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Molecular Simulations of Beta-Amyloid Protein near Hydrated Lipids (PECASE)

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Abstract

We performed molecular dynamics simulations of beta-amyloid(Aβ) protein and Aβ fragment(31-42) in bulk water and near hydrated lipids to study the mechanism of neurotoxicity associated with the aggregation of the protein. We constructed full atomistic models using Cerius^2 and ran simulations using LAMMPS. MD simulations with different conformations and positions of the protein fragment were performed. Thermodynamic properties were compared with previous literature and the results were analyzed. Longer simulations and data analyses based on the free energy profiles along the distance between the protein and the interface are on-going.

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1 INTRODUCTION

1.1 Background and Significance

Alzheimer's disease, characterized as a progressive, degenerative disorder, affects the functionality of the human brain. It is widely accepted that the deposition or aggregation of β-amyloid(Aβ) is related to AD (Hardy and Allsop, 1991; Sisodia and Price, 1995). Peptide structure and aggregation play an important role in neurotoxicity of AD. In spite of the importance of the problem, accurate information of the toxic structure and mechanism associated with the toxicity still remains unclear.

Research has been done on the structure and aggregation of Aβ experimentally (Lee et al., 1999; Mansfield et al., 1998; Mason et al., 1996; Pallitto et al., 1999; Terzi et al., 1997; Wang et al., 2003). Researchers pointed out that Aβ(17-21) fragment is critical in the aggregation of Aβ peptide (Mansfield et al., 1998; Pallitto et al., 1999). Good and coworkers reported important data on the fibril formation of Aβ (Lee et al., 2005; Wang et al., 2003). Hydrogen exchange-mass spectrometry(HX-MS) experiment revealing the distribution of species suggests that the N-terminus of the peptide does not participate in fibril formation, while the C-terminus is found to be important in fibril formation (Wang et al., 2003). Moreover, Hsp20, a novel α-crystalline protein, was found to drastically reduce Aβ toxicity to two different cells and the prevention of Aβ fibril formation was confirmed by electron microscopy in recent publication (Lee et al., 2005) .

Considerable research interest has been paid to the interaction of Aβ with lipid membranes (Ariga and Yu, 1999; ChooSmith et al., 1997; Mason et al., 1996; McLaurin et al., 1998; Terzi et al., 1997; Yoo, 2002). Result of small angle X-ray diffraction analysis of the Aβ(25-35) with liposomes implies that the peptide has strong lipophilicity and inserts into the membrane hydrocarbon core (Mason et al., 1996). It was concluded that Aβ binding occurs electrostatically to the outer envelope of polar head group region from the study on interaction of Aβ(1-40) with lipid membranes (Terzi et al., 1997). Studies show that membrane containing those molecules significantly affects the aggregation of Aβ, although the roles of both cholesterol and gangliosides are not clearly understood (Ariga and Yu, 1999; ChooSmith et al., 1997; McLaurin et al., 1998; Yoo, 2002). Experiments using membranes with gangliosides showed that gangliosides might be the Aβ binding site on the cell membrane(Yoo, 2002) and reduction in cholesterol and sialic acid content protected cells from toxic effect (Wang et al., 2001).

Biological systems consisting of lipid and water have been studied extensively via simulations, but the lipid molecules are represented as united-atom models in many studies (Chiu et al., 1995; Chiu et al., 1999; Egberts et al., 1994; Essmann et al., 1995; Leontiadou et al., 2004; Marrink et al., 1996; Mashl et al., 2001; Patra et al., 2003; Shinoda et al., 1995; Shinoda et al., 1997; Smondyrev and Berkowitz, 1999a; Smondyrev and Berkowitz, 1999b; Tieleman and Berendsen, 1996; Tieleman et al., 1997). As computing power increases greatly in recent years, more detailed description of biological systems including water and lipid bilayer with or without protein have been made (Crozier et al., 2003; Feller et al., 1997; Jang et al., 2004; Sachs et al., 2004).

Molecular simulations with Aβ as the target protein have been studied, but the system has not been resolved since then. Molecular dynamics studies mainly focused on the conformations

and the structures of Aβ(1-42) have been done by Mager and co-workers (Mager, 1998a; Mager, 1998b; Mager, 2001; Mager and Fischer, 2001; Mager et al., 2002; Mager et al., 2001). Conformations of Aβ(1-28) peptide fragment at different pH have been investigated at 298K using molecular dynamics without lipid bilayers (Kirshenbaum and Daggett, 1995). MD simulation study of Aβ peptide (25-35), another important fragment of Aβ, has been reported in aqueous trifluoroethanol(TFE) solution and it was found that TFE plays an important role in the formation of α-helix (Lee and Kim, 2004). The structure and dynamics of Aβ(10-35)-NH_2 peptide in aqueous solution has been studied via molecular dynamics simulations (Massi et al., 2001; Massi and Straub, 2003) and it was insisted that the central LVFFA hydrophobic cluster(17-21) and the VGSN turn (24-27) regions are strongly correlated with the preservation of the structure (Massi et al., 2001). MC simulation of Aβ insertion into cell membranes at amino acid level has been investigated and it was shown that most familial AD(FAD) mutations have a central effect on the insertion of Aβ peptide (Mobley et al., 2004).

Regardless of the huge amount of research on the Aβ protein, to the best of our knowledge studies on the Aβ insertion in membrane with full atomistic models have not been reported. In this work, we are interested in applying MD technique to understand the interaction behavior of Aβ with cell membranes. We will focus on the Aβ protein structure and the membrane composition which is related with the membrane fluidity and specific binding affinity.

1.2 Molecular Simulation Work Scope

We constructed model systems consisting of the protein, lipids and water molecules in different starting configurations. The protein itself is very large(~ 50 Å). Therefore, we will frequently focus on the simulation of an important fragment of the protein, Aβ(31-42), which is highly hydrophobic. Since dipalmitoylphosphatidylcholine(DPPC) is one of the best studied and abundant lipids in human body, DPPC molecules will be used as our lipid bilayer materials. Two water phases will be generated outside of the bilayer, where one of the phases encompasses the target protein or protein fragment.

First we will simulate pure water to check the applicability of our simulation tool, LAMMPS(Large-scale Atomic/Molecular Massively Parallel Simulator). We then will perform simulations of hydrated lipids together with the systems with the protein in hydrated lipids.

Considering the system size and the available computing power, the maximum real time will be on the order of several ns at best, and we probably will not be able to observe the folding/unfolding of Aβ during our simulations. We will measure the thermodynamic properties such as the kinetic and potential energies including each contribution to the potential. We will calculate other physical properties such as the specific surface area, pair correlation function, and order parameter of DPPC phase. Also, we will calculate the distance between certain atoms and dihedral angles from peptide backbones during the simulations, since the average value of the distance can be an indicator of the secondary structure of Aβ (Lee and Kim, 2004).

2 MOLECULAR SIMULATIONS

2.1 Theory

2.1.1 Free Energy Calculation

Figure 1 is the conceptual figure of Aβ protein in hydrated lipids, where half of the simulation system is represented in the figure. From the molecular dynamics simulations of Aβ protein in the hydrated lipids we obtain the thermodynamic properties such as the kinetic and potential energies of the system.

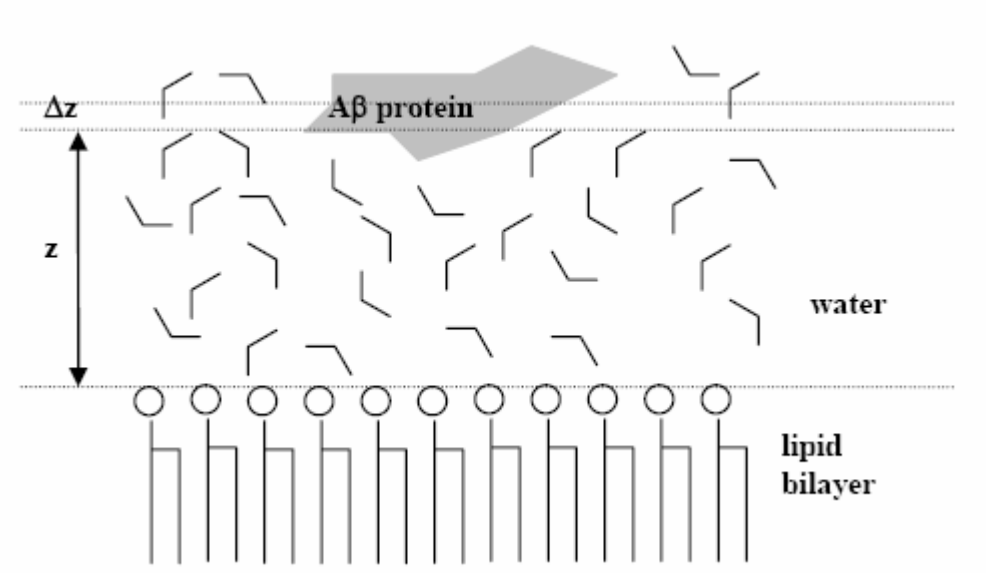


Figure 1 Conceptual figure of Aβ protein in hydrated lipids

As pointed out in the introduction section we are interested in determining the most stable or probable structure of the system and the protein position relative to the water-lipid interface. Therefore, we compare the free energy profiles of the system. Free energy difference at each separation distance can be obtained from the following expressions via the free energy perturbation technique.

$$\Delta A = A_{z+\Delta z} - A_z = -\frac{\ln\left\langle \exp(-\beta \Delta U_{z \to z+\Delta z}) \right\rangle_z}{\beta} \quad (1)$$

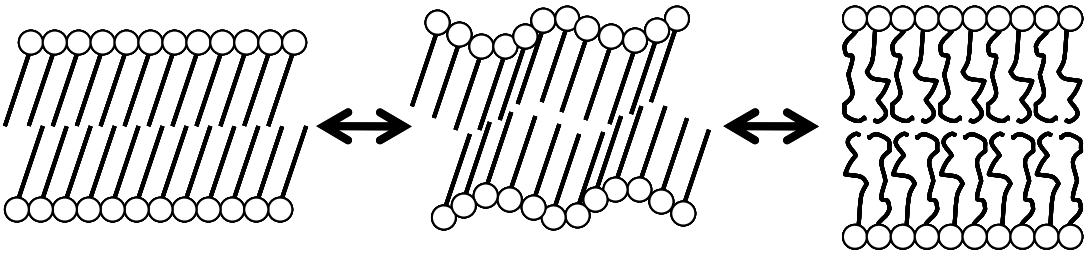
$$\Delta G = G_{z+\Delta z} - G_z = -\frac{\ln\left\langle \exp(-\beta \Delta U_{z \to z+\Delta z}) \right\rangle_z}{\beta} \quad (2)$$

Eqs 1 and 2 will be used for canonical ensemble and for isothermal-isobaric ensemble, respectively. A conceptual figure is drawn in Figure 1.

2.1.2 Phase of DPPC bilayer

The phase of lipid bilayer can be classified as a gel phase or a liquid crystalline phase, where the gel phase is more ordered. Figure 2 depicts the phase transition of phospholipids bilyer. The main transition can be considered as a disordering process by which the lipid bilayer turns into a liquid from a solid gel losing its translational order or from Lβ or Lβ' to Lα.

There are two variables to describe the nature of a phase; 1)Order in internal conformational degree of freedom of the lipid acyl chains, 2)Translational order. The main transition from a solid gel phase to liquid crystalline phase is a transition from a low temperature gel phase(so-called solid-ordered, SO), which is solid and has (quasi) long-range translational order and a high degree of conformational order within the lipid chains, to a high temperature fluid or liquid phase(so-called liquid-disordered, LD), which displays disorder in both the translational and chain conformational degree of freedom (Pasini and Zannoni, 2000).



Lβ' Pβ' Lα

Figure 2 Phase transition of DPPC bilayer

To have membrane fluidity the bilayer should be at Lα phase at the target temperature of 323 K. Temperature above T_m of 314 K, it is known that the bilayer is in the liquid crystalline phase (Mouritsen, 1991). Most studies of a lipid bilayer has been done at well above T_m, since the lipid phase resembles the physiological condition, even though that temperature is higher than the body temperature.

The pressure to give a phase change for DPPC at 314 K is know to be 40 dynes/cm, which corresponds to the difference between the surface tensions of water-air and water-lipid interfaces. We obtain the water-lipid surface tension γ_{WL} of 28 dynes/cm using the water-air surface tension γ_{WA} of 68 dynes/cm. The boundary surface tension is obtained from the following formulae.

$$\gamma = \int_{Z_1}^{Z_2} \left[P_N(Z) - P_L(Z)\right] dZ = \left(Z_2 - Z_1\right) - \int_{Z_1}^{Z_2} P_L(Z) dZ \tag{3}$$

$$\frac{\int_{Z_1}^{Z_2} P_L(Z) dZ}{\left(Z_2 - Z_1\right)} = 1 - \frac{\gamma}{\left(Z_2 - Z_1\right)} \tag{4}$$

Substitution of γ with 56 dynes/cm, due to the two interfaces, reduces to the average lateral pressure of -51.6 atm with the z-dimensional box length of 107.2 Å.

2.2 Models and Methods

2.2.1 Models

Aβ protein

Aβ protein data including fragments were downloaded from Protein Data Bank, http://www.rcsb.org/pdb/. Structures with different conformation will be obtained as found elsewhere (Mager, 1998b; Mager et al., 2001). Figure 1 is the schematic of the protein Aβ(1-42). Residues 1-16 and 22-28 are hydrophilic and residues 17-21 and 29-42 are hydrophilic. Since the hydrophobic part of the protein is of importance, our simulation works will be concentrated on the protein fragment of Aβ(31-42). Initial α-helix and β-sheet structures were generated with 3D-DOCK software.

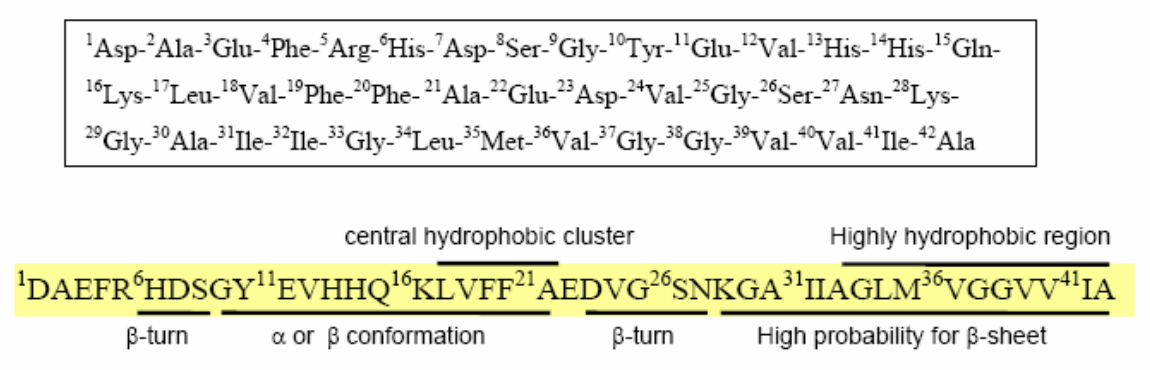


Figure 3 Aβ(1-42) protein structure with fragment features

DPPC bilayer

We constructed model membranes consisting of hydrated DPPC bilayer. We start the construction of the model system by building the lipid bilayers.

Figure 4 shows the structure of a DPPC molecule, and Figure 5 is the DPPC bilayer with 128(8×8×2) DPPC molecules. The construction of model system was done using Cerius^2 and/or Insight II. The head group area is slightly larger than the experimental value(62.9 Å, (Nagle et al., 1996)) to avoid overlap. We then perform a short molecular dynamics simulation with isothermal-isobaric ensemble to get slightly squeezed simulation box, especially in x- and y-directions. We fix the simulation box dimensions which are slightly larger than the squeezed box but same as the experimental values, and then perform the energy-minimization with fixed cell parameters: head group area = 62.9 Å, bilayer thickness of 39.3 Å, cell angles are 90 degrees.

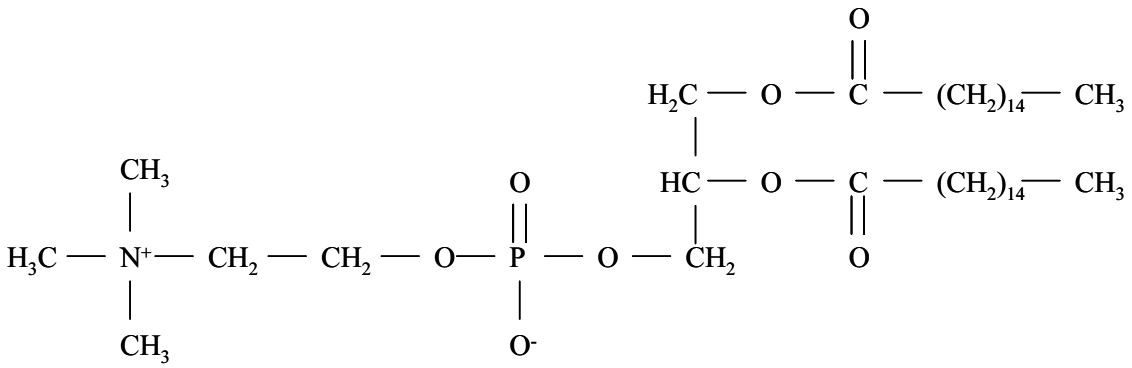


Figure 4 Structure of a DPPC molecule

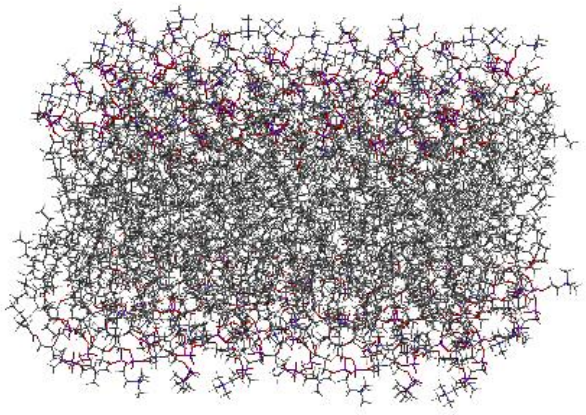


Figure 5 Construction of pure lipid bilayers

Construction of Model System

The whole system consisting of Aβ, DPPC, and water molecules are generated by either using soaking function in Insight II or adding water phase. The simulation box size of the starting configuration is 63.4×63.4×107.2 Å.

In case of change in membrane composition we remove some of DPPC molecules(mol ratios of DPPC/cholesterol and DPPC/GM1 ganglioside are the major variables) and put cholesterol and/or GM1 gangliosides on the vacant spots, then minimize the system energy.

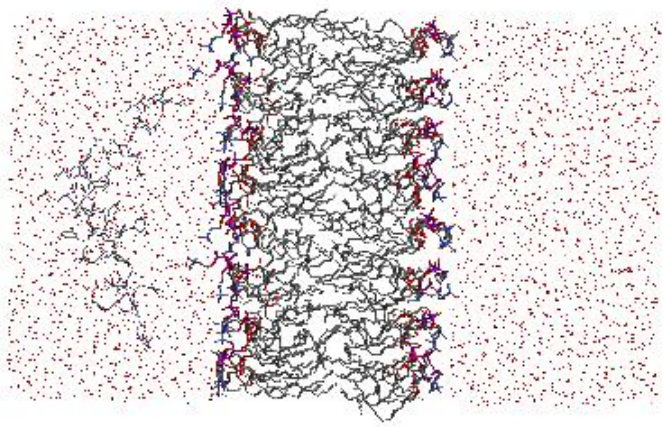


Figure 6 Aβ(1-42) protein in fully hydrated lipids; hydrogen atoms are intentionally hidden.

2.2.2 Simulation Details

Since accurate calculation of the DPPC head group area is needed and it is reasonable to give the bilayer freedom to move, we decided to use the isothermal-isobaric or NPT ensemble. We chose cvff without cross terms as our simulation force-field and fixed bond lengths for C-H and O-H in water molecules are used for the sake of saving computing load, which makes it possible to use larger time step of 2.0 fs. The water model used in this work is SPC with r_e = 0.96 Å and θ_e = 104.5 ° and the charge values of H and O atoms are +0.41e and -0.82e respectively. The simulation box has slab geometry and periodic boundary conditions are employed. The temperature and pressure damping parameters were determined from short simulations 0.1ps and 1.0ps and confirmed by the literature. Since much experimental and computational studies have been performed at around T = 323 K, simulations at the temperature are being performed for reference. The boundary condition remains periodic with constant pressure. The lateral pressure of the system or $P_{xx} = P_{yy}$ were set to -51.6 atm based on the surface tension of 28 dynes/cm or -100 atm for comparison. The normal pressure or P_{zz} was set to 1 atm. Two different methods of long-range electrostatics treatment were used and particle-particle-particle-mesh(PPPM) shows much faster simulation time without losing accuracy. Therefore, the production runs are being performed using PPPM for long-range electrostatics. To compare some of the simulation results with literature some features will be added to the LAMMPS code (c++ version), to dump out information such as the box lengths, the pair correlation function, and the order parameter calculations.

For the use of Cerius^2 and Insight II SGI machines at the department of chemistry at Texas A&M University are being used. Energy-minimizations are being done using either SGI machines or Apple G5 cluster in the Chemical Engineering department or SGI Altix 3700(COSMOS) at the Supercomputing Center at Texas A&M University. Most production runs are being performed on CAT cluster and COSMOS with multiple CPUs.

2.3 Procedure and Analysis

2.3.1 Simulation Procedure

The models of Aβ protein in hydrated lipids are created using Cerius^2 and Insight II and the data are converted into LAMMPS data file. The models systems are minimized and the production runs are being performed. Simulation time varies from several hundred ps to a few ns depending on the properties interested. Optimization of parameters such as the method of long-range treatment, applicability of multiple CPUs, and cutoff radius of the non-bond interactions is done. Target simulation time is 1-2 ns with different starting structures and positions of the protein fragment.

2.3.2 Result Analysis

We can generate movies of molecular movement which can be viewed using VMD and VideoMach. The atomic positions are written out at regular intervals, typically every 100 time steps.

The basic LAMMPS output produces the kinetic and potential energies, including the intra-molecular terms and intermolecular terms.

3 RESULTS AND DISCUSSION

3.1 Pure Water Simulations

As a first attempt to tackle the complex system of interest, MD simulations of bulk water using LAMMPS with different water models were performed. Simulations of 500 water molecules for the time span of 2 ps (with time step of 0.1 fs) yielded properties in good agreement with results from the literature.

We reached the following conclusions from the bulk water simulations: 1) With proper choice of model parameters the potential energy of water can be accurately obtained by LAMMPS and it can be used as a reliable simulation tool for bio-molecular research, 2) The potential energy of water at the temperature of 301 K and the density of 0.99567g/cm^3 was found to be –45.3 kJ/mol (HF-8, σ = 3.166Å, ε = 0.65107kJ/mol, q_O = -0.82e, q_H = 0.41e, r_e = 1.04 Å, θ_e = 104.6 ^{o}), compared with –43.8 kJ/mol (Teleman et al., 1987) Differences in the number of molecules in a simulation box, starting configurations, cutoff radii, and Ewald sum convergence criteria had little influence on the simulation results.

3.2 Aβ in Water

3.2.1 Simulation of Aβ(1-42)

A simulation box 65 Å long in each dimension was constructed. The system was minimized and a 5 ps run without fixing the bonds was performed. The time step of the system was 0.5 fs and the total potential energy of the system was found to be -72,714kcal/mol.

3.2.2 Simulation of Aβ(31-42) with α-helix conformation

The same size of simulation box as described in 3.2.1 was constructed with Aβ(31-42) α-helix conformation.

Speedup of 1.57 for the 4 CPU job compared to 2 CPU job on COSMOS was found. The 8 CPU job showed 2.32 speedup compared to 4 CPU job(not shown in this report). The potential energies on CAT with Myrinet interconnect and COSMOS were -76,654 and -76,549 kcal/mol with the same cutoffs for nonbond interactions of 10 Å for 10,000 steps with Δt of 0.5 fs.

A set of test runs of 20 ps duration, with a time step of 2 fs and the PPPM option, starting from (locally) energy-minimized structures, showed that the optimal temperature and pressure damping parameters are 0.1 ps and 1.0 ps, respectively. Figure 7 is the result chart of the test runs. These values are the choice for other researchers' work, even though they used united atom model for DPPC molecules(Tieleman and Berendsen, 1996). The potential energy of the system varies from -76,461 to -76,584 kcal/mol, which is quite close to the previous short run results.

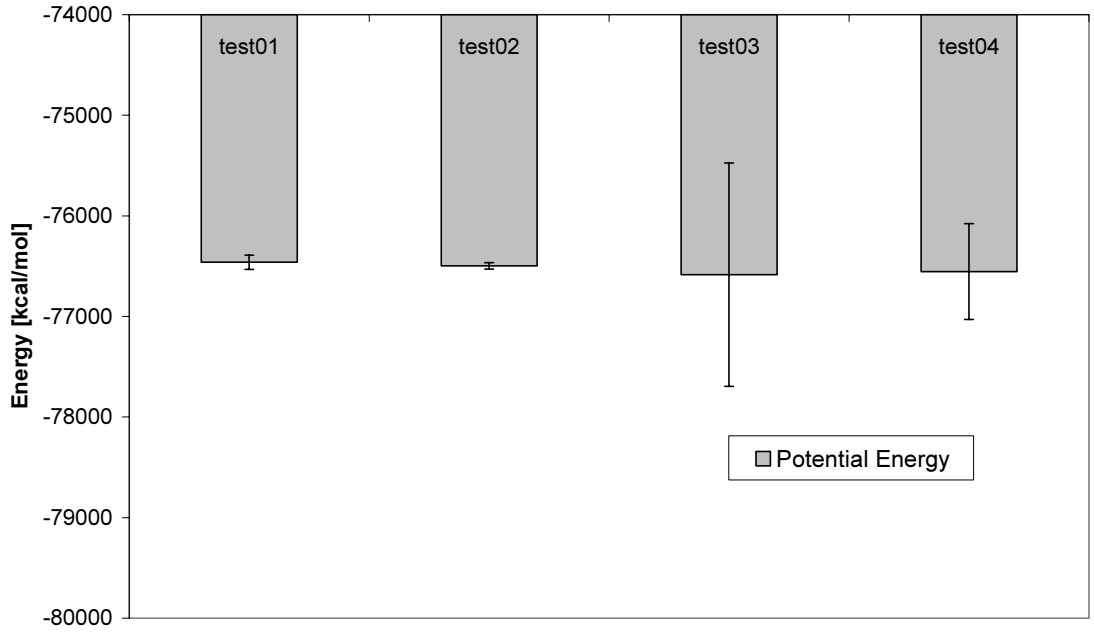


Figure 7 Damping parameters determination from the simulations of Aβ(31-42) in water; test01 represents T_{damp} = 0.1 ps, P_{damp} = 0.1 ps; test02 T_{damp} = 0.1 ps, P_{damp} = 1.0 ps; test03 T_{damp} = 1.0 ps, P_{damp} = 0.1 ps, test04 T_{damp} = 1.0 ps, P_{damp} = 1.0 ps.

Table 1 is the summary of simulation results for short MD runs with Aβ(31-42) α-helix conformation. In this table the 1st row in the 1st column is the index and the 2nd row represents the machine name and # of CPUs. Each column from the 2nd gives the energy value term by term, and the 2nd row of each production run gives the standard deviation. The temperature is well maintained at 323 K and the energy terms in each column are the same within the statistical error regardless of the choice of machine and the number of CPUs. Therefore, we can conclude that LAMMPS produces statistically similar results on those two machines.

Table 1 Comparison of short MD results for Aβ(31-42) α-helix conformation in water with different # of CPUs on COSMOS; NPT ensemble with lateral pressure of -100 atm and normal pressure of 1 atm, Δt = 2.0 fs, cutoff = 10.0 Å for both nonbond interactions and PPPM, SHAKE algorithms applied, 10000 steps or 20 ps of total time span, values are from the last 10 ps simulations.

Index/Machine	E_total	KE	Temp	PE	E_vdW	E_coul	E_long
Aβ_W-a	-55781.13	20679.78	323.01	-76460.91	10983.44	359139.84	-451988.09
CAT – 2 CPUs	±70.92	±3.94	±0.06	±70.35	±11.15	±102.76	±1.97
Aβ_W-b	-55807.63	20679.15	323.00	-76486.78	10988.05	359139.53	-451987.59
COSMOS – 1 CPUs	±42.71	±1.69	±0.03	±42.52	±32.22	±80.39	±0.93
Aβ_W-c	-55820.34	20679.33	323.00	-76499.67	10987.04	359109.28	-451988.89
COSMOS – 2 CPUs	±60.59	±6.57	±0.10	±59.40	±19.36	±54.95	±1.67
Aβ_W-d	-55827.58	20679.43	323.00	-76507.01	10991.70	359088.17	-451989.32
COSMOS – 4 CPUs	±38.86	±6.00	±0.09	±36.38	±41.79	±81.07	±0.69
Aβ_W-e	-55791.50	20677.85	322.98	-76469.35	10973.23	359180.11	-451987.73
COSMOS – 8 CPUs	±25.26	±2.58	±0.04	±24.20	±18.03	±39.42	±1.67
Aβ_W-f	-55782.53	20681.14	323.03	-76463.68	10970.95	359167.14	-451987.32
COSMOS – 16 CPUs	±31.53	±9.52	±0.15	±29.33	±25.24	±67.23	±0.92

3.2.3 Simulation of Aβ(31-42) with β-sheet conformation

As discussed in the Introduction, the β-sheet conformation is believed to play a pivotal role in the formation of Aβ aggregates. Since we probably will not be able to simulate the dynamics for a long enough period of time to observe a spontaneous conformation change from one structure, for instance α-helix, to another, for instance β-sheet, we carry out simulations with different starting structures. The protein fragment Aβ(31-42) with β-sheet conformation was hydrated and the system was minimized.

Table 2 summarizes the results from short MD runs with different starting structures. The potential energy of system with β-sheet conformation was -72,456 kcal/mol with 10.0 Å cutoff radius for the nonbond interactions, while that of the α-helix was -76,654 kcal/mol. The potential energy difference between the α-conformation and β-conformation is largely due to the Coulomb interaction or electrostatics as shown in the table. This result clearly implies that the energetic contribution to the free energy profile for different Aβ structure will be different. The magnitude of total energy for the system with α-helix conformation in this table is about 10% less than that in Table 1. And comparing the results with those in Table 1 indicates that using cutoff method for long-range electrostatics leads to simulation error and should be avoided.

Table 2 Comparison of short MD results for Aβ(31-42) in water; NVT ensemble, Δt = 0.5 fs, cutoff = 10.0 Å for both nonbond interactions, 10000 steps or 5 ps of total time span, values are from the last 2.5 ps simulations.

Index	E_total	KE	Temp	PE	E_vdW	E_coul
α-helix	-50074.15	26579.89	323.15	-76654.03	12519.58	-102189.05
	±1366.84	±58.61	±0.71	±1346.94	±35.49	±1629.12
β-sheet	-45915.41	26540.77	323.27	-72456.18	12556.42	-98068.86
	±1797.38	±107.47	±1.31	±1737.34	±89.54	±1562.28

3.3 Aβ in Hydrated Lipids

3.3.1 Simulation of Aβ(1-42)

A simulation box with 63.4×63.4×107.2 Å was created with protein hydrated in one of the water phases. We started with the Aβ(1-42) protein aligned parallel to the interface. Simulations with various parameters have been performed.

Table 3 shows the parameters and major simulation variables for this task. We tried to simulate the system with different options such as the ensemble, starting configuration, and different methods of long-range electrostatics treatment. Some simulations were not successful because of a system crash on the computer cluster.

Table 3 Parameter and conditions of production MD runs for Aβ(1-42) in hydrated lipids on CAT cluster; Δt = 2.0 fs, SHAKE algorithms applied.

Index	# of CPUs	Ensemble	Time span	Values taken	Data file	LR treatment
Aβ-A	16	NVT	140 ps	Last 80 ps	whole_run01_070105.10000	Cutoffs = 10.0 Å PPPM n=5,ε=1.0e^{-4}
Aβ-B	2	NVT	630.4 ps	Last 400 ps	data.last_mod_04	Cutoffs = 10.0 Å PPPM n=5, ε=1.0e^{-4}
Aβ-C	2	NPT Px=Py=-100, Pz=1	134.4 ps	Last 80 ps	data.last_mod_04	Cutoffs = 10.0 Å PPPM n=5, ε=1.0e^{-4}
Aβ-D	16	NPT Px=Py=-100, Pz=1	34.4 ps	Last 20 ps	data.last_mod_04	Cutoffs = 10.0 Å PPPM n=4, ε=1.0e^{-3}
Aβ-E	8	NPT Px=Py=-100, Pz=1	2 ns	Last 1 ns	data.min_091405_04	Cutoffs = 10.0 Å PPPM n=4, ε=1.0e^{-3}
Aβ-F	4	NPT Px=Py=Pz=1	22.8 ps	Last 12 ps	data.last_mod_04	Cutoffs = 15.0 Å
Aβ-G	2	NPT Px=Py=-100, Pz=1	1.8 ns	Last 1 ns	data.last_mod_04	Cutoffs = 10.0 Å PPPM n=5, ε 1.0e^{-3}

Table 4 is the corresponding result table with energy terms. There was approximately 2% difference in potential energy between NVT and NPT simulations(Aβ-B vs. Aβ-G). The comparison between the two long runs of NPT simulation(Aβ-E vs. Aβ-G) with different starting structure exhibits similar MD statistics. The PPPM accuracy criterion change effect was tested. Comparing the potential of Aβ-C with that of Aβ-D shows a difference of 2%, which is comparable to that of Aβ-E vs. Aβ-G, even though Aβ-C and Aβ-D ran for relatively short time. This indicates that the PPPM accuracy criterion of 1.0e^{-3} is acceptable choice for this study.

Table 4 Comparison of MD results for Aβ(1-42) in hydrated lipids with different simulation parameters and conditions; Δt = 2.0 fs, SHAKE algorithms applied.

Index	E_total	KE	Temp	PE	E_vdW	E_coul	E_long
Aβ - A	-34038.34	33219.00	322.98	-67257.34	9044.40	371745.90	-469204.59
	±292.22	±168.73	±1.64	±218.38	±175.86	±235.10	±11.14
Aβ - B	-34550.51	33220.60	322.99	-67771.11	9139.86	371663.14	-469206.82
	±268.21	±142.23	±1.38	±229.30	±172.95	±215.97	±10.98
Aβ - C	-31742.91	33208.39	322.88	-64951.30	9223.54	374096.68	-469152.10
	±270.01	±134.57	±1.31	±241.20	±157.60	±241.77	±10.67
Aβ - D	-30666.05	33221.83	323.01	-63887.88	9514.76	279084.41	-373745.98
	±381.30	±168.15	±1.63	±334.64	±216.70	±282.97	±8.50
Aβ - E	-32540.47	33218.82	322.98	-65759.29	8666.20	278581.76	-373236.45
	±84.65	±5.90	±0.06	±85.78	±87.55	±82.21	±2.06
Aβ - F	-27233.23	33224.16	323.03	-60457.39	8372.85	-89306.61	0.00
	±5150.77	±167.54	±1.63	±5143.66	±126.88	±5136.11	0.00
Aβ - G	-33127.37	33224.66	323.03	-66352.03	9120.39	278245.23	-373757.83
	±248.28	±147.84	±1.44	±202.24	±164.29	±250.75	±8.27

Figure 8 shows the energy profiles along the simulation time. We can conclude that 200 ps(or 10,0000 steps) of warm-up time is sufficient for the system. Moreover, several hundreds of ps simulations will definitely provide the stable thermodynamic properties.

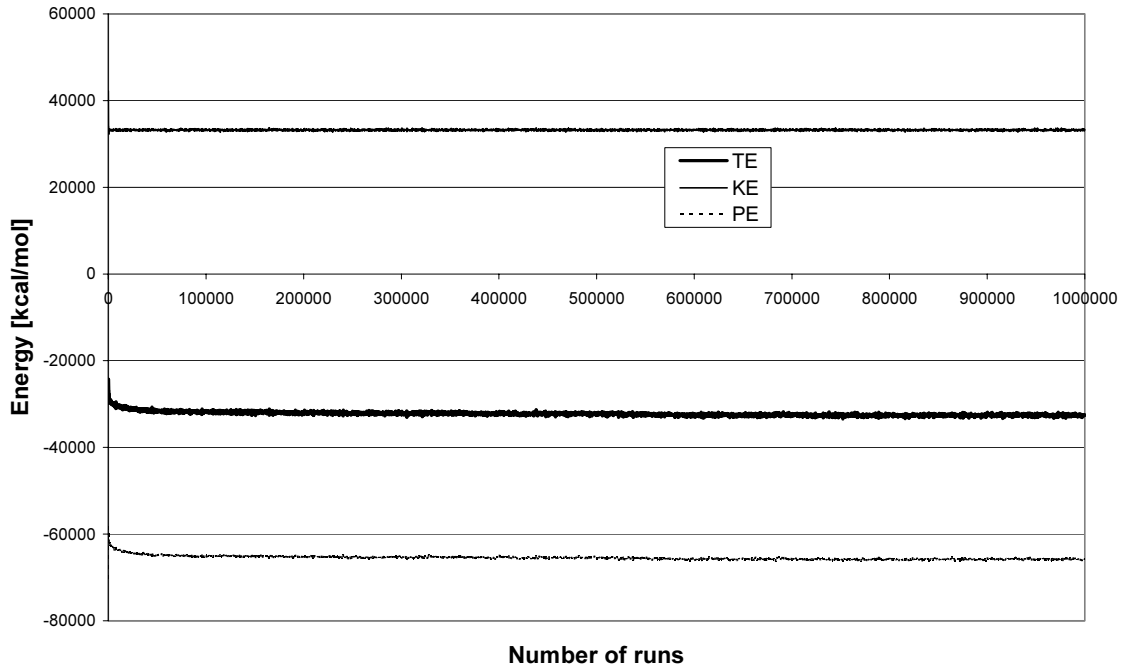


Figure 8 Energy profiles along the simulation time; Aβ-E case

3.3.2 Simulation of Aβ(31-42) with β-sheet conformation

The simulation box was created in the same way as described in the previous section. Short runs were carried out to test simulation variables. Simulations with longer runs were performed to obtain thermodynamic properties.

Figure 9 is a starting configuration of Aβ(31-42) β-conformation in the fully hydrated lipids (shown without hydrogen atoms). The protein is placed perpendicular to the water-DPPC interface.

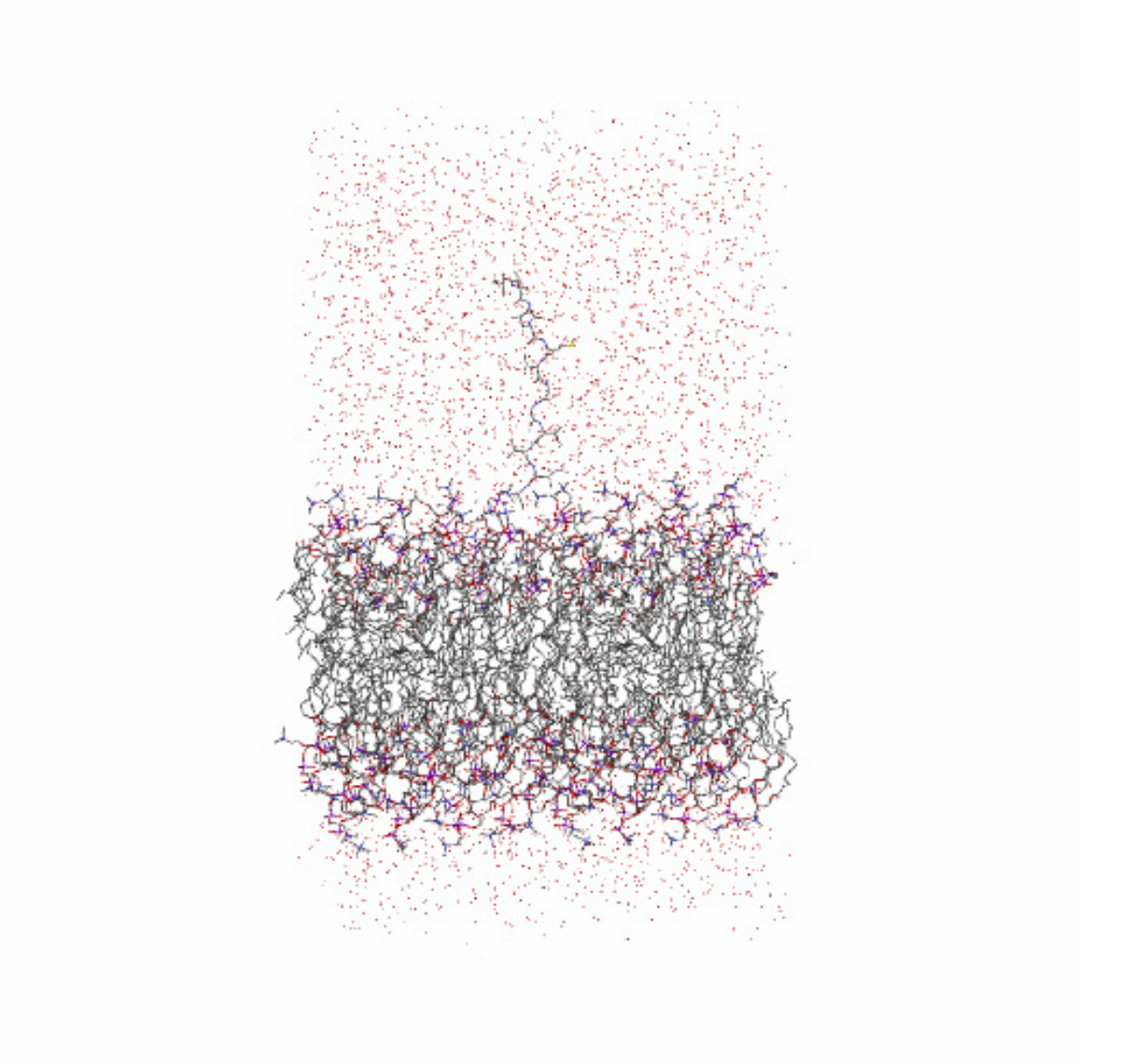


Figure 9 A snapshot of Aβ(31-42) β-conformation in hydrated lipids; the protein is close to the interface, hydrogen atoms are intentionally hidden.

Table 5 is a summary of parameters and conditions of each simulation. One of the major variables, the lateral pressure, varied from -51.6 to -100. Other major variables are the cutoff radius change and the method of long-range electrostatics.

Table 5 Parameter and conditions of production MD runs for Aβ(31-42) β-conformation in hydrated lipids; Δt = 2.0 fs, SHAKE algorithms applied.

Index	Machine # of CPUs	Ensemble	Time span	Values taken	LR treatment
Aβ-a	CAT 8	NPT Px=Py=-100, Pz=1	400 ps	Last 200 ps	Cutoffs = 10.0 Å PPPM n=4,ε=1.0e^{-3}
Aβ-b	COSMOS 8	NPT Px=Py=-51. 6, Pz=1	131.2 ps	Last 60 ps	Cutoffs = 15.0 Å PPPM n=3,ε=1.0e^{-3}
Aβ-c	COSMOS 8	NPT Px=Py=-51.6, Pz=1	20 ps	Last 10 ps	Cutoffs = 10.0 Å
Aβ-d	COSMOS 8	NPT Px=Py=-51.6, Pz=1	20 ps	Last 10 ps	Cutoffs = 10.0 Å PPPM n=4,ε=1.0e^{-4}
Aβ-e	COSMOS 8	NPT Px=Py=-51.6, Pz=1	20 ps	Last 10 ps	Cutoffs = 15.0 Å PPPM n=4,ε=1.0e^{-4}
Aβ-f	CAT 8	NPT Px=Py=-100, Pz=1	20 ps	Last 10 ps	Cutoffs = 15.0 Å PPPM n=3,ε=1.0e^{-3}
Aβ-g	CAT 8	NPT Px=Py=-100, Pz=1	20 ps	Last 10 ps	Cutoffs = 15.0 Å PPPM n=3,ε=1.0e^{-3}
Aβ-h	CAT 8	NPT Px=Py=-100, Pz=1	2 ns	Last 1 ns	Cutoffs = 15.0 Å PPPM n=3,ε=1.0e^{-3}

Data file: data.DPPC_B_frag_H2O_092705_2_min_100

Table 6 is the corresponding result summary table using the choices of Table 5. First we will focus on the short simulation results. The cutoff radius change(10 Å vs 15 Å), comparison of Aβ-d with Aβ-e, shows only about 2% difference in potential energy. The potential energy of the 10 ps to 20 ps run from Aβ-b(accuracy 1.0e^{-3}) was found to be -65,673 kcal/mol(not shown in the table), which is quite close to -64,371 kcal/mol of Aβ-e (obtained with PPPM accuracy of 1.0e^{-4}). Again, it seems that a choice of 1.0E-03 is satisfactory for the PPPM calculation.

Several production runs were done for longer simulation time, over 100 ps. The lateral pressure change doesn't affect the thermodynamic properties as can be seen from the comparison of Aβ-b and Aβ-h. This observation matches well with our physical intuition that the lipid bilayer has membrane fluidity and the system volume will not change much with the pressure change, since it is incompressible. We conclude that the cutoff radius change in the electrostatic interactions will not affect the statistics much provided that the long-range electrostatics is treated with a proper method such as PPPM. From the case simulation of Aβ-g, we found that the average head group area of DPPC is 72.55 Å^2, which is quite larger than the starting value of 62.9 Å^2. But, the result came out of very short simulation, therefore, we might see more reasonable value for longer runs. Controversy about the exact head group area has existed from both the simulations and experiments; its value from our short simulation is close to the largest value of the literature (Chiu et al., 1995; Nagle et al., 1996; Tieleman and Berendsen, 1996).

Table 6 Comparison of MD results for Aβ(31-42) β-conformation in hydrated lipids with different simulation parameters and conditions; Δt = 2.0 fs, SHAKE algorithms applied.

Index	E_total	KE	Temp	PE	E_vdW	E_coul	E_long
Aβ-a	-32979.82	33126.22	323.09	-66106.04	9249.41	281697.04	-376989.39
	±99.59	±17.88	±0.17	±83.88	±78.17	±78.35	±1.73
Aβ-b	-34049.66	33117.33	323.00	-67166.99	8573.02	152092.54	-247948.24
	±251.24	±146.52	±1.43	±204.94	±160.76	±240.35	±4.31
Aβ-c	-24172.77	33114.91	322.98	-57287.67	8081.00	-83333.57	0.00
	±1588.79	±11.20	±0.11	±1586.51	±108.15	±1806.06	0.00
Aβ-d	-29952.40	33116.74	323.00	-63069.14	9084.53	380012.16	-471449.10
	±615.06	±9.92	±0.10	±614.54	±41.95	±781.30	±15.49
Aβ-e	-31254.99	33116.44	322.99	-64371.42	8374.76	220700.08	-312734.02
	±652.59	±14.59	±0.14	±648.34	±93.96	±731.83	±4.83
Aβ-f	-31858.80	33119.21	323.02	-64978.01	8807.32	153753.40	-247941.47
	±318.67	±4.35	±0.04	±316.78	±83.02	±223.23	±1.34
Aβ-g	-31912.78	33115.71	322.99	-65028.49	8906.35	153591.42	-247941.89
	±309.00	±5.59	±0.05	±306.82	±18.51	±326.64	±2.28
Aβ-h	-34575.14	33113.96	322.97	-67689.10	8293.10	152159.13	-247944.99
	±82.86	±3.20	±0.03	±83.75	±70.72	±57.09	±0.40

Figure 10 provides the energy profiles for the total, kinetic, and potential energies. As concluded from earlier energy profile figure, we conclude that 100 ps of warm-up stage is sufficient to obtain stable MD results. To save the total computing time several hundreds of ps can be used for production runs in the future.

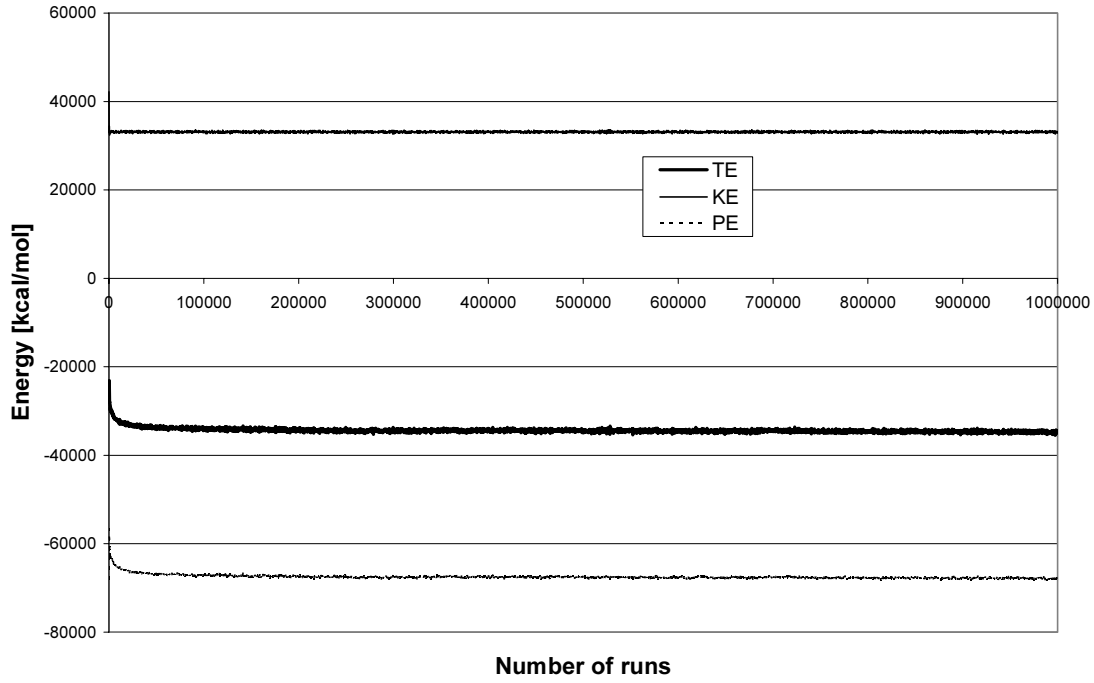


Figure 10 Energy profiles along the simulation time: Aβ-h case

4 SUMMARY AND CONCLUSIONS

From the full atomistic molecular simulation study of Aβ(1-42) protein or Aβ(31-42) protein fragment in fully hydrated lipids, we could reach the following conclusions.

(1) LAMMPS can be efficiently used for such a big system, and produces accurate results with multiple CPUs.

(2) The system can be simulated with NPT ensemble with constant surface tension boundary condition, and 500 ps of dynamics including 100 ps of warm-up produces acceptable range of MD statistics.

(3) Long-range electrostatics can be accurately treated with PPPM method combined with cutoff radius of 10 or 15 Å.

(3) Longer simulations are currently under way and the analyses including the water diffusion, the phase of lipids, etc. are under investigation.

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