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Conformational Search of Gamma Aminobutyric Acid and Monte Carlo Simulation of Allantoin.

Abstract:

GABA and Allantoin are biochemically relevant molecules to the systems we have studied in this class as well as in our previous education. Thus, fundamental and quantitative structural data is important to understanding how these molecules are formed and how they interact with enzymes, receptors, solvent molecules, and other analytes. Though experimental methods for this determination exist, using these methods at the current time is impossible and impractical. Regardless of this, we have the tools necessary to acquire this data computationally. Using provided input structures, we were able to use Monte Carlo simulations to support alternative experimental structures of allantoin. We were also able to perform a conformational search that approximated the structure of the most probable orientation of GABA. Although we did not end up performing sufficient sampling for any confident conclusions to be drawn for GABA, we still developed our skills in the process.

Introduction:

Gamma-aminobutyric acid is a neurotransmitter inhibitor that has been extensively studied for potential medicinal utility[5]. This is why it is interesting and relevant to study possible structures. One reasonable way to accomplish this is by performing a conformational search. In a conformational search the minima of the potential energy surface is calculated for a flexible molecule. This is done by generating some initial structures and then minimizing the energies of those structures. The initial structure in the case of GABA in our experiment was obtained from Pubchem and random alterations were made from this structure to generate alternative conformations using the Monte Carlo method[1, 5]. From the energies of these conformations we can determine probable structures of GABA and perform further analysis on how the molecules structure may change in various solvents. The solvent effect of GABA is particularly interesting because it is a polar molecule and thus interacts with water.

Allantoin is the final metabolite in the breakdown of purines and more specifically results from 5-hydroxyisourate. Furthermore, it is relevant to the urate oxidase enzyme, which we have studied over the past six months, as well as to quantitative study by molecular quantum

mechanics due to its relatively small size. We did not perform quantum calculations via the Schrodinger equation because of the inherent computational challenges. That being said, we used specifically designed input parameters in order to better predict geometries and energies of Allantoin using molecular mechanics and Monte Carlo simulations. Molecular mechanics uses Newton's equations of motions in order to determine spatial and kinetic properties of atomic particles[1]. The parameters are slightly beyond the scope of this class, but can be explained qualitatively. For example, the Allantoin.z file contained weighted parameters for the variability of each bond distance and bond angle. The general principles of Monte Carlo simulations; however, are particularly relevant. In Monte Carlo simulations, an algorithm randomly selects for conformations by changing one or more variables of said conformer. Energy is calculated for each new structure, yet high energy structures are omitted because they are not particularly interesting to study. This is done by what is called Metropolis sampling, where new conformations that are lower in energy than the previous conformation are accepted, but those higher in energy are randomly passed on or rejected. This causes a greater ratio of low energy conformers as is the case in reality. To account for solvent effects, a periodic box is created. A periodic box consists of many consecutive boxes containing one central molecule of interest and a few hundred solvent molecules located everywhere else. Using these methods we were able to predict the energies and thus probabilities of specific angles and distances in allantoin.

Methods:

Conformation Search for Gamma-Aminobutyric Acid:

We first downloaded a SDF structure file of GABA from PubChem. This file contained cartesian coordinates for all atoms, partial charges for all atoms, the identity of every atom and several other physical and computational parameters[2]. We ran this input file through BOSS and acquired many structures and their corresponding energies for GABA. BOSS uses Monte Carlo simulations to generate these structures. For this procedure, we specified OPLS force fields. We then used molden to visualize the structure produced by BOSS.

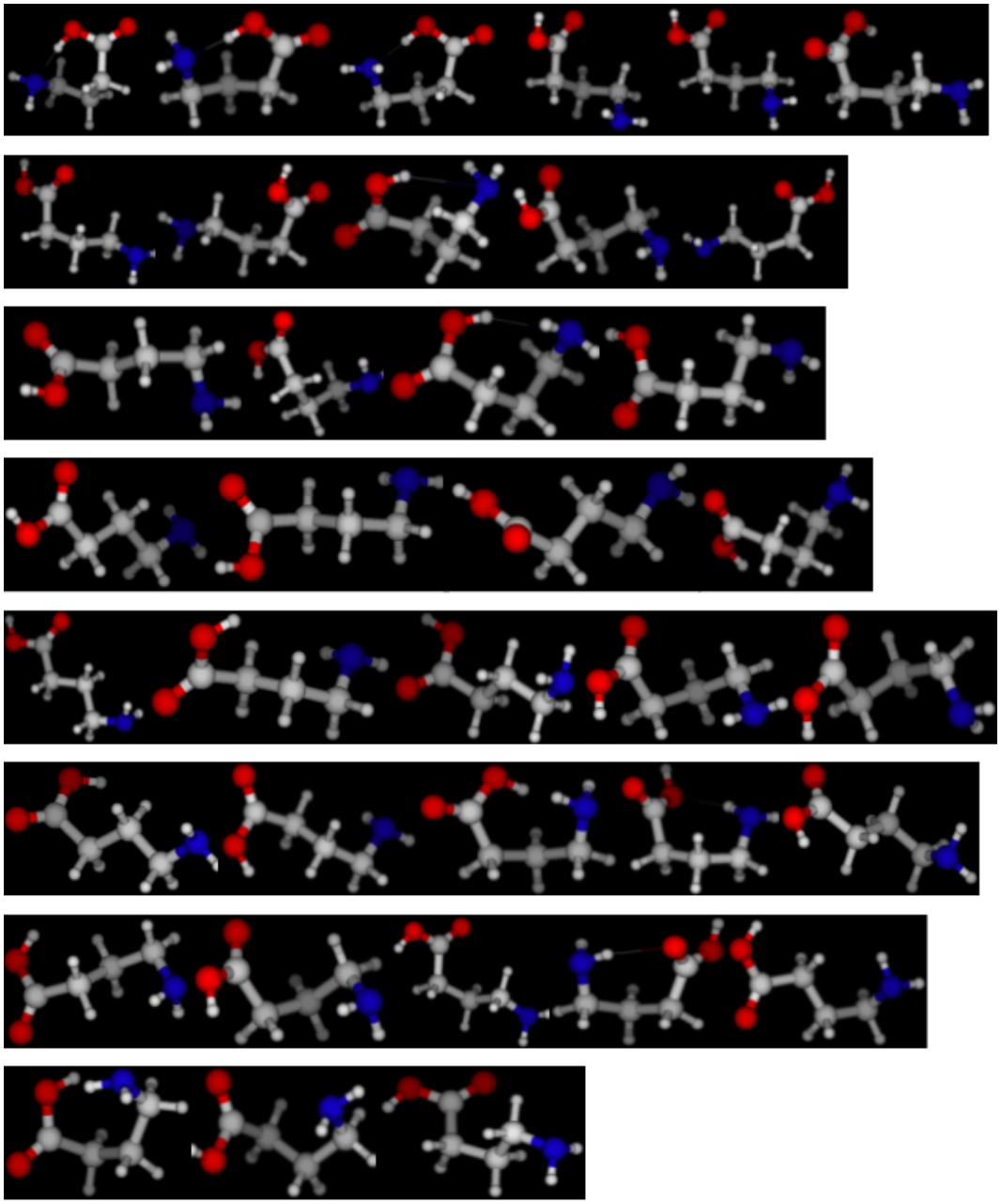
Monte Carlo Simulations of Allantoin:

We used an Allantoin z matrix file for this procedure. This file contains the structure in the Z-matrix format, which shows connectivity, atom type, force field type, the angles between atoms, and the bonds we allowed to vary (4-19)[3]. We also specified how much various angles, distances, and torsional angles were allowed to vary[3]. We used an oplsaa.sb file that defined stretching force constants, bond lengths, angle bending force constants, and natural angle widths[3]. Another file, oplsaa.par, described partial charges, Lennard-Jones interaction strength parameters, and attractive forces between atoms[3]. Critically, this file gives parameters for our desired solvent, DMSO, and also energetically defines the rotational barriers in the molecule[3]. The last three input files: MChdr, MCcmd, and xMC provide information on the setup parameters for the simulation, define the input and output files, and run a script that starts the simulation, respectively[3]. We reviewed the outputs using xmgrace and gOpenMol.

Results:

Conformation Search for Gamma-Aminobutyric Acid:

After performing our conformational search on gamma-aminobutyric acid, we recorded the structure and energy of the 37 conformations determined by BOSS in figure 1 below.



Conformer	Energy kJ/mol
1	-42.55964
2	-41.93694
3	-41.59094
4	-38.35154
5	-38.10847
6	-38.10080
7	-37.74319
8	-37.73906
9	-37.54233
10	-37.47600
11	-37.37680
12	-37.33222
13	-37.21577
14	-37.13180
15	-37.12452
16	-36.99475
17	-36.94746
18	-36.86331
19	-36.72946
20	-36.54369
21	-36.43990
22	-36.40655
23	-36.13078
24	-35.95056
25	-35.94419
26	-35.79667
27	-35.76379
28	-35.64592
29	-35.51366
30	-35.42112
31	-35.20954
32	-35.15751
33	-35.05646
34	-34.64713
35	-34.58512
36	-34.39365
37	-35.15606

Figure 1: Conformational Search for Gamma-Aminobutyric Acid

This figure highlights the energies of the 37 structures generated by the BOSS program. The conformers are presented in order with respect to the numbered list

Monte Carlo Simulations of Allantoin:

The following figures present data that was collected after performing a Monte Carlo simulation on Allantoin.

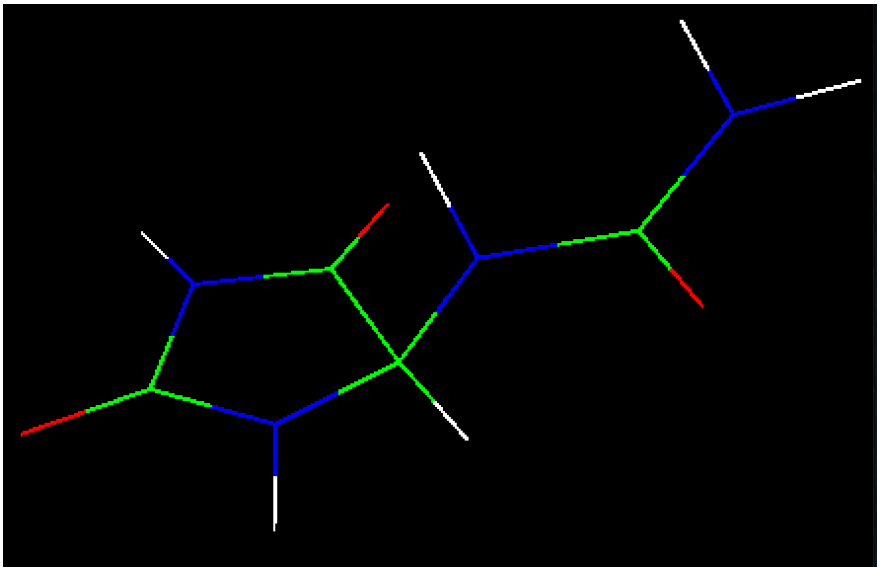


Figure 2: Monte Carlo generated structure of Allantoin

This figure highlights one of the several favorable conformers of allantoin that was generated by Monte Carlo simulations. The energy of this particular conformer is 36.818283 kj/mol.

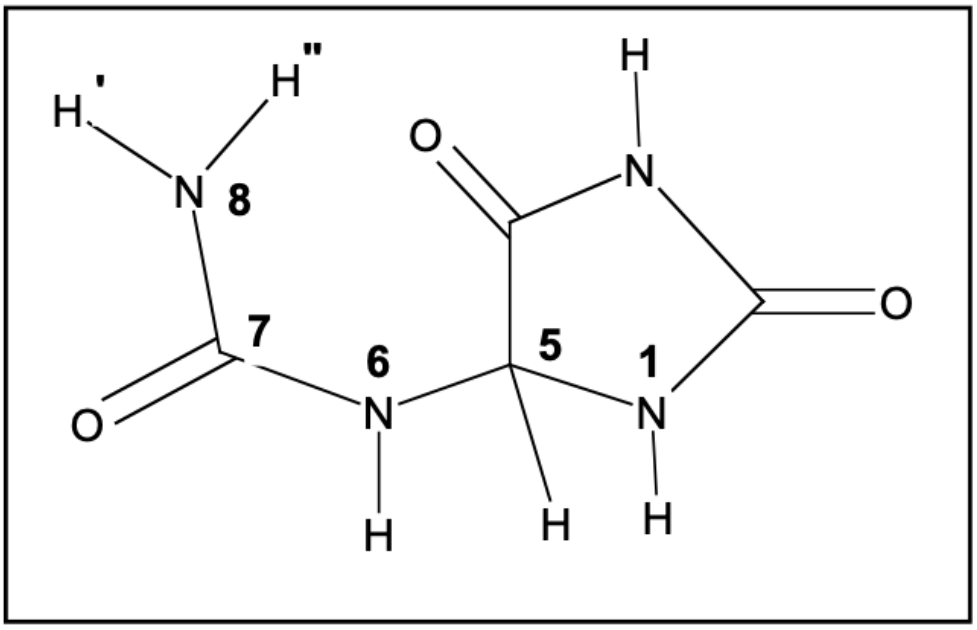


Figure 3: Labelled Allantoin

This figure shows allantoin with reference labels for the following discussion and figures.

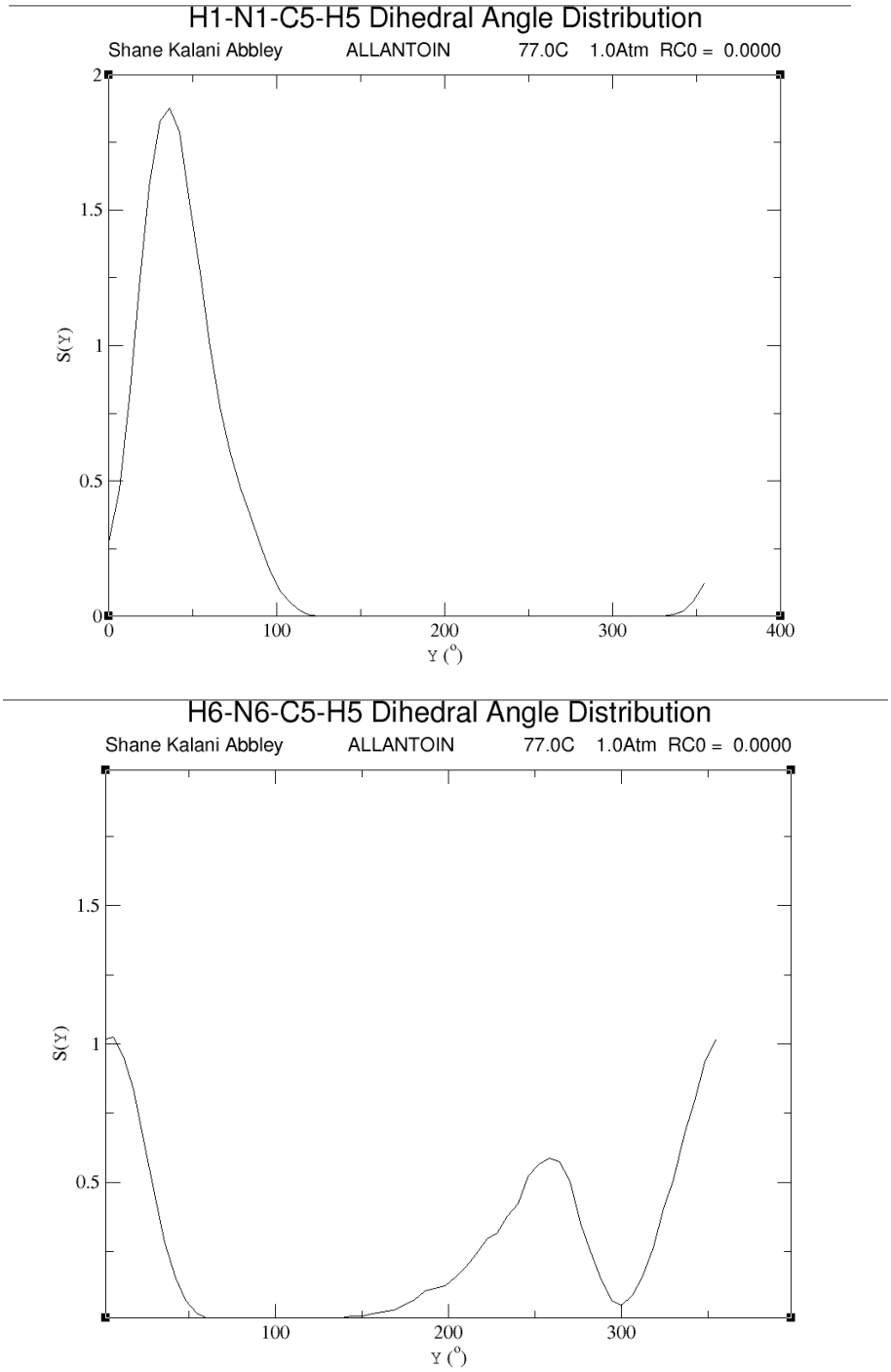


Figure 4: Dihedral Angles

This figure highlights the structural probabilities of the Monte Carlo simulation for the hindered rotation of the arm around the C5-N6 bond and the fixed position of H5 with respect to the ring.

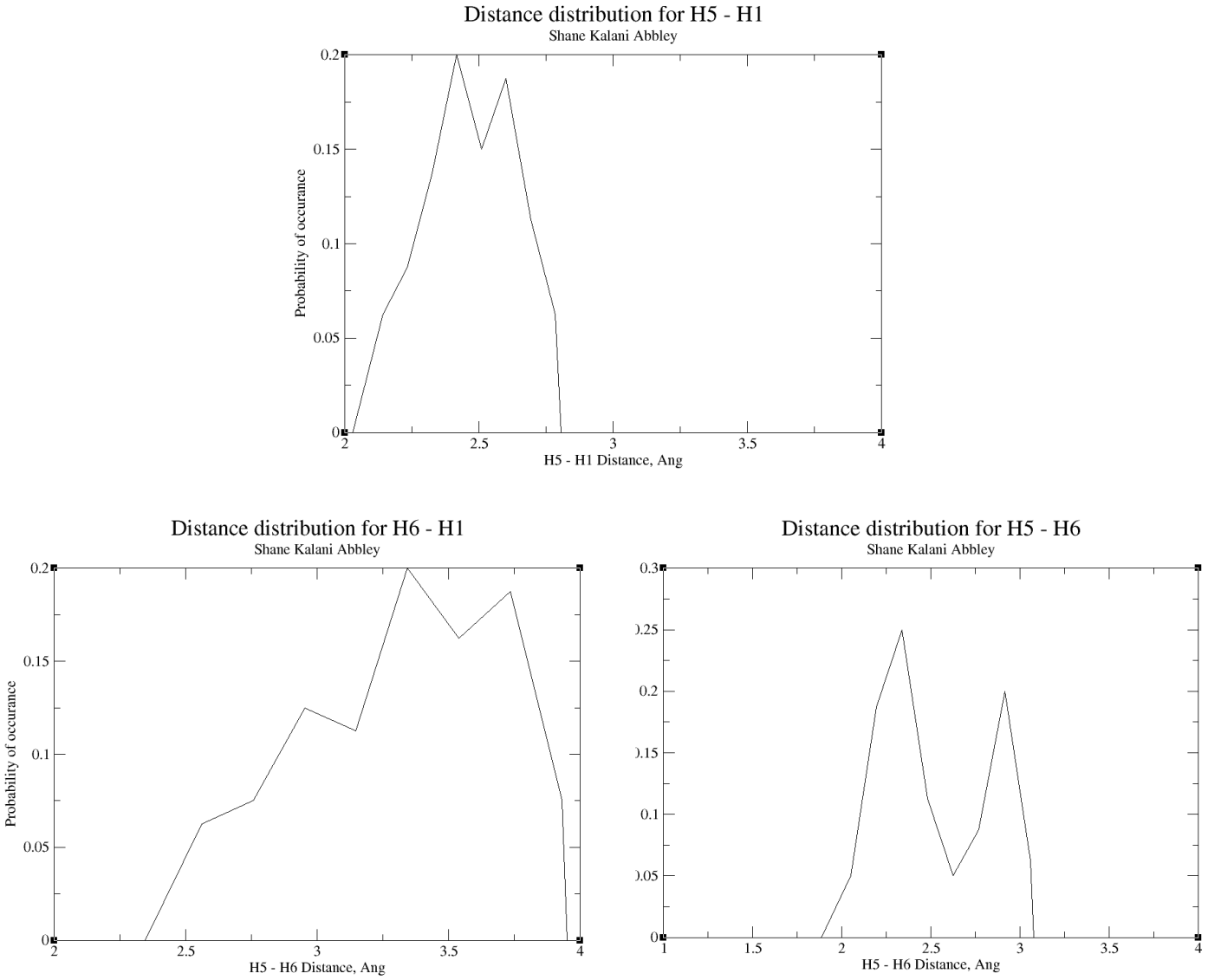


Figure 5: Ring Hydrogens Distance Distributions

These graphs provide probabilities for the distances between hydrogen atoms in the various geometries described by the Monte Carlo simulation.

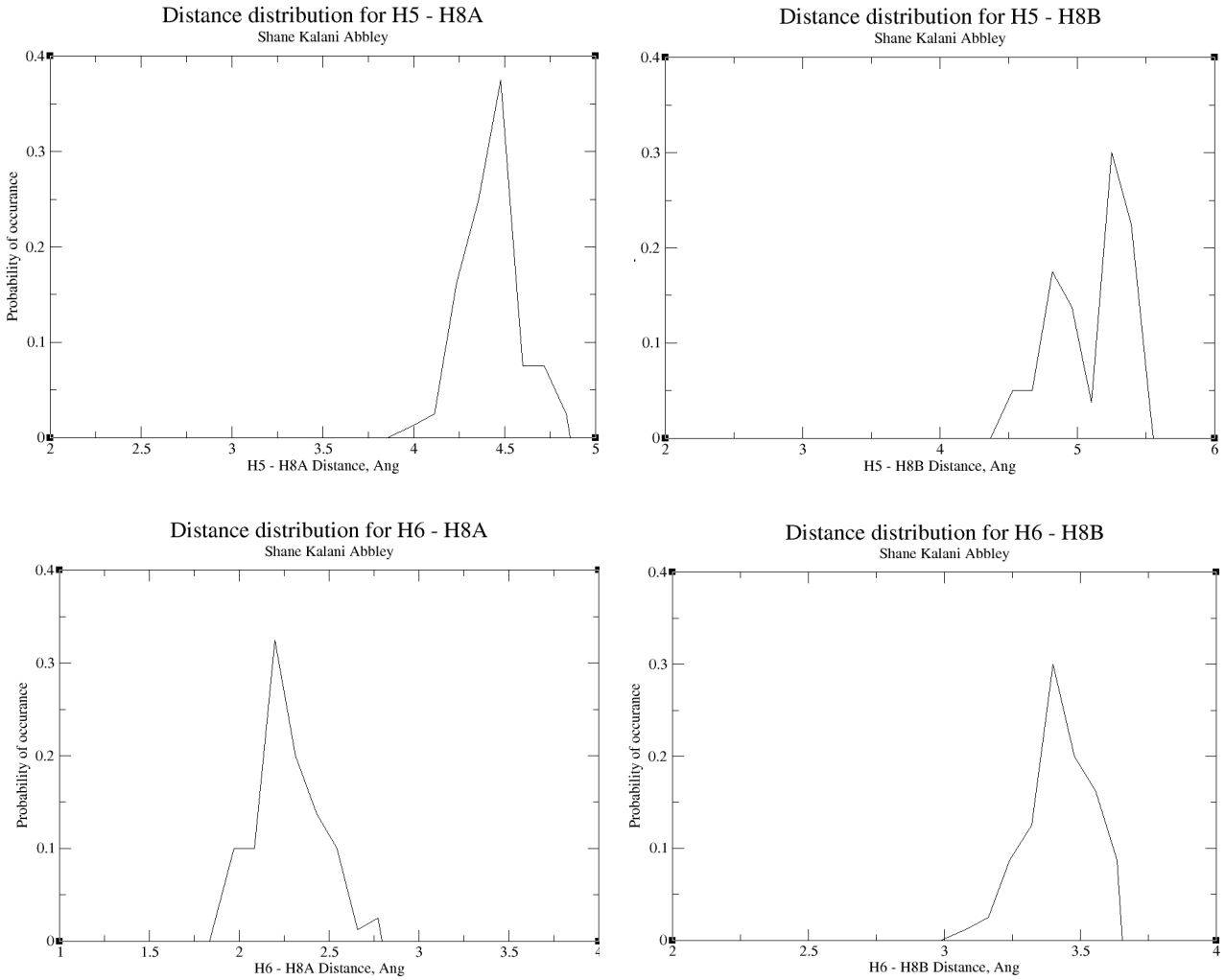


Figure 6: Distance Distributions for the Primary Amine Hydrogens
These graphs provide probabilities for various distances between the primary amine hydrogens and the ring/arm hydrogens.

Discussion:

Conformation Search for Gamma-Aminobutyric Acid:

Many of the low energy conformers in this section make logical sense with respect to their structure. For example, conformer 1 in Figure 1 highlights the stabilization caused by the intramolecular hydrogen bond between the carboxylic acid hydrogen and the nitrogen. Similarly, structures 2 and 3 have much lower energy values than any other conformers of GABA. Strangely, conformation 35 possesses this same interaction, but is one of the highest energies

sampled here. This is likely because of the sharp C-C bond angles and thus steric hindrance in the molecule. This steric hindrance is also the likely cause in the two other highest energy conformers.

In this experiment, Monte Carlo was used to generate other initial structures of GABA from the first provided structure file. This illustrated some of the possible structures of GABA that show alternative features. We could then analyze whether these features had a positive or negative effect on the favorability of the structure as a whole. Conformers 23 and 24 had nearly identical structures except for the two hydrogens on the nitrogen. Structure 23 had less overlap and was thus slightly lower in energy as our intuition would predict.

Our data collected on GABA was not sufficient to compare to literature values. That is, we can not reasonably compare our generated structure to that of legitimate research papers. This is the case for one particular reason. We did not possess sufficient sampling for our molecule. Good sampling would have every possible sample represented multiple times, whereas in our search we did not set rigorous enough collection parameters in order to provide us with representatives of each sample even once. An easy solution to this would be to increase the number of initial structures and to increase the simulation steps. This would increase the number of conformers generated and thus give us energetic information for more structures.

Monte Carlo Simulations of Allantoin:

The low energy structure of allantoin in figure 2 generated from the BOSS program has only equatorial substituents on the aromatic ring as well as linear and evenly spaced arm. This is what the expected lowest energy conformer would look like and the dihedral angles present correspond to the most probable dihedral angles described in figure 4.

Monte Carlo simulations helped to understand the structure of the molecule better by highlighting the probable ranges of dihedral angles and bond lengths. In particular, Monte Carlo simulations would allow us to predict the NMR vicinal coupling constants for H1 and H5 from the H1-N1-C5-H5 dihedral angle and for H6 and H5 from the H6-N6-C5-H5 dihedral angle. The most favorable structure shown in figure 2 is also a very practical representation of the molecule and although fairly obvious, it is nicely supported by the simulation.

Based on the results of other computational experiments performed on allantoin we can be reasonably confident in the structure of our best simulation[4]. We also did not run into the same

problem of sampling that we did earlier because we were provided with data from a complete Monte Carlo simulation of allantoin with parameters specifically designed to increase the accuracy of our generated structures. That being said, there was clearly still room for improvement. To start, it would be nice to run the simulation again in a new solvent so that we can compare it to literature results, which favored different solvents. This would require a modified input file and to rerun the entire simulation again because changing the solvent would drastically affect the interactions between allantoin and the solvent, and thus the conformation of allantoin. One other change would be to compare more bond lengths and the other two dihedral angles. This would not require a change in experimental parameters, but instead a change in data analysis.

Conclusion:

GABA was not successfully described quantitatively by our conformational search because we did not possess sufficient sampling. We did; however, acquire an approximation for the structure and energy of GABA. On the other hand, we successfully determined the conformations of allantoin using a Monte Carlo simulation. We were able to compare it to previously obtained data as well as propose a plan for improved experimental design and data analysis.

References:

1. Khan, K; Parsons, S; Bishop, J. Theory Manual: Biophysical and Analytical Laboratory. Department of Chemistry and Biochemistry University of California, Santa Barbara (2021), 12-27.
2. Khan, K. Biochemistry Laboratory Operation Manual (CHEM 125L): Conformational Analysis of Allantoin. Department of Chemistry and Biochemistry University of California, Santa Barbara (2021).
3. Khan, K. Biochemistry Laboratory Operation Manual (CHEM 125L): Monte Carlo Simulation in DMSO Solution. Department of Chemistry and Biochemistry University of California, Santa Barbara (2021).

4. Kuş, N.; Bayarı, S. H.; Fausto, R. Thermal Decomposition of Allantoin as Probed by Matrix Isolation FTIR Spectroscopy. *Tetrahedron* **2009**, *65* (47), 9719–9727.
5. gamma-Aminobutyric acid. https://pubchem.ncbi.nlm.nih.gov/compound/gamma-Aminobutyric-acid (accessed May 7, 2021).