Pymol Exercise 8
Chem 6572

Pease read the paper:
Contributions of hydrogen bonds of Thr 157 to the thermodynamic stability of phage T4 lysozyme, Alber et al., Matthews, Nature 330, 1987, 41-46.

Don't worry about understanding the x-ray diffraction details, read the paper for their description
and their interpretations of the structures.

Below is Table 2 from the paper, which shows how mutations impact the thermal stability of the lysozyme.

Table 2 Thermodynamic stability of lysozymes with different amino acids at position 157

Amino acid at position 157	ΔT_m(°C)	$\Delta\Delta G$ (kcal mol^{-1})
Thr (wild type)	—	—
Asp	−1.7	0.45
Ser	−2.5	0.66
Asp	−4.2	1.1
Gly	−4.2	1.1
Cys	−4.9	1.3
Leu	−5.0	1.3
Arg	−5.1	1.3
Ala	−5.4	1.4
Glu	−5.8	1.5
Val	−6.0	1.6
His	−7.9	2.1
Phe	−9.2	2.4
Ile (ts mutant)	−11.0	2.9

The proteins were purified as described[13], except that cell lysis was promoted with EDTA (ref. 21) instead of chloroform. ΔT_m is the change in the melting temperature of the mutant lysozyme (±0.5°) relative to wild-type lysozyme (T_m=42 °C at *p*H 2.0). $\Delta\Delta G$ is the corresponding change in free energy at 42 °C (ref. 7). Protein samples (20–30 μg ml^{-1}) were extensively dialysed against oxygen-free buffers. Ionic strength was 0.2 with KCl and *p*H was adjusted to 2.0 with HCl to avoid irreversible aggregation at high temperature. Circular dichrosim (θ_{223} nm) was measured with a Jasco J-500C instrument equipped with a Hewlett Packard 89100A thermoionic controller. The temperature of the sample was changed at a constant rate of 1 K min^{-1}. To ensure reversibility, unfolding and refolding were both monitored[5,6].

Create a directory called lysozyme
Open a Pymol window
Paste the header information below into Pymol command line

```
#-------------------------------------------------------------------------------
## Header: General Commands ##

## this file was created by ____ on ____

# 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 desktop.
config_mouse three_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 header----------
```

Turn on logging in Pymol
Save the log file to as lysozyme.pml in the lysozyme directory

The wild type protein is 2LZM.pdb and the TS mutant is 1L02.pdb
The proteins in this table are

2LZM.pdb	(THR, wild type)
1L01.pdb	(ILE, ts mutant)
1L02.pdb	(ALA)
1L03.pdb	(CYS)
1L04.pdb	(ASP)
1L06.pdb	(GLU)
1L07.pdb	(PHE)
1L08.pdb	(GLY)
1L09.pdb	(HIS)
1L11.pdb	(LEU)
1L12.pdb	(ASN)
1L13.pdb	(ARG)
1L14.pdb	(SER)
1L15.pdb	(VAL)

In PyMol fetch all of these proteins and name the objects by the amino acid at position 157. Copy the following into the Pymol command line

```
fetch 2LZM, wild_type
fetch 1L01, mut_ts_mutant
fetch 1L02, mut_ala
fetch 1L03, mut_cys
fetch 1L04, mut_asp
fetch 1L06, mut_glu
fetch 1L07, mut_phe
fetch 1L08, mut_gly
fetch 1L09, mut_his
fetch 1L10, mut_leu
fetch 1L12, mut_asn
fetch 1L13, mut_arg
fetch 1L14, mut_ser
fetch 1L15, mut_val
```

Remove everything but the protein from the objects.

```
remove resn HOH or resn BME
```

Use the cealign command to superimpose each of the mutant proteins onto the wild type protein.
https://pymolwiki.org/index.php/Align

```
cealign mut_ts_mutant ,wild_type
cealign mut_ala, wild_type
cealign mut_cys, wild_type
cealign mut_asp, wild_type
cealign mut_glu, wild_type
cealign mut_phe, wild_type
cealign mut_gly, wild_type
cealign mut_his, wild_type
cealign mut_leu, wild_type
cealign mut_arg, wild_type
cealign mut_ser, wild_type
cealign mut_val, wild_type
```

Hit <escape> to see the results of the superimposition.
Notice that the RMSD (root mean square deviation) of atomic positions for closely related protein structures like this is small, less than 0.1 Å.
Hit <escape> again to switch back to the viewer window.

Create objects with loop that contains the mutation

```
create loop1wild_type, resi 154-160 and wild_type
create loop1ts_mutant, resi 154-160 and ts_mutant
create loop1ala, resi 154-160 and mut_ala
create loop1cys, resi 154-160 and mut_cys
create loop1asp, resi 154-160 and mut_asp
create loop1glu, resi 154-160 and mut_glu
create loop1phe, resi 154-160 and mut_phe
create loop1gly, resi 154-160 and mut_gly
create loop1his, resi 154-160 and mut_his
create loop1leu, resi 154-160 and mut_leu
create loop1asn, resi 154-160 and mut_asn
create loop1arg, resi 154-160 and mut_arg
create loop1ser, resi 154-160 and mut_ser
create loop1val, resi 154-160 and mut_val
```

Set up the representations of the loops, add the hydrogen atoms, make the carbon atoms green, except for amino acid 157.

```
hide everything, loop1*
show stick, loop1*
h_add loop1*
color green, name C* and loop1*
color wheat, name C* and loop1* and resi 157 and sidechain
```

Turn off all the objects but the loops by clicking them off on the RH panel

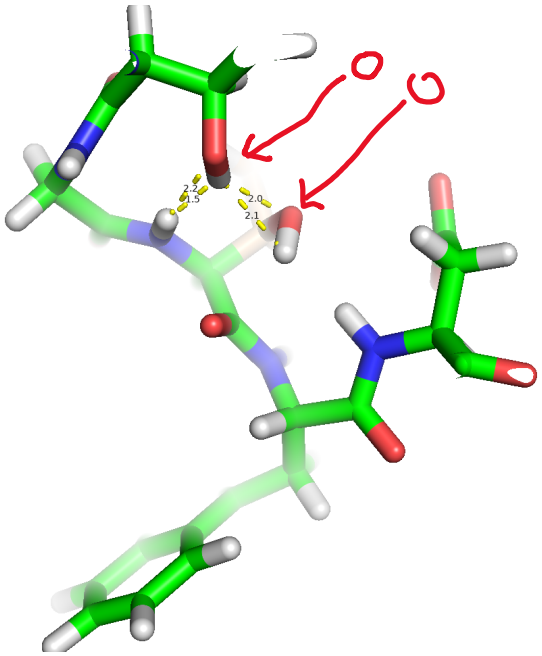
Find the hydrogen bonds to the sidechain of amino acid 157. Remember this is a simple distance calculation that does not take into account the atom types.

```
distance hbond_wild_type, (loop1wild_type and resi 157 and sidechain), (loop1wild_type and resi 155 and sidechain), 2.2
distance hbond_ ts, (loop1wild_type and resi 157 and sidechain), (loop1wild_type and not resi 157), 2.2
distance hbond_ala, (loop1ala and resi 157 and sidechain), (loop1ala and resi 155), 2.2
distance hbond_cys, (loop1cys and resi 157 and sidechain), (loop1cys and resi 155), 2.2
distance hbond_asp, (loop1asp and resi 157 and sidechain), (loop1asp and resi 158), 2.2
distance hbond_glu, (loop1glu and resi 157 and sidechain), (loop1glu and resi 155), 2.2
distance hbond_phe, (loop1phe and resi 157 and sidechain), (loop1phe and resi 155), 2.2
distance hbond_gly, (loop1gly and resi 157 and sidechain), (loop1phe and resi 155), 2.2
distance hbond_his, (loop1his and resi 157 and sidechain), (loop1his and resi 155), 2.2
distance hbond_leu, (loop1leu and resi 157 and sidechain), (loop1leu and resi resi 155), 2.2
```

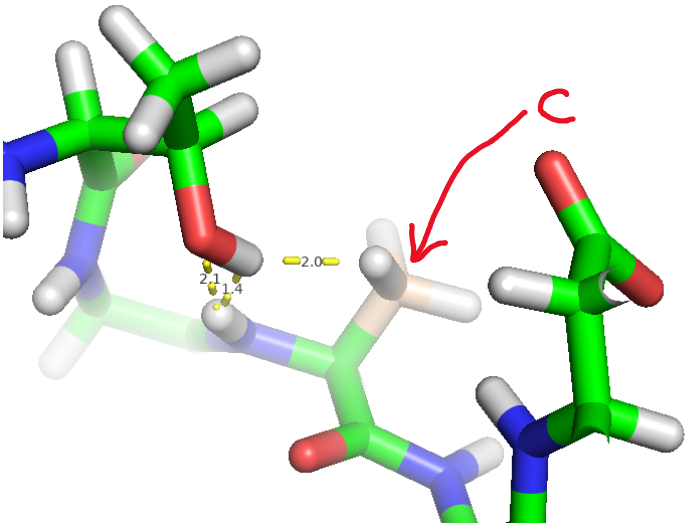
distance hbond_asn, (loop1asn and resi 157 and sidechain), (loop1asn and resi 155), 2.2
distance hbond_arg, (loop1arg and resi 157 and sidechain), (loop1arg and resi 159), 2.3
distance hbond_ser, (loop1ser and resi 157 and sidechain), (loop1ser and resi 155), 2.2
distance hbond_val, (loop1val and resi 157 and sidechain), (loop1ser and resi 155), 2.2

Turn on Loop1wild_type and hbond_wild_type. Turn everything else off.

These are potential hydrogen bonds, because the hydrogen atoms are bonded to oxygen atoms



This is not a hydrogen bond, because the hydrogen atom is bonded to a carbon atom. Hydrogen atoms bonded to carbon atoms to not form hydrogen bonds because carbon is not electronegative.



Predict the relative stability of each mutant by assuming a hydrogen bond contributes 2 kcal/mol. Make a figure of each mutant, with the relative stability.

Graph the observed relative stability from the paper versus your predicted relative stability (Please do not expect your graph to be pretty). Why is the graph so ugly?

Turn off logging. Open your log file (lysozyme.pml) in a text editor. Copy and paste the RMSDs from lysozyme.pml into your report. Clean out the necessary text from the file. Paste the header information (above) at the top of lysozyme.pml. Save lysozyme.pml as text only.

Quit your Pymol session, then double click lysozyme.pml to relaunch it. Ensure that your script works properly.