Simulation of Secondary Structure Formation of (poly)-Glycine Using a Simple Molecular Dynamics Model.

Abstract

A simple molecular dynamics model was created to simulate polymerized glycine amino acid monomers in order to determine pathways to secondary structure formation. It was found that in the case of (15)-glycine, no substantial secondary structure formation occurred in the first 100 picoseconds and that no hydrogen bonds were formed during the molecular dynamics.

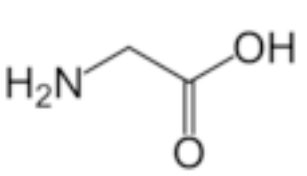
Introduction

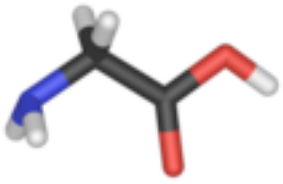
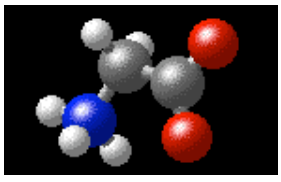
There is a great deal of interest in the chemistry of polypeptides (and the longer amino acid chain, proteins) since these macromolecules are pervasive in biological systems. The functions of polypetides are mainly determined by their individual geometric structures. Since many diseases are caused by improper polypeptide/protein folding (BSE prions, cystic fibrosis, G proteins in Parkinson's, etc.), understanding geometrical functionality of these molecules is critical for treatment.

Mechanisms for polypetide primary structure determination (amino acid sequencing) based on DNA codons is fairly well understood. However, knowledge of the amino acid sequencing does not yet translate into knowledge of biological and chemical function because the secondary and tertiary structures cannot be predicted. It is reasonable to examine the development of only secondary structures as a first approximation to total folding properties of an amino acid sequence and that is the route taken here.[1]

The most abundant protein in mammals is collagen, in which about every third amino acid monomer is glycine. Glycine is also found in large concentrations in loops, a poorly understood secondary structure of polypetides and proteins.[2] Glycine has even been detected in interstellar space supporting the theory of exogenesis.[3]

Glycine is the simplest of all the /alpha amino acids with its active group being a simple proton:



,

and


Figures 1, 2 *(from http://en.wikipedia.org/wiki/Glycine)* ***and 3*** *(from: http://www.biology.arizona.edu/biochemistry/problem_sets/aa/Glycine.html):*
Common depictions of glycine.

This means that two hydrogens will be attached to the α-carbon so that glycine lacks the isomerization found in all other α-amino acids. Of the two carbons of an amino acid that lie

along the polymerized backbone, the α-carbon is not bonded to oxygen, but to the nitrogen and an active side chain group, which for glycine is simply hydrogen.

Where most known life produces or utilizes L-isomerized amino acids (as opposed to D), glycine alone does not have this distinctive chirality because its active group is hydrogen. Therefore, computational simulations of (poly)-glycine chains have a reduced folding search space due to possible energetically equivalent ground states, and more importantly, combinatorially many more pathways by which to reach ground states.

Protein folding studies are not only intended to find stable structures, but more importantly, to find the pathway by which stable structures are achieved.[4] It may be considered the ultimate exercise in nanoscale engineering.

Secondary structures include three types of helices (alpha, 3_{10}, and π), beta sheets, loops, and more. In the short polypeptides studied here, only some kind of helix formation was sought, though the model was free to determine any final configuration.

The Molecular Dynamics Model

Atomic Coordinates

Glycine monomer atomic coordinates are entered as a 2-dimensional, flat structure with initial bond lengths matching known data. This energetically unfavorable initial arrangement is used as it is the simplest way to input polypeptides and automatically calculate bond types between first nearest neighbors, second nearest, etc (see *Figure 4*).

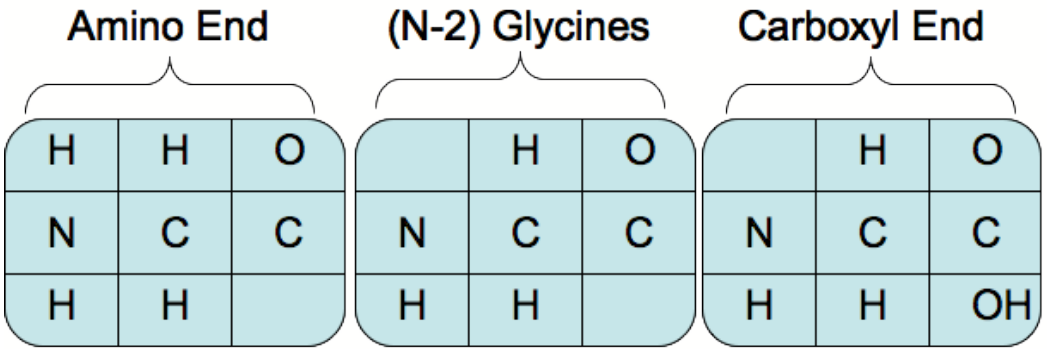


Figure 4: *Flat sequencing of input structures.*

With this input, some extraneous calculations must be done initially to move the atoms away from the flat geometry to a three dimensional structure with accurate bond angles. This occurs rapidly within the first few hundred femtoseconds of folding, and so some computational time is traded for this ease of input. Ideally, a folding program should be able to accept input data from shared international research delivered online from the Protein Data Bank (www.rcsb.org), SWISS-PROT (www.expasy.ch/sprot/) or one of many others, but this was not done here.[4]

Bonding Models

The heart of a molecular dynamics model is the choice of atomic interactions. In this study, a very simple model is desired for use on a *personal computer*. The program selected for

use is MatLab.[5] This has the feature of availability on a wide array of platforms. In fact, one may use a free versions of Octave to run the MatLab scripts on a unix-based operating system if an increase inperformance is desired.

The model developed here uses Hookeian spring bonding for nearest neighbors, but has additional features necessary to capture hydrogen bonding and bonding angles. Nearest neighbor interactions are modeled with linear restoring forces where the spring constants match measured values of the isolated bonding atoms, i.e.

$$\bar{F}(\bar{r}) = -k_{spring} \cdot \bar{r}$$

where **r** is the deviation from ideal bond length. Here and throughout this paper, **r** is the vector representing bond deviation from ideal distance for atomic pairs.

Therefore this model will not allow nearest neighbor bonds to be broken. This is desirable since it is highly unlikely that changes bonding occur without altering the primary structure of the polypeptide. The spring constant and bonding distance values used for the model are provided in *Table 1*.[6]

A	B	C	D	E	F	G		H
Atomic Endpoints [1-2]	**Mass 1 [amu]**	**Mass 2 [amu]**	**Reduced Mass [amu]**	**Reduced Mass [kg]**	**Bond Frequency [1/cm]** [6]	**k [N/cm]**	**Bond Force [N/cm = mdyne/Ang]** [6]	**Bond Length [Angstroms]** [6]
			[B]*[C] / [B+C]	**1.67E-27 * [D]**		**[E] * (2PI*3E10*[F])^2 / 100**	***as check***	
C-C	12.0110	12.0110	6.0055	9.972E-27	1854.71	12.1883	12.16	1.513
C-H	12.0110	1.0079	0.9299	1.544E-27	2858.50	4.4829	4.48	1.059
C-N	12.0110	14.0067	6.4662	1.074E-26	2068.59	16.3244	16.29	1.325
C-O	12.0110	15.9994	6.8606	1.139E-26	2169.81	19.0567	19.02	1.333
C-S	12.0110	32.0660	8.7380	1.451E-26	1285.15	8.5145	8.49	1.786
H-N	1.0079	14.0067	0.9403	1.561E-27	1926.60	2.0591	5.97	1.033
H-O	1.0079	15.9994	0.9482	1.574E-27	3737.76	7.8157	7.80	0.967
S-S (not used)	32.0660	32.0660	16.0330	2.662E-26	725.65	4.9809	4.96	2.031
H-N (hydrogenic)	1.0079	14.0067	0.9403	1.561E-27	NA	NA	0.0174	2.033

Table 1: *Parameters used in molecular dynamics.*

An unavoidable effect of the Hookeian primary bonds is the upper limit this places on time step sizes. Time steps of 1 fs generate reasonable oscillations in the nearest neighbor bonds for all systems. However, even 2 fs time steps cause wild oscillations as atoms drifted into regions of high force. Thus any attempt to model bonding with realistic parameters necessitate large computational resources.

As an example of the sensitivity of the model to time step size, examine two movies via the internet for the structural reorganization of (4)-glycine. The first movie uses a 2 fs time step and is located at http://www.physics.nau.edu/~gradweb/Leone_Home/pj/m1.html. The second movie shows smooth folding performance with a 1 fs time step at http://www.physics.nau.edu/~gradweb/Leone_Home/pj/m2.html. These movies demonstrate the key difficulty in molecular dynamics: the necessity of incredibly small time scales. Incidentally,

more accurate models require much smaller time scales on the order of attoseconds, 10^{-18} (see: http://www.gaussian.com/g_ur/k_bomd.htm).

Bond angles are often modeled with torsional forces. In order to facilitate computational speed, the number of free parameters is limited to atomic positions without the calculation of bond angles. In order to capture proper bond angles, a constant second nearest neighbor repulsion is installed that is independent of atomic separation,

$$F_{2^{nd}\ NN} = 0.04\ \frac{\text{amu} \cdot \text{Angstoms}}{\text{fs}^2}.$$

This is roughly equivalent to a 2/3 mdyne force or rather, the force between Hookeian nearest neighbors deviating from their ideal bond lengths by 4% (0.068 angstroms). This is clearly too large causing significant deviation of nearest neighbor bonding. However, this sacrifice is made to gain speed of computation and to capture accurate bonding angles.

Since nearest neighbor bonds are affected nearly uniformly in this manner, the simulated polypeptide can be considered to have simply been rescaled and so model integrity is still maintained. Of course the effect on hydrogen nearest neighbor bonds is more pronounced with about 0.1 angstrom deviations.

A smaller constant repulsion one quarter the size of the second nearest neighbor repulsion is placed on all third nearest neighbors to capture the correct bond rotational stability.

Finally, for all other atomic combinations, a linear repulsive force is initiated at distances closer than 0.5 angstroms (*Figure 5*). This prevents the model from unrealistic distant atoms overlapping. This last interaction force was witnessed to be necessary for longer polypeptide chains, but unfortunately necessitates the model to calculate interatomic distances for every atomic pair in the system thereby increasing the computational effort.

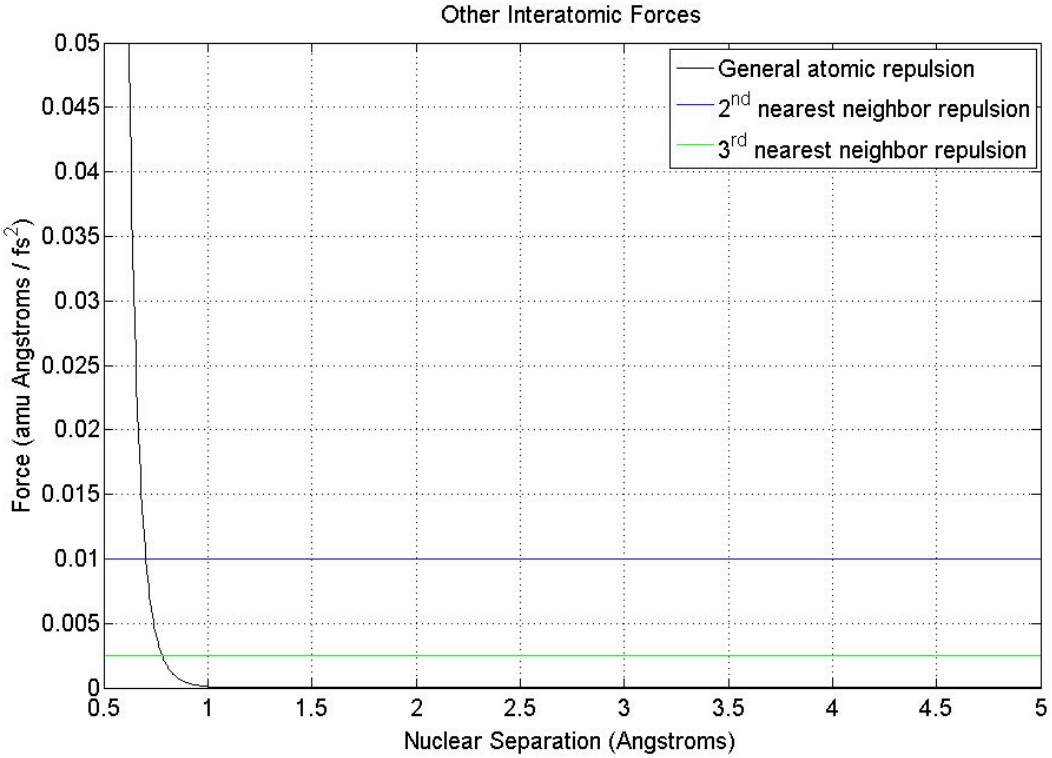


Figure 5: *Modeled nonbonding interatomic forces.*

Finally, two models for hydrogen bonding are tested, a Lennard-Jones Model and a modified Lennard-Jones model. The modified Lennard-Jones was modeled to fit experimental results showing a slight potential barrier on the path to hydrogen bond formation.[7] *Figures 6 and 7* show the model forces used along with the idealized spring forces that are generated with experimental values. These idealized linear restoring forces are the same in form to those used for first nearest neighbor bonding.

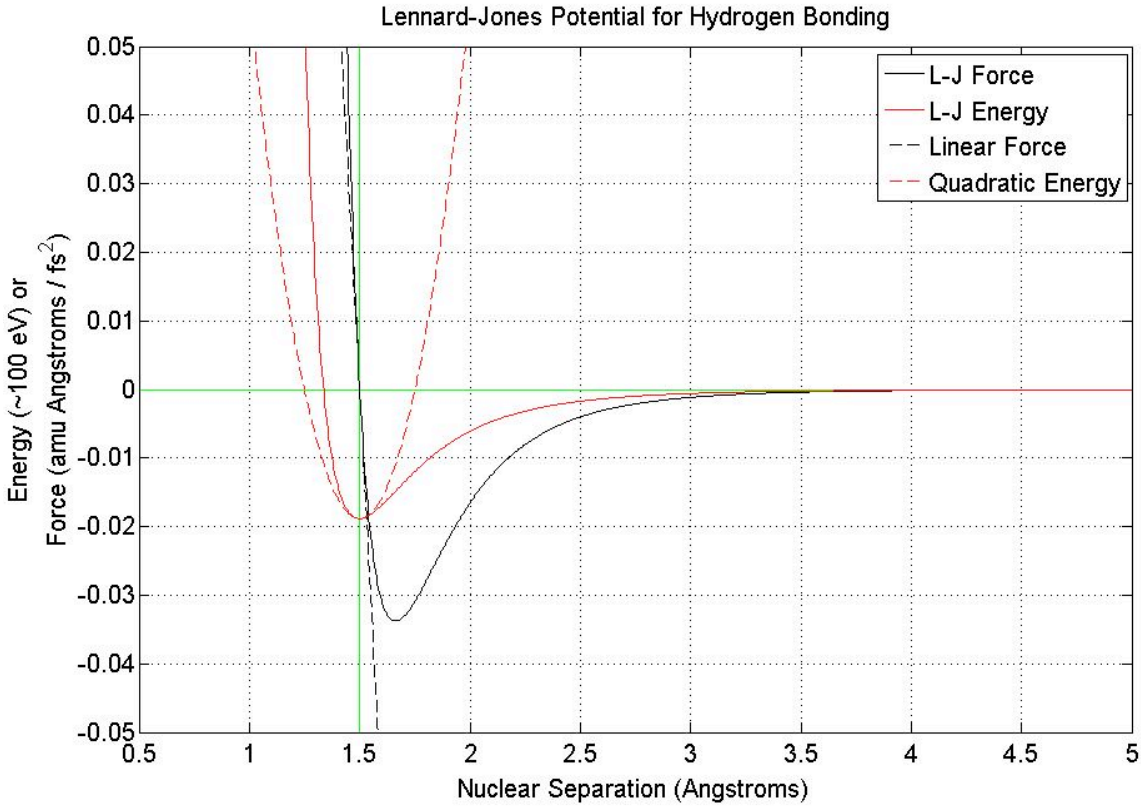


Figure 6: *Lennard-Jones potential to model hydrogen bonding.*

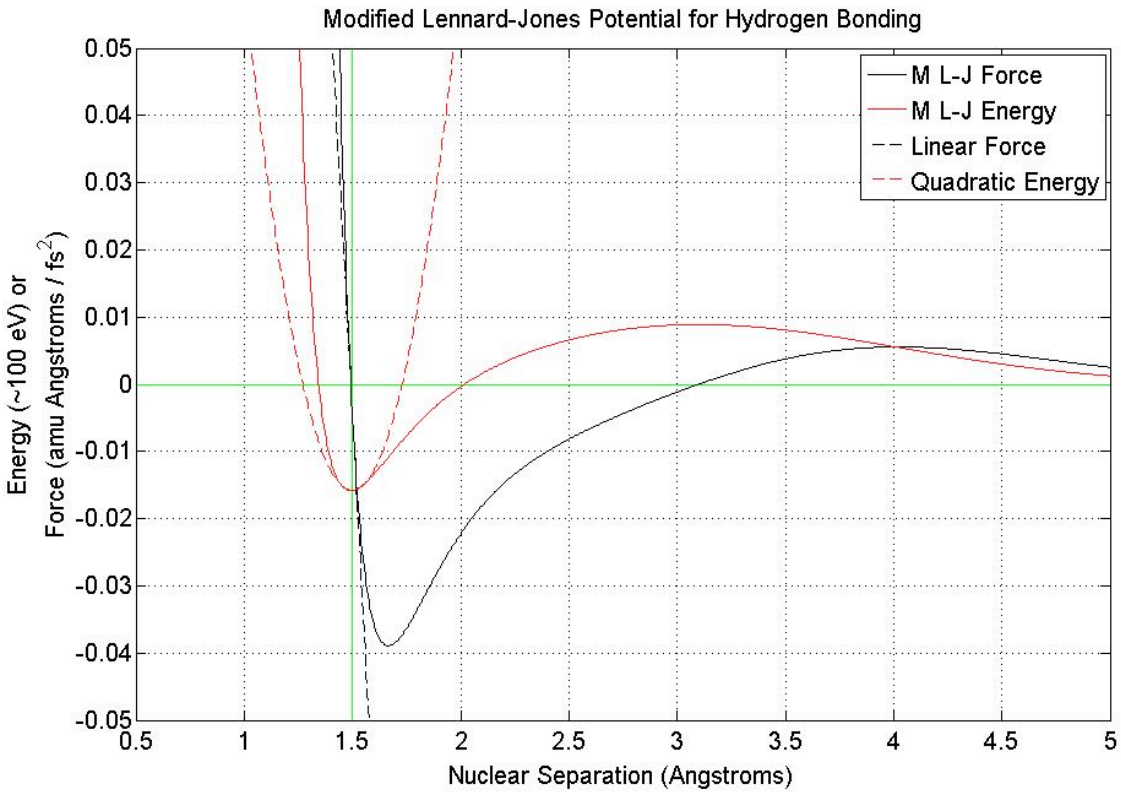


Figure 7: *Modified Lennard-Jones potential to model hydrogen bonding.*

Analysis of Units

The CRC lists force constants on the order of 10 mdyne/angstrom (= 1000 N/m). Since atomic processes occur on the angstrom scale, examination of observed vibrational frequencies shows a femtosecond time scale. Given an typical vibrational reduced mass of ~5 amu, this gives a vibrational frequency of approximately

$$f = \frac{1}{2\pi}\sqrt{\frac{k}{m}} \approx .5\text{x}10^{14}\ \text{Hz}\ \ \text{or}\ \ \omega = 3\text{x}10^{14}\ \ \text{rad/s}.$$

This is in agreement with other treatments.[8,9]

This means that velocity is measured in angstroms/fs and accelerations in angstroms/fs^2, equivalent to 10^5 m/s and 10^{20} m/s^2, respectively. This coupled with the use of atomic mass units (amu) gives forces measured in units of amu angstroms/fs^2. A reported experimental force constant of 1 mdyne/Anstrom equals 0.06024 amu/fs^2.

With a uniform time step of 1 fs, these units give an acceleration of about 0.1 to 1 times the force. In other words, the calculated numerical amounts to be added and subtracted during the simulation range from approximately 10^{-4} to 10^1 orders of magnitude. Thus the use of natural numbers is not necessary since roundoff error is insignificant in comparison to the random accelerations used every 10 time steps (described below).

Random Accelerations & Frictional Damping

A Langevin-like equation of motion was utilized to capture the dynamics of Brownian motion and damping by the solvent.[10] Specifically,

$$\ddot{r} = \frac{\left(\sum F_{\text{interaction}}\right) - \mu\dot{r}}{\text{m}} + a_{random},$$

where this equation is applied to each atom at every time step. $\Sigma F_{\text{interaction}}$ is the sum of the forces derived from the Hookeian nearest neighbor bonds, the modified or unmodified Lennard-Jones potential hydrogen bonds, and the several distant neighbor repulsive forces. The frictional damping coefficient applied to a linear velocity term is μ set to 0.1 /fs. Finally, a_{random} is a randomly generated acceleration.

This differs from the standard Langevin equation, which uses a randomly generated force to simulate Brownian motion. Both are equivalent however since the mean work is near zero over sufficient time intervals.[10] Additionally, a_{random} is only added every tenth time step to simulate molecular relaxation in the solvent and its maximum possible value is slowly decreased to simulate cooling in order to find a best ground state structure.

The random accelerations were generated by a random number between zero and an exponentially decreasing maximum value shown in *Figure 8*. This exponential decrease captures a diminishing effect of Brownian motion to simulate high initial temperatures for rapid folding followed by cooling of the solvent to freeze out any final ground state structure.

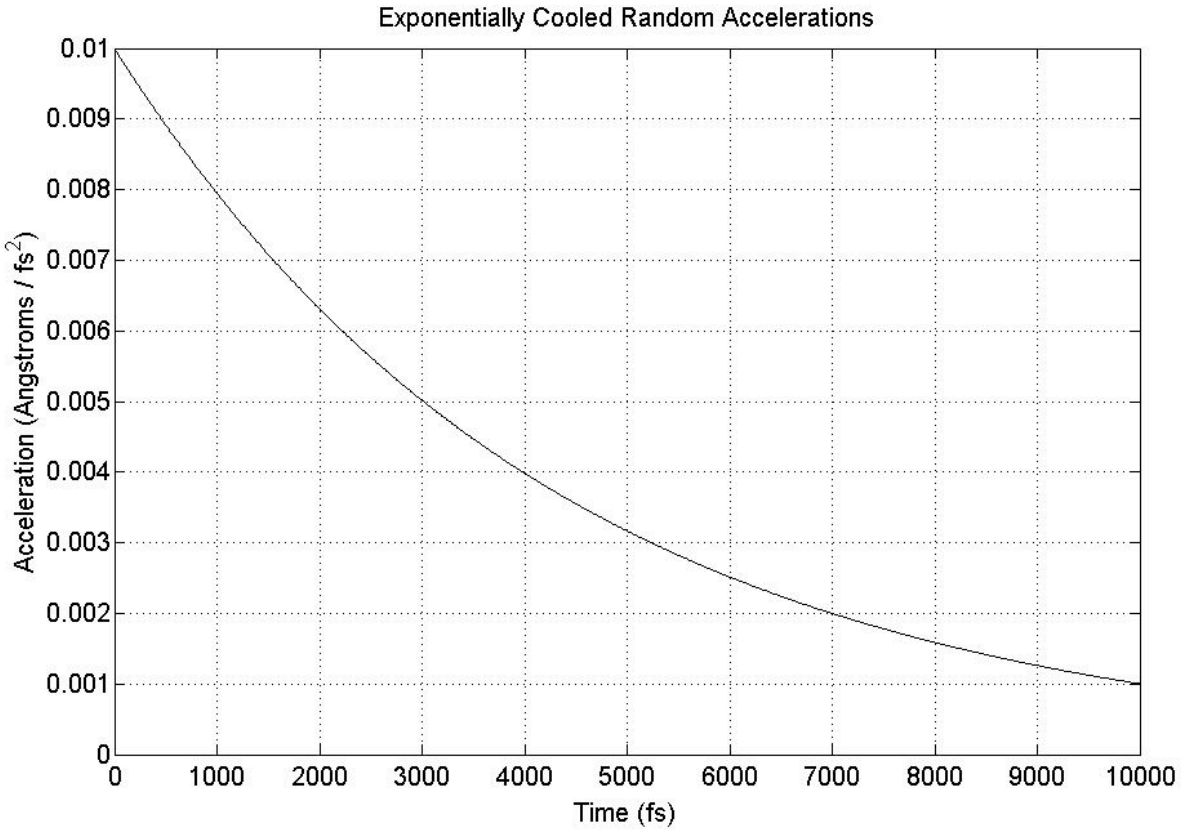


Figure 8: *Simulated Brownian motion with random accelerations.*

The maximum random acceleration is initially set to 0.01 Angstoms/fs^2. This value is chosen as it gives approximately a 10% maximum change in bond length (~0.1 angstroms) at room temperature. That is if

$$\frac{3}{2}k_{\text{Boltz}}T_{\text{emp}} = \frac{1}{2}k_{\text{spring}}(\Delta x)^2,$$

then

$$\begin{aligned}\Delta x &\approx \sqrt{\frac{3\cdot\left(1.38\text{x}10^{-23}\ \text{J/Kelvin}\right)\cdot 300\ \text{Kelvin}}{1000\ \text{N/m}}} \\ &= \sqrt{1.242\text{x}10^{-22}\ \text{J}\cdot\text{m}\cdot\text{m/N}\cdot\text{m}} \\ &= 0.1\ \text{Angstroms}\end{aligned}$$

for a spring constant of ~10 N/m at 300 Kelvin. For simulations other than 10,000 fs as shown in *Figure 8*, this function is scaled to reproduce the same endpoint values.

Another random acceleration function was attempted that multiplied the exponential term by a sinusoidal term shown in *Figure 9*. The sinusoidal oscillations model a somewhat unrealistic cooling and reheating of the polypeptide with the goal of finding any intermediate metastable structures.

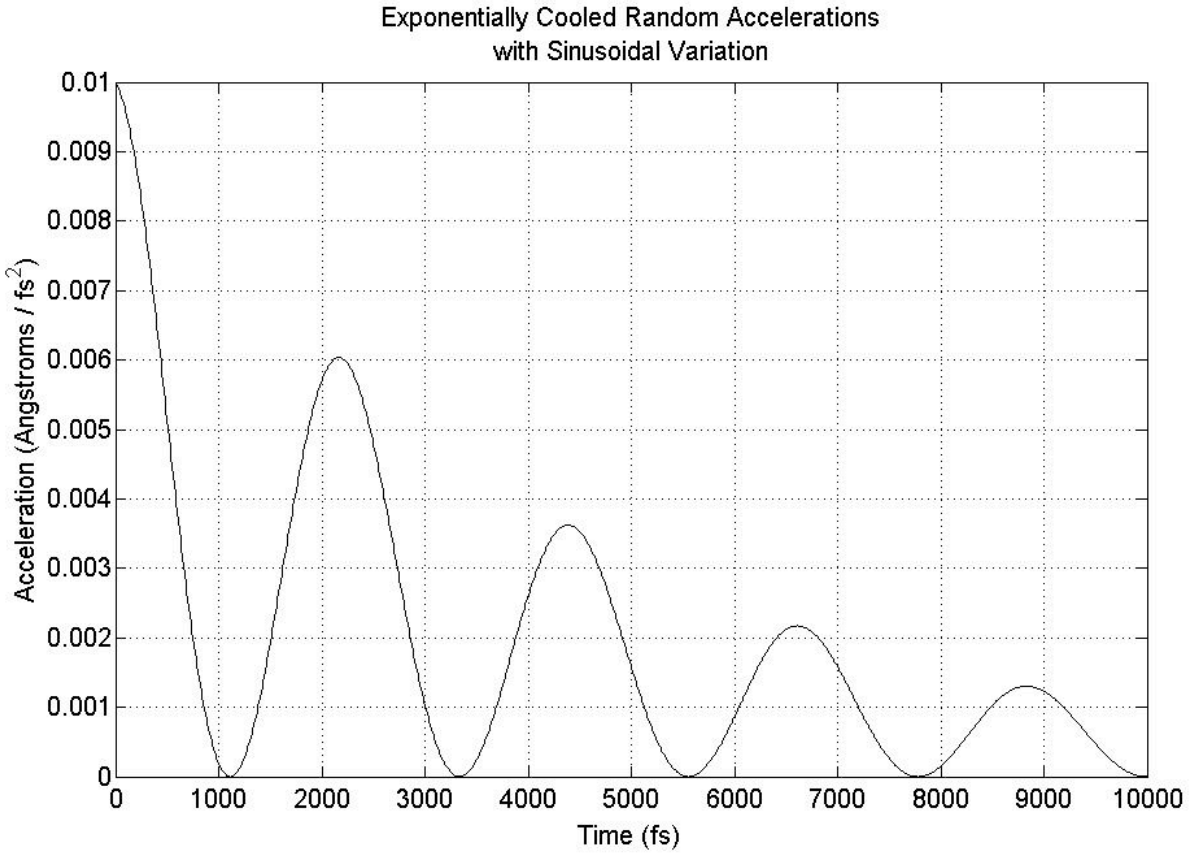


Figure 9: *Simulated Brownian motion with random accelerations.*

The use of this oscillating random acceleration term did not result in finding any intermediate metastable states was abandoned. Still, the general idea may have merit in future simulations. Perhaps metastable states could be determined by an oscillating square waveform shape for the random oscillation maximums.

Squeezing Forces

Due to the incredibly small time stepping, one simulation for (15)-glycine employed a slight attractive squeezing force applied to distant atoms to help bend the polypeptide into a more confined region in a quicker time. This force was turned off halfway through the run, but the polypeptide quickly sprung back into a more linear shape. This squeezing may have merit for future simulations as it could be said to model the physical solvent effect of hydrophobic polypeptides that is not addressed elsewhere in the model.

Dynamics

The polypeptide molecular dynamics are mathematically modeled as highly coupled system of nonlinear ordinary differential equations in time and bond separation, **r**:

$$m\ddot{r} = \left(\sum F(r)\right) - \mu\dot{r} + a_{random},$$

where this equation is applied to every atom. These equations are numerically evaluated using the Euler-Cromer method due to the voluminous oscillations about interatomic bonding.[11] Rather, once all forces and random accelerations for each atom have been determined, a new velocity for each atom is calculated by

$$\bar{v}_{next} = \bar{v}_{previous} + \left(\frac{\left[\sum \bar{F}\right] - \mu \cdot \bar{v}_{previous}}{m}\right) \cdot \Delta t.$$

This is immediately followed by the calculation of the new atomic positions with

$$\bar{p}_{next} = \bar{p}_{previous} + \bar{v}_{next} \cdot \Delta t.$$

Simulation Algorithms

Input Structure

The following sequence explains how to construct a two dimensional input file for (poly)-glycine.

1. Input the experimentally determined values for force constants, masses and bond distances in the appropriate units (as shown in *Table 1*).
2. Two dimensional atomic positions for the free amine end, free carboxyl end, and one center monomer are entered (as shown in *Figure 4*). The center monomer is duplicated to reproduce the correct total number of monomers. The atomic positions are saved as an array.
3. All bonding interaction types (1st nearest neighbor, hydrogen bonds, etc.) are computed and saved into a bonding array.

Figure 10 shows the input structure for (15)-glycine as an example.

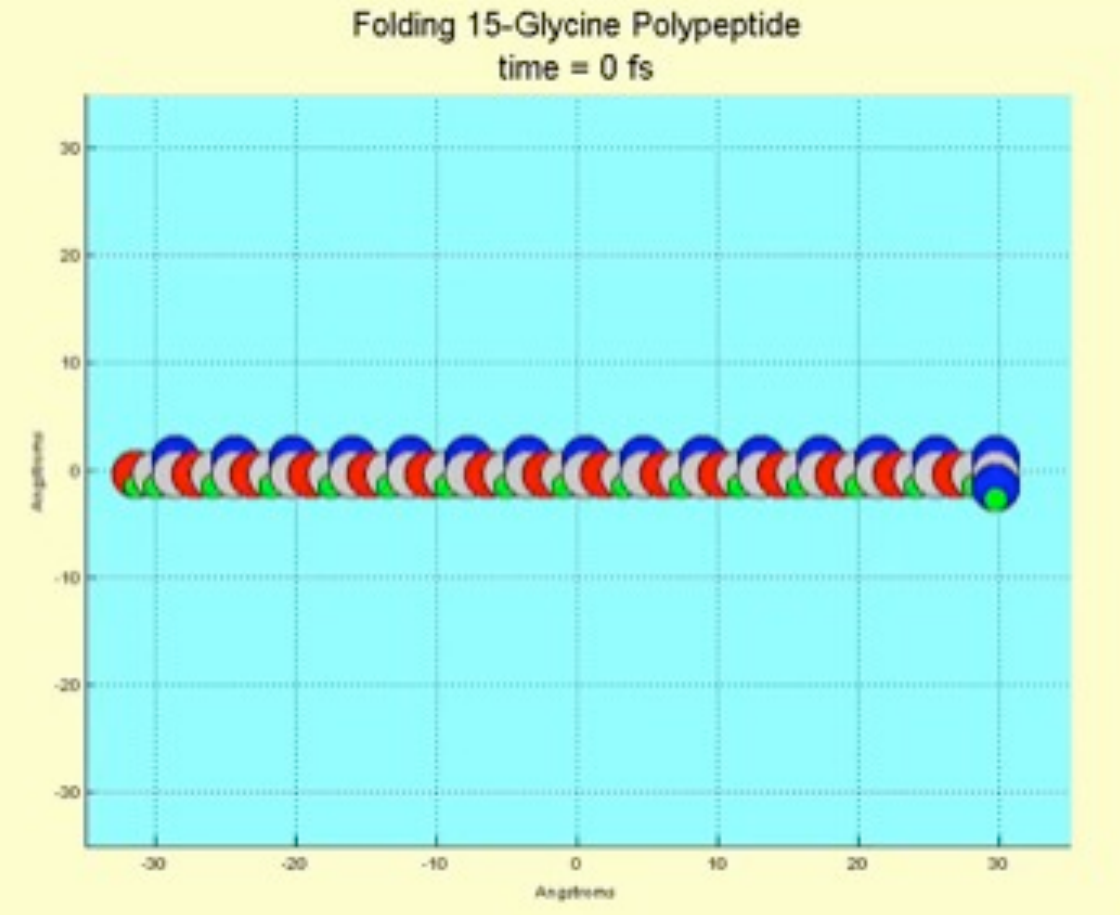


Figure 10: *Input structure for (15)-glycine.*

Molecular Dynamics

The following steps describe how the molecular dynamics simulation is conducted. Several modifications were made during testing so only the most up-to-date version is described here.

1. Load results from input program giving atomic coordinates and bonding interaction tables.
2. Select
    a. the total time of run,
    b. the time interval between recording atomic coordinates to an output file,
    c. a name for the output file,
    d. if the visualization program is to run automatically after molecular dynamics,
    e. whether or not to apply a squeezing force, and
    f. whether or not to continue folding from a previous run and if so, the file name with atomic coordinate output from a previous run.
3. Next the parameters determining the random accelerations and friction should be given.
4. The molecular dynamics calculations should be computed in the following steps
    a. Loop through all atomic pair combinations and calculate the accelerations for each atom due to interatomic forces. Here the vector connecting atomic pairs and the separation distance must be calculated.

    b. Every tenth time step (or another choice) add a random acceleration to every atom choosing the acceleration magnitude from a decreasing interval.
    c. Apply an Euler-Cromer time step to advance the atoms' positions.
    d. After the selected time interval has passed, record the atomic positions.
5. Finally, compute the distances between all nitrogen-hydrogen pairs in the polypeptide to see which if any hydrogen bonds formed in the folding process.
6. Proceed automatically to the visualization software if selected.

Visualization

The following instructions outline the program used for visualizing the polypeptide folding process. Because the result of a folding is most importantly the folding itself, a proper visualization is critical.

1. First several selections must be made. These include
    a. whether or not to read each line of the output file, i.e. to use the output time steps selected when the program was run or to expand the time interval to be viewed,
    b. whether or not to make an AVI formatted movie of the folding, the frame rate and the output file name,
    c. whether or not to plot the hydrogen bonds that may form as small red dotted lines (useful for seeing hydrogen bonds, but could make the plots a little busy),
    d. selection of plot title and axis dimensions, and
    e. if the final state of the polypeptide should be rotated so that the final structure may be viewed from many angles.
2. Import the atomic coordinate data generated by the molecular dynamics code, and measure how many atoms the polymer has (number of lines with same time stamp).
3. Loop through the time stamps (some time stamps may be skipped depending on step 1.a) completing the following for each cycle of the loop.
    a. Move the center of mass to zero for the polypeptide. This is done here rather than the molecular dynamics code for speed.
    b. Plot the bonds by drawing a black line between all 1^{st} nearest neighbors, but only to the edge of the plotted atom shell, not its center. This must be done to avoid rendering issues.
    c. If selected, plot red dotted lines for all possible hydrogen bonds to see if any form.
    d. Plot the atoms with correct colors: carbon is gray, hydrogen is green, nitrogen is red, and oxygen is blue. The colors for nitrogen and oxygen are sometimes switched in the literature. The atoms must be plotted in order of farthest to nearest to the viewer's eye in order for the rendering to be correct.
    e. If a movie is to be made, create a frame.
4. If the rotation mode has been selected, rotate the actual atomic coordinates through rotation matrix multiplication and make movie frames if selected.
5. If selected, make the movie using the *movie2avi* function.

Key Findings

The primary achievement of this simulation project was to model the folding of (15)-glycine over 100,000 single femtosecond time steps (.1 ns). The result is reported at http://www.physics.nau.edu/~gradweb/Leone_Home/pj/m3.html. This simulation shows no significant primary structure formation. Here all potential nitride hydrogen-oxide hydrogen bonds are shown with dotted red lines so that as one examines the folding they may examine any hydrogen bond formation.

The simulation of (15)-glycine shows that the first 5,000 fs yield only minor backbone oscillations, waves of a sort with amplitude about 2-3 angstroms as can be seen in *Figure 11*. After this until about 8,000 fs, the oscillations become larger at about 5-6 angstroms shown in *Figure 12*.

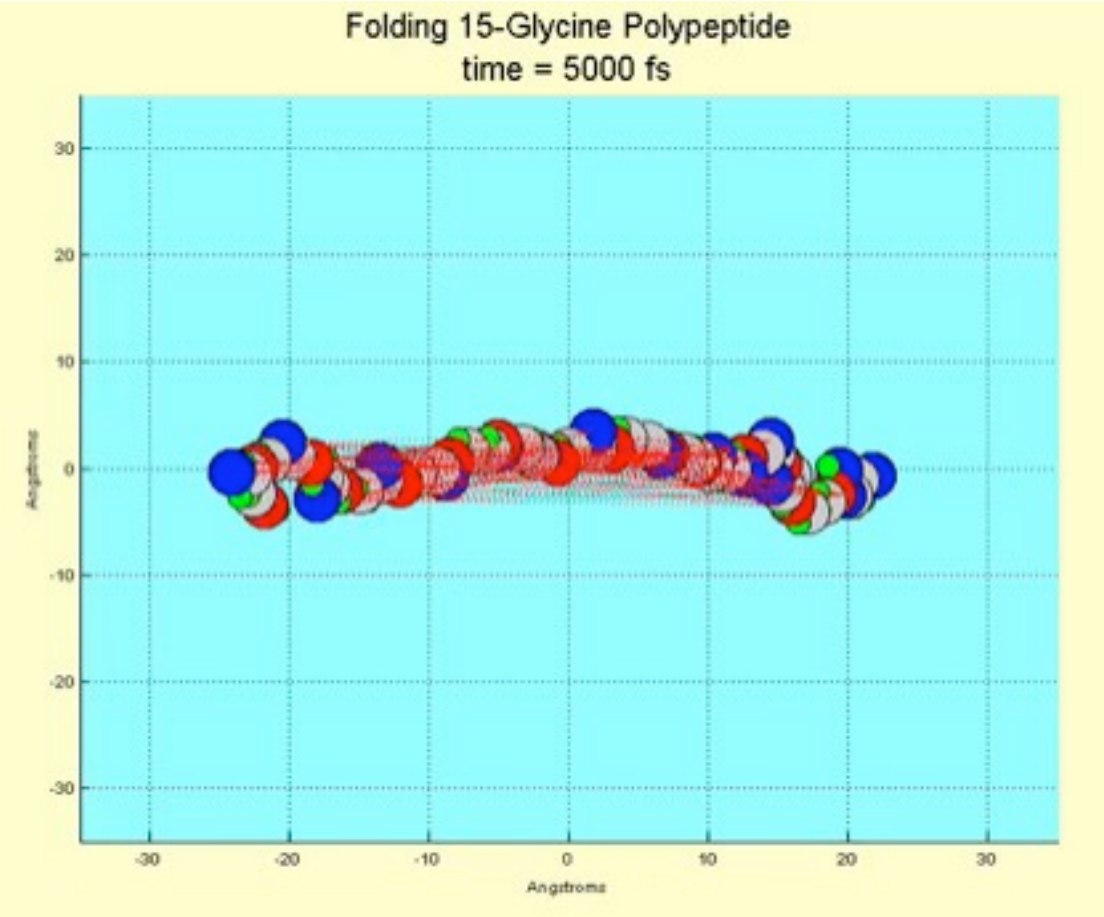


Figure 11: *(15)-glycine shows only minor backbone distortion after 5 ps.*

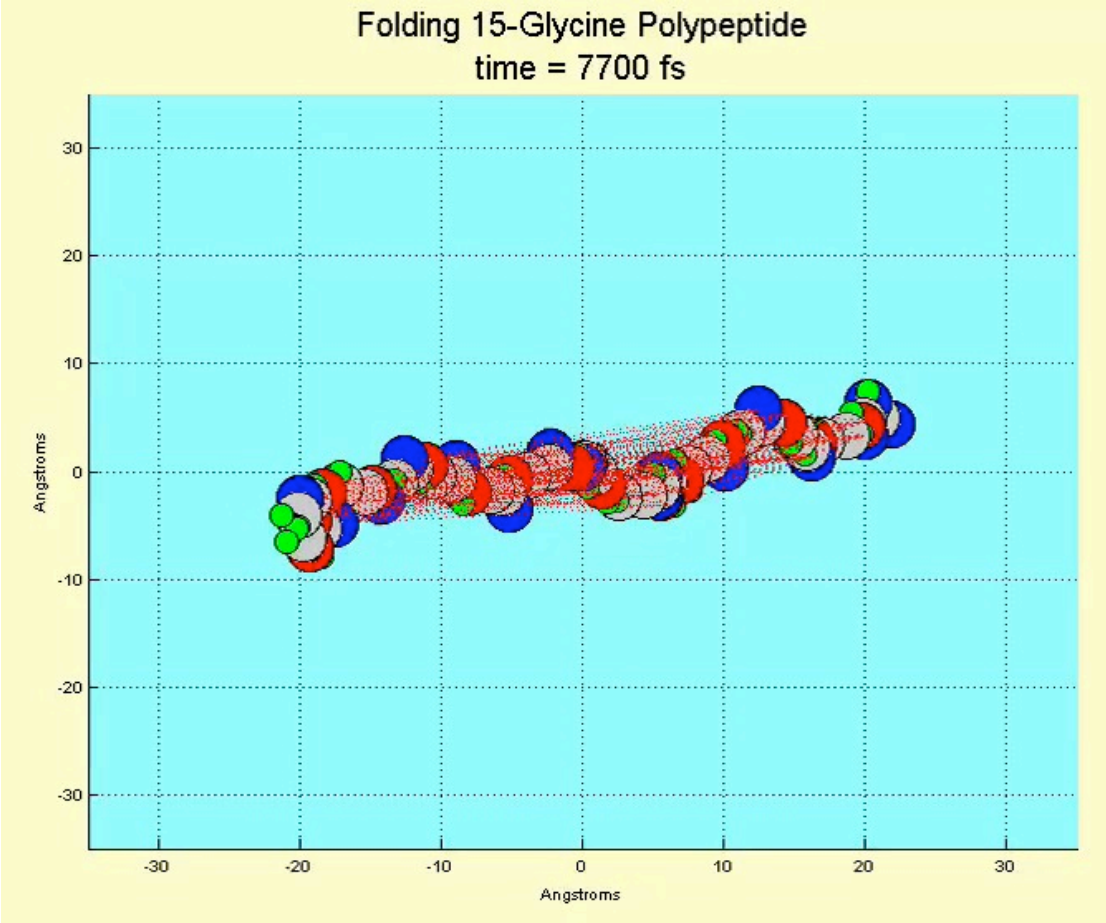


Figure 12: *(15)-glycine has larger backbone distortions from 5-8 ps.*

The is an abrupt change in behavior at after about 8,000 fs where the polypeptide experiences large, 10 anstrom backbone distortions and some end-of-chain folding (*Figure 13*). This continues until about 18,000 fs when some significant clumping begins to appear on part of the chain. This clumping shows the backbone chain folding into an S configuration that is not reported as a usual secondary structure formation as shown in *Figure 14*. It may be that it is a legitimate intermediate state during folding, or it may be an artifact of the simplistic model wherein the simulated polypeptide achieves higher energy states not reached in nature.

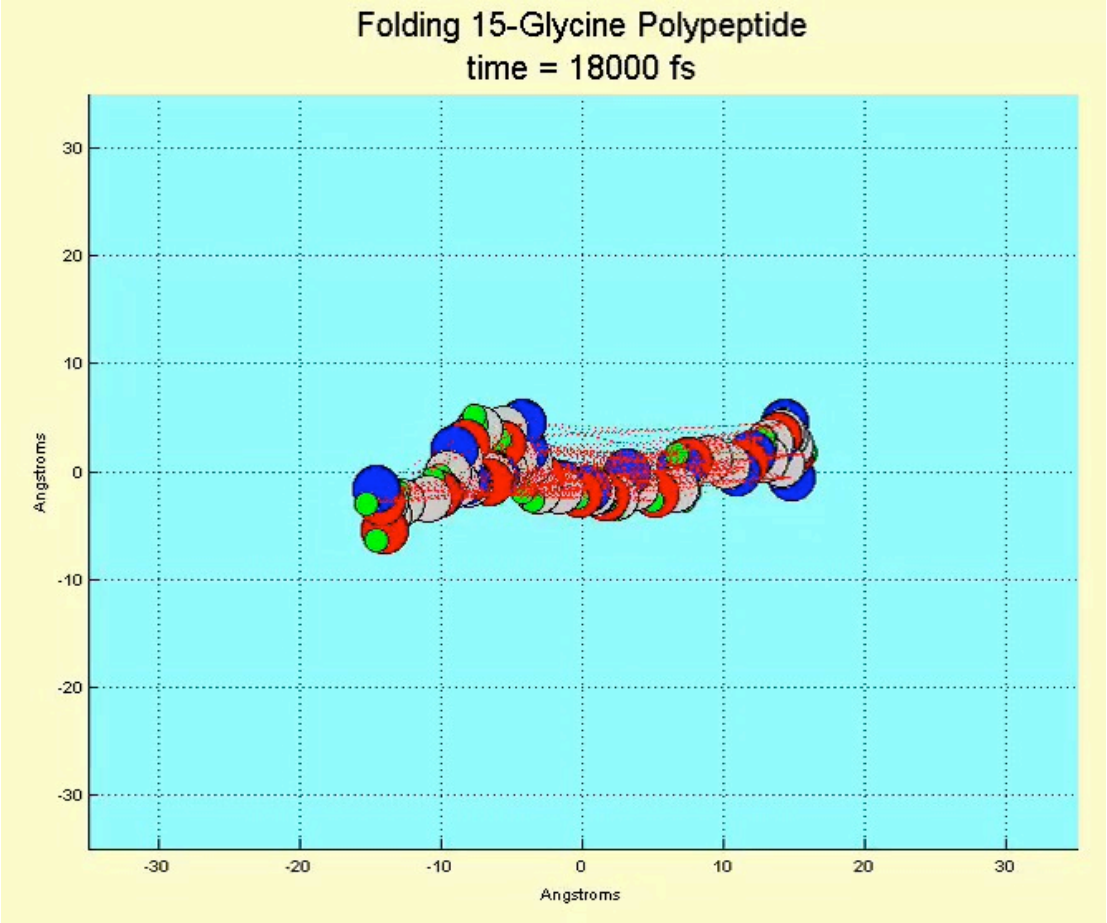


Figure 13: *(15)-glycine has severe backbone distortions and end-of-chain folding from 8-18 ps.*

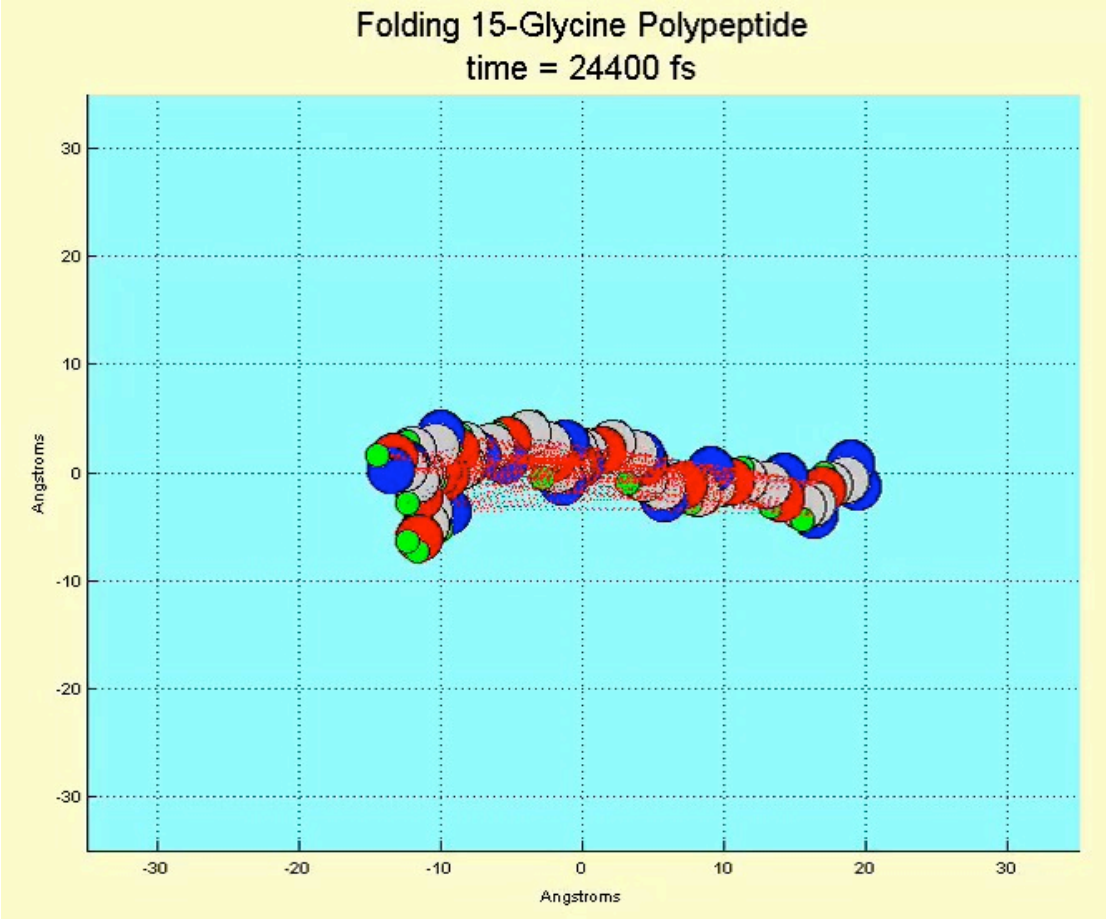


Figure 14: *(15)-glycine shows clumping on part of the chain from 8-24 ps.*

Significant end-of-chain folding is then seen from 24,000 to 40,000 fs. Since the ends of the polypeptide have the most freedom of motion, it is surprising that this was not seen until a later stage. The random accelerations follow exponential cooling and so have already dropped in maximum value by 20% at this point (*Figure 8*). This may have caused some of the backbone oscillation to pass through a critical phase so that waves do not traverse the backbone as easily though this is difficult to confirm from the results (i.e. backbone wave propagation has not been quantified). For whatever reason, the ends seem to travel more at this stage (*Figure 15*).

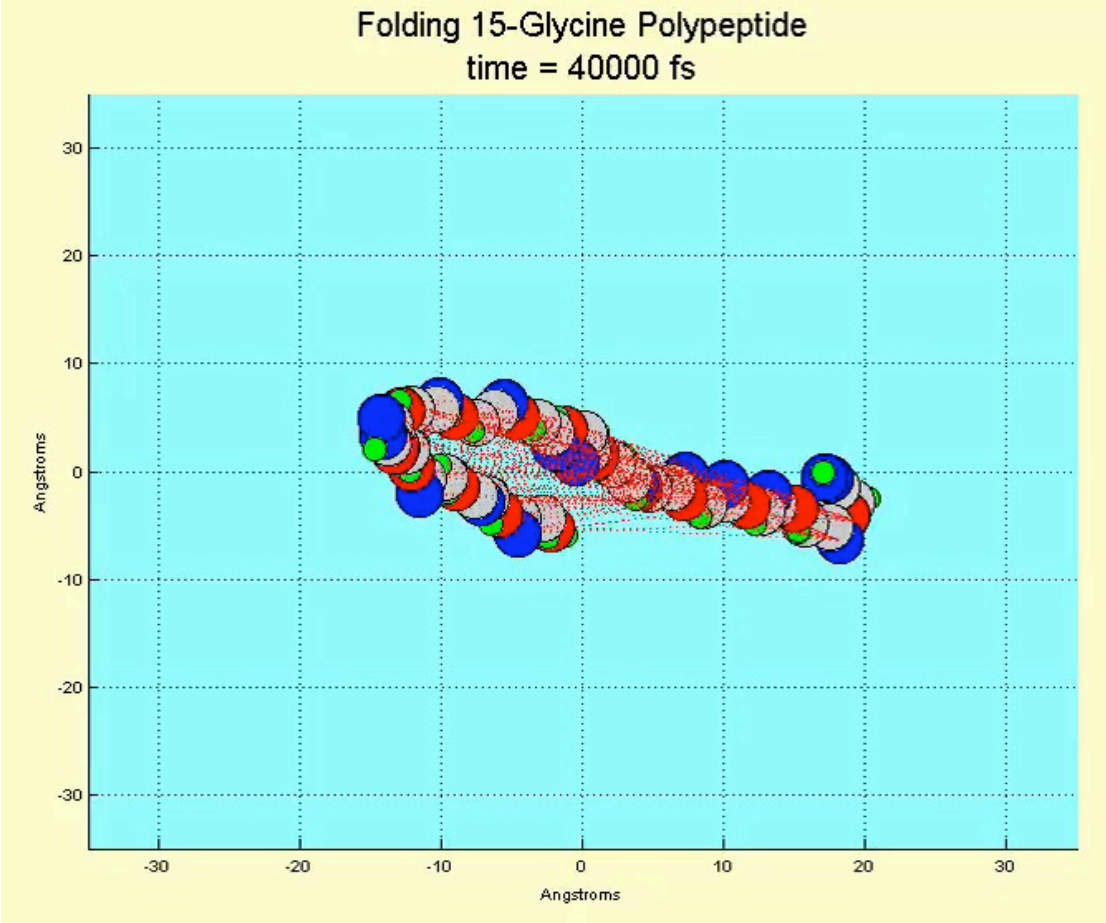


Figure 15: *(15)-glycine chain ends travel more from 24-40 ps.*

From 40,000 fs and forward, the simulated polypeptide experiences minor contracting but no other major structural changes. By the beginning of this time interval, the random accelerations have decreased by 60% in maximum value. Either due to this cooling or perhaps because the structure is trapped in a local minimum, it appears to have reached some metastable configuration (*Figure 16*).

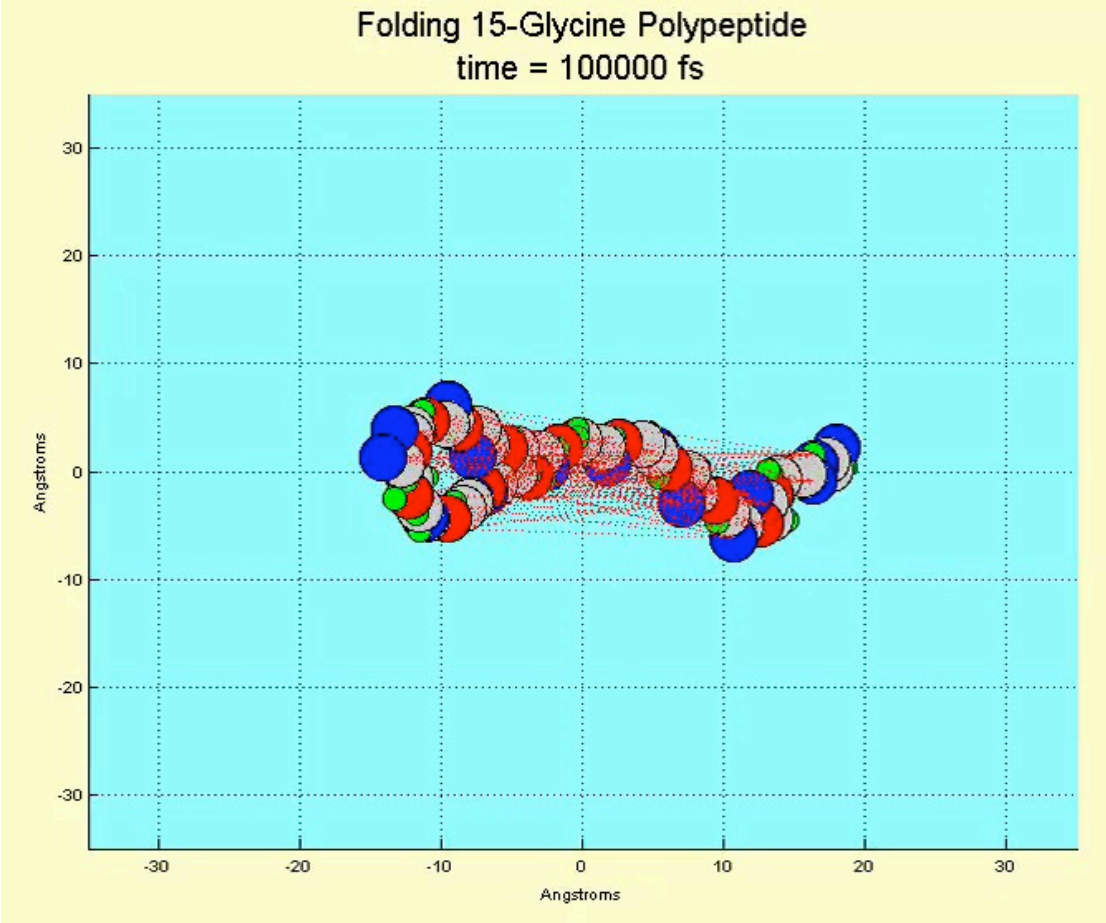


Figure 16: *Final simulated configuration of (15)-glycine.*

In this study, it was assumed that due to the lack of active side chains on the glycine amino acid, any secondary structure formation would solely be due to the formation of hydrogen bonds. *Figure 17* shows that the final simulated structure has only two candidates for hydrogen bond formation. All other nitride hydrogen-oxide possibilities are separated by more than 5 angstroms. The yellow bond candidate seen on the left side of the figure has a hydrogen-oxygen separation of 4.4 angstroms while the one on the right has a separation of 3.1 angstroms. As is seen in *Figure 6* however, these distances are too great to be considered hydrogen bonded with a Lennard-Jones interaction potential. This lack of hydrogen bonding may explain why (15)-glycine does not demonstrate significant secondary structure formation.

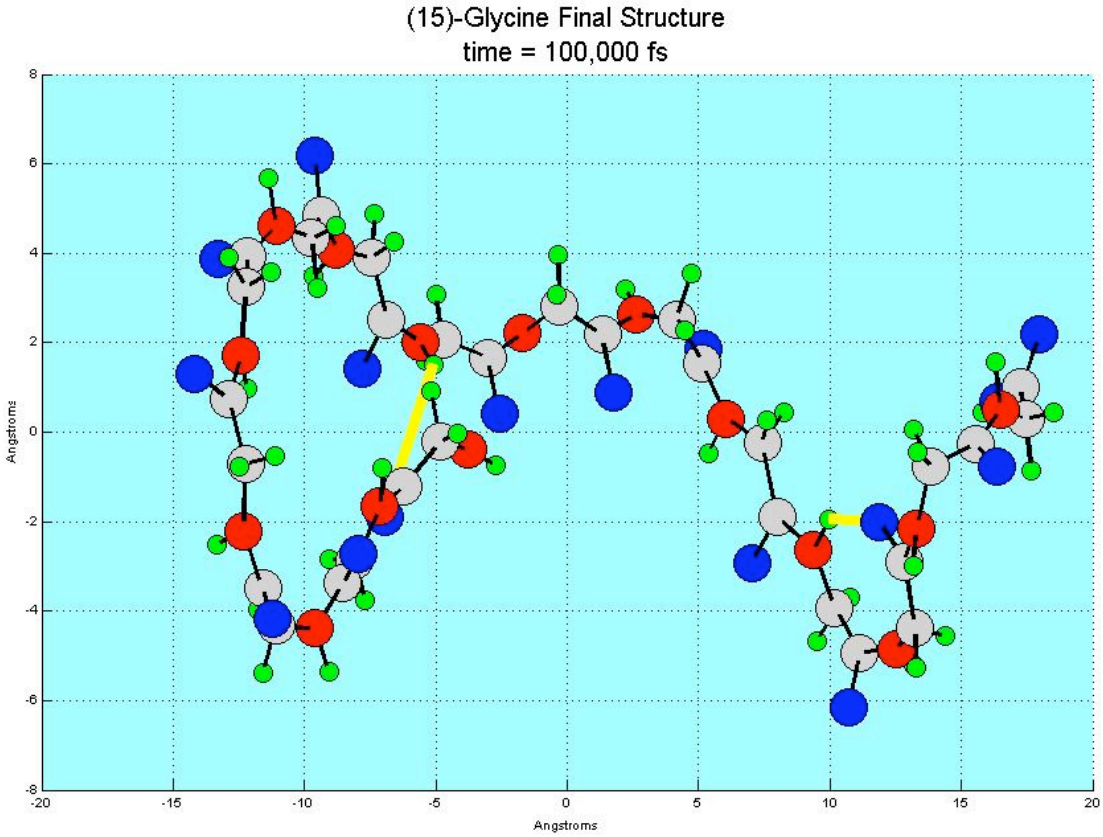


Figure 17: *Nitride hydrogen-oxide hydrogen bonding candidates.*

The length of time needed to search through substates may be on the order of 10^{-7} s, 1,000 times longer than was simulated here.[12] Such a long time scale requires much faster computing ability not typically available on a personal computer, and may account in part for the lack of significant folding observed.

The radius of gyration of the final structure was found to be 11.28 angstroms and the mean backbone separation of the ends was 8.74 angstroms. Were this an ideal Gaussian chain, these two distances would be nearly equal.[10] Instead, the simulated structure appears to be more folded than a random walking chain.

The glycine amino acid is typically found to break helix formation, and in fact a previous computational study of the thermodynamic stability of (10)-Glycine showed a hydrogen bonded structure (i.e. folded) as energetically unfavorable.[13] Another Monte-Carlo investigation was unable to observe stable secondary structure in (20)-glycine.[14] Qualitative determination of metastable states was made by viewing the output folding visualization. This proved to be too difficult to achieve reliable information. Energetics calculations although possible with minor reprogramming were not made in this study. In retrospect, this would have been an ideal method for searching for metastable states, although based upon the current results it appears that much more simulation time would be required to make this a worthwhile investigation.

Other Results and Testing

A molecular dynamics simulation was carried out for ethanol to see if bond angle and rotational configuration energy minimization was achieved through the use of 2nd and 3rd nearest neighbor repulsions. This successful trial is shown at http://www.physics.nau.edu/~gradweb/Leone_Home/pj/m4.html. Another trial was conducted with (3)-glycine that successfully shows the molecular dynamics code working correctly (at http://www.physics.nau.edu/~gradweb/Leone_Home/pj/m5.html).

Before a simulation of (15)-glycine was attempted, a 100,000 fs simulation of (5)-glycine was performed. This simulation utilized the sinusoidally decreasing maximum random acceleration shown in *Figure 9*. The results of this simulation are interesting because the final configuration contains a folded end similar to that found in the simulation of (15)-glycine. This is reported at http://www.physics.nau.edu/~gradweb/Leone_Home/pj/m6.html. The sinusoidally decreasing random accelerations did not aid in identifying intermediate metastable configurations.

Conclusion

Glycine is the simplest of amino acids to simulate due to its unique structure. However, because of its propensity to for loops rather than helices in nature, it may also poison any attempt to simulate primary structure formation. However, as an amino acid for polymerization in nanoscale engineering, this trait may be desirable for certain applications.

The simple model constructed for simulation of (15)-glycine does show some folding properties, but not due to hydrogen bonding as expected. The final configuration appears to fold more than the ideal Gaussian chain of a random walker, and even more than a self-avoiding random walk polymer chain.[15] Thus this model appears to conform to known properties of simulated (poly)-glycine that as a short polymer it does not form secondary structures. It also supports the biochemical understanding that in nature glycine monomers prevent helix formation.[16]

References

[1] J. K. Myers, C. N. Page and J. M. Scholtz, Proc. Natl. Acad. Sci. USA, **94**, 2833-2837 (1997).

[2] A. D. Nagi and L. Regan, Folding & Design, **2** 1 67-75 (1997).

[3] Y-J. Kuan, S. B. Charnley, H-C. Huang, W-L. Tseng, and Z. Kisiel, The Astronphysical J., **593** 848-867 (2003).

[4] A. M. Lesk, *Introduction to Protein Architecture*, (Oxford Univ. Press, NY, 2001) pp.23, 46, 50-51.

[5] Matlab v.7.1.0.21 (R14) Service Pack 3, August 02, 2005. The MathWorks, Inc.

[6] D. R. Lide, Ed., *Handbook of Chemistry and Physics, 81st Ed.*, (CRC Press, Boca Raton, 2000) pp.9.2-10, 9.76, 9.82-85.

[7] M. J. Sippl, J. Mol. Biol., **260** 644-648 (1996).

[8] I. G. Main, *Vibration and Waves in Physics, 3rd Ed.*, (Cambridge Univ. Press, Cambridge, 1993) p.28.

[9] R. A. Serway, C. J. Moses and C. A. Moyer, *Modern Physics, 3rd Ed.*, (Thomson Brooks-Cole, Belmont, 2005) p.382.

[10] M. Daune, *Molecular Biophysics: Structures in Motion*, (Oxford Univ. Press, NY, 1999) pp.11,165-166.
[11] N. J. Giordano and H. Nakanishi, *Computational Physics, 2nd Ed.,* (Pearson Prentice Hall, Upper Saddle River, NJ, 2006) p.152
[12] J. A. McCammon and S. C. Harvey, *Dynamics of Proteins and Nucleic Acids*, (Cambridge Univ. Press, NY, 1987) p.116.
[13] F. Pichierri, Riken Review **40** 51-52 (Oct. 2001).
[14] D. Shental-Bechor, S. Kirca, N. Ben-Tal, and T. Haliloglu, Biophysical J. **88** 2391-2402 (2005).
[15] E. A. Rietman, *Molecular Engineering of Nanosystems*, (Springer-Verlag, NY, 2001) p.192.
[16] G. C. Barret and D. T. Elmore, *Amino Acids and Peptides*, (Cambridge Univ. Press, NY, 1998) p.22.