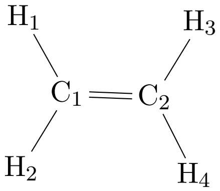
CS612 Homework Assignment 3

Due Thursday, March 30, 2023

1. **Internal and Cartesian coordinates:** The molecule ethene or ethylene looks like this:



The bond between the two carbons is double, and the molecule itself is all planar.

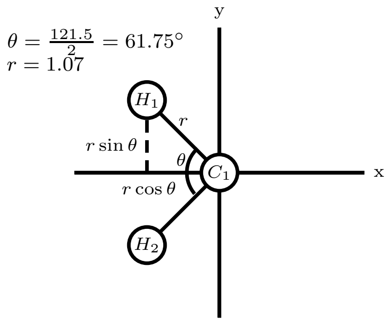
The Z-matrix looks as follows:

Atom	Bonded	Dist	Angle	Value	Dihe	Value
C						
C	1	1.31				
H	1	1.07	2	121.5		
H	1	1.07	2	121.5	3	180.0
H	2	1.07	1	121.5	3	180.0
H	2	1.07	1	121.5	4	180.0

Reconstruct the cartesian coordinates for ethylene. Have the first C (the left most in the figure) be the origin and the bond between the two carbons be the X-axis. Show your calculations. You may either calculate by hand or write a small piece of code to convert polar to cartesian coordinates. Notice that most software packages measure angles in radians, not degrees.

Note: Since the molecule is planar you can use x,y coordinates only.

In this illustration the carbons and hydrogens are numbered according to the coordinates we will assign to them (these are not official names). We can use X-Y coordinates, but it can be also X-Z, or whatever. Let's fix the origin on C_1 and the X-axis is the $C_1 - C_2$ bond. First calculate H_1 and H_2. Since the angle $H_1 - C_1 - H_2$ is 121.5° and the negative X-axis cuts it in the middle, the $C_1 - H_1$ bond created an angle of $61.75°$ with the negative X-axis, and the $C_1 - H_1$ bond created an angle of $-61.75°$ with the negative X-axis, see illustration below. H_3 and H_4 have a similar angle with respect to C_2 and the positive X-axis, but their coordinates have to be moved by 1.31 in the positive X direction.



So, the coordinates are as follows:

Atom	x	y
C_1	0	0
C_2	1.31	0
H_1	-0.51	0.94
H_2	-0.51	-0.94
H_3	1.82	0.94
H_4	1.82	-0.94

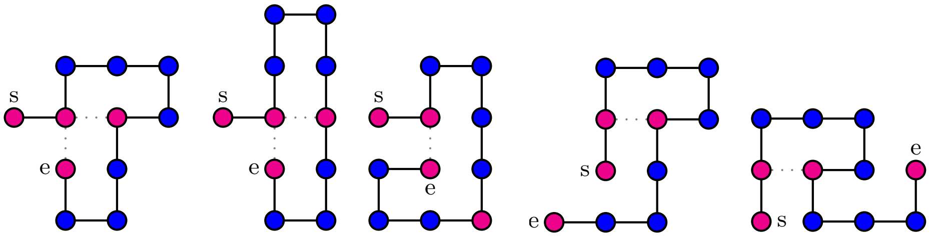
To make the molecule completely symmetric you can set the origin between the two carbons, and then subtract 0.655 from all the X values:

Atom	x	y
C_1	-0.655	0
C_2	0.655	0
H_1	-1.165	0.94
H_2	-1.165	-0.94
H_3	1.165	0.94
H_4	1.165	-0.94

2. **HP lattice model:** Given the following two sequences:
   - `S1 = HHPPPPHPPPH`
   - `S2 = HHPHPPHPHPH`

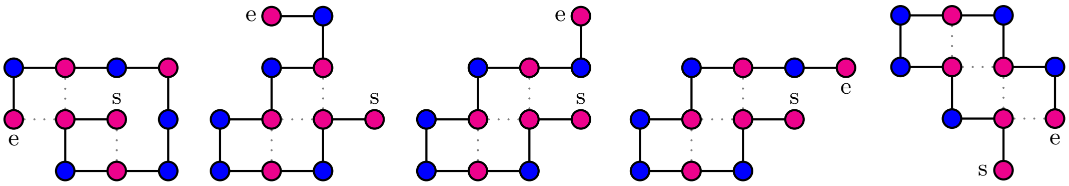
   (a) Find five possible high-scoring self-avoiding 2-D grid arrangements for S1 and calculate each one's "potential energy" according to the model discussed in class (award one point for each pair of H-H points that are one grid point apart (not diagonal), but not adjacent on the sequence. For more info look at the class notes – I'm refering to the white dashed lines. Attach the five arrangement (indicate which one is the start and which one is the end). Color P in blue and H in red or magenta, like in class. Notice that you don't have to find the absolute globally best structure, just arrangements that have a good score.

   Here are five examples. I could not find any conformation with $>$ 2 H-H interactions. The values (left to right) are 2, 2, 1, 1, 1.

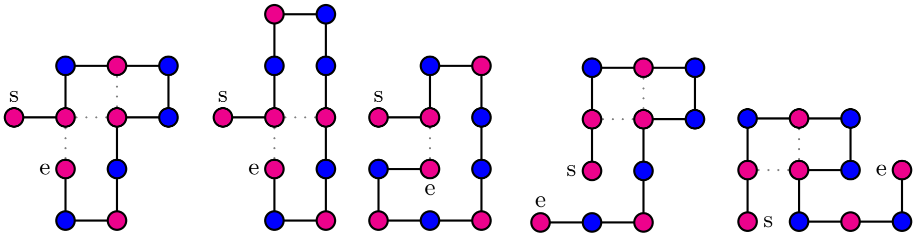


   (b) Find five possible high-scoring self-avoiding 2-D grid arrangements for S2 and calculate their score as in (a).

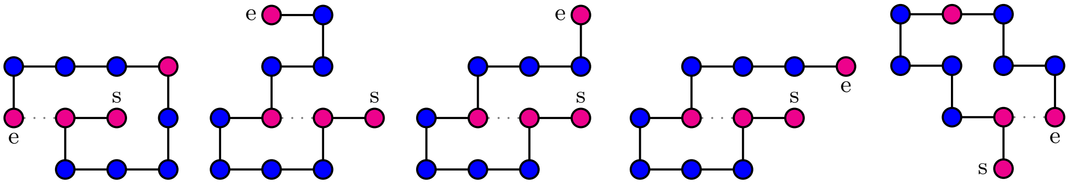
   Here are five examples. The best scoring example had 4 H-H interactions. The scores are: 3, 3, 3, 3, 4.



(c) For the same grid arrangements as in (a), “thread” S2. That is, use the same arrangements as in (a) above but the amino acid sequence in S2. Calculate the score again. The scores are, left to right: 3, 2, 1, 2, 2.



(d) For the same grid arrangements as in (b), “thread” S1. That is, use the same arrangements as in (a) above but the amino acid sequence in S1. Calculate the score again. The scores here are 1 for all the conformations.



(e) Explain the observed differences briefly.

Answer: The main observations are that: 1) S2 has more hydrophobic amino acids, so its scores generally tend to be higher. Also, the conformations that are suitable for S1 are not the ideal for S2 and vice versa.

3. **Hands-on homology modeling exercise using a template:** In this assignment you will perform homology modeling using the SwissModel server.

As a nice and easy example, let us start by modeling a short protein from a family called Crambin. Go to the SwissModel server at: https://swissmodel.expasy.org/interactive . There are three ways of doing homology modeling using the SWISS-MODEL server – a fully automated approach (called Automated mode), Alignment mode which allows you to submit a multiple sequence alignment of your target with one or more templates and a Project mode, which allows you to manually optimize your alignment. We will use the automated.

Note: SwissProt contains a rather extensive help section, please take a look if you need a clarification. I suggest you create a login with them, so that your jobs will be saved on the server for about a week.

On the top window, copy and paste the following sequence:

```
SVCCPSLVARTNYNVCRLPGTEAALCATFTGCIIIPGATCGGDYAN
```

Click on ”search for templates” and wait. It should take a couple of minutes. When you are done, you can access the templates by clicking on ”templates”. The page contains information about each template - its PDB code, its coverage of the query sequence, its sequence identity etc.

(a) How many templates did you get? What is the PDB code, GMQE and identity of the top template?

There were nine templates. The top template is an AlphaFold model of Crambin, P01542.1.A (that’s a Uniprot code, not PDB), the identity is 80.43 and the GMQE is 0.93.

(b) Now, check the boxes near the top three templates and click ”build model” on the top right. The server will now try to build three models, based on the three templates. Again, you will probably have to wait a while. What is the QMean score of each of the models? (this is a score that combines various aspects of interactions in the molecule. This statistical score is logarithmic and the lower, the worse.

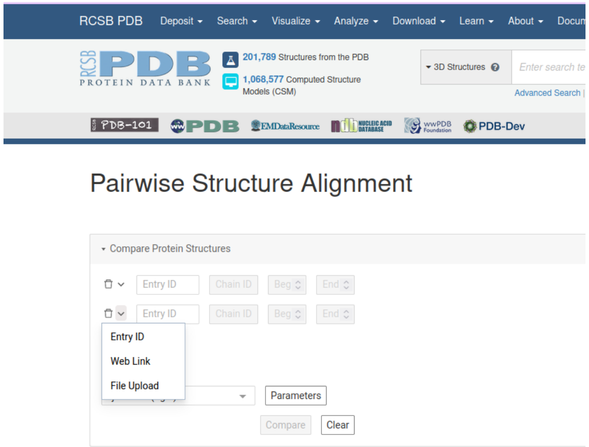
The QMean of each model is 0.83 and 0.73 (for some reason it appears not in order). The AlphaFold model did not have a QMean score.

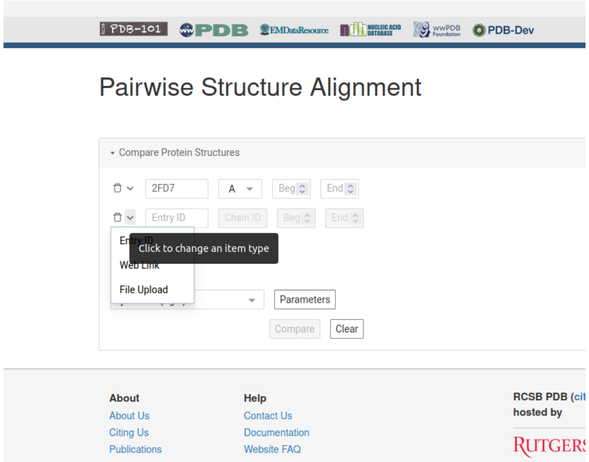
(c) What is the overall score, GMQE (Global Model Quality Estimation) of each one of the models? The scoring functions combine the energy terms used for the modeling, which are shown on the model window on a blue (good) to red (bad) scale. Notice the three superimposed models on the right window. You will notice that the protein is depicted on a blue to orange scale which correspond to model quality. As you may tell, the orange parts correspond mostly to loop regions.

The GMQE is 0.93, 0.86 and 0.78. Notice that the models may not appear in order.
At the top of the page you will see an icon of a page and if you hover the mouse over it, it will say ”one page project report”. Please attach it to your submission.

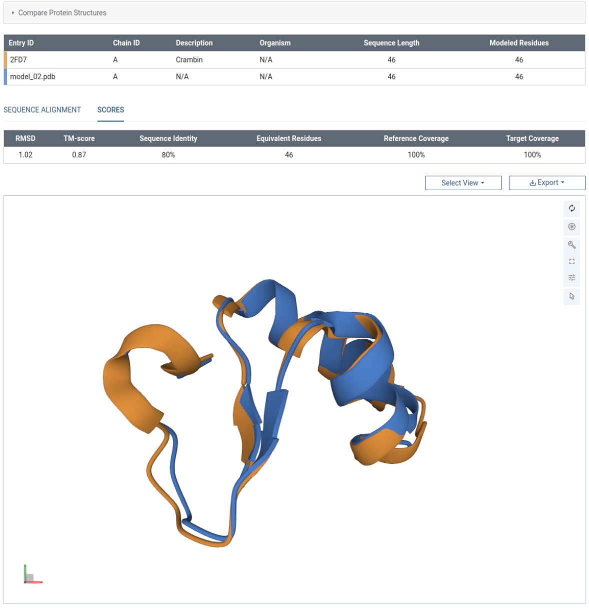
See page attached to the HW solution.

(d) Now measure the RMSD and TM scores of each model to its respective template. The easiest way IMO is to save the three models to your computer by clicking on the button near the model and save as pdb. The names of your file will be model_01.pdb, model_02.pdb and model_03.pdb. For each model do the following: Go to https://www.rcsb.org/alignment. In one window put the pdb code (only the first four number-letter-letter-letter/number), and chain A. In the second window click ”upload file”, upload your respective model and hit ”Compare” (see Figures).





Hit ”compare” and retrieve the RMSD and TM scores from the ”Scores” tab (see Figure):



Entry ID	Chain ID	Description	Organism	Sequence Length	Modeled Residues
2FD7	A	Crambin	N/A	46	46
model_02.pdb	A	N/A	N/A	46	46

RMSD	TM-score	Sequence Identity	Equivalent Residues	Reference Coverage	Target Coverage
1.02	0.87	80%	46	100%	100%

For model 1 the RMSD was 0.05 and TM score was 1. For model 2 it was 0.06 and 1 resp. and for model 3 it was 0.09 and 1, resp.

Important! Notice that even though the models have the same coverage, the same sequence identity and they are overall quite good, model 3 drops in quality with respect to model 2. The reason is that the model 3 was generated by NMR and others were generated by X-ray and ares very high-resolution. NMR structures tend to be more fuzzy, and in the case of homology modeling selecting a good quality, high-res structure can be quite important. While all three models came out OK in this case, it can be quite critical in other cases If at all possible – aim for X-ray structures when you create your models, and try not to go below a 2Å resolution.

4. **Basic protein folding exercise:** Use Protein Investigator (the software we demonstrated in class) at http://intro.bio.umb.edu/MOOC/jsPI/JsPI.html. It requires the Java running environment to run. On the upper folding window type the following sequence: IFMQSRTDAA (Ile-Phe-Met-Gln-Ser-Arg-Thr-Asp-Ala-Ala). Type ”Fold” and see the shape of the folded protein. The energy function is based on hydrophobic contacts, ionic interactions (opposite charges attract, similar charges repel each other), and hydrogen bonds between polar amino acids. For the classification of hydrophobic, charged and polar amino acids see class notes.

   (a) Create a mutant protein by changing **one** amino acid in the sequence above, such that the mutation has no effect on the shape of the mutant protein. Explain. Attach a screenshot of the resulting protein.

   (b) Create a mutant protein by changing **one** amino acid in the sequence above, such that the mutation has a large effect on the shape of the mutant protein. Explain. Attach a screenshot of the resulting protein.

   (c) Design a protein of at least 8 amino acids such that a salt bridge (an ionic interaction between charged amino acids) is critical to its shape. Explain and attach a screenshot.

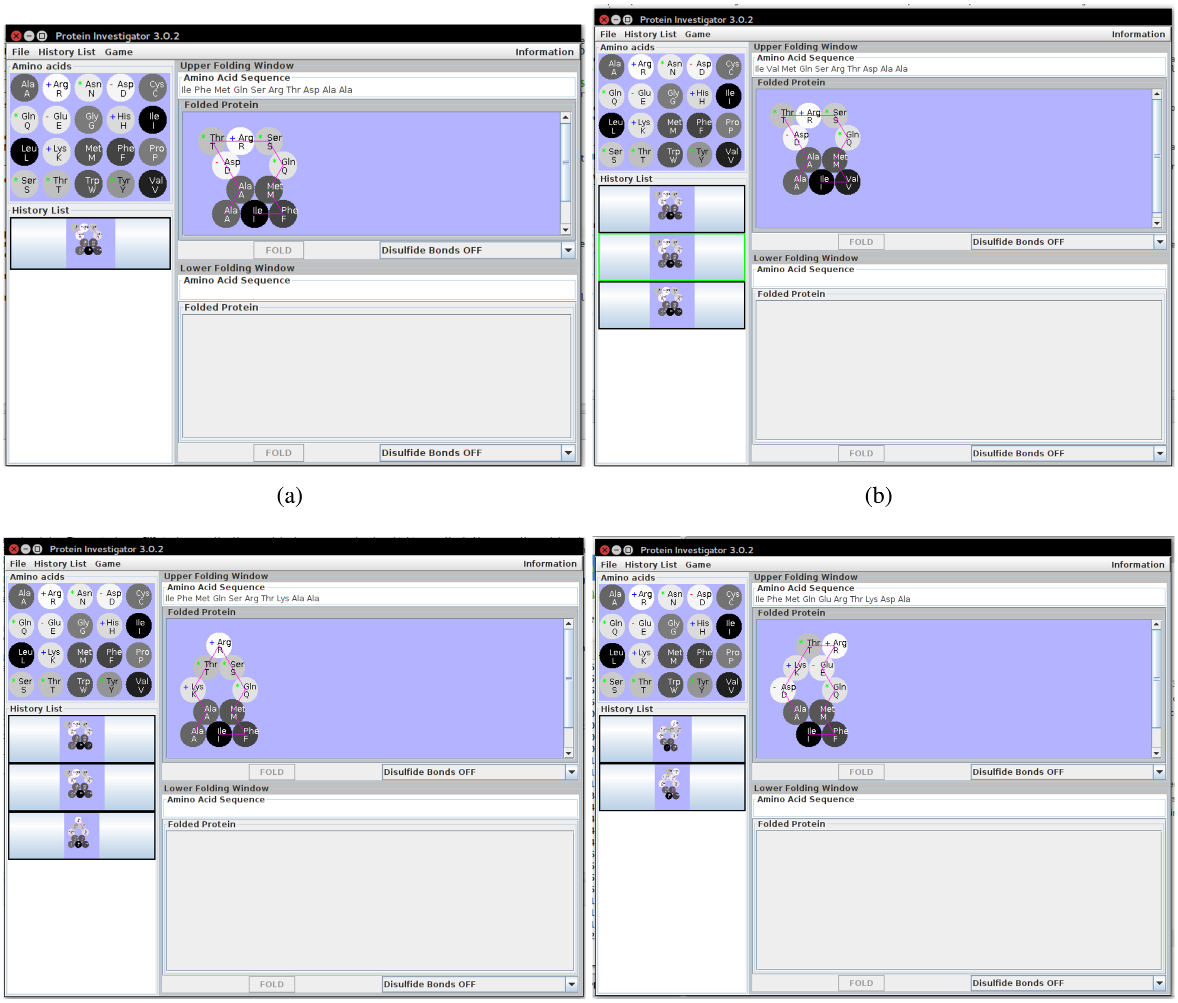
   Answer: See the figures below. This is just a suggestion. I accepted any reasonable answer. (a) The original sequence. (b) The sequence after mutating Phe (the 2nd amino acid) to Val (Valine). They are both hydrophobic and the shape doesn't change. (c) When Asp (8th amino acid) is changed into Lys, it is a change from a negative to a positive amino acid. This causes a significant change in the structure. (d) The sequence contains several oppositely charged amino acids that create several salt bridges.

5. Given the following point sets:

   Point Set A:

0.9003	-0.3258	-0.2888
-0.5377	0.2196	-0.8140
0.2137	0.8614	-0.4608
-0.0280	-0.0740	-0.9969
0.7826	0.2782	0.5569
0.5242	-0.7065	0.4755
-0.0871	0.9154	-0.3929
-0.9630	0.2336	-0.1344
0.6428	-0.6475	0.4094
-0.1106	0.7801	-0.6158

   Point Set B:



Amino Acid Sequence
Ile Phe Met Gln Ser Arg Thr Asp Ala Ala

Disulfide Bonds OFF

(a)

Amino Acid Sequence
Ile Val Met Gln Ser Arg Thr Asp Ala Ala

Disulfide Bonds OFF

(b)

Amino Acid Sequence
Ile Phe Met Gln Ser Arg Thr Lys Ala Ala

Disulfide Bonds OFF

(c)

Amino Acid Sequence
Ile Phe Met Gln Glu Arg Thr Lys Asp Ala

Disulfide Bonds OFF

(d)

-0.8842	0.4649	0.0448
-0.2943	-0.0193	-0.9555
0.6263	-0.7336	0.2636
-0.9803	0.1798	-0.0821
-0.7222	-0.6759	0.1467
-0.5945	-0.7013	0.3934
-0.6026	0.4536	-0.6566
0.2076	-0.9660	-0.1540
-0.4556	0.2610	0.8511
-0.6024	-0.3751	-0.7046

(text versions available as set1.txt and set2.txt, enclosed).

(a) Determine the RMSD between the two point sets.

(b) Determine the optimal RMSD between the point sets given that they are allowed to translate but not rotate. It can be shown that the optimal RMSD is obtained when the two point sets are translated so that their centroids are at the same point. The centroid or center of mass of a set of points is a point whose x,y,z coordinates are the average of the x,y,z coordinates of the point set, respectively. You can use any of C/C++, Java, Matlab, R, Python or any other acceptable programming language.

Please attach your source code.

Answer: See R, Matlab and python codes as separate files. The RMSD for the original point set is approximately 1.44Å and for the translated set approximately 1.27Å. The translation+rotation was 0.84Å.