Problem 1 (9 pts):

For a monomer-dimer equilibrium, let the monomer concentration in molar units be [A], then we have:

$$k_d = \frac{[A][A]}{[A_2]} \quad \text{and} \quad C_{total} = [A] + 2[A_2]$$

We need to express the total concentration in terms of monomer, therefore, the number of monomers in the dimer is of course 2, so to get the total concentration of monomer, we need to multiply the molar concentration of dimer by 2. Substituting the concentration of dimer and solving for the monomer concentration results in a quadratic:

$$[A_2] = \frac{[A]^2}{k_d} \quad \text{and} \quad C_{total} = [A] + 2\frac{[A]^2}{k_d}$$

Rearranging:

$$\frac{2}{k_d}[A]^2 + [A] - C_{total} = 0$$

For our quadratic equation, we need to solve:

$ax^2 + bx + c = 0$ with $a = 2/k_d$ and $b = 1$ and $c = -[C_{total}]$

Solving the quadratic gives us the concentration of the monomer in molar units ([A]).

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

The total concentration of the protein is measured in monomer molar units, hence the amount of monomer that is complexed in the dimer form is [C] – [A]. Since there are two monomers in each dimer, we need to divide the concentration of the dimer into 2 so we can calculate the molar ratio of mols monomer/mols dimer.

Example:

C= 6 μM

a = 2/6 = 1/3
b = 1
c = -6
Solving for [A] we get two roots, we can ignore the negative root.

Molar quantity of the monomer [A] = 3.0 μM

This makes sense, because at equilibrium (the kD concentration), there should be exactly half of all monomers in monomeric conformation and half of them should be in dimeric conformation. To get the molar concentration of monomers in the dimer, we need to subtract the concentration of monomers from the total concentration and then divide by 2 to the molar concentration of the dimer:

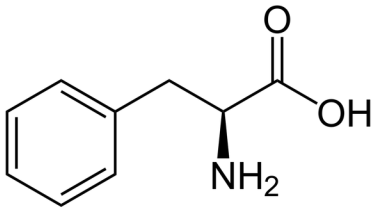
Molar quantity of the dimer $[A_2]$ = (6 – 3)/2 = 3/2 = 1.5 μM

Therefore, the molar ratio of monomer/dimer = 3/1.5 = 2:1. This means at the Kd concentration there is twice as much mols of monomer than dimer in the mixture, and equal amounts of monomers in the monomeric and dimeric form. For the other concentration, just change “c” and solve again.

At 60 nM, the protein is very dilute and mostly dissociated into monomer, and the monomer concentration is not much less than the total concentration, solving for [A] yields 0.0588457 M. Hence, the mols of dimer is (0.06-0.0588457)/2 = 0.00057715 M. The ratio is ~101.9. At 60 μM the protein concentration is above the k_D, and therefore there will be more dimer present, and the molar ratio M/D is 0.5.

Problem 2 (12 pts)

Phenylalanine is an amino acid with a benzene side chain. The side chain does not have any ionizable groups and is therefore inert to changes in pH. However, the polypeptide chain will have one ionizable carboxylic acid at the C-terminus and an amine at the N-terminus. The charge of these depends on the pKa of these groups. The table on p16 of the lecture shows the pKa for Phe to be 1.83 for the carboxylic acid, and 9.13 for the amino group.



At the pKa, half of the molecules will be protonated, the other half deprotonated. At the isoelectric point the charges from both groups will equal out and the amino acid will be neutral. In this case, the isoelectric point is simply the average of the two pKa values. At low pH, both groups become protonated, leading to a neutral charge on the carboxy terminus, and a positive charge on the amino group, and therefore the protein has a positive charge, while at higher pH, the amino group will be neutral and the carboxylic acid will be negatively charged, resulting in an overall negative charge. The degree of positive charge will hence decrease with pH:

pH 2.51 (overall positively charged) > pH 5.48 (iso-electric point, hence neutral) > pH 7.0 (slightly negatively charged) > pH 11.0 (mostly negatively charged)

Problem 3 (6 pts):

You suspect a monomer-dimer interaction is caused by electrostatic interactions, how would you change the solution properties to test this (assume an aqueous solvent)? Would the kD increase or decrease? What about the kA?

If you were to increase the salt concentrations, the positively and negatively charged ions would neutralize the charges on the protein and reduce the strength of the interaction. This would promote dissociation and increase the k_D and decrease the k_A. A method like AUC could be used to test for the shifting equilibrium.