Solution to problem set 6

Problem 1

Motion by diffusion.

a) Using the Einstein-Stokes relationship with $d_{protein} \approx 1$ nm and $d_{bacterium} \approx 1$ μm, we find for the diffusion coefficients $D_{protein} \approx 2 \cdot 10^{-10}$ m^2/s and $D_{bacterium} \approx 2 \cdot 10^{-13}$ m^2/s.

b) For diffusion in 3D, we have that the RMSD distance d_{RMSD} diffused after time t is given by $\sqrt{6Dt}$. This gives for the protein $d_{RMSD} = 1$ μm, 3 mm, and 200 mm for 1 ms, 1 h, and 1 year, respectively. Similarly, for the bacterium we find $d_{RMSD} = 0.04$ μm, 70 μm, and 6 mm for 1 ms, 1 h, and 1 year, respectively. Note that this is for pure diffusion. If there is any amount of flow/drift, the transport will by much more efficient on long time and length scales.

Problem 2

Michaelis-Menten, revisted and expanded.

a) From the quasi steady-state assumptions we have

$$\frac{d[EP]}{dt} = 0 = k_2[ES] - k_3[EP] \tag{1}$$

and

$$\frac{d[ES]}{dt} = 0 = k_1[E][S] - (k_{-1} + k_2)[ES] \tag{2}$$

Constant total enzyme concentration gives the relationship

$$[E_{tot}] = [E] + [ES] + [EP] \tag{3}$$

From equation 1, it follows that

$$[EP] = \frac{k_2}{k_3}[ES] \tag{4}$$

and from equation 2 we have that

$$[ES] = \frac{k_1}{k_{-1} + k_2}[E][S] \tag{5}$$

Plug the relationships from equation 4 and 5 into 3, we find that

$$[E_{tot}] = [E]\left(1 + \left(1 + \frac{k_2}{k_3}\right)\frac{k_1}{k_{-1} + k_2}[S]\right) \tag{6}$$

The rate of product formation is

$$\frac{d[P]}{dt} = v = k_3[EP] = k_3\frac{k_2}{k_3}[ES] = k_2[ES] = k_2\frac{k_1}{k_{-1}+k_2}[E][S] \tag{7}$$

where we have again used equations 1 and 2.

Using equation 6 and the fact that $[S] \approx [S_{tot}]$, we find from equation 7 that

$$\frac{d[P]}{dt} = v = \frac{k_1 k_2}{k_{-1}+k_2}[S_{tot}]\frac{[E_{tot}]}{\left(1+\left(1+\frac{k_2}{k_3}\right)\frac{k_1}{k_{-1}+k_2}[S_{tot}]\right)} \tag{8}$$

Collecting terms, we find

$$\frac{d[P]}{dt} = v = \frac{\frac{k_2 k_3}{k_2+k_3}[E_{tot}][S_{tot}]}{\frac{k_3}{k_1}\frac{k_{-1}+k_2}{k_2+k_3}+[S_{tot}]} \tag{9}$$

b) Comparing this result with the standard expression from the Michaelis-Menten framework (see Lecture 14), we can identify

$$k_{cat} = \frac{k_2 k_3}{k_2+k_3} \tag{10}$$

and

$$K_M = \frac{k_3}{k_1}\left(\frac{k_{-1}+k_2}{k_2+k_3}\right) \tag{11}$$

c) For that case that $k_2 \gg k_3$ the conversion to the product complex is very fast. In that case, we have

$$k_{cat} \approx k_3 \tag{12}$$

as the last step, product release from the enzyme-product complex, now becomes rate limiting. In addition

$$K_M \approx \frac{k_3}{k_1}\left(\frac{k_{-1}+k_2}{k_2}\right) \tag{13}$$

For the opposite case, $k_3 \gg k_2$, we find

$$k_{cat} \approx k_2 \tag{14}$$

and

$$K_M \approx \left(\frac{k_{-1}+k_2}{k_1}\right) \tag{15}$$

i.e. we are back to the standard Michaelis-Menten scenario. For $k_3 \gg k_2$ product release is very fast and the enzyme-product complex is only very briefly populated, which means we can essentially ignore it.

Problem 3

DNA-nucleosome interactions.

a) For an estimate of the bending energy, we can directly use the expression from problem set 5, problem 3:

$$E_{bend} \approx \frac{1}{2} k_B T L_p \frac{\theta}{R} = \frac{1}{2} k_B T \cdot 50\text{nm} \frac{2\pi \cdot 1.7}{5\text{nm}} \approx 50 k_B T \tag{16}$$

where we have used the bending persistence length of DNA ≈ 50 nm, the fact that radius of the DNA center line is ≈ 5 nm and makes 1.7 full turns.

b) To estimate the electrostatic energy contribution from bringing 14 pairs of opposite charges into a distance of $d = 3$ Å, we simply evaluate the Coulomb energy

$$E_{Coulomb} \approx 14 \frac{e^2}{4\pi\epsilon_0 \epsilon d} \tag{17}$$

For $\epsilon = 80$, this gives 34 $k_B T$, which is less than the bending energy; under these circumstances, the formation of nucleosomes would be energetically unfavorable. For $\epsilon = 2$, the electrostatic energy is 1300 $k_B T$, which is more than enough to compensate for the unfavorable bending energy. This implies that the DNA-histone interface is not fully hydrated (as in that case ϵ should be close to 80), but likely still somewhat hydrated (as 1300 $k_B T$ is a very large energy, which would make it difficult to remove/remodel nucleosomes).