1. When present at low concentrations, the non-hydrolyzable nucleotide analog CrATP activates hexokinase by binding to an allosteric site (a site distinct from the enzymatic active site). When a preformed enzyme-CrATP complex is diluted into a buffer containing substrates in a pre-steady-state quench flow experiment, a burst of glucose phosphate product formation is observed. Curiously, the amplitude of the burst is much greater than the stoichiometric concentration of the enzyme. Explain.

The observations suggest a mechanism such as this one:

\[ A \rightleftharpoons \text{CrATP} \]

\[ EA + S \rightleftharpoons EAS \xrightarrow{k_{\text{cat}}} EA + P \]

\[ k_1 \quad k_{-1} \quad k_1 \quad k_{-1} \]

\[ E + S \rightleftharpoons ES \xrightarrow{k_{\text{cat}}} E + P \]

in which

1. \( \frac{k_1}{k_{-1}} \gg 1 \) (equilibrium lies toward unactivated form)

2. \( k_{\text{cat}} \gg k_{\text{cat}}' \) (A activates)

3. \( k_1 \ll k_{\text{cat}} \)

Due to 3, there will be multiple turnovers of A-bound enzyme before the slow A dissociation step occurs, and the system reaches steady state. Thus, the burst amplitude can be \( \gg \) enzyme conc.
2. A molecule of the enzyme ribonuclease has a diffusion coefficient of $1.1 \times 10^{-6}$ cm$^2$ s$^{-1}$. How long will it take a single ribonuclease molecule to diffuse in an infinite medium a root-mean-square distance of:

a. the width of an E. coli ($\sim 1 \mu$m)?

b. the width of a "typical" animal cell ($\sim 10 \mu$m)?

c. the length of an internodal cell in the alga Nitella ($\sim 1$ cm).

d. the length of a spinal motor neuron that innervates a foot muscle in an adult human ($\sim 1$ m).

e. the width of the Atlantic Ocean ($\sim 6 \times 10^6$ m)?

In the Nitella and motor neuron cases, is diffusion adequate to transport macromolecules synthesized at one end of the cell to destinations at the opposite end?

$$\sqrt{\langle x^2 \rangle} = \sqrt{2Dt}$$

$$\Rightarrow t = \frac{\langle x^2 \rangle}{2D} = \frac{d^2}{2 \cdot 2 \times 10^{-10} \text{ m}^2/\text{s}}$$

<table>
<thead>
<tr>
<th>d (m)</th>
<th>t (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-6}$</td>
<td>$4 \times 10^{-3}$</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>$4 \times 10^{-1}$</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>$4 \times 10^4$</td>
</tr>
<tr>
<td>$10^0$</td>
<td>$4 \times 10^9$</td>
</tr>
<tr>
<td>$6 \times 10^6$</td>
<td>$2 \times 10^{-23}$</td>
</tr>
</tbody>
</table>

Even 5 days might be too slow, depending on how quickly the protein is degraded after synthesis. $10^2 \gamma$ is approximately the lifetime of the organism; that's definitely too long to wait.
3. *E. coli* RNA polymerase can transcribe a 2000 nucleotide long piece of linear DNA in about 20 s. It does this by taking unidirectional one-nucleotide-long steps along the DNA. Suppose the steps were not unidirectional but instead were an unbiased one-dimensional random walk. On average, how long would it take to transcribe this DNA if the polymerase starts random walking at one end of the DNA and stops immediately the first time it comes to the opposite end? Assume that the polymerase does not dissociate from the DNA.

Here $\delta = 1$ nt

$2000$ nt in $20$ s $\Rightarrow \tau = 0.01$ s

$D = \frac{\delta^2}{2\tau} = \frac{2000}{50} nt^2 s^{-1}$

In class we derived:

$W(u) = -\frac{u^2}{2D} + \frac{L}{D}$, in this case $L = \infty$

$\Rightarrow W(u) = \frac{1}{2} \frac{L^2}{D} = \frac{1}{2} \frac{(2000 \text{nt})^2}{50 \text{nt}^2 s^{-1}} = 40,000$ s

Problem 1 asked about how long it takes the concentration peak to spread a certain distance in an unbounded medium. Prob. 2 is different—it asks about the average time a molecule takes to travel from one end to the other of a space with boundaries (the ends of the DNA molecule). The correct answer is the same as you would get in the simple calculation of Prob. 1, but the reason is different. Be sure you understand why!
Problem Set 5/Reading Assignment 4

4. Show that in an unbiased one-dimensional random walk of constant step length, the probability that the walker will eventually visit the position \(n\) steps to the right of the starting position is one for any value of \(n\).

Hints:
a. Define a probability function:
   \[ P(n) = \text{Prob}\{\text{walker will reach } n \text{ given that it starts at the origin}\} \]
b. Clearly, \( P(n) = P(-n) \)
c. Show that \( P(n+1) = P(n)P(1) \) for \( n > 0 \)
d. Show that \( P(n) = \frac{1}{2}P(n-1) + \frac{1}{2}P(n+1) \)
e. Then, show that \( P(n) = 1 \) for all \( n \)

\( c. \) \( P(1) \) is by definition the probability of reaching \( n=1 \), having started at \( n=0 \). This must in general be the probability of reaching \( n+1 \) having started at \( n \), since all points along the axis are identical.

\[ P(n+1) = P(1) \cdot \left( \text{prob of reaching } n+1 \right) = P(1) \cdot P(n) \]

Also, by the same argument,
\[ P(n-1) = P(1) \cdot P(n) \]

\[ \frac{1}{2} P(n-1) + \frac{1}{2} P(n+1) = \frac{1}{2} P(1) \cdot P(n) + \frac{1}{2} P(1) \cdot P(n) = P(1) \cdot P(n) \]

This must be \( P(n) \) because to reach \( n \), we must either reach \( n-1 \) [prob. = \( P(n-1) \)] and then step to the right [prob. = \( \frac{1}{2} \)] or reach \( n+1 \) [prob. = \( P(n+1) \)] and then step to the left [prob. = \( \frac{1}{2} \)].

\[ P(n) = P(1) \cdot P(n) \Rightarrow P(1) = 1 \]

\( e. \) Given \( d \) and \( e \), \( P(n) = 1 \) for all \( n \) by induction.
5.

a. Estimate the diffusion coefficients (in water at room temperature) of an enzyme molecule (assume it's a sphere with radius 12 nm) and a fructose molecule (assume it's a sphere with radius 0.5 nm). Now calculate the upper (diffusion-controlled) limit on the second order rate constant for the reaction between these two molecules.

b. Now calculate what the diffusion-limited rate constant will be if the enzyme is rigidly attached to the surface of a blood cell (radius 20 μm) instead of floating free in solution.

c. Use your physical intuition, not equations (!), to explain why cell attachment does (or does not) significantly change the rate.

\( \text{(a) Stokes Law says } D = \frac{kT}{6\pi \eta r} \) 
\[ kT = 4 \times 10^{-14} \text{ erg} \quad (= 9 \text{ cm}^2/\text{s}^2) \] 
\[ \eta \text{ at room temp.} \]
\[ \eta = 0.01 \quad \text{g cm}^{-1} \text{ s}^{-1} \]

\[ \Rightarrow \quad D = \left( 2.0 \times 10^{-13} \text{ cm}^2/\text{s} \right) / r \]

enzyme \( r = 12 \text{ nm} = 1.2 \times 10^{-6} \text{ cm} \Rightarrow D = 1.8 \times 10^{-7} \text{ cm}^2/\text{s} \)
fructose \( r = 0.5 \text{ nm} = 5 \times 10^{-8} \text{ cm} \Rightarrow D = 4.2 \times 10^{-6} \text{ cm}^2/\text{s} \)
blood cell \( r = 20 \mu\text{m} = 2 \times 10^{-3} \text{ cm} \Rightarrow D = 1.1 \times 10^{-10} \text{ cm}^2/\text{s} \)

**Smoluchowski eqn.**

\( k = 4\pi (D_a + D_b)(r_a + r_b) \frac{N_A}{1000} \)

\( k = 4\pi \left( (1.8 + 4.2) \times 10^{-7} \right) \left( (120 + 5) \times 10^{-8} \right) 6.0 \times 10^{23} / 10^3 \)

\[ = 4.01 \times 10^{10} \text{ mol}^{-1} \text{ s}^{-1} \]

**Note 1**

**Note 2**

\( k = 4\pi \left( 4.2 \times 10^{-6} \right) \left( (120 + 5) \times 10^{-8} \right) 6 \times 10^{23} / 10^3 \)

\[ = 3.3 \times 10^{10} \text{ mol}^{-1} \text{ s}^{-1} \]

not much different!
Note 1: $D_{cell}$ is insignificant (9.10^{-10} \text{ cm}^2/\text{s}) compared to $D_{fructose}$ (2.10^{-6} \text{ cm}^2/\text{s})

Note 2: The sum of the radii is the distance (center to center) that the two spheres must approach to react. In this problem, that would be $R_{enz} + R_{fructose}$, NOT $R_{enz} + R_{cell}$.

\( \text{(c)} \) The relative rate of collision between $E$ & fructose depends on the rate of relative diffusion ($D_{enz}$ plus $D_{fructose}$). Since fructose moves so much faster, it doesn't matter if the enzyme moves a little or not at all. $D_{fructose} + D_{enz} = D_{fructose}$. So, the rates on and off the cell are roughly the same.