Assignment #4: Spectroscopy of the GFP Chromophore Based pH-Indicator 35Bu

This assignment is based on current research literature: *Chemically Modulating the Photophysics of the GFP Chromophore.* Conyard, J.; Kondo, M.; Heisler, I. A.; Jones, G.; Baldridge, A.; Tolbert, L. M.; Solntsev, K. M.; and Meech, S. R. (dx.doi.org/10.1021/jp111593x) *J. Phys. Chem. B* **2011**, XXX, 000–000. A hyperlink to the article is provided in the assignment section of the course web site.

The goals of this assignment include (a) to learn and to understand the structural chemistry of the **HBDI** analog **35Bu** as a function of pH, (b) to understand the pH-dependence of the equilibrium concentrations of the various indicator dye species, and (c) to regenerate Figure 3 from scratch by simulation using the information provided in Table 1.

(a) Structural Chemistry of **35Bu** Depending on pH (Scheme 1). Read the paper and create a ChemDraw scheme that shows the structures of protonated **35Bu** ("the cation", **C**), of neutral **35Bu** ("the dye", **D**), and of deprotonated **35Bu** ("the anion", **A**). Add arrows, use suitable color, provide the values of the absorption maxima and the corresponding extinction coefficients in the scheme. Import the scheme into a Word file and add a Scheme legend.

(b) Compute and Plot Concentrations of **C**, **D**, and **A** Depending on pH (Sheet #1, Figure 1). The anion has the highest extinction coefficient (Table 1). Use the dye with an initial concentration $\mathbf{D_o}$ that will result in a maximum absorbance of the anion in the range 0.4 – 0.8 if a 10 cm cell is used. Use Beer's Law to compute the initial concentration $\mathbf{D_o}$.

Using the two pK_a values (Table 1) and $\mathbf{D_o}$, compute the concentrations of **C**, **D**, and **A** as a function of pH. Column 1: pH values from 1.5 to 11.5 in steps of 0.5. Column 2: $[H^+]$. Column 3: [**C**]. Column 4: [**D**]. Column 5: [**A**].

Determine the equations for the concentrations of **C** and **A** as functions of pH using the Henderson-Hasselbalch equation. Remember that [**D**] = $\mathbf{D_o}$ - [**C**] - [**A**]. Plot the concentrations

of **C**, **D**, and **A** as a function of pH from 1.5 to 11.5 using unmarked line plots. Report the equations in the legend to your Figure 1.

(c) Compute Absorbances of **C**, **D**, & **A**; Plot Spectra for Several pH Values (Sheet #2, Fig. 2). For a given pH value, we can use the concentrations computed in **(b)** together with the extinction coefficients of Table 1 to compute the absorbances of **C**, **D**, and **A** at that pH value. Once the absorbances are determined, we can plot the simulated spectrum by drawing the sum of the three Gaussian functions for **C**, **D**, and **A** with their maxima located at the respective λ_{max} (Table 1).

The original authors plotted two species in each half of their Figure 3. You should create one plot that shows spectra over the entire pH range. Your Figure 2 should show spectra computed for at least five pH values for the range between 300 and 600 nm, and your list of pH values must include 1.5, 5.0, 7.0, 9.0 and 11.5. Your sheet #2 should contain the absorbances of **C**, **D**, and **A** at the pH values you selected to simulate (at least 5 values) in your Figure 2.

You might want to use separate sheets to compute the Gaussians for **C**, **D**, and **A** and of their sum at a given pH. Note that the Gaussians require a parameter that controls their widths. We do not have experimental data for these parameters and you should fudge these parameters so that your Figure 2 resembles their Figure 3 as much as possible.

Submission & Deadline: The assignment must be completed with MS Excel and MS WORD. Submit one WORD file "A04_'your name'.docx" and one Excel file "A04_'your name'.xlsx" on Tuesday, 02/22/11 by midnight. The Word file should contain Scheme 1, Figure 1 and Figure 2, each with its own legend and on a separate page, and your names should appear in the header. The Excel file should contain computations and graphs related to **(b)** on sheet #1, and computations and graphs related to **(c)** on sheet #2. Bring one hardcopy (pages of Word file first, then Excel pages, you might reduce Excel pages to save space, show only sheets #1 & #2 of the Excel sheet) to class on Wednesday, 02/23/11.