Principles and Applications of Systems Biology
ChE 411

# Exercise 3: Kinetics models

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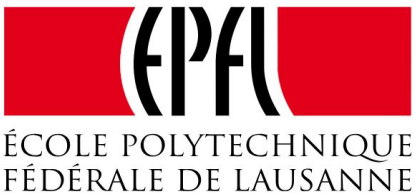
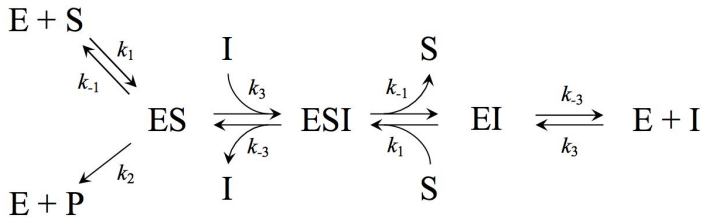


**Part 1. Michaelis-Menten Kinetics**

In this part, we will explore the impact of non competitive and competitive inhibition on the Michaelis-Menten kinetics.

We begin by considering a noncompetitive inhibition and the general reaction is represented by the following equation:




Where E is the enzyme, S the substrate, P the product, I the inhibitor. The rate constants are $k_1$, $k_{-1}$, $k_3$ and $k_{-3}$.

a) Write ODEs describing the change over time of all components of the system. Also, write a conservation of equation for the total enzyme concentration (equal to the initial concentration of free enzyme, or $[E]_0$ ), free and bound, in the system.

$$\frac{d[E]}{dt} = -k_1[E][S] + k_{-1}[ES] + k_2[ES] + k_{-3}[EI] - k_3[E][I] \quad (1)$$
$$\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[ES] + k_{-1}[ESI] - k_{-1}[EI][S] \quad (2)$$
$$\frac{d[I]}{dt} = -k_3[ES][I] + k_{-3}[ESI] + k_{-3}[EI] - k_3[E][I] \quad (3)$$
$$\frac{d[ES]}{dt} = -k_{-1}[ES] + k_1[E][S] - k_2[ES] - k_3[ES][I] + k_{-3}[ESI] \quad (4)$$
$$\frac{d[ESI]}{dt} = k_3[ES][I] - k_{-3}[ESI] - k_{-1}[ESI] + k_1[EI][S] \quad (5)$$
$$\frac{d[EI]}{dt} = k_{-1}[ESI] - k_{-1}[EI][S] - k_{-3}[EI] + k_3[E][I] \quad (6)$$
$$\frac{d[P]}{dt} = k_2[ES] \quad (7)$$

The total enzyme concentration is:

$$[E]_0 = [E] + [ES] + [EI] + [ESI] \quad (8)$$

b) Define the following dissociation constants:

$$K_D = \frac{k_{-1}}{k_1} \text{ and } K_I = \frac{k_{-3}}{k_3} \quad (9)$$

From part a)

We have $\frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]} = \frac{[EI][S]}{[ESI]}$ (10)

[ES]= $\frac{[E][S]}{K_D}$ (11)

$K_D$ is the dissociation constant of the substrate to the enzyme.

$\frac{k_{-3}}{k_3} = \frac{[ES][I]}{[ESI]} = \frac{[E][I]}{[EI]}$ (12)

From equations (11) and (12)

[ESI] = $\frac{[ES][I]}{K_I} = \frac{[E][S][I]}{K_D K_I}$ (13)

[EI] = $\frac{[E][I]}{K_I}$ (14)

$K_I$ is the dissociation constant of the inhibitor to the enzyme.

c) Substitute your equilibrium equations into the equation for $[E]_0$ . Use your equilibrium equation for [ES] to eliminate [E] from the equation. Solve this expression for [ES]

From equation (8):

$$[E]_0 = [E] + [ES] + [EI] + [ESI]$$

Then substitute equations (11), (13) and (14)

$$[E]_0 = [E] + \frac{[E][S]}{K_D} + \frac{[E][I]}{K_I} + \frac{[E][S][I]}{K_D K_I}$$

$$[E]_0 = [E]\left(1 + \frac{[S]}{K_D} + \frac{[I]}{K_I} + \frac{[S][I]}{K_D K_I}\right) \quad (15)$$

From (11)

[E] = $\frac{[ES]K_D}{[S]}$ (16)

Substituting (16) in (15)

$$[E]_0 = \frac{[ES]K_D}{[S]}\left(1 + \frac{[S]}{K_D} + \frac{[I]}{K_I} + \frac{[S][I]}{K_D K_I}\right)$$

$$[ES] = \frac{[E]_0[S]}{K_D\left(1 + \frac{[S]}{K_D} + \frac{[I]}{K_I} + \frac{[S][I]}{K_D K_I}\right)} \quad (17)$$

d) The product formation rate v is $\frac{d[P]}{dt}$ . Write an equation for v in terms of [S], [I], $K_D$ , $K_I$.

Vmax = $k_2[E]_0$ (18)

From (7)

$$\frac{d[P]}{dt} = k_2[ES]$$

$$\frac{d[P]}{dt} = k_2 \frac{[E]_0[S]}{K_D\left(1 + \frac{[S]}{K_D} + \frac{[I]}{K_I} + \frac{[S][I]}{K_D K_I}\right)}$$

$$\frac{d[P]}{dt} = \frac{V\,max\,[S]}{K_D\left(1 + \frac{[S]}{K_D} + \frac{[I]}{K_I} + \frac{[S][I]}{K_D K_I}\right)}$$

$$\frac{d[P]}{dt} = \frac{V\,max\,[S]}{(K_D + S)(1 + [I]/K_I)} \quad (19)$$

Equation 19 corresponds to non-competitive inhibition. The inhibitor changes the enzyme-substrate interaction without bonding to the active site. One proposed mechanism is that inhibitor changes the conformation of the enzyme by bonding with it in a remote location, making its active site unavailable for the substrate. The result of this type of inhibition is that the vmax and the km decrease with an increase in the inhibitor concentration.

e) Generate a plot of [S] (x axis) versus v (y axis) for varying [I].
Set the following constants:

$K_D$ = 0.25 %mM

$K_I = 1\,\%$ %mM
Vmax = 50 %mmol/min

<u>Effect of increasing [I]</u>

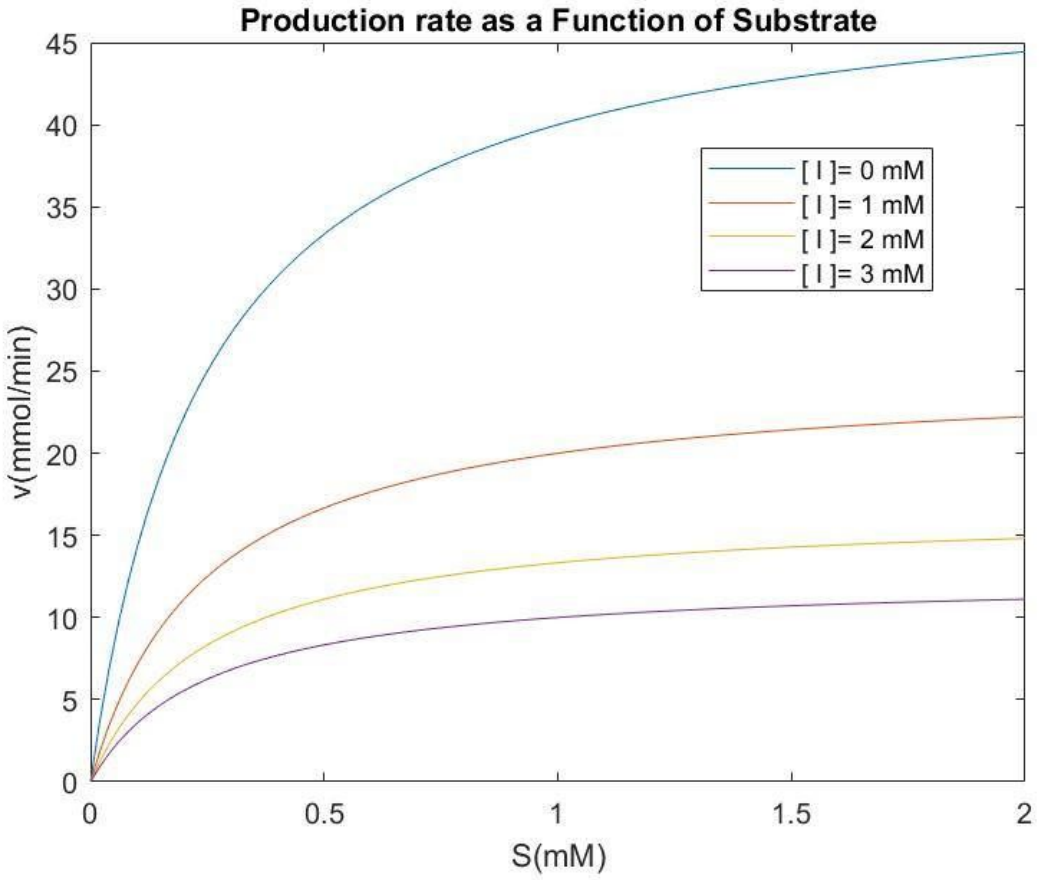



*Figure1. Production rate as a function of substrate*

The figure above describes the evolution of the production rate as a function of the substrate by varying the concentration of inhibitor [I].
One can see that, with an increase of [I], the production rate v decreases. The reason is that the inhibitor binds to the active enzyme instead of the substrate and interrupts the chemical pathway.
For example, for [I] = 0mM the production rate is maximal and v = 45 mmol/min.

Whereas for [I]= 3mM the production rate is minimal and v= 10 mmol/min.

**Part 2. Mitogen-Activated Protein Kinase (MAPK) cascades**

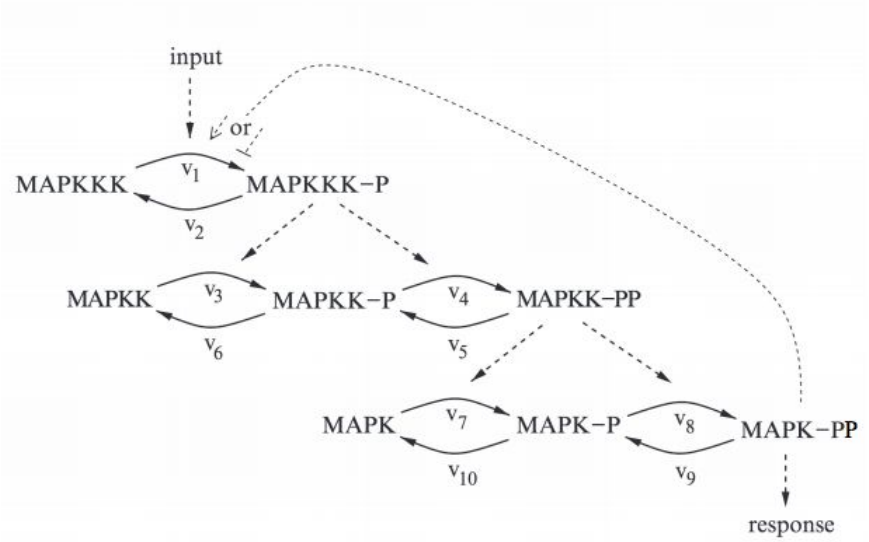



*Figure 2. MAPK cascade*

The simple model of the mammalian Ras-Raf-ERK activation cascade aims to explore dynamics behaviours of MAPK pathways.
The proteins in the cell communicates a **signal** from a receptor on the surface of the cell to its DNA in the nucleus. MAPK communicate by adding phosphate groups to a neighboring protein, which acts as an "on" or "off" switch.

Michaelis-Menten kinetics are used:

$$v_1 = V_{max,1} \frac{[MAPKKK]\,(1+K_a[MAPK-PP])}{(K_{M1}+[MAPKKK])(1+K_i[MAPK-PP])} \quad (20)$$

$$v_2 = V_{max,2} \frac{[MAPKKK-P]}{(K_{M2}+[MAPKKK-P])} \quad (21)$$

$$v_3 = k_{cat,3}\,[MAPKKK-P] \frac{[MAPKK]}{K_{M3}+[MAPKK]} \quad (22)$$

$$v_4 = k_{cat,4}\,[MAPKKK-P] \frac{[MAPKK-P]}{K_{M4}+[MAPKK-P]} \quad (23)$$

$$v_5 = V_{max,5} \frac{[MAPKK-PP]}{(K_{M5}+[MAPKK-PP])} \quad (24)$$

$$v_6 = V_{max,6} \frac{[MAPKK-P]}{(K_{M6}+[MAPKK-P])} \quad (25)$$

$$v_7 = k_{cat,7}\,[MAPKK-PP] \frac{[MAPK]}{K_{M7}+[MAPK]} \quad (26)$$

$$v_8 = k_{cat,8}\,[MAPKK-PP]\,\frac{[MAPK-P]}{K_{M8}+[MAPK-P]} \quad (27)$$

$$v_9 = V_{max,9}\,\frac{[MAPK-PP]}{(K_{M9}+[MAPK-PP])} \quad (28)$$

$$v_{10} = V_{max,10}\,\frac{[MAPK-P]}{(K_{M10}+[MAPK-P])} \quad (29)$$

a) Write down the differential equations for all species in the system using the rate equations given above

$$\frac{d[MAPKKK-P]}{dt} = v_1 - v_2 \quad (30)$$

$$\frac{d[MAPKK-P]}{dt} = v_3 - v_4 + v_5 - v_6 \quad (31)$$

$$\frac{d[MAPKK-PP]}{dt} = v_4 - v_5 \quad (32)$$

$$\frac{d[MAPK-P]}{dt} = v_7 - v_8 + v_9 - v_{10} \quad (33)$$

$$\frac{d[MAPK-PP]}{dt} = v_8 - v_9 \quad (34)$$

$$\frac{d[MAPKKK]}{dt} = v_2 - v_1 \quad (35)$$

$$\frac{d[MAPKK]}{dt} = v_6 - v_3 \quad (36)$$

$$\frac{d[MAPK]}{dt} = v_{10} - v_7 \quad (37)$$

b) Solving the differential equation for all species at all timepoints.

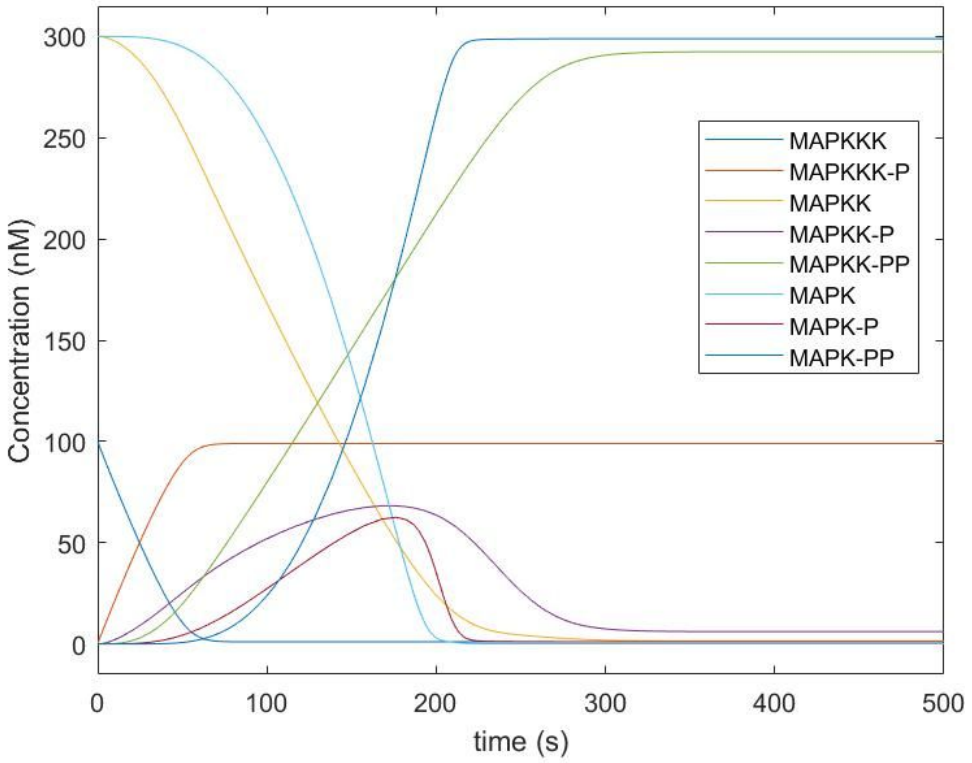



*Figure3. Concentration evolution of all species in function of time*

The figure above shows the concentration evolution for $V_{max,1} = 2.5\ nM.\ s - 1$
$K_i = K_a$ = 0, [MAPKKK] = 100 nM, [MAPKK] = 300 nM and [MAPK] = 300 nM.
The concentration of phosphorylated species increases with time until reaching the steady state, which is the point of the maximum activity.
[MAPKKK], [MAPKK], and [MAPK] decreases with time and reaches zero.

c) **Switch-Like dose-response**

$K_i = K_a$ = 0 : No feedback in the system
[MAPKKK] = 100 nM
[MAPKK] = 300 nM
[MAPK] = 300 nM
All the other concentration are set to zero.

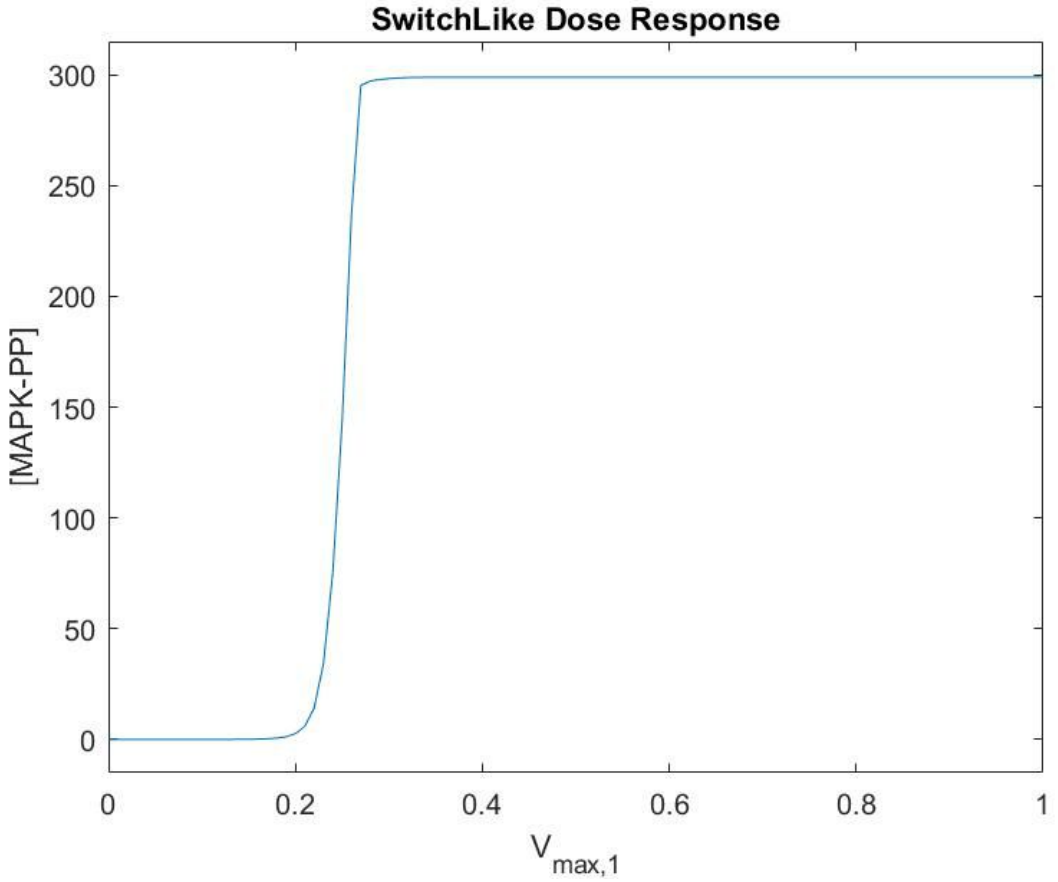



*Figure4. Switch-Like Dose Response*

It's said that $V_{max,1}$ is the input of the system and it can be changed due a ligand-receptor binding that increases the activity of the enzyme that phosphorylates MAPKKK. That means if one increases the input $V_{max,1}$, the output concentration of MAPK-PP should also increase. One can see that without any feedback loop in the MAPK cascade, the maximal amplitude phosphorylation of MAPK-PP output is reached. It is interesting to see how a system composed of Michaelis Menten rates which don't have a switch-like response individually, are able to cooperate and generate a step-like response when a certain threshold signal is reached.

d) **Kholodenko model**

$K_i$ = 0.1

$K_a$ = 0

For $V_{max,1} = 2.5\,\text{nM}.s^{-1}$

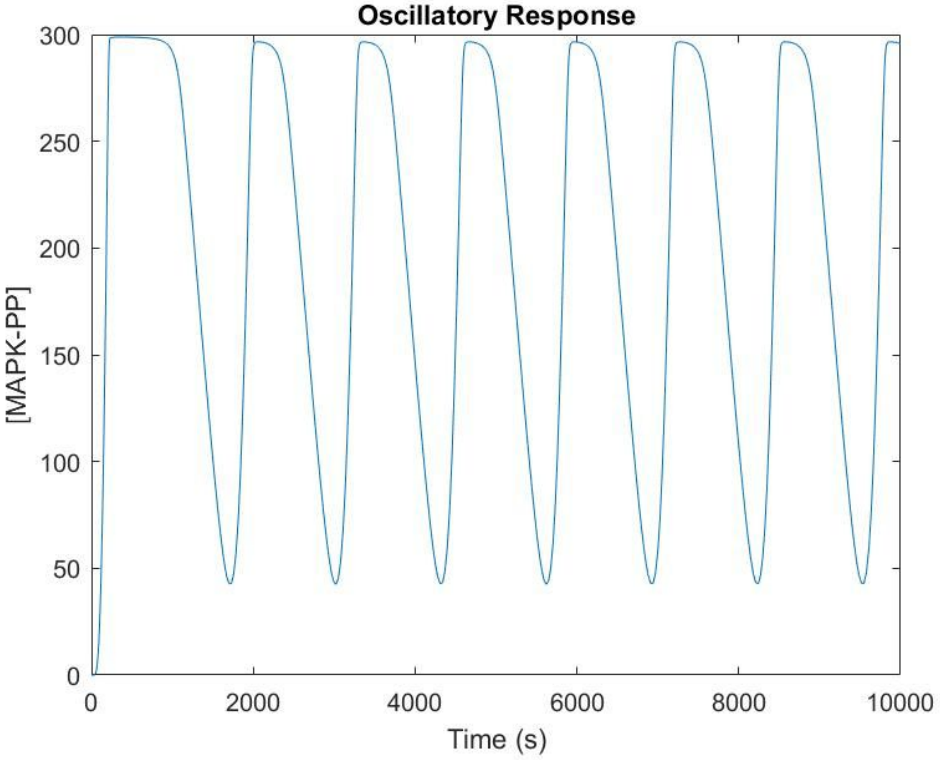



*Figure5. Oscillatory response for input* $V_{max,1} = 2.5\,\text{nM}.s^{-1}$

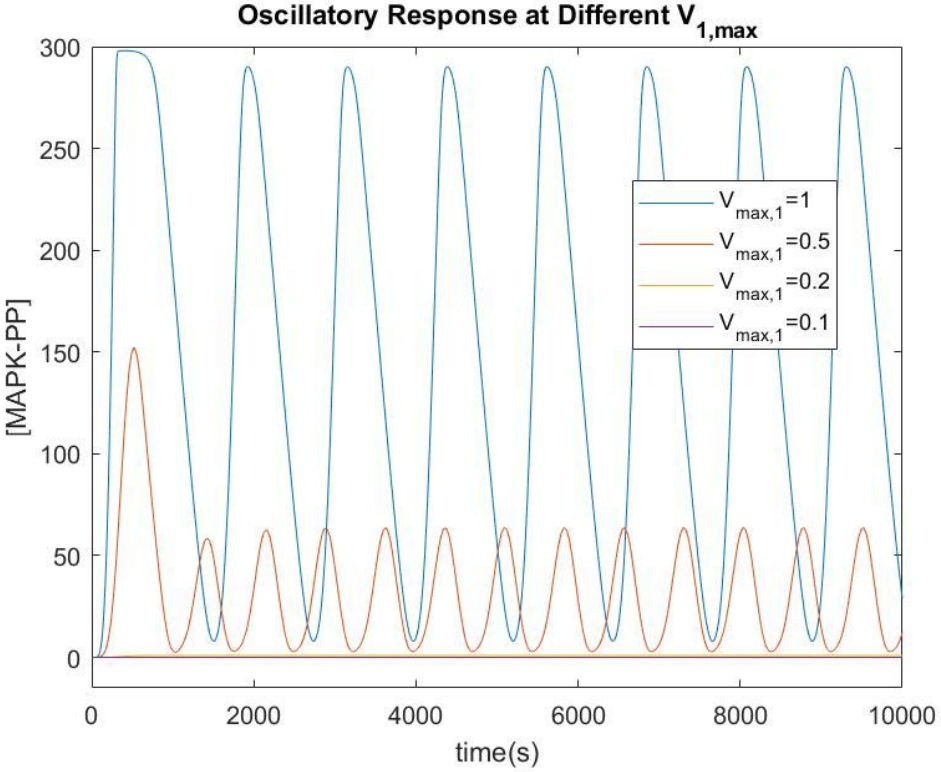



*Figure 6. Oscillatory response for* $V_{max,1} = [1.0,\ 0.5,\ 0.2,\ 0.1]$ *nM/s*

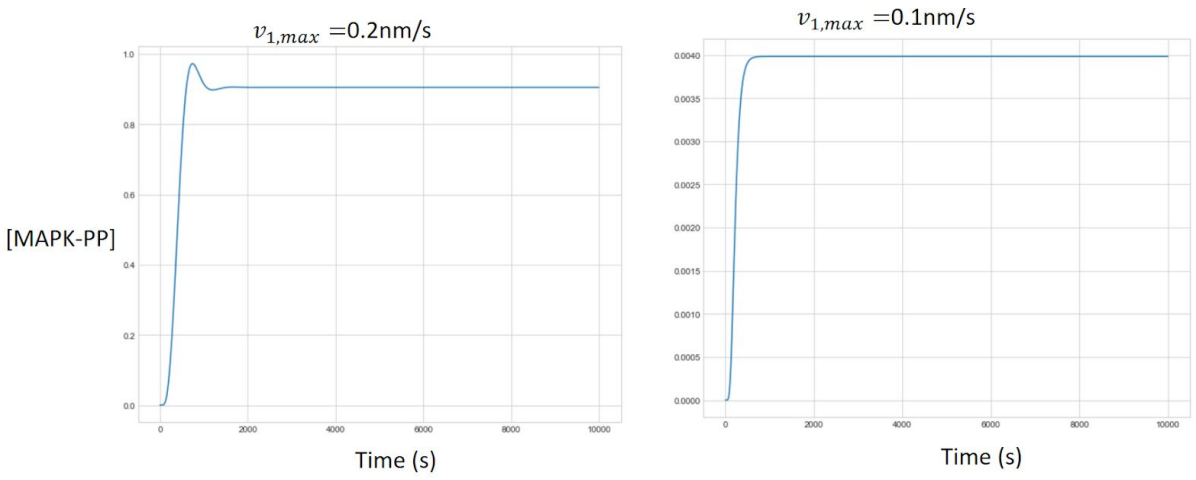



Figure 7: Dampened Response at for $V_{max,1} = [\,0.2,\ 0.1\,]$ *nM/s*

The coupled negative and positive feedbacks lead to an oscillation response for high enough Vmax,1. Varying the input $V_{max,1}$ shows that the oscillations do not persist at lower input level of $V_{max,1}$ and the maximum amplitude is attained at $V_{max,1}$ = 1 nM. $s^{-1}$ (Figure 6). From figure (5), for $V_{max,1}$ = 2.5 nM. $s^{-1}$ the period of oscillation is about 1300 s, and the amplitude is slightly lower than for Vmax,1=1nM/s. The following table shows the comparison of periods and amplitudes as a function of vmax:

| V1max [nm/s] | Period [s] | Amplitude [nM] |
|---|---|---|
| 2.5 | 1300 | 125 |
| 1.0 | 1230 | 140 |
| 0.5 | 720 | 30 |

Table 1: Comparison of Oscillatory Response for Different V1,max

Table 1 shows that the period seems to decrease with v1max and the amplitude as well, although the amplitude seems to be in the same range when comparing 2.5nm/s and 1nm/s. This is perhaps due to both v1max being above the 'swtich-like' threshold, leading to a similar oscillatory response. As v1max decreases to 0.5, the frequency of the oscillations increase, yet the amplitude decreases greatly. When comparing this response to the response without the inhibition (Figure 5), it is clear that the inhibition shifted the 'switch' threshold to the left. Moreover, when the feedback is on, a combination of the switch-like response and the oscillatory response are observed. At low vmax, if the threshold is not met, the oscillatory response will be dampened and the product concentration will be close to 0. At high vmax, the average product concentration raises and the feedback effect controls the upstream production of phosphorylated species when MAPK-PP is in excess.