Problem 1 (12 pts):

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

$[A] + 2\,k_a[A]^2 - [C] = 0$

$k_a = 1/k_d$, therefore: $[A] + 2/k_d[A]^2 - [C] = 0$

For our quadratic equation, we need to solve:

$ax^2 + bx + c = 0$ with $a = 2/k_d$ and $b = 1$ and $c = -[C]$

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

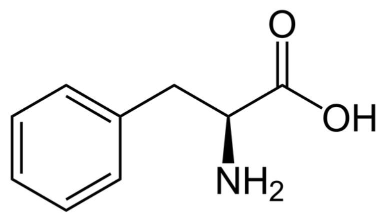
a = 0.3333
b = 1
c = -6
Molar quantity of the monomer [A] = 3.0 μM
Molar quantity of the dimer $[A_2]$ = (6 – 3)/2 = 3/2 = 1.5 μM

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

For 60 nM, the monomer/dimer molar ratio is ~102:1, and for 60 μM the molar ratio is 0.5.

Problem 2 (8 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 typically 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)