

Problem Set IV - Due Monday, October 21

1) (15 points) On the web, go to the PDB site (go to lecture 16, there is a link there), and find a structure of HIV protease (there are many - anyone will do). Download the structure file (.ent or .pdb file), and using your browser or RasMol answer the following questions:

- i) Is this protein a monomer or a dimer?
- ii) How many residues does this protein has?
- iii) How many α -helices are present in this protein? How many residues do they have? How long (in Å) are they?
- iv) Is this protein mostly α , mostly β , / or $\alpha + \beta$?

2) (15 points) Consider a ligand **L** in equilibrium with a protein **P**:



- i) Write the mathematical definition of the dissociation constant, K_d , as a function of the species present in solution (**P**, **L**, and **PL**).
- ii) **Derive** the mathematical expression for the occupancy factor (θ) as a function of the species in solution (**P**, **L**, and **PL**).
- iii) **Derive** from ii) the mathematical expression for θ as a function of [**L**] and K_d .

3) (25 points) You have a solution with the same concentration of two proteins that bind glucose with different affinities, **GB1**, with a K_d of 10 mM, and **GB2**, with a K_d of 100 mM.

- i) Which protein has higher affinity for glucose?
- ii) What will be the % occupancies of **GB1** and **GB2** at glucose concentrations of 0.05M, 0.1M, 0.5M, and 1M?

4) (25 points) Suppose you have two proteins, **P1** and **P2**, both of which have the same K_d for Kriptonite. The only difference is that **P1** shows no cooperativity for Kriptonite binding, while protein **P2** shows cooperative Kriptonite binding. Draw a sketch of the binding curves (θ vs [Kriptonite]), *but be sure to draw them both in the same graph*. Indicate clearly what will be the only similarity between the two curves. In other words, at what [Kriptonite] will both proteins behave the same?

- 5) (20 points) You have found several hemoglobin variants as part of your PhD research, and have started to analyze their structural differences to native hemoglobin. You have found the following for one of the variants you found, **V2**:
- i) **V2** binds O_2 cooperatively, but has a slightly poorer binding O_2 affinity than native hemoglobin.
 - ii) Carbon monoxide (CO) binds 2000 times better than oxygen (O_2) to variant **V2**. (remember that in native hemoglobin CO binds only 200 times better than O_2).
 - iii) Sequence analysis shows that in all the α and β subunits there is a **basic residue** missing, and a glycine residue too many.

Based on these observations, what residue has been replaced by glycine in the subunits of variant **V2**, and what is its relative position in the subunits' sequence? Your answer has to be fully justified, and you have to account for all the observations presented.