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Stability of diphenylalanine pepide [i.e. peptide] nanotube studied by molecular dynamics simulation

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STABILITY OF DIPHENYLALANINE PEPIDE NANOTUBE STUDIED BY MOLECULAR DYNAMCIS SIMULATION

By

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A REPORT

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This thesis, "Stability of Diphenylalanine peptide nanotube studied by Molecular Dynamics Simulation," is hereby approved in partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE IN PHYSICS.

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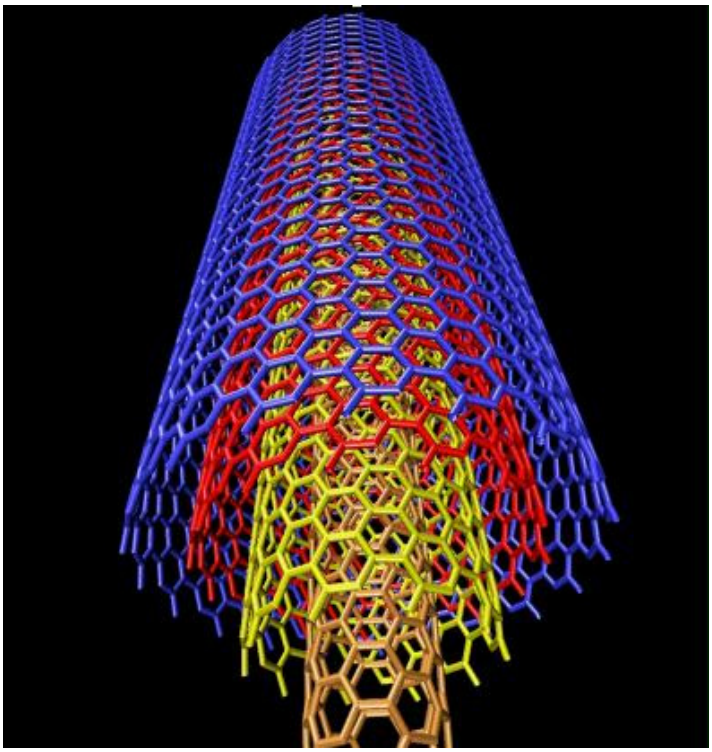
1. INTRODUCTION

In recent years, nanotubular structures have proved their ability and potential for many sorts of application in both scientific and technological aspects. A new subject of how to prepare different types of nanotubes has been given considerable research. The term nanotube is normally used to refer to the carbon nanotubes, which have received much attention from researchers over the last few years.

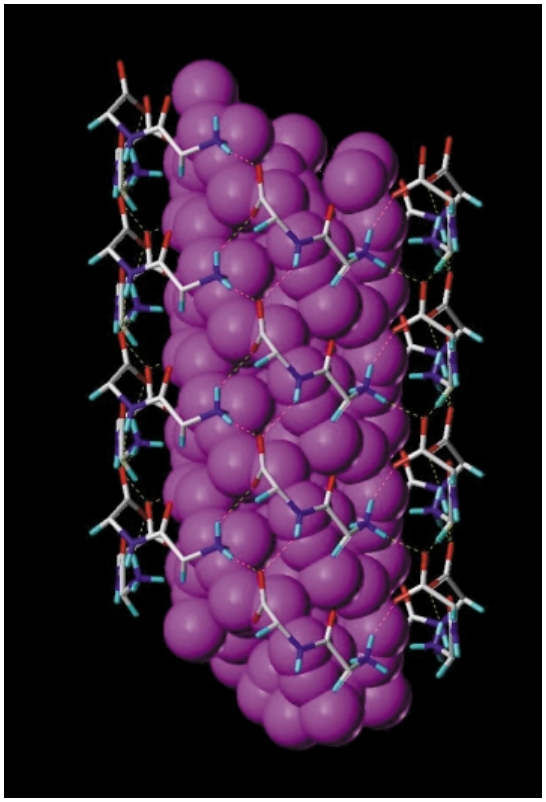


(Figure 1. Carbon nanotube*, CMP Científica, Paul Holister, January 2003*)

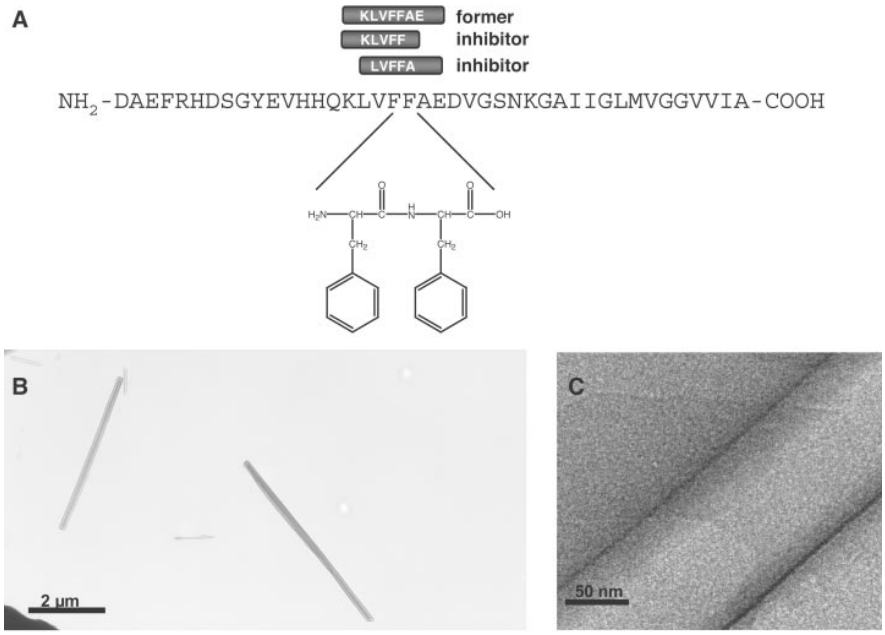
However, the basic building block can be formed from various materials where include carbon, inorganic, synthetic polymers, and biological units such as amino acids, nucleic acids, or lipids. One attractive branch among the alternative routes is the biomimetic self-assembly peptides. Due to their chemical variety, biocompatibility and ability to associate spontaneously, peptide-based nanotube are given particular interest from a biological point of view as application for ion channels, membrane pores, and more. Pi-pi Stacking interaction between two neighbor layers of cyclic molecules contributes together with, hydrogen bonds between neighbor molecules. Those two contributions make a stable tube-like nano structure. (*C. Görbitz. Nanotube Formation by Hydrophobic Dipeptides, Chemistry - A European Journal, 12/03/2001*)

In 1974 De Santis from theoretical analysis, and in 1993 Ghadiri from experiment, found that an even number of alternating D- type amino acid and L- type amino acid residues with equivalent β-type dihedral angles would form stacking closed rings through backbone–backbone hydrogen bonds.

In *Science 2003*, *Casting Metal Nanowires Within Discrete Self-Assembled Peptide Nanotubes,* E. Gazit first observed the formation of peptide nanotubes when he was studying the formation of amyloid fibrils consisting of aromatic peptides. As known gradually, self-assembly aggregation of amyloid fibrils are associated and resulted mostly in many amyloid diseases such as Alzheimer's disease, and Parkinson's disease. Especially for Alzheimer's disease, the minimal core recognition motif of the amyloid-protein consists of two aromatic phenylalanine residues. Diphenylalanine (FF) has been shown its ability to self-assemble to peptide nanotubes (PNTs).



(Figure 2. Amino acid based peptide nanotube, *Self-assembling peptide Nanotubes, Nanotoday, JUN-AUG 2008*)

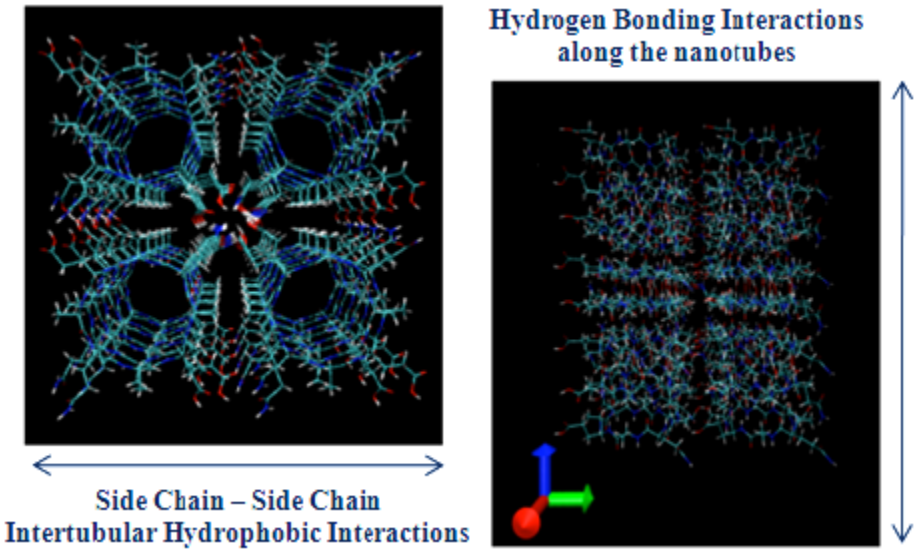


(Figure 3. The molecular structure of FF. *Casting Metal Nanowires Within Discrete Self-Assembled Peptide Nanotubes Science 2003*)

On the basis of both experimental and theoretical analysis, the stacking of aromatic residues plays a key role in the process of molecular recognition and self-assembly that leads to the formation of amyloid fibrils.

In this context, we focus on diphenylalanine based nanotubes which have received numerous attentions. One reason for this is that these peptide nanotubes are believed to be stable both thermally and chemically.

In *Thermal and chemical stability of diphenylalanine peptide nanotubes: implications for nanotechnological applications* (*Phys. Biol. 2006),* Gazit E. reported that this phenylalanine dipeptide nanotube has remarkable chemical stability in a wide range of organic solvents and pH. In solvent conditions, the circular dichroism spectra performs limited change when temperature keeps increasing even to 90°C. For dried ones, the tubes even more stable till heating to 150°C while it degrades at 200°C.



(Figure 4. *Thermo-mechanical stability and strength of peptide nanostructures from molecular dynamics: self-assembled cyclic peptide nanotubes, J. Diaz et al. Nanotechnology, 2010*)

Piezoelectric coefficients were unexpectedly high, reaching 60-70 pm/V in shear mode. By pressing of self-assembled PNTs into pellets and thus to confirm ferroelectric-like properties in aromatic peptide investigate this irreversible phase transition in PNTs, A. J. Lovinger discovered the existence of irreversible phase transition at about 140-150 °C from polar hexagonal to non-polar orthorhombic structure.

In summary, experimentally much work about the production and properties of Diphenyalanine peptide nanotubes has been done. But the mechanism behind the rigid structure and special Ferro electricity property is not understood. We tried to use molecular dynamics and Monte Carlo simulation to study the conformational dynamics.

Diphenyalanine, is reported to have the property of self-aggregation as reported by the E. Gazit Group *in Science 2003*. In their experiments, the peptide is highly soluble in the organic solvent and one can observe a rapid assembly into ordered semi-crystalline structures within seconds after dilution into the aqueous solution at a final μM concentration range. However, the detailed dynamics behind the well-ordered structures is still unknown. Through computational methods, we simulated this biological or material system, in order to find some truth to understand the properties of assemblies of Diphenyalanine in terms of their structure and the microscopic interactions between them.

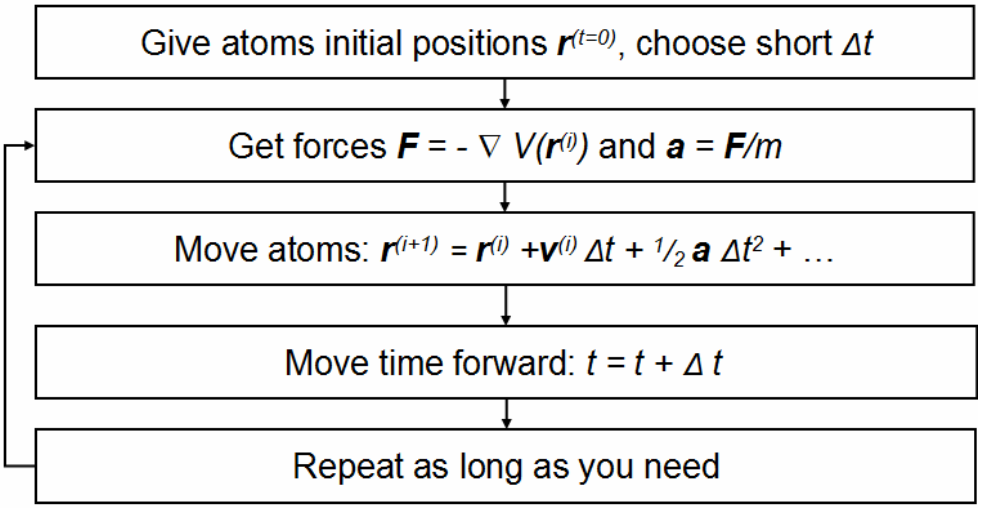
2. APPROACHES

(1) Introduction to simulation methods

Computational biophysics simulation locates between purely theoretical studies and biochemical reactions. We can say that it serves as theoretical experiment, providing strong proofs for potential movements or laws that experiment unable to observe. It is popular to carry out this method in probing folding, aggregation, binding and other fundamental processes in cells.

Among those biophysical simulation techniques, Molecular dynamics (MD) and Monte Carlo (MC) occupies the two Master positions. Briefly speaking, MD solves the trajectories assuming after enough relax time for every conformation. It gives insight to dynamical properties of the system. MC, comes more from statistical background. It generates all possible and random conformations, energy micro-states with given energy, through which folding states are analyzed by seeking the lowest potential energy conformation. In one word, how powerful the optimization technique matters.

In this lecture we shall concentrate on MD simulation. With respect to MD, it begins with an experimental structure and an assigned initial velocity. Then force fields, in the form of potential energy, are defined to describe the forces between the atoms, and apply the Newton's law to the system at the atomic level to propagate the system's motion. By numerically solving those Newton's equations of motion for this system, the trajectories of molecules and atoms are determined. This provides clear clue for dynamical properties if a sufficiently long MD trajectory have been carried out. The method was originally conceived within theoretical physics in the early 1960s.



(Figure 5. *Maximally simplified molecular dynamics algorithm, 6.12.2007, Knordlun*)

Especially, for Amber 99 SB force field, the potential consists of two parts: the bond part and the nonbond part like:

$$V = V_{bond} + V_{nonbond}$$

As shown next, for the bond potential, harmonic (ideal spring) potential is the choice used as a good approximation near the equilibrium bond length, bond angle and dihedral. The first and second terms are the energy by covalent bond length and the energy by bond angle due to the geometry of electron orbitals involved in covalent bonding. The third term is related to the dihedral contribution, where a bond twisting due to bond order and neighboring bonds or lone pairs of electrons.

$$V_{bond} = \sum_{r} \frac{1}{2} K_r (r - r_0) + \sum_{\theta} \frac{1}{2} K_\theta (\theta - \theta_0) + \sum_{\phi} V_n \cos(n\phi - \gamma)$$

Coulomb and van der Waals energy make up the nonbond interactions. Lennard-Jones potential is used in the form of vdw energy.

$$V_{nonbond} = V_{VDW} + V_{elec}$$

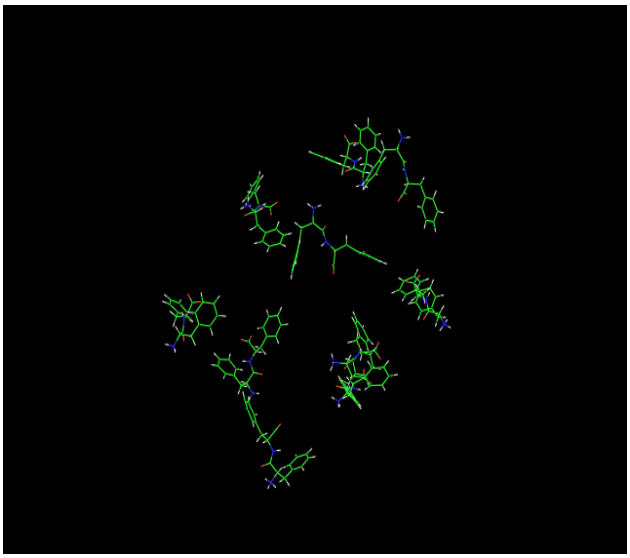
$$V_{elec} = \sum_{i} \sum_{i<j} \frac{q_i q_j}{r_{ij}}$$

$$V_{VDW} = \sum_{i} \sum_{i<j} 4\varepsilon_{ij} [(\frac{\delta_{ij}}{r_{ij}})^{12} - (\frac{\delta_{ij}}{r_{ij}})^{6}]$$

(2) Self-assembly from random distribution at room temperature

For this study, our first simulation strategy is to begin with random distributed dipeptides. As described in the above context, of Diphenylalanine peptide nanotube self-assembly is a pronounced property. Generally speaking, this process of proteins and peptides involves several weak, non-covalent bonds, such as ionic, van der Waals, hydrogen bonding,

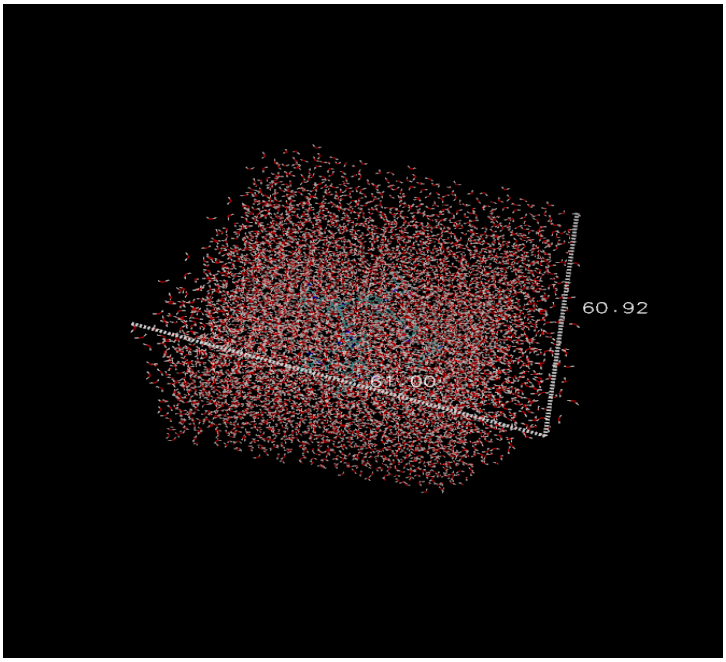
hydrophobic, and π-stacking. We built a simulation with 10 Di-phenylalanine peptide, randomly located in a 6nm*6nm box, with explicit water. Here is the simulation procedure I carried out.



(Figure 6. Initial conformation, the random distribution of ten diphenylalanine peptides)

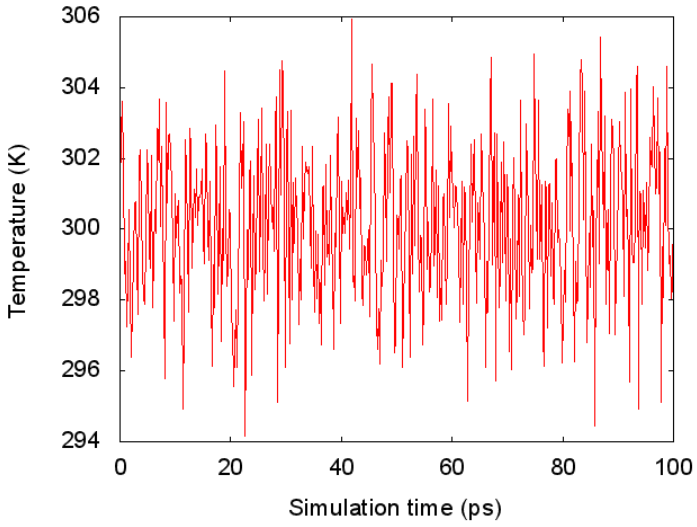
The detailed procedure for this simulation is:

Firstly, we need to create the molecular model and prepare for other input files. LEAP program in the AMBER 10.0 package was used to obtain the start configuration, with the amino-acid sequence C-F-F-N as input, leading to a total of 43 atoms in one Di-phenylalanine peptide. We put ten Dipeptides into one pdb file, and chose the AMBER99 SB force field (detailed description is shown in last section) to describe this peptide system. GROMACS 4.5.3 program package are used for next preparation and the main simulation. By pdb2gmx subroutine, we transferred the pdb file into the gro and top file. By editconf subroutine, we define a cubic water box with 1.0 nm from the farthest peptide to the edge of box. This way the molecular is solvated with 17407 water molecules in a cubic box of length 6.00 nm. The water model here we used is TIP3P. Subsequently we have the initial coordinate with explicit water box and parameter/topology input file.

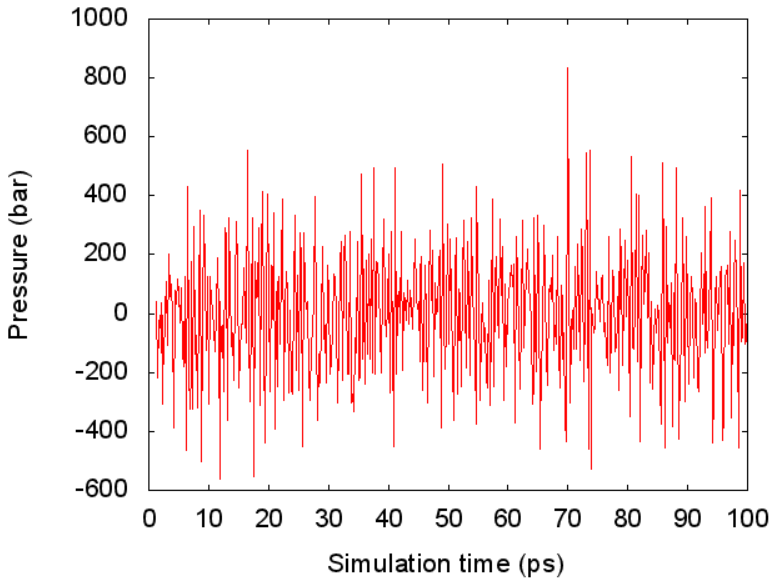


(Figure 7. Final simulation box, with TIP3P water model solvated.)

In the second step, we run energy minimization (EM) to relax the structure to ensure there are no steric clashes or inappropriate geometry. Grompp subroutine of Gromacs package suit, was used to create simulation parameters like the topology. Later, subroutine mdrun, are implemented. The whole system is minimized with the steepest decent method. Then we run two equilibration simulations with two successive molecular dynamic runs of 500 ps length each. The first one is at constant pressure (1 atm). The second is NVT, to make a constant volume.

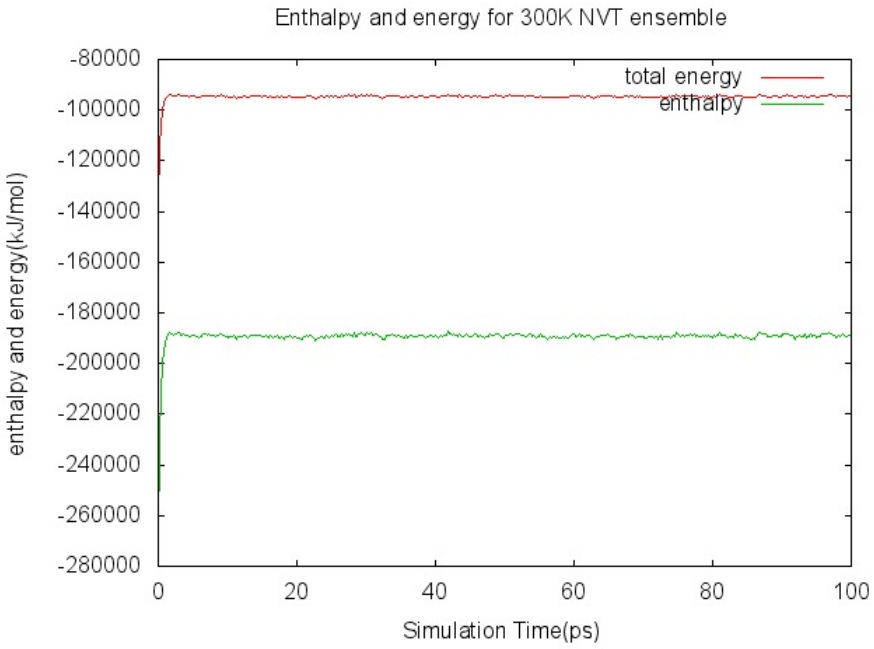


(Figure 8. Temperature in NVT equilibration simulation)

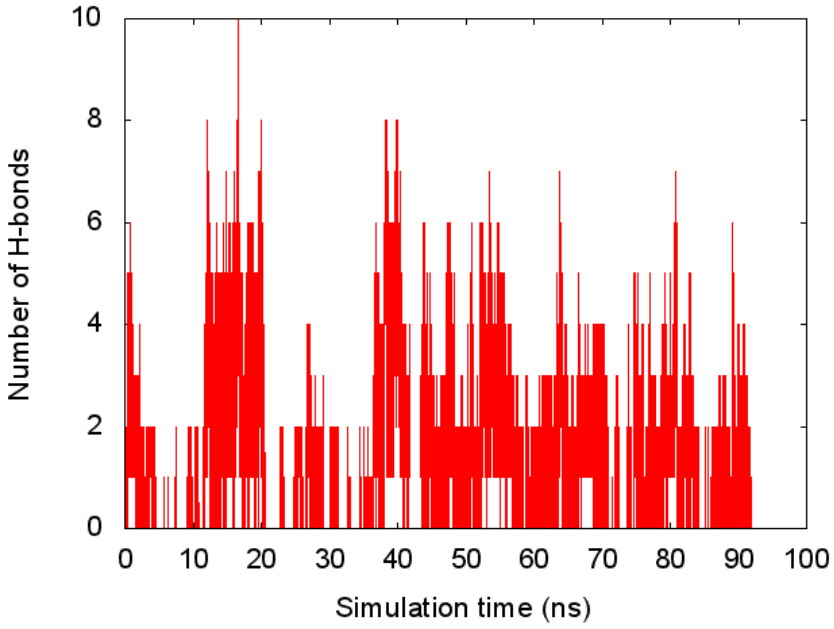


(Figure 9. Pressure NPT equilibration simulation, plot with simulation steps)

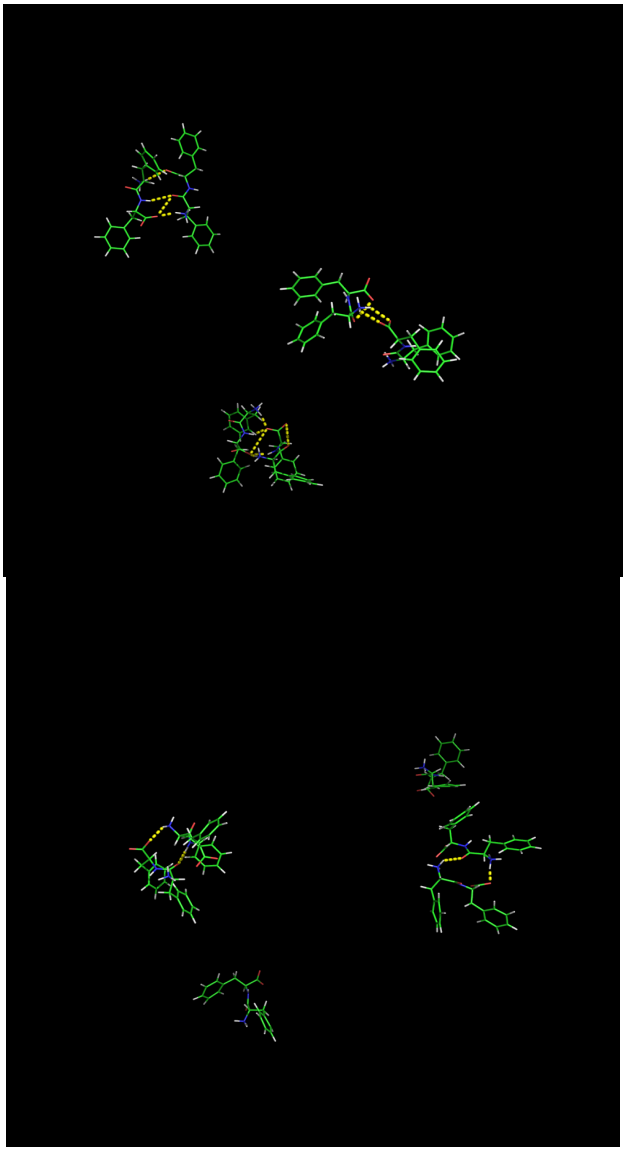
After energy minimization and equilibration, it is time to carry out the MD production run. After the first production run, we calculated the energy and enthalpy, in the expectation of phase transition between two states. Knowing it is almost a flat line, we used the hydrogen bonds to help analyze how many phenylalanine peptides are interacted with another one, from the direct geometry perspective. However, it does not looks as transition-like when we analysis the trajectory. Only small parts of cluster are gathered even after 100ns run, as shown below.



(Figure 10. Enthalpy and energy for 300K NVT ensemble)



(Figure 11. Number of hydrogen bonds for 300K NVT simulation)

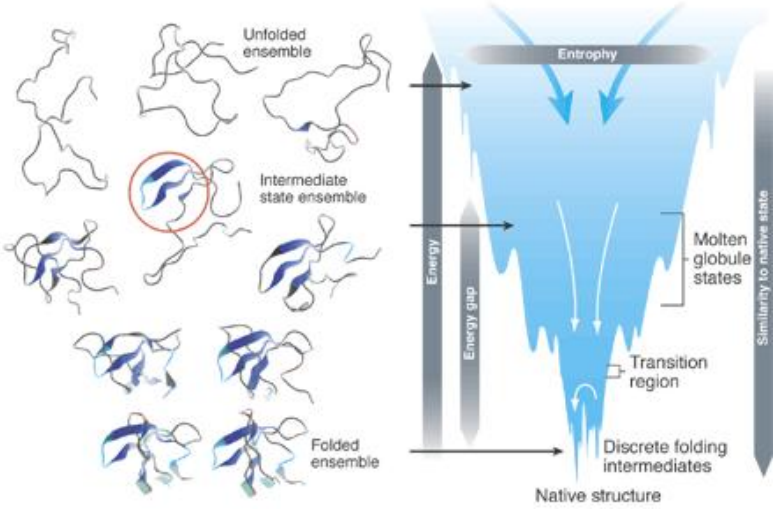


(Above: figure 12, Below: figure13. Two snapshots for high numbers of hydrogen bonds, yellow marked)

(3) Seeking the relevant temperature

Temperature decides the kinetic energy of the system, which is related mainly the free energy landscape. In last simulation, what we did is carried out at the room temperature. However, since we all know from thermodynamics, the temperature is the reason to

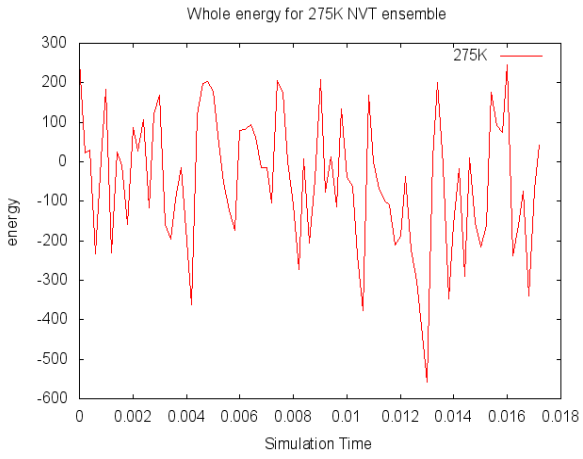
provide particle's kinetic energy. It has been an important factor to overcome the free energy landscape and get a good sampling of conformations. With this consideration, we designed canonical ensembles (NVT) with different temperatures in order to seek the relevant temperature for the nanotube's formation.

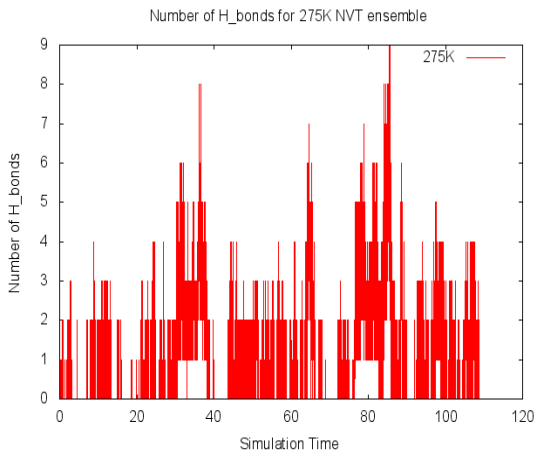


(Figure 14. The famous Wolynes free energy landscape. *Statistical Thermodynamics: taking a walk on a landscape. J Onuchic, ACS meeting, Jan. 2007*)

After position restrain on the backbone of molecular, we are ready to release the system to the production run. As analyzed above, here we applied different temperatures, using the same procedure.

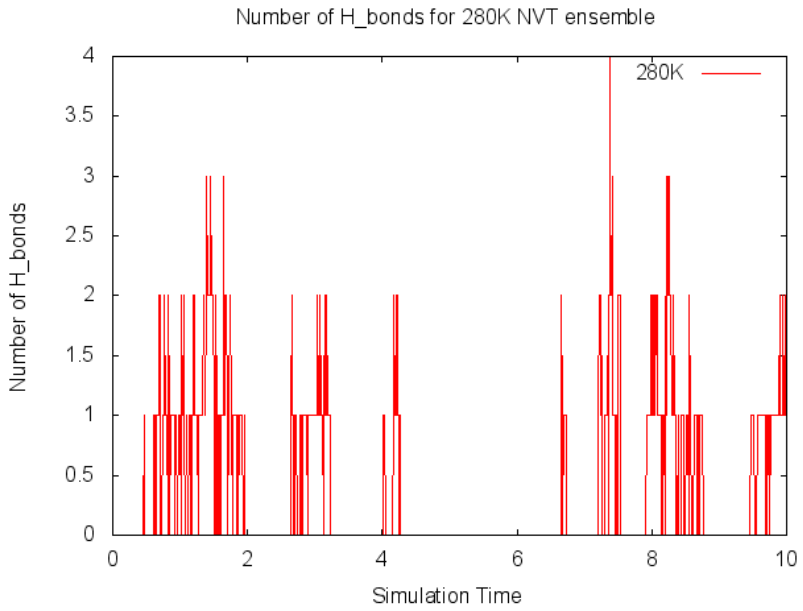
275K





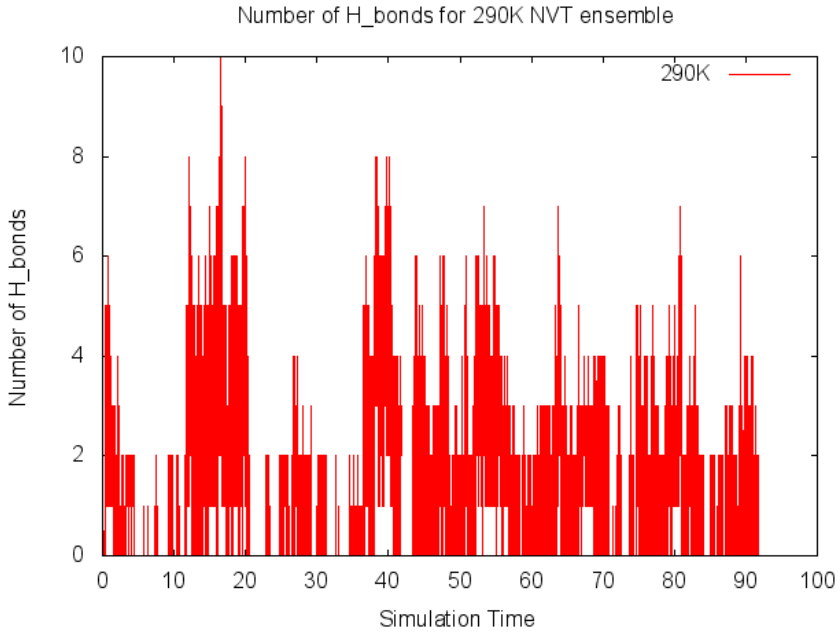
(Left: figure 15. Energy for 275K NVT; Right: figure 16. Number of hydrogen bonds for 275K NVT)

280K,



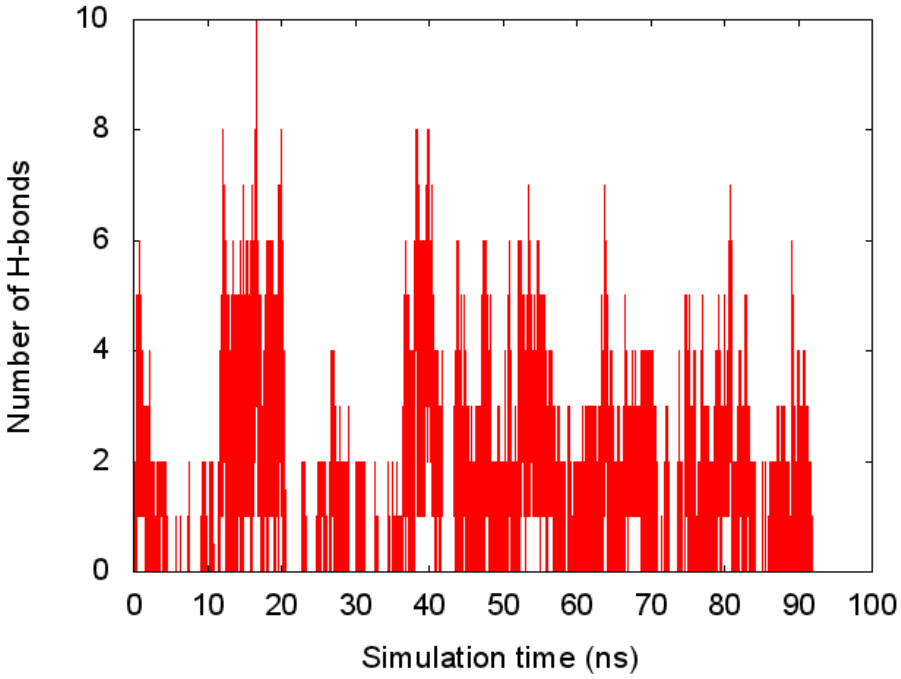
(Figure 17. Hydrogen bonds analysis for 280K NVT)

290K,



(Figure 18. Hydrogen bonds analysis for 290K NVT)

300K,



(Figure 19. Hydrogen bonds analysis for 300K NVT)

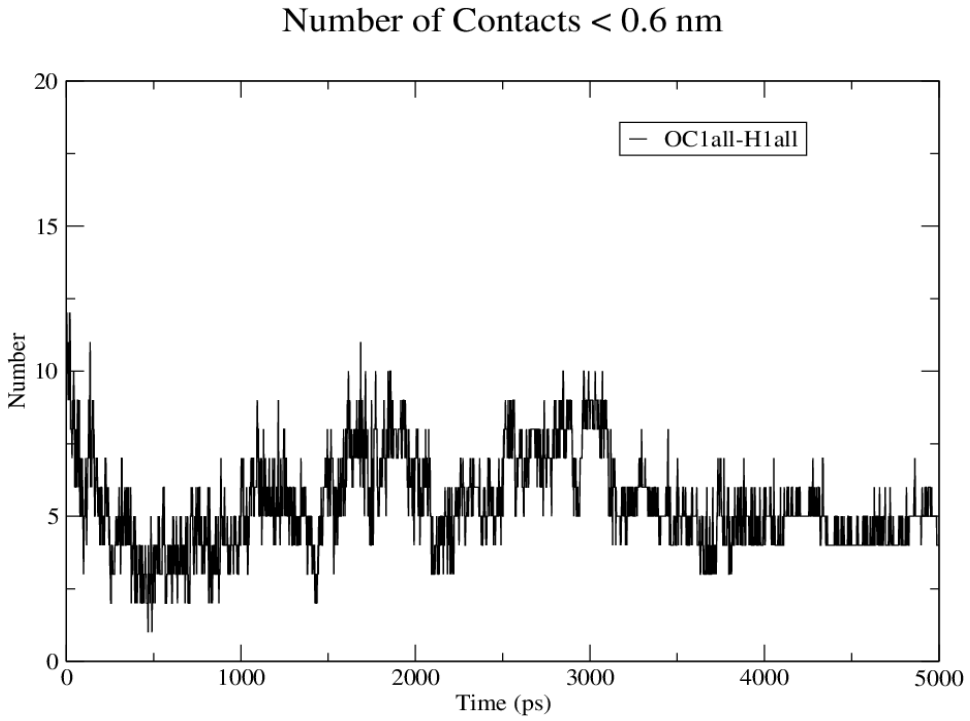
The above data shows that this discontinue temperature strategy cannot make long lasting hydrogen bonds, regardless of low temperature of 275K, and 100ns long run. Then we turned to simulated annealing.

(4) Simulated Annealing

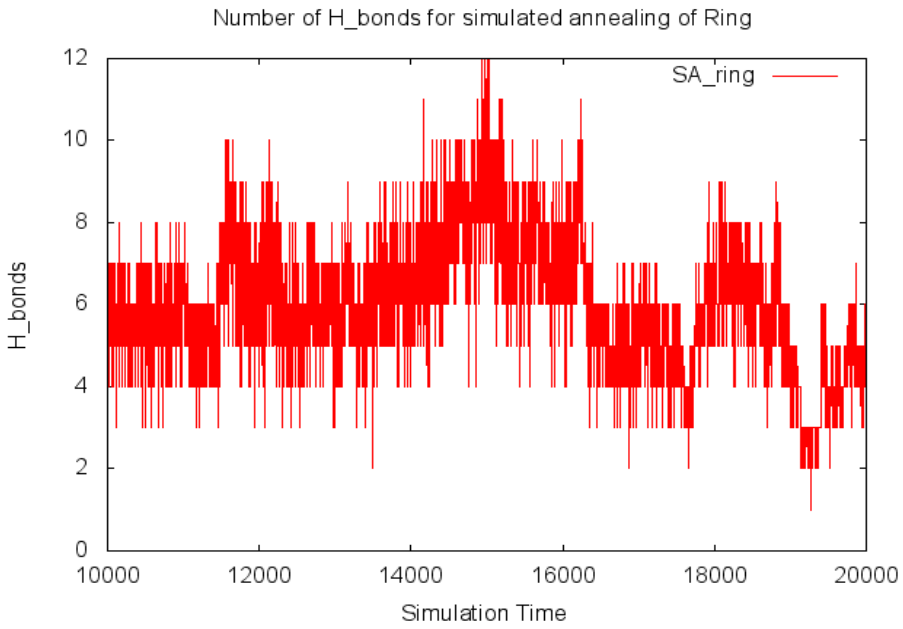
For global optimization, simulated annealing helps to overcome the energy wall and get more samples. The concept of "annealing" itself means heating and controlled cooling of one material so as to increase the size of crystals, and reduce internal defects. It is easy to understand since the heat leads the particles more active, and can randomly jump through either higher or lower energy state. This way more chance of getting lower energy configurations can be resulted from a slow cooling. (*H. Tang et al. Combining simulate*

anneal algorithm with support vector regression to forecast wind speed, 2nd IITA Conference, 08/2010)

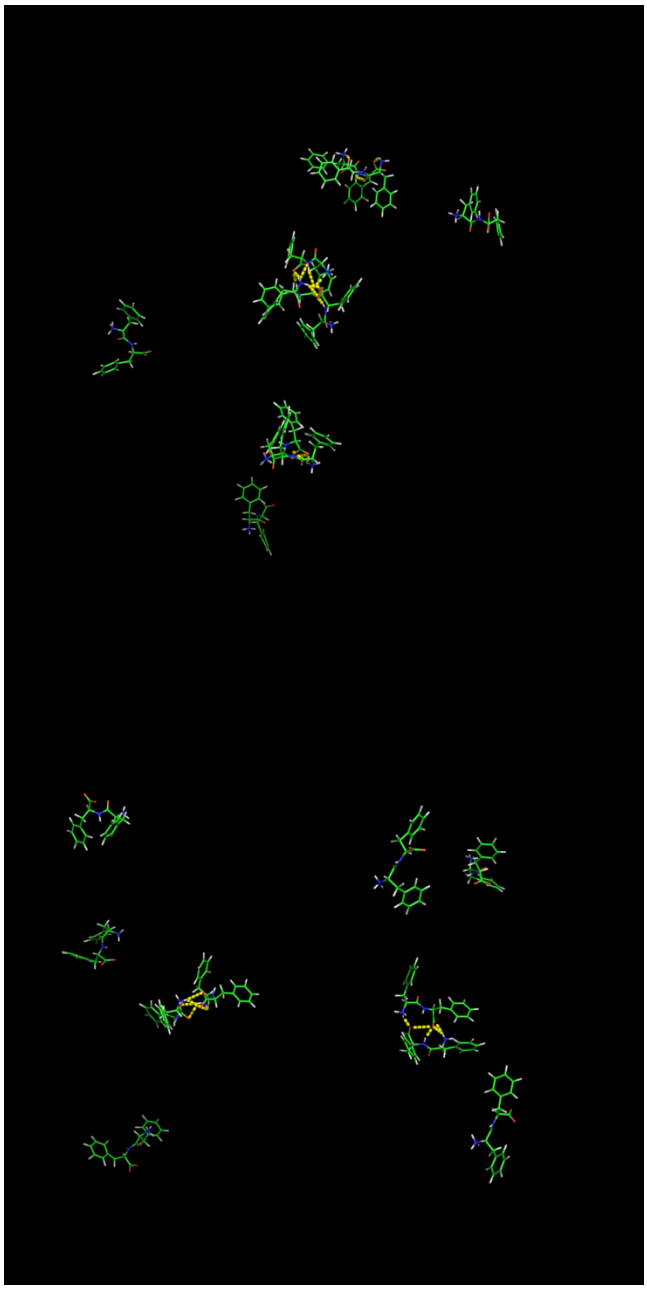
My setup for annealing is, to use linear relation to control the temperature increase and decrease. First 500K is decreased to 250K in 10ns, and increased to 500K in the second 20 ns, then going back to 250K using the same speed.



(Figure 20. Contacts analysis in simulated annealing simulation)



(Figure 21. Hydrogen bonds analysis in simulated annealing simulation)



(Above: figure 22; below: figure 23. The best structure we found in simulated annealing, which shows a four- units made ring, but the other two are far way.)

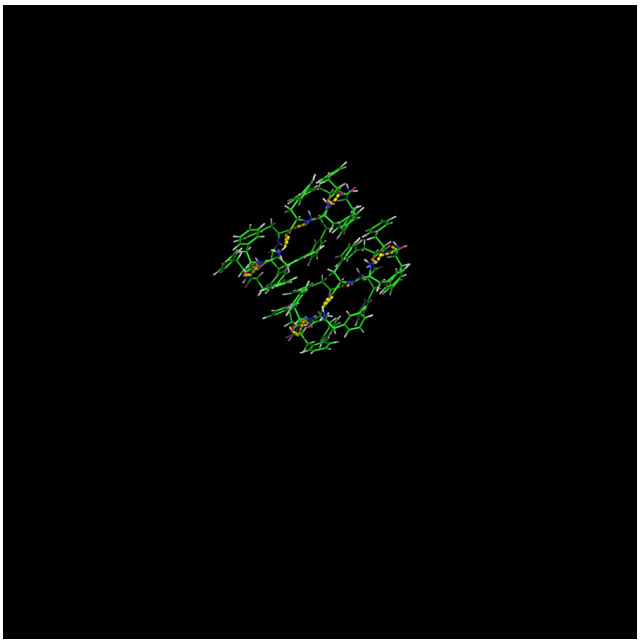
As shown from above contacts and hydrogen bonds analysis, it is clear that, more stable hydrogen bonds are formed in long relax time of annealing procedure. The direct trajectory also reflects this result. However, it is still far away from a closely linked peptide ring.

(5) Stability of handed structures

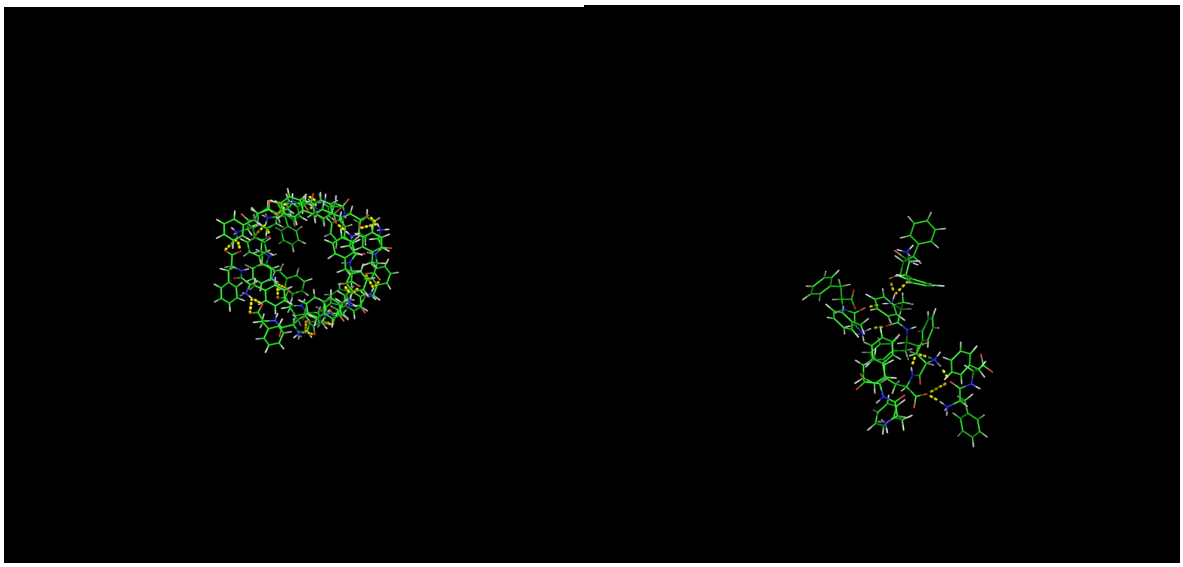
We prepared the structure by Pymol, for one layer's and double layers' diphenylalanine ring. About the simulation strategies, here we improved by two new ideas:

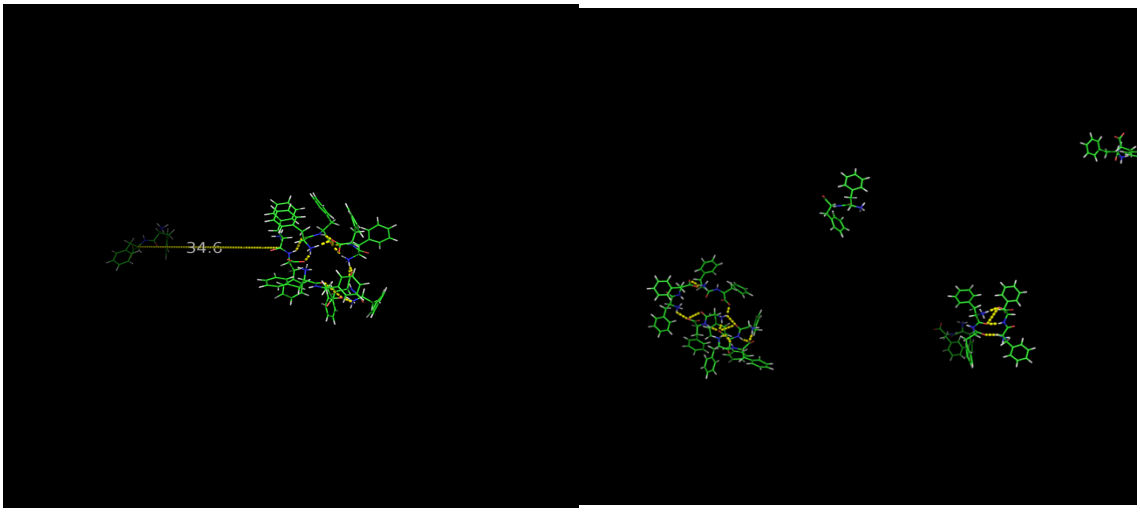
(a) Concentration referenced box size. After discussion with experimental chemical group, we changed the box size with 0.75nm to the edge, from concentration perspective. As described in review paper, Self-assembling peptide nanotubes (*S. Scanlon et al. Nanotubes review, 2008*), in experiment, phenylalanine's dipeptide is dissolved with hexafluoro-2-propanol and water at a final concentration less than 2 mg/ml. By a simple calculation we found for twelve diphenylalanine molecules, the distance of 0.75nm to the edge in setting is proper.

(b) Distance Restrain for the ring layers. We keep the distance of neighbor phenylalanine's so as to promise the hydrogen bonds between them.

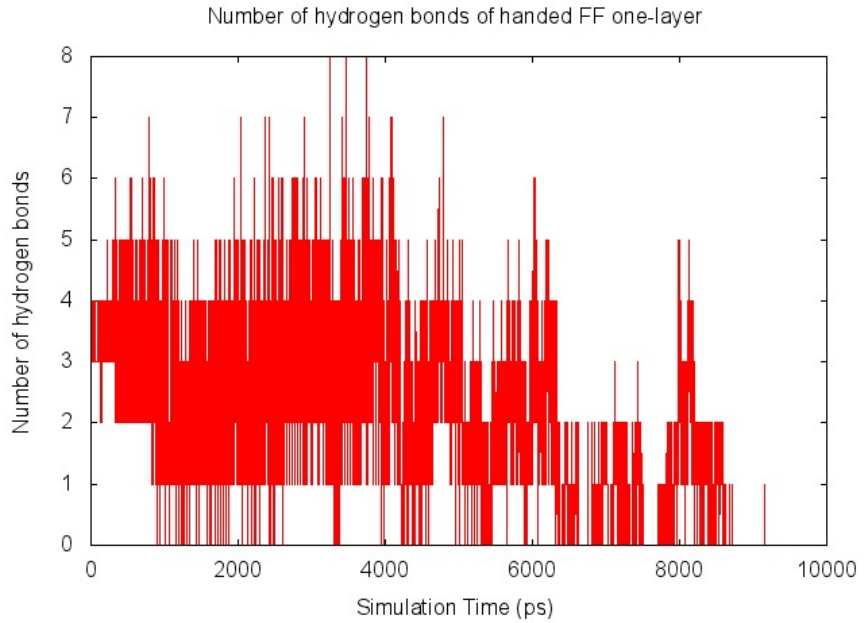


(Figure 24. The hand constructed two layers diphenylalanine peptide nanotube.)

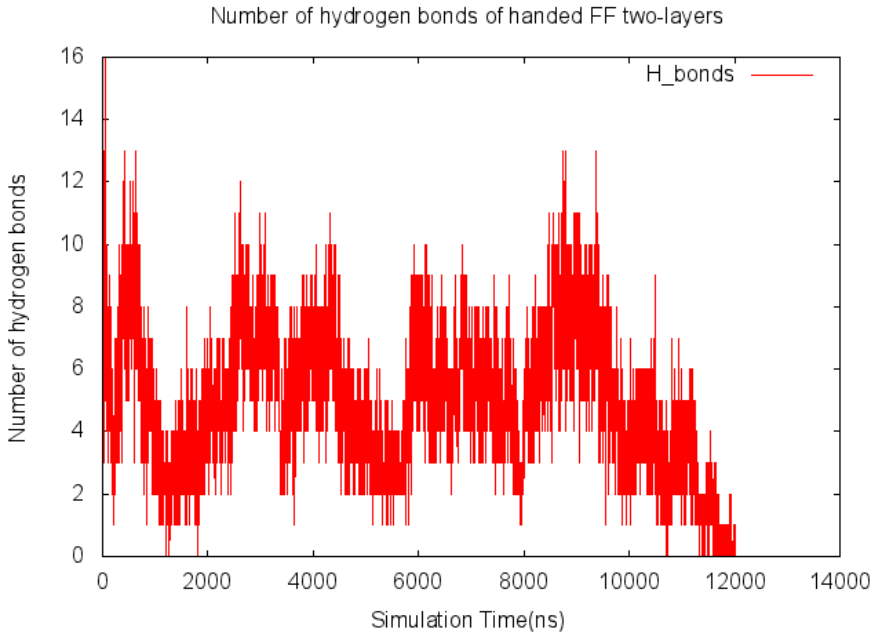




(Left above: figure 25. The initial handed two layers' peptide nanotube; right above: figure 26. Conformation after 1ns; left below: figure 27. Conformation after 2ns; right below: figure 28. Conformation after 10ns)



(Figure 29. Hydrogen bonds analysis for one layer handed peptide ring)



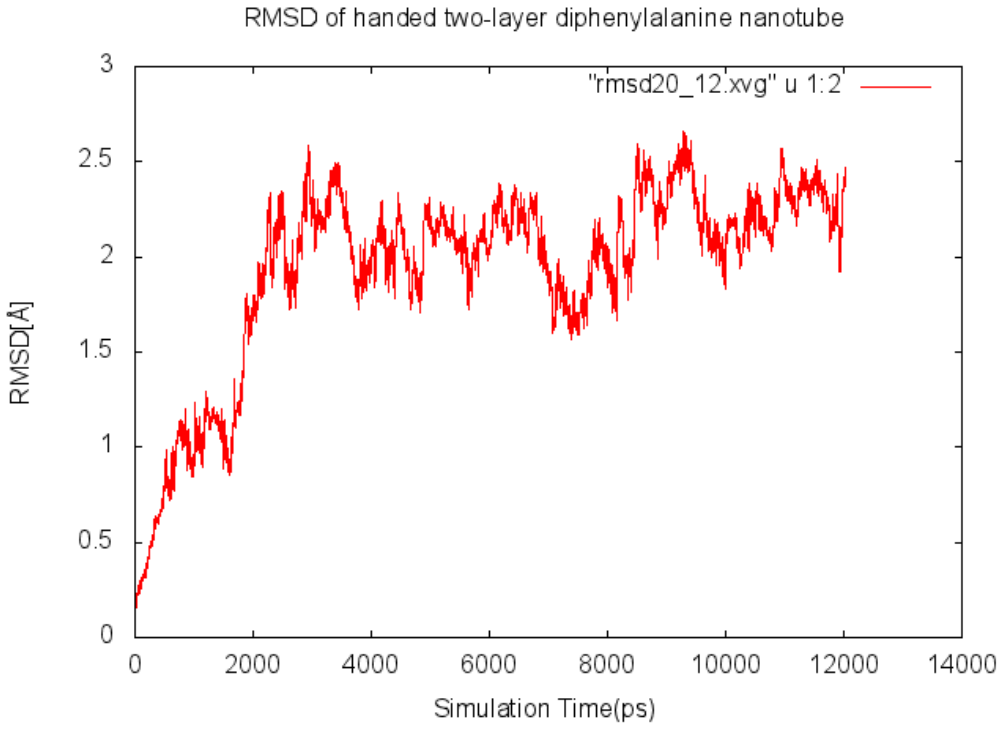
(Figure 30. Hydrogen bonds analysis for two layers handed peptide nanotube)

To analysis, besides hydrogen bonds, we took use of the root-mean-square deviation analysis (RMSD) analysis since we have a relative standard structure as the reference.

As the equations shows,

$$\mathrm{RMSD} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\delta_i^2}$$

(where δ is the distance between N pairs of equivalent atoms), RMSD compares differences between two atoms that may vary, neither of which is accepted as the "standard".



(Figure 31. Root-mean-square deviation analyses for two-layer structure)

In summary of the handed structure simulation, it intends that the reason for the challenging growth of Di-phenylalanine peptide nanotube could be, the structure itself is not stable. That means it could be a local minimum in free energy landscape. The trajectory shows apparently the break-up of our handed one layer and two layers peptide nano structures. RMSD graph fits our guess that it becomes bigger with the longer simulation time.

3. CONCLUSIONS

With an explicit solvent we have studied all-atom simulations for the formation process and stability of Di-phenylalanine peptide nanotube. At the beginning, we try to fit the experimental condition, setting a NVT ensemble, at room temperature 300K. However, from the direct trajectory after 100ns, we did not see any rings forming. In this case, we lower down the temperature, in the hope of overcoming the local minimum of the free energy landscape. The following 275K, 280K, 290K are simulated one by one. This time we modified out method to analyze the simulation result since we realized that peptide nanotube cannot be obtained easily. The number of hydrogen bonds become into our consideration. We compared those results coming from different temperatures. Against proper expectation, even under the lowest temperature, we cannot see a number of hydrogen bonds, over 6 constantly. Either these direct manual controlled temperatures or the dedicated simulated annealing strategy could help us to obtain a circular interacting peptide ring.

Facing this sampling challenge, we could not identify the Di-phenylalanine peptide nanotube, which produced from experiments, as the free energy minimum state. Referring to the experimental results' parameters, we build a similar structure, use distance restraint to run the energy minimization run before performing the production run. However, it turns out this simulated products can be easily broken up once they are released from distance restraints.

To be concluded, we think the reason why we cannot observe the growth of nanotube like structure, is that the temperature strategy is still far from experimental conditions, which use P.C.R for more than 30 hours. The pH environment could be a factor blocking here since in experiment people use buffer to keep the pH in a relatively acid one. Considering the expensive computation time and space, we did not put enough Nano rings in the last simulation (only two handed rings as described). Actually multi-layers structures have possibility to be more stable as aromatic interactions between every two layers can contribute more.

Responding to those analyses, one way to alleviate this problem are generalized ensemble simulations. In future, one can use replica exchange molecular dynamics (REMD) to control temperature more detailed and more efficient. At the same time, more constructed Di-phenylalanine peptide nano layers, are expected to put into the system.

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