Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

***The workshop is done as a group/class. This assignment is an individual homework assignment. Type your answers, paste in images as required (with word wrap). Keep images to a reasonable size!***

***Day 1 – Bioinformatics – Exploring Sequence***

1. From your initial investigation of NCBI and RCSB, answer the following: **(3 pts)**
2. Are there different variants of MDH1 and MDH2 listed in the nucleotide database? If so, what types can you identify?
3. Find an MDH isoform not explicitly mentioned in the workshop and identify the species, along with its nucleotide and protein accession numbers for the sequences.
4. Search the nucleotide, protein, and structure databases for malate dehydrogenase. How many hits do you find in each? **(1 pt)**

***For the rest of this exercise:***  *Use the UniProt human MDH1v3, the non-transit human MDH2 (the cannonical version)and watermelong glyoxosomal MDH UniProt ID P19446 (MDHG\_CITLA) the mature form with 1SMK or 1SEV PDB files to carry out this assignment. Use the databases and the papers you worked with in the workshop to answer the questions*

1. Align the nucleotide sequences for two different MDH records (ie. watermelon glyoxysomal and MDH1 or MDH2 NOT MDH1 and MDH2, you did this in the exersize). Use one of the human MDH sequences as reference. Capture the image with a screen shot of the result. **(2 pt)**
2. Identify the 90th to the 100th amino acid for the reference MDH and report the corresponding (number and identity) in the second sequence. Do the same for the nuclotide binding region of the reference and second sequences **(3 pts)**

***Bioinformatics – Exploring Structure***

**NOTE:** For the following questions, you will be asked to create and capture protein structure images. *To export images from PyMOL, use File → Save Image As → PNG. These .png picture files can be directly inserted into your typed answers.* *All images should annotated with a figure title and description of the image and its highlighted features.*

1. Create a complete cartoon image of the protein and ligand (using one of the human MDH structures from UniProt that has a ligand in the structure). Insert the image into this typed answer word document and annotate to explain your picture. **(4 pts)**
2. Zoom in on and display the ligand binding site. (hint – With the ligand showing, shift and drag a box to select the protein region all around the ligand. For the selected region, then under A(action), choose preset → ligand sites, and then choose how to display). Display to highlight all the binding/interacting/interesting amino acid residues on the screen. Capture, insert, and annotate this image in your answer. **(4 pts)**
3. Using a homologous protein (use either the non-ligand MDH or a different isoform of MDH (not a different splice varient)), perform an overlay of both structures. Capture, insert, and annotate the image as before. **(2 pts)**
4. Compare the overall binding energy of two of the wild-type MDH-CS PDB files linked on our class webpage. Partner with another classmate and each of you pick a different wild-type MDH-CS dock model and share the overall free energy of association. One of you should use one of the “\* published MDH-CS docks” and the other one of the two human MDH-CS docks not published. Interpret the possible reasons for the difference. **(5 pts)**
5. Using ONE of the wild-type MDH-CS docks, measure the distance from 4 or 5 of the best binding residues in your protein dock in the interface of MDH-CS using the Measurement Wizard. Capture, insert, and annotate the image as before. **(2 pts)**
6. Using the Mutagenesis Wizard, mutate an interesting or critical amino acid in the interface to a) an amino acid with the opposite chemical characteristics and one of the possible phosphorylated Ser or Thr to an Asp. Use the domain handout from Day 4. You may need to check the alignments of those numbers versus your structure numbering – yep, confusing. While not the best, a last choice would be a posphorylated Tyr to an Glu. Describe the changes in structure when you perform each mutation including differences in binding partners and distances. This type of mutation is called a [phosphomimetic](https://en.wikipedia.org/wiki/Phosphomimetics). Capture the more dramatic instance, then insert and annotate the image as before. **(5 pts)**
7. Create a publication quality image to highlight an interesting feature in the MDH-CS interface. Use a white background and have some fun with this image. Capture, insert, and annotate the image as before. **(4 pts)**
8. Create a movie in PyMOL with your protein – see the PyMOL wiki. Upload file to your notebook, if possible. **(5 pts)**