THE EFFECT OF 3,4-DIHYDROXYMANDELIC ACID AND NORMETANEPHRINE ON AMYLOID-BETA 40 MONOMER AGGERGATION IN ALZHEIMER'S DISEASE USING MOLECULAR MODELING

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LIST OF SYMBOLS AND ABBREVIATIONS

3,4-Dihydroxymandelic Acid	DHMA
Normetanephrine	NMN
Amyloid Beta 40	Ab40
Root Mean Square Deviation	RMSD
Define Secondary Structure of Protein	DSSP

SUMMARY

Alzheimer's disease is the one of the most common types of degenerative dementia. It is known to cause memory loss and loss of other intellectual abilities. The formation of neurotoxic plaque composed of amyloid beta fibrils has been found in a relatively high portion of Alzheimer patient's brains. Investigation into the mechanism of beta amyloid protein aggregation discovered that the initial amyloid beta monomer structure misfolds to form oligomers and fibrils as the disease progresses.

3,4 – Dihydroxymandelic acid and normetanephrine are metabolites of norepinephrine found in the brain. These chemical have been found to have an effect on fibril formation and aggregation through *in vitro* experiments. In this study, molecular dynamic modeling methods will be used to discover the effect of 3,4 – dihydroxymandelic acid and normetanephrine have on the amyloid beta 40 monomer as well as try to understand its mechanism.

CHAPTER 1

INTRODUCTION

Alzheimer's disease is a form of dementia that causes memory loss and loss of other intellectual abilities. A potential cause of Alzheimer's disease is the formation of amyloid beta plaques. It was found that the mechanism of amyloid beta protein aggregation starts with the amyloid beta monomer. This structure, which is originally in the alpha helix form, then misfolds to form soluble oligomers and insoluble fibrils. The insoluble fibrils can be associated with beta sheet structures and can result in the formation of plaques. These plaques can increase neurotoxicity.[1] It was found in Dr. Jin Ryoun Kim's lab at the Tandon School of Engineering at New York University that 3,4 dihydroxylmandelic acid (DHMA) and noremetanephrine (NMN) had an effect on amyloid beta 40 fibril formation and aggregation. Further research is being conducted to test the extent of it's efficiency. These chemicals are a metabolite of norepinephrine and are found in the brain. In this study, we will look at DHMA and NMN's ability to inhibit the formation of these insoluble fibrils and maintain the alpha helix structure and try to discover the mechanism of these chemicals.

Our work, along with other efforts in drug screening for targeting amyloid beta fibril formation, will hopefully advance our understanding of the role of amyloid beta fibrils in the pathogenesis of Alzheimer's disease and could one day contribute to the rational design of new therapeutics.

CHAPTER 2

MATERIALS AND METHODS

The amyloid beta 40 protein will be downloaded from the pdb bank online (PDB ID: 1BA4). DHMA will be created using Cerius2. The chemical will then be run through Jaguar for DFT analysis and charge optimization. The following conditions were set for simulation: Functional theory: B3LYP, Basis: 6-31G**, Charge Analysis: Mulliken, Total Charge: -2, and Spin Multiplicity: 1. After DFT, the approximate binding sites of the drug and protein will be found using AutoDock. MD simulations will be done using those binding sites and using the AMBER 99SB and drieding forcefield. All analysis will be done using GROMACS.[3]

CHAPTER 3

RESULTS

RMSD

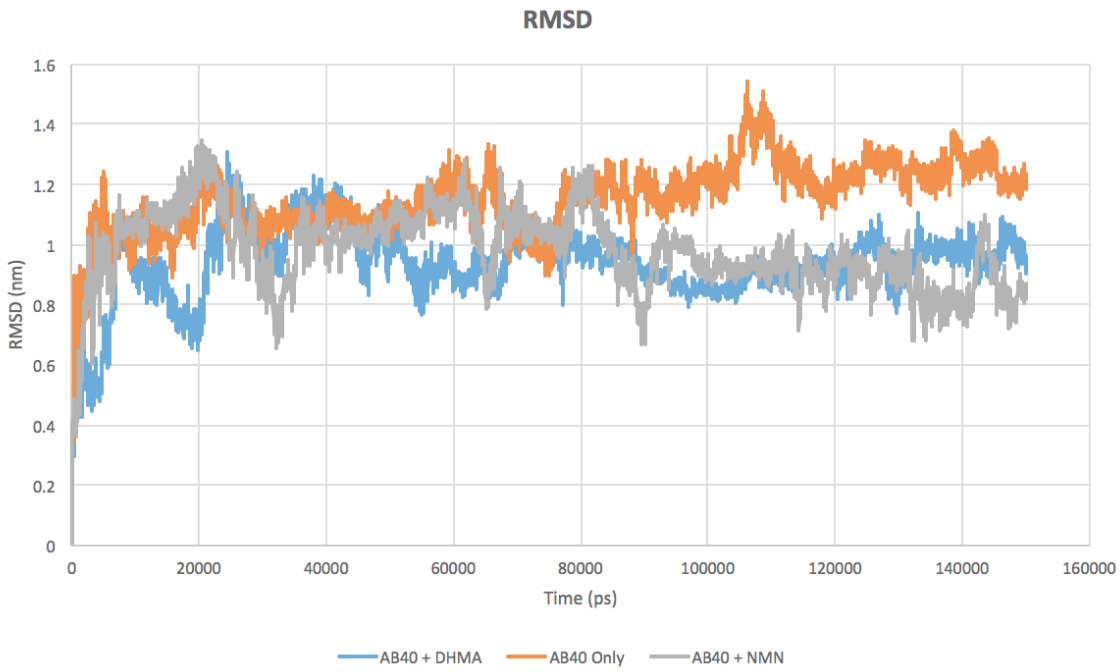

Figure 1. RMSD for Amyloid beta 40 only and Amyloid beta 40 with DHMA and NMN after 150 ns of simulation time

The RMSD for amyloid beta 40 only shows an initial increase in values and then fluctuates around the value 1.2. The RMSD for DHMA and Amyloid Beta 40 shows an initial increase in values and then fluctuates around the value 1. The RMSD for NMN and Amyloid Beta 40 shows a larger decrease in RMSD value.

TRAJECTORY ANALYSIS

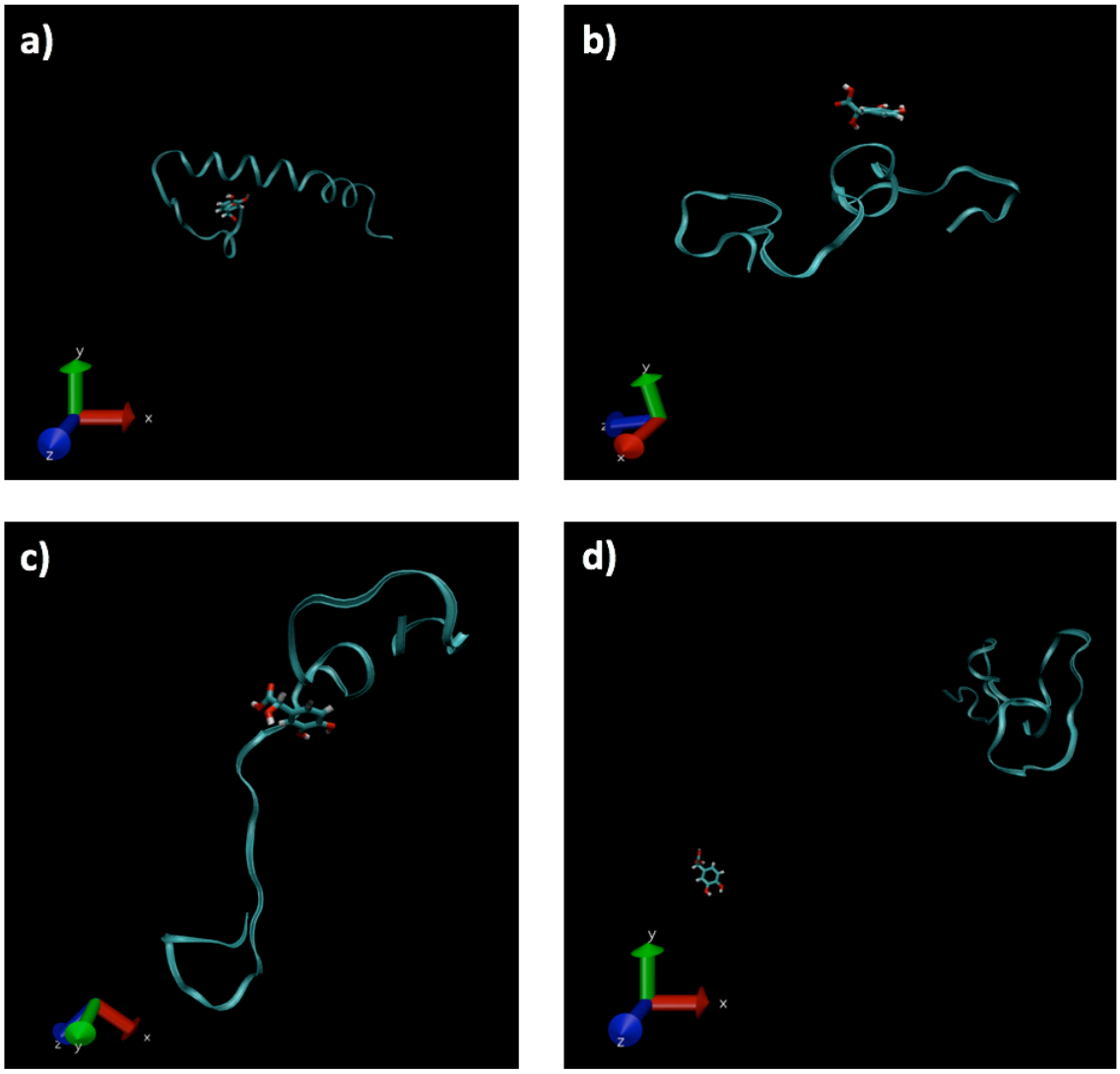

Figure 2. Trajectories of DHMA and Amyloid beta 40. A) 0 ns B) 50 ns C) 100 ns D) 150 ns

The trajectories of Amyloid Beta 40 with DHMA show an initial loss of structure but the maintenance of some of the alpha helical shape of the in the original protein structure.

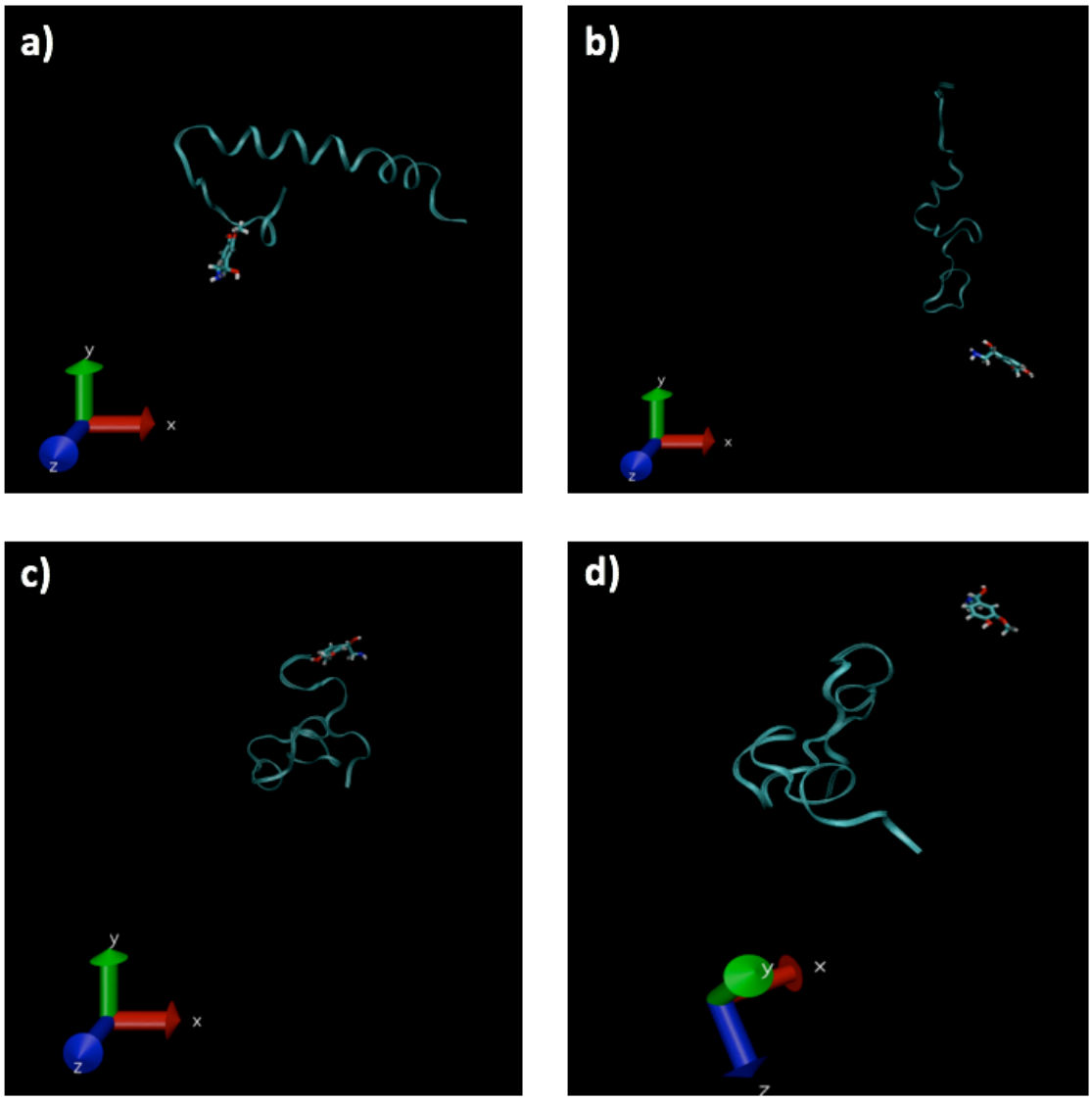

Figure 3. Trajectories of NMN and Amyloid beta 40. A) 0 ns B) 50 ns C) 100 ns D) 150 ns

The trajectories of Amyloid Beta 40 with NMN show a loss of the amyloid beta structure.

DSSP

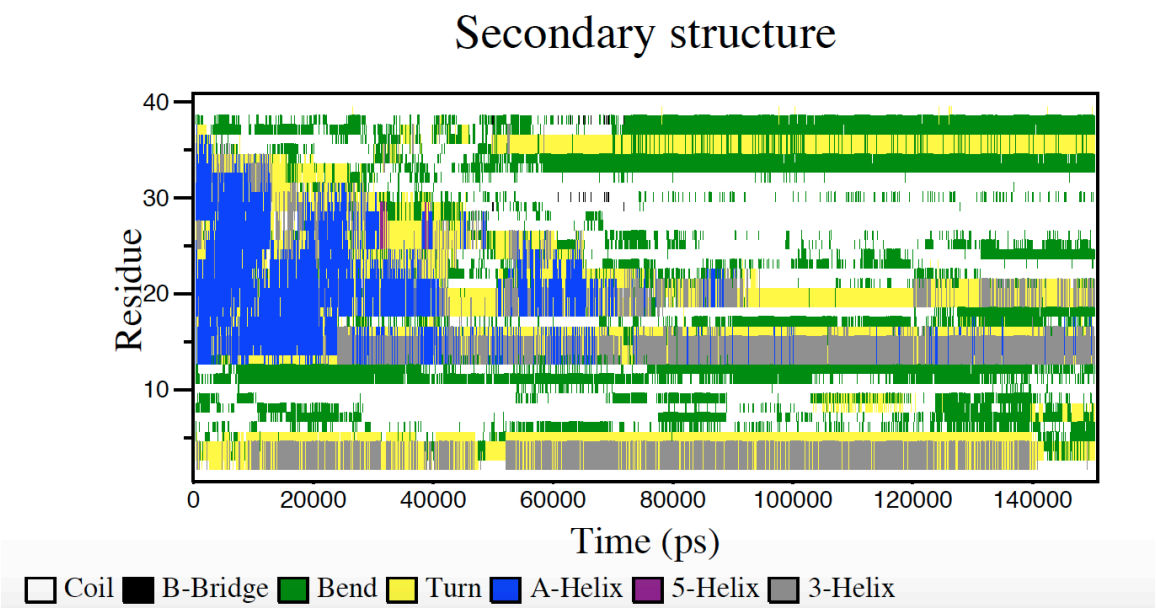

Figure 4. DSSP of DHMA and Amyloid beta 40 after 150ns of simulation.

The DSSP for DHMA with amyloid beta 40 shows that the alpha helix structure of the protein was not maintained and that the formation of beta sheets was prevented.

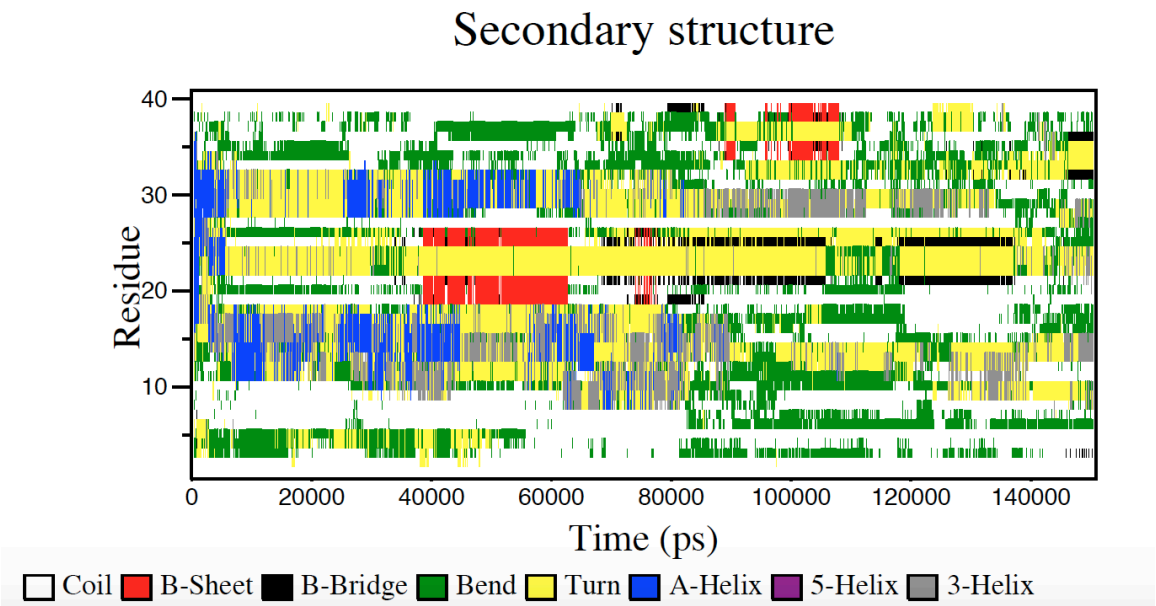

Figure 5. DSSP of NMN and Amyloid beta 40 only after 150ns of simulation.

The DSSP for NMN and Amyloid beta 40 shows loss of the alpha helix structure and the formation of beta sheets around 4000 ns and again at 9000 ns. These beta sheets are not maintained through out the remainder of the simulation time.

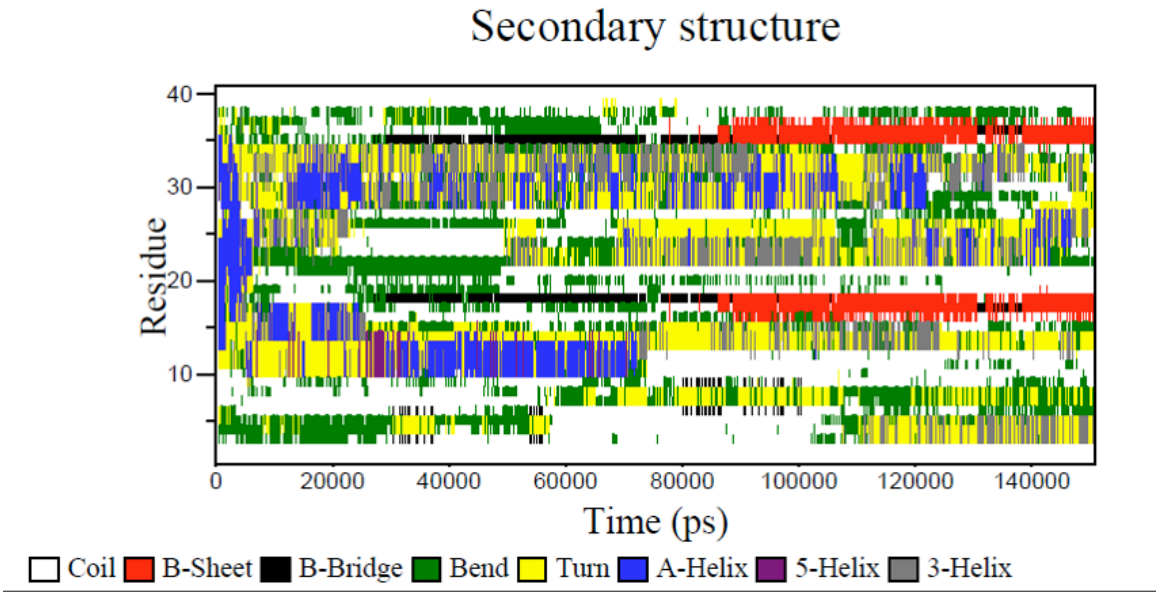

Figure 6. DSSP of Amyloid beta 40 only after 150ns of simulation.

The DSSP for Amyloid beta 40 only shows the formation of beta sheets and the loss of the alpha helix structure.

MINIMUM DISTANCE

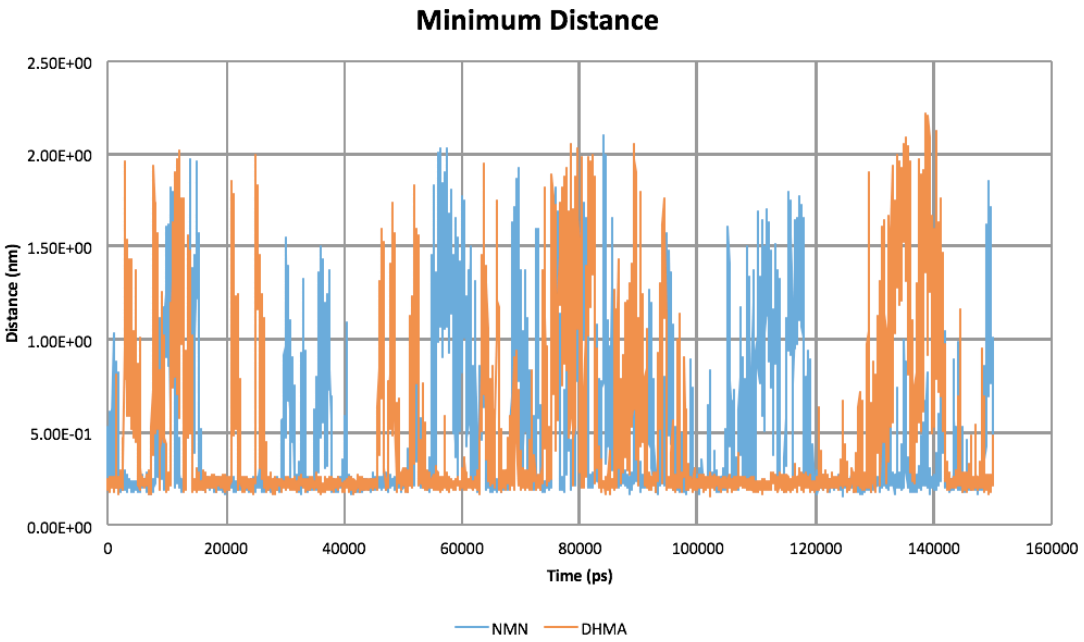

Figure 7. Minimum distance of DHMA and NMN to Amyloid beta 40 after 150ns of simulation.

The minimum distance shows that for both chemicals there is a large fluctuation in the minimum distance. At times it is bound very closely and some portions of the simulation time show that the distance is 2 nm. The average minimum distance for DHMA was 0.493 nm and for NMN it was 0.483 nm.

CHAPTER 4

DISCUSSION AND CONCLUSIONS

The RMSD results for DHMA show a small difference between the control, where as the results for NMN show an even larger difference. A change in the RMSD means that there was a change in the structure of the backbone. The RMSD data shows that there is a decrease in RMSD when comparing the experimental groups to the control, meaning that there was less change in the backbone. This difference is not significant, but does show that DHMA and NMN may have capabilities of stabilizing the structure and preventing more changes to occur. These results also show that NMN may have stronger capabilities of changing the backbone than DHMA.

The DSSP results show that DHMA is effectively able to prevent the formation of beta sheets. However, it is unable to maintain the alpha helix structure. The trajectory images supplement the DSSP data by showing that there is no formation of beta sheets within the simulation. The DSSP results for NMN show that it does not maintain the alpha helix structure and that beta sheets were formed. These beta sheets were not maintained through out the simulation time. Based on these results, DHMA is better at preventing the formation of beta sheets and was able to maintain the alpha helix structure longer than NMN.

The minimum distance results show how close the chemicals were to the protein. The values for both chemicals were very similar and show strong binding to the protein. Both chemicals also show fluctuations in minimum distance. More analysis would have to be done to fully understand the fluctuations.

In conclusion, DHMA is better able to prevent the formation of beta sheets, while NMN was able to maintain a more stable structure with respect to the backbone. More analysis needs to be done to better understand which chemical would be better at treating Alzheimer's disease.

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