Synergy or Transport in Enzyme Assembly

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Abstract

Multi-step enzymatic reactions play a critical role in many cellular functions and have numerous applications in the metabolic engineering of natural product biosynthesis. The enzymes in metabolic pathways do not work independently, but synergistically form supramolecular complexes. Based on the research work conducted by Park et al., where cellulose hydrolysis was used as a model system to show that a single protein scaffold can be used to sequentially assemble multiple enzymes and generate nanoscale outer membrane vesicles. To study the enzyme synergism, the system was considered diffusion controlled first and the reaction kinetics was simulated by random walk theory. To simulate the kinetically controlled mechanism tau leaping algorithm along with Michaelis Menten kinetics was used. The work was concluded by studying the combined effects of diffusion controlled and kinetic controlled mechanisms.

1.Introduction

Enzymes play a vital role in controlling the rate of chemical reactions in living organisms and their application include but not limited to protein synthesis by means of replication, transcription, and translation; primary and secondary metabolism, developing a cell's immune system etc (Palmer and Bonner). Oftentimes their work is done by an assembly of multiple enzymes working together throughout multistep reactions or cascade processes. Here, the enzymes synergistically form supramolecular complexes where the product of one enzyme-substrate reaction acts as a substrate for the other enzyme, the major reason behind the effectiveness of this system is based on the high substrate specificity of enzymatic reactions (Schoffelen and van Hest).

To implement these multistep or cascade reactions several research works based on enzyme encapsulation have been conducted. Efforts have been made to encapsulate enzymes in liposomes made out of phospholipids or polymers (i.e. polymersomes). The main reason that propelled the use of these vesicles, is that the hydrophobic bilayer membranes of these vesicles are similar to that of the phospholipid bilayer of a cell and they also act as a barrier. However, the major challenge related to the use of liposomes includes its structural instability, the phospholipid bilayer in the liposomal structure is not rigid enough by itself and often time needs the addition of cholesterol or other amphiphilic molecules. Polymersomes have challenges related to low membrane permeability and fixation of this problem requires the inclusion of proton pumps and channel proteins in the bilayer to increase membrane permeability (Nomura et al., Van Dongen et al., Peters et al.)

In their work Park et al. used Outer membrane vesicles (OMVs) as nano-bioreactors. OMVs are proteo-liposomes and obtained from the surface of some gram-negative bacteria. By genetic modification of the gram-negative bacteria, several functional proteins can be attached to the surface of the OMVs and thus makes them a suitable candidate for use as a nano-bioreactor (Lee et al.). The study by Park et al., took advantage of the cohesin-dockerin interaction strategy and used cellulose hydrolysis as a model system to show that a single protein scaffold can be used to sequentially assemble multiple enzymes and generate nanoreactors capable of performing biocatalysis. Figure 1 shows a simplified schematic of this system. In an effort to understand the reactivity of a system consisting of three enzymes and a substrate such as the one presented in the paper by Park et al. 2014, our study aims to investigate the influence of both diffusion and chemical kinetics to determine if the 23-fold increase in conversion of cellulose to glucose that was observed by OMVs carrying all three enzymes is truly a result of synergy developed between the three enzymes as the paper states. To investigate this further, the present investigation walks through 3 simulations: diffusion-controlled, kinetic-controlled, and a combination system. Through these simulations, our goal is to determine whether the result by Park et al. is truly a result of synergy or simply special organization.

2. Methodology

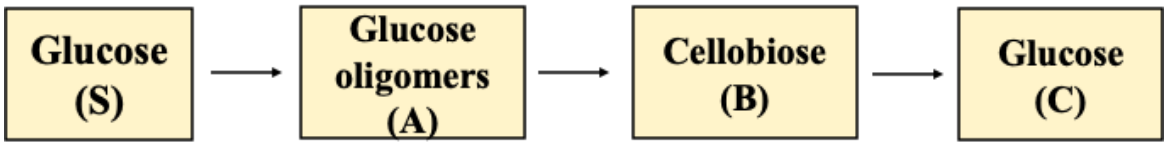
The conversion of substrate cellulose (S) to glucose (C) explained in the paper of Park et al. can be further simplified into following reactions:

S in presence of E1 → A, (S + E1 → A) (in our model, the moment of A formation is t=0) A in presence of E2 → B, (A + E2 → B)

B in presence of E3 → C, (B + E3 → C)

Overall Reaction: A → B → C

Where E1 = Enzyme 1, A = glucose oligomer, E2 = enzyme 2, B = cellobiose, and E3 = enzyme 3.



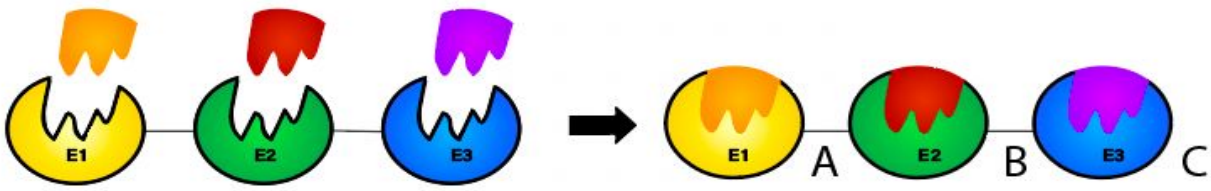


Figure 1. Schematic representation of the work done by Park el al., Here, S, is the initial substrate cellulose, E1 is enzyme 1, A is glucose oligomer, E2 = enzyme 2, B is cellobiose, E3 is enzyme 3, and C is the final product glucose.

To simplify the analysis part of this work and make it less code expensive, the following assumptions were made: (i) No side reactions can occur due to the enzymes' high specificity to their intended substrates (ii) An enzyme will react with only their corresponding substrate if an enzyme's active site is occupied by another substrate it will be considered as inactivated. Based on these assumptions, detailed descriptions of the implementation of the diffusion-controlled system, kinetic controlled system, and combined system have been given below:

Diffusion-controlled system: Random walk theory was used to simulate the diffusion-controlled system. Random walk theory correlates well with diffusion effects since diffusion can be related to Brownian motion. Based on this correlation, the reaction rate constant of the system can be determined by

$$k = \frac{1}{average\ reaction\ time} = \frac{average\ steps\ to\ the\ reaction}{time\ per\ step}$$

Since we are only interested in the effects of diffusion, another assumption was made that the reaction is instantaneous. Through this assumption, we are implying that step of the reaction occurs as soon as a molecule reaches its enzyme and then diffuses to continue to the next step of the reaction. This also means we are considering the diffusion time to be much longer than reaction time and therefore reducing the system to where reaction rates only depend on the physical movements of the molecules. Using this knowledge, a 2D walk simulation was developed. This script assumes a walk with user-defined steps and locations of E2 and E3, where A forms B and B forms C, respectively. The walk is periodically bounded by a 1000 unit square from the initial position of A which prevents the molecules from traveling too far out while dually simulating the presence of duplicate enzymes a molecule can encounter if it diffuses out far enough. We also implemented a set of successes and failures to inform the system of whether it should continue to the next reaction step or stop. Here, we defined a success as maneuvering successfully from A to B then C within the user-defined number of steps. Failures are defined as:

(i) No molecule of C was produced even after the use of a maximum number of user-defined steps. (ii) A interacted with E3 before interacting with E2, deactivating E3, preventing the reaction from continuing. This second failure is implemented based on our assumptions that no side reactions can occur due to the enzymes' high specificity to their intended substrates and if an enzyme's active site is occupied by another substrate it will be inactivated. These successes and failures are set to document separately in output and provide the number of steps for each. Below is a sample output. (Henderson et al., Mak et al.)

```
Walk  1 completed in  1000000  steps, no reaction

Walk  2  completed in  7400  steps, A reached E3 before E2. Simulation failed :(

Successful! A+E2 -> B completed in 660230 steps
Walk  3 completed in  1000000  steps, no reaction

Successful! A+E2 -> B completed in 425338 steps
Successful! B+E3 -> C
Walk  4  completed in  812028  steps.

Walk  5 completed in  1000000  steps, no reaction
```

In our actual model, we used step numbers n=10000000 and then n=50000000 for larger spatial distances between A-E2/B and E2/B-E3/C steps, and we did 15 walks for each distance variation.

Kinetic-controlled system: This system is explored to model and assist in familiarizing us with the fundamental kinetics and creating code for such a system. Here, an additional assumption was made that the reaction times are long enough that conversion can be considered instantaneous. Since diffusion rates are negligible in a kinetically controlled system, reaction rates from this system were calculated but ignored in the final comparison between our initially controlled systems and our combination system. The reaction rate equation is still useful, however, since an analysis of enzymatic kinetics will be required for the combination system. The reaction rate equation in a Michaelis-Menten functional form was used within the tau leaping script (Cornish-Bowden. Cao et al.)

$$rate = \frac{V_{max} \cdot [S]}{K_M + [S]}$$

Here, Vmax is the maximum achieved reaction rate, Km is substrate concentration when the reaction velocity is equal to one half of the maximal velocity for the reaction, and [S] is the concentration of the substrate for the reaction. Tau leaping method was chosen for this system

because it uses a stochastic algorithm to predict the progression of a reaction scheme by random selection of a specific reaction followed by a decision on whether this reaction proceeds or not (Cao et al.). Using the modified (nonnegative) Tau Leaping steps from Cao et al., Tau Leaping was implemented into our simplified code in the following way: first, current critical conditions were identified by estimating maximum number of times (L) a reaction is fired before exhaustion of one of the reactants in the system. In this current study L was set to a value of 5. For critical reactions during a leap, kj=1 and for non-critical reactions kj is modeled as a Poisson random variable sample with a mean of aj(x) tau in the script. The final requirement for our script was a stochastic matrix representing our elementary reactions. We believed the inclusion of both enzymes' degradation was the most accurate representation of the full system, therefore, this is what we used for both the kinetic controlled and combined system.

R1: A + E2 -> B

R2: B + E3 -> C

R3: E2 degrades

R4: E3 degrades

$$\begin{array}{cc} & \text{REACTIONS} \\ & \begin{array}{cccc} R1 & R2 & R3 & R4 \end{array} \\ \text{ELEMENTS} \begin{array}{c} A \\ B \\ C \\ E2 \\ E3 \end{array} & \begin{bmatrix} -1 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ -1 & 0 & -1 & 0 \\ 0 & -1 & 0 & -1 \end{bmatrix} \end{array}$$

Combined System: In this system it was assumed that the system would be equally diffusion and reaction controlled. This implies when the molecule reaches the appropriate enzyme site, it has a fixed amount of time to react, equal to the diffusion time, before it diffuses away without reacting. This was implemented by starting from the random walk algorithm utilized for diffusion control and then inserting the tau leaping algorithm wherever a reaction occurs. We were unfortunately naive in believing our ability to code and debug such a system and were unable to get this code to work as there was an error somewhere that we could not figure out. We believe this could also be due to our lack of expertise in this area which may have caused us to make inaccurate assumptions. Sample output is shown below where our walk immediately went straight to the second reaction in one step

```
Successfully reacted B with E3 to form C
Walk  1 completed in 1 steps, congratulations

Successfully reacted B with E3 to form C
Walk  2 completed in 1 steps, congratulations

B diffused away from E3 before reacting
Walk  3 completed in  1  steps, no reaction

Successfully reacted B with E3 to form C
Walk  4 completed in 2 steps, congratulations

Successfully reacted B with E3 to form C
Walk  5 completed in 1 steps, congratulations
```

In this combined system we also allowed for the possibility that the substrate could diffuse away before reacting with an enzyme. If we got this code to work, we would have followed a similar protocol as the Diffusion limited to graph the reaction rate constant vs variations in distances between A-E2/B and E2/B-E3/C steps

3. Result and discussion

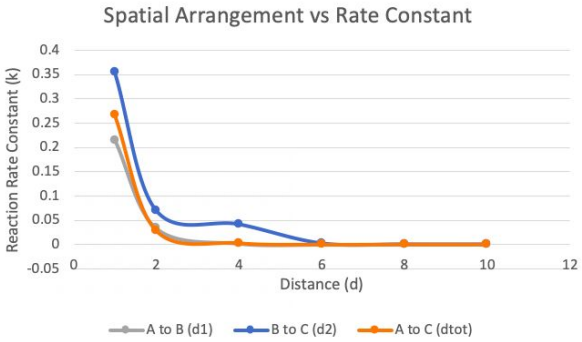


Figure 2. Influence of spatial arrangement of A-E2/B and E2/B-E3/C on the formation of B and C.

In figure 2, the reaction rate constant for all sub reactions follow a similar decreasing trend. The initial average reaction rate constants for d1, d2, and dtot are 0.214, 0.354, and 0.266 respectively. The rate constants shown were calculated and normalized by the rate constant for the initial point or the shortest number of steps the system took to reach complete each subreaction. The normalized values were then averaged for each distance. Further explanation of this can be found in the excel sheet of our repository. This drastic decrease in rate constants is fundamentally comparable to the results achieved by Park et. al as our system shows a 1 to 3,000, 4,000, and 20,000 decrease between the 1-10 increase in distances for d1, d2, and dtot, respectively.

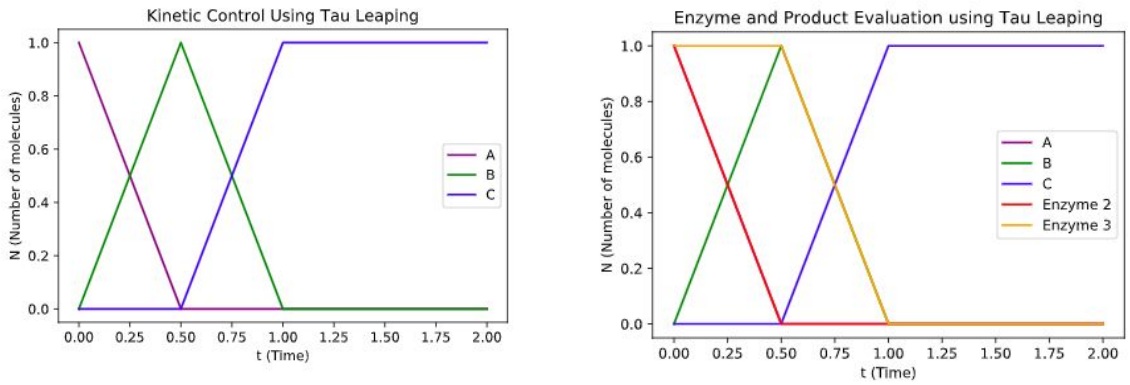


Figure 3. Change in (a) substrate and (b) enzyme concentration over time, each component's concentration has been normalized to 1.

Figure 3 (a) demonstrates how the concentration of a substrate (i.e. A, B, or C) is varying over time as the tau leaping algorithm decides the fate of a reaction whether it's going to proceed or not. Formation of B happens instantaneously while the second sub-reaction doesn't start up until 0.5-time units in the reaction following the degradation of B. In Figure 3 (b) concentration variation for enzymes have been demonstrated along with the substrates. In the first reaction state, E1 gets used up along with A and production of C requires the degradation of B and E3. We are aware that enzymes are just catalyzing a reaction and after their specific catalysis activity they are considered to be regenerated. However, we also know that regeneration of an enzyme after it completes its specific reaction has no implications on the completion of the subsequent sub-reactions. For instance, if a regenerated E2 gets deactivated during formation of B, this study owing to its one-substrate nature will consider that E2 has already completed its requirement of forming substrate B and is no longer required to form the product C for the success of the simulation process. We chose not to include this regeneration. It is also important to not that while the y-axis only goes up to 1, this does not imply that our system only has one molecule. We chose to normalize the molecules by the largest number of molecules for simplicity and believe this fundamental result would equally apply to any x number of molecules.

4. Conclusion

The above-mentioned analyses demonstrate how enzyme synergism works in a multi-step cascade reaction. While we were not able to get the combination system to work, we believe based on the response of our diffusion controlled system that it would be a fair prediction that the 23-fold increase achieved by Park et al. could be a result of solely diffusion effects based on the increase in reaction rate constants as spatial arrangement was decreased. These models shown here are simplified versions of a complex system and further research is required to make it more inclusive. Namely achieving working code for a combined, accurate system.

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