Heating and Cooling Strategy to Improve Estimates of CYFIP1p-Derived Peptides' Helicity Probabilities in Biased Molecular Dynamics Simulations

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Summary of Progress

In computational biophysics, it is important to sample a broad population of conformations of a given molecule in order to understand the behavior of the molecule. While computational models of molecular interactions are very advanced, it may take longer than 12 hours for a simulation that is only 100 ns to run on a GPU. How do we enable the molecule to take on a high range of likely conformations in simulations lasting days or weeks, rather than months?

This is a challenge we ran into while working to estimate the probability of helicity of the eIF4E binding region of CYFIP1p, the peptide involved in Fragile X Syndrome and implicated in cancers. It is important to estimate the probability of helicity of this binding region, since it only binds eIF4E while in a helical conformation. When bound to both eIF4E and FMRP, it forms an mRNA translation inhibition complex that limits protein synthesis and cellular proliferation to healthy levels.

Since the binding region of CYFIP1p can be used as a model for a precursor molecule for the development of a drug to treat Fragile X syndrome and cancer, we wanted to calculate the probability of finding the peptide corresponding to this binding region in a helical conformation. Furthermore, we were looking to expand on a project previously done by Megan Wang [1,2], under mentorship of Dr. Gallicchio. They started to examine several modified peptides, similar to the wild type, but likely to exhibit greater helicity. One of these peptides was named Peptide 17 and differed only by an alanine in place of a serine residue.

Thus, the following peptide, corresponding to the binding region of CYPIF1, was our Wild Type Peptide:

LLDKRLRSECKNQ (Amino acids 666 to 678 of CYPIF1)

The following was our Peptide 17:

LLDKRLRAECKNQ (Amino acids 666 to 678 of CYPIF1)

We examined/ manipulated the following 5 bonds, at the listed positions of the CYPIF1 binding region:

L 667 – L 671

D 668 – R 672
K 669 – S 673 (A 673 in Peptide 17)
R 670 – E 674
L 671 – C 675

Wang also previously measured the probability of helicity starting from a bent conformation of the Wild Type Peptide using a molecular simulation run using Desmond and OpenMM and a biasing potential, according to the 4-2 Lennard Jones potential formula [2]:

$4\varepsilon(\sigma/r)^2[(\sigma/r)^2-1]$

ε is the potential well depth which tunes the bias force, σ is the Van Der Waals radius between particles, and r is the distance between particles' nuclei, applied to selected backbone hydrogen bonding contact in the helical conformation.

This form of biasing/ umbrella sampling increases the probability of occurrence of helical conformations and allows for a range of samples to be collected within days that might otherwise take months given their low probabilities (i.e. less than 1% of the time). The results of the biased simulations were unbiased by means of the thermodynamic re-weighting formula (also known as the "umbrella sampling formula"):

$$p_{unbiased} = \frac{\langle \theta(x) e^{U_{bias}(x)} \rangle}{\langle e^{U_{bias}(x)} \rangle}$$

$\theta(x)$and $U_{bias}(x)$ are the helical indicator function and biasing potential energy, respectively, of conformation x.

Our goal was to likewise use biasing to compare the probabilities of helicity of the Wild Type Peptide and Peptide 17, but we first needed a method to measure probability values for each peptide precisely. Because we were dealing with a structure of infrequent helicity, we needed to ensure that the probability estimate did not depend on the initial conformation. Wang's estimate of the Wild Type's helicity at 6.25 kJ/mol bias force starting from a random bent conformation was only 0.18% helicity after unbiasing.

To test this further, we ran joint simulations for both of our peptides, starting from both helical and bent conformations (four conditions total), using the convergence of these simulations as a guide as to whether we were getting precise probabilities of helicity. First, we extended Wang's simulation time from 150 ns to a total of 1.5 ms (for a series of 10 simulations at 150 ns each) for all four conditions, and used the last nine simulations for helicity estimates. This resulted in possible convergence of Wild Type Bent/ Helical at 0.2 – 0.3% helicity, which did correspond to Wang's estimate. However, our Peptide 17/ Helical had about an order of magnitude higher probability of helicity than Peptide 17/ Bent, at 3% vs. 0.3%.

Our first concern was that our bias force may be too great for Peptide 17, since biased probability of helicity was about 50% for Peptide 17/ Helical. However, lowering the bias force to 5 kJ/ mol resulted in similar probability values for Peptide 17/ Helical and Bent, and still no convergence.

Next, we chose to alternate temperatures in order to help our peptides fully break free from their initial conformations. Thus, we performed a series of simulations at 6.25 kJ/mol bias force, where we alternated temperatures between 400 K and 300 K for succeeding simulations of 15 ns each and collecting data only at 300 K. The total run time was still 1.5 ms, and we sampled our values once biased probabilities of helicity appeared to begin converging: after the first 0.3 ms. We did observe convergence this time, at a probable helicity of about 0.04 – 0.05%, which was about 5 times less than our prior helicity for the Wild Type Peptide.

Then the question arose: could we trust our prior values for the WT? Was it possible that our starting conformation just happened to find a highly helical conformation early on through random chance? Performing the same heating/cooling experiment seemed like a good way to continuously disrupt its structure and get a more accurate estimate of helicity.

We repeated the heating cooling/ experiment with Wild Type and saw that it still showed possible convergence in these simulations, but now at values of 0.1 – 0.2% vs. 0.2 – 0.3% initially. We intend to do statistical analysis of this data to better understand its significance.

Our results so far suggest that alternating heating and cooling is an effective way to help peptides break out of their initial conformations, and form/ fully break alpha helices at a reasonable rate over 1 ms. In these simulations with Wild Type and Peptide 17, all of our conformations both formed and completely broke helicity (continued at 0% helicity for at least 15 ns) at least twice per microsecond.

Our next step is to perform statistical analysis on our results so far and to test the possible convergence we have seen through our new heating/ cooling method. We will start with simple analysis methods like adding error bars based on standard deviations to our plots and possibly doing block averaging. Then, we will analyze our data further through a combination of backward plots and correlation function analysis. If statistical analysis confirms convergence for heating/ cooling results, this will indicate that we can rely on our ΔG (penalty) values, and we can then start on simulations to estimate our ΔG (excess free energy of binding) of CYFIP1-derived peptides binding to EIF4E (see Figure i).

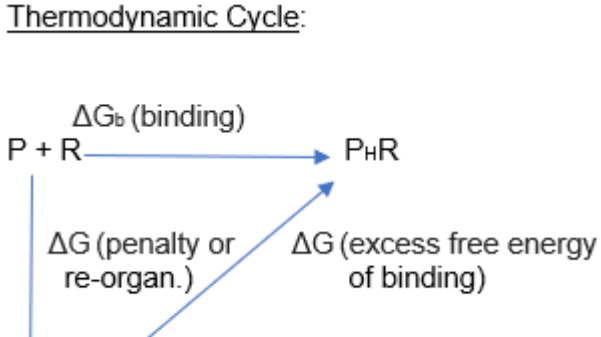


Figure i. Our experiments so far have aimed at determining ΔG (penalty) of the binding region of CYFIP1p. Further

experiments can be done to calculate ΔG (excess free energy of binding) to EIF4E to then sum up to the final value of ΔG (binding). P refers to the CYFIP-1 derived peptide, P_H to the peptide in helical form, R to EIF4E and P_HR to the helical peptide bound to EIF4E.

Methods

We ran simulations on the Wild Type Peptide and Peptide 17 listed in Table 1 of Methods of Wang's "Possible Computational Evidence…" [1] and set up the bent and helical conformation for each in Desmond in Maestro 2013-3/ Schrödinger, in the same way as described by Wang. The exception was that while we used about 7,000 SPC like Wang as our solution in our starting bent conformations, we only used about 5,500 SPC in our starting helical conformations. This was because we used the "Buffer" box size calculation method in System Builder, along with the "Minimize Volume" option, and the sizes of these systems were created automatically.

Like Wang, we applied a bias force to our peptides using OpenMM version 7.2, to the same bond pairs (listed in the Introduction), and required the middle three of our five bonding pairs to have a bond length of 2.5 Å or less in order to consider a conformation helical, measured with VMD (see Figure ii for an example). We also used the same two python scripts as those attached to her "Use of Umbrella Sampling…" [2] in order to run and analyze our simulations (while varying parameters), and performed most of our experiments at the bias force determined to be optimal by Wang: 6.25 kJ/mol.

Wang's experiment was done on the Wild Type Peptide in the bent conformation at 6.25 kJ/mol for 150 ns and temperature of 300 K, and we did this as part of Experiment 1, for both the WT Peptide and Peptide 17, each for both the bent and helical starting conformations. We also expanded this experiment into a series of 10 simulations under these conditions for each of the four cases, for a total run time of 1.5 ms for each series. Then, we performed Experiment 3 the same as Experiment 2 on Peptide 17, but lowered our bias force to 5 kJ/ mol.

In Experiment 3, we performed a series of 100 simulations on Peptide 17 Bent/ Helical at 15 nm each, a bias force of 6.25 kJ/ mol, 400 K for each odd numbered simulations, and 300 K for each even numbered simulation. The total run time was 1.5 ms.

As our simulations completed, we started sampling our results only at even numbered/ 300 K simulations, since we were only concerned with the behavior of our peptides at temperatures relatively close to physiological. When sampling our simulations, we observed that our peptides tended to lose all helicity for a high number of consecutive simulations: as many as 8 for Helical and as many as 5 for Bent. That is why we decided to only sample our results at simulations divisible by four. We could have restarted the experiment with runtimes of 30 nm for each simulation, but did not do this because the simulation series was already far along.

We then determined the unbiased probability of helicity for simulations 4 – 100, and also for 24 – 100, seeing as it took about the first 1/5 of the simulation series to begin to observe convergence . The total runtime for simulations 4-100 was 1.5 ms, and for for 24-100 was 1.2 ms.

Figure ii. Peptide 17 Bent in VMD: calculated bond
lengths (Å) are shown as white dashed lines.

Having completed this for Peptide 17 Bent/ Helical and finally observed convergence, we also performed the same experiment for WT Bent/ Helical, and again sampled at every four simulations, to see how our results would compare to those from Experiment 1. We used two different starting points of the bent and helical conformations, taken from different equilibrating simulations, but the initial Bent/ Helical conformations were still the same.

Finally, we calculated ΔG (penalty) as follows: ΔG (penalty) = -RT(lnP), where R is the universal gas constant, T is the temperature and P is the unbiased probability of helicity as a fraction. For example, for a probability of helicity of 0.002, $\Delta G = -8.31 \times 10^{-3}$ kJ/(mol × K) × (300K)[ln(0.002] = 15 kJ/mol.

Results

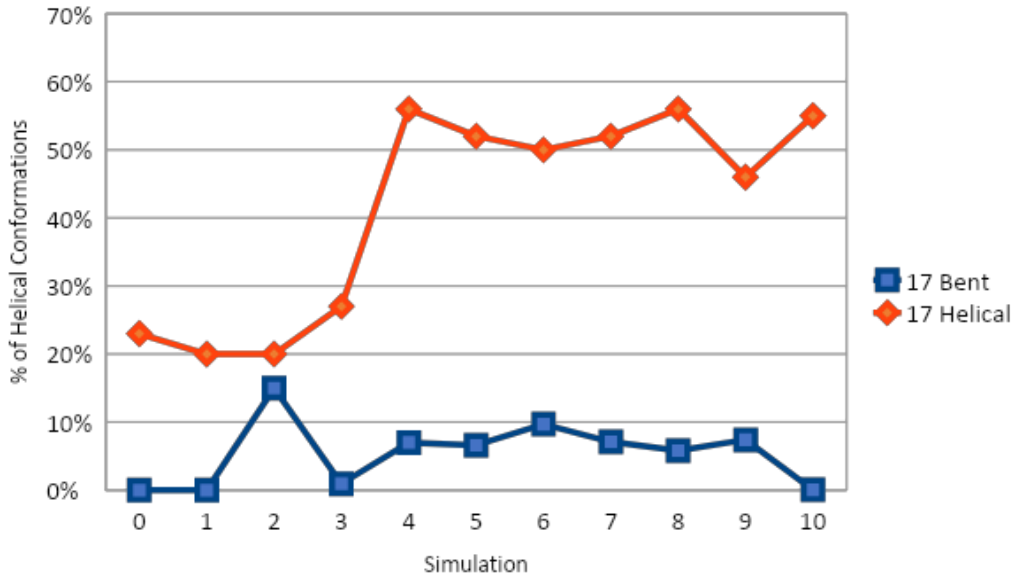
Experiment 1: Same Temperature and Bias Force (Wild Type and Peptide 17)

Table 1: Probabilities of Helicity at 300 K and Bias Force = 6.25 kJ/mol

Structure and Starting conformation	Probability of Helicity: Biased	Probability of Helicity: Unbiased	Simulations for Prob. Values	Total Time for Prob. Values	ΔG Penalty (kJ/mol)
Wild Type Bent	6%	0.2%	2 - 10	1.35 ms	15
Wild Type Helical	9%	0.3%	2 - 10	1.35 ms	14
Peptide 17 Bent	7%	0.3%	2 - 10	1.35 ms	14
Peptide 17 Helical	50%	3%	2 - 10	1.35 ms	9

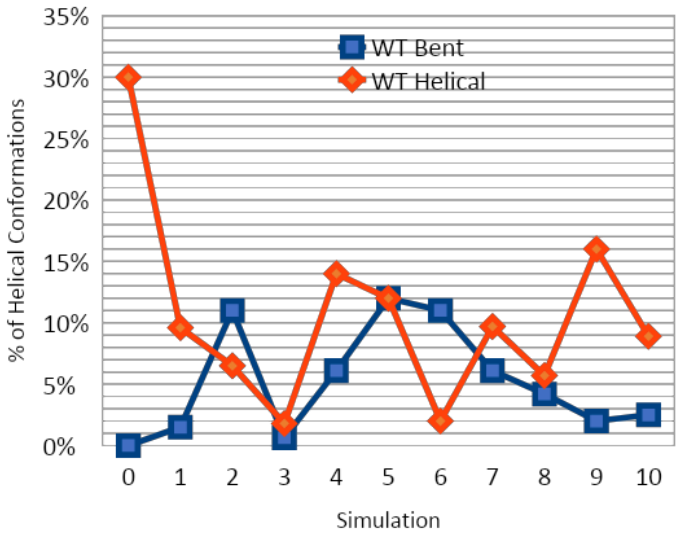
For each structure/ starting conformation, 10 consecutive simulations were run for 150 ns each. Total simulation time for each series = 1.5 ms. Probability values exclude the first simulation for each series, as it was most influenced by the starting conformation.

Chart 1.1: *Peptide 17* - Helicity at 300 K and Bias Force = 6.25 kJ/mol



10 consecutive simulations were run for both bent and helical starting conformations for 150 ns each with a total simulation time = 1.5 ms. Simulation "0" is first 15 ns of simulation 1. This chart shows probabilities of helicity prior to unbiasing.

Chart 1.2: *Wild Type* – Helicity at 300 K and Bias Force = 6.25 kJ/mol



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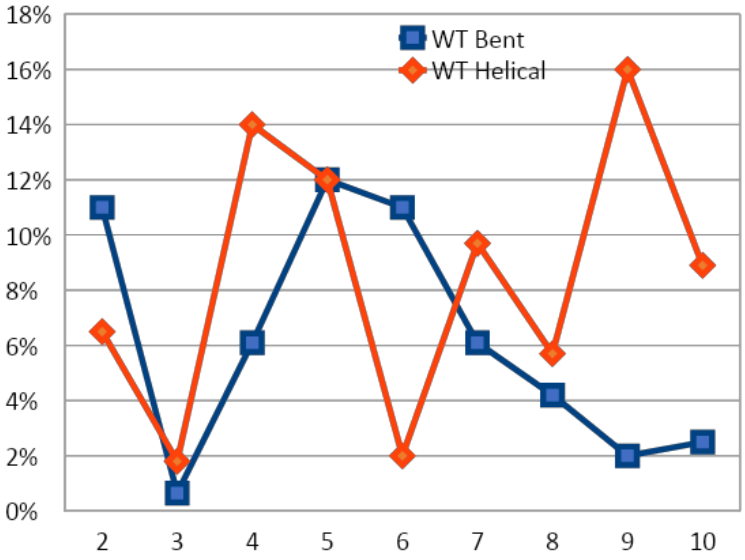


Chart 1.2 Zoomed In:

Zoomed in for simulations which were used to calculate helicity. Total time = 1.35 ms.

Summary: Running 1.5 ms simulations on both bent ("random") and helical conformations of both Wild Type and Peptide 17 at 300 K and 6.25 kJ/ mol bias force only showed possible convergence for Wild Type (see Table 1). Wild Type Bent and Helical had 0.2 – 0.3% unbiased probabilities of helicity, and Peptide 17 Bent also had an unbiased 0.3% probability of helicity. Peptide 17 Helical, on the other hand, had an unbiased probability of helicity greater by an order of magnitude from the rest at 3%. Statistical analysis is required to further test for convergence.

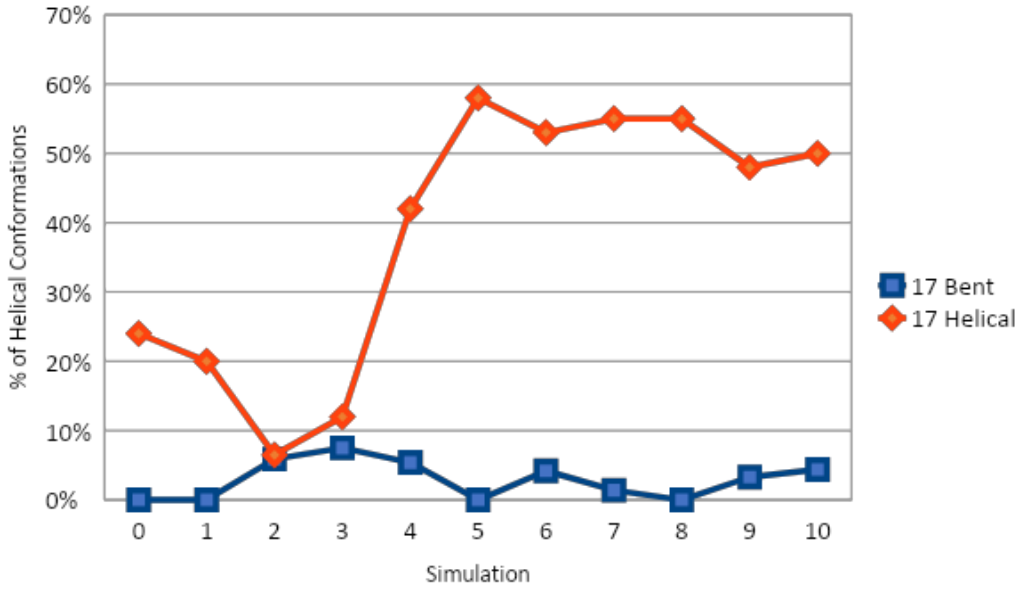
Experiment 2: Varying Bias Force (Peptide 17)

Table 2: Peptide *17* - Probabilities of Helicity at 300 K and Different Bias Forces

Bias Force (kJ/mol)	Starting conformation	Probability of Helicity: Biased	Probability of Helicity: Unbiased	Simulations for Prob. Values	Total Time for Prob. Values	ΔG Penalty (kJ/mol)
5 (Exp. 2)	Bent	4%	0.2%	2 - 10	1.35 ms	15
	Helical	40%	5%	2 - 10	1.35 ms	7
6.25 (Exp. 1)	Bent	7%	0.3%	2 - 10	1.35 ms	14
	Helical	50%	3%	2 - 10	1.35 ms	9

For each structure/ starting conformation, 10 consecutive simulations were run for 150 ns each. Total simulation time for each series = 1.5 ms. Values at bias force = 5 are from Experiment 2, and values at 6.25 kJ are from Experiment 1 and are included for comparison.

Chart 2: Peptide *17* - Helicity at 300 K and Bias Force = 5 kJ/mol



10 consecutive simulations were run for both bent and helical starting conformations for 150 ns each with a total simulation time = 1.5 ms. Simulation "0" is first 15 ns of simulation 1. This chart shows probabilities of helicity prior to unbiasing.

Summary: Running simulations same as before but changing bias force to 5 kJ/ mol did not increase overall convergence between Peptide 17 Bent and Helical, as unbiased probabilities of helicity still differed by an order of magnitude (see Table 2). Compared to the simulations at bias force at 6.25 kJ/ mol, Helical now began exploring biased conformations of lower than 20% helicity per simulation, however, Bent now stopped exploring biased conformations of higher than 10% helicity/ simulation (see Charts 1 & 2).

Experiment 3: Varying Temperature (Wild Type and Peptide 17)

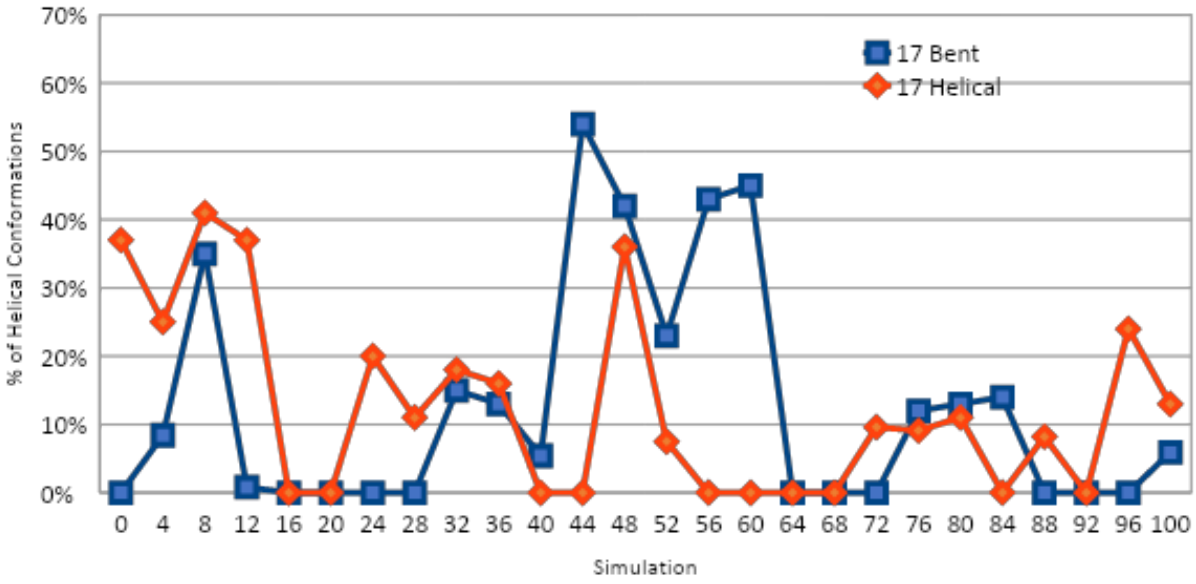
Table 3: Probabilities of Helicity at 300 K, Alternated with 400 K, and Bias Force= 6.25 kJ/mol

Structure and Starting Conformation	Probability of Helicity: Biased	Probability of Helicity: Unbiased	Sims for Probability Values	Total Time for Probability Values	ΔG Penalty (kJ/mol)
Wild Type Bent 1	7%	0.05%	24 - 100	1.20 ms	19
Wild Type Helical	9%	0.2%	24 - 100	1.20 ms	15

1					
Wild Type Bent 2	10%	0.08%	24 - 100	1.20 ms	18
Wild Type Helical 2	8%	0.2%	24 - 100	1.20 ms	15
Peptide 17 Bent	10%	0.04%	24 - 100	1.20 ms	20
Peptide 17 Helical	9%	0.05%	24 - 100	1.20 ms	19

For each structure/ starting conformation, 96 consecutive simulations (100 for Peptide 17) were run for 15 ns each. Odd simulations were run at 400 K, even simulations were run at 300 K, and data was taken every 4 simulations beginning with simulation 4. Total simulation time = 1.44 ms (1.50 ms for Peptide 17).

Chart 3.1: Peptide *17* - Helicity at 300 K, Alternated with 400 K, and Bias Force = 6.25 kJ/mol



100 consecutive simulations were run for both bent and helical starting conformations for 15 ns each with a total simulation time = 1.5 ms. Simulation “0” is the last 15 ns of an equilibrating simulation prior to simulation 1. Odd simulations were run at 400 K, even simulations were run at 300 K, and data was taken every 4 simulations.

Chart 3.2: *Wild Type 1* - Helicity at 300 K, Alternated with 400 K, and Bias Force = 6.25 kJ/mol

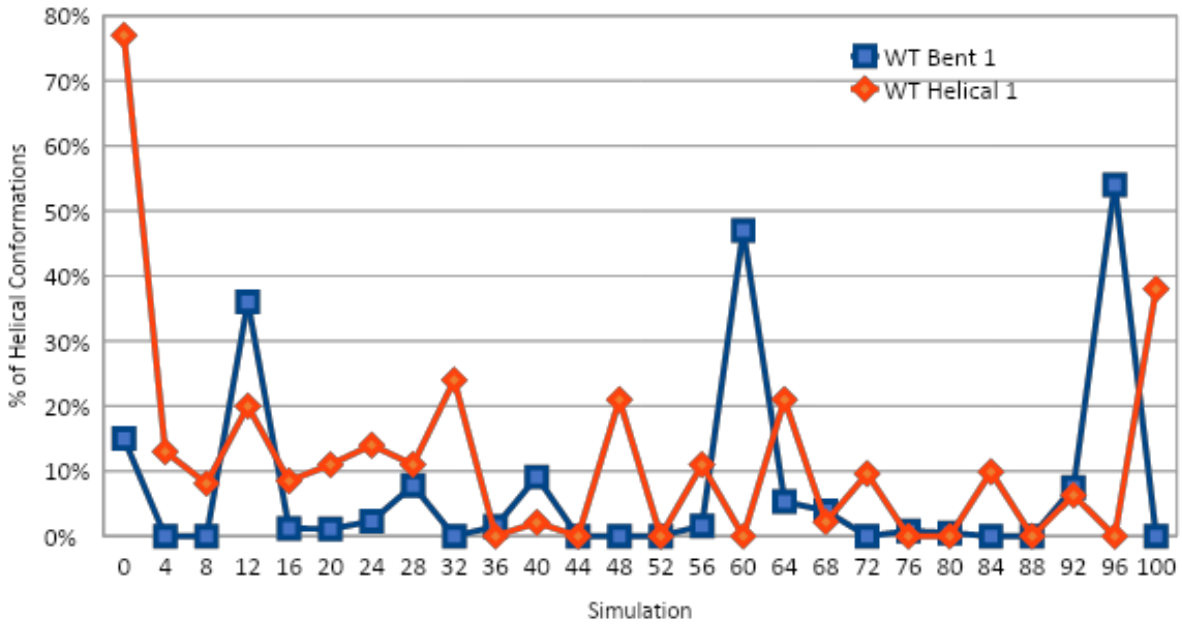
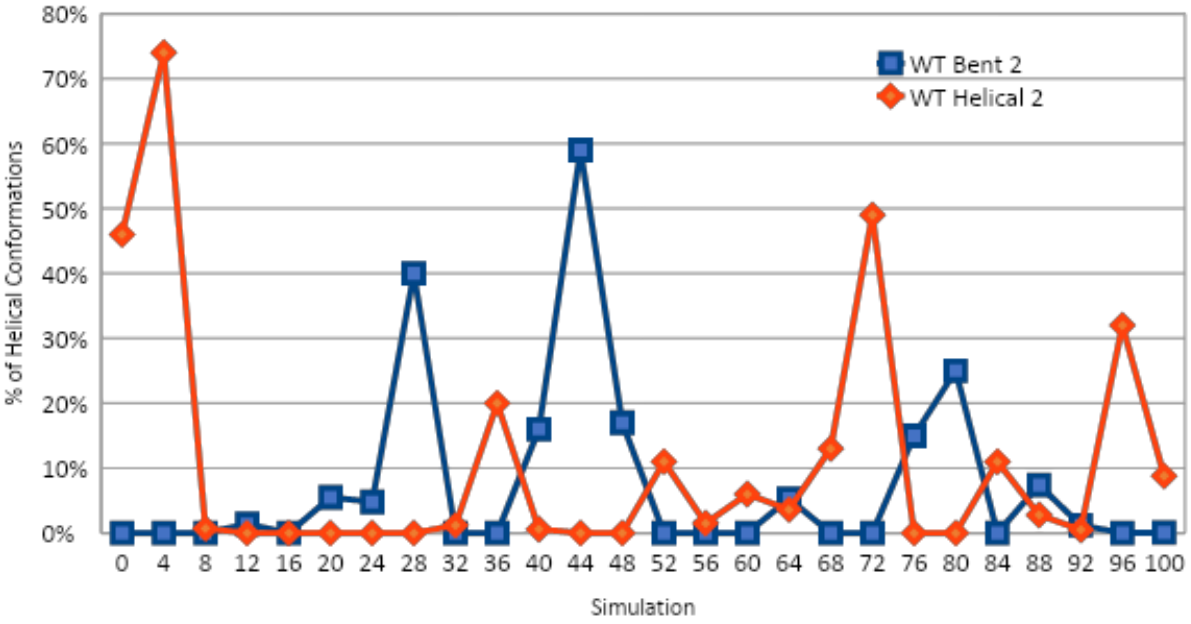


Chart 3.3: *Wild Type 2* - Helicity at 300 K, Alternated with 400 K, and Bias Force = 6.25 kJ/mol



Charts 3.2 – 3.3:

100 consecutive simulations were run for two bent and two helical starting conformations for 15 ns each with a total simulation time = 1.5 ms. Simulation "0" is always the last 15 ns of an equilibrating simulation prior to simulation 1. Odd simulations were run at 400 K, even simulations were run at 300 K, and data was taken every 4 simulations. The first bent and helical series are shown together on Chart 3.2, and the second two are shown on Chart 3.3. Also, see next page, zoomed in for simulations used to calculate helicity.

Chart 3.2 Zoomed In: *Wild Type 1* - Helicity at 300 K, Alternated with 400 K, and Bias Force = 6.25 kJ/mol

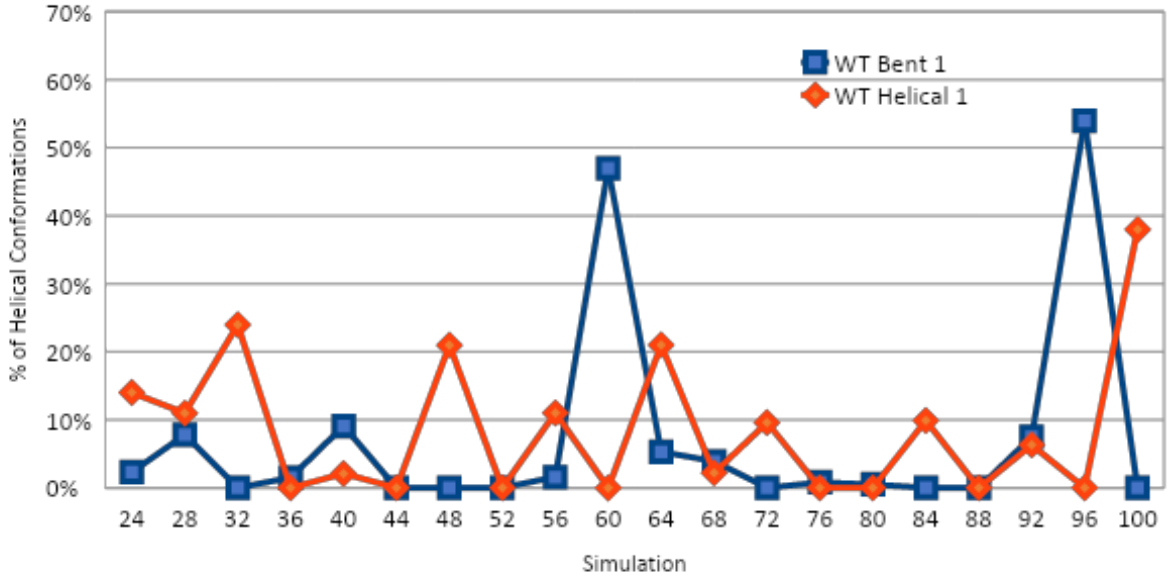
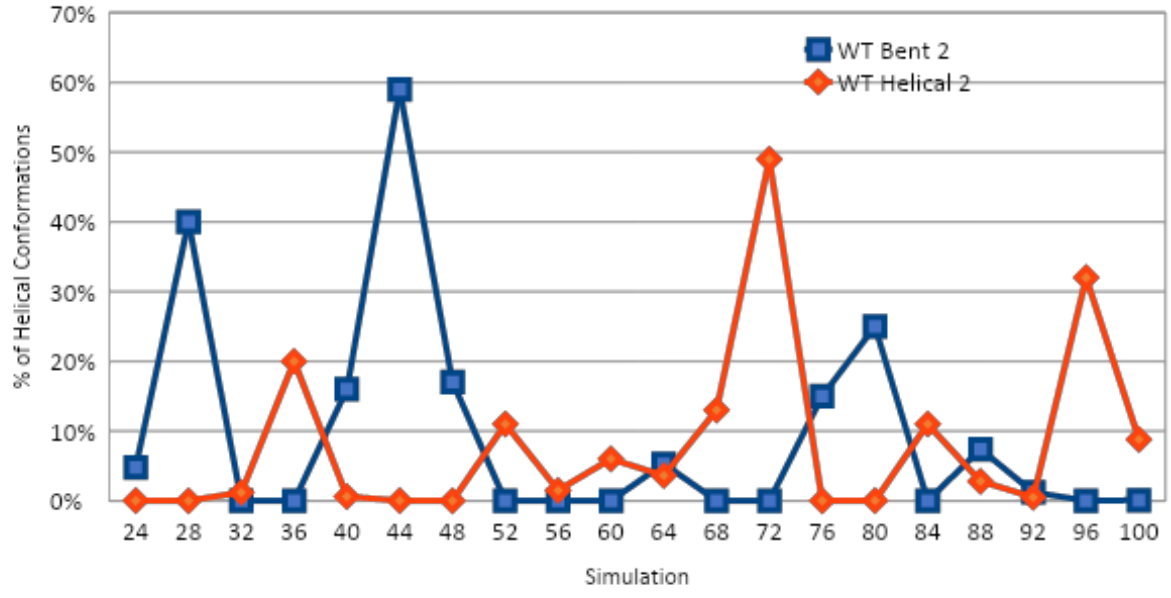


Chart 3.3 Zoomed In: *Wild Type 2* - Helicity at 300 K, Alternated with 400 K, and Bias Force = 6.25 kJ/mol



Summary: Running simulations at a bias force of 6.25 kJ/ mol, while varying temperatures from 300 K to 400 K for each consecutive simulation, and sampling 25 times in 1.5 ms (vs. 10 times), showed possible convergence between both Wild Type Bent vs. Helical (1 and 2) and Peptide 17 Bent vs. Helical (see Table 3). Peptide 17 Bent and Helical had unbiased probabilities of helicity of 0.04 – 0.05%, while Wild

Type Bent and Helical had unbiased probabilities of helicity of 0.1 – 0.2%. Statistical analysis is needed to confirm possible convergence between different conformations of these same two peptides, and to test for possible convergence between Wild Type and Peptide 17.

Works Cited

[1] http://www.compmolbiophysbc.org/research/research-blog/possiblecomputationalevidenceforenhanceda-helixinmodifiedcyfip1-derivedpeptides

[2] http://www.compmolbiophysbc.org/research/research-blog/useofumbrellasamplingmethodstoestimateprobabilityofcyfip1phelicalconformation