

Assignment 6

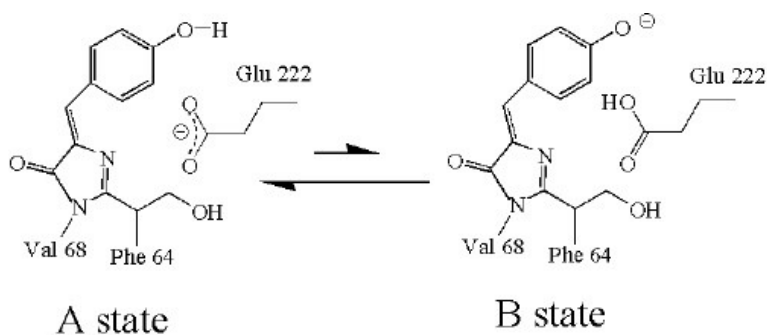
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Physics G731

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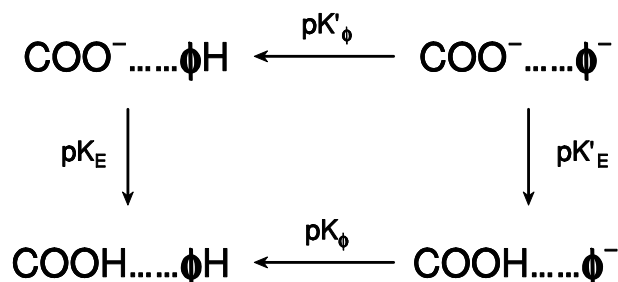
Due Friday, Oct. 19.

Interacting protonation sites. Green fluorescent protein (GFP) was originally found in jellyfish, but has been widely adopted by molecular biologists as a tool for cellular imaging. The fluorescence results from spontaneous post-translational modification of a group of three amino acids (Ser-Tyr-Gly). The resulting chromophore, shown below, can exist either in a neutral state (A) or in an anionic state (B) produced by deprotonation of the oxygen on the six-carbon phenol ring. Electrostatic interaction with a nearby glutamic acid, which is also ionizable, leads to an anomalous pH-dependence, which you will work out.

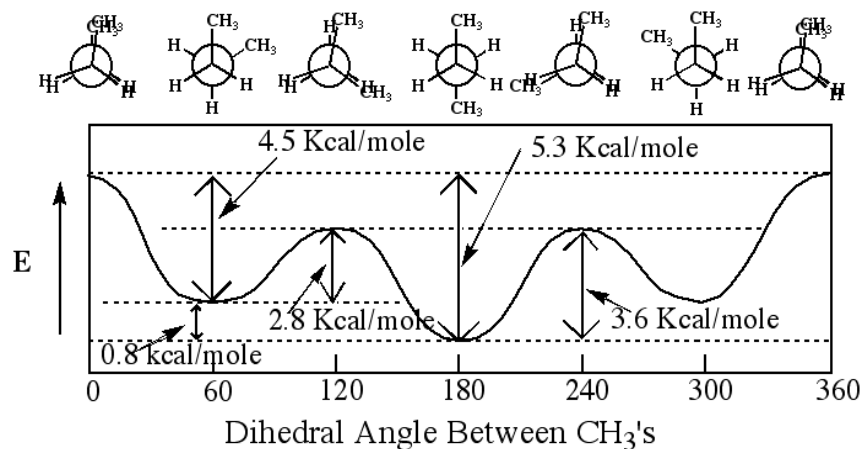


- (a) Roughly estimate the net charge on this protein at pH 8. Identify all titratable (potentially charged) amino acids in the protein by downloading the PDB structure file 1emd for GFP, and sum the average charges expected on the basis of the nominal pK values given for each side chain in the class notes.

In fact, these pK values are modified by the presence of nearby charges and the extent to which each amino acid is exposed to the solvent. You will now consider how electrostatic interaction between the chromophore and Glu 222 modify their protonation equilibria.



- (b) Take pK'_ϕ to describe ionization of the chromophore in the presence of the neutral glutamic acid and pK_E to describe ionization of the glutamic acid in the presence of the neutral chromophore. (E is the one-letter abbreviation for glutamic acid.) Call the electrostatic interaction energy between the two ionized groups ΔE . Show that a new value $pK'_\phi = pK_\phi + \Delta pK$, with $\Delta pK = \Delta E / (k_B T \ln 10)$, describes protonation of the chromophore in the presence of the ionized glutamic acid. Show that the same titration shift $pK'_E = pK_E + \Delta pK$ applies to the chromophore in the presence of the charged glutamate.
- (c) Consider the four possible charge states for this pair of residues. Find a general expression for the net population of the anionic chromophore, with Glu 222 either charged or uncharged.
- (d) Plot the expression that you found in part 4 as a function of pH for parameter values $pK_\phi = 6.3$, $pK_E = 6.0$, and $\Delta E = 12k_B T$, and show that there are two transitions separated by a plateau region.
- (e) The color of the chromophore changes when it is protonated, and the relative area of the corresponding peaks in the absorption spectrum at pH 8 indicates a 6 : 1 ratio between the populations of the neutral and anionic chromophores. From this ratio, estimate the value $pK_\phi - pK_E$. (Assume that pH 8 lies in the plateau region of your model.)



Torsional potentials. The figure sketches the energy of the butane molecule, $\text{CH}_3\text{-CH}_2\text{-CH}_2\text{-CH}_3$, as a function of the dihedral angle χ about the central C-C bond. Model this potential as accurately as you can using the functional form

$$V = \sum_n \frac{1}{2} V_n [1 + \cos(n\chi + \delta_n)],$$

by specifying values for the parameters V_n and δ_n . that reproduce the extremal energy differences indicated on the sketch. Use either kJ/mol or eV, rather than kcal/mol, as the energy unit. Identify the angles at which your model potential has local minima and maxima.