**\*\* ONLY TEXT AND IMAGES APPEARING INSIDE THE RED BOXES WILL BE GRADED\*\***

**Part I (****a-Helical Wheel and a-Helical Structure)**

1. Previously you converted your name and city of birth to an amino acid sequence of length 14.
   1. Insert that sequence into the box:

|  |
| --- |
| INSERT SEQUENCE HERE |

1. An α-helix has a rise of 1.5 Å per amino acid and amino acids per turn. The periodicity (rise per helical turn) is 5.4 Å. The turn per amino acid is 100 degrees.  
   Make helical wheels using the [Helical Wheel Generator](https://tcdb.org/progs/helical_wheel.php)
   1. Make a helical wheel of the sequence ABCDEFGABCDEFGABCDEFG (copy and paste this). Study this wheel and insert a screen grab of it into the box. Resize the image to fit on this page.

|  |
| --- |
| INSERT ABCDE.. HELICAL WHEEL HERE |

List 3 pairs of amino acids that are near each other in 3D space but not in 1D space (1D space means sequence).

* 1. Make a helical wheel of your 14 amino acid sequence. Include an image of the helical wheel in the box below. Resize the image to fit on this page.

|  |
| --- |
| INSERT THE HELICAL WHEEL of YOUR SEQUENCE HERE |

1. Is the α-helix of your peptide sequence hydrophobic, hydrophilic, amphipathic or none of these? Explain why in one sentence.

|  |
| --- |
| INSERT HERE |

1. List 3 pairs of amino acids that are close together in 3D space in your 14 amino acid α-helix.  
     
   Omit the next section (section e) of this problem set, as there appears to be a bug in the recent version of PyMol. Section II, which starts on page 3, is required.
2. Build your helix in 3D in PyMol  
   First copy the command below, all at once, into the PyMol Command line, and hit return  
   #-----first line in copy------------------------------------------------------------------------

## Header: General Commands ##

# delete all objects and reset pymol

reinitialize

# set the background color to white

bg\_color white

# make the background transparent for ray trace

set ray\_opaque\_background, 0

# set the ray trace mode

# normal color

set ray\_trace\_mode, 0

# normal color + black outline

#set ray\_trace\_mode, 1

# black outline only

#set ray\_trace\_mode, 2

# turn off shadows during ray trace

set ray\_shadows, 0

# set the mouse mode for laptop.

config\_mouse one\_button

# get rid of double bonds and skinny bonds to H

set stick\_h\_scale, 1

set valence, 0

# high quality surfaces

set surface\_quality, 3

## End of Header: General Commands ##

#-------last line in copy---------------------------------

The follow the instructions here to build your helix.

https://www.youtube.com/watch?v=lbBGZMKLycA

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h)

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**Part II (Protein Analysis)**

1. Launch Pymol
2. Set up Pymol by copying and pasting the following (all at once) into the command line

**PyMOL>**

#-----first line in copy------------------------------------------------------------------------

## Header: General Commands ##

# delete all objects and reset pymol

reinitialize

# set the background color to white

bg\_color white

# make the background transparent for ray trace

set ray\_opaque\_background, 0

# set the ray trace mode

# normal color

set ray\_trace\_mode, 0

# normal color + black outline

#set ray\_trace\_mode, 1

# black outline only

#set ray\_trace\_mode, 2

# turn off shadows during ray trace

set ray\_shadows, 0

# set the mouse mode for laptop.

config\_mouse one\_button

# get rid of double bonds and skinny bonds to H

set stick\_h\_scale, 1

set valence, 0

# high quality surfaces

set surface\_quality, 3

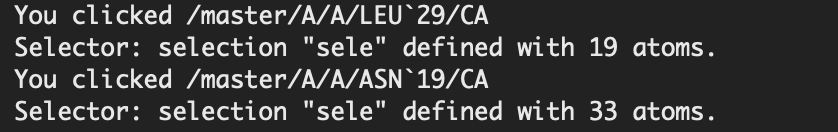
## End of Header: General Commands ##

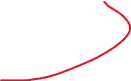
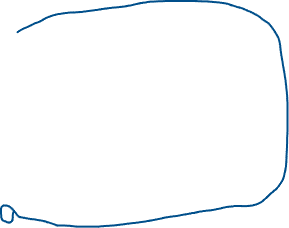
#-------last line in copy---------------------------------

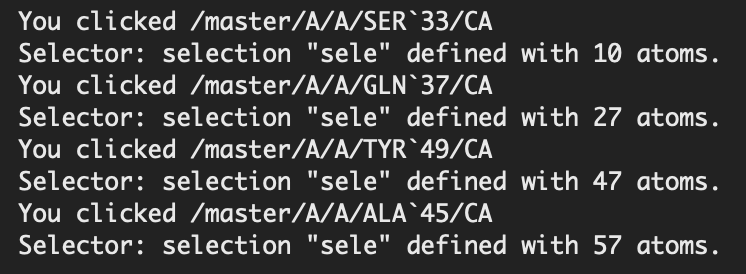
1. Get your protein, using your PDB Entry Code. After you have run the commands above, type “fetch ZZZZ, main” at the prompt (**PyMOL**>). ZZZZ is the pdb code of your protein. Do not actually type ZZZZ. This is a slight variation of what we did previously with the fetch command. The command as used here downloads your coordinates into PyMol and puts them into an object called ‘main’)
2. Use the commands you previously learned to  
   (i) display your protein (the main object) as a cartoon (this is the default),  
   (ii) color it by secondary structure (Color by ss)  
     
   (iii) create an object (called helix1) that contains an a-helix

If your protein does not have an a-helix (rare but not impossible) see \* at the end of section h.

**Set the Mouse.** You will need to be able to zoom and pan the protein in the viewing window to do the next steps. On Mac (when in 1 button viewing mode as was set when you entered the script above), hold option on the keyboard and the left mouse button to pan (drag) the molecule in the viewing window. Similarly, hold command on the keyboard and the left mouse button to zoom the molecule in the viewing window. If this does not work for you (or you have a fancy mouse), try **PyMOL**> config\_mouse three\_button.

To create the object, first identify an a-helix by looking at the ribbon structure in PyMol,  
then click on the ribbon on either end of the helix.  
Look just above the command line and you will see something like this  
  
**PyMOL**>  
create helix1, resi 19-29 (creates an object called helix1 from resi 19-29)  
(your residue identifiers (amino acid numbers) and residue names (amino acid types) will not be the same as what I have typed here)  
(PyMol is case sensitive, and the syntax (commas, etc) must be exact).  
  
If the command above selects multiple helices, change it to  
**PyMOL**>



create helix1, resi 19-29 and chain A  
  
(iv) create an object (called sheet1) that contains a 2 stranded b-sheet  
for this you will have to identify both strands and click on a total of four places to identify the start and stop of each of the two strands  
  
  
**PyMOL**>  
create sheet1, resi 33-37 or resi 45-49  
(note the boolean operator “**or**”. Do not use “and” here, it won’t select anything for your object.)

If the command above selects multiple 2 stranded b-sheets, change it to

**PyMOL**>

create sheet1, resi 33-37 and chain A or resi 45-49 and chain A

(operator ‘or’ is addition and ‘and’ is intersection, e.g., if the atoms are in resi 33-37 and in chain A or are in 45-49 and in chain A, put them in sheet1)  
  
(v) create an object (loop1) that contains a loop region of at least 7 amino acids

1. In this section we will study your a-helix.   
   The commands below convert all of your sidechains to alanine (easier to see), add hydrogens and draw hydrogen bonds. Please look through the commands and get a general sense of what each is doing. You will need to use them later on.  
   Copy and paste the following

**PyMOL**>  
#---------start copy --------------  
# center the display on helix1  
zoom helix1

# strip away the sidechains  
﻿remove helix1 and not (backbone or name CB)  
# add hydrogen atoms to object helix1  
h\_add helix1  
# find helix1 backbone atoms (either O or H) that are within hydrogen bonding distance of each other (2.3 Å). Put these distances in an object called hbond1)  
distance hbond1, (name O\* and helix1), (name H\* and helix1), 2.3  
# color the hydrogen bonds and set the dash width etc  
color gray, hbond1  
set dash\_width, 3, hbond1  
set dash\_gap, 0.4, hbond1   
# Set the atom colors  
color wheat, name C\* and (helix1)  
color red, name O\* and (helix1)  
color blue, name N\* and (helix1)  
# set the display as stick

hide everything, helix1  
show sticks, helix1  
#---------end copy --------------  
  
Save one image of each object, and insert it in table (i) below. Copy and paste the text below into PyMol

#ray 1000,1000  
save main\_pdbid\_view1.png

#ray 1000,1000

disable all

enable helix1

zoom helix1

#ray 1000,1000

save helix1\_pdbid\_view2.png

disable all

enable sheet1

zoom sheet1

#ray 1000,1000

save sheet1\_pdbid\_view1.png

disable all

enable loop1

zoom loop1

#ray 1000,1000

save loop1\_pdbid\_view1.png

\* If your protein does not have one of the secondary elements required for this assignment (i.e., it does not contain any a-helix or b-sheet) then use PDB entry 3IBF just for the missing secondary element. Use your protein for as much of this assignment as possible.

1. Insert the one image of each object from the last section. Resize the images to fit within the margins of this page.

|  |
| --- |
| Insert main\_pdbid\_view1.png |
| Insert helix1\_pdbid\_view1.png |
| Insert sheet1\_pdbid\_view1.png |
| Insert loop1\_pdbid\_view1.png |

1. Indicate if your two stranded sheet1 is parallel or antiparallel.

|  |
| --- |
| INSERT HERE |

1. Insert the sequence of loop1. (Hint: **PyMOL>** set seq\_view, 1)

|  |
| --- |
| INSERT HERE |

1. Insert the sequence of helix1.

|  |
| --- |
| INSERT HERE |

1. Compare the sequence composition of loop1 and helix1. Does either contain proline? Glycine? Is this consistent with expectations for this secondary structure? Why? Hint: See Lehninger Section 4.2.

|  |
| --- |
| INSERT HERE |

1. Hydrogen Bonding Distances   
     
   Make a table of 5 hydrogen bonding distances, O to H, within the a-helix. These distances should already be labels in the ‘hbond1’ object. Determine the average and standard deviation. An example is shown but your numbers will be different.

|  |  |  |
| --- | --- | --- |
| **O resn resi** | **H resn resi** | **Distance ( Å )** |
| Asp 207 | Leu 211 | 1.8 |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
| Average | |  |
| Standard Deviation | |  |

1. Hydrogen Bonding Angles, N to H to O

Measure and record 5 hydrogen bonding angles, N to H to O (you need the wizard for this), within the a-helix. (For this you will need to use Wizard -> Measurement (on the top menu), then go to the pull-down Measurement menu (on the bottom right) and select Distances -> Angles (in the popup menu).

Make a table of these angles and determine the average and standard deviation. An example is shown but your numbers will be different.

|  |  |  |
| --- | --- | --- |
| **N-H resn resi** | **O resn resi** | **Angle ( ° )** |
| Arg 212 | Lys 208 | 148.8 |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
| Average | |  |
| Standard Deviation | |  |

1. Hydrogen Bonding Angles, C’ to O to H

Measure and record 5 hydrogen bonding angles, C’ to O to H (note that C’ is usually called C in coordinate files), within the a-helix.

Make a table of these values and record the average and standard deviation. An example is shown but your numbers will be different.

|  |  |  |
| --- | --- | --- |
| **C’-O resn resi** | **H resn resi** | **Angle ( ° )** |
| Lys 208 | Arg 212 | 144.8 |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
| Average | |  |
| Standard Deviation | |  |

Go to the pull-down Measurement menu (on the bottom right) and click ‘Done’.

1. Phi and Psi Torsion angles

Phi and psi. For this you will need to use Wizard -> Measurement (on the top menu), then go to the pull-down Measurement menu (on the bottom right) and select Distances -> Dihedrals (in the popup menu). A torsion angle is the same thing as a dihedral angle). A torsion angle is defined by 4 atoms, so you will have to click on 4 atoms for each torsion angle.   
  
Measure and record 5 phi torsion angles in your a-helix in the table below. Phi is defined by C’-N-Ca-C’. Coordinate files will usually call these atoms C-N-CA-C. The numbering is C(j)-N(j+1)-CA(j+1)-C(j+1)   
  
Measure and record 5 psi torsion angles in your a-helix in the table below. Psi is defined by N-Ca-C’-N. The numbering is N(j)-CA(j)-C(j+1)-N(j+1). An example is shown but your numbers will be different.

|  |  |  |
| --- | --- | --- |
| **resn resi** | **Phi (C’-N-Ca-C’)** | **Psi (N-Ca-C’-N)** |
| Asn 209 | -63.6 | -45.5 |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
| Average |  |  |
| Standard Deviation |  |  |

1. Graph the phi/psi pairs of your a-helix using the layout of a standard Ramachandran plot. Use MS Excel, MATLAB, or hand draw your plot.

|  |
| --- |
| INSERT HERE |

1. Save the document Assignment\_12\_lastname.docx.