

Stochastic Simulation of a Substrate-Enzyme Reaction

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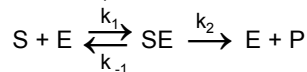
Goal:

- To use a stochastic simulation of an enzyme-catalyzed metabolic process and compare a model with two explicit reactions to a model which uses a single rate equation derived from the two reactions

Background:

- Stochastic Simulation computes a reacting system in a series of random, discrete steps as opposed to a deterministic, continuous process. This is appropriate when modeling small numbers of discrete entities such as molecules in certain biological systems.

- The Michaelis-Menten equation describes the rate at which a saturation reaction can occur. It is derived from the following reaction of a substrate molecule and an enzyme in order to form a product.



Equation 1: (S - substrate, E - enzyme, SE - substrate/enzyme combination, P - product) k_1 is the rate of the forward reaction of S + E to SE, k_{-1} is the backward reaction rate of SE back to S + E and k_2 is the rate of the reaction from SE to P.

- The Michaelis-Menten equation is:
$$\text{Rate} = \frac{V_{\max} [S]}{K_m + [S]}$$

Equation 2: V_{\max} is the maximum rate dependent on the number of enzymes, K_m is a constant dependent on k_1 , k_{-1} and k_2 of equation 1 and $[S]$ is the number of substrate molecules available. The derivation of this equation from the preceding two enzyme reactions assumes that the concentration of $[SE]$ is constant and that the concentration of $[S]$ is greater than the concentration of total enzyme.

The Experiment: (write two separate computer programs)

- Simulation 1** considers the intermediary reaction with an enzyme (using equation 1).

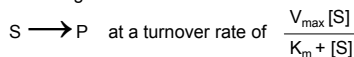
Rates k_1 , k_{-1} and k_2 are related to K_m of the first simulation by:
$$K_m = \frac{(k_{-1} + k_2)}{k_1}$$

V_{\max} corresponds to the second simulation by:
$$V_{\max} = k_2 [E_T]$$

The rate of product formation is:
$$\text{rate}_{[P] \text{ formation}} = k_2 [SE]$$

The rate $[SE]$ disappearance is:
$$\text{rate}_{[SE] \text{ disappearance}} = (k_{-1} + k_2) [SE]$$

- Simulation 2** describes the reaction of a substrate to product directly by using the Michaelis-Menten equation to give a saturation effect.



Results and Discussion:

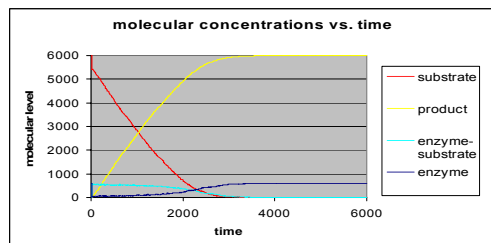
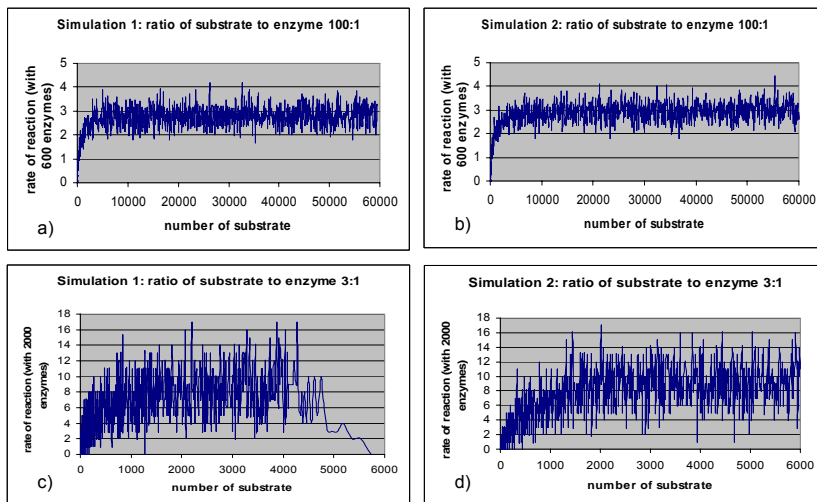


Figure 1: This graph illustrates the change of concentration in the various molecules through time in a reaction governed by equation 1.

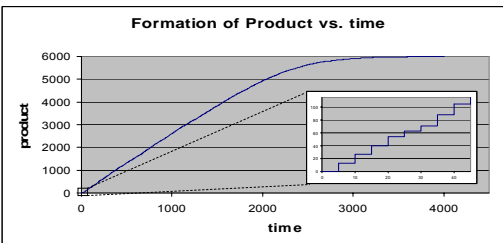


- When the number of total enzymes and substrates are set the same in both simulations the resulting V_{\max} is approximately the same value regardless of the ratio of substrate to enzyme.

- When the ratio between substrate molecules and enzymes is lowered, the fluctuation of the reaction rate increases (It is an assumption that the initial value of substrate molecules is larger than the total number of enzymes).

- In graph c) (simulation 1 with low ratio of $[S]$ to $[E_T]$) one of the assumptions used to derive equation 2 and produce graph d) is violated, namely that $[SE]$ is constant. When the simulation is started, $[E] = 2000$ and $[SE] = 0$, thus it takes time for $[SE]$ to stabilize, which accounts for the data at high substrate levels in graph c).

- Equation 1 is exact and applicable to all situations; equation 2 is derived from the reactions of equation 1 using several assumptions. However, in simulations, using equation 2 requires less time to compute. The following times were required to run each simulation: graph a), 16 min; graph b), 3 min; graph c), 3 min; graph d), < 1 min.



- The change in product over the change in time, $\Delta P/\Delta t$, computed for every data point on the curve gives the rate at which product is being formed.

- The curve is not smooth and continuous because the product is formed discretely and the amount formed each time step can vary.

Future Goals:

- To fit curves to the reaction rate vs. substrate graphs in order to determine values for V_{\max} and K_m and compare these values in both simulations.
- To study additional cases in which the two reaction approach of simulation 1 may violate assumptions made in the Michaelis-Menten equation, simulation 2.
- To explore how these two simulations and the stochastic approach relate to other reactions and events inside a cell.

Acknowledgments for support and funding:



National Science Foundation



Physics REU Program



Department of Defense