BIO-212. Lecture 7. Exercises

Question 1.

The Boltzmann distribution describes the most probable distribution of particles in different energy states for a given system. Thinking about each particle as being a protein and remembering that each different possible conformation of a protein has a different energy, use the Boltzmann distribution to explain why at extremely high temperatures proteins are unfolded.

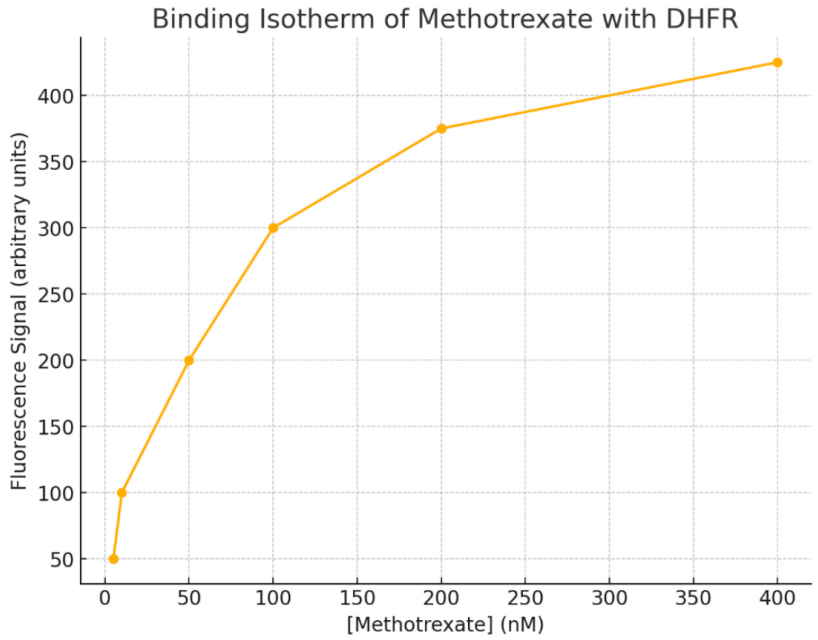
Question 2.

The two accessible conformations of a protein differ by 2 kJ/mol. What percentage of protein molecules will be in the higher energy state at 270K?

Question 3.

Methotrexate is a potent inhibitor of dihydrofolate reductase (DHFR), binding to the active site and preventing the reduction of dihydrofolate to tetrahydrofolate. Below is a binding isotherm showing the interaction between DHFR and methotrexate. The fluorescence signal (arbitrary units) is measured as a proxy for binding. At the highest concentration of methotrexate tested (500 nM), the fluorescence signal saturates at 425 units.

a) Estimate the dissociation constant (K_d) for methotrexate from the binding curve.



b) Calculate the ΔG^0_{bind} at 298 K for methotrexate's interaction with DHFR.

Question 4.

The drug jafrasitor (MW = 540 Da) binds the histone deacetylase enzyme Sir2 with a dissociation constant of 0.1 nM. What mass of jafrasitor should be administered to a patient with a blood volume of 5.5 L such that Sir2 is at least 91% inhibited?

Question 5.

At 298 K, the enzyme lysozyme binds to a specific polysaccharide substrate with a dissociation constant of 20 μM. What are the values of association constant and ΔG^0_{bind}?

Question 6.

The binding between the drug Ritonavir and the protein HIV-1 protease is measured, giving a
binding free energy of −45 kJ/mol at human body temperature. What is the value of K_d?

Question 7.

Which of the following statements are TRUE, and which are FALSE?
a. The binding affinity of a molecule increases as the dissociation constant (K_d) decreases.

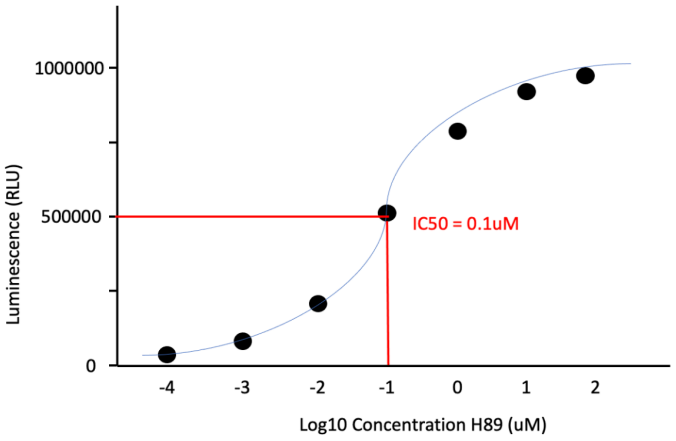
b. Hydrophobic interactions play a major role in molecular recognition by reducing the entropy loss upon ligand binding.
c. The Gibbs free energy (ΔG) of binding is independent of temperature.
d. The binding of a ligand to a receptor often involves an enthalpy-entropy trade-off.
e. The equilibrium constant (K_a) is inversely proportional to the dissociation constant (K_d).

f. Hydrogen bonds in molecular recognition are stronger in a non-polar environment than in a polar environment.
g. The entropy (ΔS) of binding is always positive for favorable binding interactions.

Question 8.

ATP consumption assays are commonly used to identify kinase inhibitors. In brief, a kinase, its respective substrate, and a fixed amount of ATP are added to several reactions with several different concentrations of a potential inhibitor. The reaction (transfer of phosphate from ATP to substrate) is allowed to take place for a fixed amount of time, at which point a mixture of a luciferase and its respective substrate are added. Therefore, the measured luminescence (in units of RLU - Relative Light Units) is inversely related to the kinase' activity. Below are the results from a recent assay you have conducted and measured using the PKA (Protein Kinase A) kinase and an inhibitor H89.

concentration H89 [mM]	*RLU*
0	100
0.0001	120
0.001	80000
0.01	200000
0.1	500000
1	750000
10	900000
100	1000000



a) If the ATP concentration was increased 1000 fold and the measurement under the same conditions had 125 RLU with 10 mM H89 present, what does this tell us about the type of inhibitor H89 is?
b) What is the inhibitor dissociation constant (K_I) of H89 in relation to PKA if 1 mM of ATP is present and the ATP has a 10 µM affinity for PKA?
c) Another known inhibitor of PKA is PKI. The Gibbs free energy change (ΔG) associated with this interaction is −32 *kJ/mol*. Under standard conditions, calculate the K_D for the binding of PKI to PKA
d) What is the fractional saturation of PKI at a concentration of 1 µM.