

SIMULATIONS OF INTERACTIONS BETWEEN FULLERENE MONOADDUCTS AND β -AMYLOID PROTOFIBRILS VIA MOLECULAR DYNAMICS

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Resumen

La causa de la enfermedad neurodegenerativa conocida como la enfermedad de Alzheimer involucra la agregación de péptidos β -amiloide en el cerebro. Las investigaciones demuestran que el buckminsterfullereno tiene la capacidad de inhibir esta agregación, proporcionando una oportunidad para un tratamiento preventivo a la enfermedad. Aunque se han explorado las cualidades inhibitoras y las interacciones entre esta molécula de carbono y el amiloide, hay información limitada sobre las interacciones de la proteína con monoadductos de fullereno que permiten que la molécula sea soluble en agua y por lo tanto es más aplicable como un tratamiento potencial. Esta investigación profundiza en esta área de interés con la dinámica molecular, que involucró el uso de programas como GROMACS y Grace. Las simulaciones sugieren que los monoadductos de fullereno probados posiblemente tenía un efecto obstaculizaba sobre las protofibrillas del amiloide y su agregación.

Abstract

The cause of the neurodegenerative illness known as Alzheimer's Disease involves the aggregation of β -amyloid peptides in the brain. Research shows that the buckminsterfullerene has the ability to inhibit this aggregation, providing an opportunity for a preventative treatment to the disease. Although the inhibiting qualities and interactions between this carbon molecule and the amyloid have been explored, there is limited information on the protein's interactions with fullerene monoadducts that allow the molecule to be soluble in water and therefore more applicable as a potential treatment. This research delves into this area of interest with Molecular Dynamics, which involved the use of programs such as GROMACS and Grace. The simulations suggest that the the tested fullerene monoadducts had a possible hindering effect on the amyloid protofibrils and their aggregation.

Keywords

Alzheimer's Disease; Modeling; Buckminsterfullerene; GROMACS; Peptide aggregation

INTRODUCTION

Alzheimer's Disease is a neurodegenerative illness that afflicts millions of people worldwide. It can produce symptoms such as memory loss, mood swings, and difficulty speaking. No current cures to the ailment exist, but research on the development of the disease and ways to restrict its advancement is being developed. Studies have shown that patients with Alzheimer's have an accumulation of senile plaque in their brains that hinder normal cerebral functions and can be attributed to the aggregation of β -amyloid peptides in the brain. [1] The C₆₀ buckminsterfullerene has recently been made a subject of intense research in the context of Alzheimer's treatment. The unique chemical properties of this carbon-based nanoparticle allows it to be used in a variety of biological applications. Previous studies have demonstrated the efficacy of fullerenes in inhibiting the accumulation of β -amyloid plaques in the brain, establishing their use as a promising treatment for individuals who suffer from Alzheimer's disease. [2] Due to the possible applications of this molecule, the bonding sites and interactions between the buckminsterfullerene and β -amyloid protofibrils have been examined [3]. However, fullerenes are not soluble in water. Therefore, the focus of this research is on monoadduct molecules that are soluble and hence able to be utilized in the human body. Molecular Dynamics were applied in order to explore the interaction of this category of fullerene molecule with the β -amyloid fibrils and acquire more information on what makes the fullerene so effective in inhibiting the amyloid aggregation.

MATERIALS AND METHODS

Modification of Buckminsterfullerene

Previous studies have shown that the Buckminsterfullerene can effectively inhibit the aggregation of β -amyloid fibrils [3]. However, its poor solubility in water renders it ineffective in aqueous environments. In this study we predict that the fullerenes inserted into the amyloid system will have a noticeable effect on the shape of the protein, causing distortion that would make it more difficult for the peptides to aggregate. Monoadducts of the C₆₀ fullerene were produced using Avogadro, a cross-platform molecular editor and visualizer. Two distinct molecules were created for use in subsequent simulations: a C₆₀ monoadduct with a diethyl malonate substituent attached and the sodium salt of this monoadduct (Figure 1). The structure of each of these molecules was optimized and subjected to energy minimization. Version 2.2 of the Automated Topology Builder (ATB) and Repository was used to produce topology files for each monoadduct. Building block files for these molecules (including united atom and all atom models) were produced, as well as optimized geometries and interaction parameter files for the appropriate force field. The CHARMM36 force field was employed for both monoadducts and an all-atom model was applied.

Solvation of β -Amyloid Peptide (1-42)

The behavior of a single β -amyloid peptide in water was simulated using Molecular Dynamics (MD) to provide a basis for its intramolecular and intermolecular interactions. A protein database file of a 42-residue pentameric unit of β -amyloid peptide was used. It was noted that residues 1-17 are disordered, however residues 18-26 and 31-42 each form a beta sheet [4]. Together, these two residue chains produce a beta strand-turn-beta strand motif. This structure allows for intermolecular sidechain contacts between beta1 (formed by residues 18-26) and beta2 (formed by residues 31-42), which results in partially unpaired beta strands where the fibrils terminate [4]. The interaction pattern observed here provides insight into the point of contact for inhibitors of fibril growth.

A rectangular box with dimensions 5 nm x 2 nm x 2 nm was generated to accommodate the elongated structure of the β -amyloid peptide. The cut-off between the molecule and the box side was designated to be 0.5 nm and the box was solvated with TIP3P water. Energy minimization was performed to prevent structural distortion and remove artificially large forces produced by excess hydrogens and the fragmented hydrogen bond network in water. In order to neutralize the negative charge of the system, Na⁺ ions were added to the solvated box. An equilibration run was executed to prevent distortion of the amyloid upon

initiation of the MD simulation. This allows the solvent to equilibrate around the molecule as the heavy atoms of the amyloid are fixed at their initial positions. The settings used in this step are identical to those used in energy minimization. However, during equilibration pressure and temperature were constrained with the Berendsen and v-scale weak coupling algorithms.

Following equilibration, a production simulation was executed during which the pressure coupling was turned off and the position restraints were removed. A series of analyses were performed after the production simulation was complete. The root-mean-square displacement (RMSD) of the heavy atoms was measured with respect to the x-ray structure to analyze the stability of the amyloid and determine how closely it resembles the experimental structure. Graphs for the potential energy of the system as well as the RMSD values were rendered using Grace. A movie was rendered in order to visualize the trajectory of the molecules in the system.

Insertion of Fullerene Molecules

The creation of two distinct C_{60} monoadducts necessitated the execution of two separate simulations. For the C_{60} monoadduct with a diethyl malonate substituent, a box containing one β -amyloid pentamer was generated in which two identical monoadduct molecules were inserted. An identical procedure was performed for the sodium salt of this monoadduct. The box used in the equilibration of water around the amyloid molecule was replicated and modified to fit 3 nm x 3 nm x 3 nm dimensions. The energy of the new amyloid-fullerene system was minimized, solvated, and equilibrated using the methods outlined in the previous section. A production simulation was run and results were visualized in VMD.

RESULTS AND DISCUSSION

Through an analysis of the potential energy, root-mean-square deviation (RMSD), and visual representation of the Molecular Dynamics simulation, it may be concluded that both the C_{60} monoadduct with a diethyl malonate substituent and that with a sodium salt substituent had a hindering effect on the aggregation of the β -amyloid fibrils. The potential energy data verified that the simulation performed a successful minimization of the energy. It also allowed for the RMSD data prior to energy minimization to be discarded for more relevant results (Figures 5.A, 6.A, 7.A). The RMSD created a quantitative comparison of the protein at the beginning of each respective simulation to its state at a given time. In the case of the amyloid with no fullerenes present, the RMSD values fluctuated. The longest decline at approximately 6000-9000 picoseconds may be due to the inherent flexibility of protein protofibrils [5]. However, the system tended to maintain a value close to 1.3 nm (Figure 5.B). For the system including the amyloid and C_{60} monoadduct with a sodium salt substituent, the RMSD value increased over the course of the production simulation to approximately 0.7 nm (Figure 6.B). The RMSD for the system including the amyloid and C_{60} monoadduct with a diethyl malonate substituent appeared to increase as well before stabilizing at approximately 0.5 nm (Figure 7.B). The lower RMSD values in the systems with fullerene monoadducts may be attributed to the fullerene's inhibition of the protofibrils that prevented their ability to deviate from their form at the beginning of the simulation. In addition, qualitative analysis of the video simulation of the molecules over time (Figures 2-4) provides evidence of greater distortion of the amyloid in the presence of either fullerene. This change in shape that is induced by the fullerene presents yet another possible cause for inhibition of aggregation of the amyloid. The number of hydrogen bonds formed between the amyloid and solvent was calculated for each system (Figure 8). In every case, when the amyloid was in the presence of fullerenes fewer hydrogen bonds were quantified in between the water and the β -amyloid as compared to the β -amyloid system in the absence of fullerenes. This indicates a non-negligible interaction in between the fullerene monoadducts and the β -amyloid peptide. In order to support this conclusion, simulations with more than just one amyloid present could be implemented. A quantitative analysis of the interactions of these amyloids with and without the company of fullerenes might accompany and verify experimental results [1].

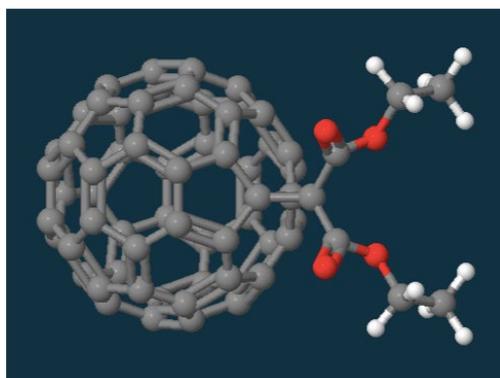
CONCLUSIONS

The accumulation of β -amyloid plaques and the presence of neurofibrillary tangles have been implicated as key components in the progression of Alzheimer's disease. Numerous studies have been conducted to address the aggregation of β -amyloid peptides and present viable treatment options. The use of fullerenes to inhibit the accumulation of these peptides has recently come into focus as a promising subject of treatment research. In this study, the behavior of a pentameric amyloid peptide in aqueous solution as well as two distinct amyloid-fullerene systems was simulated via molecular dynamics. The resulting RMSD values, along with the qualitative analysis of the simulation video and the quantification of hydrogen bonds, suggest that the fullerenes formed notable interactions with the β -amyloid peptide that may explain their inhibitory action against the accumulation of β -amyloid plaques. While these results are not conclusive, they do support previous studies that identify the use of fullerenes to impede the accumulation of β -amyloid plaques as a potential treatment for Alzheimer's disease.

APPRECIATIONS

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(A)



(B)

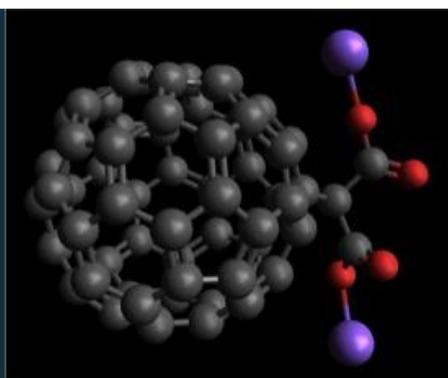
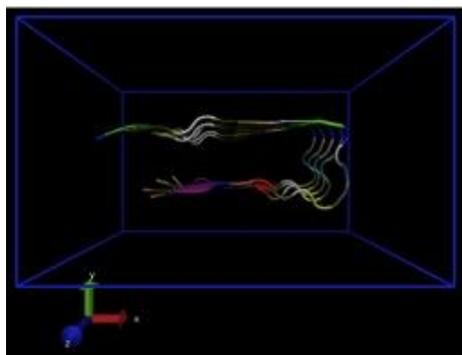


Figure 1: (A) Molecular structure of C60 monoadduct with diethyl malonate substituent. (B) Molecular structure of corresponding sodium salt

(A)



(B)

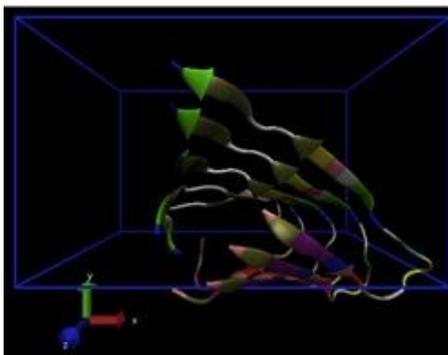


Figure 2: Depiction of the 42-residue β -amyloid pentamer in aqueous solution. (A) Representation of this protein in the initial position of the simulation. (B) Representation of β -amyloid at the end of the production simulation.

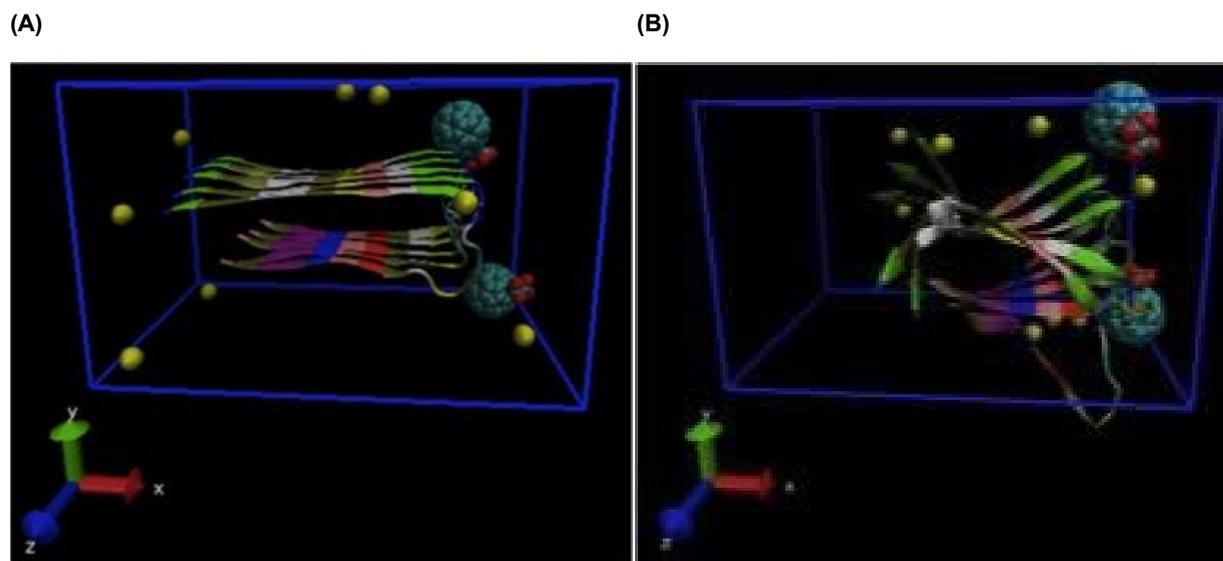


Figure 3: Depiction of the β -amyloid and C_{60} monoadducts with a sodium salt substituent in aqueous solution. The yellow atoms are representative of sodium ions that both neutralized the negative charge of the amyloid and accompanied the fullerene substituent. (A) Representation of the initial configuration of the system. (B) Representation of the system at the end of the production simulation.

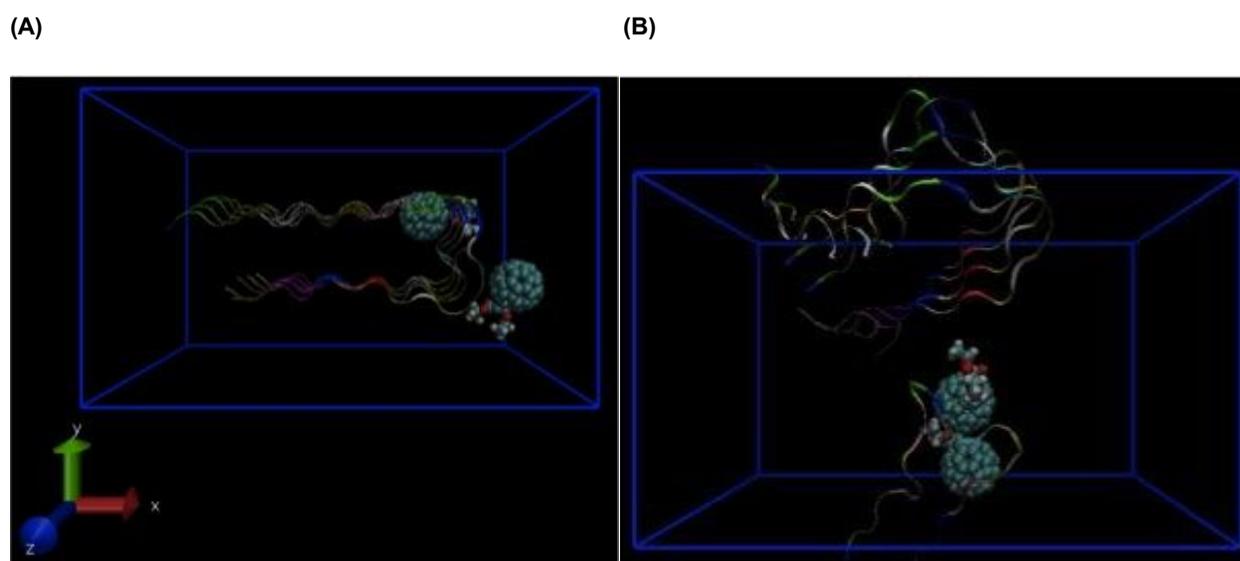


Figure 4: Depiction of the β -amyloid and C_{60} monoadducts with a diethyl malonate substituent in aqueous solution. (A) Representation of the initial configuration of the system. (B) Representation of the system at the end of the production simulation.

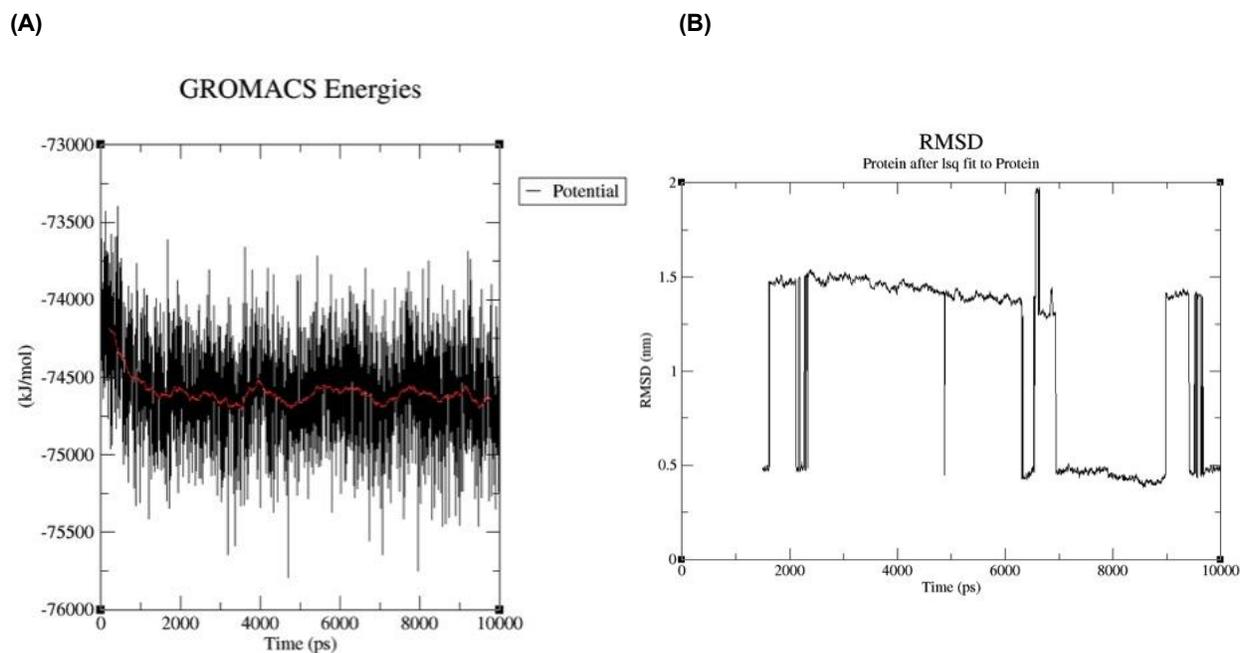


Figure 5: (A) Potential energy plot for β -amyloid with no fullerenes inserted. Running averages of these values are depicted by the red line. (B) Plot of the root-mean-square deviation (RMSD). RMSD values collected before energy minimization were removed.

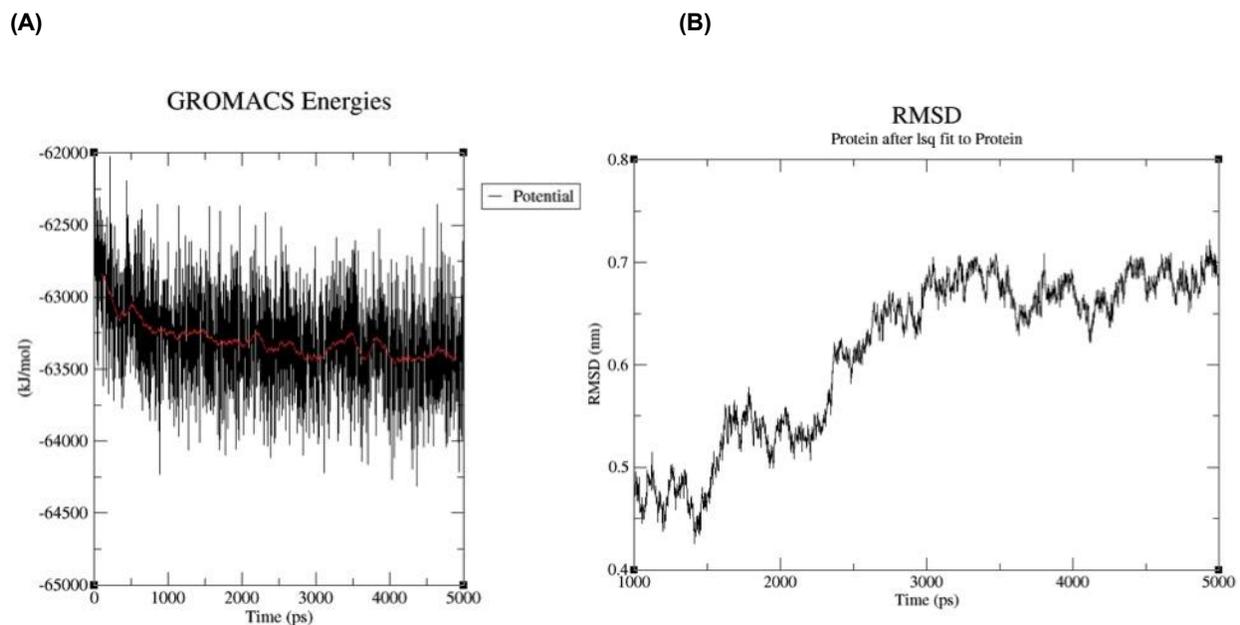


Figure 6: (A) Potential energy plot for β -amyloid and C_{60} monoadducts with a sodium salt substituent. Running averages of these values are depicted by the red line. (B) Plot of the root-mean-square deviation (RMSD). RMSD values collected before energy minimization were removed.

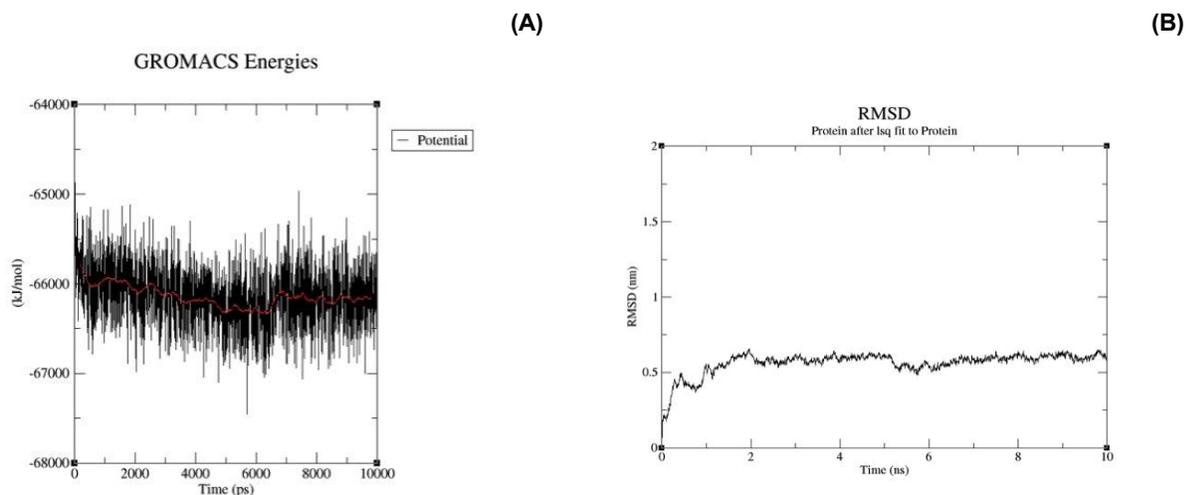


Figure 7: (A) Potential energy plot for β -amyloid and C_{60} monoadducts with a diethyl malonate substituent. Running averages of these values are depicted by the red line. (B) Plot of the root-mean-square deviation (RMSD).

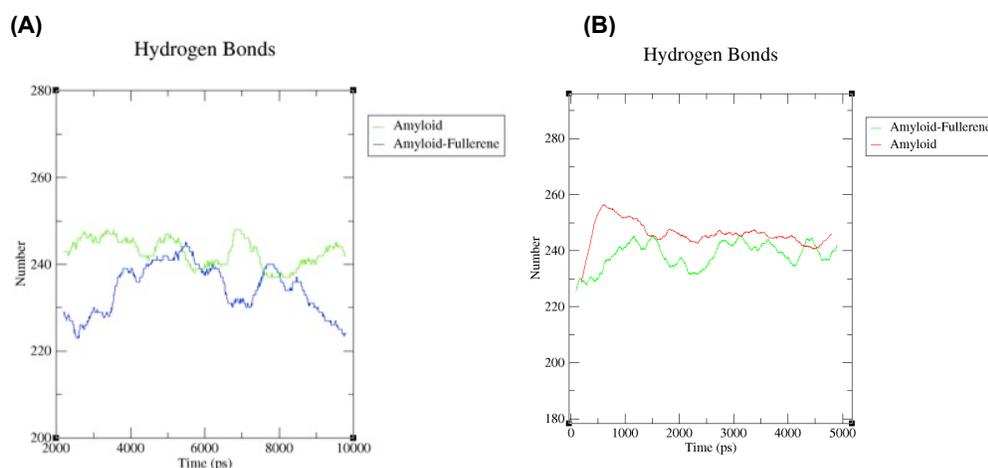


Figure 8: Plot of hydrogen bonds formed within the amyloid system. The number of hydrogen bonds formed between the β -amyloid peptide and water both in the presence and absence of fullerenes was quantified over the course of the simulation. (A) Plot for the system of β -amyloid and C_{60} monoadducts with a diethyl malonate substituent. (B) Plot for the system of β -amyloid and C_{60} monoadducts with a sodium salt substituent.

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