Assignment 3: Construction and Analysis of the Pentapeptide Met-enkephalin with the Insight II Program

1. **Building a Pentapeptide**. To build the molecule met-enkephalin, whose amino acid sequence is **Tyr–Gly–Gly–Phe–Met**, invoke the **Biopolymer** module. From Residue select Append.
   Specify the molecule's name and choose Extended to specify the structural motif of the backbone. (At this stage, do not worry whether the structure is correct). Select the first residue, **Tyr**. Then add each of the remaining four residues in turn.
   You can *center* the molecule on your screen by clicking on it with the center mouse button and dragging it to the desired position. You can also *rotate* the molecule by pressing the right mouse button and dragging the molecule. By pressing both (center and right) mouse buttons and dragging the molecule you can change its position along the z-axis, perpendicular to the screen. You can rotate the molecule around the z-axis by dragging the mouse while both left and right mouse buttons are pressed. To change the representation style of the molecule, select Molecule/Render and choose any of the options. Note how the speed of executing commands (e.g., translation, rotation) is affected by the representation display.
   Now you must amend the ends of the structure you built. Switch to Protein and choose Cap. Change both the N-terminus and C-terminus moieties to the zwitterionic form (NH_3^+ and COO^-) to get a proper oligopeptide.

2. **Measurements of Met-enkephalin's Structural Parameters**. Generate a table listing all the dihedral angles in your met-enkephalin molecule by using Protein/List from the **Biopolymer** module. Select the appropriate command (Dihedrals) and direct the data to a file (List_to_file button on). This file can be viewed and edited later.
   To measure individual bond lengths or distances, bond angles, dihedral angles, etc., use Measure. Select the atoms for the measurement by clicking on them with the left mouse button.

3. **Rotameric Structures**. By a *rotamer*, Insight II refers to a different conformational arrangement of a side chain of a given amino acid. The rotameric structures identified in Insight II are correlated with the ϕ and ψ angles for a given residue (i.e., are sterically compatible).
   Select Manual_Rotamer from Residue of the **Biopolymer** module. Press the Evaluate_Energy button. Energy for a given rotameric structure will be printed in the information window at the bottom of the screen (you can scroll by pressing on arrows to its right). Keep the Nonbond Cutoff value — an option which is displayed on the screen once Evaluate_Energy is chosen — at the default value of 8.0 Å.
   Select a residue by clicking on it and sweep through all the rotamers

of that residue (while holding other rotamer conformations fixed). Next will trigger the execution of the command. In a table, report the rotamer structures for each residue by specifying the dihedral angles χ_1, χ_2, χ_3, ... (Protein /List), along with associated energies.
Now assemble the pentapeptide structure with the lowest rotamer energy and save it. Thus far we have ignored a global optimization of the structure and have only built it up from low-energy conformations of its components. *We will return to optimization later in the course, after studying minimization techniques.*

4. **Main-chain Structure**. Choose Protein /Secondary from the **Biopolymer** module. Change the main-chain configuration by selecting different motifs (Alpha_R_Helix, Alpha_L_Helix, 3–10_Helix, etc.). For each motif:

   (a) prepare a table with the dihedral angles ϕ and ψ for each of the residues (Protein /List),
   (b) list all the hydrogen bonds present in the structure.
   Measure /HBonds and Molecule /Label will be helpful here.

5. **Torsional Rotation**. Bring back your Met-enkephalin's backbone structure in the extended form. You can use the structure saved in part 3 of this assignment (File /Restore_Folder). Change the ψ dihedral angle on the second residue, **Gly**, to $60°$ and the ϕ dihedral angle on the forth residue, **Phe**, to $-60°$.
   Torsional motion around a chosen dihedral angle can be performed with Transform /Torsion command or by pressing the Torsion button on the left of the Insight II screen.
   First, click with the left mouse button on the bond which constitutes the axis of rotation, then press the Torsion button. A little cone, defining the direction of the torsion, will pop up on the screen at one of the bond ends. Now you can change the torsion angle by horizontally dragging the mouse with the center button pressed in. To exit the torsion mode press the Torsion button again (the cone will disappear).
   Calculate the distance between the N-terminus (N atom) and C-terminus (C atom) atoms. Use the Measure /Distance command. Keep the Monitor button on, and select Monitor Mode/Add. Atom 1 and Atom 2 can be selected by clicking on them with the left mouse button.
   Print a picture of your molecule (keep the distance between the N-terminus and C-terminus atoms on the screen). To save ink, the background color should be changed to white every time you print a color or black/white picture. To do so, go to Session /Environment, press the Background button and change the color to white. Now go to File and choose Export_Plot.

Select postscript, Gray_Scale and optionally Ball_and_Stick. Save the file as postscript by using Save_Device_File (the file will have the ".ps" extension). This file can be printed on any postscript printer. Hand in your printout as part of the homework.

Summary of Items to Hand In:

(a) The table with the dihedral angles for met-enkephalin.
(b) The table with the dihedral angles and energies for the rotameric structures of met-enkephalin.
(c) The table with the $\{\phi, \psi\}$ dihedral angles for each different backbone motif of met-enkephalin.
(d) The listing of the hydrogen bonds for each of the backbone motif for met-enkephalin.
(e) Printout of the met-enkephalin structure with the end-to-end link marked.

Background Reading from Coursepack

- G. Némethy and H. A. Scheraga, "Theoretical Determination of Sterically Allowed Conformations of a Polypeptide Chain by a Computer Method", *Biopolymers* **3**, 155–184 (1965).
- P. Y. Chou and G. D. Fasman, "Prediction of Protein Conformation", *Biochemistry* **13**, 222–245 (1974).
- M. Levitt and C. Chothia, "Structural Patterns in Globular Proteins", *Nature* **261**, 552–558 (1976).