Quantum Biology? From DNA Point Mutations to Fibril Formation in Alzheimer Disease

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QUANTUM BIOLOGY? FROM DNA POINT MUTATIONS TO FIBRIL FORMATION IN ALZHEIMER DISEASE

by

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M.Sc University of Central Florida 2017
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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry of the College of Sciences at the University of Central Florida Orlando, Florida

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Major Professor: Florencio Hernández
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ABSTRACT

In the ‘60s, Löwdin explored the realm of Quantum Biology. Löwdin defined Quantum Biology as the use of quantum mechanics to study the activity and molecular properties of biologically active molecules such as DNA, RNA, and proteins. This dissertation aims to describe molecules of biological interest such as DNA base pairs and amyloid-beta peptides and treat them using a quantum mechanical approach. We used electronic structure methods to achieve the quantum biological nature of these molecules under our computational power, tools, and techniques. In chapter 2, we present the quantum mechanical description of the spontaneous mutations in DNA base pairs using Wigner's tunneling corrections. We demonstrated that tunneling corrections are essential for the mutation description, where the GC complex is more likely to mutate, showing a larger mutation rate in a low polarity media. Following the principles of quantum biology, in chapter 3 and chapter4, we provide a compressive study on the amyloid-beta 1-42 (Aβ1-42) and Aβ25-35 fibril formation, respectively. Our findings show that the monomers present an L-S and hairpin-like topology, respectively, and an intense out of the plane dipole moment. The protofibrils present a perfectly axial dipole moment centered in the center of the pore-like structure. Based on our findings, we hypothesize that the aggregation mechanism is electric dipole assisted and follows a nucleated polymerization and a conformational conversion route, respectively. In chapter 5, we introduce the calcium ion-channel capability of Aβ1-42 and Aβ25-35 peptides using molecular dynamics simulations. Our research demonstrated that both peptides could form ion-conducting channels. Though in the case of Aβ1-42, only the protofibrils can create the channels in the gas, condensate, and membrane incrustated conditions, while Aβ25-35
can only form channels in its monomeric form. The recent results show an additional pathway for cytotoxicity in Alzheimer’s disease that agrees with experimental findings.
To My Family, Science, and the World.

Making a better place for all of us!
ACKNOWLEDGMENTS

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# TABLE OF CONTENTS

LIST OF FIGURES  ........................................................................................................ X

LIST OF TABLES ........................................................................................................ XIV

LIST OF ACRONYMS (OR) ABBREVIATION ................................................................ XV

CHAPTER 1. INTRODUCTION .................................................................................. 1

1.1 Fundamentals of Electronic Structure Methods .................................................. 7
  1.1.1 The Hamiltonian ....................................................................................... 7
  1.1.2 The Born-Oppenheimer Approximation .................................................... 9
  1.1.3 The Hartree-Fock Formalism .................................................................. 9
  1.1.4 The Density Functional Theory .............................................................. 12
  1.1.5 The Quantum Tunneling Effect .............................................................. 15
  1.1.6 The Polarizable Continuum Model ........................................................ 22

1.2 Molecular Optical Properties .......................................................................... 24
  1.2.1 One-Photon Absorption ....................................................................... 24
  1.2.2 Electronic Circular Dichroism .............................................................. 25
  1.2.3 Two-Photon Absorption ....................................................................... 26
  1.2.4 Two-Photon Circular Dichroism .......................................................... 30

1.3 References ......................................................................................................... 33

CHAPTER 2. SOLVENT EFFECT ON THE INTERMOLECULAR PROTON TRANSFER OF THE WATSON AND CRICK GUANINE-CYTOSINE AND ADENINE-THYMINE BASE PAIRS: A POLARIZABLE CONTINUUM MODEL STUDY ...................................... 40

2.1 Introduction ...................................................................................................... 40

2.2 Computational Methods ................................................................................. 47

2.3 Results and Discussion .................................................................................... 49
  2.1.1 AT and GC Gas-phase. ....................................................................... 49
  2.1.2 AT and GC Condensed-phase. ............................................................ 53

2.4 Conclusions ..................................................................................................... 69

2.5 References ...................................................................................................... 69

CHAPTER 3. ROLE OF THE AMYLOID-β(1-42) ELECTRIC DIPOLE MOMENT ON FIBRILS FORMATION ............................................................... 73
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Introduction</td>
<td>74</td>
</tr>
<tr>
<td>3.2</td>
<td>Theoretical Methodology</td>
<td>77</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Structures Optimization</td>
<td>77</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Energy and Dipole moment</td>
<td>78</td>
</tr>
<tr>
<td>3.2.3</td>
<td>Molecular Dynamics Simulations</td>
<td>80</td>
</tr>
<tr>
<td>3.3</td>
<td>Results and Discussion</td>
<td>81</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Monomer</td>
<td>81</td>
</tr>
<tr>
<td>3.3.2</td>
<td>Stacked Oligomers</td>
<td>84</td>
</tr>
<tr>
<td>3.3.3</td>
<td>Protofibrillar Arrangements</td>
<td>87</td>
</tr>
<tr>
<td>3.3.4</td>
<td>Fibril Formation Mechanism</td>
<td>92</td>
</tr>
<tr>
<td>3.4</td>
<td>Conclusions</td>
<td>99</td>
</tr>
<tr>
<td>3.5</td>
<td>References</td>
<td>99</td>
</tr>
</tbody>
</table>

**CHAPTER 4.** AXIAL ELECTRIC DIPOLE MOMENT AND ITS ROLE OF THE AMYLOID-
β_{25-35} CYTOTOXICITY | 108 |
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Introduction</td>
<td>109</td>
</tr>
<tr>
<td>4.2</td>
<td>Theoretical Methodology</td>
<td>114</td>
</tr>
<tr>
<td>4.2.1</td>
<td>Structures Optimization</td>
<td>114</td>
</tr>
<tr>
<td>4.2.2</td>
<td>Energy and Dipole Moment</td>
<td>115</td>
</tr>
<tr>
<td>4.2.3</td>
<td>Molecular Dynamics Simulations</td>
<td>116</td>
</tr>
<tr>
<td>4.2.4</td>
<td>One, Two-photon (OPA, TPA), ECD and Two-photon Circular Dichroism (TPCD) Simulations</td>
<td>116</td>
</tr>
<tr>
<td>4.3</td>
<td>Results and Discussion</td>
<td>119</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Monomer</td>
<td>119</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Stacked Oligomers</td>
<td>125</td>
</tr>
<tr>
<td>4.4</td>
<td>Conclusion</td>
<td>141</td>
</tr>
<tr>
<td>4.5</td>
<td>References</td>
<td>142</td>
</tr>
</tbody>
</table>

**CHAPTER 5.** ROLE OF THE AMYLOID β_{1-42} AND β_{25-35} DIPOLE MOMENT ON THE FORMATION OF SELECTIVE ION CHANNELS | 152 |
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Introduction</td>
<td>153</td>
</tr>
<tr>
<td>5.2</td>
<td>Theoretical Approach</td>
<td>156</td>
</tr>
<tr>
<td>5.3</td>
<td>Results and Discussion</td>
<td>157</td>
</tr>
<tr>
<td>5.3.1</td>
<td>AB_{1-42} Pore formation</td>
<td>157</td>
</tr>
<tr>
<td>Section</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>5.3.2</td>
<td>Aβ_{25-35} Pore Formation</td>
<td>171</td>
</tr>
<tr>
<td>5.4</td>
<td>Conclusions</td>
<td>177</td>
</tr>
<tr>
<td>5.5</td>
<td>References</td>
<td>178</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 1-1. Quantum Rectangular Barrier. The barrier energy is larger than the particle kinetic energy. The incident reflected, and transmitted wavefunctions are shown in black, gold, and red, respectively. The green curve shows the exponentially decaying wavefunction in the interior of the barrier. 15

Figure 1-2. Tunneling in a chemical reaction. Lines indicated in red and green are the vibrational levels of the reagents and products, respectively. A purple line shows the approximated wavefunction of the atom tunneling. 18

Figure 1-3. Arrhenius plot of classical and a quantum tunneled reaction. The green dotted line represents the classical Arrhenius plot, while the solid red line represents a chemical reaction with tunneling at low temperatures. 20

Figure 1-4. Simplified Jablonski diagram showing one and two-photon processes. 24

Figure 1-5. Open aperture Z-scan setup. 27

Figure 1-6. Open aperture Z-Scan curve. 28

Figure 1-7. Experimental L-Scan setup. 30

Figure 2-1. Double proton transfer (DPT) reaction in the AT base pairs. This reaction could take place via a concerted (CDPT) or through a stepwise mechanism with two different proton transfer steps (SP1 and SP2) achieving the DPT tautomers A* and T* (AT2 complex). 43

Figure 2-2. Double proton transfer (DPT) reaction in the GC base pairs. This reaction could take place via a concerted (CDPT) or through a stepwise mechanism with two different proton transfer steps (SP1 and SP2) achieving the DPT tautomers G* and C* (GC2 complex). 45

Figure 3-1. Structures of the Aβ(1-42) peptide. (A) Monomer. (A/A) Stacked parallel dimer. (A/B) Stacked antiparallel dimer. (A/A/A) Stacked parallel trimer. (AA') Parallel protofibril. (AB) Antiparallel protofibril. (AB/A) Prototibril trimer. (AB/AB) Protofibril tetramer. A blue and green arrow represents individual dipole moments. The center Bottom figure represents the sphere surface of the AB/AB tetramer. 77

Figure 3-2. Structure of Aβ1-42 monomer. (A) Cartoon backbone structure of Aβ1-42 monomer (Top view), hydrogen atoms are omitted. (B) Cartoon Structure of Aβ1-42 monomer (Front view). (C) Sphere surface of Aβ1-42 monomer (Front view). The electric dipole moment is represented by a red cylinder arrow. 82

Figure 3-3. Structure of Aβ1-42 stacked parallel dimer (A/A). (A) Cartoon and sphere representation of A/A (Top view), hydrogen atoms are omitted. (B) Cartoon Structure of A/A (Front view). (C) Sphere surface of A/A (Front view). The net electric dipole moment is shown by a red cylinder arrow. Single dipoles are indicated by Blue (Bottom) and Green (Top) cylinder arrows. 86

Figure 3-4. Structure of Aβ1-42 antiparallel protofibril (AB). (A) Cartoon backbone structure of AB (Top view), hydrogen atoms are omitted. (B) Cartoon Structure of AB (Side perspective-view). (C) Sphere surface of AB (Side perspective-view). The net electric dipole moment is shown by a
red cylinder arrow. Individual dipole moments are indicated by blue (Front chain) and green (Back chain) cylinder arrows.-----------------------------------------------89

Figure 3-5. Structure of Aβ1-42 protofibril tetramer (AB/AB). (A) Spheres and cartoon structure of AB/AB (Top view), hydrogen atoms are omitted. (B) Cartoon Structure of AB/AB (Front view). (C) Sphere surface of AB/AB (Front view). A red cylinder arrow shows the net electric dipole moment. The lower layer dipole moment is indicated with a blue cylinder arrow, and the high layer dipole moment is indicated with a green cylinder arrow. -----------------------------------------------92

Figure 4-1. Structures of the Aβ(35-35) peptide. (A) Monomer. (A/A) Stacked Parallel Dimer. (A/B) Stacked Antiparallel Dimer. (A/A') Parallel Protofibril. (AB) Antiparallel Protofibril. (AB2) Protofibril Dimer. (AB3) Protofibril Trimer. (AB4) Protofibril Tetramer. (AB5) Protofibril Pentamer. -----------------------------------------------113

Figure 4-2. Structure of Aβ25-35 monomer. (A) Cartoon backbone of Aβ25-35 monomer (Top view), hydrogen atoms are omitted. (B) Cartoon structure of the Aβ25-35 monomer (Front view). (C) Sphere surface of Aβ25-35 monomer (Front view). A red cylinder arrow represents the electric dipole moment. (D) Ramachandran plot of the Aβ25-35 monomer.-----------------------------------------------121

Figure 4-3. (A) Cartoon backbone of Aβ35-35 monomer (HF/STO-3G) (ice blue chain) in contrast with the of Aβ1-42 (PM6) (green chain). (B) Aβ25-35 MD snapshot at 0 ns in gas phase (C) Aβ25-35 MD snapshot at 20 ns in gas phase. (D) Energy profile during the 20 ns of MD simulation of Aβ25-35 in gas phase. (E) RMSD change during 20 ns of MD simulation of Aβ25-35 in gas phase. ----123

Figure 4-4. (A) Aβ25-35 MD snapshot at 0 ns in the condensate phase with TIP3P water molecules (B) Aβ25-35 MD snapshot at 28.8 ns in the condensate phase with TIP3P water molecules. (C) Energy profile during the 28.8 ns of MD simulation of Aβ25-35 in the condensate phase with TIP3P water molecules. (D) RMSD change during 28.8 ns of MD simulation of Aβ25-35 in the condensate phase with TIP3P water molecules. -----------------------------------------------124

Figure 4-5. Structure of Aβ25-35 Parallel stacked dimer A/A. (A) Cartoon backbone of A/A (Top view), hydrogen atoms are omitted. (B) Cartoon structure of A/A (Front view). (C) Sphere surface of A/A (Front view). (D) Ramachandran plot of A/A. A red cylinder arrow represents the electric dipole moment.-----------------------------------------------127

Figure 4-6. Structure of Aβ25-35 Antiparallel protofibril AB. (A) Cartoon backbone of AB (Top view), hydrogen atoms are omitted. (B) Cartoon structure of AB (Front view). (C) Sphere surface of AB (Front view). (D) Ramachandran plot of AB. A red cylinder arrow represents the electric dipole moment.-----------------------------------------------129

Figure 4-7. (A) Aβ25-35 Protofibril MD snapshot at 0 ns in gas phase (B) Aβ25-35 Protofibril MD snapshot at 20 ns in gas phase. (C) Energy profile during the 20 ns of MD simulation of Aβ25-35 Protofibril in gas phase. (D) RMSD change during 20 ns of MD simulation of Aβ25-35 Protofibril in gas phase. -----------------------------------------------132

Figure 4-8. (A) Aβ25-35 Protofibril MD snapshot at 0 ns in condensate phase with TIP3P water molecules (B) Aβ25-35 Protofibril MD snapshot at 20 ns in condensate phase with TIP3P water molecules. (C) Energy profile during the 20 ns of MD simulation of Aβ25-35 Protofibril in condensate phase with TIP3P water molecules. (D) RMSD change during 20 ns of MD simulation of Aβ25-35 Protofibril in condensate phase with TIP3P water molecules. -----------------------------------------------133
Figure 4-9. Structure of Aβ25-35 Protofibril Dimer AB2. (A) Cartoon backbone of AB2. (Top view), hydrogen atoms are omitted. (B) Cartoon structure of AB2. (Front view). (C) Sphere surface of AB2. (Front view). (D) Ramachandran plot of AB2. A red cylinder arrow represents the electric dipole moment. 135

Figure 4-10. Spectra of Aβ25-35. (A) Theoretical UV-Vis spectra of Aβ25-35. (B) Theoretical ECD spectra of Aβ25-35. (C) Experimental UV-Vis spectrum of Aβ25-35 in PBS buffer (~ 1x10^-5 M) over a period of nine days. (D) Theoretical TPA spectra of Aβ25-35. 136

Figure 4-11. Aβ25-35 Protofibrils aggregation MD snapshot at 0 ns in gas phase and in condensate phase with TIP3P water molecules (B) Aβ25-35 Protofibrils aggregation MD snapshot at 20 ns in gas phase. (C) Aβ25-35 Protofibrils aggregation MD snapshot at 20 ns in condensate phase with TIP3P water molecules. (D) Energy profile during the 20 ns of MD simulation of Aβ25-35 Protofibrils aggregation in gas phase. (E) Energy profile during the 20 ns of MD simulation of Aβ25-35 Protofibril aggregation in condensate phase with TIP3P water molecules. 139

Figure 5-1. MD pore simulation of the Aβ1-42 protofibril with Ca2+ ions at both sides of the pore in the gas phase. (A) Snapshot of the Aβ1-42 protofibril at 0 fs, hydrogen atoms is omitted. (B) Snapshot of the Aβ1-42 protofibril at 120 fs. (C) Snapshot of the Aβ1-42 protofibril at 200 fs. (D) Snapshot of the Aβ1-42 protofibril at 600 fs. 158

Figure 5-2. MD pore simulation of the Aβ1-42 protofibril with Ca2+ ions at both sides of the pore in the condensate phase with TIP3P water molecules. (A) Snapshot of the Aβ1-42 protofibril at 0 fs, hydrogen atoms is omitted. (B) Snapshot of the Aβ1-42 protofibril at 100 fs. (C) Snapshot of the Aβ1-42 protofibril at one ps. (D) Snapshot of the Aβ1-42 protofibril at 5.9 ps. 160

Figure 5-3. MD pore simulation of the Aβ1-42 protofibril with Ca2+ ions at a single side of the pore in the condensate phase with TIP3P water molecules. (A) Snapshot of the Aβ1-42 protofibril at 0 fs, hydrogen atoms is omitted. (B) Snapshot of the Aβ1-42 protofibril at one ps. (C) Snapshot of the Aβ1-42 protofibril at ten ps. (D) Snapshot of the Aβ1-42 protofibril at 54.14 ps. 162

Figure 5-4. Protofibril dimer Aβ1-42 with Ca2+ ions at a single side of the pore in the condensate phase with TIP3P water molecules and Prototibril stability in a POPC membrane. (A) Total energy per simulation step of protofibril dimer with Ca2+ ions. (B) RMSD per simulation step of prototibril dimer with Ca2+ ions. (C) Total energy per simulation step of Aβ1-42 in a POPC membrane (D) RMSD per simulation step of Aβ1-42 prototibril in a POPC membrane. The small pictures in each plot show the configuration of the system at the starting and endpoints of the simulation. 164

Figure 5-5. Aβ1-42 Prototibril embedded in a POPC membrane with Ca2+ ions on site I and II. A) Total energy per simulation step of prototibril in a POPC membrane with Ca2+ ions site I. B) RMSD per simulation step of prototibril in a POPC membrane with Ca2+ ions site I. C) Total energy per simulation step of prototibril in a POPC membrane with Ca2+ ions site II.D) RMSD per simulation step of prototibril in a POPC membrane with Ca2+ ions site II. The small pictures in each plot show the configuration of the system at the starting and endpoints of the simulation. 168

Figure 5-6. Aβ25-35 Monomer embedded on a POPC membrane with Ca2+ ions and Aβ25-35 prototibril dimer stability in a POPC membrane. A) Total energy per simulation step of the Aβ25-35 prototibril dimer in a POPC membrane. B) RMSD per simulation step the Aβ25-35 prototibril dimer in a POPC membrane. C) Total energy per simulation step of the Aβ25-35 monomer in a POPC
membrane with Ca\textsuperscript{2+} ions site I. D) RMSD per simulation step of the Aβ\textsubscript{25-35} monomer in a POPC membrane with Ca\textsuperscript{2+} ions site I. The small pictures in each plot shows the configuration of the system at the starting and endpoint of the simulation.----------------------------------- 172

Figure 5-7. Aβ\textsubscript{25-35} protofibril embedded on a POPC membrane with Ca\textsuperscript{2+} ions A) Total energy per simulation step of the Aβ\textsubscript{25-35} protofibril in a POPC membrane with Ca\textsuperscript{2+} ions site I. B) RMSD per simulation step of the Aβ\textsubscript{25-35} protofibril in a POPC membrane with Ca\textsuperscript{2+} ions site I. The small pictures in each plot shows the configuration of the system at the starting and endpoint of the simulation.----------------------------------- 175
LIST OF TABLES

Table 2-1. AT interbase hydrogen bond (Å) distances in the Gas Phase calculated with Several Theoretical Schemes.----------------------------------------------- 50
Table 2-2. GC Interbase hydrogen bond (Å) distances in the Gas Phase calculated with Several Theoretical Schemes.----------------------------------------------- 51
Table 2-3. Relative Gibbs Free energies (ΔG0/Kcal mol⁻¹), equilibrium constant and rate constants (s⁻¹) of the concerted double proton transfer in the gas phase with different theoretical levels using the 6-311++G(d,p) basis set at 298.15K for the GC complex.  ----------------------------------------------- 52
Table 2-4. Hydrogen bond (Å) distances of the AT complex in water at different theoretical levels using PCM and 6-311++G(d,p) basis set. ----------------------------------------------- 54
Table 2-5. Hydrogen bond distances (Å) of the GC in water at different theoretical levels using PCM and 6-311++G(d,p) basis set. ----------------------------------------------- 55
Table 2-6. Hydrogen bond distances (Å) of AT complex during its double proton transfer in several solvents at the theoretical level CAM-B3LYP/6-311++ G(d,p). ----------------------------------------------- 58
Table 2-7. Relative Gibbs Free energies (Kcal mol⁻¹), transition state frequencies (cm⁻¹), equilibrium constant, and rate constants (s⁻¹) of the double proton transfer of the AT complex in solution with different solvents using the theoretical level Cam-B3LYP/6-311++G(d,p) at 298.15K. ----------------------------------------------- 59
Table 2-8. Hydrogen bond distances (Å) of GC complex during its double proton transfer in several solvents at the theoretical level M05-2x/6-311++ G(d,p). ----------------------------------------------- 61
Table 2-9. Relative Gibbs Free energies (Kcal mol⁻¹), transition state frequencies (cm⁻¹), equilibrium constant and rate constants (s⁻¹) of the double proton transfer of the GC complex in solution with different solvents using the theoretical level M05-2X/6-311++G(d,p) at 298.15K. ----------------------------------------------- 65
Table 3-1. Energy, Complexation Energy, and electric dipole moment of the studied Aβ(1-42) at HF/STO-3G theoretical level. ----------------------------------------------- 84
Table 4-1. Energy, Complexation Energy and electric dipole moment of the studied Aβ25-35 at B3LYP/6-31G(d) theoretical level. ----------------------------------------------- 122
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ</td>
<td>Amyloid beta peptide</td>
</tr>
<tr>
<td>Aβ₁₋₄₂</td>
<td>Amyloid beta 1-42 peptide</td>
</tr>
<tr>
<td>Aβ₂₅₋₃₅</td>
<td>Amyloid beta 25-35 peptide</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s Disease</td>
</tr>
<tr>
<td>AT</td>
<td>Adenine-Thymine Base Pair</td>
</tr>
<tr>
<td>AT₁</td>
<td>Adenine-Thymine Center Proton Tautomer</td>
</tr>
<tr>
<td>AT⁺</td>
<td>Adenine-Thymine First Transition State</td>
</tr>
<tr>
<td>AT₁⁺</td>
<td>Adenine-Thymine Second Transition State</td>
</tr>
<tr>
<td>AT₂</td>
<td>Adenine-Thymine two proton tautomer</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>c</td>
<td>Light Speed in Vacuo</td>
</tr>
<tr>
<td>DFT</td>
<td>Density Functional Theory</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DPT</td>
<td>Double Proton Transfer</td>
</tr>
<tr>
<td>dₐ</td>
<td>Nuclear Position</td>
</tr>
<tr>
<td>ECD</td>
<td>Electronic circular dichroism</td>
</tr>
<tr>
<td>E</td>
<td>Energy</td>
</tr>
<tr>
<td>Ė</td>
<td>Energy operator</td>
</tr>
<tr>
<td>Eₓc[n(r)]</td>
<td>Exchange-Correlation Energy functional</td>
</tr>
<tr>
<td>e</td>
<td>Electron Charge</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full Width at Half Maximum</td>
</tr>
</tbody>
</table>
\( f_{0f} \) Oscillator strength

GC Guanine-cytosine base pair

GC1 Guanine-cytosine center proton tautomer

GC‡\(^+\) Guanine-cytosine first transition state

GC1‡\(^+\) Guanine-cytosine second transition state

GC2 Guanine-cytosine two-proton tautomer

\( g(\omega,\omega_{0f},\Gamma) \) Lorentzian line shape

HF Hartree-Fock

\( \hat{\mathcal{H}} \) Hamiltonian

\( \hbar \) Plank constant

\( \hbar \) Plank constant divided by two \( \pi \)

\( l_0 \) Irradiance

IEFPCM PCM with the integral equation formalism variant

\( K_{eq} \) Equilibrium Constant

\( k \) Rate Constant

\( k_0 \) Wavenumber

\( k_B \) Boltzmann constant

MAD Mean absolute deviation

MD Molecular dynamics

m Mass

\( N_A \) Avogadro's number

\( n(t) \) Number of reactants particles
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n(r)$</td>
<td>Electron density</td>
</tr>
<tr>
<td>OPA</td>
<td>One photon absorption</td>
</tr>
<tr>
<td>PCM</td>
<td>Polarizable Continuum Model</td>
</tr>
<tr>
<td>$p$</td>
<td>Linear Momentum</td>
</tr>
<tr>
<td>$\hat{p}_i$</td>
<td>Linear Momentum Operator</td>
</tr>
<tr>
<td>QT</td>
<td>Quantum Tunnel</td>
</tr>
<tr>
<td>$q_{ts}$</td>
<td>Partition function</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>RMSD</td>
<td>Root Mean Standard Deviation</td>
</tr>
<tr>
<td>$R_{0f}$</td>
<td>Rotatory Strength</td>
</tr>
<tr>
<td>$R_{0f}^{TPCD}(\omega_{0f})$</td>
<td>Two-photons rotatory strength</td>
</tr>
<tr>
<td>$r$</td>
<td>Reflection Coefficient</td>
</tr>
<tr>
<td>$r_i$</td>
<td>Particle position</td>
</tr>
<tr>
<td>$T$</td>
<td>Temperature</td>
</tr>
<tr>
<td>TD-DFT</td>
<td>Time dependent density functional theory</td>
</tr>
<tr>
<td>TST</td>
<td>Transition State Theory</td>
</tr>
<tr>
<td>TPA</td>
<td>Two-Photon Absorption</td>
</tr>
<tr>
<td>$T[n(r)]$</td>
<td>Kinetic Energy functional</td>
</tr>
<tr>
<td>$t$</td>
<td>Transmission Coefficient</td>
</tr>
<tr>
<td>$V_0$</td>
<td>Potential Energy</td>
</tr>
<tr>
<td>$Z_i$</td>
<td>Nuclear charge</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Two-photon absorption coefficient</td>
</tr>
</tbody>
</table>
Γ  
\[ \text{FWHM} \]

\( \nabla_i \)  
Gradient Operator

\( \Delta G_{f/r}^{\pm} \)  
Gibbs free activation energy for the forward or reverse barrier

\( \Delta T(z) \)  
Transmissivity

\( \Delta V^{\pm} \)  
Transition state potential energy barrier

\( \delta_{0f}^{TPA}(\omega) \)  
TPA cross-section

\( \bar{\delta}_{bf}^{TPA}(\omega_{0f}) \)  
Orientation Averaged Two-Photon Transition Probability

\( \varepsilon_0 \)  
Vacuum Permittivity

\( \varepsilon(\omega) \)  
Molar absorptivity

\( \varepsilon(\vec{r}) \)  
Position-Dependent Permittivity

\( \theta(x - 0) \)  
Heaviside-theta function

\( \kappa(T) \)  
Transmission Coefficient in TST

\( \mu \)  
Transmission Coefficient in TST

\( \mu \)  
Dipole moment

\( \mu_{0f} \)  
Electric transition dipole moment

\( \nu \)  
Frequency

\( \rho(\vec{r}) \)  
Charge density

\( \sigma \)  
TPA cross-section

\( \sigma(\vec{s}) \)  
Superficial Charge Distribution

\( \psi \)  
Wavefunction

\( \omega_{TS,i} \)  
Angular Frequency
CHAPTER 1. INTRODUCTION

Since ancient times humans have tried to understand the workings of nature and how we are so different from other species inhabiting the planet. Civilizations have attempted to use their immediate resources to cure disease, accumulate wealth, and acquire the power to improve lifestyles. Since our primordial beginnings, man has tried to manipulate the external surroundings for the betterment of humanity. Improvements seem to have been obtained mostly by trial and error for the lack of pre-fundamental knowledge. In the early times, alchemy was one the most mystical sciences due to the belief that any material could be transformed into others by just thermal or chemical modifications (e.g., conversion of copper into gold)\(^1\).

In contrast with alchemy, mathematics and physics provided developments that allowed the quantification of natural and geometrical processes. It is not until the systematic quantification of chemistry by Lavoisier that alchemy became a pure science (Chemistry)\(^1\). After the creation of the periodic table by Mendeleev\(^2\) in 1869, chemistry started to grow and thrive rapidly. Although many elements were discovered, their characteristics were not well explored just by using empirical models of that era. By integrating chemistry, physics, and mathematics, fundamental understandings of atomic and molecular characteristics became quickly unveiled.

The improvement in the understating of how chemical reactions occurred and their structural bases, including light-matter interactions using the fundamentals of the quantum theory\(^3\), yielded to a better understanding of biological processes, comprising small and relatively sizeable
biological molecules\textsuperscript{4}. The latter shows that chemistry is, in fact, the central science, which is fundamental and also multidisciplinary\textsuperscript{5}.

The development of quantum mechanics has brought advancements to many interdisciplinary realms by allowing us to understand the properties of atoms and molecules, which would not be followed by only the use of empirical knowledge\textsuperscript{6}. Many of the studied systems go beyond our common sense and practical conceptions of the macroscopic world. Moreover, a theoretical framework to explain these unintuitive observations of the “quantum world” allows us to understand how quantum systems can produce the macroscopic properties that are quantifiable with our senses and instruments.

The advancement of readily attainable powerful computers has allowed researchers to create and implement theoretical quantum approaches in the electronic structure of small and complex molecules such as DNA and peptides\textsuperscript{7, 8}. These methods are mostly based on simple quantum-mechanical strategies, as is the case of ab-initio\textsuperscript{9}, density functional theory (DFT)\textsuperscript{10}, and post-Hartree-Fock methods\textsuperscript{11}. It is, however, a much more complicated task to simulate large molecular systems (e.g., metabolism webs, membranes, and DNA) using a simple quantum approach due to the high computational cost that these simulations require. To solve this problem, researchers have proposed different approaches to treat these systems in an approximative way. The most accurate methods are known as semi-empirical methods\textsuperscript{12}. These methods consist of the use of empirical data such as ionization energies, electron affinity, and energies of formation to estimate the one and two-electrons integrals of the Hartree-Fock method. In this method, the calculations of energies of formation, structures optimization, and thermodynamics are faster than
ab-initio or DFT approaches. Despite that, the semi-empirical methods are fast and are based on empirical and highly accurate ab-initio data. The basis set of the method is designed based on local minimal slater functions that are minimized in the function of a particular training set. The latter could make that the method is not accurate enough in specific molecular systems. As a consequence, the semi-empirical methods are not designed to be accurate in molecules with specific characteristics that exhibit well-defined quantum effects such as electron exchange and electron energy correlation.

Notwithstanding the limitations of the semi-empirical methods, it has been demonstrated that they can be useful to describe large molecular systems such as proteins performing in some cases at the same level than ab-initio and DFT approaches\textsuperscript{13-17}. However, the inclusion of quantum effects in some molecular systems is crucial for the correct characterization of the system and the complete description of its interactions. Though in larger systems containing millions of atoms, the use of semi-empirical methods is not possible due to its high computational cost\textsuperscript{18}. It is for this reason that more simple classical approaches have been developed to describe large systems that are impossible to simulate under ab-initio or semi-empirical approaches.

One of these simple approaches is known as molecular mechanics\textsuperscript{19}. This method consists of classical approaches to describe simple quantum interactions such as bond length forces, steric interaction, conformational changes, and dispersion interactions. To achieve this, researchers train computational objects known as “force fields” by using ab-initio electronic structure data of simple molecular systems. Based on the previous data, the parameters for the “force fields” are tuned to describe with the most considerable possible accuracy these molecular interactions observed in
quantum simulated systems. This approach allows us to simulate extensive systems in a short time and with a lower computational cost. In this way, however, a small fraction of accuracy is lost, but this factor tends to be insignificant in very complex systems.

In addition to the force field approaches, the dynamic behavior of the molecular systems is essential in large and very dynamic molecular systems such as proteins and cell organelles. The previous allows us to understand the critical chemical and biological mechanism such as oxidation processes, protein folding, an ion-conducting pore dynamic. To observe these dynamical processes, researchers have approached this problem by the mere use of Newton’s Second Law of Motion, which is valid in the quantum and classical world. However, to simulate the dynamics of quantum systems, it is necessary to solve the Newton equation of motion and determine the solution of the system in short periods that are between 0.1 and 1 femtoseconds per step. Nevertheless, extensive simulations are required to observe the entire dynamics of the system in considerably large molecular systems such as proteins (i.e., 1-100 ms) and short simulation times for ion mobility or small molecules docking (e.i., 0.1-100 ns)\textsuperscript{20}.

Despite the accuracy of the modern electronic structure methods and the accessibility to high performing computers, it is still impossible to simulate ideal large molecular systems. Simulating large molecular systems with high accuracy using quantum mechanical approaches resides in the fact that quantum effects are significant to elucidate specific proteins of biological importance completely\textsuperscript{21-23}. The first person to suggest this approach to explain the spontaneous mutation of DNA was Löwdin in the early sixties, and he defined it as Quantum Biology\textsuperscript{24-26}.
Quantum Biology is defined as the use of quantum mechanical tools and effects to describe the function of biologically active molecules, organelles, and cells. Most of the highly accurate methods tend to scale with the number of atoms of the system, making it impossible to study large biological molecules promptly under our actual computational power conditions. However, by using quantum computers, they can mimic the real quantum states of the molecular systems speeding up the structure elucidation and function of such complex systems.

Despite the limitations of the quantum biological approach, we can study specific molecular systems at a quantum theoretical level under certain conditions. In exemplar, low molecular mass biological molecules such as DNA base pairs and small peptides as amyloid-beta peptides can be studied and described under this approach, which is the primary goal of this dissertation. In addition to the structural quantum biological approach for the elucidation of the properties and interactions of biological systems of interest, the light-matter interactions of this type of molecular systems are also of high interest, including one-photon absorption (OPA) and electronic circular dichroism (ECD). The utilization of these techniques will give crucial structural information of chiral systems and proteins. On the other hand, the development of powerful light sources such as lasers has allowed researchers to obtain not only molecular information through light-matter linear interactions, but also observe non-linear effects as two photon-absorption. The use of non-linear optical techniques presents several advantages. First, the required photon energy required for the excitation of the molecule is lower than in the case that OPA, which avoids molecule damage. Second, the light scattering of the sample is minimized because of the longer photon wavelength. Lastly, the non-linear absorption techniques present tremendous penetration depth; this allows its use for bioimaging and photodynamical therapy.
Experimental demonstration of the two-photon circular dichroism (TPCD) technique developed by Hernandez and co-workers\textsuperscript{28} has shown to be more effective than ECD for the structural characterization of proteins\textsuperscript{29, 30}. Generally, the conformational information of proteins is hidden in the problematic ultraviolet region of the electromagnetic spectrum, making it difficult for the characterization of these structures by just ECD. TPCD offers a more simplistic approach to solve the previous problem, decreasing the needed ECD photon energy to the half, this allows the characterization of the chiral properties of proteins, and it is exceptionally sensible to small conformational changes.

Based on the previously described information, this dissertation sought to explore the use of quantum biological approaches on the spontaneous mutations in DNA base pairs using quantum tunneling corrections. Furthermore, the cytotoxic pathways of aggregation and ion-conducting pore formation of the highly toxic Alzheimer’s peptides $\text{A} \beta_{1-42}$ and $\text{A} \beta_{25-35}$ analyzed in function of the structure’s electric dipole moment. The fundamentals of the performed theoretical models and measured properties will be described in the following paragraphs.
1.1 Fundamentals of Electronic Structure Methods

1.1.1 The Hamiltonian

Erwin Schrödinger, in 1925\(^3\) postulated his famous equation quantizing the classical total energy of a system using as a fundament the wave-particle duality found experimentally and its theoretical description\(^{31,32}\). The energy of the classical system (E) can be described in terms of its linear momentum (p) and potential energy (V) by equation 1-1.

\[
E = \sum_{i=1}^{n} \frac{p_i^2}{2m_i} + V
\]  

(1-1)

Quantizing equation 1-1 we obtain,

\[
\hat{E} = \sum_{i=1}^{n} \frac{\hat{p}_i^2}{2m_i} + \hat{V}
\]

(1-2)

Where \(\hat{E}\) and \(\hat{p}\) are defined by equations 1-3 and 1-4 respectively as follows,

\[
\hat{E} = i\hbar \frac{\partial}{\partial t}
\]

(1-3)

\[
\hat{p}_i = -i\hbar \frac{\partial}{\partial x_i}
\]

(1-4)

Introducing equation 1-3 and 1-4 on equation 1-2 and applying it to the N-body wavefunction (\(\Psi\)) of the particle, we obtain the time-dependent Schrödinger equation that is giving by:
\[ i\hbar \frac{\partial \psi}{\partial t} = \sum_{i=1}^{n} -\frac{\hbar^2}{2m_i} \frac{\partial^2 \psi}{\partial x_i^2} + V \quad (1-5) \]

The time-dependent Schrödinger equation can be rewritten when the potential energy operator is independent using the ansatz given in equation 1-6 and obtaining the time-independent Schrodinger equation:

\[ \Psi = \psi e^{-iE\hbar t} \quad (1-6) \]

\[ \hat{H}\psi = E\psi \quad (1-7) \]

\( \hat{H} \) in equation 1-7 is the Hamiltonian of the system where E is the steady-state energy of the system. In the case of a molecular system, the molecular Hamiltonian is given by:

\[ \hat{H} = \sum_{i=1}^{n} \left( -\frac{\hbar^2}{2m_i} \nabla_i^2 + \frac{Z_i^2 e^2}{4\pi \varepsilon_0 |r_i - r_j|} \right) + \frac{1}{2} \sum_{i=1}^{N} \sum_{j=1}^{N} \frac{Z_i Z_j e^2}{4\pi \varepsilon_0 |r_i - r_j|} \quad (1-8) \]

Any problem in Chemistry can be simulated using the Schrödinger equation. However, the equation only has an exact solution for monoelectronic atoms. To use the Schrödinger equation for polyelectronic atoms, controlled and well-understood approximations are used to reduce the complexity of the equation to a mailable level. Solving the equation allows us to calculate many properties from the wavefunction. A considerable error in the obtained properties can be present, depending on the approximations used to solve the equation. For this reason, it is necessary to understand the complexity of the approximations made and their implication to verify if they can be applied to the studied system.
1.1.2 The Born-Oppenheimer Approximation

A commonly used approximation to solve the Schrödinger equation in molecular systems is the Born-Oppenheimer approximation\(^{33}\). In molecular systems, the electrons and the nuclei interact via Coulombic interactions; however, the individual linear moments of the particles are considerably different due to the large mass of the nuclei in comparison with the electrons. It is for the previous reason that the electrons will travel at larger velocities in contrast with the nuclei, this allows us to separate the nuclear motion from the electronic motion in the Schrodinger equation as follows:

\[
\mathcal{H} = \sum_{i=1}^{n} \left( -\frac{\hbar^2}{2m_i} \nabla_i^2 \right) + \sum_{i=1}^{N} \sum_{\alpha} \frac{Z_{\alpha}e^2}{4\pi\varepsilon_0 |r_i - d_{\alpha}|} + \frac{1}{2} \sum_{i=1}^{N} \sum_{j \neq i} \frac{e^2}{4\pi\varepsilon_0 |r_i - r_j|} + \frac{1}{2} \sum_{\alpha=1}^{N} \sum_{\beta \neq \alpha} \frac{Z_{\alpha}Z_{\beta}e^2}{4\pi\varepsilon_0 |d_{\alpha} - d_{\beta}|} \tag{1-9}
\]

In equation 1-9, the nuclear motion has been neglected, allowing us to separate the Hamiltonian in two parts. The first is known as the electronic Hamiltonian, which includes the first three terms of equation 1-9, while the second term is known as nuclear Hamiltonian and only consists of the nuclei Coulombic repulsion term. The Z terms indicate the nuclear charge while d indicates the position of the nuclei in the molecule. Despite the simplified nature of the Hamiltonian using this approximation, it still extremely difficult to solve, where analytical solutions only exist in systems that contain a single electron.

1.1.3 The Hartree-Fock Formalism

The Hartree-Fock formalism is one of the simplest approximations to solve the Schrödinger equation in many-body problems such as molecular Hamiltonians\(^{34,35}\). The approximation consists
of treat the total molecular wave function as an N-particles decoupled wave function (Eq. 1-10). The decoupled wavefunction approximation can be used if we consider that the electron-electron repulsion interactions as constant on average. This approximation makes it possible to write the general wavefunction of the system as pure products of one-electron wavefunctions.

\[
\Psi(x_1, x_2, x_3, x_4, \ldots) = \psi_1(x_1)\psi_2(x_1)\psi_3(x_1)\psi_4(x_4) \ldots \tag{1-10}
\]

As a consequence, that electrons are indistinguishable particles, and they can be exchanged. A simple product of mono-electronic wavefunctions does not represent the whole wavefunction. Also, the fermionic nature of the electrons which follow Pauli’s uncertainty principle makes the Hartree product (Eq. 10) antisymmetric under electron exchange (see Eq. 1-11).

\[
\Psi(x_1, x_2, x_3, x_4, \ldots) = -\Psi(x_1, x_3, x_2, x_4, \ldots) \tag{1-11}
\]

To overcome the mentioned problem, the N-body wavefunction can be described as a single slater determinant given by equation 1-12 where \(\epsilon^{ij,k,m,\ldots}\) is the antisymmetric Levi-Civita tensor that takes a value of +1 for symmetric permutations, -1 for antisymmetric permutations and cero for repeated indices.

\[
\Psi(x_1, x_2, x_3, x_4, \ldots) = \frac{1}{\sqrt{N!}} \sum_{i,j,k,m,\ldots=1}^{N} \epsilon^{ij,k,m,\ldots} \psi_1(x_1)\psi_2(x_2)\psi_k(x_1)\psi_m(x_3) \ldots \tag{1-12}
\]

Applying this wavefunction and minimizing the molecular Hamiltonian respect, the wave function (Eq. 1-13) simplifies the Schrödinger equation considerably to one-electron equations given by equation 1-14.
\[
\frac{\delta}{\delta \psi} \left[ \langle \mathcal{H} \rangle - \sum_j E_j \int |\psi_j|^2 \right] = 0
\]  
(1-13)

\[
f(x_1)\psi_i(x_1) = E_i\psi_i(x_1)
\]  
(1-14)

The equation 1-14 is the compact Hartree-Fock equation expressed by the Fock operator \( f(x_1) \) which is defined by equation 1-15. \( h(x_1) \) is the simplified molecular Hamiltonian that includes the electrons kinetic energy and the nuclei-electron potential energy defined by equation 1-16. \( J_i(x_1) \) is known as the Coulomb operator who determines the average local potential at point \( x_1 \) due to the charge distribution of the electrons in the orbital \( \psi_i(x_1) \) and is given by equation 1-17. On the other hand, \( K_i(x_1) \) is known as the exchange operator; this operator does not present a classical analog and arises from the asymmetric nature of the wavefunction. The operator is similar to the Coulomb operator, with the exception that it switches or exchanges one-electron spin-orbital and is defined by equation 1-18.

\[
f(x_1) = h(x_1) + \sum_i J_i(x_1) - K_i(x_1)
\]  
(1-15)

\[
h(x_1) = -\frac{\hbar^2}{2m} \nabla_i - \sum_\alpha \frac{Z_\alpha}{r_{i\alpha}}
\]  
(1-16)

\[
J_i(x_1) = \int \frac{|\psi_i(x_2)|^2}{r_{12}} dx_2
\]  
(1-17)

\[
K_i(x_1) = \int \frac{\psi_i^*(x_2)\psi_i(x_2)}{r_{12}} dx_2
\]  
(1-18)
Using the previous equations, to solve the Schrödinger equation, this becomes just a linear algebra eigenvalue problem that is solved iteratively. That is why this method is often called the self-consistent field procedure.

1.1.4 The Density Functional Theory

The Density Functional Theory (DFT) is a compelling theory founded in the function of the electron density of the N-body system and presents an exact nature\textsuperscript{36-41}. However, the difficulty in defining functionals to describe the correlation and exchange energy makes it to a certain level an approximative theory. Also, DFT has been demonstrated to present a relatively low computational cost when it is compared with traditional wavefunction based methods such as exchange only Hartree-Fock and post-Hartree-Fock methods.

DFT presents its origin in the Thomas-Fermi model on a non-interacting electron gas for the description of the kinetic energy in molecular systems. Though, it is not until the development of the Hohenberg-Kohn theorems that DFT was defined as an exact theory for the description of the electronic structure of atoms, molecules, and solids.

The Hohenberg-Kohn theorem shows that if a group of N-interacting electrons move in an external potential $V_{\text{ext}}(r)$ the electronic density of the ground state $n_0(r)$ minimizes the energy functional and is given by equation 1-19.

$$E[n] = F[n] + \int n(r)V_{\text{ext}}(r) \, dr$$ \hspace{1cm} (1-19)
F[n] is a universal function of the electron density given by equation 1-20, and it minimized respect the system electron density as follows.

\[
F[n] = \min_{\Psi \rightarrow n(r)} |\Psi| \hat{F} |\Psi >
\]

(1-20)

So that \( \hat{F} \) is given by:

\[
\hat{F} = \sum_{i=1}^{n} \left( -\frac{\hbar^2}{2m_i} \nabla_i^2 \right) + \frac{1}{2} \sum_{i=1}^{N} \sum_{j \neq i}^{N} \frac{e^2}{4\pi\varepsilon_0 |r_i - r_j|}
\]

(1-21)

Starting by the demonstration of the Hohenberg-Kohn theorem, Kohn and Sham later derived a set of equations that allow the finding of the ground state electron density. To achieve this objective Kohn and Sham separated the F[n] into three parts as follows.

\[
E[n(r)] = T[n(r)] + \frac{1}{2} \int \int \frac{n(r)n(r')}{|r-r'|} dr'dr + E_{xc}[n(r)] + \int n(r) V_{ext}(r) dr
\]

(1-22)

The first term in equation 1-22, is defined as the kinetic energy of non-interacting electron gas, the second corresponds to a pure electron exchange term and \( E_{xc}[n(r)] \) as the exchange-correlation energy functional. Introducing the constraint of the normalization of the electron density and minimizing the energy functional against this will yield to the one-electron Schrödinger equation (Eq. 1-22). The one-electron Schrödinger equation has to be solved self
consistently with the system electron density described in the function of the one-electron wavefunction (Eq. 1-25).

\[
\left( -\frac{1}{2} \nabla_i^2 + V_{\text{eff}}(r) - E_i \right) \psi_i(r) = 0 \tag{1-23}
\]

\[
V_{\text{eff}}(r) = V_{\text{ext}}(r) + \int \frac{n(r')}{|r-r'|} dr' + V_{\text{XC}}(r) \tag{1-24}
\]

\[
V_{\text{XC}}(r) = \frac{\delta E_{\text{xc}}[n(r)]}{\delta n(r)} \tag{1-25}
\]

\[
n(r) = \sum_{i=1}^{N} |\psi_i(r)|^2 \tag{1-26}
\]

The equations showed above permit to find the exact ground state energy of any N-body particle system if the \(E_{\text{xc}}\) of the system is given. Unfortunately, the precise form of \(E_{\text{xc}}\) is not known and is treated in an approximative way. It is common to approximate the energy exchange-correlation functional using the local density approximation or the generalized gradient approximation. For simplicity, the \(E_{\text{xc}}\) functional can be described by just correlation energy or just exchange energy functional.

However, hybrid functionals seem to be more accurate due to the representation of these two essential quantum interactions in the functional (e.g., B3LYP). Modern DFT functionals contain local and non-local interactions, which are mainly described by generalized-gradient
approximations of the electron density and the inclusion of Hartree-Fock exchange, making some functionals as accurate as benchmark methods such as CCSD(T) and MP-X.

1.1.5 The Quantum Tunneling Effect

Quantum tunnel or tunneling is one of the quantum effects that do not present a classical analog. When particles move into a classical potential barrier with a kinetic energy that is smaller than the barrier potential, the particles get reflected. However, at the subatomic level, an interesting effect occurs; the particles present a certain probability to penetrate the barrier despite that these do not present enough energy to penetrate the potential barrier. Tunneling aid in the understanding of physical phenomena such as nuclear fusion and quantum tunneled chemical reactions.\textsuperscript{42, 43}

\textbf{Figure 1-1. Quantum Rectangular Barrier. The barrier energy is larger than the particle kinetic energy. The incident reflected, and transmitted wavefunctions are shown in black, gold, and red, respectively. The green curve shows the exponentially decaying wavefunction in the interior of the barrier}

Tunneling effect can be explained by using a unidimensional rectangular barrier and solving the Schrödinger equation for a rectangular potential defined by equation 1-27:
\[ V(x) = V_0[\Theta(x - 0) - \Theta(x - a)] \]
\[ (1-27) \]

In equation 1-27, \( V_0 \) is the potential energy of the barrier, and \( \Theta(x - 0) \) is the Heaviside theta function. The function is defined as 0 for \( x < 0 \) and 1 for \( x > 0 \). Observing the potential barrier in Fig.1-1, three regions are present in the system. The regions corresponding to the boundaries of the barrier can be represented in the function of free particles moving waves, while the barrier region is represented by a quasi-free particle wavefunction as follows.

\[ \psi_L(x) = A_r e^{ik_0t} + A_l e^{-ik_0t} \quad x < 0 \quad (1-28) \]
\[ \psi_B(x) = B_r e^{k_1t} + B_l e^{-k_1t} \quad 0 < x < a \quad (1-29) \]
\[ \psi_R(x) = C_r e^{ik_0t} + C_l e^{-ik_0t} \quad x > 0 \quad (1-30) \]

Where the wavenumbers are given by:

\[ k_0 = \sqrt{\frac{2mE}{\hbar^2}} \quad (1-31) \]
\[ k_1 = \sqrt{\frac{2m(V_0 - E)}{\hbar^2}} \quad (1-32) \]

The wavefunction requires to be finite, single evaluated, and continuous. Generating family of four boundary conditions that are needed to solve the problem. The set of boundary conditions are given as follows:

\[ \psi_L(0) = \psi_c(0) \quad (1-33) \]
\[
\frac{d \psi_L(0)}{dx} = \frac{d \psi_L(0)}{dx}
\]  
\[
\psi_C(a) = \psi_R(a)
\]  
\[
\frac{d \psi_C(a)}{dx} = \frac{d \psi_R(a)}{dx}
\]

Using the boundaries conditions showed in equation 1-33 to 1-36, it is possible to express the transmission and reflection coefficient as follows:

\[
t = \frac{4k_0k_1 e^{-ia(k_0-k_1)}}{(k_0+k_1)^2 - e^{-2iak_1(k_0-k_1)^2}}
\]  
\[
r = \frac{(k_0^2-k_1^2)Sin(k_1a)}{2ik_0k_1Cos(k_1a)+(k_0^2+k_1^2)Sin(k_1a)}
\]

Even though tunneling is most common in fundamental particles such as electrons, this effect can also occur in chemical reactions; especially at low temperatures (Fig 1-2 and 1-3). However, it is of significant importance in astrochemistry\textsuperscript{44-48}, and reactions at cryogenic temperatures\textsuperscript{49-51}; though proton tunneling has also been observed even at room temperature\textsuperscript{52-54}. For this reason, it is imperative to understand if this process can occur under physiological conditions in enzymes, protein, and biologically active molecules, which is part of the aim of quantum biology and this dissertation.

One of the principal goals of quantum chemists is to calculate rate constants. The reaction rates are based on the change of reagents during reaction time. For a first-order reaction, the rate is given by:
\[
\frac{dn(t)}{dt} = -kn(t)
\]  

(1-39)

In equation 1-39 \((k)\) is the rate constant of the reaction, while \(n(t)\) is the number of reactant particles at a given time. At high temperatures, the reagents can be converted into products easily. This is because reaction barriers are being overcome by thermal energy (Fig. 1-2). Also, the reaction kinetics is often well described by the transition state theory (TST)\(^{55}\).

![Figure 1-2. Tunneling in a chemical reaction. Lines indicated in red and green are the vibrational levels of the reagents and products, respectively. A purple line shows the approximated wavefunction of the atom tunneling.](image)

The rate constants in TST are the probability of finding the system at the transition state multiplied by the particle flux, which can be written as follows:

\[
k = \frac{1}{2} < |\dot{x}| > \frac{q_{ts}}{q_r}
\]  

(1-40)

Equation 1-40 describes the rate constant \((k)\) of a chemical reaction, where \(q_{ts}\) and \(q_r\) are the partition function of the transition state and reactants, respectively. Besides, \(< |\dot{x}| >\) is the average thermal velocity of the transition state. The latter can be correctly described assuming that
the thermal energy is large enough to be at the same energetic level of the principal vibrational frequency of the transition state \( k_B T = h\nu \), where \( \nu = \langle |\dot{x}| \rangle \). The previous equation allows us to write the velocity flux of the TS as \( \frac{k_B T}{h} \). In the previous equations \( k_B \) is the Boltzmann’s constant, \( T \) the absolute energy, and \( h \) the Planck’s constant.

The transition state theory presents certain assumptions that may limit its application to non-classical chemical reactions. First, the system is in thermal equilibrium in the reactant state. Second, the reactions do not present reversibility once the system is at the transition state. Third, classical mechanics and thermodynamics laws are completely fulfilled (i.e., no tunneling). Finally, the Born-Oppenheimer approximation is valid at any point in the reaction. These approximations may cause a considerable error in the prediction of the rate constants in molecular systems that do not fulfill the four assumptions mentioned above.

On the other hand, to describe better the rate constants and its dependence with temperature, the transition state potential can be expanded in Taylor’s series using the harmonic approximation. In the harmonic approximation, Taylor’s series is truncated in the quadratic term, resulting in the Harmonic transition state theory. In this theory, the vibrational degree of freedom at the transition state is treated as a quantum mechanical harmonic oscillator, without considering the imaginary frequency at the transition state, yielding to the following rate constant:

\[
\kappa = \frac{k_B T}{2\pi h} \frac{1}{\prod_{i=1}^{N-1} \sinh(\frac{\hbar \omega_{R,i}}{2k_B T})} e^{-\frac{\Delta V}{k_B T}}
\]

(1-41)
In equation 1-41, $\omega_{TS,i}$ and $\omega_{R,i}$ are the vibrational frequencies of the transition state and the reactant, respectively. $\Delta V^\ddagger$ on the other hand, is the potential energy barrier from reactant to the transition state. This approximation considers the zero-point harmonic vibrational energies of the reagents and products but neglects the tunneling effect. Also, the central temperature dependence in equation 1-41 is given by the exponential factor; Arrhenius exponential factor. It is for that reason that when the $\text{Ln}(k)$ is plotted against $1/T$, it results in a straight line (Fig.1-3). However, in systems that present tunneling in the limit of low temperature, they show that there is no dependence of the rate constant with temperature. This is a consequence that the tunneling rate for the decay of a bond is independent of temperature. In low temperatures, the reaction rate is dominated by the ground vibrational state; therefore, it is temperature independent (see Figure 1-3).

![Figure 1-3. Arrhenius plot of classical and a quantum tunneled reaction. The green dotted line represents the classical Arrhenius plot, while the solid red line represents a chemical reaction with tunneling at low temperatures.](image)

One of the most straightforward approaches to incorporate tunneling corrections into the TST consists of: approximating the potential barrier as a function of the parabolic barrier where
the transmission coefficient can be calculated and multiplied to equation 1-41. The correction
factor is known as Wigner correction\(^{56}\) and is given by:

\[
\kappa(T) = \frac{\hbar \omega_b}{2k_BT \sin(\frac{\hbar \omega_b}{2k_BT})}
\]  

(1-42)

\(\omega_b\) is the imaginary part of the transition state imaginary frequency. The transmission
coefficient \(\kappa\) diverges at a particular system-dependent temperature. The divergence of equation
1-42 can be avoided truncating Taylor’s series expansion at the high-temperature limit around
\(\frac{1}{k_BT} = 0\), by doing so, the transmission coefficient results in:

\[
\kappa(T) = 1 + \frac{1}{24} \left(\frac{\hbar \omega_b}{k_BT}\right)^2
\]  

(1-43)

This correction is often used in the limit of high temperature with surprising success due
to its inherent assumptions and approximations. In contrast with Wigner corrections, the Transition
State Theory has an approximated energy profile along with multidimensional potential energy
(MPE) surface that can be simulated by more accurate methods that include vibrations
perpendicular to the transition state. The most straightforward conception of this approximation is
known as Eckart barrier\(^{57}\). More sophisticated techniques require treatment of the transition state
MPE curvature, as in the case of Truhlar’s, zero, short, and long curvature tunneling corrections\(^{58}\).
1.1.6 The Polarizable Continuum Model

Solvents' effects play a fundamental role in chemical reactions, light-matter interactions, and biological function. For this reason, researchers have looked to include solvents explicitly in simulated molecular systems. However, the number of solvent molecules needed to enclose the solute in a cavity is considerably large. The number of solvent molecules makes quantum chemical calculations in these systems exceptionally computationally expensive. Moreover, most of the computational resources are used to describe the solvent and not the molecular system of interest\textsuperscript{59}. To overcome this problem, Miertus et al. proposed one of the most useful approaches to describe solvents that can be combined with any quantum chemical calculation; known as the polarizable continuum model (PCM)\textsuperscript{60, 61}.

The formulation of PCM requires the solution of a classical electrostatic problem, which is known as Poisson problem given by equation 1-44.

\[
-
\vec{V} \times [\epsilon(\vec{r})\vec{V}(\vec{r})] = 4\pi\rho(\vec{r})
\]  

(1-44)

Were $\rho(\vec{r})$ is the solute charge density and $\epsilon(\vec{r})$ is the general position-dependent permittivity. Assuming that solute charge density is contained in a molecular cavity of appropriated shape (C) to include all the atoms present in it, $\epsilon(\vec{r})$ assumes a simple form, where $\epsilon$ is the dielectric constant of the solvent:

\[
\epsilon(\vec{r}) = \begin{cases} 
1 & \vec{r} \in C \\
\epsilon & \vec{r} \notin C
\end{cases}
\]  

(1-45)
Using the approximation given by equation 1-45 and the appropriated boundary conditions, the electrostatic Poisson problem (Eq. 1-44) can be solved in terms of the potential $V$. This potential is expressed in function of the contribution of the solute and the contribution of the superficial charge distribution generated by the fictitious solvent and is given by equation 1-46 as shown below:

$$V(\mathbf{r}) = V_s(\mathbf{r}) + V_\sigma(\mathbf{r}) \quad \text{(1-46)}$$

$$V_\sigma(\mathbf{r}) = \int \frac{\sigma(\mathbf{s})}{|\mathbf{r} - \mathbf{s}|} d^2 s \quad \text{(1-47)}$$

Despite the remarkable simplification of the problem, the integration of the equation 1-47 on the surface is challenging and computationally expensive. The integral in equation 1-47 is usually solved by dividing the cavity into small pieces, which are known as a discrete grid. Once the surface charge distribution is known, the whole electrostatic problem is solved using the solvation Gibbs free energy as follows:

$$G^{sol} = \frac{1}{2} \int \sigma(\mathbf{s}) [\int \frac{\rho(\mathbf{r})}{|\mathbf{r} - \mathbf{s}|} d^3 r] d^2 s \quad \text{(1-48)}$$

The description of the superficial charge distribution $\sigma(\mathbf{s})$ is calculated nowadays under different formulations. The most commonly used are DPCM, CPCM, and IEFPCM, where the latter method is implemented in this dissertation for structure optimization and the calculation of optical properties.
1.2 Molecular Optical Properties

1.2.1 One-Photon Absorption

One photon absorption is one of the simplest light-matter interaction processes, as shown in figure 4-1. When one photon with enough energy ($S_0$ to $S_1$) collides with the electron density of a molecule, its electric field disturbs the electron density generating an electric dipole moment, which allows the molecular system to go to a short-lived metastable state. When this happens, the electromagnetic field of the light reduces its intensity, consequently, that the photon energy is absorbed and converted into kinetic energy in the electron density of the molecule.

![Simplified Jablonski diagram showing one and two-photon processes.](image)

**Figure 1-4.** *Simplified Jablonski diagram showing one and two-photon processes.*

The experimental OPA is usually reported in terms of absorption or molar absorptivity ($\epsilon$). The molar absorptivity is proportional to the oscillator strength ($f_{0f}$) of the electronic transition which is directly proportional to the square module of the electric transition dipole moment ($\mu_{0f}$),
using time-dependent approaches such as TD-DFT, it is possible to calculate the oscillator strengths of electronic transitions. The molar absorptivity is then written as 62:

\[
\epsilon(\omega) = \frac{2\pi^2\omega N_A}{1000 \ln(10)(4\pi\varepsilon_0)hc} \times \sum_{\omega} \frac{g(\omega, \omega_{0f}, \Gamma) f_{0f}}{\omega_{0f}}
\]  

(1-49)

In equation 1-49, \(\omega\) is the angular frequency of the incident radiation, \(c\), \(\varepsilon_0\) are the speed of the light and the permittivity in the vacuum, respectively, and \(N_A\) is Avogadro’s number. In addition, \(f_{0f}\) is calculated from the electric transition dipole moment \(\mu_{0f}\) and is given by:

\[
f_{0f} = \frac{2m\omega_{0f}|\mu_{0f}|^2}{3hc^2}
\]  

(1-50)

In addition, the line shape, \(g(\omega, \omega_{0f}, \Gamma)\) can be represented in the function of a Lorentzian function centered at \(\omega = \omega_{0f}\) and with a full width at half maximum (FWHM) \(\Gamma\) given by:

\[
g(\omega, \omega_{0f}, \Gamma) = \frac{1}{\pi} \frac{\Gamma}{(\omega_{0f}-\omega)^2 + \Gamma^2}
\]  

(1-51)

1.2.2 Electronic Circular Dichroism

Electronic Circular Dichroism (ECD) is a useful technique for the characterization of chiral molecules. It is known that enantiomers present the same physical properties because one molecule is the specular image of the other. However, their response to the circularly polarized light is
different in each enantiomer, making possible its characterization\textsuperscript{63}. Also, ECD is beneficial for protein characterization because it gives valuable information regarding the protein secondary structure.

ECD can be calculated using standard time-dependent quantum chemical methods such as DFT, where its response is based on the magnetic (m) and electric (µ) transition dipole moments that are represented by the rotatory strength of the transition ($R_{0f}$), this rotatory strength is zero for achiral molecules showing the same response in absorption for both polarization states of the light. The differential ECD molar absorptivity is given by\textsuperscript{62}:

$$\Delta \epsilon (\omega) = \epsilon_L (\omega) - \epsilon_r (\omega) = \frac{64 \pi^2 \omega N_A}{9 \times 1000 \ln (10) (4 \pi \epsilon_0) \hbar c^2} \times \sum_f g (\omega, \omega_{0f}, \Gamma) R_{0f} \quad (1-52)$$

The optical rotatory strength $R_{0f}$ is given by:

$$R_{0f} = \frac{3}{4} Im [< 0 | \mu | f > < f | m | 0 >] \quad (1-53)$$

1.2.3 Two-Photon Absorption

In 1931 Maria Goeppert-Mayer proposed other mechanisms of light-matter interaction in her doctoral dissertation\textsuperscript{64} using second-order time-dependent perturbation theory\textsuperscript{65}. She found that there is a certain probability that photons with an integer energy fraction of the required first-
order photon energy can excite atoms and molecules to other electronic levels. However, this electronic transition is highly dependent on the magnitude of the photon electric field. In the small field approximation, it is not possible to observe multiphoton absorption unless the system presents a substantial multi-photon absorption cross-section. After the invention of the Laser, high electric fields or intensities were achieved, allowing the third-order two-photon absorption (TPA) process to be discovered in a europium-doped crystal.\textsuperscript{66}

TPA consists of the absorption of two degenerated photons simultaneously. As in the case of OPA, TPA depends on the electric transition dipole moment of each photon, but in this case, the transition occurs into a virtual electronic state (see Figure 1-4) and is highly dependent on the magnitude of the photon electric field. TPA can be determined by multiple techniques where the Z-scan technique\textsuperscript{67} is one of the more popular.

\textbf{Figure 1-5.} \textit{Open aperture Z-scan setup}

To achieve TPA in a molecular system, two ingredients are needed, high electron delocalization, and a powerful light source with high intensity. One optical element able to increase
the intensity of a light source in a particular spot is a convergent lens. Adding a convergent lens to a powerful pulsed laser allows us to measure TPA in inorganic and organic molecules.

![Diagram](image)

**Figure 1-6.** Open aperture Z-Scan curve.

The Z-scan technique is supported by the idea that lenses can focalize light in a tiny spot, increasing the intensity of the light source considerably. This increases the probability of the simultaneous absorption of two photons, where the maximum absorption is observed at the focal point of the lens. The experiment consists of a pulsed laser source with a gaussian beam that presents a fixed energy and is focused on the sample. The sample is moved in and out of the focal plane of the convergent lens in Z direction, where the intensity distribution induces the non-linear process giving a typical open aperture Z-scan curve (see figure 1-6). The open aperture Z-scan technique allows the computation of the TPA cross-sections, thereby fitting the normalized Z-scan curve using:
\[ \Delta T(z) \approx 1 + \frac{\beta I_0 L_{\text{eff}}}{2\sqrt{2}} \left( \frac{1}{1 + \frac{z}{z_0}} \right) \]  

(1-54)

In equation 1-54, \( \beta \), is the two-photon absorption coefficient of the medium, \( I_0 \) is the maximum irradiance at the focus, \( L_{\text{eff}} \) is the effective sample length, \( z \) is the position of the sample with respect to the focal point, and \( z_0 \) is the Rayleigh range. The TPA cross-section can be derived using \( \beta \) as follows, where \( N_0 \) are the number of excited molecules per unit volume and \( \omega \) is the two-photon frequency:

\[
\sigma = \frac{\beta \hbar \omega}{N_0} \]  

(1-55)

Using quadratic response calculations by the implementation of the TD-DFT formalism, it is possible to obtain the TPA theoretical two-photon cross-section as follows:

\[
\delta_{0f}^{\text{TPA}}(\omega) = \frac{4\pi a_0^5}{c} \sum_f (\hbar \omega_f)^2 \delta_{0f}^{\text{TPA}}(\omega_{0f}) g(2\omega, \omega_{0f}, \Gamma) \]  

(1-56)

\[
\overline{\delta}_{0f}^{\text{TPA}}(\omega_{0f}) = \frac{1}{30} (2 \sum_{a,b} S_{aa} S_{bb}^* + 4 \sum_{a,b} S_{ab} S_{bb}^*) \]  

(1-57)

In Eq. 1-56, \( c \) is the speed of the light in vacuum, \( \alpha \) is the fine structure constant, \( E=h\omega \) is the photon energy (in the degenerate case half of the transition energy), \( a_0 \) is the Bohr radius, and \( \overline{\delta}_{0f}^{\text{TPA}}(\omega_{0f}) \) is the orientation averaged two-photon transition probability for the degenerate case defined by Eq. 1-57. A Lorentzian line-shape \( g(2\omega, \omega_{0f}, \Gamma) \) was used to broaden the electronic transitions (Eq. 1-51). The obtained TPA cross-sections are represented in Göpper-Mayer units.
(GM), i.e., $10^{50} \text{cm}^4 \text{s.mol}^{-1} \text{photon}^{-1}$ when atomic units are used for $\delta_{0f}^{TPA}(\omega_{0f})$, $\omega$, and $\Gamma_{gf}$ and cgs units are used for $a_0$ and $c$.

1.2.4 Two-Photon Circular Dichroism

TPCD is defined as the difference of two-photon cross sections, when right and left circularly polarized light is used to excite a chiral molecule. TPCD was predicted theoretically by Tinoco\textsuperscript{69} and Power\textsuperscript{70} independently using the work developed by Maria Göpper-Mayer\textsuperscript{64} as a starting point. The first experimental determination of pure TPCD was achieved by Hernandez and coworkers using the Z-scan technique and was then refined by the implementation of the L-Scan technique\textsuperscript{28} (Figure 1-7).

![Experimental L-Scan setup.](image)

The L-Scan technique is similar to the circularly polarized Z-scan method with the difference being that twin-pulses with different circular polarization states are used. In the experiment, the lenses are moved into the direction of the sample simultaneously, where the
sample is located at an angle of 45 degrees to each beam. These twin pulse configurations would allow one to account for power and noise fluctuations in the beam by considerably increasing its sensitivity to the single beam Z-scan technique. Moreover, TPCD is a powerful technique for the characterization of chiral molecules due to high sensitivity to small conformational changes\textsuperscript{29, 30}, which is of vital importance in the study of biologically active molecules such as proteins. The TPA cross-sections for each light polarization state are calculated as in the Z-scan technique by use of the L-Scan measured curves and equations 1-54 and 1-55.

Besides, as well as in TPA it is possible to calculate TPCD differential two-photon cross-section (Eq.1-58) by the use of quadratic response calculations and the TD-DFT formalism as follows\textsuperscript{69, 70}:

$$\Delta \delta^{TPCD}(\omega) \approx 4.87555 \times 10^{-5} \times \omega^2 \sum f \, g(2\omega, \omega_{0f}, \Gamma) \cdot R_{0f}^{TPCD}(\omega_{0f})$$

(1-58)

$g(2\omega, \omega_{0f}, \Gamma)$ is defined by the Lorentzian curve in Eq.1-51. and $R_{0f}^{TPCD}(\omega_{0f})$ is the two-photon rotatory strength that is given by:

$$R_{0f}^{TPCD}(\omega_{0f}) = -b_1 B_{1Tf}^{TI}(\omega_{0f}) - b_2 B_{2Tf}^{TI}(\omega_{0f}) - b_3 B_{3Tf}^{TI}(\omega_{0f})$$

(1-59)

$b_1$, $b_2$, and $b_3$ are scalars that depend on the experimental setup. For our experimental setup of two colinear photons traveling in the same direction with opposed circular polarization state, $b_1 = 6$, $b_2 = 2$, and $b_3 = -2$. The theoretical TPCD parameters comprising the TPA rotatory
strength $B_1^{TI}, B_2^{TI},$ and $B_3^{TI}$ are defined in function of the generalized tensors $P_{\rho\sigma}^{P,0f} (\omega_{0f}), M_{\rho\sigma}^{P,0f} (\omega_{0f})$ and $T_{\rho\sigma}^{P,0f} (\omega_{0f}).$ The first tensor corresponds to the two-photon electric transition dipole moment in the velocity gauge. While the second tensor corresponds to the two-photon magnetic transition moment in the length gauge. The third tensor accounts for the two-photon electric quadrupole transition moment in the velocity gauge.
1.3 References


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CHAPTER 2. SOLVENT EFFECT ON THE INTERMOLECULAR PROTON TRANSFER OF THE WATSON AND CRICK GUANINE-CYTOSINE AND ADENINE-THYMINE BASE PAIRS: A POLARIZABLE CONTINUUM MODEL STUDY


In this chapter, we present our results on the study of the double proton transfer (DPT) mechanism in the adenine-thymine (AT) and guanine-cytosine (GC) base pairs, both in the gas phase and in solution. The latter was modeled using the polarizable continuum method (PCM) in different solvents. According to our DFT calculations, the DPT may occur for both complexes in a stepwise mechanism in the condensate phase. In the gas-phase only, the GC base pair exhibits a concerted DPT mechanism. Using Wigner’s tunneling corrections to the transition state theory, we demonstrate that such corrections are essential for the prediction of the rate constants of both systems in gas and the condensate phase. We also show that i) as the polarity of the medium decreases the equilibrium constant of the DPT reaction increases in both complexes, and ii) that the equilibrium constant in the GC complex is four orders of magnitude larger than in AT. This observation suggests that the spontaneous mutations in DNA base pairs are more probable in GC than in AT.

2.1. Introduction

From the beginning of the understanding of the biological sciences, there has been an intriguing question about how organisms can inherit the characteristics of their progenitors. This question was answered by Watson and Crick in their seminal article in 1953 when they proposed that deoxyribonucleic acid (DNA) was responsible for the storage and transfer of genetic
information\textsuperscript{1}. DNA consists of two helices of nucleotides composed of a sugar-phosphate backbone and four nitrogenous bases, where the helices are bonded together through the formation of hydrogen bonds between adenine-thymine (AT) and guanine-cytosine (GC) nitrogenous base pairs (see Figures 2-1 and 2-2). Although DNA is known to be a very stable molecule, the original genetic information can be modified through mutations, which are responsible for new variations of a trait\textsuperscript{2}. The mutations in DNA can happen in several ways, including exposure to radiation (fields, ionizing radiation), free radicals (Hydroxyl radicals), metallic centers (Mg), or mutagenic compounds (benzene) and spontaneous mispairing of base pairs\textsuperscript{3-7}. A base mispairs of the double-strand DNA in one, or several positions can produce malformation of proteins, which can lead to an adaptive improvement, or a total malfunction of the cell causing cellular death or metastasis\textsuperscript{8-10}.

In 1963, Löwdin suggested that base pairs in DNA could be responsible for the spontaneous mutation of DNA, where this mutation may occur as a consequence of the DPT reaction between the Watson and Crick base pairs forming rare tautomers such as AT2 GC2\textsuperscript{11-13} (see Figures 2-1 and 2-2). These tautomers can subsequently cause base mispairing during the replication process that can lead to a mutation in the DNA producing the loss or modification of the genetic information. Löwdin also suggested that the DPT does not follow a classical reaction path because the protons behave more like a quantum particle. Consequently, these particles can quantum tunnel (QT). Even though Löwdin’s mechanism of QT seems reasonable, the DPT mechanism in DNA has not been observed yet under physiological conditions. Nevertheless, an experimental study performed by Limbach and co-workers suggests that these rare tautomers can exist at low concentrations, thus under physiological conditions, DNA is indeed a very stable molecule\textsuperscript{14}. 
Although Löwdin presents an exciting possibility for point mutations in DNA, more theoretical work needs to be done to understand how this process occurs entirely. Because these macromolecules are not isolated under physiological conditions, there is a great need to understand the effects of the environment of the DNA on the DPT reaction of its base pairs.

It is well known that 30% of water by weight is essential for DNA to maintain its native configuration stable\textsuperscript{15,16}. It is also known that under biological conditions, nucleic acids are considered charged electrolytes because of the presence of deprotonated phosphate groups in the DNA backbone on the lateral chains. The neutrality of these macromolecules, as well as the cellular environment, is achieved by the presence of monovalent and divalent cations such as Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+}, and Mg\textsuperscript{2+}, which can stabilize or destabilize the double helix structure of DNA\textsuperscript{17,18}. Among these ions, magnesium is considered the most important because it plays a role in most of the nucleic acid activation processes such as RNA three-dimensional folding, DNA replication, and protein codification\textsuperscript{19-22}.

The simplest model to understand the mechanism of spontaneous mutation in DNA due to DPT between base pairs consists of studying the pairs in “isolation,” i.e., without the DNA backbone. In this model, the Van Der Walls interactions (stacking) between base pairs can increase or decrease the spontaneous mutations in DNA\textsuperscript{6}. However, the activity of spontaneous mutations outweighs the contribution of stacking effects. To get a better picture of these and other mechanisms of spontaneous mutation in DNA, in the first approximation, the physiological conditions (determinant for the stability of DNA) are simulated through the inclusion of water molecules in the system\textsuperscript{23,24}. The addition of solvent effects is typically performed following one
of the two methods described next: 1) A gas-phase micro-hydrated model where water molecules are added around the base pairs. This approach requires the direct comparison of bonds lengths with the experimental values to accurately describe the final geometry, which depends on the number of water molecules, added to the system where the base pair hydrogen bond interaction happens. 2) A simulated solvation medium – separated multiple solvent spheres – of non-interacting molecules surrounding the substrate of interest. This model is called the polarizable continuum model (PCM) and has been successfully used to simulate solvated environments in chemical and biochemical systems\textsuperscript{25,26}.

**Figure 2-1.** Double proton transfer (DPT) reaction in the AT base pairs. This reaction could take place via a concerted (CDPT) or through a stepwise mechanism with two different proton transfer steps (SP1 and SP2) achieving the DPT tautomers \(A^*\) and \(T^*\) (AT2 complex).
In the 1960s, Löwdin suggested that the DPT mechanism was a concerted mechanism where both protons in the GC or AT complex are transferred from one molecule to another through tunneling\cite{12}. This approach is only sound in the gas phase, where the environment does not weaken the hydrogen bonds between the base pairs. However, in solution, the surrounding water molecules can interact with atoms that present lone pairs, thus causing the weakening of the inter-base hydrogen bonds. While in the GC complex the oxygen atoms O1(C) and O2(G) can interact with the water molecules in its surroundings via hydrogen bonds, in the AT complex the interaction occurs at the O4(T) oxygen atom. The interaction between the solvent molecules and the base pairs dramatically affects how the proton transference occurs in both systems. The transfer can happen via i) a concerted mechanism where, in a single step, two protons are exchanged, forming the AT2 and GC2 complexes (see Figures 2-1 and 2-2) or ii) a stepwise mechanism where only one proton is transferred at a time forming AT1 and GC1.
Figure 2-2. Double proton transfer (DPT) reaction in the GC base pairs. This reaction could take place via a concerted (CDPT) or through a stepwise mechanism with two different proton transfer steps (SP1 and SP2) achieving the DPT tautomers G* and C* (GC2 complex).

It is already known that the presence of water molecules in the neighborhood of the base pairs weakens the hydrogen bonds\textsuperscript{5,6,27,28}. Consequently, the interaction between the base pairs becomes less critical. This effect results in a decrease in the probability of spontaneous mutations in DNA, which depends on the proximity and interaction between the neighboring water molecules and the base pairs. There are two well-known mechanisms based on the interaction between the water molecules and the base pairs that describe the proton transference reaction\textsuperscript{27,28}. The first one comprises the direct proton transference between the base pairs without any assistance from the water molecules near the base pairs. This scenario is highly probable when the water molecules are not in direct contact with the base pairs due to spatial interference of the DNA backbone and
stacking interactions. This mechanism is typically and satisfactorily modeled using PCM\textsuperscript{22}. The second one considers the assistance of the water molecules in the transfer of exposed protons in the base pairs in a concerted fashion. This type of reaction tends to require more energy compared to the first mechanism because it needs the breaking and formation of bonds in the partaking water molecules.

Using DFT in the Gaussian 03 (G03) suite, Cerón \textit{et al.} \textsuperscript{24,25} proposed a third mechanism of DPT in DNA in AT and GC simulating a micro-hydrated environment without considering stacking interactions (not in the DNA double-strand). Based on the relative free energy of the tautomers with respect to the transition state at 298K and without considering tunneling corrections in the rate constants, Cerón and co-workers’ results suggest that the only base pair vulnerable to spontaneous mutation in DNA is the GC base pair. In their work, the authors were able to achieve a local minimum for the GC2 structure in a micro-hydrated medium, which exhibits a concerted mechanism. They rationalized that during the reaction, the proton H1(G) is transferred in a first step to the N2(C) of the cytosine, and the water-assisted, simultaneously, the transfer of the second proton from N3(C) to O2(G). On the other hand, the DPT on the AT base pair happens in a stepwise mechanism where H6(T) is transferred from Thymine to Adenine in a first step, and then H2(A) is water-assisted transferred from adenine to thymine in a second step.

To pursue a deeper understanding of the actual mechanisms of spontaneous mutation in DNA due to DPT between base pairs in solution, we decided to study the effect of solvent polarity on this process. In this dissertation, we report on the study of DPT of AT and GC base pairs using
PCM and density functional theory at different levels of theory. The study was carried out in water, methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, and 1-heptanol.

2.2. Computational Methods

The structure of all molecules were optimized using the hybrid BP86\textsuperscript{27, 28}, coulomb attenuated method (hybrid CAM-B3LYP)\textsuperscript{29}, range-separated dispersion corrected ωB97x-D\textsuperscript{30}, and the meta-hybrids M05-2x\textsuperscript{31} and M06-2x\textsuperscript{32} functional, with the Pople 6-311++G(d,p) basis set (a basis set considered large enough for DNA description)\textsuperscript{19, 24, 25, 33-35}. To validate the accuracy of our calculations, we compared our results directly with gas-phase data reported previously in the literature using the cc-pVQZ and cc-pVTZ basis set\textsuperscript{36-38} and with Cerón and co-workers results in the gas phase and solution\textsuperscript{24, 25}. The optimizations were performed without any symmetry restrictions (C1 symmetry group). The stationary points corresponding to a minimum or transition state were checked by analyzing the vibrational frequencies at the same level of theory - the absence of imaginary frequencies denoted a minimum of energy, and the presence of an imaginary frequency denoted a transition state.

The condensed phase DPT study was done using the same theoretical level mentioned above in PCM with the integral equation formalism variant (IEFPCM) using water as a solvent. To determine which functional best represented the hydrogen bonds of the AT and GC complex in condensed phase using the 6-311++G(d,p) basis set, we compared the theoretical distances for hydrogens bonds with those reported in the literature\textsuperscript{39, 40}. After finding the best method (the CAM-B3LYP and M05-2X) for both complexes in the condensate phase, the systems were studied in a
series of alcohols that included methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, and 1-pentanol.

To find the equilibrium constant \((K_{eq})\), forward \((k_f)\) and reverse \((k_r)\) rate constants for each step of the mechanism, which are related with the frequency of spontaneous mutations in the base pairs, we computed the energies and the free Gibbs energies at 298.15K for the reactants, transition states and products of DPT using the CAM-B3LYP and M05-2X functional.

Tunneling effects were considered in the calculations including the Wigner's correcting term into the classical transition state theory\(^{41,42}\):

\[
k_{f/r} = \left( 1 + \frac{1}{24} \left( \frac{h|\nu|}{k_B T} \right)^2 \right) \frac{k_B T}{h} e^{-\Delta G_{f/r}^{\ddagger}/RT}
\]  \hspace{1cm} (2-1)

Where \(|\nu|\) is the module of the imaginary frequency of the transition state, \(k_B\) is the Boltzmann’s constant, \(h\) is the Planck’s constant, \(R\) is the ideal gas constant, \(T\) is the temperature in Kelvin and \(\Delta G_{f/r}^{\ddagger}\) is the Gibbs free activation energy for the forward or reverse barrier. These corrections have been considered in the gas phase as well as in the condensed phase. Even though equation (2-1) only describes approximately the nature of the tunneling effect, it usually provides a correction of the same order of magnitude as other more refined approaches such as the Eckart, Skodje and Thuhlar’s barrier methods that include all the vibrational frequencies perpendicular to the transition state\(^{26,43}\). For that reason, the equation (2-1) was used to determine the effect of the
solvent on the rate constant. All calculations were carried using Gaussian 09, a well-known and established computer program for computational chemistry\textsuperscript{44}.

2.3. Results and Discussion

2.1.1 AT and GC Gas-phase.

First, we validated the theoretical schemes employed by comparing the geometric parameters of these molecules, in particular, the hydrogen bonds, with the benchmark MP2 values reported in the literature\textsuperscript{36-38}. Tables 2-1 and 2-2 show the geometric parameters involved in the hydrogen bonds of the AT and GC complexes, respectively. Examining the mean absolute deviation (MAD) calculated from the inter-base hydrogen bond distances, it is clear that our DFT calculations are in excellent agreement with the MP2 and CCSD results. In the AT base pair (Table 2-1), it can be observed that most of the functionals overestimate the hydrogen bond distances in the gas phase, especially in the N2(A)-O1(T) bond distance. This result suggests that neither large Hartree-Fock (HF) exchange (M05-2x and M06-2x), i.e., long-distance interactions (CAM-B3LYP, ωB97x-D), nor long-range and dispersion interactions (ωB97x-D) play a specific role on the geometry prediction of the isolated complex in the gas phase. Consistent with the literature\textsuperscript{24}, the best functional to describe the AT complex in the gas phase is the BP86 functional - BP86 presents a much smaller deviation (0.014 Å) compared to the other functionals. The geometry of the GC complex indicates that the best functional to represent this complex in the gas phase is also the BP86 functional. Nevertheless, the small MAD value (less than 0.03 Å) found in CAM-B3LYP and ωB97x-D compared to the benchmark values suggests that long-range interactions and non-
covalent interactions (dispersion) can be used to study a gas phase DNA fragment which consists of three or more GC base pairs. This as a direct consequence that such interactions should be important in bulk DNA and are not considered in the BP86 functional.

**Table 2-1.** AT interbase hydrogen bond (Å) distances in the Gas Phase calculated with Several Theoretical Schemes.

<table>
<thead>
<tr>
<th>Theoretical level</th>
<th>N2(A)-O1(T)</th>
<th>N1(A)-N6(T)</th>
<th>MAD(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RI-MP2/aug-cc-pVQZ/RI-MP2/cc-pVTZ(^a)</td>
<td>2.86</td>
<td>2.83</td>
<td></td>
</tr>
<tr>
<td>B3LYP(^b)</td>
<td>2.93</td>
<td>2.88</td>
<td>0.060</td>
</tr>
<tr>
<td>BLYP(^b)</td>
<td>2.94</td>
<td>2.90</td>
<td>0.075</td>
</tr>
<tr>
<td>BP86(^b)</td>
<td>2.87</td>
<td>2.83</td>
<td>0.005</td>
</tr>
<tr>
<td>M05-2x(^c)</td>
<td>2.97</td>
<td>2.86</td>
<td>0.069</td>
</tr>
<tr>
<td>M06-2x(^c)</td>
<td>2.96</td>
<td>2.83</td>
<td>0.050</td>
</tr>
<tr>
<td>CAM-B3LYP(^c)</td>
<td>2.93</td>
<td>2.85</td>
<td>0.043</td>
</tr>
<tr>
<td>ωB97x-D(^c)</td>
<td>2.93</td>
<td>2.85</td>
<td>0.035</td>
</tr>
<tr>
<td>BP86(^c)</td>
<td>2.89</td>
<td>2.83</td>
<td>0.014</td>
</tr>
</tbody>
</table>

\(^a\) Benchmark Value from ref 38. \(^b\) From ref 36 with the cc-pVTZ basis set. \(^c\) Data obtained with the 6-311++G(d,p) basis set. \(^d\) Mean absolute deviation of the theoretical distances with respect to the benchmark values.

Afterward, we investigated the DPT reaction between base pairs AT and GC using several theoretical levels and with the Pople’s basis set 6-311++G(d,p). According to Villani, there is only one possible mechanism for the DPT in the AT complex, i.e., a stepwise mechanism. In a first step, the hydrogen H6(T) (see Figure 2-1) migrates from N6(T) to N1(A) generating the AT1 complex where the H2(A)-O1(T) hydrogen bond remains almost unperturbed. Then, in a second step, H2(A) moves from N2(A) to O1(T), achieving the final DPT product AT2 (see Figure 2-1 for detailed mechanism). Our calculations performed at the BP86/6-311++G(d,p) level, showed a global minimum for the canonical structure of the AT complex only where neither the product of
the first proton transference (AT1) nor the DPT product AT2 was found as stable structures in a global minimum. The procedure was repeated with five other functionals - M05-2X, M06-2X, CAM-B3LYP, and ωB97x-D - using the same basis set, no AT1 or AT2 stable structure was attained. These results can be explained considering that in the AT1 complex, the transfer of the H6(T) from Thymine to Adenine creates a very unstable ionic pair-like complex in the gas phase. The same explanation can be used for the AT2 complex, though it presents no charge separation; its charge distribution is unstable in the gas phase. Our results are consistent with those reported by Cerón and coworkers24.

Table 2-2. GC Interbase hydrogen bond (Å) distances in the Gas Phase calculated with Several Theoretical Schemes.

<table>
<thead>
<tr>
<th>Geometry</th>
<th>Theoretical level</th>
<th>O2(G)-N3(C)</th>
<th>N1(G)-N2(C)</th>
<th>N6(G)-O1(C)</th>
<th>MADf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benchmark Valuesa</td>
<td>2.75</td>
<td>2.90</td>
<td>2.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP86b</td>
<td>2.73</td>
<td>2.90</td>
<td>2.89</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>BP86c,d</td>
<td>2.75</td>
<td>2.91</td>
<td>2.90</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>BP86c,e</td>
<td>2.77</td>
<td>2.92</td>
<td>2.92</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>M05-2Xf</td>
<td>2.82</td>
<td>2.96</td>
<td>2.94</td>
<td>0.057</td>
<td></td>
</tr>
<tr>
<td>M06-2Xf</td>
<td>2.80</td>
<td>2.94</td>
<td>2.92</td>
<td>0.042</td>
<td></td>
</tr>
<tr>
<td>Cam-B3LYPc</td>
<td>2.78</td>
<td>2.93</td>
<td>2.91</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>ωB97x-Dc</td>
<td>2.78</td>
<td>2.92</td>
<td>2.91</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>BP86f</td>
<td>2.75</td>
<td>2.91</td>
<td>2.91</td>
<td>0.009</td>
<td></td>
</tr>
</tbody>
</table>

a MP2/aug-cc-pVQZ//MP2/cc-pVTZ and CCSD/aug-cc-pVTZ values obtained from references 38 and 37, respectively. b cc-pVTZ basis set from ref 36. c 6-311++G(d,p) basis set. d Geometry calculated using basic set superposition error (BSSE) from ref 24. e Geometry calculated without BSSE from ref 24. f Mean Absolute deviation from the theoretical benchmark values.

In the case of the GC complex, the mechanism does not exhibit a stepwise mechanism, as suggested by Villani for the AT complex45. At the BP86/6-311++G(d,p) level of theory, the proton transference occurs through a concerted and synchronous DPT mechanism (see Figure 2-2) where
the H1(G) and H3(C) are transferred simultaneously from Guanine to Cytosine and vice versa, respectively. These results agree with the vibrational analysis of the transition state (GC$^+$ complex), which exhibits an imaginary frequency mode at 980.86 cm$^{-1}$ (see Table 2-3). This frequency can be associated with the simultaneous stretching mode of N1(G)-H1(G) and H3(C)-N3(C) bonds.

Table 2-3. Relative Gibbs Free energies (ΔG$_{0}$/Kcal mol$^{-1}$), equilibrium constant and rate constants (s$^{-1}$) of the concerted double proton transfer in the gas phase with different theoretical levels using the 6-311++G(d,p) basis set at 298.15K for the GC complex.

| Theoretical level | ΔG$_{0}$| |ν| |μ$^b$ | k$_f$$^c$ | k$_r$$^c$ | K$_{eq}$ |
|------------------|--------|---|----|---|---|---|---|
| BP86$^a$         | 0      | 9.72 | 8.28 | 970 | 1.67 | 5.27 x 10$^4$ | 2.06 x 10$^{10}$ | 8.4 x 10$^{-7}$ |
| M05-2X           | 0      | 12.40 | 9.51 | 833.12 | 2.16 | 3.69 x 10$^5$ | 4.90 x 10$^{10}$ | 2.6 x 10$^6$ |
| M06-2X           | 0      | 10.31 | 6.99 | 1093.98 | 2.06 | 1.05 x 10$^4$ | 9.76 x 10$^{10}$ | 7.5 x 10$^6$ |
| CAM-B3LYP        | 0      | 12.40 | 9.51 | 1044.25 | 2.06 | 1.05 x 10$^4$ | 9.76 x 10$^{10}$ | 7.5 x 10$^6$ |
| oB97x-D          | 0      | 13.15 | 9.78 | 821.21 | 1.65 | 2.36 x 10$^3$ | 3.48 x 10$^{10}$ | 6.8 x 10$^8$ |
| BP86$^a$         | 0      | 8.69 | 8.56 | 980.86 | 1.93 | 5.13 x 10$^6$ | 9.66 x 10$^{12}$ | 5.3 x 10$^{-7}$ |

$^a$ From ref 24. $^b$ transmission coefficient, including the Wigner’s tunneling correction. $^c$ rate constants, including tunneling corrections.

Next, we calculated the transmission coefficient (μ) for this reaction at 298.15 K, considering Wigner’s tunneling correction to the classical transition state theory$^{41}$. The significant transmission coefficient (1.93) indicates that the reaction has a significant probability of occurring by simultaneous double hydrogen tunneling. This mechanism is particularly curious because the hydrogens H1(G) and H3(C) move in opposite directions simultaneously, locally annulling the forces that oppose the movement of the protons as suggested by Löwdin. The calculated forward and reverse rate constants (k$_f$, k$_r$) are in the range of 10$^6$ and 10$^{12}$, respectively. These values show that the reverse reaction is faster than the forward because the GC complex is 8.6 kcal/mol more stable (lower) than the GC2 complex with an equilibrium constant in the range of 10$^{-7}$. Subsequently, we repeated our calculations with M05-2X, M06-2X, CAM-B3LYP, and oB97x-D, and using the same basis set cited in Table 2-3. Our results reveal that the most stable GC2
complex, with an energy of 6.99 kcal/mol, is achieved with the M06-2x functional. Nevertheless, its transition state (GC\textsuperscript{1}) is less stable than the one obtained with the BP86 functional, the most promising functional in our theoretical scheme. The stability of the GC\textsuperscript{2} using this functional suggests that the exchange interactions can stabilize the complex. Under these conditions, the reaction exhibits an equilibrium constant in the range of $10^{-6}$, i.e. an order of magnitude larger than the one obtained using the BP86 functional. Based on these results, the BP86 is still the most reliable functional to study our system based on the geometrical configuration of the GC complex in gas phase compared to the benchmark values (see Table 2-2).

It is worth noting that in all the schemes considered so far, Wigner’s tunneling corrections cause the transmission coefficient ($\mu$) to be larger than one. Therefore, they should be considered in the rate constants where transmission coefficients in the range 1.65 to 2.16 double the rate constants with respect to the classical transition state theory. The ωB97x-D functional shows the lowest transmission coefficient and presents an equilibrium constant in the order of $10^{-8}$. This suggests that as the amount of non-covalent interactions increases, the tunneling probability and the stability of the DPT reaction product decreases. This factor is important when stacking is significant.

2.1.2 AT and GC Condensed-phase.

In the previous section, we found that the BP86/6-311++G(d,p) is a reliable level of theory to describe the structure and the hydrogen bond distances of the AT and GC Watson and Crick base pairs in the gas phase (the calculated values were very good agreement with the standard).
However, the gas phase geometry did not fit with the experimental data reported in the literature\textsuperscript{39, 40}. This is attributed to deficiencies in the gas phase model. To overcome this challenge, one can incorporate the effects of the molecular environment, such as adding water molecules to the AT and GC complexes. It has been shown that an micro-solvated climate improves the geometries of these complexes\textsuperscript{36}.

\textit{Table 2-4}. Hydrogen bond (Å) distances of the AT complex in water at different theoretical levels using PCM and 6-311++G(d,p) basis set.

<table>
<thead>
<tr>
<th>Theoretical level</th>
<th>N2(A)-O1(T)</th>
<th>N1(A)-N6(T)</th>
<th>MAD\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental\textsuperscript{a}</td>
<td>2.95/2.93</td>
<td>2.82/2.85</td>
<td></td>
</tr>
<tr>
<td>M05-2x</td>
<td>2.97</td>
<td>2.90</td>
<td>0.052/0.047</td>
</tr>
<tr>
<td>M06-2x</td>
<td>2.96</td>
<td>2.87</td>
<td>0.028/0.023</td>
</tr>
<tr>
<td>CAM-B3LYP</td>
<td>2.92</td>
<td>2.89</td>
<td>0.050/0.025</td>
</tr>
<tr>
<td>\textit{ω}B97x-D</td>
<td>2.93</td>
<td>2.87</td>
<td>0.037/0.012</td>
</tr>
<tr>
<td>BP86</td>
<td>2.89</td>
<td>2.88</td>
<td>0.060/0.035</td>
</tr>
</tbody>
</table>

\textsuperscript{a}From ref 40. \textsuperscript{b}Mean absolute deviation respect the experimental values.

To simulate a more realistic biological environment, we extended our gas-phase methodology into the condensate phase using PCM\textsuperscript{22}. To describe the DPT reaction in the condensate phase, we proceeded to select the best theoretical method to reproduce the typical hydrogen bond distances of the AT and GC complexes in solution (see Tables 2-4 and 2-5). The X-ray Crystallographic measurements of Seeman and co-workers on sodium adenylyl-3',5'-uridine hexahydrate\textsuperscript{40}, display two different values for the hydrogen bond length due to the two different environments the AT pairs undergo in the RNA dimer. In Table 2-4 the MAD of these hydrogen bonds estimated using functionals BP86, M05-2x, M06-2x, CAM-B3LYP, and \textit{ω}B97x-D with the Pople’s 6-311++G(d,p) basis set are shown. The direct comparison of the calculated theoretical values with the experimental distances reported in both environments revealed that the
best functionals are the M06-2x (MAD of 0.028 Å) and ωB97x-D (MAD of 0.012 Å) for the first and second environment, respectively. On average, one can certify that the latter works better than the former in AT pairs. However, since the computational cost is significantly higher with the ωB97x-D (Usually the double of time respect M06-2x) depending on the resources available, and stacking interactions are not as important as inter-base interactions, we chose the M06-2x functional to represent the structure of the AT complex in condensate phase. Afterward, we repeated the same procedure, and theoretical scheme explained above, using the M06-2x functional, with the GC complex using PCM. In this part, we compared our theoretical geometry (only one chemical environment contrary to the AT complex) with that obtained by Rosenberg and co-workers on guanylyl-3',5’cytidine nonahydrate via X-ray crystallography\textsuperscript{39}. In Table 2-5 one can observe that the best functionals - lower MAD with respect to the experimental values – are those that contain higher HF exchange, i.e., M05-2x (56%) and M06-2x (54%). This result indicates that exchange interactions in this complex are more important than long and short-range interactions, which can be included with CAM-B3LYP and ωB97x-D.

**Table 2-5.** Hydrogen bond distances (Å) of the GC in water at different theoretical levels using PCM and 6-311++G(d,p) basis set.

<table>
<thead>
<tr>
<th>Theoretical level</th>
<th>O2(G)-N3(C)</th>
<th>N1(G)-N2(C)</th>
<th>N6(G)-O1(C)</th>
<th>MAD\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental\textsuperscript{a}</td>
<td>2.91</td>
<td>2.95</td>
<td>2.86</td>
<td></td>
</tr>
<tr>
<td>M05-2x</td>
<td>2.91</td>
<td>2.96</td>
<td>2.88</td>
<td>0.011</td>
</tr>
<tr>
<td>M06-2x</td>
<td>2.89</td>
<td>2.94</td>
<td>2.87</td>
<td>0.014</td>
</tr>
<tr>
<td>CAM-B3LYP</td>
<td>2.87</td>
<td>2.92</td>
<td>2.85</td>
<td>0.020</td>
</tr>
<tr>
<td>ωB97x-D</td>
<td>2.87</td>
<td>2.92</td>
<td>2.85</td>
<td>0.028</td>
</tr>
<tr>
<td>BP86</td>
<td>2.85</td>
<td>2.91</td>
<td>2.85</td>
<td>0.039</td>
</tr>
</tbody>
</table>

\textsuperscript{a}From ref 39. \textsuperscript{b}Mean absolute deviation respect the experimental values.
It is worth highlighting that the PCM calculations reproduced the base pairs geometries obtained by Cerón and coworkers using the microhydrated model employed for the AT and GC complexes\textsuperscript{24, 25}, where the GC complex exhibits a better geometry when it is compared to the experimental values. This outcome demonstrates the reliability of using PCM, a less complicated approach, to study our systems (at least from the geometry point of view) without specifying the presence of surrounding water molecules on the pairs and, consequently, in a shorter computational time.

Next, we studied the DPT reactions for both complexes, including Wigner’s tunneling corrections in the rate constants. This was done in seven different solvents (methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, and 1-heptanol) using PCM. To begin, we studied the AT complex at the M06-2x/6-311++G(d,p) level of theory. We found a global minimum for the canonical Watson and Crick AT complex and the DPT complex, AT2. After several attempts, we were not able to find a stable AT1 complex and a transition state between the AT and AT2 complex (concerted reaction path, see Figure 2-1). Perhaps the AT1 complex does not become stable, ignoring long-distance interactions due to the lack of a third hydrogen bond in comparison with the GC complex. Long-range interactions, however, could easily be included using the CAM-B3LYP or the ωB97x-D. To overcome this challenge, we decided to study the DPT reaction using CAM-B3LYP/6-311++G(d,p). This scheme displayed global minima for the AT, AT1, and AT2 complexes. The DPT reaction stepwise mechanism (see Figure 2-1) consists of i) the proton H6(T) is transferred from N6(T) to N1(A) with an imaginary transition state frequency of 607.77i cm\textsuperscript{-1} and ii) the transference of the proton H2(A) from N2(A) to O1(T) with a transition state imaginary frequency of 204.52i cm\textsuperscript{-1} in water.
In Table 2-6 we present the hydrogen bond distances for the DPT reaction in AT complex in several solvents. To start our analysis, we chose water as the medium. In this solvent, the first transition state (AT$^\dagger$ complex) exhibits shorter distances in all the hydrogen bonds with the most drastic change is found in the N1(A)-N6(T) distance. This result suggests that a decrease in the length of the hydrogen bonds is needed for the reaction to proceed. Then, the gap between the N1(A) and the H6(T) also decreases significantly with respect to the AT complex. This indicates that in the transition state, the hydrogen H6(T) is more likely to be near to the Adenine compared to the canonical structure, which presents a relative Gibbs energy of 6.842 kcal/mol (see Table 2-7). After the first transition state, a global minimum with shorter hydrogens bond distances than the transition state (except in the case N1(A)-N6(T) hydrogen bond) is located in the AT1 complex. The hydrogen H6(T) is bonded to the Adenine by the N1(A) atom. The observed decrease in hydrogen bond distances, as well as the charge separation between the base pair, causes this state to be less stable than the canonical structure, which presents a relative free Gibbs energy of 6.170 kcal/mol (see Table 2-7). Our results indicate that the transition state (AT$^\dagger$ complex) is structurally more similar to the AT1 complex than the AT complex. The second transition state (AT1$^\dagger$ complex) shows a shorter distance in the N2(A)-O1(T) bond compared to the AT1 complex, where the hydrogen H6(A) is closer to the Thymine. It is also notable that the N1(A)-N6(T) distance becomes comparable with that of the canonical AT complex. The increase in range causes the N1(A)-H6(T) bond to increase in strength, thus making the bond shorter with respect to the AT1 complex, which presents a relative Gibbs free energy of 14.798 kcal/mol.
Table 2-6. Hydrogen bond distances (Å) of AT complex during its double proton transfer in several solvents at the theoretical level CAM-B3LYP/6-311++ G(d,p).

<table>
<thead>
<tr>
<th>Bond</th>
<th>Water</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>1-Propanol</th>
<th>1-Butanol</th>
<th>1-Pentanol</th>
<th>1-Hexanol</th>
<th>1-Heptanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2(A)-O1(T)</td>
<td>2.9228</td>
<td>2.9226</td>
<td>2.9222</td>
<td>2.9222</td>
<td>2.9225</td>
<td>2.9226</td>
<td>2.9227</td>
<td>2.9240</td>
</tr>
<tr>
<td>N1(A)-N6(T)</td>
<td>2.8931</td>
<td>2.8919</td>
<td>2.8913</td>
<td>2.8905</td>
<td>2.8898</td>
<td>2.8892</td>
<td>2.8881</td>
<td>2.8876</td>
</tr>
<tr>
<td>H2(A)-O1(T)</td>
<td>1.9058</td>
<td>1.9056</td>
<td>1.9052</td>
<td>1.9053</td>
<td>1.9056</td>
<td>1.9058</td>
<td>1.9059</td>
<td>1.9072</td>
</tr>
<tr>
<td>N1(A)-H6(T)</td>
<td>1.8524</td>
<td>1.8511</td>
<td>1.8504</td>
<td>1.8495</td>
<td>1.8488</td>
<td>1.8468</td>
<td>1.8481</td>
<td>1.8462</td>
</tr>
<tr>
<td>AT†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2(A)-O1(T)</td>
<td>2.7869</td>
<td>2.7852</td>
<td>2.7840</td>
<td>2.7830</td>
<td>2.7820</td>
<td>2.7812</td>
<td>2.7797</td>
<td>2.7788</td>
</tr>
<tr>
<td>N1(A)-N6(T)</td>
<td>2.5968</td>
<td>2.5977</td>
<td>2.5982</td>
<td>2.5987</td>
<td>2.5993</td>
<td>2.5998</td>
<td>2.6008</td>
<td>2.6015</td>
</tr>
<tr>
<td>H2(A)-O1(T)</td>
<td>1.7559</td>
<td>1.7539</td>
<td>1.7524</td>
<td>1.7512</td>
<td>1.7498</td>
<td>1.7489</td>
<td>1.7470</td>
<td>1.7458</td>
</tr>
<tr>
<td>N1(A)-H6(T)</td>
<td>1.1840</td>
<td>1.1815</td>
<td>1.1801</td>
<td>1.1788</td>
<td>1.1774</td>
<td>1.1760</td>
<td>1.1735</td>
<td>1.1719</td>
</tr>
<tr>
<td>AT‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>N2(A)-O1(T)</td>
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<td>1.1243</td>
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<tr>
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<td>2.5202</td>
<td>2.5207</td>
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<td>2.8142</td>
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<td>2.8131</td>
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<td>2.8110</td>
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<tr>
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<td>1.0462</td>
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<td>1.0465</td>
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<tr>
<td>AT2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N2(A)-O1(T)</td>
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<td>2.5509</td>
<td>2.5509</td>
<td>2.5509</td>
<td>2.5509</td>
<td>2.5509</td>
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<td>2.5511</td>
</tr>
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<td>N1(A)-N6(T)</td>
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<td>2.8290</td>
<td>2.8286</td>
<td>2.8281</td>
<td>2.8278</td>
<td>2.8271</td>
<td>2.8262</td>
<td>2.8262</td>
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<tr>
<td>H2(A)-O1(T)</td>
<td>1.0765</td>
<td>1.0766</td>
<td>1.0767</td>
<td>1.0767</td>
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</tr>
<tr>
<td>N1(A)-H6(T)</td>
<td>1.0434</td>
<td>1.0434</td>
<td>1.0435</td>
<td>1.0435</td>
<td>1.0435</td>
<td>1.0436</td>
<td>1.0437</td>
<td>1.0436</td>
</tr>
</tbody>
</table>
In summary, in the second step, a global minimum is reached for the AT2 complex were the N1(A)-N6(T) bond distance lies close to the canonical AT complex. Nevertheless, the N2(A)-O1(T) is smaller compared with the canonical AT complex, which creates instability in the molecule due to the minor charge separation compared with the AT complex. This one also contains a relative Gibbs free energy of 13.843 kcal/mol (see Table 2-7), indicating that the second transition state (AT1‡ complex) resembles more the AT2 complex than the AT1.

*Table 2-7.* Relative Gibbs Free energies (Kcal mol$^{-1}$), transition state frequencies (cm$^{-1}$), equilibrium constant, and rate constants (s$^{-1}$) of the double proton transfer of the AT complex in solution with different solvents using the theoretical level Cam-B3LYP/6-311++G(d,p) at 298.15K.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>AT</th>
<th>AT$^\dagger$</th>
<th>AT1</th>
<th>AT1$^\dagger$</th>
<th>AT2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
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<td>6.842</td>
<td>6.170</td>
<td>14.798</td>
<td>13.843</td>
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<tr>
<td>Methanol</td>
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<td>6.849</td>
<td>6.150</td>
<td>14.773</td>
<td>13.821</td>
</tr>
<tr>
<td>Ethanol</td>
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<td>7.610</td>
<td>6.136</td>
<td>14.757</td>
<td>13.811</td>
</tr>
<tr>
<td>1-Propanol</td>
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<td>7.614</td>
<td>6.122</td>
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<td>13.802</td>
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<td>7.623</td>
<td>6.107</td>
<td>13.794</td>
<td>13.794</td>
</tr>
<tr>
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<td>7.624</td>
<td>6.084</td>
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<td>13.786</td>
</tr>
<tr>
<td>1-Hexanol</td>
<td>0</td>
<td>7.654</td>
<td>6.064</td>
<td>14.702</td>
<td>13.772</td>
</tr>
<tr>
<td>1-Heptanol</td>
<td>0</td>
<td>7.667</td>
<td>6.043</td>
<td>14.694</td>
<td>13.765</td>
</tr>
</tbody>
</table>

| Solvent | |ν1|/|ν2| | μ1/μ2$^a$ | k$_{f1}$/k$_{f2}$ x 10$^7$ | k$_{r1}$/k$_{r2}$ x 10$^{12}$ | K$_{eq}$ x 10$^{-11}$ |
|---------|-----|-----|-----|----------|----------------|----------------|----------------|----------|
| Water   | 607.77/204.52 | 1.36/1.04 | 8.14/0.306 | 2.71/1.28 | 7.13 |
| Methanol| 564.95/203.7 | 1.31/1.04 | 7.76/0.309 | 2.50/1.30 | 7.39 |
| Ethanol | 550.11/206.14 | 1.28/1.04 | 2.10/0.310 | 0.662/1.31 | 7.52 |
| 1-Propanol | 519.12/197.39 | 1.26/1.04 | 2.05/0.300 | 0.632/1.28 | 7.63 |
| 1-Butanol| 493.05/204.56 | 1.24/1.04 | 1.98/0.305 | 0.594/1.32 | 7.74 |
| 1-Pentanol| 466.92/210.63 | 1.21/1.04 | 1.91/0.306 | 0.551/1.35 | 7.85 |
| 1-Hexanol| 420.9/209.2 | 1.17/1.04 | 1.78/0.302 | 0.497/1.35 | 8.04 |
| 1-Heptanol| 389.78/207.47 | 1.15/1.04 | 1.71/0.295 | 0.459/1.35 | 8.14 |

$^a$. Transmission coefficient with Wigner’s tunneling correction.
To continue with our analysis, we studied the effect of different solvents in the hydrogen bond distance during the DPT reaction of the AT complex. In Table 6, one can observe how the hydrogen bond distance in N1(A)-N6(T) decreases as the polarity of the medium decreases. In AT and AT2 complexes, however, N2(A)-O1(T) did not exhibit any particular trend as still observed in the rest of the structures. In Table 2-7, it can be noted in all solvents that the first forward rate constant ($k_{f1}$) is larger than the second forward rate constant ($k_{f2}$) by approximately one order of magnitude. The observed difference indicates that the second step is the determining step of the reaction. This behavior can also be noticed in the transition state imaginary frequencies, which are more significant in the first proton transference and not the second. Therefore, the Wigner’s transmission coefficient ($\mu$) becomes considerably more significant than one for the first barrier. In a scenario like this, the tunneling becomes more probable during the first step of the reaction.

On the other hand, the effect of the solvent causes a progressive decrease of the first Wigner’s transmission coefficient ($\mu$) as the polarity of the solvent decreases. This effect can also be observed in the first forward and reverse rate constants but not in the second rate constants or second Wigner’s transmission coefficient. The latter remain virtually unchanged demonstrating that the first step is the most affected by the environment in all solvents. Overall the AT1 and AT2 complexes are more stable with respect to the canonical AT complex when the polarity of the medium decreases. The translation of this effect into a larger equilibrium constant would indicate an increase in the spontaneous mutation of the AT when the solvent polarity decreases. An equilibrium constant in the range of $10^{-11}$ certifies the proposition of spontaneous mutations in DNA$^{46}$
Table 2-8. Hydrogen bond distances (Å) of GC complex during its double proton transfer in several solvents at the theoretical level M05-2x/6-311++ G(d,p).

<table>
<thead>
<tr>
<th>Bond</th>
<th>Water</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>1-Propanol</th>
<th>1-Butanol</th>
<th>1-Pentanol</th>
<th>1-Hexanol</th>
<th>1-Heptanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O2(G)-N3(C)</td>
<td>2.9102</td>
<td>2.9081</td>
<td>2.9069</td>
<td>2.9052</td>
<td>2.9049</td>
<td>2.9031</td>
<td>2.9018</td>
<td>2.9012</td>
</tr>
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<td>2.9603</td>
<td>2.9602</td>
<td>2.9606</td>
<td>2.9602</td>
<td>2.9603</td>
<td>2.9605</td>
<td>2.9608</td>
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<td>O2(G)-H3(C)</td>
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<td>1.8905</td>
<td>1.8892</td>
<td>1.8874</td>
<td>1.8870</td>
<td>1.8850</td>
<td>1.8835</td>
<td>1.8829</td>
</tr>
<tr>
<td>H1(G)-N2(C)</td>
<td>1.9301</td>
<td>1.9302</td>
<td>1.9302</td>
<td>1.9306</td>
<td>1.9303</td>
<td>1.9304</td>
<td>1.9307</td>
<td>1.0306</td>
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<tr>
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</tr>
<tr>
<td>O2(G)-N3(C)</td>
<td>2.7252</td>
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<td>2.7116</td>
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<td>N1(G)-N2(C)</td>
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<td>1.6573</td>
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<tr>
<td>H1(G)-N2(C)</td>
<td>1.2153</td>
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<td>1.2109</td>
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<td>1.2082</td>
<td>1.2067</td>
<td>1.2046</td>
<td>1.2034</td>
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<tr>
<td>GC1</td>
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<td></td>
</tr>
<tr>
<td>O2(G)-N3(C)</td>
<td>2.6443</td>
<td>2.6349</td>
<td>2.6298</td>
<td>2.6254</td>
<td>2.6208</td>
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<td>2.7881</td>
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<td>1.5559</td>
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<td>1.5405</td>
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<td>1.0728</td>
<td>1.0739</td>
<td>1.0746</td>
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<tr>
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<tr>
<td>O2(G)-N3(C)</td>
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<td>2.4809</td>
<td>2.4806</td>
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<td>N1(G)-N2(C)</td>
<td>2.8423</td>
<td>2.8397</td>
<td>2.8382</td>
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<td>2.8356</td>
<td>2.8344</td>
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<td>2.8309</td>
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<tr>
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<td>2.6253</td>
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<td>1.0353</td>
<td>1.0346</td>
<td>1.0337</td>
<td>1.0330</td>
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<td>1.0311</td>
</tr>
<tr>
<td>H1(G)-N2(C)</td>
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<td>1.0330</td>
<td>1.0331</td>
<td>1.0331</td>
<td>1.0332</td>
<td>1.0332</td>
<td>1.0333</td>
<td>1.0334</td>
</tr>
</tbody>
</table>
After completing the study of the DPT mechanism of the AT complex in several solvents, we proceeded to study the DPT mechanism in GC complex employing M05-2X/6-311++G(d,p), which yielded global minima for the GC, GC1 and GC2 complexes in all solvents. The global minima point towards a stepwise mechanism in the condensate phase (see Figure 2-2). In the first step, the hydrogen H1(G) is transferred from the N1(G) to the N2(C). In water, the transition state of step one (GC\(^1\)) has an imaginary vibrational frequency of 812.26\(i\) cm\(^{-1}\) (see Table 2-9). Table 2-8 shows that in this transition state, the N1(G)-N2(C) presents the largest decrease in the bond distance with respect to the canonical GC complex. This causes the hydrogen H1(G) to be closer to the Cytosine, i.e., the transition state is more like the product. This transition state presents a relative free Gibbs energy of 7.427 kcal/mol (see Table 2-9), a slightly larger value than in the case of the AT complex.

Then, a global minimum is reached in the GC1 complex after the first transition state. The relative Gibbs free energy of 7.409 kcal/mol confirms that the transition state and the product of the first proton transference in GC1 are very similar. In this complex, the N1(G)-N2(C) bond distance increases with respect to the first transition state (GC\(^1\)) and remains smaller than the GC complex. Also, the O2(G)-N3(C) bond distance decreases more significantly with respect to the first transition state than in the case of the N2(A)-O1(T) hydrogen bond in the AT1 complex. Therefore, better interaction between the H3(C) and the O2(G) atoms is anticipated. In the second step of the reaction, the proton H3(C) is transferred from the N3(C) to O2(G). In water, the transition state of this second step (GC1\(^2\)) has a relative Gibbs free energy of 9.256 kcal/mol with an imaginary vibrational frequency of 1142.12\(i\) cm\(^{-1}\). The O2(G)-N3(C) bond distance decreases with respect to the GC1 complex, thus making the proton H3(C) closer to the Guanine than in the
GC complex. This complex also presents an increase in the N1(G)-N2(C) bond length, causing an increase in the H1(G)-N2(C) bond strength. Afterward, the second step ends with a global minimum in the GC2 structure, which presents a relative Gibbs free energy of 8.609 kcal/mol. The fact that this value is closer to the second transition state rather than to the GC1 complex makes the transition state resembles more the GC2 complex. Moreover, the O2(G)-N3(C) bond distance is larger than in the presiding transition state and remains smaller than in the canonical GC complex. Consequently, this bond length causes the destabilization of the GC2 complex with respect to the GC due to small charge separation. On the other hand, the N1(G)-N2(C) bond distance is slightly smaller than in the canonical GC complex making this structure more prone to hydrogen bond formation. The shorter N1(G)-N2(C) bond may have an effect in the shape of the DNA double strand as well as the neighbor interbase distances.

In Table 2-9, we present the thermodynamic data of the reaction. One can notice that the first forward rate constant is three orders of magnitude smaller than the second forward rate constant. This difference in value makes the first step the rate-limiting step in the reaction, which involves a close interaction between the N1(G) and N2(C) atoms contrary to the AT complex where the second step is the rate-determining step. Conversely, both reverse reaction rates are of the same order of magnitude, the first one being slightly larger than the second. It is also notable that the Wigner’s transmission coefficient is significantly larger in the second step compared to the first. This indicates that tunneling is more probable in the second step of the reaction. Nevertheless, both transmission coefficients are larger than in the case of the AT complex, suggesting that the GC DPT reaction is more likely to proceed by tunneling than in the AT complex with an equilibrium constant that is four orders of magnitude larger (on the order of $10^{-7}$) than the
one reported by Cerón and coworkers\textsuperscript{25}. In Cerón’s model, the water molecules assist the DPT directly following a concerted path, which requires more energy in comparison with the two-step mechanism proposed herein. We should highlight that in this case, the surrounding water molecules are not directly involved in the mechanism of the reaction. The type of mechanism taking place in DNA depends on how DNA allows the proximity of water molecules to the base pairs. Following the Florian and Leszczynski estimation on the delivered lifetime ($< 10^{-10}$ s) based on the characteristic base-pair opening\textsuperscript{47}, it is possible to determine if the chemical model employed will yield to a permanent mutation or not. Although the previous prediction is based on a classical picture, the molecular potential during the inter-base opening may stabilize the rare tautomeric species. Another scenario to be considered is the quantum picture where these rare tautomers can be formed by a successive single proton tunneling process near the DNA replication stage, generating a point mutation in the successive cloned DNA fragments. Nevertheless, neither the classical picture (molecular potential stabilization) nor the quantum picture has been observed experimentally yet, making it difficult to confirm these hypotheses. The experimental evidence of the DNA mutation rate (base pair opening time) shows that the quantum chemical model employed in this dissertation does not meet the characteristics needed to achieve a permanent mutation at the biological level. This requires reverse barrier energy of at least 3 Kcal mol\textsuperscript{-1} following the Florian and Leszczynski estimation on the delivered lifetime.
Table 2-9. Relative Gibbs Free energies (Kcal mol$^{-1}$), transition state frequencies (cm$^{-1}$), equilibrium constant and rate constants (s$^{-1}$) of the double proton transfer of the GC complex in solution with different solvents using the theoretical level M05-2X/6-311+G(d,p) at 298.15K.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>GC</th>
<th>GC$^2$</th>
<th>GC1</th>
<th>GC1$^2$</th>
<th>GC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0</td>
<td>7.427</td>
<td>7.409</td>
<td>9.256</td>
<td>8.609</td>
</tr>
<tr>
<td>Methanol</td>
<td>0</td>
<td>7.583</td>
<td>7.548</td>
<td>9.287</td>
<td>8.583</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0</td>
<td>7.663</td>
<td>7.618</td>
<td>9.305</td>
<td>8.574</td>
</tr>
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$^a$. Transmission coefficient with Wigner’s tunneling correction.

The effect of the polarity of the solvent in the medium was also studied for the GC complex during the DPT reaction [see Tables 2-8 (geometry) and 2-9 (thermodynamics and kinetics)]. First, the geometry of the complexes involved in the DPT reaction of the GC complex was studied, evidencing that in GC complex, the O2(G)-N3(C) bond length decreases with the polarity of the medium. As expected, the reduction of O2(G)-N3(C) makes the hydrogen bonds stronger. Nevertheless, the N1(G)-N2(C) bond does not follow any particular trend with the solvent polarity. In the latter bond there is not a direct interaction of the solvent with it as was observed in O2(G)-N3(C) hydrogen bond. Therefore, the strength of the N1(G)-N2(C) hydrogen bond depends only
on the covalent and non-covalent interactions between the base pairs. In this case, the base pairs are modified by the interaction of the system boundaries with the solvent.

In the first transition state (GC\textsuperscript{‡} complex) one can observe the same trend of the O2(G)-N3(C) hydrogen bond distances as in the GC complex. Contrary to the GC complex the N1(G)-N3(C) hydrogen bond distance presents a particular trend where this bond distance increases inversely with the solvent polarity causing the destabilization of the transition state (see Table 2-9) and reducing the distance between the H1(G) and N2(C) with the polarity of the solvent.

In the GC1 and GC1\textsuperscript{‡} complexes, one can observe the same trend in the O2(G)-N3(C) hydrogen bond. In contrast to the first transition state (GC\textsuperscript{‡}) the N1(G)-N2(C) bond distance decreases as the polarity of the solvent decrease. This dependence destabilizes the GC1 complex due to small charge separation and the GC1\textsuperscript{‡} complex because the transition state requires a stronger interaction between the O2(G) and N3(C) than between the N1(G)-N2(C) (see Table 2-9). The GC2 complex, however, shows an opposite trend compared to the GC1 and GC1\textsuperscript{‡} complexes: as the polarity of the solvent decreases the N1(G)-N2(C) and in the O2(G)-N3(C) hydrogen bond increases. This effect causes the stabilization in the GC2 complex.

Finally, the kinetic and thermodynamic data (Table 2-9) shows that the $k_{f1}$, $k_{r1}$, and $k_{r2}$ rate constants, with Wigner’s transmission coefficient tunneling corrections, increase with the polarity of the solvent. However, the second forward rate constant ($k_{f2}$) decreases with the polarity of the solvent. This dependence makes the first step the determining step of this reaction in all the studied solvents. Therefore, the equilibrium constant of the global reaction increases inversely with the
polarity of the solvent, a phenomenon that was also observed for the AT complex. Nevertheless, the GC equilibrium constant is four orders of magnitude larger than the AT equilibrium constant, showing that under these conditions, the spontaneous mutations in DNA due to DPT are more likely to occur in the GC complex under low polarity conditions. The justification of the significant difference between the equilibrium constant and rate constants in AT and GC proton transfer is due to the third hydrogen bond located in the GC complex (N6(G)-O1(C) bond). This bond is responsible for the stabilization of proton transfer product GC2. Even though this complex is relatively unstable respect to the GC complex, the extra hydrogen bond makes it more stable to respect the AT2 complex (relative to AT) (see tables 2-7 and 2-9). It can also be observed stabilization in the second transition state, which is translated in a larger rate constant. On the other hand, the GC1 complex is slightly unstable when it is compared with the AT1. This effect is a consequence of the charge separation during the first proton transfer that destabilizes the third Hydrogen bond. Nevertheless, this instability is significantly shorter to the gained in the GC2 complex due to charge stabilization for that reason we have a large equilibrium constant in the successive proton transfer of the GC complex when it is compared with AT. On the other hand, the GC complex exhibits an equilibrium constant that exceeds for at least in one order of magnitude the spontaneous mutation equilibrium constant estimated by Topal and coworkers. This difference can be attributed to the proximity of the water molecules used in our model based on the standard parameters of the PCM in G09 showing once more that the effect of water molecules proximity is very important when these complexes are studied.

Despite the ability of the proposed model in this work to reproduce the experimental geometry of the DNA base pairs GC and AT in biological conditions, the studied double proton
transfer mechanisms will not lead to a permanent biological mutation if the rough reverse barrier limit (3 Kcal mol\(^{-1}\)) of Florian and Leszczynski is followed\(^{47}\). There is insufficient experimental evidence to show that the Florian’s and Leszczynski’s estimate is the only minimal condition for such mutation to occur. For that reason, other scenarios may be considered during the DNA mutation process such as the quantum behavior of the protons. This attractive hypothesis suggests that even though the classical reaction rate is not faster than the DNA opening time, the quantum tunneling rate may be faster than the classical reaction rate inducing a permanent mutation in DNA. Such a mutation will present a small probability and should be related to Topal’s equilibrium constant interval\(^{46}\).

The simplicity of our model limits the interactions that DNA base pairs may have in a biological environment. For that reason, more accurate methods can be used to simulate the correct DNA environment that can include dynamics effects such as molecular dynamics (MD), Carl Parrinello molecular dynamics, and reactive force field (ReaxFF) that will allow the rearrangement of the surrounding water molecules as a function of the molecular potential during the DPT process. The addition of stacking interactions is also crucial since they tune the double proton process\(^{19}\). In summary, our study showed that the polarity of the solvent is essential during the DPT reaction process exhibiting significant changes in rate constants, Wigner’s transmission coefficients, and equilibrium constant when the polarity of the medium is modified. In particular, the DPT transfer mechanism studied for the GC, and AT base pairs exhibited an increase in the formation of the GC2 and AT2 complexes when the polarity of the medium decreased trend that we expect to observe in the real biological system.
2.4. Conclusions

We demonstrated that the AT complex is less prone to react in a concerted or stepwise mechanism in the gas phase. The GC complex displays a concerted reaction mechanism thermodynamically allowed with an equilibrium constant in the range of $10^{-7}$.

Using Wigner’s tunneling corrections to the transition state theory, we demonstrate that such corrections are essential for the prediction of the rate constants of both systems in gas and condensate phase. We showed that DFT and PCM are able to reproduce the hydrogen bond distances of the AT and GC complexes when water is used as a solvent. We demonstrated that the AT and GC complexes exhibit a stepwise mechanism in all the studied solvents with no direct involvement of the solvent in the DPT process. We validated the effect of solvent in the rate and equilibrium constants and showed an inverse dependence of the latter with the polarity of the solvent in both complexes. The GC DPT reaction exhibits the larger equilibrium constant which is about four orders of magnitude larger than in the case of the AT complex, indicates that the spontaneous mutations in the studied base pairs are more likely to occur in the GC complex rather than in the AT complex when PCM is used to simulate the solvation environment during the double proton transfer reaction.

2.5. References


CHAPTER 3. ROLE OF THE AMYLOID-β(1-42) ELECTRIC DIPOLE MOMENT ON FIBRILS FORMATION

In the previous chapter we studied point mutations of DNA base pairs in different environments using tunneling corrections demonstrating the importance of quantum effects in biological systems. In order to exert the benefits of quantum biology on complex biological systems in this chapter we study the aggregation mechanism and cytotoxic pathway of the Amyloid-β_{1-42}. This peptide is one of the primary peptides found in senile plaques in patients with Alzheimer’s disease (AD). Though, the mechanism of fibril formation associated with AD is still unknown. To help answer this question, we performed the theoretical study of the Aβ_{1-42} monomer, stacked oligomers, and protofibrillar structures, optimized at the PM6 theoretical level in the gas phase. Our findings show that the monomers exhibit an L-S shaped topology with the same remarks confirmed by L. Gremer et al. experimental results and present an intense localized electric dipole moment in all three arrangements that can promote fibril formation through a cascaded dipole-dipole interaction. The protofibril exhibits the most localized dipole moment, oriented parallel to the fibril axis. We hypothesize that the resultant dipole moment assists in the formation of fibrils found in AD via kinetic control. The stabilization energies, as well as electric dipole orientation, suggest that the fibril formation mechanism follows a nucleated polymerization route where oligomers are thermodynamically stable after achieving nucleation.
3.1 Introduction

Alzheimer’s Disease (AD) is one of the most common chronic neurodegenerative diseases. AD is responsible for 60-70% of dementia cases worldwide\(^1\) and is pathologically characterized by extracellular deposits of Amyloid-\(\beta\) (A\(\beta\)) in senile plaques (typically containing 40 to 42 residues), intraneuronal neurofibrillary tangles, and reduced brain function that eventually leads to neuronal death\(^4\). Researchers believe that the pathogenesis of the disease is associated with the accumulation of A\(\beta\) which are small peptides prone to form aggregates\(^5, 6\). Although the potential neurotoxic behavior of A\(\beta\) is known\(^11-13\), it is still unknown what is the direct correlation between the accumulation of A\(\beta\) and the progression of the disease\(^8\). Additionally, A\(\beta\) is present in both cerebrospinal fluid and plasma of healthy individuals, indicating that A\(\beta\) plays a role in normal physiology\(^14\), in particular, the synapsis regulation by depressing neuronal activity\(^4, 8, 15-17\).

The proteolytic process leading to the formation of A\(\beta\) from the amyloid precursor protein (APP) is well characterized\(^18\). APP is transported to the outer membrane, where it is subject to proteolysis by \(\alpha\)-secretase. Failure of APP cleavage by \(\alpha\)-secretase causes the breakup of the APP by \(\beta\)-secretase and \(\gamma\)-secretase generating A\(\beta\)\(^4, 6-8, 19-21\). A\(\beta\) can also be produced by APP rupture in the Golgi apparatus and later secreted into the extracellular space\(^22\). The most abundant secreted A\(\beta\) peptide is 40 amino acids in length (A\(\beta\)\(_{1-40}\)). However, the smaller secreted fraction of 42 amino acids (A\(\beta\)\(_{1-42}\)) receives significant attention due to the propensity of these A\(\beta\) peptides to nucleate and yield to the production of fibrils\(^23\). Though the neurotoxic mechanism of A\(\beta\) is not entirely understood, experimental evidence shows that small oligomers are more neurotoxic than fibrils\(^4, 7\).
as a result of their large surface area, and their possible interaction with the neuronal membrane. Fibrils are also attributed to structural modification of the synapses and even neuronal death, indicating that small oligomers are responsible for pathological and neuronal changes during the early stages of AD while the fibrils are the cause of the chronic stages of the disease. The formation of fibrils and its later accumulation (plaques) causes irreversible damage to the brain. Therefore, it is essential to understand the mechanism of fibril formation, starting from the monomers, to uncover the mechanism of action of AD and try to propose a potential treatment for neurodegenerative diseases like AD, including Parkinson’s and Huntington's disease that is also characterized by the presence of Aβ plaques.

Fibrils tend to be heterogeneous, making the production of highly ordered samples difficult. The literature describes Aβ fibrils as protofilaments tangled together, forming a helical structure. The structures exist in several polymorphs showing different cross-sections, widths, helical pitches, and interactions between protofilaments. The existence of different isomorphs within the fibril makes its characterization demanding. Despite the preceding limitations, researchers have constructed models of fibrils at atomic resolution using solid-state nuclear magnetic resonance (NMR), which have partially uncovered part of the geometry of several amino acid fragments within the fibril. Even though solid-state NMR is a powerful technique for the elucidation of peptides, it fails to resolve the structure of fibrils by itself.

In contrast to solid-state NMR, cryo-electron microscopy (cryo-EM) and atomic-force microscopy, offer additional capabilities that contribute to Aβ fibrils elucidation. Recently L.
Gremer et al. \cite{Gremer2017} elucidated the complete structure of Aβ1-42 fibrils by cryo-EM at a resolution of 4.0 Å. They showed that the monomers within the protofibril exhibit an L-S topology and stack in parallel with an approximated 21 screw symmetry. Based on L. Gremer et al. experimental structure of Aβ1-42 fibrils, in this publication, we present the theoretical electronic structure study of Aβ1-42 in the monomeric and oligomeric (stacked and protofibril) form using semi-empiric Parametric Method 6 (PM6)\cite{ParametricMethod6}. Our results show that monomers and oligomers present highly oriented electric dipole moments that can contribute to the formation of Aβ1-42 fibrils. This outcome gives insight into the potential mechanism of fibril formation from the thermodynamic and kinetic point of view. Eight structures were optimized at the theoretical level PM6 (Fig. 3-1) starting from the Aβ1-42 monomer, including arrangements with protofibrillar interactions, stacking interactions, or both.
Figure 3-1. Structures of the $\text{A}\beta(1-42)$ peptide. (A) Monomer. (A/A) Stacked parallel dimer. (A/B) Stacked antiparallel dimer. (A/A/A) Stacked parallel trimer. (AA') Parallel protofibril. (AB) Antiparallel protofibril. (AB/A) Protofibril trimer. (AB/AB) Protofibril tetramer. A blue and green arrow represents individual dipole moments. The center Bottom figure represents the sphere surface of the AB/AB tetramer.

3.2 Theoretical Methodology

3.2.1 Structures Optimization

The $\text{A}\beta_{1-42}$ monomer was drawn following the Gremer’s reported structure$^{36}$ and initially optimized using molecular mechanics with the universal force field (UFF), followed by structure refinement at the semi-empirical theoretical level PM6 using the G09 suite$^{38}$ in the gas phase. It has been shown that PM6 semi-empirical level can reproduce experimental data of small and big biochemical molecules and performing outstandingly better than the HF and B3LYP theoretical levels with the Pople’s basis set 6-31G(d)$^{37}$. Also, it has been shown that PM6 outperforms previous semi-empirical methods (e.g., PM3, AM1), where one of its principal advantages is a
better description of hydrogen bonds, molecule polarization, and systems with small electron correlation\textsuperscript{37, 39, 40}. Recently researchers have shown that the semi-empirical method PM6 is suitable for the determination of charges in proteins and ligands that improve considerably force fields for molecular dynamics simulations. Also, the method is good enough to optimize proteins and determine correct docking of ligands into these proteins\textsuperscript{41-44}, which makes it a perfect candidate for the description of beta amyloids with a better description of quantum interactions than molecular dynamics (Force fields), but comparable performance with ab-initio or DFT methods\textsuperscript{45}. Due to the size of the monomer (including 630 atoms), no further high accuracy optimizations were performed using ab-initio or DFT approaches for the sake of comparison between oligomeric species and the lack of enough computational resources though, the theoretical level is enough for a semi-quantitative description of the system. Using the previous optimized structure (monomer), oligomeric species with up to four monomers were constructed, including three stacked structures and four fibrillar structures. The structures were optimized at the theoretical level PM6 in the G09 suite in the gas phase.

\subsection*{3.2.2 Energy and Dipole moment}

Even though PM6 is an excellent semi-empirical method for the optimization of proteins\textsuperscript{44}, it may not be entirely accurate for the quantitative determination of energy and dipole moment as an ab-initio or DFT electronic structure methods. To overcome the previous problem and collect semi-quantitative data for quantitative and qualitative comparison of the optimized structures. A single point calculation was performed using the PM6 optimized structures at the theoretical level HF/STO-3G. The method includes quantum interactions that may not be enterally described by
the PM6 semi-empirical method and its approximations, which also presents a minimal basis set
description of the molecular wavefunction. The latter was done to evaluate the energy and dipole
moments of structures containing up to 2520 atoms to give the reader a quantitative description of
the structure’s stability, dipole moments, and their role in the mechanism of fibril formation.

The dipole moments of the studied molecules were calculated using the expectation value
of the dipole moment with the aid of the peptide wave function for both PM6 and HF methods
as follows:

\[
\mu = \langle \Psi | \hat{\mu} | \Psi \rangle = \int \Psi^* \hat{\mu} \Psi d^3 \vec{r}
\]  

(3-1)

The dipole moment operator is given by:

\[
\hat{\mu} = \sum_k Z_k \vec{e} \vec{R}_k - \sum_i e \vec{r}_i
\]  

(3-2)

Inserting the dipole moment operator \(\hat{\mu}\) in Eq. 3-1 leads to:

\[
\mu = \langle \Psi | \hat{\mu} | \Psi \rangle = \langle \Psi | \sum_k Z_k e \vec{R}_k - \sum_i e \vec{r}_i | \Psi \rangle
\]  

(3-3)

Rearranging Eq. 3-3 yields to:

\[
= \sum_k Z_k e \vec{R}_k \langle \Psi | \Psi \rangle - e \int |\Psi|^2 \sum_i \vec{r}_i \cdot d^3 \vec{r}
\]  

(3-4)
Using the orthonormal properties of the wavefunction we obtain:

\[
\sum_k Z_k e \mathbf{R}_k - e \int \rho(\mathbf{r}) \mathbf{r} d^3 \mathbf{r}
\]

(3-5)

\( \rho(\mathbf{r}) \) in Eq. 3-5 describes the electron probability density for a continuous charge distribution within the molecule.

The previous method described in this dissertation to determine the dipole moment of a molecule is not unique. Some researchers calculate the dipole moments founded on the formal charges of the atoms constituting the molecules. However, the charge of an atom in a molecule is not a physical observable and is usually ambiguously or arbitrarily defined, which makes it highly basis set depended\textsuperscript{48-53}. For this reason, the expectation values of the dipole moment are anticipated to be more accurate than the dipole moment calculated by the formal charges’ formalism. Though discrepancies in the calculation of the dipole moment expectation values have been found for the Hartree-Fock and DFT methods where the former tends to overestimate dipole moments while the later underestimate them\textsuperscript{54}, however the basis set dependence in more important than the method. Nevertheless, from the qualitative point of view, the dipole moment direction and trend verges to be very well described for both methods\textsuperscript{55}.

3.2.3 *Molecular Dynamics Simulations*

To supply additional insight into the aggregation mechanism of amyloid \( \beta_{1-42} \). We performed the molecular dynamics simulations of the \( \beta_{1-42} \) peptide. The monomer, protofibril, protofibril
dimer were simulated using the amber 14 protein force field. The simulations were carried in the python package openmm \cite{56} at a temperature of 310 K using a Langevin's integrator. The integrator was set with a friction coefficient of 2 ps\(^{-1}\) and an integration time of 2 fs running for up to 5 ns. For the sake of simplicity, water molecules bond angles and bond lengths were restricted when TIP3P waters molecules are added to the model, while the nonbounded interactions are treated without a cutoff value, and hydrogen bonds within the proteins are constrained. Additionally, the aggregation of \(\beta_{1-42}\) was assessed in the condensed and gas phase using the same simulation parameters mentioned above, where six randomly placed protofibrils within 98 Å box were left to interact over time.

### 3.3 Results and Discussion

#### 3.3.1 Monomer

First, the optimized structure of the monomer (A) presents the experimentally found L-S topology (Fig. 3-2) reported by L. Gremer et al. \cite{36}. This structure presents three hydrophobic regions, i) Val\(^{36}\), Phe\(^4\), and Leu\(^{34}\); ii) Ile\(^{31}\), Leu\(^{17}\), and Phe\(^{19}\); and iii) Ala\(^{30}\), Ile\(^{32}\), Met\(^{35}\), Val\(^{40}\), and Ala\(^{42}\), which makes the predicted structure to display a more compact packaging in comparison with L. Gremer et al. structure \cite{36}. Additionally, in the L region of the A\(\beta_{1-42}\), one can observe an extended beta-sheet while in the S region, the amyloid contains three beta-sheets. The beta-sheets have a separation of 7.01 Å between Val\(^{39}\)-Ile\(^{32}\) and 6.64 Å between Ile\(^{32}\)-His\(^{14}\). The structure also shows the same features observed in the 20–25 turn region of L. Gremer et al. structure\cite{36}, where only Phe\(^{19}\) is facing the hydrophobic core (Fig. 3-2A). These matching features are not in
agreement with previous solid-state NMR data\textsuperscript{31, 33, 34}, that indicate that both Phe\textsuperscript{19} and Phe\textsuperscript{20} are facing the hydrophobic core. These differences can be explained by the existent increase in steric repulsion in the interior of the hydrophobic core, which favors the alternating conformer in the real structure of the A\textsubscript{β}\textsubscript{1-42}.

\textbf{Figure 3-2.} Structure of A\textsubscript{β}\textsubscript{1-42} monomer. (A) Cartoon backbone structure of A\textsubscript{β}\textsubscript{1-42} monomer (Top view), hydrogen atoms are omitted. (B) Cartoon Structure of A\textsubscript{β}\textsubscript{1-42} monomer (Front view). (C) Sphere surface of A\textsubscript{β}\textsubscript{1-42} monomer (Front view). The electric dipole moment is represented by a red cylinder arrow.
Next, structure A exhibits the presence of salt bridges located between Arg$^{5}$ and Asp$^{7}$; and Glu$^{11}$ and His$^{6}$ and His$^{13}$ (Fig. 3-2). These interactions are also in agreement with those reported by L. Gremer et al. $^{36}$. However, the calculated structure displays two new salt bridges located between His$^{14}$ and Lys$^{16}$, and His$^{13}$ and Gln$^{15}$. We found that in these new salt bridges (never reported before), the former stabilizes the latter, generating a hydrophilic cluster on the top of the first hydrophobic region. This contribution is responsible for the observed curvature on the predicted structure of this specific peptide (Fig. 3-2). Additionally, structure A displays an angle of residues Glu$^{11}$ to Ala$^{21}$ with respect to the fibril axis of 12.27°, which is comparable with the ~10° angle observed in the experimental structure $^{36}$. This result indicates that PM6 predicts, remarkably well, the structure of the Aβ$_{1-42}$ peptide.

Finally, the calculated Aβ$_{1-42}$ monomer presents a significant electric dipole moment of 6.43 D (Table 3-1), which is partially oriented orthogonal to the peptide plane that follows the peptide curvature (Fig. 3-2 and S5). We hypothesize that the alignment of individual dipole moments present in monomers facilitates their stacking, thus contributing to the formation and growth of fibrils. To verify our hypothesis, we study different arrangements of Aβ$_{1-42}$. 
Table 3-1. Energy, Complexation Energy, and electric dipole moment of the studied Aβ(1-42) at HF/STO-3G theoretical level.

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<td>AB/AB</td>
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<td>129.2</td>
<td>12.81</td>
</tr>
</tbody>
</table>

$^1$Energy in Hartrees. $^2$Complexation Energy in kcal/mol. $^3$Electric dipole moment in Debye.

3.3.2 Stacked Oligomers

First, we modeled the formation of stacked dimers in the parallel (A/A) and antiparallel (A/B) arrangement (Fig. 3-1). A/A dimer exhibits a pure electrostatic interaction between monomers without the formation of intermolecular hydrogen bonds or direct $\pi$-$\pi$ stacking between aromatic rings (Fig. 3-3). The dimer preserves the L-S shape of each monomer, maintaining the same number of salt bridges, and their curvature (Fig. 3-3). The S region of the individual stacked monomers exhibit $\beta$-sheet distances of 7.09 Å and 7.08 Å between Val$^{39}$-Ile$^{32}$ residues, and 6.63 Å and 6.62 Å between Ile$^{32}$-His$^{14}$ residues, in each monomer in the dimer. This result, which is consistent with the distances observed in an isolated monomer, indicates a mild distortion of the structures in A/A.

Next, A/A presents an angle between the centers of masses (COM) of the two monomers of 0.19°, indicating that the monomers are not entirely aligned with each other (see Fig. 3-3). Also, it shows a monomer COM inter-chain distance of 19.11 Å, which is not in total agreement with
the reported X-ray crystallographic distance of 4.67 Å. The small discrepancy between the theoretical and experimental distances can be attributed to the lack of dispersion corrections in the theoretical calculations or the number of fragments presented in the oligomer that may improve the dipolar interaction between monomers. It can also be attributed to the specific experimental conditions used in X-ray crystallography, which are different than considering an isolated dimer in the gas phase. Nevertheless, the dimeric structure shows an intense electric dipole moment partially oriented perpendicular to the peptide plane and with a magnitude of 11.73 D (Table 3-1). This result implies that the dipole moments of both monomers in an A/A dimer add up directly, thus increasing the magnitude of the resulting dipole moment (Fig. 3-3 and S6). On the other hand, A/A is electronically less stable than the two isolated monomers by 17.8 kcal/mol (Table 3-1). This result is consistent with the proposed mechanism that sustains the hypothesis that the formation of plaques in the early stages of AD is not very likely due to low peptide concentrations.
Figure 3-3. Structure of Aβ_{1-42} stacked parallel dimer (A/A). (A) Cartoon and sphere representation of A/A (Top view), hydrogen atoms are omitted. (B) Cartoon Structure of A/A (Front view). (C) Sphere surface of A/A (Front view). The net electric dipole moment is shown by a red cylinder arrow. Single dipoles are indicated by Blue (Bottom) and Green (Top) cylinder arrows.

In the second stacked dimer A/B (see Appendix D) we observed complexation energy of 31.2 kcal/mol (Table 3-1) (more significant than the A/A dimer), and a net electric dipole moment of 5.83 D pointing perpendicularly to the fibril axis (Fig S2 and S7). The latter makes difficult the dipole-dipole interaction between A/B and new monomers, reducing the kinetic control of the aggregation considerably. To prove this assertion, we studied the parallel stacked trimer A/A/A (Fig. 3-1). This triplet Aβ_{1-42} peptide structure has complexation energy of 32.8 kcal/mol (Table 3-1), which is comparable with the A/B dimer. Structure A/A/A also presents a total dipole
moment partially oriented parallel to the fibril axis. The aforementioned suggests that the monomeric species are prone to aggregate in parallel dimeric $A/A$ and trimeric $A/A/A$ structures as a consequence of the thermodynamic stability presented by these complexes in contrast with the $A/B$ dimer. Furthermore, the orientation of the structure electric dipole moment has a crucial role on the dipole-dipole interaction between monomers, which allows for the correct orientation an interaction between the complexes ($A/A$, $A/A/A$) and new monomers. This extended interaction aids, in turn, the oligomer growth and improves the efficiency of the complexation by electric dipole assisted kinetic control. For further analysis of these complexes, please refer to Appendix D.

3.3.3 Prototypor Arrangements

After studying the effect of monomer orientation and stacking of oligomers, the focus of our study moved into the analysis of prototypor structures with different arrangements. The most straightforward prototypor assembly consists of two monomers interacting with each other in the fibril plane. The interaction was then simulated in two different scenarios: 1) when the chains interact with the same external fragments (parallel arrangement, $AA'$) and 2) when the chains interact with different outer chain fragments (antiparallel arrangement, $AB$ – L. Gremer et al. experimental structure $^{36}$).

The second prototypor arrangement $AB$ (Fig. 3-4) exhibits a more symmetric in-plane structure in comparison with $AA'$ (see Appendix for further discussion) and maintains the monomer's curvature. The structure has two intermolecular salt bridges between Asp$^1$ and Lys$^{28}$ (Fig. 3-4A), which is stabilized by an intramolecular salt bridge between Lys$^{28}$ and Ala$^{42}$. These
Salt bridges are not present in AA’ where the interaction is merely dipolar (Fig. S3). Interesting to highlight is the fact that, the additional stabilization noticed in the AB protofibril due to the Lys$^{28}$-Ala$^{42}$ salt bridge, might be the reason for the observed difference in cytotoxicity between Aβ$_{1-42}$ and Aβ$_{1-40}$ - the latter does not present the additional salt bridge stabilization. The salt bridges show a separation of 4.84 Å for Asp$^{1}$-B and Lys$^{28}$-A; and 5.17 Å for Asp$^{1}$-A and Lys$^{28}$-B. The difference between the salt bridges distances is intriguing, for one should expect an even interaction between both links in a C$_2$ symmetry. However, this discrepancy explains why in the experimental fibrillar structure AB protofibrils present ridge and groove ends, and an approximated 2$_1$ screw symmetry$^{36}$.

The inter-chain distance between salt bridges in AB have separations that are consistent with the X-ray diffraction pattern of the fibril (4.65 Å), but the experimental distance is often assigned to the stacking separation between protofibrils. The findings shown above reveal that the AB protofibril does not present an evenly salt bridge dimeric interaction, as already demonstrated experimentally in the literature$^{36}$. One remarkable feature of the AB protofibril is that it has a total electric dipole moment utterly parallel to the fibril axis and located at the COM. The dipole moment orientation occurs by the cancelation of the on-plane dipole moment components of the individual monomers generating a perfect alignment of the dipole moment with the fibril axis. The magnitude of the dipole moment of the AB protofibril is 5.88 D (Table 3-1), which is smaller than in A due to the cancelation of the individual on-plane components (Fig. 3-4 and S10). However, the impeccable orientation of the dipole through the protofibril COM can produce noteworthy effects. First, the protofibrils can couple via dipole-dipole interaction, facilitating their approach,
and adjusting their alignment. The dipole moment orientation can drive the interaction between two protofibrils, thus playing a fundamental role in the kinetic control of the fibril formation. Second, the protofibril dipole moment magnitude and direction added to the stacking of multiple monomers will significantly increase the magnitude of the electric dipole moment in the fibril, modifying the fibril formation rate and facilitating its aggregation and the subsequent formation of plaques.

**Figure 3-4. Structure of Aβ1-42 antiparallel protofibril (AB).** (A) Cartoon backbone structure of AB (Top view), hydrogen atoms are omitted. (B) Cartoon Structure of AB (Side perspective-view). (C) Sphere surface of AB (Side perspective-view). The net electric dipole moment is shown by a red cylinder arrow. Individual dipole moments are indicated by blue (Front chain) and green (Back chain) cylinder arrows.
The protofibril is less stable than two isolated monomers by 31.4 kcal/mol (Table 3-1), which is surprisingly less stable than the stacked parallel dimer A/A and the AA’ protofibril - the instability of the protofibril compared with A/A and AA’ was not expected. Hence, we anticipate that the intermolecular salt bridges between the chains should stabilize the AB protofibril. Nevertheless, this scenario could change if dispersion interactions were included during the optimization process, allowing us to discard the AA’ structure (Appendix D) as a possible protofibril candidate due to the lack of intermolecular salt bridges and an axial electric dipole moment. Finally, AB presents a COM interchain distance of 25.13 Å, where the two monomers in AB have β intra-strand distances of 7.08 Å and 7.08 Å for Val^{39}-Ile^{32} residues and, 6.61 Å and 6.68 Å for Ile^{32}-His^{14} residues, in each monomer A or B in the dimer. This result is consistent with β intra-strand distances observed in A and A/A fragments.

Aiming to understand further the kinetic and thermodynamic control of the fibril formation, we simulated at the same theoretical level as all previous structures, the structure of a protofibril dimer with four monomers (AB/AB). The AB/AB fibril (Fig. 3-1 and 3-5) exhibits both protofibrillar and stacking interactions. The structure preserves the monomer’s L-S topology, curvature, and shows a stepwise shift of one protofibril respect to another, as observed experimentally. AB/AB presents two salt bridges within each protofibril with distances of 4.34 Å Asp^{1}-A and Lys^{28}-B, 2.53 Å Asp^{1}-B and Lys^{28}-A, 2.63 Å Asp^{1}-C and Lys^{28}-D, and 2.53 Å Asp^{1}-D and Lys^{28}-C. These results agree with those found in the AB protofibril, where the intermolecular salt bridge distances within the structure are uneven, although they contain the same type of residues. This outcome explains the presence of ridges and grooves in the experimental
structure and its approximated \(2_1\) screw symmetry. The \(2_1\) screw symmetry is associated not only with a semi \(C_2\) symmetry but also to the stepwise shift of individual protofibrils, as shown in our theoretical calculations (see Fig. 3-5). \(AB/\overline{AB}\) presents monomers \(\beta\) intra-strand distances of 7.04 Å, 7.08 Å, 7.06 Å, and 7.12 Å for Val\(^{39}\)-Ile\(^{32}\) residues, and of 6.69 Å, 6.68 Å, 6.70 Å, and 6.69 Å for Ile\(^{32}\)-His\(^{14}\) residues, in each monomer in the dimer. These results are in good agreement with those found in \(AB\) in three out of four chains – the D chain presents an enlarged Val\(^{39}\)-Ile\(^{32}\) \(\beta\) intra-strand distance. Next, the fibril displays an intermolecular chain COM distance of 24.97 Å between chains A and B, and 24.93 Å between chain C and D, which are shorter than in the \(AB\) protofibril. The stacked separations are of 19.37 Å between chain A and C, and 19.38 Å between chains B and D, which are comparable with the \(A/A\) oligomer. Both results show that stacking interactions are similar to the stacked \(A/A\) dimer. However, these interactions have a substantial effect on the salt bridge stabilization within the protofibril, as observed in the \(AB/A\) trimer (refer to Appendix D).

The net dipole moment in \(AB/\overline{AB}\) is partially oriented parallel to the fibril axis and has a magnitude of 12.81 D (Table 3-1, Fig. 3-5, and Fig. S12). The dipole size is considerably larger than in the \(AB\) protofibril, confirming that the addition of multiple protofibrils will yield to a stronger oriented dipole moment, which is critical for the fibril growth. The latter is a direct consequence of the strong dipole-dipole interaction of fibrillar structures with free oligomers or protofibrils. Although \(AB/\overline{AB}\) presents an intense dipole moment, it does not align perfectly with the fibril axis. The reason for this feature is that both protofibrils display a stepwise displacement, as shown experimentally \(^{36}\). Lastly, the complexation energy of \(AB/\overline{AB}\) is 129.2 Kcal/mol (Table 3-1), which shows that the tetramer is less stable than the protofibril \(AB\); this makes the formation
of the tetramer more challenging than its protofibril counterpart from the thermodynamic point of view.

**Figure 3-5. Structure of Aβ₁₋₄₂ protofibril tetramer (AB/AB).** (A) Spheres and cartoon structure of AB/AB (Top view), hydrogen atoms are omitted. (B) Cartoon Structure of AB/AB (Front view). (C) Sphere surface of AB/AB (Front view). A red cylinder arrow shows the net electric dipole moment. The lower layer dipole moment is indicated with a blue cylinder arrow, and the high layer dipole moment is indicated with a green cylinder arrow.

### 3.3.4 Fibril Formation Mechanism

So far, our study has given us a comprehensible indication of how the fibril of Aβ₁₋₄₂ peptide may form and grow. In general, two lines of thought exist, i.e., the thermodynamic control...
and the kinetic control of the fibril formation. From the thermodynamic point of view, the spontaneous formation of oligomers and fibrils does not seem possible, at least in the gas phase (Table 3-1). Nevertheless, the optimized PM6 structures suggest that stacked oligomers are more likely to be formed than protofibrils when energies are calculated at the theoretical level HF/STO-3G, which indicates that an initial step in the fibril formation could include the nucleation of multiple stacked oligomers followed by their protofibril binding. The dipole-dipole interaction between two adjacent stacked oligomers may assist the protofibrillar link, yielding to the active formation of fibrils. From the kinetic point of view, a sizeable well-localized dipole moment can facilitate the interaction and orientation of new monomers or oligomers with a seed (protofibril), thus improving the fibril development and increasing its rate of formation. This kinetic control has been observed experimentally as the monomers in the presence of fibrils, aggregate faster than those that are isolated 57. This process is known as secondary nucleation. The sizeable oriented dipole moment in the fibrils can also explain the difference in fibril growing rate. The fibril dipole facilitates the alignment and interaction of the fibril with other monomers and oligomers, proliferating the formation of fibrils at a more significant rate than without the presence of a fibril seed.

Three possible fibril formation mechanisms can be established based on the thermodynamic and kinetic control of the fibril growth.

I. The stacking of multiple monomers assisted via dipole-dipole interactions. This process leads to filaments that will link later, forming fibrillar structures.
II. The formation of small stacked oligomers (two to four fragments) via dipole-dipole interactions. These fragments can arrange into protofibrillar structures via intermolecular salt bridges.

III. The growth of protofibrils via the dipolar interaction between nearby monomers, and the establishment of salt bridges between these structures. These protofibrils will subsequently stack via dipole-dipole interaction, producing mature fibrils.

As mentioned previously, the parallel stacking of monomers generates structures that are thermodynamically more stable than the protofibrillar ones, where the dipolar interaction between the monomers can assist its growth. Although our theoretical approach is not sophisticated enough to allow for a complete analysis of such massive structures due to the lack of dispersion and long-range interactions, the observed results will enable us to make some significant remarks.

Although the parallel stacking of monomers is thermodynamically favorable compared with the formation of protofibrils, it does not present an entirely localized dipole moment as it occurs in the protofibril to assist aggregation via dipole-dipole interaction. Second, small parallel stacked oligomers require a significant rearrangement to form a protofibril, thus a large amount of energy to break the initial dipolar interaction. This mechanism also favors the formation of protofibril at the early stages of aggregation. Third, salt bridges in the protofibrils are strong interactions due to their ionic character. Therefore, mechanism III should be the dominant process that leads to the formation of fibrils in neurodegenerative diseases like AD. We estimate that the inclusion of dispersion and long-range interactions in our calculations would help stabilize these structures, making their energies comparable with those observed in the stacked oligomers. Of
course, more powerful computational methods need to be developed first. Additionally, the protofibril presents a perfectly axial dipole moment at the COM (Figure 3-3) that makes the dipole-dipole interaction and alignment with nearby protofibrils extremely effective. Therefore, among the three mechanisms described above, we believe that mechanism III is more likely to generate mature fibrils due to the strong dipolar interaction between the protofibrils perfectly axial electric dipole moments, and the possibility of inter-protofibril coupling without the need of energy-demanding rearrangement of the structures.

Before we jump into any conclusions about the potential aggregation routes for Aβ1-42, it is important to revisit the amyloid hypothesis that suggests three significant pathways for protein aggregation\textsuperscript{21}, the first route, known as the nucleated polymerization\textsuperscript{23, 58}, consists of the accumulation of high-energy species, generally oligomers, that eventually combined to form a nucleus that can subsequently add more monomers to ensemble a thermodynamically stable structure. The second path, also called the nucleated conformational conversion\textsuperscript{59}, consists of an existing equilibrium between monomers and structurally heterogeneous oligomers that are generally more stable than the free monomers. The third mechanism, known as downhill polymerization\textsuperscript{60}, consists of the direct formation of oligomers without the presence of a seed, with increasing stability as the oligomers grow. This last pathway can be discarded based on experimental evidence that established that fibrils nucleation-centers enhances the formation of mature fibrils through monomer aggregation\textsuperscript{57}. However, the nucleated polymerization and the nucleated conformational conversion routes present important similarities to the mechanism described in our highly ordered fibrils structures (\textit{AB}, \textit{AB/A}, \textit{AB/AB}, \textit{A/A}, \textit{A/A/A}), i.e.,
accumulation of high-energy species, nucleation of oligomers and conformational conversion. Due to the presence of intense and oriented dipole moments in all of them, the nucleation of oligomers takes place in an ordered fashion. Although the aggregation of monomers and oligomers lead to the formation of larger structures that are thermodynamically less stable than smaller fragments, we believe that beyond a specific fibril size-threshold, the dipole-dipole interaction would induce a cascaded aggregation effect. This presumption is supported by the experimental evidence provided by Knowles and co-workers who showed that in the presence of nucleated seeds (fibril fragments) fibrils grow at a higher rate.  

In Addition to the previously discussed results, molecular dynamics simulations (MD) made in A, AB, and AB/AB (see Appendix D) gives us additional insight into the dynamics of fibril formation in Alzheimer’s disease. The simulated monomer in vacuo (Fig S13) at 310K shows that the L-S structure of the monomer is preserved even after 5ns of simulation. Though, the S region tends to lose part of its beta-sheet conformation and acquires an alpha helix-like confirmation, which we expect in a highly hydrophobic environment such as membranes. On the other hand, the condensed phase simulation (Fig S14) tends to preserve the L-S topology, but out of plane distortions are observed after one ns of simulation. This indicates that the preservation of a hydrophobic core is more likely to maintain the L-S topology of the monomer. Simulations on the protofibril structure in the condensed phase at 310K (Fig. S15) show small structural distortions after 1.5 ns of simulation; this demonstrates that the protofibrillar interaction by salt bridge formation allows a tighter interaction between monomers, preserving their geometry and hydrophobic properties. The same behavior is observed for the protofibril dimer (AB/AB) (Fig.
S16) in the condensed phase. The stacking interaction between protofibrils reduces the effective cross-section of the solvent in the peptide, making that the hydrophobic interaction is preserved. The strong hydrophobic interaction preserves the L-S topology after a simulation time of 345 ns.

The MD simulations have shown that the formation of a hydrophobic core yields to better preservation of the L-S topology. It also guarantees a better interaction between the protofibrils and monomers. This result shows that the fibril simulation does not require to include solvent effects if a seed is already formed due to the solvent will not be entirely accessible to the peptide surface. In table 3-1 is shown that some complexation energies are enormous and can go beyond the energy required for the formation of a chemical bond. This effect can be attributed to the lack of a large basis set or the need for a method that includes a better description of the electron correlation energy, long-range interactions, and dispersion corrections. Though, the theoretical level HF/6-31G(d) shows for the formation of the protofibril AB that the complexation energy is lower than in the case of the HF/STO-3G. AB complexation energy is 25.54 kcal/mol indicating that in the Hartree-Fock limit of the system, the complexation energies will be below the formation of a chemical bond. More refined calculations beyond this work are required to accurately predict these complexation energies, which need extensive computational resources for ab-initio and DFT calculations.

Moreover, MD simulations have also shown that the solvent in the media tend to be important in the initial aggregation stage of the Aβ1-42 peptide, where a repulsive behavior between protofibrils is observed in vacuum (see Fig S17), while in a solvated medium (Fig S18) an
attractive interaction is observed yielding to the aggregation of the peptide, once a seed is formed the concentrated and robust dipole moment will enhance the aggregate of the fibrils as it has been observed experimentally by Knowles and co-workers\textsuperscript{57}, this observation can explain why the complexation energies are so considerable at the theoretical level HF/STO-3G, indicating that the presence of solvent stabilizes the system considerably, and should be considered in further studies.

Dipole moment calculations tend to be highly dependent on the used basis set rather than the employed theoretical method. To verify the accuracy of the dipole moments and its qualitative implications, the dipole moment of the monomer and protofibril AB were determined at different theoretical levels (see Fig S19). All the employed methods PM6, HF/STO-3G, and HF/6-31G(d) showed differences in magnitude but small differences in the direction of the resulting dipole moments (Fig S19) indicating that the employed method is reliable. The dipole moments in the studied structures are more likely to be geometry dependent instead that basis set or method depended at least from the qualitative point of view, which is enough in our opinion to give support amyloid cascade aggregation hypothesis.

Despite that, indeed, the simulations presented in this work do not entirely resemble the real biological system in the realm of neurobiology, the approximation given shows new insight on how to “quantumly” treat the system and obtain reliable quantitively and qualitative structural and dipole moment data. This, of course, is more effective than the mere use of force fields to predict structures and properties that do not include direct quantum effects as does ab-initio, semi-empirical, and DFT methods. The latter can help us to fully understand how the aggregation of
Aβ_{1-42} occurs and find pathways to prevent the aggregation of this peptide in a living system, which is the ultimate goal to treat Alzheimer’s disease effectively.

### 3.4 Conclusions

Performing semi-empirical calculations on Aβ_{1-42} monomers, and oligomers we were able to reproduce the L-S shaped topology recently uncovered, experimentally by L. Gremer et al., and to discover the presence and intense localized electric dipole moment in monomers, stacked oligomers and protofibrils that can promote fibril formation through a cascaded dipole-dipole interaction. The latter is kinetically controlled and follows a nucleated polymerization route where oligomers are thermodynamically stable after achieving a fibril size-threshold. Though, further experimental evidence is required to certify our conclusions, the discovery of a highly oriented dipole moment of Aβ_{1-42} monomers and the proposed assisted dipole interaction between fragments as a formation mechanism of fibrils found in AD opens a new path to the understanding, treatment, and control of Alzheimer’s disease and alike neurodegenerative related-disorders.

### 3.5 References


CHAPTER 4. AXIAL ELECTRIC DIPOLE MOMENT AND ITS ROLE OF THE AMYLOID-β25-35 CYTOTOXICITY

In the previous chapter, we studied the aggregation mechanism of Amyloid-β1-42 using the semi-empirical theoretical level PM6, where we found that the aggregation is driven by dipole-dipole interactions by the out-of-the-plane dipole moment of the studied structures. In this chapter, we assess the dipole-driven aggregation mechanism and cytotoxicity of Amyloid-β25-35. The peptide is typically found in senile plaques of subjects with Alzheimer’s disease and is known to be more cytotoxic than the full-length Amyloid-β1-42. To understand the mechanism of aggregation of the Amyloid-β25-35 peptide, we performed the theoretical study of the Aβ25-35 monomer, stacked, and protofibrillar oligomers. The structures were optimized at the theoretical level HF/STO-3G in gas phase. We found that the monomers present a hairpin-like conformation; which agrees with experimental measurements. Many of these studied structures have an intense out of the plane dipole moment that can assist with the aggregation of the peptide by dipole-dipole interactions. The monomer and protofibrillar structures contain the most localized dipole moments, oriented perpendicular to the peptide plane. The magnitude of the dipole moment and the pore-like topology of the oligomers explains why the Aβ25-35 peptide is more cytotoxic than the full-length Aβ1-42. We hypothesize that the resulting dipole moment aids the nucleation step of the aggregation mechanism, followed by a conformational change yielding to mature fibrils as our molecular dynamic simulations suggest, indicating that the aggregation is likely to follow a nucleated conformational conversion pathway.
4.1 Introduction

Neurogenerative diseases have caught researchers’ attention due to their impact on society and the quality of life of our elders. In particular, Alzheimer’s disease is one of the most common neurodegenerative diseases which is characterized by memory loss, yielding to brain dysfunction, and eventually death in its final stage\(^1-3\). This disease is commonly found in elders, but with some exceptions, it can be developed in young individuals in their early forties or even before\(^4\). The disease is frequently developed in female individuals indicating that there exists a relationship between the biological gender and the etiology of the disease. However, the direct cause or the particular genetic expression of the disease in females still unknown\(^5\).

Morphologically, Alzheimer’s disease is characterized by the formation of plaques in the patients’ brains\(^6-10\). Nevertheless, not a direct correlation between the advance of the disease and the number of brain plaques have been found\(^9\). Further, perhaps the formation of plaques in the patients’ brains is not the direct cause of the neurodegenerative process, but a response to it.

The senile plaques are composed of extracellular deposits of Amyloid-\(\beta\) peptides, whose typically contain 40 to 42 amino acids prone to aggregate and disrupt the neural activity\(^6-10\). In general, two lines of thought exist. i) small oligomers are responsible for the cytotoxicity in Alzheimer’s disease\(^11, 12\). ii) Fibrillar aggregates are responsible for the cytotoxicity in Alzheimer’s disease\(^13\). The cytotoxic pathway can be described in the function of these two hypotheses. In the early stages of the disease, the small oligomers are the main driving force for the progression of the disease\(^7-10, 14\). In contrast, with the chronic stages of the disease, the fibrillar
aggregates disrupt the neural activity by the cleavage of the synapsis, yielding eventually to neuronal death\textsuperscript{6, 15}. Even though the neurotoxic potential of A\textsubscript{ß} is known\textsuperscript{15-17}, its cytotoxicity pathway still not completely understood. Researchers suggest that two possible pathways of cytotoxicity are present. i) the formation of ion channels that suppress or overexcites neurons (small oligomers)\textsuperscript{18, 19}, and ii) the mechanic cleavage of the neuronal synapses by the mechanical stress induced by the extracellular deposits of A\textsubscript{ß} peptides\textsuperscript{20}.

The A\textsubscript{ß} peptides are usually formed by the proteolysis of the amyloid precursor protein (APP) after being transported into the neuronal membrane\textsuperscript{21}. In the membrane, APP is subjected to cleavage by \(\alpha\)-secretase. However, failure to do so causes the breakage of APP by \(\beta\)-secretase and \(\gamma\)-secretase yielding to A\textsubscript{ß} \textsuperscript{6-9, 22-24}. Additionally, A\textsubscript{ß} is also produced at the Golgi apparatus by APP cleavage and secreted into the extracellular space \textsuperscript{25}. The presence of an excess of A\textsubscript{ß} amyloids is indicative of Alzheimer’s disease. The basic human physiology uses it for regulation processes such as neural depression to control neuronal overexcitation\textsuperscript{6, 9, 26, 27}. For this reason, the presence of A\textsubscript{ß} is in the cerebrospinal fluid and plasma of healthy individuals.

Moreover, the cleavage of APP protein generates A\textsubscript{ß} peptides containing 40 to 42 amino acids, where the most abundant secreted A\textsubscript{ß} peptide is A\textsubscript{ß}\textsubscript{1-40}, which includes 40 amino acids in length. Nevertheless, the poor selectivity of the APP cleavage also yields smaller secreted peptides such as the 42 amino acids long A\textsubscript{ß}\textsubscript{1-42} and the 11 amino acid long A\textsubscript{ß}\textsubscript{25-35}. These A\textsubscript{ß} receive particular attention due to their high cytotoxicity and their fast aggregation yielding to the production of fibrils\textsuperscript{19, 28, 29}.
In the previous chapter, we showed that the presence of an out-of-the-plane dipole moment in the monomer triggers the aggregation of Aβ1-42, which is mostly driven by protofibrils and their axial dipole moment. The axial dipole moment aids to the aggregation of multiple protofibrils, yielding to mature fibrils. The pore-like structure of the Aβ1-42 protofibril, as well as the localized dipole moment, suggest the possibility that Aβ1-42 may also behave as a precursor for ion channel formation in neuronal membranes as it is proposed experimentally\textsuperscript{30, 31}.

The previously mentioned properties of the Aβ1-42 protofibril demonstrates why this peptide is more likely to aggregate than other Aβ peptides. This further unravels that the two possible cytotoxic pathways of Alzheimer’s disease can be covered by the Aβ1-42 peptide, explaining its chronic cytotoxicity in neurons yielding to neuronal death. Therefore, it is imperative to understand the mechanism of aggregation and fibril formation in Aβ peptides, beginning with the monomers up to small size oligomers. This can help to disentangle the mechanism of cytotoxicity in AD and find potential treatments. Since AD shares similarities with other neurogenerative diseases such as Parkinson’s and Huntington’s, finding a potential therapy for AD will also contribute to the treatment of the conditions mentioned above, improving the lifestyles of millions of persons.

Even though Aβ1-42 is known to be highly cytotoxic, the undecapeptide Aβ25-35 has shown to be equivalent to/more cytotoxic than the Aβ1-42 peptide \textsuperscript{29, 32-35}. The complete cytotoxic pathway of Aβ25-35 still unknown, however preliminary data has demonstrated that Aβ25-35 cytotoxicity is exerted by damage into the mitochondrial membrane and leakage of proteins that trigger apoptotic cellular death\textsuperscript{36-39}. Aβ25-35 is also involved in the overexpression of proteins related to the
mitochondrial permeability transition pore increasing ion mobility toward the mitochondria, which could trigger cell death\textsuperscript{40,41}. In addition to this, substantial evidence suggests that Aβ\textsubscript{25-35} is capable of binding into membranes forming ion conduction pores in biological and synthetic lipid membranes showing high permeability to calcium ions\textsuperscript{18,42-49}, where the peptide is more likely to bind to anionic lipid membranes \textsuperscript{43,50}. Also, it has been demonstrated that the oxidation of methionine 35 fragment and the N-methylation of the peptide inhibits its cytotoxicity and aggregation by disrupting the formation of hydrogen bonding\textsuperscript{51,52}. This shows that there is a compromise between structural shape and external residues on the cytotoxic strength of the peptide. The previously mentioned characteristics make of Aβ\textsubscript{25-35} a perfect candidate and model for the study of aggregation of Aβ peptides and the study of their cytotoxicity.

Despite the high cytotoxicity of Aβ\textsubscript{25-35} and fast aggregation rate, researchers have not been able to elucidate the structure of the peptide completely. The lack of structural evidence is the main consequence of the disordered and inhomogeneous state of the fibers and its transcendent nature\textsuperscript{53}. NMR studies have shown that the structure of Aβ\textsubscript{25-35} follows a beta hairpin-like beta-turn, or U-shaped conformations \textsuperscript{54-56}, which agrees with ECD measurements\textsuperscript{54,56-59} and molecular dynamic simulations\textsuperscript{53,55,60}. Researchers believe that the cytotoxic characteristics of Aβ\textsubscript{1-42}, are mostly due to the 25-35 region of the peptide, which made this study interesting particularly in understanding the driving forces of aggregation. Within our previous results on Aβ\textsubscript{1-42}, we present our theoretical electronic structure study of Aβ\textsubscript{25-35} in monomeric and oligomeric (Stacked and protofibril like structures) forms using the self-consistent field Hartree-Fock approach\textsuperscript{61-63}. Our results show that the monomers and oligomers presented a large out of the plane dipole moment. The dipole moment presents a stronger and purer axial component than the Aβ\textsubscript{1-42} fragment, which explains its high
cytotoxicity and aggregation rate in comparison with the full-length Aβ₁₋₄₂ peptide. This outcome gives insight into the potential aggregation mechanism of the fibril and its role in the toxicity of the Aβ₁₋₄₂ fragment. Ten structures were optimized at the theoretical level HF/STO-3G (Fig.4-1) beginning with the Aβ₂₅₋₃₅ monomer and continuing with structures with stacking and protofibrillar interactions or both.


113
4.2 Theoretical Methodology

4.2.1 Structures Optimization

The neutral charged Aβ<sub>25-35</sub> monomer was drawn starting with a linear chain configuration with Ramachandran angles $\phi = -130^\circ$ and $\psi = +140^\circ$ for a beta-sheet configuration<sup>64</sup> and initially optimized in order to obtain a better guess structure using the universal force field (UFF)<sup>65</sup>. Starting from the UFF guess, the structure was refined at the theoretical level HF<sup>62, 63</sup> with the Pople’s basis set STO-3G<sup>66</sup> using the G09 suite<sup>67</sup> in the gas phase where the methodology was repeated for the oligomeric species. It has been demonstrated that the Hartree-Fock method as an ab-initio approach is accurate enough to reproduce protein structure and thermodynamics<sup>68-70</sup>. Furthermore, Hartree-Fock usually performs better, or at the same level, than a semi-empirical calculation because it is less accurate than the former as it neglects some quantum effects<sup>71</sup>. Additionally, molecular force fields were optimized using Hartree-Fock, and DFT approaches, producing suitable protein structures. The prior optimization indicates that self-consistent-field Hartree-Fock is a fair method for the protein structure determination at medium-to-high computational cost as a consequence of its O(N<sup>4</sup>) system size scaling<sup>68</sup>.

Also, the presence of long-range interactions and electron exchange energy in the Hartree-Fock method increases its performance in protein systems in comparison with semi-empirical and force field approaches; which is significantly important in highly interacting systems. One particular issue with the Hartree-Fock approach is its lack of electron correlation due to the electron-electron repulsion potential, which is taken as an average, and can underestimate hydrogen bonding and bond lengths in highly correlated systems<sup>72, 73</sup>. However, for larger systems
such as DNA and proteins, small variations in the strength of hydrogen bonding or bond lengths will no yield to extended protein structural changes.

On the other hand, due to the size of the studied systems, the Pople’s basis set STO-3G was selected for the geometry optimization of the system due to the following reasons: i) computational cost of the calculations that scales as $N^4$ with the increase of the system size. ii) basis set superposition error, which can be significantly broad in systems containing a large number of atoms nearby, as is the case of a protein\textsuperscript{74, 75}. iii) it has been shown that STO-3G performs reasonably in large molecular systems, which includes DNA and proteins\textsuperscript{74, 76, 77}.

4.2.2 Energy and Dipole Moment

While HF/STO-3G is a reasonable method for the optimization of proteins, the method is not entirely reliable for the correct determination of energy and dipole moment as in DFT or post-Hartree-Fock electronic structure approaches. Also, a small basis set yields significant errors in the determination of the structure energy and local electronic density, which is indispensable for the determination of accurate dipole moments in order to obtain accurate data for quantitative and qualitative comparison of the optimized structures. A single point calculation was performed using the previously optimized HF/STO-3G structures at the B3LYP/6-31G(d) theoretical level. This was done to evaluate the energies and dipole moments of structures containing up to 1540 atoms and to obtain a quantitative description of the structures’ stability and dipole moment and their role within the aggregation mechanism and cytotoxicity. The dipole moments were calculated using
the expectation value on the dipole moment\textsuperscript{78, 79} with the aid of the peptide wavefunction as it was shown in the previous chapter.

4.2.3 \textit{Molecular Dynamics Simulations}

To supply additional information regarding the aggregation mechanism of the amyloid β\textsubscript{25-35} peptide, the monomer and protofibril were simulated using the amber 14 protein force field (ff14sb). The simulations were run in the python package OpenMM using a Langevin’s integrator at 310 K. The integrator was offset with a friction coefficient of 5ps\textsuperscript{-1} and an integration time of 2 fs running up 30 ns. Due to computational constraints and the sake of simplicity in the simulation, water molecules, bond angles, and bond lengths were fixed when TIP3P water molecules were included in the model. Also, nonbonded interactions were treated without a cut off distance, constraining the carbon-hydrogen bond distances within the protein. For further insight, the aggregation of Aβ\textsubscript{25-35} was assessed in the condensate phase and in the gas phase using the same simulation conditions mentioned above, where four randomly placed protofibrils were at a center of mass distanced larger than 10 Å and were left to interact over time.

4.2.4 \textit{One, Two-photon (OPA, TPA), ECD and Two-photon Circular Dichroism (TPCD) Simulations}

The OPA and ECD calculations were carried using the TD-DFT formalism on G09 using the theoretical level B3LYP/6-31G(d) over then electronic states, for the monomer, parallel stacked dimer, and protofibril. The OPA spectrum was compared with the experimental UV-Visible spectrum of Aβ\textsubscript{25-35} (~1x10\textsuperscript{-5} M) measured during a time-lapse of nine days. The sample
was prepared dissolving the pure Aβ25-35 peptide purchased from peptide 2.0 and dissolved in hexafluoropropanol (HFIP) to reach a concentration of ~1×10^3 M. A small aliquot of the previous solution was nitrogen dried and re-dissolved in PSB buffer 25 mM in order to reach a concentration of ~1×10^{-5} M.

The TPA and TPCD spectrum for the degenerate case was modeled using the theoretical level B3LYP, and ten electronic excitations were obtained. The monomer was simulated using the 6-31G(d) basis set, but due to computational constraints, the stacked dimer and protofibril were simulated with the STO-3G minimal basis set. The simulation was carried in the Dalton 18 suite.

The TPA spectra were obtained using:

\[
\delta_{0f}^{TPA}(\omega) = \frac{4\pi c}{c} \sum f (\hbar \omega_f) \delta_{0f}^{TPA}(\omega_0f) g(2\omega, \omega_0f, f), \tag{4-1}
\]

\[
\delta_{0f}^{TPA}(\omega) \approx 1.25273 \times 10^{-2} \times (\hbar \omega)^2 \sum f g(2\omega, \omega_0f, f) \delta_{0f}^{TPA}(\omega_0f), \tag{4-2}
\]

In Eq. 4-1, c is the speed of the light in vacuum, α is the fine structure constant, E=\hbar \omega is the photon energy (in the degenerate case half of the transition energy), a_0 is the Bohr radius and \delta_{0f}^{TPA}(\omega_0f) is the orientation averaged two-photon transition probability for the degenerate case defined by Eq. 4-3. A Lorentzian line-shape \( g(2\omega, \omega_0f, f) \) was used in order to broaden the electronic transitions (Eq. 4-4). Where Γ is the full width at half maximum (FWHM).
\[ \overline{\delta}_{0f}^{TPA}(\omega_{0f}) = \frac{1}{30} \left( 2 \sum_{a,b} S_{aa} S_{bb} + 4 \sum_{a,b} S_{ab} S_{bb}^* \right), \]  

(4-3)

\[
g(2\omega, \omega_{0f}, \Gamma) = \frac{\Gamma_{gf}^2}{\pi (\omega_{gf} - 2\omega)^2 + (\Gamma_{gf}/2)^2},
\]

(4-4)

A value of \( \Gamma = 0.1 \) eV was applied to reproduce the broadening of the experimental spectra. Solving part of the Eq. 4-1 yield to Eq. 4-2, which includes the necessary conversion factors to obtain the TPA spectra in Göpper-Mayer units (GM), i.e., \( 10^{-50} \text{cm}^4 \text{s.mol}^{-1} \text{ photon}^{-1} \), when atomic units are used for \( \delta_{0f}^{TPA}(\omega_{0f}), \omega, \) and \( \Gamma_{gf} \) and the cgs units are used for \( a_0 \) and \( c \).  

The TPCD cross section spectra (In GM) is given by:

\[ \Delta \delta^{TPCD}(\omega) \approx 4.87555 \times 10^{-5} \times \omega^2 \sum_f g(2\omega, \omega_{0f}, \Gamma). R_{0f}^{TPCD}(\omega_{0f}). \]

(4-5)

\[ g(2\omega, \omega_{0f}, \Gamma) \] is defined by the Lorentzian curve in Eq.4-4. and \( R_{0f}^{TPCD}(\omega_{0f}) \) is the two-photon rotatory strength that is given by:

\[ R_{0f}^{TPCD}(\omega_{0f}) = -b_1 B_1^{TI}(\omega_{0f}) - b_2 B_2^{TI}(\omega_{0f}) - b_3 B_3^{TI}(\omega_{0f}) \]

(4-6)

b1, b2, and b3 are scalars that depend on the experimental setup. For our experimental setup for two colinear photons traveling in the same direction with identical circular polarization state, \( b_1 = 6, b_2 = 2, \) and \( b_3 = -2. \) The theoretical TPCD parameters comprising the TPA rotatory
The optimized structure of the monomer (A) (Fig 4-1) presents high similarity with the experimentally reconstructed NMR structure of the Aβ25-35 monomer reported by D’Ursi and coworkers\(^5^4\). The monomer presents an outstanding hairpin-like conformation in the region located between the residues Lys\(^{28}\) and Gly\(^{33}\). The hairpin structure matches with ECD experimental measurements and molecular dynamics simulations reported in the literature\(^5^4, 57-59, 86\). On the contrary, the NMR structure reported by D’Ursi and coworkers is mostly defined as an α-helix protein conformation as a consequence of the helix-like turns observed in two regions of the peptide. The first α-helix region is located between the residues Gly\(^{25}\) and Lys\(^{28}\), while the second region is located between Gly\(^{33}\) and Met\(^{35}\). Figure 4-1 shows the Ramachandran plot of the monomer. The plot agrees with the peptide shape analysis mentioned above, where the peptide presents a considerable character of a random coil and beta-sheet conformations indicating a hairpin or U-shaped structure. On the other hand, the α-helix region of the Ramachandran plot is
also populated, showing a match between our point of view and the D’Ursi reported structure analysis. The structure information is enriched with experimental ECD data which reached the same conclusions as in this dissertation.

In contrast with the Aβ1-42 peptide, the monomer presents a single hydrophobic core located in the interior of the hairpin core that includes the residues Ile32, Ala3, Met35, and Leu34 which partially agrees with our previous results for the Aβ1-42 in its S region. The hairpin region contains a beta-sheet character exhibiting a distance between the fragments Lys28-Gly33 of 7.42 Å and a random coil distance in the U-shaped region of the hairpin region between the fragments Ala30 and Ile32 of 5.81 Å. In addition to the hydrophobic core and the boundaries of the hairpin; the monomer also presents hydrophilic regions that are located out of the hairpin plane, which will be highly interacting with the physiological environment, including the fragments Asp27 below the hairpin plane and Lys28 above the hairpin plane.
Figure 4-2. Structure of Aβ25-35 monomer. (A) Cartoon backbone of Aβ25-35 monomer (Top view), hydrogen atoms are omitted. (B) Cartoon structure of the Aβ25-35 monomer (Front view). (C) Sphere surface of Aβ25-35 monomer (Front view). A red cylinder arrow represents the electric dipole moment. (D) Ramachandran plot of the Aβ25-35 monomer.

In highly polar environments, the peptide is expected to close the hairpin even further. This is due to better solvation of the structure and the formation of a salt bridge between the extremes of the hairpin. As a most relevant characteristic, the monomer presents its perfect axial dipole moment (Figure 4-1) with a magnitude of 7.68 D (Table 4-1), which is smaller in comparison with the Aβ1-42 full-size peptide. However, the dipole moment magnitude is not just the direct cause of the cytotoxicity of these peptides. On the other hand, the absolute orientation of the dipole moment respects the peptide plane, which is remarkably superior in the case of the Aβ25-35, explaining its high cytotoxicity and aggregation rate. Besides the imponent direction of the dipole moment on the Aβ25-35 monomer, the hairpin-like conformation makes that monomer resemble a pore-like...
structure. The latter gives us insight regarding the cytotoxic pathway of the amyloid Aβ_{25-35}, which can follow the two cytotoxic pathways suggested by the amyloid hypothesis.

### Table 4-1. Energy, Complexation Energy and electric dipole moment of the studied Aβ_{25-35} at B3LYP/6-31G(d) theoretical level.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Energy(^a)</th>
<th>ΔE(^b)</th>
<th>P(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-3926.81835542</td>
<td></td>
<td>7.68</td>
</tr>
<tr>
<td>A/A</td>
<td>-7853.64096079</td>
<td>-2.67</td>
<td>15.30</td>
</tr>
<tr>
<td>A/B</td>
<td>-7853.65493397</td>
<td>-11.44</td>
<td>13.52</td>
</tr>
<tr>
<td>A/A/A</td>
<td>-11780.48395390</td>
<td>-18.13</td>
<td>24.99</td>
</tr>
<tr>
<td>AA'</td>
<td>-7853.64707766</td>
<td>-6.50</td>
<td>4.21</td>
</tr>
<tr>
<td>AB</td>
<td>-7853.67202377</td>
<td>-22.16</td>
<td>15.65</td>
</tr>
<tr>
<td>(AB)(^2)</td>
<td>-15707.34680470</td>
<td>-46.04</td>
<td>32.96</td>
</tr>
<tr>
<td>(AB)(^3)</td>
<td>-23561.01994150</td>
<td>-68.91</td>
<td>49.42</td>
</tr>
<tr>
<td>(AB)(^4)</td>
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<td>65.85</td>
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<tr>
<td>(AB)(^5)</td>
<td>-39268.36519630</td>
<td>-113.98</td>
<td>82.28</td>
</tr>
<tr>
<td>A(^d)</td>
<td>-15846.45115110</td>
<td></td>
<td>10.42</td>
</tr>
<tr>
<td>AB(^d)</td>
<td>-31692.88391710</td>
<td>11.54</td>
<td>5.88</td>
</tr>
</tbody>
</table>

\(^a\)Energy in Hartrees. \(^b\)Complexation Energy in kcal/mol. \(^c\)Electric dipole moment in Debye.\(^d\) Amyloid β 1-42

Researchers believe that most of the cytotoxic characteristics of amyloid Aβ_{1-42} are inherited from the highly cytotoxic Aβ_{25-35} region. Most of the amyloid fragments that do not contain the 25-35 region are deficient in cytotoxicity. In figure 4-3, we show the comparison between the 25-35 region of the Aβ_{1-42} peptide and the Aβ_{25-35} monomer. Both peptides show matching similarities in the hairpin-like region between Ile\(^{31}\) and Met\(^{35}\), but differ in the α-helix region from the lack of additional residues interactions in the Aβ_{25-35} monomer, which tend to look for the complete closure of the hairpin as MD simulation models suggest\(^{60}\).
Figure 4-3. (A) Cartoon backbone of Aβ25-35 monomer (HF/STO-3G) (ice blue chain) in contrast with the of Aβ1-42 (PM6) (green chain) (B) Aβ25-35 MD snapshot at 0 ns in gas phase (C) Aβ25-35 MD snapshot at 20 ns in gas phase (D) Energy profile during the 20 ns of MD simulation of Aβ25-35 in gas phase. (E) RMSD change during 20 ns of MD simulation of Aβ25-35 in gas phase.

Additionally, figure 4-3 shows the MD simulation of the Aβ25-35 monomer in the gas phase in a time frame of 20 ns. The simulation shows fast energy stabilization reaching an asymptote in the first ten nanoseconds of simulation with an oscillating root mean standard deviation, which recovers in multiple simulation steps as the starting-point structure. One of the most stable structures is observed at 20 ns, exhibiting a more compact hairpin structure (Fig 4-3) with an optimized minimum with a broader hydrophobic region; which is expected in the gas phase.
**Figure 4-4.** (A) Aβ25-35 MD snapshot at 0 ns in the condensate phase with TIP3P water molecules (B) Aβ25-35 MD snapshot at 28.8 ns in the condensate phase with TIP3P water molecules (C) Energy profile during the 28.8 ns of MD simulation of Aβ25-35 in the condensate phase with TIP3P water molecules. (D) RMSD change during 28.8 ns of MD simulation of Aβ25-35 in the condensate phase with TIP3P water molecules.

On the other hand, the condensate phase MD simulation of the Aβ25-35 monomer using TIP3P water molecules (Figure 4-4) show an enlargement of the hairpin-like structure as a consequence of the solvation of the oppositely charged extremes of the peptide, which is translated in a hairpin with shorter curvature, presenting a c-shaped topology. Distortions in the hydrophobic core are also observed where the Ile$^{31}$ and the Ala$^{30}$ fragments move into the hydrophobic core while the Ile$^{32}$ fragment moves out of the hydrophilic core. In addition, the Met$^{35}$ and Lys$^{28}$ fragments show great distortion respect the Hartree-Fock optimized structure.

These fragments tend to interact with the surrounded TIP3P water molecules causing the Met$^{35}$ to leave the hydrophilic core, while the Lys$^{28}$ fragment is bent toward the peptide plane. The
exposure of the hydrophilic fragments of the peptide may also change in the presence of nearby monomers. This is a consequence of their aggregation or the formation of salt bridges between monomers. This is why it is not easy to obtain reliable NMR structures of the monomers as the peptide is highly dynamical in both gas phase and condensate phase. Based on the total energy profile of the simulation in condensate phase (Fig 4-3), the starting gas-phase structures are more stable in solution than the solvated dynamical state reaching stability after 20 ns.

As mentioned above, the exposure of the hydrophilic regions to the solvent destabilizes the hairpin conformation due to the charge solvation at the extremes of the peptide chain. On the other hand, the RMSD show variations in the peptide coordinates that are as large as 40 Å. This significant RMSD variation is not entirely attributed to modifications in the bond lengths and bond angles of the peptide in the condensate, but also the molecule diffusion through the solvation sphere. The RMSD shows that the peptide presents three possible metastable states located between 2-5 ns, 8-16 ns, and 20 and 28 ns of simulation, which cannot be observed in the function of the simulation energy. This can be attributed to the fact that small distortions in the peptide yield a significant increase in the peptide energy despite the fact that the RMSD does not change over a particular time-lapse.

4.3.2 Stacked Oligomers

Aiming to understand the aggregation mechanism of Aβ25-35, we characterized the effect of monomer parallel stacking on the magnitude and direction of the electric dipole moment of the resulting structure. The structure was optimized at the same level of theory as the monomer. The monomers A/A preserve their hairpin-like conformation (Fig. 4-5), exhibiting a more significant
beta-sheet character, which can be observed in the Ramachandran plot in figure 4-5. The hairpin distances for the lower layer monomer Lys$^{28}$-Gly$^{33}$ is 7.32 Å and for Ala$^{30}$-Ile$^{31}$ is 5.82 Å, while in the upper layer monomer the distances are Lys$^{28}$-Gly$^{33}$ 7.50 Å and Ala$^{30}$-Ile$^{31}$ 5.80 Å. The more dramatic changes in distances are present toward the outer part of the hairpin (Lys$^{28}$-Gly$^{33}$) in comparison with the isolated monomer. The opening of the hairpin at the extremes can be related to the decrement of the electron density between the outer fragments due to the stacking interaction. Moreover, the monomers center of mass is at 16.93 Å with an angle of 1.80º indicating a stepwise shift between the monomers.
Figure 4-5. Structure of Aβ25-35 Parallel stacked dimer A/A. (A) Cartoon backbone of A/A (Top view), hydrogen atoms are omitted. (B) Cartoon structure of A/A (Front view). (C) Sphere surface of A/A (Front view). (D) Ramachandran plot of A/A. A red cylinder arrow represents the electric dipole moment.

In contrast to the isolated monomer, the A/A dimer presents a more significant dipole moment with a magnitude of 15.30 D (Table 4-1). The dipole moment is not perfectly axial, as in the case of the monomer (see fig 4-5), due mostly to a stepwise displacement and tilt between the monomers. However, the structure keeps its pore-like topology, where the dipole increased by a factor of two respect the isolated monomer. Additionally, the structure presents a relatively larger stability than two isolated monomers by -2.67 Kcal/mol. Further indicating that the stacking interactions of this peptide within gas phase are stable. The previous result is not in agreement with the results obtained for the full-length Aβ1-42 peptide, where the oligomeric structures are less stable than the isolated monomers.
Even though the stacked parallel dimer presents a stable conformation, it is needed to understand if the parallel stacked configuration is more stable than its antiparallel configuration (A/B). The simulation was carried optimizing the A/B structure at the same theoretical level used previously. During the optimization, the antiparallel monomer flips back into a distorted parallel conformation, maintaining the hairpin conformation of its monomers. The structure exhibits a dipole moment mostly axial in comparison with the A/A structure with a magnitude of 13.52 D. The dipole is better localized than in the case of the A/A structure but weaker. This decreases its effectivity to aggregate in comparison with A/A. Also, the pore-like topology of the stacked dimer is mostly lost in the case of the A/B structure, reducing its ion channel like cytotoxic capability.

The structure is more stable than in the case of the A/A, where A/B is more stable than two isolated monomers by -11.44 Kcal/mol due to the strong interaction between aliphatic carbon chains. The A/B structure present a center of mass distance between monomers of 14.16 Å, which is considerably larger than in the case of the A/A oligomer. The center of masses presents an angle of 1.07 °, which is noticeably smaller than in the case of the A/A oligomer, which can explain why the dipole moment of the structure is almost entirely axial.

Based on the previous result, we have demonstrated that the parallel stacking configuration yields to a more significant dipole moment and retains the ion pore topology of the structure. These are two critical elements for the cytotoxicity of the peptide. For that reason, we studied the parallel stacked trimer A/A/A in order to explore its stability and dipole moment. The monomers in A/A/A preserve their hairpin-like conformations with an average center of mass distance between monomers of 14.35 Å. This distance is considerable shorter than in the case of A/A. The properties
mentioned above can be related to the increase of the structure dipole moment to a magnitude of 24.99 D. Besides, the dipole-dipole interaction between monomers enhances the stability of the complex to -18.13 Kcal/mol, more stable than three isolated monomers, which is a considerably stabilized in comparison to the A/A structure.

Even though the dipole moment strength is larger than the addition of three individual monomer dipole moments, the direction of the dipole is not perfectly axial. However, it is tilted in respect to the aggregation axis. This can be explained in the function of the stepwise shift and tilt between the monomers in the structure that present an average center of mass angle of 68.14°. The structure exhibiting an ion channel-like topology added to the dipole moment of the structure which further explains the high cytotoxicity of these small oligomers.

![Figure 4-6. Structure of Aβ25-35 Antiparallel protofibril AB. (A) Cartoon backbone of AB (Top view), hydrogen atoms are omitted. (B) Cartoon structure of AB (Front view). (C) Sphere surface of AB (Front view). (D) Ramachandran plot of AB. A red cylinder arrow represents the electric dipole moment.](image-url)
In the previous chapter, we have demonstrated that the Aβ1-42 peptide can form protofibrils by the interaction of two monomers in an antiparallel arrangement which are held by salt bridges located between Lys28 and Asp1 of both monomers, which agrees with experimental evidence supplied by Gremer and coworkers. The protofibril structure resembles a pore-like topology with a dipole moment utterly aligned with the fibril axis. Making this protofibril a perfect candidate for the formation of ion channels in neuronal membranes. Aiming in the same direction, we decided to explore the possibility of protofibril formation in the Aβ25-35 peptide.

In figure 4-6, we show the structure of the Aβ25-35 protofibril AB, which is comprised of the interaction of two antiparallel Aβ25-35 monomers side by side. The monomer in AB shows a hairpin-like shape with α-helical extremes as it was observed in the isolated monomers. In contrast with the Aβ1-42 protofibrils, the monomers are not bonded by pure salt bridges but are held together by hydrogen bonds between the residues Gly33-Lys24 and a with a partial salt bridge between Asn27-Lys24. The hairpin distances of the left monomer are Lys28-Gly33 by 6.96 Å and Ala30-Ile31 by 5.83 Å, while for the second monomer the distances are Lys28-Gly33 of 7.83 Å and Ala30-Ile31 of 5.79 Å. Again, the significant distortions are observed in the outer region of the hairpin, where the first monomer (left) presents a compacted hairpin in comparison with the second monomer (right). Besides, the peptide presents a distance between the center of masses of 13.32 Å.

Moreover, the lack of salt bridges in the gas phase structure may change when dynamical effects are considered, which can be weakened by the presence of the solvation environment. One of the more relevant characteristics of the protofibril is the presence of an utterly align dipole moment with the fibril axis. This was also observed in the case of the Aβ1-42 protofibril. The
magnitude of the electric dipole moment in the protofibril is of 15.65 D. This magnitude is considerably large than in the case of the Aβ1-42 protofibril. This could explain the fast aggregation rate of the Aβ25-35 peptide and its high cytotoxicity. The dipole moment of the structure is more significant than in the case of the stacked dimer. This indicates that the protofibril is more likely to aggregate than the stacked dimer due to the strength of the dipole moments. Moreover, the structure is more stable than two isolated monomers by -22.16 Kcal/mol, which is significantly more stable than the stacked configuration reaffirming the structure stability and setting it up as a possible candidate for the formation of fibers of Aβ25-35; which has not been reported yet in literature. Furthermore, the Ramachandran plot in figure 4-6 shows that the protofibril structure presents a larger β-sheet character than the isolated monomer and stacked dimer with certain α-helix and random coil conformation, which agrees with ECD experimental measurements.
**Figure 4-7.** (A) Aβ_{25-35} Protofibril MD snapshot at 0 ns in gas phase (B) Aβ_{25-35} Protofibril MD snapshot at 20 ns in gas phase. (C) Energy profile during the 20 ns of MD simulation of Aβ_{25-35} Protofibril in gas phase. (D) RMSD change during 20 ns of MD simulation of Aβ_{25-35} Protofibril in gas phase.

In figure 4-7, the MD simulation of Aβ_{25-35} protofibril AB in the gas phase is shown. The dynamical relaxation of the protofibril yields to a more stable structure, which is stabilized by the formation of salt bridges between Lys^{28} and Asn^{27} fragments of both monomers. In addition, the structure gets more compact due to the formation of salt bridges and the interaction between aliphatic hydrocarbon chains. This scenario may change under solvated conditions. Observing the energy profile of the simulation, we notice that the minimum energy profile is reached within the first 2 ns of simulation, which agrees with the RMSD data in the first 2 to 5 ns of simulation. The system oscillates close to 10 Å with amplitudes variations of about 8 Å, which are typical in gas phase MD simulations due to the large velocities of the individual atoms and the low viscosity of the medium.
Moreover, the condensate phase MD simulation with TIP3P water molecules after 20 ns of simulation also shows the formation salt bridges between Lys$^{28}$ and Asn$^{27}$, showing a more compact hairpin conformation restricting the exposure of the solvent to the hydrophobic core. This conformation increases the steric repulsion between the aliphatic chains, which can be observed in the energetic profile of the simulation. The RMSD shows significant variations in the first 2.5 ns of simulations observing a large RMSD stable state from 5 to 15 ns. Which does not resemble a metastable state in the function of the energetic profile of the simulation. The RMSD reaches a threshold of about 12 Å after 20 ns of simulation, where a metastable state is reached in the energetic profile.
Furthermore, the parallel protofibril conformation AA’ (figure 4-1) preserves the hairpin conformation of each monomer with a distance between the center of masses of 13.35 Å. The dipole moment magnitude of the AA’ structure is 4.21 D, where the direction of the dipole moment lies in the peptide plane, which is not effective for the aggregation by dipole-dipole interactions. Moreover, the AA’ structure is more stable than two monomers by -6.50 Kcal/mol. This structure is more stable than two stacked monomers but less stable than the antiparallel protofibril. This indicates that the antiparallel protofibril is more likely to be the building block of fibrillar structures rather than stacked structures or parallel protofibrils.

Figure 4-9 shows the structure of the protofibril Dimer AB2. The monomers within the protofibril dimer preserve the hairpin structure with a significant β-sheet character and certain α-helix and random coil character, as is shown in the Ramachandran plot. In contrast with the Aß1-42 double protofibril, the structure presents a large, highly localized dipole moment, which is perfectly axial to respect the fibril plane. The dipole moment presents a magnitude of 32.96 D (table 4-1), and the structure is more stable than for isolated monomers by -46.04 Kcal/mol. The protofibrils present an average stacking distance between the center of masses of 20.69 Å. The average distance between monomers in the protofibril is 13.35 Å.
The distances between the center of masses of the monomer within the double protofibril are similar to the protofibril. Also, the stacking distances between the double protofibril are larger than in the case of the stacked dimer A/A. The latter can be explained by the reduction of the electron density in the individual monomer in order to achieve an effective protofibrillar interaction weakening the stacking between the monomers in comparison to the A/A stacked dimer. It is worth to mention that the protofibril dimer resembles a pore-like topology as occurs in the isolated protofibril.

**Figure 4-9. Structure of Aβ25-35 Protofibril Dimer AB₂.** (A) Cartoon backbone of AB₂. (Top view), hydrogen atoms are omitted. (B) Cartoon structure of AB₂. (Front view). (C) Sphere surface of AB₂. (Front view). (D) Ramachandran plot of AB₂. A red cylinder arrow represents the electric dipole moment.
The pore-like topology, the axial direction, and magnitude of the dipole moment make the Aβ25-35 peptide more cytotoxic than the Aβ1-42 oligomers. The former and latter peptides can potentially exert the two cytotoxic pathways suggested by the amyloid hypothesis. The large magnitude of the dipole moment in the Aβ25-35 peptide makes it a perfect candidate for fast aggregation and ion channel strength in comparison with the full-length Aβ1-42 peptide. The formation of larger protofibril structures (Fig 4-1 and Table 4-1) yield to oligomers whose dipole moment increases linearly with the number of protofibril fragments. This produces more stable structures that can speed up the aggregation mechanism as the fiber grows, explaining the high aggregation rate of the peptide.

**Figure 4-10.** Spectra of Aβ25-35. (A) Theoretical UV-Vis spectra of Aβ25-35. (B) Theoretical ECD spectra of Aβ25-35. (C) Experimental UV-Vis spectrum of Aβ25-35 in PBS buffer (~ 1x10⁻³ M) over a period of nine days. (D) Theoretical TPA spectra of Aβ25-35. (E) Theoretical TPCD spectra of Aβ25-35.
The theoretical OPA spectrum for the Aβ-25-35 monomer and its stacked and protofibril dimer (\(A\), \(A/A\), and \(AB\), respectively). Figure 4-10 shows one band when ten electronic states are considered. The states within the region between 210 and 270 nm. The monomer band presents the bluest shifted band, which is centered at 234.4 nm with a molar extinction coefficient of 3763.4 M\(^{-1}\).cm\(^{-1}\). The stacked dimer band is redshifted with respect to the monomer and centered at 243 nm with a molar extinction coefficient of 2381.72 M\(^{-1}\).cm\(^{-1}\). The peak exhibits not just a redshift respect the monomer peak, but also a reduction in the molar absorptivity, which makes it possible to track the aggregation process by just UV-Vis.

Additionally, the Aβ-25-35-protofibril presents the farther redshifted band to respect the monomer, which is centered at 245.8 nm with a molar extinction coefficient of 1567.2 M\(^{-1}\).cm\(^{-1}\). This makes possible their distinction respects the monomer and stacked dimer not just by its characteristic redshift but by its molar absorptivity. The theoretical results discussed above agree with experimental UV-Vis measurements of amyloid-β-25-35 (~1.10\(^{-4}\) M) where a clear redshift of the principal band is observed, and the intensity of this band decreases over time as the aggregation of the peptide occurs.

On the other hand, the theoretical ECD spectrum of Aβ\(_{25-35}\) (Fig 4-10) also shows a similar trend to respect the UV-Vis spectrum of its oligomers. The monomer presents the most blue-shifted bands of the set with ten electronic states. It presents a double negative band that are centered at 236.55 nm and 225.1nm with differential molar extinction coefficients of -57.413 M\(^{-1}\).cm\(^{-1}\) and -4.714 M\(^{-1}\).cm\(^{-1}\), respectively. The Aβ-25-35-stacked dimer presents a single red-shifted band with respect to the monomer centered at 241.2 nm with a larger differential molar extinction coefficient.
than the monomer, which has a value of $-61.217 \text{ M}^{-1}\text{cm}^{-1}$. The protofibril exhibits a red-shifted band with respect to the previously analyzed species, which is centered at 244.6 nm and is considerably smaller differential molar extinction coefficient of $-34.097 \text{ M}^{-1}\text{cm}^{-1}$ This makes possible the identification of the monomer and oligomers not just by their red-shift in the ECD spectrum, but also by the magnitude of their CD. It is clear from the obtained theoretical ECD spectrum that the beta-sheet or beta-turn conformation are the most prominent characteristics of the Aβ$_{25-35}$ peptide, as the experimental evidence suggests$^{54,56-59}$.

Moreover, the theoretical TPA spectrum of the Aβ$_{25-35}$ monomer and oligomers present a single band. The monomer band is the bluest shifted band as it occurred in OPA. The monomer shows a TPA band centered at 484.3 nm with a TPA cross-section 1.57 GM. The stacked protofibril dimer band, on the other hand, presents a redshifted peak with a larger two-photon cross-section, which is centered at 510.3 nm with a TPA cross-section of 79.18 GM. The protofibril configuration presents a red-shifted band with respect to the monomer, which was virtually indistinguishable from the stacked dimer. The band is centered at 511.2 nm with a TPA cross-section of 50.39 GM. The proximity between the stacked dimer and protofibril bands makes it extremely difficult to distinguish them by just band shift; however, it possible to identify them by TPA cross-section. This indicates that OPA can resolve these peaks slightly better than TPA.

Furthermore, the theoretical TPCD spectrum (Fig 4-10) shows an entirely different picture than the TPA spectrum, where it is even easier to distinguish the oligomers and the monomer of the Aβ-25-35 peptide not just by shifts but by band shape and sign. The monomer presents a double band structure centered at 489 nm and 472.7 nm with TPCD of 0.012 GM and 0.013 GM,
respectively. On the other hand, the stacked dimer also presents a double band structure that is red-shifted with respect to the monomer but presents positive and negative bands that are centered at 513.5 nm and 497.2 nm with TPCD cross-sections of 0.082 GM and - 0.012 GM, respectively. The protofibril presents a single TPCD positive band that virtually overlaps with the positive band of the stacked dimer. The band is centered at 513 nm with a TPCD cross-section of 0.06 GM. The difference in the TPCD band position and sign can make easily distinguishable this species in comparison with just ECD. This demonstrates the versatility of TPCD as a tool for the distinction of conformational changes as well as aggregation configurations in chiral systems.

**Figure 4-11.** (A) Aβ25-35 Protofibrils aggregation MD snapshot at 0 ns in gas phase and in condensate phase with TIP3P water molecules (B) Aβ25-35 Protofibrils aggregation MD snapshot at 20 ns in gas phase. (C) Aβ25-35 Protofibrils aggregation MD snapshot at 20 ns in condensate phase with TIP3P water molecules. (D) Energy profile during the 20 ns of MD simulation of Aβ25-35 Protofibrils aggregation in gas phase. (E) Energy profile during the 20 ns of MD simulation of Aβ25-35 Protofibrils aggregation in condensate phase with TIP3P water molecules.
In Figure 4-11, we show the aggregation of four Aβ_{25-35} protofibrils in the gas-phase and condensate phase using TIP3P water molecules. In the gas phase, the structures repeal one another, which can be attributed to the net positive charges of the monomers. This behavior was also observed for the Aβ_{1-42} protofibril aggregation. Also, the energy profile of the aggregation shows that as the protofibrils get farther apart, the net energy of the system reaches stabilization, keeping the hairpin-shape of the monomers and the protofibril interactions.

On the other hand, the MD simulation in the condensate phase showed after 20 ns of simulation the Aβ_{25-35} protofibrils get in closer contact. Although they get into closer proximity, the protofibrillar interaction is destroyed as a consequence of the close interaction between monomers and multiple protofibrils. Many structures at 20 ns of simulation have hairpin-like conformations, some of them showing parallel hairpin dimeric structures. The confinement of one of the hairpin structures between multiple oligomers makes that the structure loses its hairpin conformation and becomes an extended beta-sheet.

The model of aggregation has been already reported in the literature theoretically and is proposed as a possible pathway of fibril formation. However, this scenario can change in highly hydrophobic environments, as is the interior of the neuronal membrane. Please note that classical MD simulations do not include quantum effects such as exchange and correlation energies that may lead to a completely different aggregation pathway. The denaturation of the hairpin structure only occurs in environments with multiple protofibrils. In contrast, a single protofibril is shown to be stable during that simulation time frame (see figure 8), which will not necessarily occur in hydrophobic environments. Also, in comparison with the full-length Aβ_{1-42}, the Aβ_{25-35} exhibits a
smaller interaction surface area that may yield to the formation of linear peptides, as mentioned above. However, more evidence is required to prove this hypothesis entirely.

Furthermore, based on the model of aggregation of the amyloid hypothesis, three possible mechanisms of aggregation\textsuperscript{24} are suggested. i) Downhill polymerization\textsuperscript{88}, ii) nucleated polymerization \textsuperscript{89, 90}, and iii) nucleated conformational conversion\textsuperscript{91}. Our results suggest that as an initial step, the nucleation of the peptides is required, which allows us to discard the first mechanism. Our MD simulations suggest that after the initial nucleation of protofibrillar species in water, a conformational change occurs in the peptides, which eventually yield mature fibrils conformed by linear beta-sheets. However, this scenario may change in the interior of a membrane in the case of the formation of ion channels.

### 4.4 Conclusion

In summary, the optimization of the monomer and oligomeric structures at the theoretical level HF/STO-3G, showed that the monomer presents a hairpin-like structure with certain alpha-helix character as is reported within literature. The monomer also presents a perfectly axial dipole moment, which is weaker than in the case of Aβ\textsubscript{1-42} monomer but better oriented. The presence of an intense localized electric dipole moment in the monomer and oligomers can promote the formation of fibrils through a cascade of dipole-dipole interactions, especially in the protofibril arrangement. The aggregation cascade is controlled kinetically and follows a nucleated conformational conversion aggregation pathway. More evidence is needed to elucidate the
aggregation mechanism of Aβ25-35 completely. The out of the plane dipole moment in the Aβ25-35 peptide opens the door to new possibilities and routes of treatment for neurodegenerative diseases.

4.5 References


33. Behensky, A. A.; Yasny, I. E.; Shuster, A. M.; Seredenin, S. B.; Petrov, A. V.; Cuevas, J., Stimulation of Sigma Receptors with Afobazole Blocks Activation of Microglia and Reduces Toxicity Caused by Amyloid-β.


CHAPTER 5. ROLE OF THE AMYLOID \( \beta_{1-42} \) AND \( \beta_{25-35} \) DIPOLE MOMENT ON THE FORMATION OF SELECTIVE ION CHANNELS

In the previous chapters, we have studied the aggregation mechanism of the peptides \( \text{A} \beta_{1-42} \) and \( \text{A} \beta_{25-35} \). We have demonstrated that the aggregation mechanism of these peptides is mostly electric dipole driven by a cascade of dipole-dipole interactions being the protofibrils the structures more likely to aggregate due to their strong and axial dipole moment. The protofibrils in these structures present a pore-like topology that added to their strong axial dipole moment can make them perfect candidates for ion-conducting pores. In this chapter, we explore the ion channel capability of these peptides using calcium as a pore-permeable ion. Strong experimental evidence suggests the formation of ion-conducting channels in neurons by these peptides may be the cytotoxic pathway of action of the AD. This methodology aimed to help answer this fundamental question. We performed molecular dynamics simulations of the gas phase optimized structures of \( \text{A} \beta_{1-42} \) and \( \text{A} \beta_{25-35} \) monomers and oligomers in the gas phase, condensate phase, and in the membrane incrusted configuration using as pore permeable ion calcium. Our results show that the \( \text{A} \beta_{1-42} \) protofibril and its dimer can function as an ion-conducting pore in gas, condensate, and incrusted membrane conditions. At the same time, in the case of the \( \text{A} \beta_{25-35} \), only the monomer presents this property when calcium ions are used. Also, we have demonstrated that the presence of calcium ions in \( \text{A} \beta_{25-35} \) monomeric and protofibrillar pore disrupts the integrity of the membrane opening a new pathway of cytotoxicity in this peptide.
5.1 Introduction

The amyloid β peptide is known to play a fundamental role in the development and progression of Alzheimer’s disease\(^1\)\(^-\)\(^5\). Aß is generated by the membrane proteolysis of the amyloid precursor protein (APP) by γ- and β-secretase\(^1\)-\(^4\), \(^6\)\(^-\)\(^8\). The poor selectivity of these secretases produces Aß molecules of different amino acids sequence lengths. In particular, the undecapeptide Aß\(_{25-35}\) is known to be more cytotoxic than others Aß peptides and is naturally occurring in Alzheimer’s disease patients\(^9\)-\(^11\).

Researchers suggest that two cytotoxic pathways are possible in Alzheimer’s disease. However, the mechanism of cytotoxicity is still unknown. Strong evidence suggests that the small oligomers can bind into the neuronal membrane and form ion-conducting pores\(^12\)-\(^14\). Though, Aß is also present in the brain of healthy individuals, showing that Aßs have a fundamental role in normal human physiology, mostly depressing the neural activity when neurons are overexcited\(^1\), \(^4\), \(^15\)-\(^16\). Meaning that the presence of an excessive amount of Aßs is not the initial cause of the disease, but a response to it.

Substantial evidence suggests that the Aß\(_{1-42}\) can form ion-conducting pores in membranes and presents high permeability to calcium ions, which is known to control the releasing of neurotransmission in neurons\(^12\), \(^17\)-\(^20\). The suppression of the binding ability of the peptide to the neuronal membrane reduces the cytotoxicity of the peptide considerably. Just the oxidation of the methionine amino acid, the methylation of the terminal amines, or the blockage of the ion-conducting pore reduces the cytotoxicity of the peptide considerably\(^18\), \(^21\)-\(^23\). Based on our previous
theoretical findings, the protofibril of the Aß1-42 peptide is more likely to exhibit ion-conducting channel capabilities. This is due to the pore-like topology, and more critical of all, its axial electric dipole moment, which is maintained by salt bridges between the amino acids Lys28 and Asp1. Inhibiting the formation of salt bridges between monomers on the Aß1-42 depletes the possibility of protofibril formation and the ion-conducting channel capability of the peptide. The mentioned properties can facilitate the diffusion of ions through the pore, creating an ionic imbalance in neurons producing neuronal death, which agrees with experimental evidence.

The Aß1-42 peptide is not the only Aß peptide able to exhibit ion-conducting pores. It is known that the most segregated Aß peptide Aß1-40 is capable of forming ion-conducting pores24. However the evidence is controversial due to a recent study suggesting that this peptide is not capable of the creation of ion-conducting pores, while Aß1-42 can do it spontaneously25. On the other hand, the highly cytotoxic Aß25-35 peptide is known to exert its cytotoxic capabilities through the damage of the mitochondrial membrane, causing leakage of proteins such as cytochrome c, citrate synthase, and malate dehydrogenase along with others21, 26-28. Besides, the presence of Aß25-35 has been shown to increase the expression of proteins associated with the mitochondrial transition pore. Aß25-35 was shown to bind into anionic lipid membranes mostly by electrostatic interactions28. The peptide is known to form voltage-dependent channels but also can diffuse ions through the membrane without the application of potential difference to the membrane14, 26, 29-31.

The morphology of the membrane conducting ion channels has not been elucidated yet. To the best of our knowledge, there is no reported attempt to explain the structure of the pores in real or artificial membranes using atomic-resolution capable techniques. In the case of the Aß25-35,
theoretical research has been done to elucidate the mechanism of membrane pore formation. Researchers using molecular dynamic (MD) simulations propose that the pore formation is achieved by the formation of beta-barrel pores comprised of eight stranded monomers in a mixed parallel and antiparallel configuration with an inner pore diameter of 3.5-4.0 Å. Nevertheless, the proposed structures are highly dependent on the initial guess structure during the simulation, and there is no experimental evidence that this structural configuration is more likely to be appropriate for the description of the ion conduction channels formed by Aβ25-35.

Our recent findings shown in the previous chapters suggest that the axial dipole moment of the Aβ1-42 and Aβ25-35 monomers and oligomers plays a fundamental role in the aggregation mechanism of these peptides. More important of all, the dipole could have an essential role in the formation of ion channels. Based on this evidence, we simulated and explored the ion channel capabilities of the Aβ1-42 and Aβ25-35 peptides using molecular dynamic simulations in the gas, condensate, and zwitterionic POPC lipid membranes.

Our results show that the Aβ1-42 protofibril allows the diffusion of calcium ions toward the interior of the membrane in the gas, condensate, and membrane incrusted configuration. The calcium ions are only able to diffuse in one direction, demonstrating that the diffusion in the pore is assisted by the direction and magnitude of the axial dipole moment of the peptide. On the other hand, the Aβ25-35 ion pore formation is entirely different. The membrane-incrusted protofibrils cause membrane damage in the presence of calcium ions, while the monomers can act as pores by themselves, showing a preferred diffusion direction. The monomer tends to work more like an ion tramp rather than as an ion channel under zero potential membrane conditions. The results obtained
in this dissertation demonstrate that the axial dipole moment of these Aβ structures plays a fundamental role in their cytotoxicity by both cytotoxic pathways acknowledged in the literature.

5.2 Theoretical Approach

The gas-phase optimized structures of the Aβ25-35 and Aβ1-42 peptides were obtained using the theoretical level HF/STO-3G and the semi-empirical theoretical level PM6, respectively. The structures of interest, protofibril, protofibril dimer of the Aβ1-42, and the monomer, protofibril, and protofibril dimer of the Aβ25-35 were optimized under the theoretical conditions mentioned above using the G09 suite.

Molecular dynamics simulations of the ion channel capability of the peptides mentioned above were simulated using the amber 14 protein force field (ff14sb) using calcium as the transported ion of interest. Calcium was selected as permeable pore ion, due to its role in the release of neurotransmitters under normal neuronal function in nerve cells or neurons. The simulations were run in the python package OpenMM using a Langevin’s integrator at a temperature of 310 K. The integrator was configurated with a friction coefficient parameter of 5ps⁻¹ and an integration time of 2 fs running for up to 30 ns. Due to computational constraints and the sake of simplicity in the simulation, water molecules bond angles and bond lengths were fixed when TIP3P water molecules were included in the model. Also, nonbonded interactions were treated without a cut off distance, constraining the carbon-hydrogen bond distances within the protein. Also, the POPC membrane simulations were set by incrusting the gas phase optimized structures in a membrane with minimum padding of 1nm with the aid of PDBfixer from the OpenMM python suite. The
RMSD of the peptide distances was obtained frame by frame using the molecular graphical viewer VMD\textsuperscript{38}.

5.3 Results and Discussion

5.3.1 Aβ\textsubscript{1-42} Pore formation

Fig 5-1. Shows the results of the MD simulation of the amyloid Aβ\textsubscript{1-42} protofibril ion-conducting pore in the gas phase. Starting with the PM6 optimized geometry of the Aβ\textsubscript{1-42} protofibril, we placed positive charged calcium ions (Ca\textsuperscript{2+}) at both sides of the pore of the structure to determine if there exists a preferred diffusion direction and verify if the structure can behave as an ion-conducting channel. As a consequence of the absent solvent Ca\textsuperscript{2+} ions tend to repeal to each other, making its diffusion fast. After 120 fs of simulation, the Ca\textsuperscript{2+} ions on top of the pore diffuse to the outer region of the channel, exhibiting no affinity to the entrance pore. The Ca\textsuperscript{2+} ions on the bottom region of the channel diffuse towards the pore entrance, where one calcium ion is approaching to the center of the pore. The ions movement toward the channel from the bottom of the pore follows the dipole moment direction of the protofibrillar structure. The monomers preserve the L-S topology in the structure without apparent modification of the salt bridges between the two protofibrils.
Figure 5-1. MD pore simulation of the Aβ1-42 protofibril with Ca^{2+} ions at both sides of the pore in the gas phase. (A) Snapshot of the Aβ1-42 protofibril at 0 fs, hydrogen atoms is omitted. (B) Snapshot of the Aβ1-42 protofibril at 120 fs. (C) Snapshot of the Aβ1-42 protofibril at 200 fs. (D) Snapshot of the Aβ1-42 protofibril at 600 fs.

In the following 200 fs of simulation, one calcium ion from the bottom region of the pore reaches the center of the pore, while the ions ion the top of the protofibril get repealed further away. On the other hand, ions closer to the bottom region of the protofibril are attracted to the entrance pore. Moreover, the ions farther from the pore entrance are repealed due to the repulsion force between the positively charged calcium neighbors. Though the repulsion between the calcium ions does not affect either the L-S topology of the monomers nor the salt bridges between the monomers.

At the 600 fs of simulation, one calcium ion from the bottom layer has fully passed the pore, while the calcium ions closer to the bottom layer of the channel get closer to its center. With this simulation, we have shown that Ca^{2+} ions are permeable to the pore in the Aβ1-42 protofibril.
Also, it has been demonstrated that the Aβ1-42 protofibril can behave as an ion conductive pore. The pore function is not based only on the structure topology, but also on its localized dipole moment of the peptide. The channel exhibits one preferred direction of diffusion without the application of a difference of potential in the extremes of the pore. The demonstration of ion diffusion through the pore in the gas phase allows us to speculate that in the condensate phase, it is also possible, and of course, the diffusion rate will change. However, the repulsion character of the ions will decrease as a consequence of the ion solvation.

To assess the previously mentioned hypothesis, we simulated the ion channel capability of the Aβ1-42 with the same conditions mentioned above. However, the peptide and surrounding calcium ions were solvated using TIP3P water molecules (Fig 5-2). This MD simulation was running for 5.9 ps, which was the time required for one calcium ion to diffuse through the pore under the initial set of conditions. In the first 100 fs of simulation, we observe that some of the calcium ions present repulsion due to the non-equilibrated solvation of the system. However, the repulsion is not as dramatic as in the gas phase simulation due to the medium viscosity and the charge solvation.

During this time frame, the calcium ions in the top layer of the pore feel repulsion as it was shown in the gas phase, while the calcium ions in the bottom layer do not diffuse towards the opposite region of the pore. This shows that the protofibril dipole and the charge separation fomented by the medium causes that the bottom calcium ions to experience an attractive Coulombic force towards the pore. The repulsion force is not entirely overcome by the repulsion between the positively charged calcium ions. Also, the L-S shaped topology of the monomers is
maintained as well as the salt bridges between the monomers indicating that the solvated ions do not denaturalize the geometry of the peptide in this short simulation time. In this time step, one solvated calcium ion is approaching the pore entrance indicating that the water molecules surrounding the calcium ion do not reduce the selectivity of the pore permeability.

Figure 5-2. MD pore simulation of the Aβ₁₋₄₂ protofibril with Ca⁺² ions at both sides of the pore in the condensate phase with TIP3P water molecules. (A) Snapshot of the Aβ₁₋₄₂ protofibril at 0 fs, hydrogen atoms is omitted. (B) Snapshot of the Aβ₁₋₄₂ protofibril at 100 fs. (C) Snapshot of the Aβ₁₋₄₂ protofibril at one ps. (D) Snapshot of the Aβ₁₋₄₂ protofibril at 5.9 ps.

In the first picosecond of simulation, the outer calcium ions of the bottom layer repulse due to the proximity between the solvated ions. However, the closest ions to the pore attract towards it. This attraction causes one calcium ion to get trapped in the center of the pore at this simulation time frame. On the other hand, the top layer calcium ions suffer repulsion from the entrance pore, which is driven by the repulsion between the positively charged calcium ions and the direction of
the protofibril dipole. Additionally, the ion mobility follows just one particular direction, as it was seen in the gas phase obeying the dipole moment direction of the peptide.

After 5.9 ps of simulation, one calcium ion has completely passed the center of the pore and diffused towards the top layer. The rest of the top layer solvated calcium ions stay near the pore entrance but cannot enter the pore accentuating the conclusion that there is a preferred diffusion direction without the application of a potential difference to the extremes of the pore. The bottom layer solvated calcium ions get in closer proximity to the entrance pore, indicating that the ion-dipole force interaction is still in the medium, despite the ions that have diffused towards the entrance of the pore.

The results obtained in the simulation confirm three points. First, the protofibril presents the capability of forming ion-conducting channels in the condensate phase. Second, the dipole moment direction of the protofibril defines the mobility of the solvated calcium ions toward the center of the pore under zero potential conditions property that is also observed in the gas phase. Third, the ion mobility in the condensate phase occurs at a lower rate. However, the ion's solvation causes more ions to get in proximity to the pore assisting the mobility of the calcium ions toward its interior. This occurs not just by the dipole moment of the peptide, but also the repulsive interaction between the calcium ions. These two effects occur in conjunction aiming to minimize the forces in the system. Hereby decreasing the net energy of the system. A small perturbation by just thermal fluctuations causes an imbalance in the electrostatic interactions that eventually yield to the expulsion of the calcium ions one by one at the time.
All these variables can explain why the cytotoxicity of this peptide is so high. In particular, the presence of a preferred ion mobility direction can yield to the decrement or increase of the calcium ions in the interior of the neurons, making them lose their biological function and die eventually. The alignment of the protofibrils with the neuronal membrane will depend mostly on the direction of the membrane electric field. The minimal energetic condition is achieved when the dipole moment of the protofibril is parallel with the direction of the membrane electric field.

![Figure 5-3](image)

**Figure 5-3.** MD pore simulation of the Aβ1-42 protofibril with Ca^{2+} ions at a single side of the pore in the condensate phase with TIP3P water molecules. (A) Snapshot of the Aβ1-42 protofibril at 0 fs, hydrogen atoms is omitted. (B) Snapshot of the Aβ1-42 protofibril at one ps. (C) Snapshot of the Aβ1-42 protofibril at ten ps. (D) Snapshot of the Aβ1-42 protofibril at 54.14 ps.

We simulated the Aβ1-42 protofibril in the condensate phase with Ca^{2+} ions in the bottom layer for 54.14 ps (Figure 5-3). In the first picosecond of simulation, one of the calcium ions reaches the pore and penetrates it. The diffusion through the pore is considerably faster in contrast when the ions are on both sites of the pore. There is significant repulsion between the ions, most of them remain in proximity to the pore, showing a better interaction of the ions and the pore in contrast with the previous simulation. Also, the repulsive character between the solvated ions does
not distort the L-S topology of the monomer nor the salt bridges of the peptide during the one ps simulation time frame.

After ten ps of simulation, the excreted calcium ion remains in the top layer of the peptide but near the entrance pore. On the other hand, multiple solvated calcium ions get into proximity to the pore even though simulation was not done in the presence of an external electric field. Indicating that the ion diffusion direction is entirely determined by the direction of the dipole moment of the protofibril. Moreover, the presence of multiple solvated ions close to the pore does not alter the L-S topology of the monomers nor their salt bridges, indicating that the complex is very stable in the presence of calcium ions and exerts the functionality of ion-conducting pore.

After 54.14 ps of simulation, a second calcium ion gets in proximity to the pore, making the previously excreted calcium ion repeal from the pore exit. Indicating that the Aβ1-42 protofibril ion channel works in a fashion where an ion is excreted one at the time as many naturally occurring ATP driven ion-conducting channels. In these channels, one ion is excreted at the time and then is substituted by the next one by a cascaded ordered mechanism until the pore is closed\textsuperscript{39}. In the Aβ\textsubscript{1-42} protofibril, the pore closure cannot occur as a consequence of the topology of the protofibril. The pore, and the dipole moment, work as a highway for the diffusion of ions from one side of the membrane to the other, which is maintained until the neuron dies by apoptosis. We hypothesize that the formation of ion channels is the main cytotoxic pathway for neuronal death when the cytotoxic Aβ\textsubscript{1-42} protofibril is present in excess in the neuronal membrane.
In the previous chapters, we showed that the formation of protofibrillar oligomers of Aβ yields to the increment of the structure dipole moment, wherein the case of the Aβ₁₋₄₂ protofibril the dipole moment tends to tilt. The increase of the strength in the dipole moment may yield to fast ion mobility and diffusion selectivity, however, the presence of this tilted dipole moment may produce intricate pathways of ion mobility in the interior of the pore. Although the preferred diffusion direction is kept due to the dipole moment of the structure is mainly axial. For this reason, we explored the capability of the Aβ₁₋₄₂ protofibril dimer to form ion-conducting channels in the condensate phase using positively charged calcium ions in the bottom layer of the peptide (Fig 5-4).
In the first 0.5 ns of simulation, some of the calcium ions have entered through the pore. Also, the protofibrils get in closer proximity than in their gas phase. This agrees with the aggregation simulations showed in the previous chapters where the Aβ1-42 protofibrils strongly aggregate in the condensate phase. Despite the substantial aggregation of the protofibrils, and the penetration of calcium ions, the L-S topology and the salt bridges of the monomers is conserved. The fast ions diffusion toward the pore is indicative of the increment of electrostatic interactions between the ions and the structure. This is a direct consequence of the overall strength and direction of the dimer electric dipole moment.

Also, within the first 0.25 ns of simulation, the system reached its equilibrium energetic state. However, the Root Mean Square Deviation (RMSD) of the simulation did not reach an equilibrium point due to the individual monomers’ strong affinity to one another. Also, the salt bridges between the monomers get strengthened by their low exposure to the solvent (see Fig 5-4). It is worth mentioning that in the gas phase, the dipole moment of the Aβ1-42 protofibril dimer is tilted toward the left side of the structure. At 0.5 ns of simulation, it is possible to observe most of the calcium ions close to the pore entrance as they move into the left side of the pore. This confirms that not just the strength of the dipole moment is necessary, but also its direction.

In the first nanosecond of simulation, the protofibrils get in closer proximity and exhibit stronger salt bridges. Furthermore, more of the calcium ions move further inside the pore, except for one calcium ion that diffuses by the exterior of the protofibril dimer but not by the pore. Most of the calcium ions in the first 0.5 ns of simulation diffuse toward the left side of the protofibril dimer. This uncovers that the dipole moment of the protofibril dimer is tilted to the fibril axis.
Moreover, the energetic profile of the dimer exhibits an energetic minimum. However, the RMSD does still show considerable changes indicating that the structure has not achieved complete dynamical stability.

Furthermore, after 2.6 ns of the simulation, the RMSD reaches an asymptotic point where small structure structural fluctuation is present. In this simulation time, two calcium ions have arrived at the exterior of the pore. Besides, the protofibrils get closer one to each other, showing an essential strengthening of their salt bridges. Despite the significant structural changes of the protofibril, the L-S topology of the monomers is conserved. They are exacerbating its high stability even under the transport of calcium ions toward the interior of the pore.

The majority of the non-excreted calcium ions are not repelled into the extracellular region but are trapped and attracted to channel interior from the bottom layer of the peptide to the pore exit. This indicates that the attractive character of the Aβ1-42 protofibril dimer is stronger than the case of the monomer. This agrees with experimental results and the proposed hypothesis that suggests that small oligomers are more cytotoxic than the monomers and fibrils.

The previous results ultimately demonstrate that the dipole moment of the protofibrils is the driving force for the formation of ion-conducting channels in the Aβ1-42 peptide. It is for that reason that oligomeric protofibril aggregates may form more efficient ion channels. Nevertheless, an intense dipole moment is not the only essential element needed for the transport of ions toward the pore but also its direction. A perfectly axial dipole moment is more likely to be more effective than a tilted dipole moment, which can limit the number of the protofibril oligomers that can
transport the ions effectively toward the interior or exterior of the neuronal membrane. This number of protofibrils in principle should not exceed the thickness of the neuronal membrane; otherwise, the transport of ions toward the interior or exterior of the membrane would be considerably longer. It is making the oligomer work like an ion trap rather than as an ion channel.

In the previously reported results, we have studied the stability of the Aß1-42 protofibril in the gas and condensate phase as well as its ion channel capability in both media. Nevertheless, a question to be answered is: is a protofibril is stable enough in a membrane? To answer this question, we simulated the stability of an Aß1-42 protofibril in a POPC membrane, which is on top, and the bottom is layered with TIP3P water molecules (Fig 5-4).

Our results show that in the first ten ns of simulation, the monomers get in closer proximity due to the presence of the hydrophobic membrane while maintaining their protofibrilar interactions by the salt bridges formed between Lys28 and Asp1. Also, the membrane is affected by the protofibril going from a rectangular topology into a cylindrical one. In the following 20 ns of simulation, the membrane gets tighter to the protofibril closing the pore due to the increase of the salt bridge stabilization maintaining a cylindrical topology without further evidence of the partial excretion of the peptide from the membrane or further membranal disruption.

Each monomer in the protofibril preserves their L-S topology showing that the structure is dynamically stable, not just in the gas but also in the condensate phase and embedded in a membrane. They are indicating that the structure is a perfect candidate for the exact composition of the peptide under physiological conditions. The energy vs. time plot shows us that the proximity
between protofibrils causes great attraction, which agrees with the significant RMSD changes in the first five ns of simulation. After this five ns period, small variations in the RMSD are observed (about 1 Å) (see Fig. 5-4), which are mainly caused by the membrane dynamics. It can also be found that the system starts to stabilize, reaching a metastable state in about 15 ns as a consequence of the dynamics of the membrane.

The results showed above demonstrated that the protofibril is stable when it is embedded in a POPC. The structure shows exceptional retention of the topology of each monomer and stronger salt bridges. For that reason, we decided to explore the ion channel capability of Aβ-1-42 protofibril in a POPC membrane using calcium as pore permeable ion.

![Figure 5-5. Aβ1-42 Protofibril embedded in a POPC membrane with Ca²⁺ ions on site I and II. A) Total energy per simulation step of protofibril in a POPC membrane with Ca²⁺ ions site I. B) RMSD per simulation step of protofibril in a POPC membrane with Ca²⁺ ions site I. C) Total energy per simulation step of protofibril in a POPC membrane with Ca²⁺ ions site II. D) RMSD per simulation step of protofibril in a POPC membrane with Ca²⁺ ions site II. The small pictures in each plot show the configuration of the system at the starting and endpoints of the simulation.](image-url)
The simulation was carried during a time frame of 1 ns, including the Aß\textsubscript{1-42} protofibril embedded in a bilayer POPC membrane, including eight calcium ions. In the first 0.4 ps of simulation, the monomers get in closer proximity to each other due to the strengthening of their salt bridges. This occurs as a consequence of the hydrophobic interior of the membrane and the high hydrophobicity of the peptide.

In this time frame of 0.4 ps, one calcium ion has fully passed through the pore, where the diffusion time is comparable with the isolated protofibril in the condensate phase. In the subsequent simulation times, the ions get in closer proximity to the entrance pore, allowing the entrance of a second calcium ion into the pore. The presence of ions near the pore destabilizes the protofibril in the interior of the membrane. This yields a metastable equilibrium after 100 ps. The RMSD, on the other hand, shows a significant variation on the first 100 ps of simulation, which afterward show small fluctuations that are within 1 Å.

This simulation demonstrates that the protofibril can behave as a calcium ion channel when it is embedded in a membrane. This evidence added to the stability of the protofibrils in the membrane makes it a perfect candidate for a neuronal ion channel, which can be an additional mechanism of action in Alzheimer’s disease. This simulation also confirms that protofibrillar structures are responsible for the formation of ion-conducting pores in biological or artificial membranes. This explains the mechanism of pore action and its dependence with the topology, magnitude, and direction of the electric dipole moment in the peptide confirming experimental observations of Aß\textsubscript{1-42} oligomeric ion-conducting pores. This study also can be taken as a starting
point to completely elucidate the experimental pore structure of Aβ1-42, which could lead to effective treatment and control of Alzheimer’s disease.

To determine the diffusion selectivity of the Aβ1-42 ion channel in the membrane, we studied the ion channel capability of the Aβ-1-42 embedded in a POPC membrane with eight Ca\(^{2+}\) ions located toward the protofibril dipole end, which is opposed to the ion diffusion mechanism expanded above.

The simulation was run in a time frame of 2.4 ns. In the first 20 ps of simulation, the monomers get nearby due to the hydrophobic character of the membrane strengthening the salt bridges between monomers in the protofibril. However, the initial interspace between the membrane and the peptide allows the pass of a calcium ion. Nevertheless, this does not occur through the center of the pore. This is mainly caused due to the membrane not in complete contact with the peptide because PDBfixer allows us to construct the membrane of the system with a default minimum padding of 1 nm.

In the subsequent nanoseconds of simulation, the ions start to get repelled from the pore entrance toward the water layer located at the hydrophilic region of the membrane. During the latter process, some ions reach the center of the pore due to the repulsive interactions between calcium ions. None of the calcium ions can pass through the pore during the simulation time frame of 2.4 ns. The presence of ions near the pore does not destabilize the protofibril in the interior of the membrane, reaching a stable state after 100 ps, which is not consistent with the results obtained with reverse ion flow. Moreover, the RMSD show relatively essential variations in the first 100 ps.
of simulation, while in the next simulation steps, the deviations are within 1 Å. This demonstrates that the peptide is mostly unaffected by the presence of ions on this side of the membrane.

During the simulation time, each monomer preserves their L-S topology, showing that the structure is dynamically stable. In this configuration, calcium ions are not able to cross the pore. This indicates that there is a preferred direction of ion calcium flow under zero potential conditions. This single flow direction can substantially increase the cytotoxicity of the ion channel due to the creation of an ionic imbalance in the interior of the neuron. The aforementioned can trigger overexcitation or depression of the neural activity. This evidentially will cause neuronal death and describe the high cytotoxicity of the peptide.

5.3.2 Aβ25-35 Pore Formation

In the previous chapter of this dissertation, we have shown that Aβ25-35 can exhibit pore-like characteristics due to the perfectly axial dipole moment of monomer and protofibril. In contrast with the Aβ1-42, the Aβ25-35 monomer exhibits pore-like topology and a perfectly axial dipole moment respect to the peptide plane, making it a perfect candidate for the formation of an ion-conducting channel. Furthermore, as a consequence of the small cross-section of the Aβ25-35 peptides, a gas or condensate phase simulation will be more likely to diffuse calcium ions through the surroundings of the peptide than to the center of the pore. The latter is mainly due to the repulsion of the positively charged calcium ions, which is more dramatic in the gas phase. To avoid this issue, we decided to approach the problem from a more realistic point of view, simulating the ion-conducting channel capability of the peptide embedded in a POPC membrane.
As a first step, the stability of the membrane-embedded Aβ25-35 peptide was assessed. We simulated the stability of the Aβ25-35 protofibril dimer in a POPC membrane, which is bottom and top layer with TIP3P for a simulation time of 1.02 ns (Fig 5-6). In the first 100 ps of the simulation, the monomers within the protofibrils interact strongly, getting close to each other. Also, there is an apparent strengthening of the intermonomer hydrogen bonds within each protofibril. Moreover, the stacking interaction between the protofibrils is stronger than in their gas-phase equilibrium geometries, which can be attributed to the highly hydrophobic character of the interior of the membrane. On the other hand, the membrane suffers distortion due to the presence of the peptide and adopts a cylindrical topology.

![Figure 5-6. Aβ25-35 Monomer embedded on a POPC membrane with Ca^{2+} ions and Aβ25-35 protofibril dimer stability in a POPC membrane. A) Total energy per simulation step of the Aβ25-35 protofibril dimer in a POPC membrane. B) RMSD per simulation step the Aβ25-35 protofibril dimer in a POPC membrane. C) Total energy per simulation step of the Aβ25-35 monomer in a POPC membrane with Ca^{2+} ions site I. D) RMSD per simulation step of the Aβ25-35 monomer in a POPC membrane with Ca^{2+} ions site I. The small pictures in each plot shows the configuration of the system at the starting and endpoint of the simulation.](image-url)
Furthermore, the energy profile and RMSD of the simulation suggest that the dynamical equilibrium is reached in the first 100 fs of the simulation. This result is not in agreement with the membrane stability of the Ab1-42, which requires a considerably longer relaxation time. This mostly caused by the broad cross-section of the Ab1-42 protofibril in comparison with Ab25-35 protofibril. Also, because of the small cross-section of the Ab25-35 the RMSD, does not exhibit substantial structural changes in contrast with the Ab1-42 peptide indicating that the hairpin-like structure of the monomers and the protofibrillar geometries in the gas phase are in relative closeness to the membrane-embedded geometries.

In the subsequent simulation steps, no significant structural variations are observed. The protofibrils exhibit small rotations and strengthening of the hydrogen bonds between monomers as well as a strong stacking interaction. Even though the membrane suffers deformation from a rectangular to a cylindrical topology, there is no evidence either that the peptide is not stable in the membrane nor that the peptide is excreted from the membrane. The latter assures that the peptide may behave as a stable membrane-embedded protein in the absence of positively charged ions. However, this scenario may change when ions are added to the simulation.

To verify the ion-conducting channel capability of the Ab25-35 and its permeability with positive charge calcium ions, we simulated the Ab25-35 monomer embedded in a POPC membrane. Ca^{2+} ions were added into the bottom layer of the protofibril in the negative region of the structure dipole moment. This direction was shown as the preferred diffusion direction of the Ab1-42 protofibril.
In the first 1.32 ns of simulation, two calcium ions were able to pass the center of the monomer pore trapping one calcium ion in the center of the hairpin. In addition, the hairpin structure gets more compact after trapping one calcium ion in the center of the pore. Though its geometry preserves certain beta-sheet and alpha-helix character as ECD experimental measurements suggest (POPC, POPG, and cholesterol vesicle)\(^{32, 40}\). In contrast, to the monomer structural changes, the membrane also exhibits significant distortion in the presence of the peptide and the calcium ion. The cylindrical distortion is more pronounced than in the case of the calcium-free protofibrils, exhibiting parabolical distortion into the top and bottom layer of the membrane. Which may lead to membrane damage which eventually will disrupt the biological function of the neuron. Also, the excess of calcium ions in the bottom layer of the peptide suffer repulsion but do not abandon the membrane cavity.

In the following simulation times, the peptide does not change its topology. However, the repealed calcium ions in the interior of the pore get in closer proximity to the peptide after 2.12 ns of simulation. Additionally, no further calcium ions abandoned the pore. The membrane suffers significant distortion in comparison with the first nanoseconds of simulation. Confirming that the presence of calcium ions with the peptide in the membrane allows the ion mobility toward the pore but also traps some of the calcium ions in the pore entrance distorting the integrity of the membrane considerably. This may explain why A\(\beta\)\(_{25-35}\) tends to break the mitochondrial membrane and increase the size of lipid vesicles in the presence of high concentrations of A\(\beta\)\(_{25-35}\). Moreover, the energy profile of the simulation (Fig 5-6) shows that the system reaches a stable state in the first 200 ps of the simulation. Also, the RMSD exhibits substantial structural changes of the peptide in
the first 1.5 ns of simulation, which agrees with the compact hairpin conformation of the Aβ25-35 monomer described above.

To verify the ion-conducting channel capability of the Aβ25-35 protofibril, we simulated the protofibril embedded in a POPC membrane top and bottom layered by TIP3P water molecules. Ca²⁺ ions were added to the bottom layer region of the membrane following the dipole moment assisted preferred diffusion direction. Additionally, the simulation ran for 20 ns in order to verify the integrity of the membrane after significant simulation times. In the first 5 ns of simulation, the protofibrillar interaction was broken as a consequence of the lack of salt bridges between the protofibrils. Furthermore, the calcium ions can interact strongly with the monomers getting trapped closer to the monomer pore; however, no calcium ions were able to trespass into the pore. The membrane suffers from a significant cylindrical distortion, which may affect its integrity seriously. Also, the top and bottom membrane layers exhibit a paraboloid distortion similar to the one observed with the membrane incrusted Aβ25-35 monomer.

![Figure 5-7](image)

**Figure 5-7.** Aβ25-35 protofibril embedded on a POPC membrane with Ca²⁺ ions. A) Total energy per simulation step of the Aβ25-35 protofibril in a POPC membrane with Ca²⁺ ions site I. B) RMSD per simulation step of the Aβ25-35 protofibril in a POPC membrane with Ca²⁺ ions site I. The small pictures in each plot shows the configuration of the system at the starting and endpoint of the simulation.
At 15ns of the simulation, the individual monomers present significant repulsion moving toward the sides of the membrane. The monomer repulsion disrupts the integrity of the membrane completely, making the membrane lose its bilayer characteristics and the separation between the hydrophobic and hydrophilic regions of the peptide. No calcium ion was able to abandon the membrane nor the pore, where more of them are trapped close to each monomer; this as a consequence of the intense a highly localized dipole moment that these peptides present.

After 20 ns of the simulation, the integrity of the membrane is importantly compromised. The monomers are repealed to the boundaries of the membrane. In addition, no calcium ion has escaped from the pore, and most of them are trapped at monomer positions (see Figure 5-9). The energy profile of the simulation shows that the systems reach a semi-stable equilibrium in about one ns of simulation. Nevertheless, the energetic profile is highly oscillating; this is mostly due to the movement of the calcium ions that causes repulsion between the protofibrils and the structural changes in the membrane.

Moreover, the RMSD concurs with the large displacement of the individual monomers in the membrane, which reaches a stable maximum of around 15 ns of simulation—agreeing with the point where the monomers are in contact with the boundaries of the membrane. The obtained results demonstrate that αβ25-35 protofibril is not an efficient ion-conducting channel due to the lack of salt bridges between the monomers. The αβ25-35 protofibril is more likely to trap ions in the interior of the pore rather than act as an ion channel. Furthermore, the most relevant observed characteristic of the protofibril membrane incrusted peptide is its ability to disrupt the integrity of the membrane adding cytotoxic pathway to the αβ25-35 peptide. The latter agrees with the
The destruction of the mitochondrial membrane in neurons will trigger a faster cellular death than in the case of large ionic imbalances, as is the case of the Aß_{1-42} peptide. The large ionic imbalance creates considerable stress in the cell as a consequence that most of the ion transporting channels in the cell require ATP to open, this makes that the cell start to consume more energy than what is supplied. Triggering apoptosis or the overwork of the mitochondria. The disruption of the mitochondrial membrane produces a faster cellular death. The mitochondria are the highly efficient molecular factories that transform the energy of hydrocarbons into ATP, which is fundamental for all cellular processes. In short, the damage to the mitochondria causes the cell to starve to death. This is a process that is faster than in the case of ionic imbalance where the mitochondria still doing its normal biological function.

5.4 Conclusions

In this chapter, we have demonstrated that both Aß_{1-42} and Aß_{25-35} can except cytotoxicity in Alzheimer’s disease by the formation of ion-conducting pores. The ion selectivity of these pores depends highly on the topology of the peptides. The ion mobility permeability and direction depend mostly on the axially localized electric dipole moment of the peptide. Also, we have shown that in contrast with the Aß_{1-42}, Aß_{25-35} can excerpt and additional cytotoxic pathway, which consists of the damage of the neuronal membrane by the presence of calcium ions in the medium. The latter explains the larger cytotoxicity of the peptide respect the 42 amino acid long Aß_{1-42}
peptide. The simulations shown agree with old and new research that claims that these peptides are capable of forming ion-conducting pores.

In the case of the Aβ25-35, it does not only form ion-conducting pores but disrupts membranes and lipid vesicles. The understanding of the cytotoxic pathways of Aβ is a fundamental step to treat and control Alzheimer’s disease and other similar neurogenerative diseases. The results shown in this dissertation explain the importance of the axial dipole moment in these peptides and their role in the cytotoxicity and aggregation of the peptides. The disruption of the pore by chemical modification of the structure (e.g., oxidation) or the mechanical blockage of the pore will provide control of the disease. However, more work needs to be constructed to fully understand the initiators of the disease rather than control their symptoms.

5.5 References


Conclusions and Future Work

The research presented in this dissertation constitutes a comprehensive approach to quantum biology. The quantum biology approach is richer in comparison with simple molecular mechanics simulations giving us additional information required to understand such complex systems. Using this approach, we have found that tunneling corrections are required to explain DNA point mutations accurately and to predict that a decrement in the medium polarity yields to a higher mutation rate.

Following the quantum biology approach, we have also shown that the aggregation mechanism and the formation of ion-conducting pores in the Aβ1-42 and Aβ25-35 peptides are driven by the axial dipole moment in these structures result, that was uncovered in this dissertation and explained the fast aggregation and high cytotoxicity of these peptides. Although considerable progress has been made in this dissertation regarding the description of the point mutation in DNA, and the aggregation and cytotoxic mechanisms of Aβ peptides, further research can be done to continue this study including:

1. Assess the effect of DNA base pair stacking on point mutations and tunneling corrections.

2. Determine the effect of the DNA sequence on the mutation process.

3. Explore the effect of molecular intercalation on the DNA point mutations.
4. Investigate the effect of Aβ peptides non-familial mutations on the direction and magnitude of the peptide’s dipole moment.

5. Simulate the aggregation and pore-formation mechanism of Aβ peptides by the use of QM-MD, which contains quantum effects that traditional force fields do not possess.
APPENDIX A: PUBLICATIONS FROM DISSERTATION WORK

APPENDIX B: CONTRIBUTION TO CONFERENCES AND RESEARCH FORUMS FROM DISSERTATION WORK


APPENDIX C: AUTHORIZATIONS FROM EDITORIAL OFFICES FOR USE OF COPYRIGHTED MATERIAL
Solvent effect on the intermolecular proton transfer of the Watson and Crick guanine—cytosine and adenine—thymine base pairs: a polarizable continuum model study

DOI: 10.1039/C7CP05356H

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APPENDIX D: ADDITIONAL STRUCTURAL ANALYSIS OF CHAPTER 3
1. A/B and A/A/A Structural analysis

The A/B dimer (Fig. S1) presents an arrangement where both chains are oriented in opposite directions (antiparallel), while the monomers within the structure preserve their L-S shape and curvature. However, essential differences are found with respect to the A/A dimer (Fig. 3). First, the electric dipole moment is not localized towards the peptide axis but to the peptide plane, as a consequence of the dipole moment orientation of the individual monomers (see Fig. S1 and S7). The magnitude of the electric dipole moment in the structure is 5.83 D which is smaller than in the A/A dimer. Second, the complexation energy of the dimer is 31.2 kcal/mol less stable than two isolated monomers and less stable than the A/A dimer (Table 1). Third, the COM distance between the chains is 33.39 Å, showing a less efficient packing in the A/A dimer. A/B presents an \( \beta \) intra-strand distance of (7.09 Å, 7.09 Å) Val\(^{39}\)-Ile\(^{32}\) and (6.61 Å, 6.61 Å) Ile\(^{32}\)-His\(^{14}\) (Fig. S1), which does not vary significantly with respect to A/A and A, indicating that at the PM6 theoretical level the topology of the monomers is not affected by their stacking interaction in both dimers. Nonetheless, the dipole moment orientation can contribute to the formation of oligomeric species where an axial dipole (A/A) is more likely to assist oligomeric formation rather than an equatorial one (A/B).

Next, the structure of a trimer in parallel orientation was optimized (A/A/A) at the same theoretical level (Fig.1 and Fig. S2). The A/A/A trimer preserves the L-S topology and the peptide curvature in its monomers, with an inter-chain COM separation of 18.99 Å (chains A-B) and 20.52 Å (chains B-C) (see Fig. S2). The inter-chain distance exhibits a reduction between chains A and
B and an increase between chains B and C in comparison with the dimer A/A. This result shows that the number of fragments in the oligomer affects their relative separation. This effect contributes to the oligomers packaging, the reduction of their surface area, and the increase of their hydrophobic character.

A/A/A presents a significant localized electric dipole moment partially oriented parallel to the fibril axis of magnitude 11.42 D (Table 1, Fig. S2 and S8). This value is comparable with dimer A/A. The similarity between dipole moments is associated with the displacement between the individual chains, which is supported by the experimental $2_1$ screw symmetry of the fibril, and certifies the reduction of the total dipole moment in the trimer. The trimer presents a complexation energy of 32.8 kcal/mol (Table 1) showing that the structure is less stable than three isolated monomers. These results show that the complexation of monomers is not stable, making their formation, from the thermodynamic point of view, rather difficult. However, the dipole orientation may contribute to the kinetic control of the aggregation and creation of stacked oligomers. Also, A/A/A presents a structure stabilization similar to A/B. The aforementioned suggests that monomers are more likely to form dimeric A/A and trimeric A/A/A structures rather than antiparallel dimeric structures A/B as a consequence of their thermodynamic stability and the kinetic control assisted by the dipole moment orientation of individual monomers. The $\beta$ intra-strand distance of A/A/A is more extensive in comparison to the dimer for the fragments Ile$^{32}$-His$^{14}$ (6.70 Å, 6.70 Å, 6.72 Å) but similar for the Val$^{39}$-Ile$^{32}$ fragments (7.19 Å, 7.05 Å, 7.07 Å), except for chain A.
2. AA’ and AB/A Structural analysis

AA’ is a protofibril dimer in a parallel arrangement (Fig. 1 and Fig. S3). The L-S topology and the curvature of the monomers are preserved in the complex, but intermolecular salt bridges and intermolecular hydrogen bonds are not present in the structure (Fig. S3). The monomers COM separation is 33.74 Å, which is larger than in A/A and A/A/A but similar to that observed in A/B. The total electric dipole moment of the structure is located in the fibril plane (Fig. S3 and S9) with a magnitude of 8.19 D (Table 1) The magnitude of the net dipole is greater than A/B. AA’ is less stable than two isolated monomers by 23.2 kcal/mol. AA’ it is more stable than A/B, but less stable than A/A, indicating that the formation of this protofibril structure is less favorable than the parallel stacking of monomers. AA’ exhibits an β intra-strand distance of (7.12 Å, 6.68 Å) Val39-Ile32 and (6.26 Å, 5.86 Å) Ile32-His14. It is notable that the intra-strand separations are not consistent between the monomers, where chain B exhibits a reduction in comparison with chain A and the Ile32-His14 distances are significantly smaller in comparison with the previously studied models.

To understand the differences between the protofibrillar and stacked arrangements, a three monomers structure (AB/A) was built, using as a base an AB protofibril and one parallel stacked monomer. AB/A is the simplest arrangement of a protofibril with stacking interactions (Fig. 1 and Fig. S4). Chains A and B exhibit a typical AB protofibril behavior with salt bridges located at Asp1 and Lys28 and stabilized by Ala42-Lys28 salt bridges. These salt bridges have distances of 2.56 Å Asp1-A & Lys28-B; and 2.53 Å Asp1-B & Lys28-A. The salt bridges within AB/A are more packed than in AB demonstrating that the stacking of subsequent monomers strengthens the
intermolecular salt bridges within each protofibril and therefore it increases the hydrophobicity of the oligomer through its surface area reduction. The compacting within AB/A can also be observed in the COM distances between chain A and B (protofibril), i.e. 25.04 Å, and between chain B and C (stacked side) 15.67 Å. The protofibril region shows a small distance change respect the AB structure. However, the stacking interaction distance is considerably smaller than in the A/A structure. The structure packaging is assisted by the rightly localized dipole moment in the protofibril that enhances the interaction of monomers through stacking. On the other hand, the stacked monomer strengthens the salt bridges within the protofibril, indicating that the cooperative interaction between protofibrils (stacking and salt bridges) are crucial for the fibril formation and its compacting.

AB/A preserves the L-S topology in all its monomers with an β intra-strand distances of (6.27 Å, 7.02 Å, 7.12 Å) and (6.67 Å, 6.67 Å, 6.59 Å) Ile32-His14, this result shows that the β intra-strand distance of Val39-Ile32 fragments is strongly affected by both stacking and inter-chain interactions within the protofibril. AB/A presents a long electric dipole moment that is partially oriented parallel to the fibril axis with a magnitude of 8.51 D (Table 1), which is more significant than in AB. The tilt of the dipole moment with respect to the fibril axis is caused by the additional stacked monomer which does not present a completed oriented dipole moment and to the shift between the stacked structures (see Fig. S4 and S11) as observed in A/A and A/A/A (see Fig. 3 and Fig. S3). The AB/A protofibril trimer is less stable than three monomers by 94.8 kcal/mol, which is less stable than the protofibril AB and the stacked oligomer A/A/A. The stability indicates that the stacked oligomers are prone to be formed by thermodynamic control while the protofibrils
are more likely to be created by kinetic control with the assistance of the oriented electric dipole moment of individual monomers.

3. Structures electric dipole moment analysis

The Monomer A (Fig. S5) presents an L-S topology that does not lie on the peptide plane as a consequence of its curvature. It exhibits an electric dipole moment partially oriented perpendicular to the peptide plane with a magnitude of 6.43 D. This dipole moment is centered in the region containing the amino acid fragments 25-45. The orientation of the dipole moment in the monomer may have a substantial impact during the formation of oligomers and fibrils, due to the dipolar interaction within monomers. The formation of oligomers not just facilitate the ordering and alignment of the monomers in the structures, but the rate of aggregation driven by an electric dipole assisted kinetic control. As a result of the dipole moment orientation in the monomers, it is natural to think that the stacking of multiple monomers will yield to structures that present a strong net dipole moment that will interact actively with nearby monomers increasing the rate of aggregation. The A/A, A/B, and A/A/A structures show proof of the previously mentioned statements. A/A Presents an electric dipole moment partially oriented perpendicularly to the dimer plane with a magnitude of 11.73 D (Fig. S6) showing that both monomers dipole moments add up forming a stronger net electric dipole moment.

A/B dimer shows a weaker electric dipole moment with a magnitude of 5.83 D that lies in the peptide plane (Fig S7). This occur because of the cancelation of the out of plane components of the individual monomers indicating that in effect the dipolar interaction is essential in these structures. On the other hand, the A/A/A trimer exhibits an electric dipole moment with similar
characteristics to the A/A dimer, where the dipole moment is partially oriented toward the peptide axis with a magnitude of 11.42 D (Fig. S8). The similarities between the stacked dimer A/A and trimer A/A/A is caused by the stepwise shift of the monomers within the structure that reduces the magnitude but not the orientation of the dipole. However, additional monomers in the structure can potentially increase the magnitude of the net dipole moment in the oligomer as was seen in A/A. This in turn will facilitate the interaction of other monomers with the oligomers, thus increasing the rate of aggregation.

The second way to form structures that interact strongly via dipole-dipole interaction is by their contact side by side, which in the case of the Aβ1-42 peptide yields to the formation of a protofibril. The interaction between the chains can be dipolar and stabilized by salt bridges or hydrogen bonds. The parallel disposition of the protofibril dimer AA’, where each monomer presents their out of plane dipole moment components in opposing directions, generates a structure with a resulting dipole moment that lies in the fibril plane. This is a consequence of the cancelation of the out of plane dipole components of the individual monomers. The dipole moment of AA’ is (8.19 D) is caused by the tilt of the monomers respect each other (Fig. S9) and is more significant than A and A/B. In contrast to the AA’ protofibril, the AB antiparallel protofibril presents the monomers out of plane dipole moments pointing in the same direction. These dipoles orientation makes the resulting net electric dipole moment utterly align on the fibril axis by the cancelation of the individual on plane dipole moment components of the monomers. This interaction generates a net dipole moment of 5.88 D which is slightly weaker than in the monomer (Fig S10). The perfectly axial dipole moment in AB makes possible a powerful dipole-dipole interaction with nearby
protofibrils. It is for that reason that the experimental fibrillar structure is built by AB protofibrils rather than just by stacked oligomers and AA’ protofibrils.

Furthermore, not just the dipolar interaction is vital for the protofibril formation stability, but the strong bonds between monomers through intermolecular salt bridges is required. The salt bridges get stronger as more fragments (monomers and protofibrils) stacks on the AB protofibril. This effect was observed in structures AB/A and AB/AB. The structures present net dipole moment magnitudes of 8.5 D and 12.81 D respectively. The former presents a tilted net dipole moment due to the small dipole moment misalignment and the stepwise shift of the monomer respect to the protofibril (Fig S11). The latter shows a stronger dipole moment than in the A/A dimer. The dipole moment in AB/AB is slightly oriented toward the fibril plane due to the stepwise displacement of the protofibrils.
Table S1. Heat of formation, Complexation Energy and electric dipole moment of the studied Aβ(1-42) at PM6 theoretical level.

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<th>Structure</th>
<th>$\Delta H_f^1$</th>
<th>$\Delta E^2$</th>
<th>$P^3$</th>
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<td>7.46</td>
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<tr>
<td>A/A</td>
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<td>A/B</td>
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<td>AA'</td>
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<td>-15.5149</td>
<td>8.75</td>
</tr>
<tr>
<td>AB</td>
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<td>-19.4373</td>
<td>3.80</td>
</tr>
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<tr>
<td>AB/AB</td>
<td>-11433.2752</td>
<td>-122.0396</td>
<td>7.54</td>
</tr>
</tbody>
</table>

$^1$Heat of formation in kcal/mol.  
$^2$Complexation Energy in kcal/mol.  
$^3$Electric dipole moment in Debye.
Fig. S1.

Structure of Aβ1-42 stacked antiparallel dimer (A/B). (A) Cartoon and sphere representation of A/B (Top view), hydrogen atoms are omitted. (B) Cartoon Structure of A/B (Front view). (C) Sphere surface of A/B (Front view). The net electric dipole moment is shown by a red cylinder arrow. Individual dipoles are indicated by Blue (Bottom chain) and Green (Top chain) cylinder arrows.
Fig. S2.

Structure of Aβ_{42} stacked parallel trimer (A/A/A). (A) Cartoon and sphere structure of A/A/A (Top view), hydrogen atoms are omitted. (B) Cartoon Structure of A/A/A (Front view). (C) Sphere surface of A/A/A (Front view). The net electric dipole moment is shown by a red cylinder arrow. Individual dipole moments are shown by Blue (Bottom chain), ice blue (Center chain) and light blue (Top chain) cylinder arrows.
Fig. S3.

Structure of Aβ_{1-42} parallel protofibril (AA'). (A) Cartoon backbone structure of AA' (top view), hydrogen atoms are omitted. (B) Cartoon Structure of AA' (Front view). (C) Sphere surface of AA' (Front view). The net electric dipole moment is shown by a red cylinder arrow. Individual dipole moments are indicated by Blue (Left chain) and green (Right chain) cylinder arrows.
Fig. S4.

*Structure of Aβ-α protofibril trimer (AB/A).* (A) Sphere and cartoon structure of AB/A (Top view), hydrogen atoms are omitted. (B) Cartoon Structure of AB/A (Front view). (C) Sphere surface of AB/A (Front view). The net electric dipole moment is shown by a red cylinder arrow. Lower layer dipole moment is indicated with a blue cylinder arrow and the high layer is indicated with a green cylinder arrow.
Fig. S5.

Multiple views of Aβ_{1-42} monomer. A) Top view, B) Front view, C) Right view, D) Back view, E) Left view. The net electric dipole moment is shown by a red cylinder arrow and is partially oriented perpendicular to the monomer plane.
Fig. S6.

*Multiple views of* Aβ-α* stacked parallel dimer (A/A).* A) Top view, B) Front view, C) Right view, D) Back view, E) Left view. The net electric dipole moment is shown by a red cylinder arrow and is partially oriented perpendicular to the dimer plane. Individual dipoles are indicated by Blue (Bottom) and Green (Top) cylinder arrows.
Multiple views of Aβ42 stacked antiparallel dimer (A/B). **A**) Top view, **B**) Front view, **C**) Right view, **D**) Back view, **E**) Left view. The net electric dipole moment is shown by a red cylinder arrow and is oriented in the dimer plane. Individual dipoles are indicated by Blue (Bottom) and Green (Top) cylinder arrows.
Fig. S8.
Multiple views of Aβ42 stacked parallel trimer (A/A/A), A) Top view, B) Front view, C) Right view, D) Back view, E) Left view. The net electric dipole moment is shown by a red cylinder arrow. Individual dipole moments are shown by Blue (Bottom chain), ice blue (Center chain) and light blue (Top chain) cylinder arrows.
Fig. S9.

Multiple views of \( \alpha \beta_{42} \) parallel protofibril (AA'). A) Top view, B) Front view, C) Right view, D) Back view, E) Left view. The net electric dipole moment is shown by a red cylinder arrow and is oriented in the fibril plane. Individual dipole moments in Fig. A are indicated by Blue (Left chain) and green (Right chain) cylinder arrows.
Fig. S10.

Multiple views of Aβ42 antiparallel protofibril (AB). A) Top view, B) Front view, C) Right view, D) Back view, E) Left view.
The net electric dipole moment is shown by a red cylinder arrow and is oriented in the fibril axis. Individual dipole moments are indicated by blue (Top chain, Fig A) and green (Bottom chain, Fig A) cylinder arrows.
Fig. S11.

Multiple views of $\alpha\beta_42$ three fragments protofibril (AB/A). A) Top view, B) Front view, C) Right view, D) Back view, E) Left view. The net electric dipole moment is shown by a red cylinder arrow and is oriented towards the fibril axis. Lower layer dipole moment is indicated with a blue cylinder arrow and the high layer is indicated with a green cylinder arrow.
Fig. S12.

Multiple views of $\text{A}\beta_{42}$ four fragments protofibril (AB/AB). A) Top view, B) Front view, C) Right view, D) Back view, E) Left view. The net electric dipole moment is shown by a red cylinder arrow and is partially oriented parallel to the fibril axis. Lower layer dipole moment is indicated with a blue cylinder arrow and the high layer dipole moment is indicated with a green cylinder arrow.
Fig. S13.

Molecular dynamics simulation of the amyloid β1-42 with the amber 14 force field in vacuo. Snapshots of the system were taken at 500 fs, 500 ps, 2.5 ns and 5 ns. The potential energy of each structure is shown for each snapshot.
Fig. S14.

Molecular dynamics simulation of the amyloid β_{1-42} with the amber 14 force field with explicit TIP3P water molecules. Snapshots of the system were taken at 500 fs, 50 ps, 1.57 ns and 3.32 ns.
Fig. S15.

*Molecular dynamics simulation of the amyloid ß1-42 protofibril (AB) with the amber 14 force field with explicit TIP3P water molecules.* Snapshots of the system were taken at 500 fs, 50 ps, 500 ns and 1.57 ns.
Fig. S16.

Molecular dynamics simulation of the amyloid β42 protofibril dimer (AB/AB) with the amber 14 force field with explicit TIP3P water molecules. Snapshots of the system were taken at 500 fs, 50 ps, 100 ps and 345.5 ps.
Fig. S17.

Molecular dynamics simulation of six amyloid β42 protofibrils with the amber 14 force field in vacuo. Snapshots of the system were taken at 500 fs, 10 ps, 100 ps and 271 ps.
Fig. S18.

Molecular dynamics simulation of six amyloid β-42 protofibrils with the amber 14 force field with explicit TIP3P water molecules. Snapshots of the system were taken at 500 fs, 65.5 ps, 144 ps and 219 ps.
Fig. S19.

Dipole moment of the β1-42 monomer (A) and protofibrils (AB) at different levels of theory. PM6, HF/STO-3G and HF/6-31-G(d) dipole moments are indicated by purple, red and black cylinder arrows respectively.