International Institute of
Information Technology, Hyderabad

Systems Biology

Adaptation Mechanisms in Phosphorylation Cycles

Project Notes

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Introduction

The project is based on the paper 'Adaptation mechanisms in phosphorylation cycles by allosteric binding and gene autoregulation'. The aim of this paper was to study adaptation mechanics in a class of phosphorylation cycles under regulation by allosteric binding and gene autoregulation methods.

Mass Action Kinetics

The paper has done all the math using the mass action kinetics which is described below.

Definition 0.1

For the reaction

$$\mathrm{E} + \mathrm{S} \xrightarrow{\mathrm{k}_f} \mathrm{P}$$

The rate of reaction (v) is given by :

$$\begin{aligned} v &= \frac{d[P]}{dt} \\ &= k_f[E][S] \end{aligned}$$

This is known as the mass action kinetics.

Michaelis-Menten Kinetics

The project attempts to replicate the paper using Michaelis–Menten kinetics, which is defined as below.

Definition 0.2

For the reaction

$$\mathrm{E} + \mathrm{S} \underset{\mathrm{k}_b}{\overset{\mathrm{k}_f}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{\mathrm{k}_{\mathrm{cat}}} \mathrm{P}$$

The rate of reaction (v) is given by :

$$\begin{aligned} v &= \frac{d[P]}{dt} \\ &= V_{max} \frac{[S]}{[S] + K_d} \end{aligned}$$

In this, the definitions are as follows :

$$V_{max} = k_{cat}[E]_0$$
$$K_d = \frac{k_b}{k_f}$$

This is known as the Michaelis–Menten kinetics.

Limitations of Michaelis–Menten Kinetics

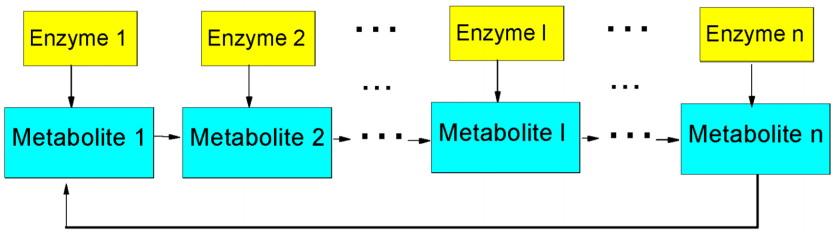
This is a different result as compared to mass action kinetics. The Michaelis–Menten kinetics operate under the assumption that $K_d \ll 1$, and in general operate under the rule that the second reaction is non reversible, which is applicable under the condition that $[S] \gg [P]$ or $\Delta G \ll 0$.

Thus, the conditions under which Michaelis–Menten kinetics operate are :

- $K_d \ll 1$
- $[S] \gg [P]$
- $\Delta G \ll 0$

Results of the Original Paper

The original paper does mathematical analysis of the phosphorylation system given below :



This system is described by the following set of equations :

$$X_i + E_i \underset{K_{i,2}}{\overset{K_{i,1}}{\rightleftharpoons}} X_i E_i \longrightarrow X_{i+1} + E_i$$

The paper then uses multiple Lemmas and Propositions to describe the behavior of the system. The following analyses are done on the system :

- Robustness Analysis
- Stability Analysis
- Adaptation to Biological Rhythms

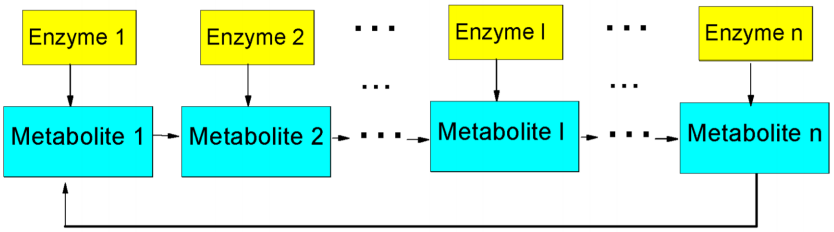
The aim of the project is to replicate results of the above analysis using Michaelis–Menten kinetics. Due to me not completely understanding the complete math of paper, the main aim is to replicate and compare Michaelis–Menten kinetics on robustness.

Mass Action Results

This chapter contains all the analysis done in the original paper and the conclusions that it came up with. This is very similar to the original paper itself but is redone again to make the compare and contrast easier to understand and make the steps followed easier to understand.

System Description

The following phosphorylation system is used for studying :



Rate Equations

This corresponds to the following set of reactions :

$$\mathrm{X}_i + \mathrm{E}_i \underset{\mathrm{K}_{i,\,2}}{\overset{\mathrm{K}_{i,1}}{\rightleftharpoons}} \mathrm{X}_i\mathrm{E}_i \longrightarrow \mathrm{X}_{i+1} + \mathrm{E}_i$$

Which then corresponds to the following rate equations :

$$\begin{aligned}
\frac{d[X_i]}{dt} &= -K_{i,1}[X_i][E_i] + K_{i,2}[X_iE_i] + K_{i-1,3}[X_{i-1}E_{i-1}] \\
\frac{d[E_i]}{dt} &= -K_{i,1}[X_i][E_i] + (K_{i,2} + K_{i,3})[X_iE_i] \\
\frac{d[X_iE_i]}{dt} &= K_{i,1}[X_i][E_i] - (K_{i,2} + K_{i,3})[X_iE_i]
\end{aligned}$$

As the amount of enzyme remains constant in the system, the equations can be rewritten as follows :

$$\begin{aligned}
\frac{d[X_i]}{dt} &= K_{i,1}[X_i][X_iE_i] + K_{i,2}[X_iE_i] + K_{i-1,3}[X_{i-1}E_{i-1}] - K_{i,1}[X_i][E_i]_0 \\
\frac{d[E_i]}{dt} &= -K_{i,1}[X_i][X_iE_i] + (K_{i,2} + K_{i,3})[X_iE_i] + K_{i,1}[X_i][E_i]_0 \\
\frac{d[X_iE_i]}{dt} &= -K_{i,1}[X_i][X_iE_i] - (K_{i,2} + K_{i,3})[X_iE_i] + K_{i,1}[X_i][E_i]_0
\end{aligned}$$

This is due to the fact that $[E_i]_0 = [E]_i + [X_iE_i]$, which can be used to eliminate the $[E_i]$ term from the equations.

Allosteric Binding and Negative Autoregulation

The paper then goes on to describe the phosphorylation cycle with allosteric binding and negative autoregulation. The reaction taken to exhibit this phenomenon is the DNA $\longrightarrow$ mRNA $\longrightarrow$ Proteins, where negative autoregulation is described.

The paper assumes that autoregulation is done by a reversible binding/releasing of free and bound promoters, which is described below.

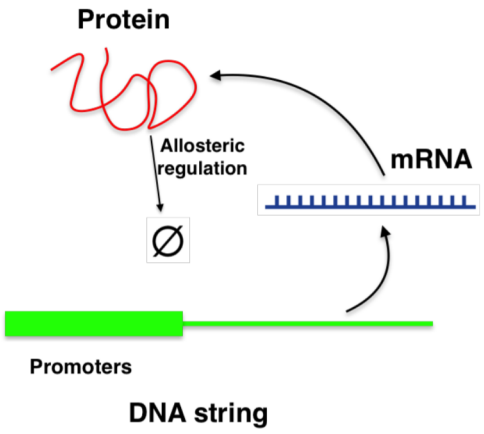
$$\mathrm{FP} + \mathrm{E_{1total}} \underset{\mathrm{K_{rel}}}{\overset{\mathrm{K_{allo}}}{\rightleftharpoons}} \mathrm{BP}$$

In this FP is the free promoter and BP is the bound one. The assumption that number of binding sites on promoters is very small as compared to concentration of proteins, to neglect the effect of binding/releasing reaction on the dynamics of the enzyme, is also made.

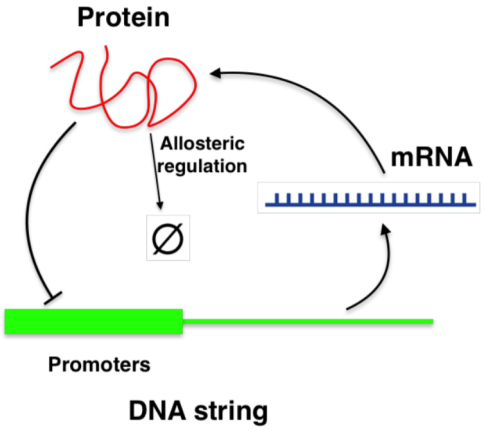
The state equations for these reactions are as follows :

$$\begin{aligned}
\frac{d[FP]}{dt} &= K_{rel}([FP]_{max} - [FP]) \\
\frac{d[mRNA]}{dt} &= K_{pro}[FP] - K_d[mRNA] \\
\frac{d[E1]_{total}}{dt} &= K_{tran}[mRNA] - K_{allo}\frac{[X_l][E_1]_{total}}{K_{half} + [E_1]_{total}}
\end{aligned}$$

These equations are for the following reaction :



Another mechanism with negative autoregulation is also described by the paper, which is given as follows :

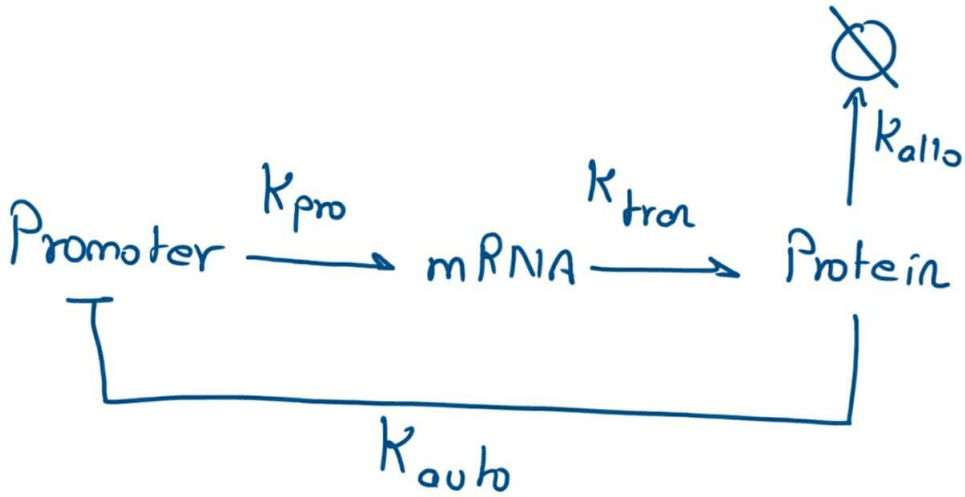


This system is described by the following equations :

$$\begin{aligned}
\frac{d[FP]}{dt} &= K_{rel}([FP]_{max} - [FP]) - K_{auto}[E_1]_{total}[FP] \\
\frac{d[mRNA]}{dt} &= K_{pro}[FP] - K_d[mRNA] \\
\frac{d[E1]_{total}}{dt} &= K_{tran}[mRNA] - K_{allo}\frac{[X_l][E_1]_{total}}{K_{half} + [E_1]_{total}}
\end{aligned}$$

In these, $[FP]_{max}$ is the total concentration of all the promoters.

The reaction with all the rate constants is given as below :



The other constants are as follows :

- K_{half}
  Concentration of $[E_1]_{total}$ when reaction rate is half-maximum.
- $[FP] + [BP] = [FP]_{\max}$

Closed Loop Systems

Thus using the math done above, the rate equations are as follows :

DNA Cycle

$$\begin{aligned}
\frac{d[FP]}{dt} &= K_{rel}([FP]_{max} - [FP]) - K_{auto}[E_1]_{total}[FP] \\
\frac{d[mRNA]}{dt} &= K_{pro}[FP] - K_d[mRNA] \\
\frac{d[E1]_{total}}{dt} &= K_{tran}[mRNA] - K_{allo}\frac{[X_l][E_1]_{total}}{K_{half} + [E_1]_{total}}
\end{aligned}$$

Phosphorylation Cycle

$$\begin{aligned}
\frac{d[X_i]}{dt} &= K_{i,1}[X_i][X_iE_i] + K_{i,2}[X_iE_i] + K_{i-1,3}[X_{i-1}E_{i-1}] - K_{i,1}[X_i][E_i]_0 \\
\frac{d[X_iE_i]}{dt} &= -K_{i,1}[X_i][X_iE_i] - (K_{i,2} + K_{i,3})[X_iE_i] + K_{i,1}[X_i][E_i]_0
\end{aligned}$$

Conclusion

In the above section, the system was properly defined and in the next section, various analysis will be done.

Analysis

This is analysis which is done under equilibrium and various aspects such as robustness will be analysed.

Robustness

The robustness of X_l species is checked under both the metabolism regulation mechanisms as discussed before. Robustness can be examined by using the elasticity metric.

A $[S]^*$ is defined, which is the concentration of given species S at equilibrium.

Elasticity Coefficient

Elasticity Coefficient (ε) is defined as :

$$\varepsilon = \frac{d \ln [X_l]^*}{d \ln [E_1]^*_{\text{total}}}$$

Thus, the closer ε is to 0, the more robust the system is, as it will try to remain at equilibrium.

Derevation

The deravative of the latent state variable $[FP]+\frac{K_{rel}}{K_{pro}}[mRNA]+\frac{K_d K_{rel}}{K_{tran}K_{pro}}[E_1]_{total}$ is as follows :

$$\begin{aligned}
&\frac{d}{dt}([FP] + \frac{K_{rel}}{K_{pro}}[mRNA] + \frac{K_d K_{rel}}{K_{tran}K_{pro}}[E_1]_{total}) \\
=& - \frac{K_d K rel K_{allo}}{K_{tran}K_{pro}} \frac{[X_l][E_1]_{total}}{K_{half} + [E_1]_{total}} + K_{rel}[FP]_{max} - K_{auto}[E_1]_{total}[FP] \\
=& - K_{gain}([X_l] - R - \frac{K_{half}[X_l]}{K_{half} + [E_1]_{total}} + \frac{K_{auto}}{K_{gain}}[E_1]_{total}[FP])
\end{aligned}$$

In this, the defined constants are :

- $K_{gain} = \frac{K_d K_{rel} K_{allo}}{K_{tran} K_{pro}}$
- $R = \frac{K_{rel}[FP]_{max}}{K_{gain}}$

Thus, at equilibrium

$$\begin{aligned}[X_l]^* &= (R - \frac{K_{auto}}{K_{gain}}[E_1]^*_{total}[FP]^*)\frac{K_{half} + [E_1]^*_{total}}{[E_1]^*_{total}} \\ &= R(\frac{K_{rel}}{K_{auto}[E_1]^*_{total} + K_{rel}})\frac{K_{half} + [E_1]^*_{total}}{[E_1]^*_{total}}\end{aligned}$$

Thus, elasticity coefficient is :

$$\varepsilon = -\frac{K_{auto}[E_1]^*_{total}}{K_{auto}[E_1]^*_{total} + K_{rel}} + \frac{K_{half}}{K_{half} + [E_1]^*_{total}}$$

Conclusion

Thus, it can be seen that two scenarios occur, which are as follows :

- $\varepsilon \approxeq 0$
  $K_{half} \ll [E_1]^*_{total} \ll \frac{K_{rel}}{K_{auto}}$
- $\varepsilon \approxeq -1$
  $[E_1]^*_{total} \gg \max(K_{rel}/K_{auto}, K_{half})$
  $[E_1]^*_{total} \ll \min(K_{rel}/K_{auto}, K_{half})$

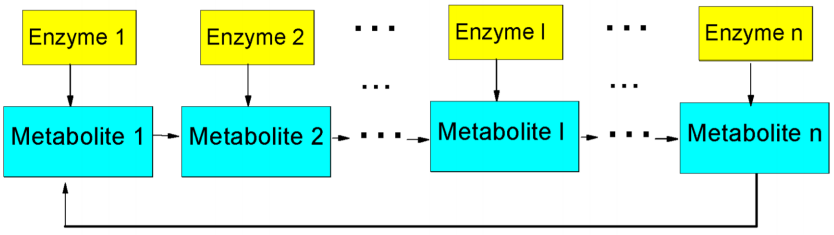
This suggests that regulator is highly robust at medium levels of $[E_1]^*_{total}$ and fragile at extreme levels of it.

Michaelis-Menten Results

In this section, Michaelis-Menten kinetics is applied to the system and the results are compared with the original results given in the original paper.

System Description

The system reaction is given below, which is same as the one given above :



Rate Equations

According to Michaelis-Menten kinetics, the rate equations are as given :

$$\begin{aligned}\frac{d[X_i]}{dt} &= V_{\max}\frac{[X_{i-1}]}{K_M + [X_{i-1}]} \\ &= K_{i,3}[E_i]_{\text{total}}\frac{[X_{i-1}]}{\frac{K_{i,1}}{K_{i,2}} + [X_{i-1}]}\end{aligned}$$

$$\begin{aligned}\frac{d[E_i]}{dt} &= -K_{i,1}[E_i][X_i] + K_{i,2}[E_iX_i] + K_{i,3}[E_iX_i] \\ &= -K_{i,1}[E_i][X_i] + (K_{i,2} + K_{i,3})\frac{[E_i]_{\text{total}}[X_i]}{K_d + [X_i]}\end{aligned}$$

$$\begin{aligned}\frac{d[X_iE_i]}{dt} &= k_{i,1}[X_i][E_i] - K_{i,2}[E_iX_i] + K_{i,3}[E_iX_i] \\ &= K_{i,1}[E_i][X_i] - (K_{i,2} + K_{i,3})\frac{[E_i]_{\text{total}}[X_i]}{K_d + [X_i]}\end{aligned}$$

These can be further simplified, when replacing $[E_i]$ with $[E_i]_{\text{total}} - [X_iE_i]$. Thus the modified equations are :

$$\begin{aligned}\frac{d[X_i]}{dt} &= K_{i,3}[E_i]_{\text{total}}\frac{[X_{i-1}]}{\frac{K_{i,1}}{K_{i,2}} + [X_{i-1}]} \\ \frac{d[E_i]}{dt} &= -K_{i,1}[E_i]_{\text{total}}[X_i] + (K_{i,2} + K_{i,3} + [X_i])\frac{[E_i]_{\text{total}}[X_i]}{K_d + [X_i]} \\ \frac{d[X_iE_i]}{dt} &= K_{i,1}[E_i]_{\text{total}}[X_i] - (K_{i,2} + K_{i,3} + K_{i,1}[X_i])\frac{[E_i]_{\text{total}}[X_i]}{K_d + [X_i]}\end{aligned}$$

Allosteric Binding and Negative Autoregulation

The equations in the allosteric binding and negative autoregulation remain the same and thus are as follows :

$$\begin{aligned}\frac{d[FP]}{dt} &= K_{rel}([FP]_{max} - [FP]) - K_{auto}[E_1]_{total}[FP] \\ \frac{d[mRNA]}{dt} &= K_{pro}[FP] - K_d[mRNA] \\ \frac{d[E1]_{total}}{dt} &= K_{tran}[mRNA] - K_{allo}\frac{[X_l][E_1]_{total}}{K_{half} + [E_1]_{total}}\end{aligned}$$

Closed Loop Systems

Thus the rate equations for the systems are as follows :

DNA Cycle

$$\frac{d[FP]}{dt} = K_{rel}([FP]_{max} - [FP]) - K_{auto}[E_1]_{total}[FP]$$
$$\frac{d[mRNA]}{dt} = K_{pro}[FP] - K_d[mRNA]$$
$$\frac{d[E1]_{total}}{dt} = K_{tran}[mRNA] - K_{allo}\frac{[X_l][E_1]_{total}}{K_{half} + [E_1]_{total}}$$

Phosphorylation Cycle

$$\frac{d[X_i]}{dt} = K_{i,3}[E_i]_{\text{total}}\frac{[X_{i-1}]}{\frac{K_{i,1}}{K_{i,2}} + [X_{i-1}]}$$
$$\frac{d[X_iE_i]}{dt} = K_{i,1}[E_i]_{\text{total}}[X_i] - (K_{i,2} + K_{i,3} + K_{i,1}[X_i])\frac{[E_i]_{\text{total}}[X_i]}{K_d + [X_i]}$$

Conclusion

Thus it can be seen that only the phosphorylation cycle has had any effect by changing the mechanism of calculating kinetics.

Analysis

This is analysis which is done under equilibrium and various aspects such as robustness will be analysed.

Robustness

We will use the same metric of elasticity coefficient to determine the robustness of the system. Thus calculation of $[X_l]^*$ is required which can be done by calculating the derivative of the latent state variable $[FP] + \frac{K_{rel}}{K_{pro}}[mRNA] + \frac{K_dK_{rel}}{K_{tran}K_{pro}}[E_1]_{total}$ which is as follows :

$$\begin{aligned}
&\frac{d}{dt}([FP] + \frac{K_{rel}}{K_{pro}}[mRNA] + \frac{K_dK_{rel}}{K_{tran}K_{pro}}[E_1]_{total}) \\
=& -\frac{K_dKrelK_{allo}}{K_{tran}K_{pro}}\frac{[X_l][E_1]_{total}}{K_{half} + [E_1]_{total}} + K_{rel}[FP]_{max} - K_{auto}[E_1]_{total}[FP] \\
=& -K_{gain}([X_l] - R - \frac{K_{half}[X_l]}{K_{half} + [E_1]_{total}} + \frac{K_{auto}}{K_{gain}}[E_1]_{total}[FP])
\end{aligned}$$

It can be seen that it yields the same result and thus, from this, it is clear that measure of robustness will remain same. Thus the $[X_l]^*$ is :

$$
\begin{aligned}
[X_l]^* &= (R - \frac{K_{auto}}{K_{gain}}[E_1]^*_{total}[FP]^*)\frac{K_{half} + [E_1]^*_{total}}{[E_1]^*_{total}} \\
&= R(\frac{K_{rel}}{K_{auto}[E_1]^*_{total} + K_{rel}})\frac{K_{half} + [E_1]^*_{total}}{[E_1]^*_{total}}
\end{aligned}
$$

Which then leeds to the following elasticity coefficient :

$$
\varepsilon = -\frac{K_{auto}[E_1]^*_{total}}{K_{auto}[E_1]^*_{total} + K_{rel}} + \frac{K_{half}}{K_{half} + [E_1]^*_{total}}
$$

Conclusion

And hence the same conclusion is drawn, which gives claim to the hypothesis given by the original papers' authors that it would remain the same.

Future Work

Due to understanding and time constrains, only one metric was tested by me, which came out give the same result.

Further work on the paper can be done, where even more testing is done to claims and the reason for the results being same can be tested in greater detail.