## Assignment #4: Solubilization of Native Integral Membrane Proteins (IMP)

This assignment is based on current research literature: *Solubilization of Native Integral Membrane Proteins in Aqueous Buffer by Noncovalent Chelation with Monomethoxy Poly(ethylene glycol) (mPEG) Polymers*. T. K. Janaratne, L. Okach, A. Brock, and S. A. Lesley* (dx.doi.org/10.1021/bc200019x) *Bioconjugate Chem.* **2011**, *22*, 1513–1518. A hyperlink to the article is provided in the assignment section of the course web site.

The goals of this assignment include (i) to understand conceptually the solubilization of a native IMS by attachment of a hexa-histidine tag and a hydrophilic polymer, (ii) to learn and to understand the structural chemistry of attachments between the IMP, the hexa-histidine tag, and a specific hydrophilic polymer, and (ii) to regenerate the black spectrum of Figure 1 from scratch by simulation.

**(a)** Structural Chemistry of **Hexa Histidine Tag** (Scheme 1). Read the paper and learn that the His-Tag is attached to the IMP. Search the literature to learn about the chemical structure of His-Tag and to learn how His-Tag is attached to the protein. Create a ChemDraw scheme that shows the structure of His-Tag and its connection to the protein. Import the scheme into a Word file and add a Scheme legend.

**(b)** Structural Chemistry of Association of **His-Tag** and **mPEG-NTA$_3$(Ni$^{2+}$)$_3$** (Scheme 2). Read the paper and learn how the hydrophilic polymer mPEG is converted to mPEG-NTA$_3$(Ni$^{2+}$)$_3$. Search the literature to learn how mPEG-NTA$_3$(Ni$^{2+}$)$_3$ binds to the His-Tag. Create a ChemDraw scheme that shows the structure of His-Tag and its connection to mPEG-NTA$_3$(Ni$^{2+}$)$_3$. Import the scheme into your Word file and add a Scheme legend.

**(c)** Simulation of **far-UV Circular Dichroism Spectrum** of DGK-mPEG(10K)-NTA$_3$(Ni$^{2+}$)$_3$ of Figure 1 (black curve). The black curve in Fig. 1 can be approximated as the sum of a number of Gaussian functions $f_i(\lambda)$ that are determined by the positions of their maxima $\lambda_{max,i}$ and their elipticities $\varepsilon_i$; $f(\lambda) = \Sigma\ f_i(\lambda)$. At least three Gaussians are needed in this simulations; you should

start with $i = 3$ and that should work. (You may try $i > 3$.) Start with reasonable initial guesses for $\lambda_{max,i}$ and $\varepsilon_i$ and adjust the parameters to obtain a good fit. You may choose a fitting procedure of any sophistication; i.e., from visual inspection to mathematical regression. In a plot with wavelength on the abscissa (160 – 260 nm) and mean residual ellipticity on the ordinate (from -30,000 to +30,000), plot the functions $f_i(\lambda)$ in three colors and plot the simulated function $f(\lambda)$ in black.

On one sheet, list discrete values of $\lambda$ in column A (i.e., 160 – 260 nm in steps of 10 or better) starting in row 3, list the parameters $\lambda_{max,i}$ and $\varepsilon_i$ in rows 1 and 2, respectively, of columns B, C and D for the three Gaussians, list the computed values of the Gaussians in rows 3ff of columns B, C and D, and compute the $f(\lambda)$ values in column E. Create the plot. Import the Figure into your Word file and add a Figure legend. Report the equations of the three Gaussians in the legend to your Figure 1.

**(d)** Write Text with Schemes and Figure embedded. Your names should appear in the header. Page 1 of the Word file should contain a brief and concise description (no more than 1 page, double-spaced, Times New Roman, 12 pt, 1 inch margins) of the method used to achieve the solubilization of the IMP, and you should cite the Schemes and the Figure in your text. Schemes 1 and 2 and Figure 1 follow the text, each with its own legend and on separate pages.

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, 02/21/12 by midnight. Bring one hardcopy (pages of Word file first, then Excel pages, you might reduce Excel pages to save space) to class on Wednesday, 02/22/12.