**7.36/7.91/20.390/20.490/6.802/6.874**
**PROBLEM SET 3. Gibbs Sampler, RNA secondary structure, Protein Structure with PyRosetta, Connections (25 Points)**

**Due: Thursday, April 3th at noon in the dropbox labeled with 7.36/7.91 outside of the Biology Education Office on the ground floor of Building 68.**

**Python Scripts**
All Python scripts must work on athena using /usr/athena/bin/python. You **may not assume availability of any third party modules** unless you are explicitly instructed so. You are advised to test your code on Athena before submitting. Please only modify the code between the indicated bounds, with the exception of adding your name at the top, and remove any print statements that you added before submission.

Electronic submissions are subject to the same late homework policy as outlined in the syllabus and submission times are assessed according to the server clock. **All python programs must be submitted electronically, as .py files** on the course website using appropriate filename for the scripts as indicated in the problem set or in the skeleton scripts provided on Stellar. To submit a file electronically:
1. Go to
http://stellar.mit.edu/S/course/7/sp14/7.36/homework/index.html
2. Click on the corresponding problem set.
3. On the Assignment Details page, click the Add Submission link.
4. On the Add Submission page, select the appropriate file on your computer. **Do not use the paste box, do not zip files**
5. Click Submit when ready.

**P1. Gibbs Sampler (10 Points).**

You are studying longevity in two species, A and B. A study was recently published showing that a transcription factor called AGE is involved in regulating many aging- and stress-related pathways in both species A and B. AGE is known to affect transcription by binding to the promoters of its target genes. You have a list of aging-related genes whose expression changed (as measured by RNA-seq) in *age*(-) mutants relative to wild-type in species A. From a similar experiment, you obtained a list of genes whose expression changed in *age(-)* in species B. You want to look at the promoters of these genes to see if you can find any enriched sequence motif that might be a recognition site for AGE. You have two lists of sequences – `seqsA.fa` contains the 30bp upstream from the AGE target genes in A, and `seqsB.fa` contains 30bp upstream from the AGE target genes in B. To do this analysis, you will implement a Gibbs sampler! Once you are done modifying the script for parts A-D, please submit your completed `gibbsSampler.py` script to the Homework Submissions dropbox on the course website.

**(A – 6 points)** Download the skeleton code `gibbsSampler.py`. The script requires two inputs: the name of a FASTA file containing sequences believed to share a common motif, and the length of the motif. The main function `run` is called at the very bottom of the script; the argument `x` passed to `run(x)` is the number of iterations of the Gibbs sampler that will be run (initially set to 1).

You can run the script with the sequences from A as the input file and motif length 7 by running

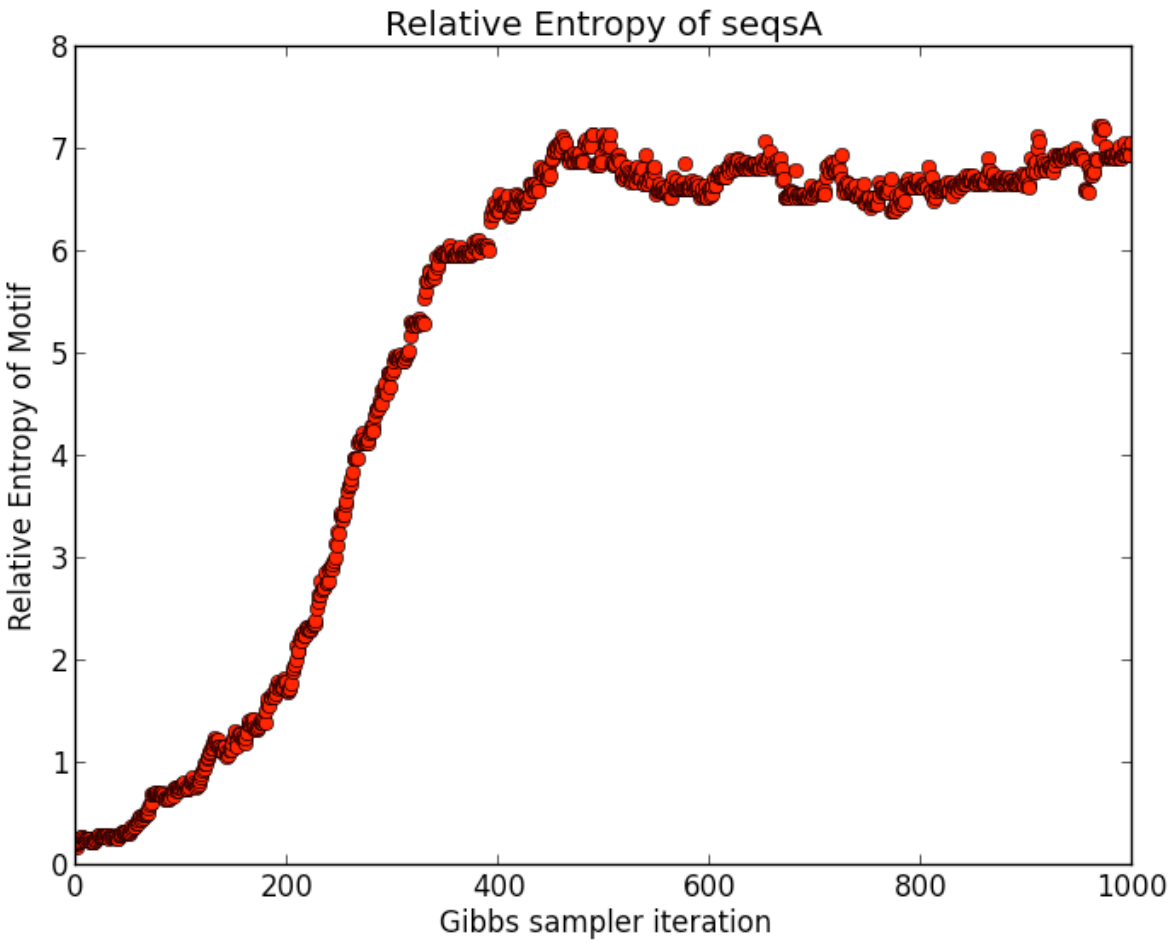
```
% python gibbsSampler.py seqsA.fa 7
```

The skeleton code should run without errors, though it will mostly be telling you that various sub-functions haven't been implemented yet. Now fill out the skeleton code so that it successfully implements the Gibbs Sampler. Though you can use whatever approach you find best when completing the script, you may find the suggestions in `GibbsSamplerHelp.txt` helpful to walk you through which sub-functions you need to complete.

Once you've completed the code, set your Gibbs Sampler to run 1000 iterations and run it on the `seqsA.fa` file. Run the algorithm several (~10) times and pick the highest scoring run. For that run, report the background distribution, final weight matrix, motif score and relative entropy (you can just copy and paste from the output of the script as long as your solution is in readable table form; .doc provided on course website if helpful). Also, plot the relative entropy of the motif after each iteration. What is the shape of this graph? What is the consensus motif? (Hint: the highest scoring motif has a score over 600).

```
Final weight matrix:
Pos  A    C    G    T
0    0.782051282051    0.0384615384615    0.0897435897436    0.0897435897436
1    0.0384615384615    0.141025641026    0.166666666667    0.653846153846
2    0.0641025641026    0.807692307692    0.115384615385    0.0128205128205
3    0.025641025641    0.0128205128205    0.0128205128205    0.948717948718
4    0.0641025641026    0.846153846154    0.0512820512821    0.0384615384615
5    0.897435897436    0.025641025641    0.0641025641026    0.0128205128205
6    0.782051282051    0.0769230769231    0.025641025641    0.115384615385
```

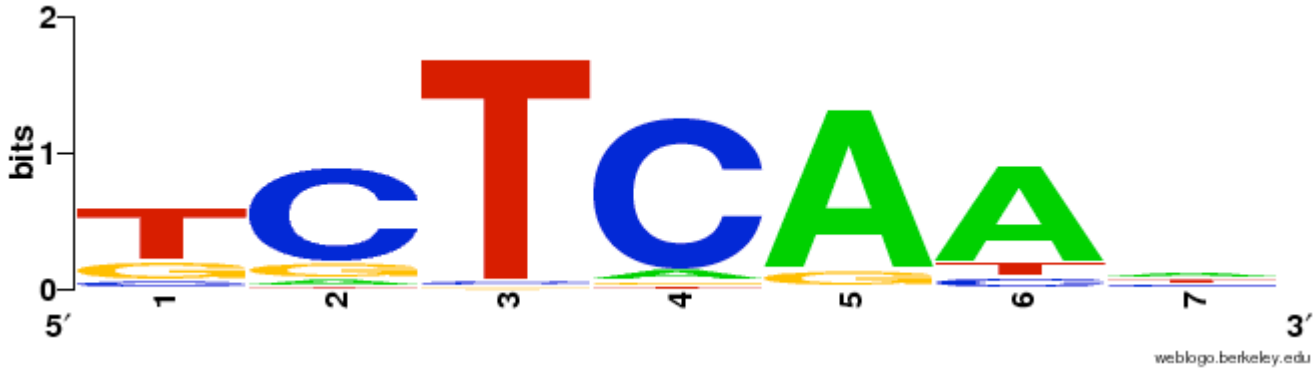
```
Background weight:
{'A': 0.29777777777777775, 'C': 0.23466666666666666, 'T': 0.28844444444444445, 'G':
0.1791111111111111}
Motif score = 588.503373641
Final relative entropy = 7.04401657361
```




The shape of the relative entropy graph after each iteration increases almost linearly until eventually converging to a constant value

**(B – 1 point)** Weight matrices are not very visually informative for understanding a motif – Sequence Logos are more human friendly. Run your code again, using the `printToLogo()` function provided in the code to print out the final motifs for each sequence after the 1000 iterations are complete (you may want to comment out the other outputs). Go to http://weblogo.berkeley.edu/logo.cgi and paste the list of aligned motifs into the input box and hit Create Logo (note that the number of bits of information at each position doesn't take into account the background distribution of the sequences). Try this at least 3 different times with different runs of your Gibbs sampler, and print off or include a screenshot of each logo as well as the relative entropy and final score of that logo. Compare the results of your various runs. Briefly explain what types of differences you observe and why the Gibbs sampler returns these different motifs.

Trial 1

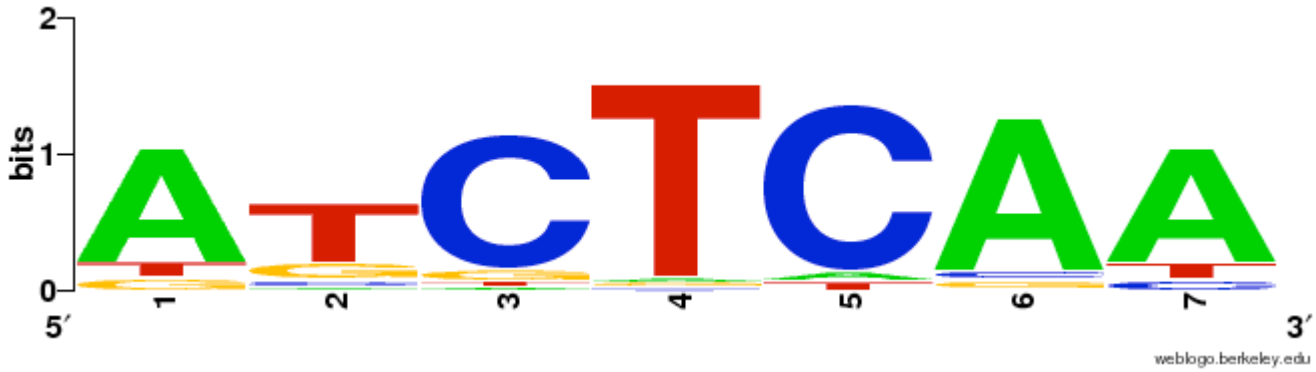



```
Motif score = 494.856523075
Final relative entropy = 5.76045628829
```

Trial 2


```
Motif score = 324.785845306
Final relative entropy = 3.68945339671
```

Trial 3




```
Motif score = 576.82089463
Final relative entropy = 6.69400918008
```

The motifs are all some subset of the motif ATCTCAA but with different confidences. Trial 3 has the highest motif and entity scores and it has a high probability for one base in each of the seven positions of the motif, whereas trial 2 has the lowest motif and entropy scores and it only determined five bases with a high probability. Trial 1 is an intermediate between Trial 2 and Trial 3 as it determined six of the bases with a high confidence. You see these differences because the Gibbs sampler is a sampler and doesn't look at all the same information on every run but rather is a randomized Monte Carlo algorithm that sometimes converges well upon a motif and other times doesn't find a motif at all.

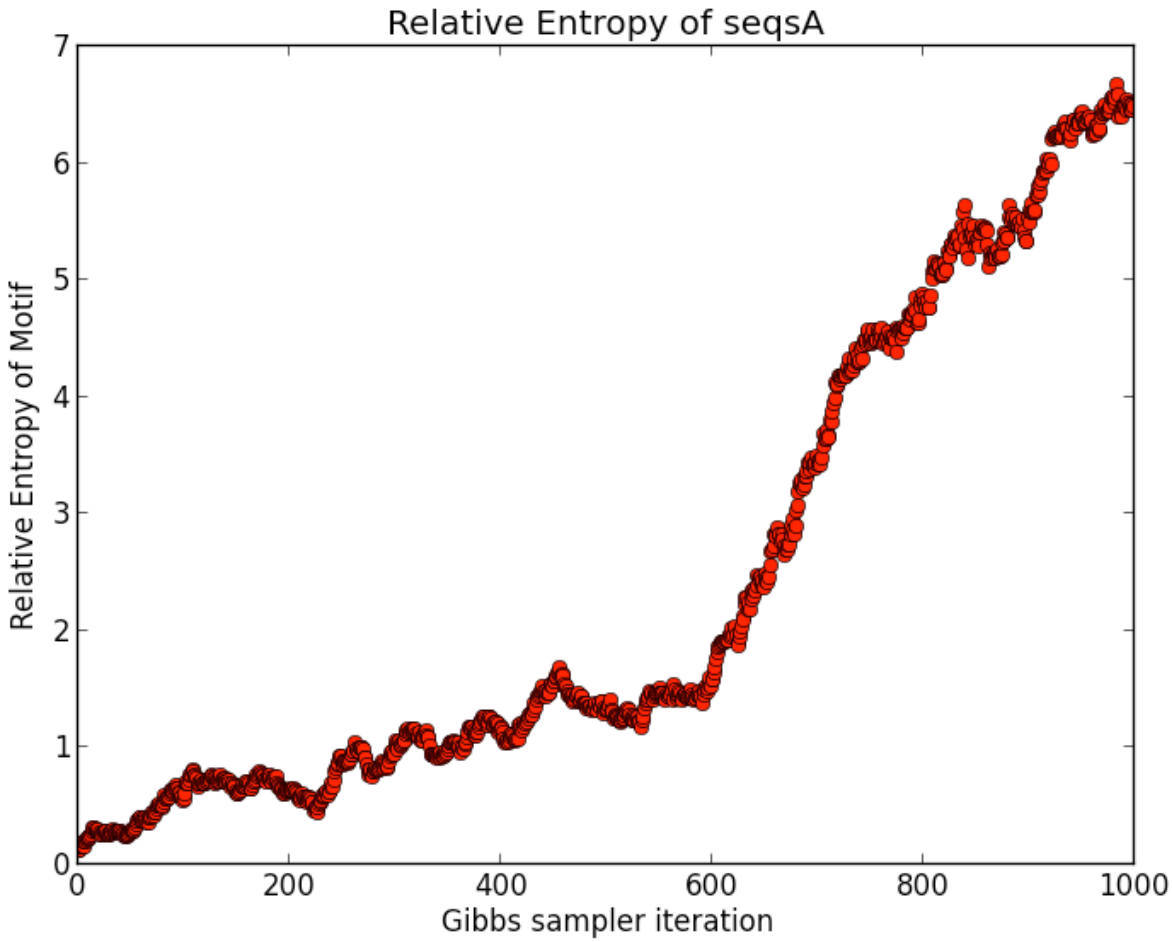
**(C – 2 points)** We now want to look for motifs in species B. Run the algorithm for length 7 and `seqsB.fa` several times and report the background distribution, final weight matrix, motif score and relative entropy (you don't need to plot RelEnt at every iteration) from a representative run. How does the relative entropy of the motif in species B compared to species A? Does this mean that the AGE motif is easier or harder to find in species B? Why?

```
Final weight matrix:
Pos  A    C    G    T
0    0.820512820513    0.0641025641026    0.0384615384615    0.0769230769231
1    0.0897435897436    0.0641025641026    0.217948717949    0.628205128205
2    0.025641025641    0.807692307692    0.153846153846    0.0128205128205
3    0.0512820512821    0.025641025641    0.0128205128205    0.910256410256
4    0.0897435897436    0.820512820513    0.0384615384615    0.0512820512821
5    0.897435897436    0.0384615384615    0.0384615384615    0.025641025641
6    0.769230769231    0.115384615385    0.0128205128205    0.102564102564
Background weight matrix:
{'A': 0.16533333333333333, 'C': 0.35777777777777775, 'T': 0.1368888888888889, 'G':
0.34}
Motif score = 756.872519361
Final relative entropy = 9.46654577713
```

The relative entropies and motif scores are pretty consistently higher than the average run from series A. This means that the motif is stronger in species B and as such is easier to find since more of the runs are converging on this strong motif.

**(D – 1 point)** Assuming there was still one occurrence of the motif in every sequence, what would happen if we increased the lengths of the sequences we are searching through? Run your Gibbs sampler on `seqsAext.fa`, which contains sequences of length 90 instead of 30. Run it several times and plot the relative entropy as a function of the number of iterations for a high scoring run. How is this plot different from part (a)? Can you explain the difference?

For the longer sequences the relative entropy plot increases consistently and eventually reaches similar entropy levels as the shorter sequences but it doesn't converge as quickly. These graphs don't reach the final entropy quickly and then level out but rather steadily increase with each iteration. This difference can be explained by the fact that the sampling space is now larger and the likelihood of stumbling upon the motif is now lower, but it doesn't on average change the final motif and entropy scores because the sequences, while longer, still contain the same strength motif as the shorter ones.



Cristina Lozano

**P2. RNA secondary structure prediction (5 points).**

**(A - 3 points)** Use the Nussinov algorithm to find the secondary structure that maximizes the number of Watson-Crick base-pairs in the following RNA sequence (show your work):

CGAGUCGGAGUC

( ( ( . ) ) )( ( . ) )

**(B – 2 points)** Run this sequence through the mfold RNA folding server (default parameters) at:

http://mfold.rit.albany.edu/?q=mfold/RNA-Folding-Form

Examine the top two structures it produces by looking at the “pdf”s under “View Individual Structures:”. Notice that they don’t match the structure predicted by the Nussinov algorithm. Examining the examples of real RNA secondary structures shown in lecture (slides 12, 32, 39 of Lecture 11), generate a hypothesis for which criteria used by the mfold algorithm to describe RNA thermodynamics prevents this algorithm from predicting the structure returned by the Nussinov algorithm in part (A). Test your hypothesis by strategically inserting adenosines at locations (e.g. in loops, between stems, across from bulges) necessary in the sequence above and finding the minimum number that must be inserted so that the top mfold-predicted structure has pairs between the same bases as in your Nussinov base pair-maximization structure.

The structure predicted by the Nussinov algorithm has stems that end in loops that are just one base but in reality the loop has to be at least three base pairs due to restrictions on backbone angles.

Adding adenosines so that the sequence is CGA**A**AG**A**UCGGAGUC gets the first stem to form correctly because there is now a loop of three base pairs but adding two more adenosines around the other loop causes the whole sequence to form one larger stem and does not mimic the shape sent back by the Nussinov algorithm.

**P3. Protein structure with PyRosetta (6 points).**

In this problem, you will explore protein structure with PyRosetta, an interactive Python-based interface to the powerful Rosetta molecular modeling suite.

In the following, we will provide instructions on how to complete this problem on Athena's Dialup Service, where PyRosetta has previously been installed as a module for another class – you may install PyRosetta (http://www.pyrosetta.org/dow) locally on your own computer and complete the problem there, but we will not provide help for installation or operating system-specific Python issues, so we highly recommend you complete it on Athena.

Log onto Athena's Dialup Service (http://kb.mit.edu/confluence/pages/viewpage.action?pageId=3907166), either at a workstation on campus or from a Terminal on your personal machine:

```
ssh <your Kerberos username>@athena.dialup.mit.edu
```

Once logged on, load the PyRosetta module with the following 2 commands:

```
cd /afs/athena/course/20/20.320/PyRosetta

source SetPyRosettaEnvironment.sh
```

If you log onto Athena to complete the problem at a future time, you will have to execute these two commands again, otherwise you will get the following error:

```
ImportError: No module named rosetta
```

Now, head to the course's Athena directory, copy the `pyRosetta_materials.zip` folder of files for this problem to your home directory (~), and then unzip it in your home directory with the following commands:

```
cd /afs/athena/course/7/7.91/sp_2014

cp pyRosetta_materials.zip ~

cd ~

unzip pyRosetta_materials.zip

cd pyRosetta_materials
```

You should now be able to edit any of these files. Most of the code you'll need is contained in the script `pyRosetta_1YY8.py` that you'll work with below, but for any additional functions you want to use you can reference the PyRosetta documentation: http://graylab.jhu.edu/~sid/pyrosetta/downloads/documentation/PyRosetta_Manual.pdf, particularly Units 2 (Protein Structure in PyRosetta), 3 (Calculating Energies in PyRosetta), and 6 (Side-chain Packing and Design).

Cristina Lozano

**(A – 1 point)** Go to the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) and search for the protein we'll be working with – 1YY8. What is this molecule? By looking at the "3D View" tab of this protein, what is the predominant secondary structure (α-helix or β-sheet)?

This molecule is a Cetuximab Fab Light Chain protein which is a monoclonal antibody found in the immune system of humans and mice.

Its predominant secondary structure is β-sheet

**(B – 1 point)** In order to open and edit the `pyRosetta_1YY8.py` file, you will need to use a text editor on Athena's Dialup Service, which doesn't register commands from the mouse. If you're familiar with emacs or vim, these are great options, otherwise we recommend nano, a friendly text editor that can be navigated with the arrow keys. Open the file for editing by typing:

```
nano pyRosetta_1YY8.py
```

You can scroll with the arrow keys, and additional commands are shown at the bottom of the Terminal, where `^` is the Control key. Once you've made any changes, to exit and save, type "`Control` + `x`" followed by "`y`" and then Enter to signify yes, you want to save your changes.

Look over how the code for `part_b()` loads the PDB file `1YY8.clean.pdb` (which has been cleaned to remove non-atomic annotation information) and prints out the protein's angles and energy score. Then run the script from the command line with `python pyRosetta_1YY8.py` (note that it may take up a couple of minutes to load the module the first time you run it; it will also print out many lines of initialization warnings before printing out what is in the code). What is the energy of the structure, and what categories are the primary contributors (weighted score) for and against the total energy? (describe the categories in words as on the lecture slides, not just the shortened names that appear)

The total weighted score of the energy for the structure is 204.257

The primary contributors for the total energy are solvation(Lazaridis-Karplus) and the Van der Waals net repulsive energy

The primary contributors against the total energy are the Van der Waals net attractive energy and the Hydrogen long range bonds in the backbone

**(C – 1 point)** In the code for `part_c()`, Monte-Carlo side-chain packing is implemented. At the indicated region, add code to print out the post-packed energy score. At the bottom of the script, uncomment `part_c()` and run the script. What is the energy of the structure after packing, and which two categories have decreased the most compared to part (b)? (describe the categories in words as on the lecture slides, not just the shortened names that appear)

The total weighted score of the energy for the structure is -972.226

The Van der Waals net repulsive energy and the Dunbrack Rotamer Probability have decreased the most.

**(D – 1 point)** The `1YY8.rotated.pdb` structure is the same as `1YY8.clean.pdb`, except that one residue's phi or psi angle has been changed. Fill in the code for `part_d()` to load the rotated structure and perform side-chain packing on it, printing out the energy of the structure before and after side-chain packing. What is the energy before and after? Why is the post-side-chain packing energy not the same as that of part (c)?

The energy for the rotated structure before packing is 59826.941 and the energy post-side-chain packing is 38938.914. The post side chain packing energy is not the same as part (c) because that one rotation caused the Van der Waals net repulsive energy to be an order of magnitude larger.

**(E – 1 point)** Add code to `part_e()` to determine which angle (phi or psi, as well as which residue number this angle belongs to) is changed in the rotated structure. Which residue/angle is it?

Residue 50 has a different phi the original protein has a phi of 52.84 and the rotated residue has a phi of -0.01

**(F – 1 point)** Using the residue and angle you determined in part (e), add code to `part_f()` to determine the energy of the structure with that angle changed to each of the possible angles [-180,-179,….,180]. Which of these angles has the lowest energy, and does this agree with the corresponding angle in the original structure from part (b)?

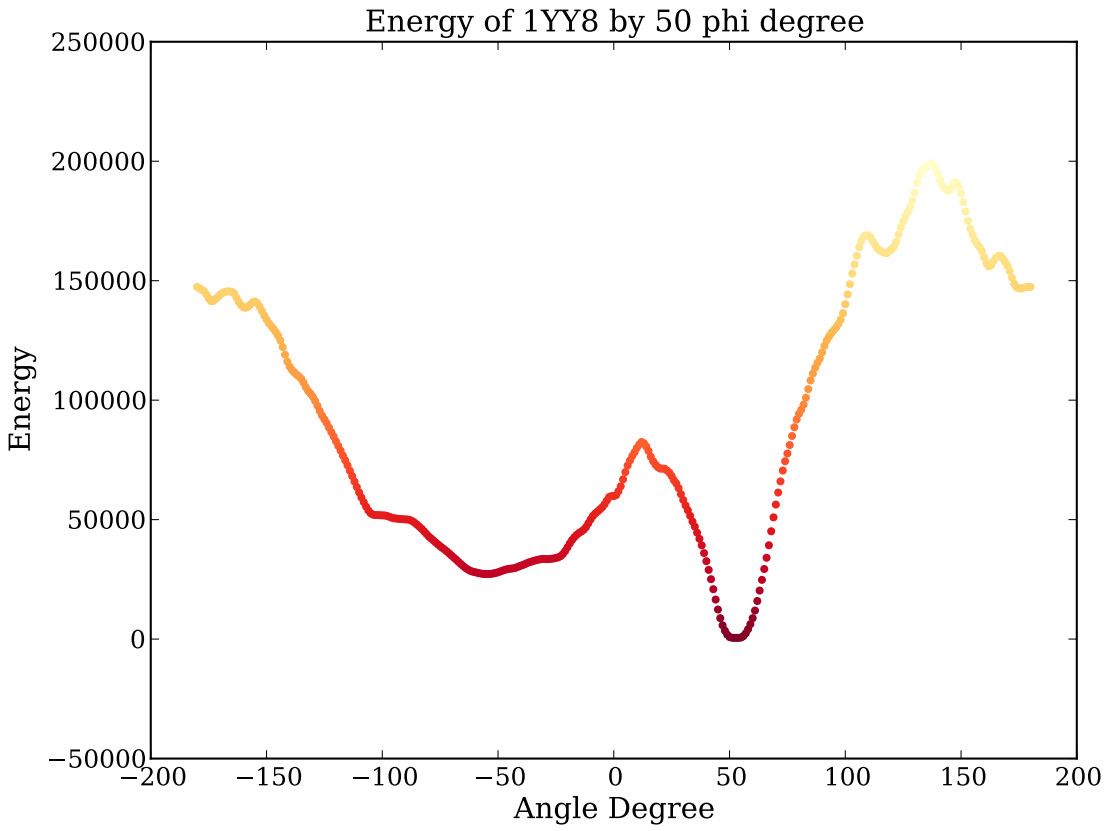
Add each of these energies to the `energies_of_angle` list, and then modify `set_title` to include the residue and angle that you changed. If you've done this correctly, an `energy_vs_angle.pdf` plot will be written to the directory where the script is. If you're at an Athena workstation, you can view this PDF directly (Home Folder > pyRosetta_materials); if you've ssh'ed into Athena from a Terminal on your local computer, you can copy this PDF to your local computer and then open it in your computer's PDF viewer with the following commands using `scp` (secure copy):

```
<in a new Terminal on your computer, cd into your local computer's directory where you want to download the PDF>

scp <your Kerberos username>@athena.dialup.mit.edu:~/pyRosetta_materials/energy_vs_angle.pdf .
```

Upload your completed `pyRosetta_1YY8.py` code to the Homework Submissions dropbox on the course website (you can do this directly if at an Athena workstation, or first download it to your computer via `scp` if ssh'ed into Athena). Also upload your `energy_vs_angle.pdf` or include a printout of it with your write-up.

The lowest energy angle is a phi of 53 which does correspond with the original structure's angle which was 52.84



**P4. Queuing theory/connections (4 points).**

A small bank hires a management consultant to figure out if they can afford to advertise free checking for one year (a $150 value) to any customer who has to wait in line more than 15 minutes. The consultant observes that, each minute that the bank is open, the waiting line gets longer by one customer with probability ¼, and – if there is a line – the line gets shorter by one customer (because a customer is served by a teller) with probability ¾. Let *X* be the probability that the line never gets longer than 10 people in a 12-week period (this is a reference case, whose empirical frequency is known to the bank), and let *Y* be the probability that the line never gets longer than 15 people in a 12-week period (the proposed duration of the promotion). The bank is open 2400 minutes every week.

Use an equation that was covered in class to calculate $\frac{\ln(X)}{\ln(Y)}$.