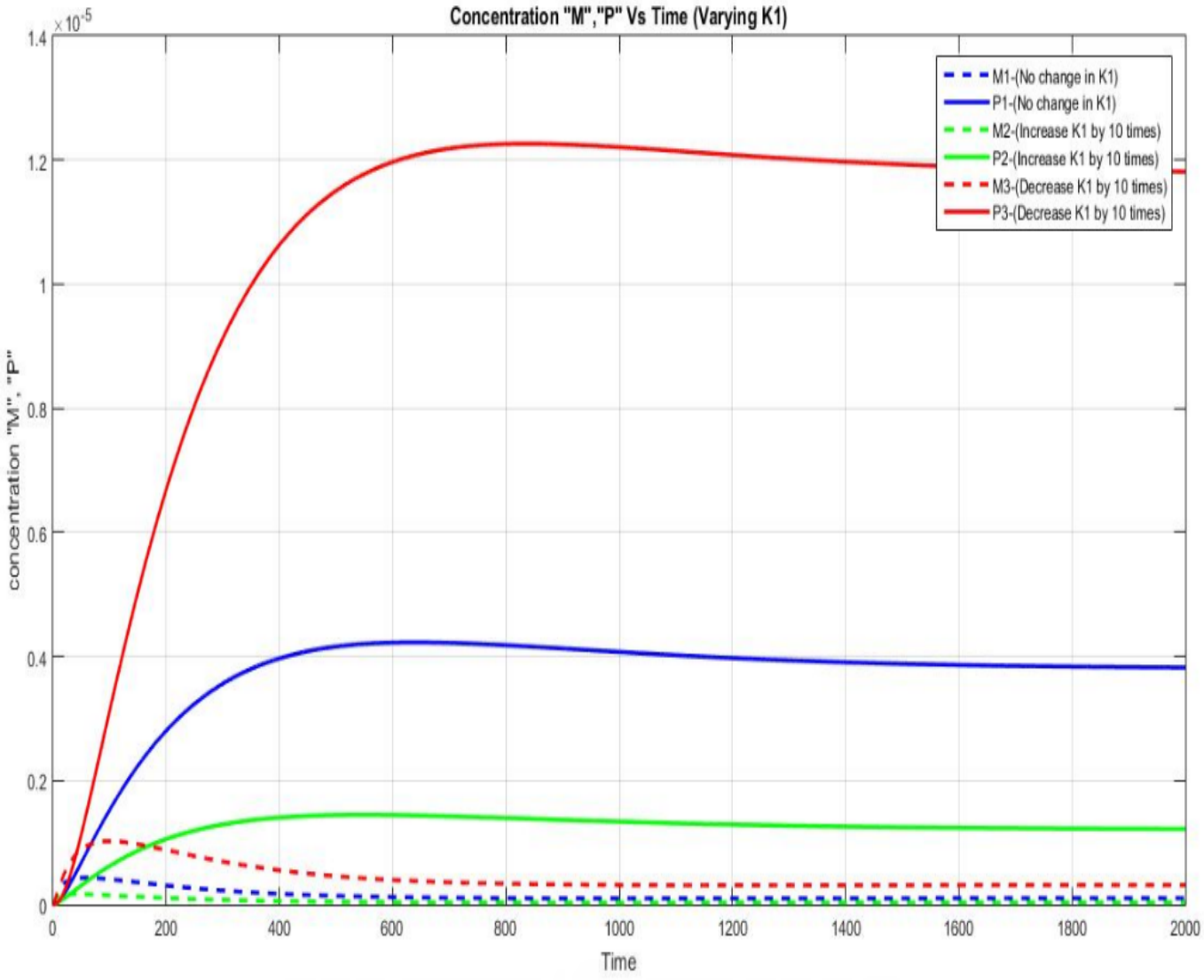

Fig 2.5; Time (0-2000) Seconds.

1. *M1=mRNA, P1=Protein:* Parameter values: Alpha1= $5.7X10^{-3}$, Alpha2= $1.15X10^{-3}$, Beta1= $0.6X10^{-9}$, Beta2= $4.3X10^{-2}$, K1= $1.2*10^{7}$, k2= 0.9.
2. *M2=mRNA, P2=Protein:* Where K1 is increase by 10 times and the rest are same.
3. *M3=mRNA, P3=Protein:* Where K1 is decrease by 10 times and the rest are same.

Varying K1:

In autoregulatory system the concentrations is induced by reducing **K1** and delayed by increasing the **K1** (as shown in fig. 2.5).

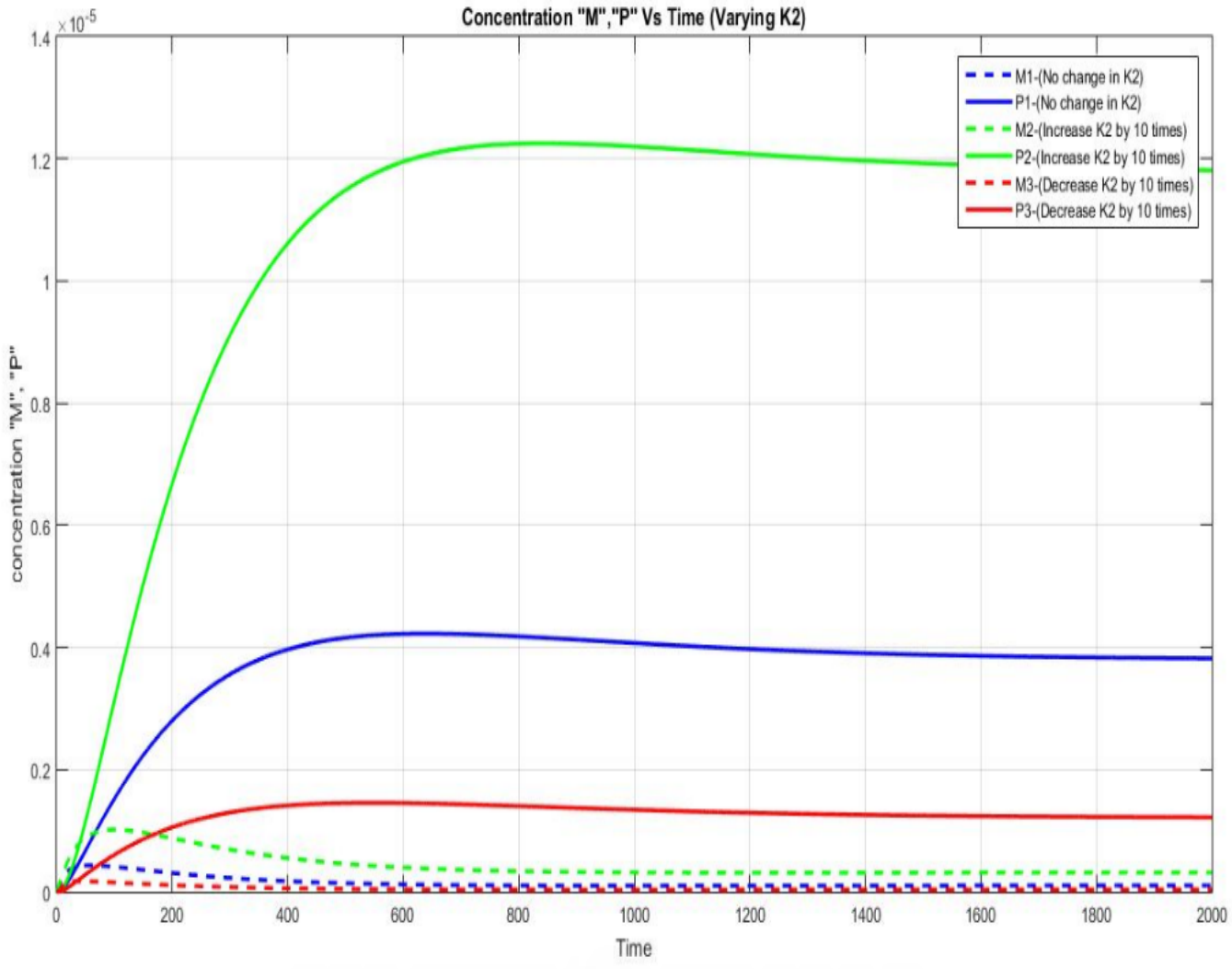

Fig 2.6; Time (0-2000) Seconds.

1. *M1=mRNA, P1=Protein:* Parameter values: Alpha1= $5.7X10^{-3}$, Alpha2= $1.15X10^{-3}$, Beta1= $0.6X10^{-9}$, Beta2= $4.3X10^{-2}$, K1= $1.2*10^{7}$, k2= 0.9.
2. *M2=mRNA, P2=Protein:* Where K2 is increase by 10 times, the rest are same.
3. *M3=mRNA, P3=Protein:* Where K2 is decrease by 10 times, the rest are same.

Varying K2:

In autoregulatory system the concentrations is induced by increasing **K2** and delayed by reducing the **K2** (as shown in fig. 2.6).

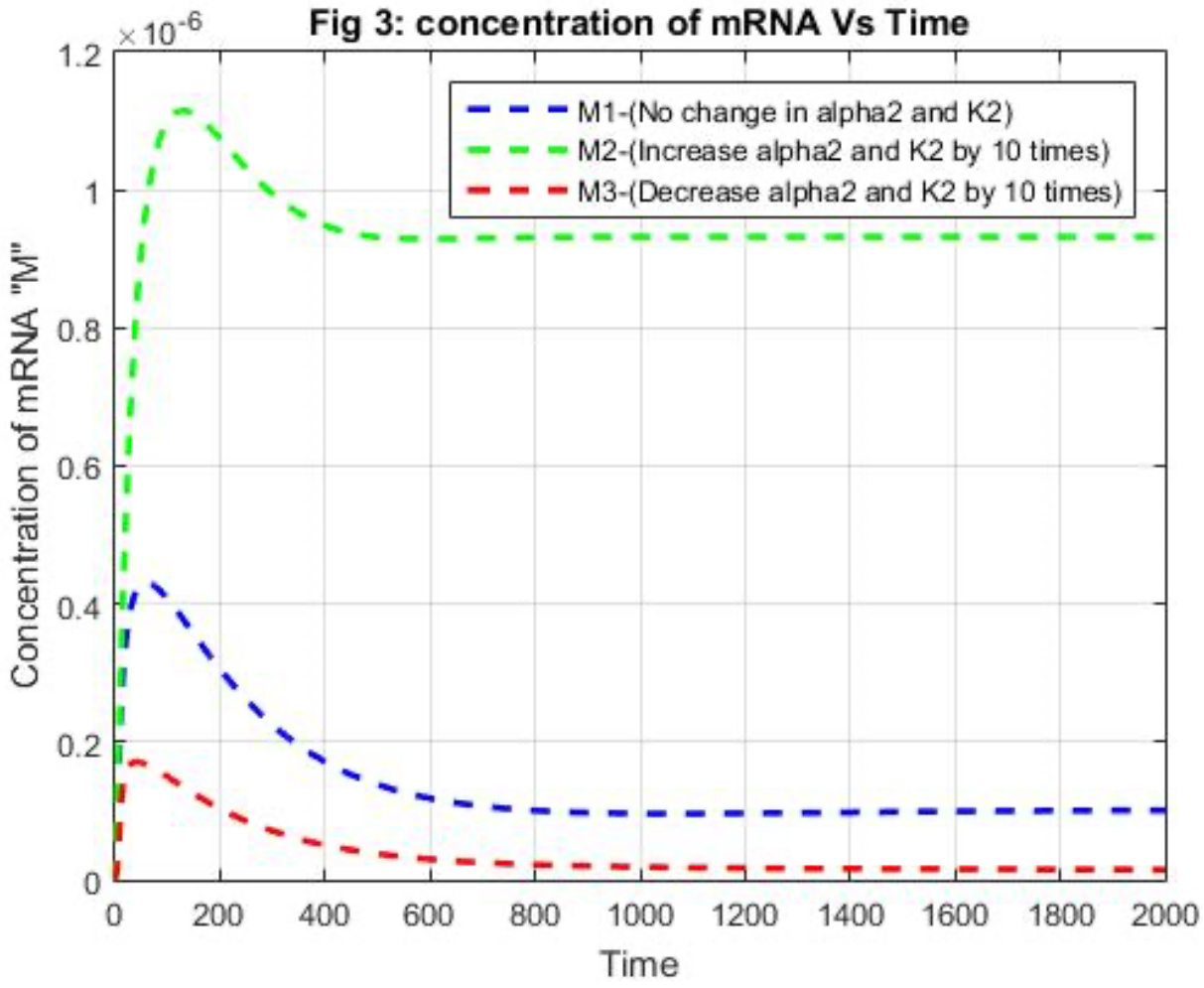

Fig 3; Time (0-2000) Seconds.

1. *M1=mRNA:* Parameter values: Alpha1= $5.7X10^{-3}$, Alpha2= $1.15X10^{-3}$, Beta1= $0.6X10^{-9}$, Beta2= $4.3X10^{-2}$, K1= $1.2*10^{7}$, k2= 0.9.
2. *M2=mRNA:* Parameters value Alpha2 and K2 is increase by 10 times, the rest are same.
3. *M3=mRNA:* Parameters value Alpha2 and K2 is decrease by 10 times, the rest are same.

Varying Alpha2 & K2 (mRNA):

In autoregulatory system the concentrations is induced by increasing **α2 & K2** *(Steady state is achieved at faster rate)* and delayed by reducing the **α2 & K2** (as shown in fig. 3).

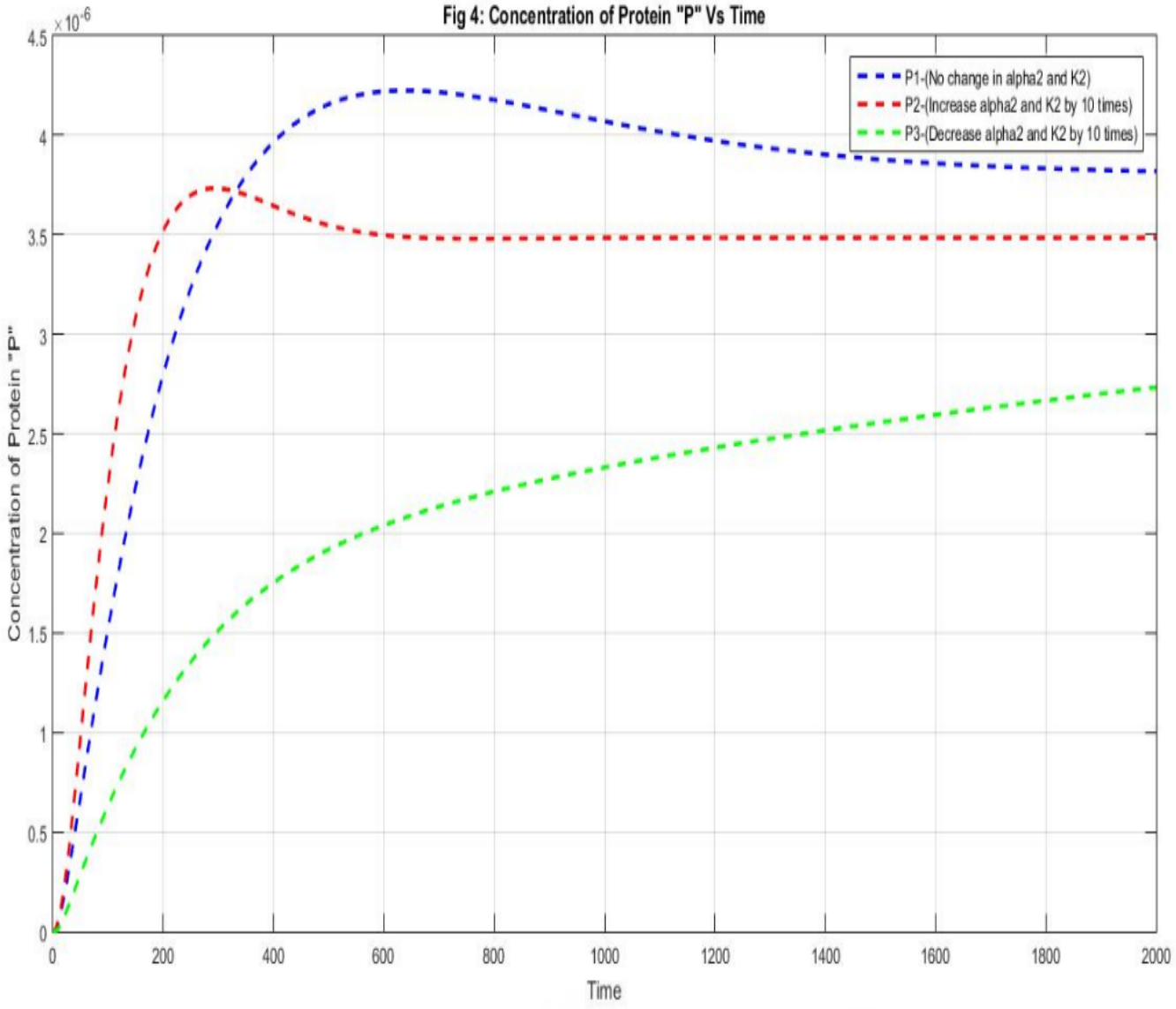

Fig 4; ; Time (0-2000) Seconds.

1. *P1=Protein:* Parameter values: Alpha1= $5.7X10^{-3}$, Alpha2= $1.15X10^{-3}$, Beta1= $0.6X10^{-9}$, Beta2= $4.3X10^{-2}$, K1= $1.2*10^{7}$, k2= 0.9.
2. *P2=Protein:* Parameters value Alpha2 and K2 is increase by 10 times, the rest are same.
3. *P3=Protein:* Parameters value Alpha2 and K2 is decrease by 10 times, the rest are same.

Varying Alpha2 & K2 (Protein):

In autoregulatory system the concentrations is induced by increasing **α2 & K2** *(Steady state is achieved at faster rate)* and delayed by reducing the **α2 & K2** (as shown in fig. 4).

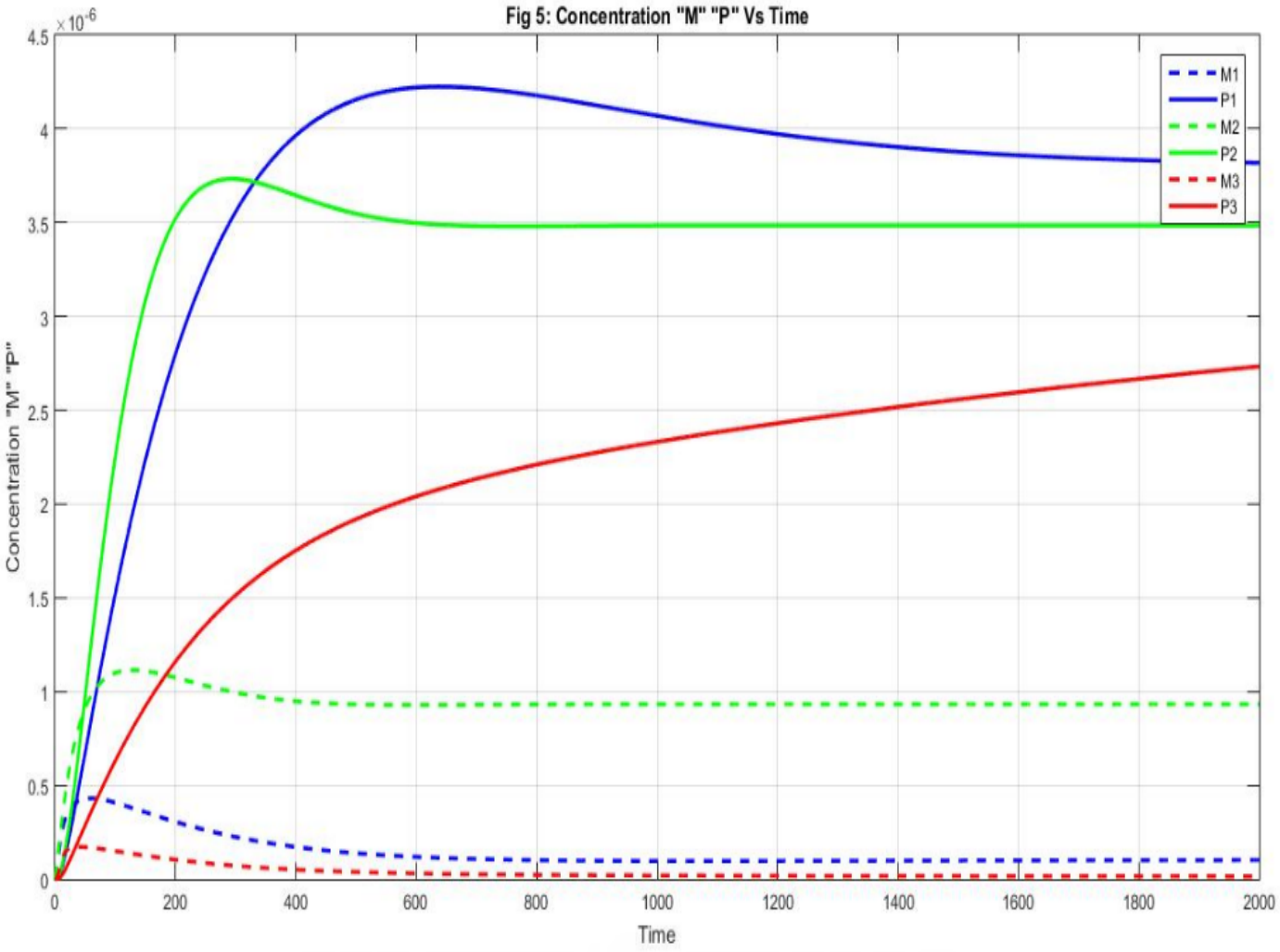

Fig 5; Time (0-2000) Seconds.

1. ***M1=mRNA (dash blue), P1=Protein (blue):*** Parameter values: Alpha1= $5.7X10^{-3}$, Alpha2= $1.15X10^{-3}$, Beta1= $0.6X10^{-9}$, Beta2= $4.3X10^{-2}$, K1= $1.2*10^{7}$, k2= 0.9.
   (No Change in the Parameters)

2. ***M2=mRNA (dash green), P2= Protein (green):*** Where Alpha2 and K2 are increases by 10 times, the rest are same.
   (Increase in Alpha2 and K2 parameters)

3. ***M3=mRNA (dash red), P3=Protein (red):*** Where Alpha2 and K2 are decreases by 10 times, the rest are same.
   (Decrease in Alpha2 and K2 Parameters)

(N.B: Steady state is achieved at much faster rate if* K2 & α2 *are increased).

RESULTS:

There are two steady state observed in mRNA ('M') and Protein ('P'), when 'M'=0 & 'P'=0 i.e (0,0) and when $'M' = \frac{\beta 1G}{\alpha 1}$ and $'P' = \frac{\beta 2M}{\alpha 2}$ i.e $(\frac{\beta 1G}{\alpha 1}, \frac{\beta 2M}{\alpha 2})$.

Varying the Parameters	Observation (where 't'=time)
Without Change in Parameters	When there is no change in the given parameters the mRNA Concentration rises rapidly at t=0-50 and degrade at t=50-600. At t=600 the steady state is achieved. In case of Protein the concentration rises rapidly at t=0-600 and degrade at t=600-1400. At t=1400 the steady state is achieved.
Alpha1	Decrease in **α1** induces the rate both in mRNA & Protein production. With increase in **α1** delay's the rate mRNA and Protein production.
Alpha2	Decrease in **α2** induces the rate both in mRNA & Protein production. With increase in **α2** delay's the rate mRNA & Protein production.
Beta1	Increase in **β1** induces the rate both in mRNA & Protein production. With decrease in **β1** delay's the rate mRNA & Protein production.
Beta2	Increase in **β2** induces the rate in Protein & delay's in mRNA production. With decrease in **β2** delay's the rate in Protein & induces mRNA production.
K1	Increase in **K1** induces the rate both in mRNA & Protein production. With decrease in **K1** delay's the rate mRNA & Protein production.
K2	Increase in **K1** induces the rate both in mRNA & Protein production. With decrease in **K1** delay's the rate in mRNA & Protein production.
Alpha2 &K2	Increase in **K2** & **α2** induces the rate both in mRNA & Protein production. With decrease in **K2** & **α2** delay's the rate in mRNA & Protein production ***(Steady state is achieved at much faster rate if* K2 & α2 *are increased).***

Discussion:

Negative autoregulation suggest that it can reduce the delays in transcription and translation factors. If a strong promoter with negative autoregulation is design, it will show faster rise-time to the same steady-state in comparison with the relatively weak promoter with no negative autoregulation. The rise-time is expected to decrease with increased cooperativity in the binding of the repressor to its own promoter.

Even if a strong non-autoregulated promoter is carried out in a gene circuit, it will reach any given concentration faster, but will stabilize at a much higher steady-state.

In Negative autoregulation the rise-time is expected to decrease with increased cooperativity in the binding of the repressor to its own promoter. Produced repressor shuts off its own production to reach the required steady-state concentration. This study contributes to the emerging understanding of genetic regulatory networks.

Conclusion:

Given the transcription of the repressor gene (G) yields mRNA molecules (M), which are translated to produce the repressor protein (P). The repressor protein acts as a negative regulator of its own transcription by binding to the operator-promoter complex. Thus the protein represses its own transcription.

Protein represses its own expression when it binds to its own promoter to inhibit the production of mRNA. At the early times Protein is very low and there is no degradation. Then the level of protein rises rapidly which will lead to stopping the production of mRNA and ultimately Protein too.

The stronger the promoter activity, the shorter the response time is. Negative autoregulation can therefore use a stronger promoter to give an initial faster transcription rate and then use autoregulation to stop the production at the desired steady state.

References:

1. Rosenfeld, N., Elowitz , M. B. & Alon, U. (2002), *Negative Autoregulation Speeds the Response Times of Transcription Networks*, J. Mol. Biol., **323**, 785–793.
2. Murugan, R., & Kreiman G., (2011), *On the Minimization of Fluctuations in the Response Times of Autoregulatory Gene Networks,* Biophysical Journal, **101**, 1297-1306.
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4. *https://in.mathworks.com/help/simbio/gs/-model-a-gene-regulation-pathway.html (access date 11-03-2017).*