Filiz CAMCI

M.S. Thesis In Physics

June - 2010

STRUCTURAL AND ENERGETIC INVESTIGATION OF EFFECTS OF ANTI-CANCER MOLECULE TOPOTECAN ON THE MECHANISM OF HUMAN TOPOISOMERASE I BY MEANS OF MOLECULAR DYNAMICS SIMULATIONS

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APPROVAL PAGE

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

Prof. Dr. Mustafa KUMRU
Head of Department

This is to certify that I have read this thesis and that in my opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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June 2010

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ABSTRACT

Topoisomerases (Topos) are enzymes that regulate topology of DNA, by adjusting the so called topological invariant 'linking number'. DNA Topology is independent of geometrical deformations, and therefore one or two strands need to be nicked to change the topological states. Topos are the enzymes that accomplish these crucial topological functions. In this study, we have applied constant forces on different regions of the lips of the enzyme in the ternary complex (Human Topoisomerase-DNA-Topotecan). By applying forces in the lips region of the protein, we have searched for possible mouth openings of the enzyme. The theoretical approach here is a kind of theoretical AFM (Atomic Force Microscopy) experiment where forces are applied on atomic resolution. Molecular dynamic simulations are done on DNA-protein complex with and without the anti-cancer drug molecule, and structural and energetic differences have been analyzed, as well as the effect of existence of TPT on the possible mouth openings. The most important result of this study is the finding that the mouth of the enzyme does not open up in the presence of anti-cancer drug molecule TPT, while it opens up in the wild type mechanism.

When we analyzed the TPT-protein interactions, we observed that the direct hydrogen bond between Asp533 and E-ring hydroxyl of TPT is the most crucial interaction, and when this interaction breaks, the lips of the enzyme starts to open up.

Keywords: Human Topoisomerase I, Topotecan, Molecular Dynamics, AFM

ANTİ-KANSER MOLEKÜL TOPOTEKANIN HUMAN TOPOİZOMERAZ I İN MEKANİZMASINA OLAN ETKİSİNİN YAPISAL VE ENERJETIK YÖNDEN MOLEKÜLER DİNAMİK SİMÜLASYONLARI İLE İNCELENMESİ

Filiz CAMCI

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ÖZ

Topoizomerler, topolojik bir değişmez olan `içiçe geçme sayısı`nı.ayarlayarak DNA topolojisini düzenleyen enzimlerdir.DNA topolojisi geometrik deformasyondan bağımsızdır, bu yüzden topolojik durumunu değistirmek için bir ya da iki iplikçiğinin kesilmesi gerekir.Topoizomerler bu kritik işi başaran enzimlerdir. Bu çalışmada, biz DNA –topoizomer-topotekan `dan oluşan üçlü yapıdaki topoizomer enziminin dudak bölgesinin farklı kısımlarına sabit kuvvetler uyguladık. Bu kuvvetleri uygulayarak enzimin ağız kısmında olabilecek açılmaları araştırdık. Teorik AFM (Atomic Force Microscopy) de diyebilecegimiz, Moleküler Dinamik simulasyonlarımız DNA-protein kompleksine ilaç molekülü varken ve yokken uygulandı ve ilaç molekülünün proteinin ağız kısmında açılmaya etkisi olup-olmadığı analiz edildi. Bu çalışmadan elde ettiğimiz en önemli sonuç, TPT li sistemlerde proteinin ağız kısmının açılmaması, fakat TPT nin olmadığı sistemlerde ise proteinin ağız kısmının açılmasıdır. Bunun sebebini araştırdığımızda, TPT ile Asp533 amino asit arasındaki etkileşinin çok önemli olduğunu gözlemledik. Bu direkt hidrojen bağ, proteinin açılmasını engellemektedir. Bu bağın kopması durumunda, proteinin açıldığını gözlemledik.

Anahtar Kelimeler: Human Topoizomeraz I, Topotekan, Molekuler Dinamik.

To my husband, daughter, and son

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I express sincere appreciation to my advisor, Assist. Prof. Levent SARI for his support and guidance throughout this research.

I express my thanks and appreciation to my husband for his understanding, motivation and patience.

Also I want to thank to my kids for their patience.

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LIST OF SYSMBOLS AND ABBREVIATIONS

SYMBOL/ABBREVIATION

DNA	DeoxyriboNucleic Acid
ccDNA	Closed Circular DNA
Lk	Linking number
Tw	Twist
Wr	Writhe
Tyr	Tyrosine
Lys	Lysine
Arg	Arginine
His	Histidine
Asp	Aspartic acid
ε_{ij}	Depth of potential well in Lennard-Jones potential
ε_0	Dielectric constant
k_b	Force constant for bond energy
k_θ	Force constant for bond angle potential energy
k_ϕ	Force constant for dihedral potential energy
k_ω	Force constant for improper potential energy
k_u	Force constant for Urey-Bradley potential energy
CPT	Camptothecin

TPT	Topotecan
MD	Molecular Dynamics
MC	Monte Carlo
VMD	Molecule Visualization Program
DFT	Density Functional Theory
$n(r)$	Electron density distribution in DFT
CHARMM	Chemistry at HARvard Macromolecular Mechanics
PSF	Protein Structure File
RTF	Residue Topology File
LSF	Load Sharing Facility
CMU	Cluster Management Utility
RMSD	Root Mean Square Deviation

CHAPTER 1

INTRODUCTION

1.1 A MIRACLE MOLECULE: DNA

A DNA (deoxyribonucleic acid) is the most important molecule of life. It contains the genetic information of the cell and transfers this information to daughter cells by replicating itself before cell division. It stores information for a long time and this is the main role of DNA molecule. All cells except some viruses have this molecule.

DNA has double helical structure that was introduced by Watson and Crick in 1953 [1]. It has two strands which are running in opposite directions. The repeating units are called nucleotide (Adenine, Thymine, Guanine and Cytosine) and they form complementary base pairs. If there is A on one strand then there is T on the other side and same for G and C. Complementary base pairing is the most important property of DNA molecule. Because of this complemantarity, each preexisting strand acts as a template for the new strand. Hence genetic information can be passed to the daughter cells without changing. These nucleotides bind to each other with hydrogen bonds inside and the sugar-phosphate backbone outside. Double helical structure repeats on every about 10 base pairs. This structure is called B-DNA and also there are A-DNA, Z-DNA and closed circular DNA (ccDNA) [2].

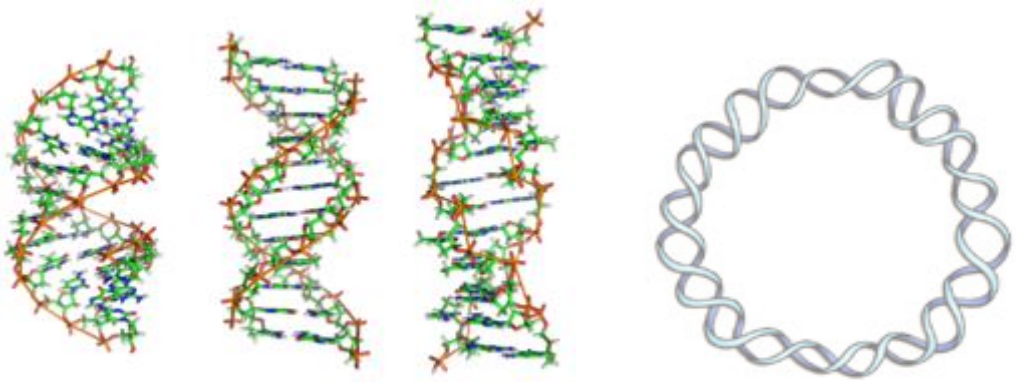

Figure 1.1 Structures of A-DNA, B-DNA, Z-DNA [3] and ccDNA [4]

1.1.1 Supercoiling of DNA

Since length of DNA molecule is very larger than length of a cell, the term supercoiling has crucial importance for DNA molecule to be packed into cell (nucleus for eukaryotes). Coiling of a coil is called supercoiling. We can twist DNA molecule like a rope on the direction or on the opposite direction of the helix, these are called positive and negative supercoiling respectively. We can describe supercoiling by a topological invariant called “Linking number” (Lk). Lk has two components: twist and writhe. First one is the number of times that two strands twist about each other and second one is the number that DNA helix coils about itself.

$$Lk = Wr + Tw \text{ [5]} \tag{1.1}$$

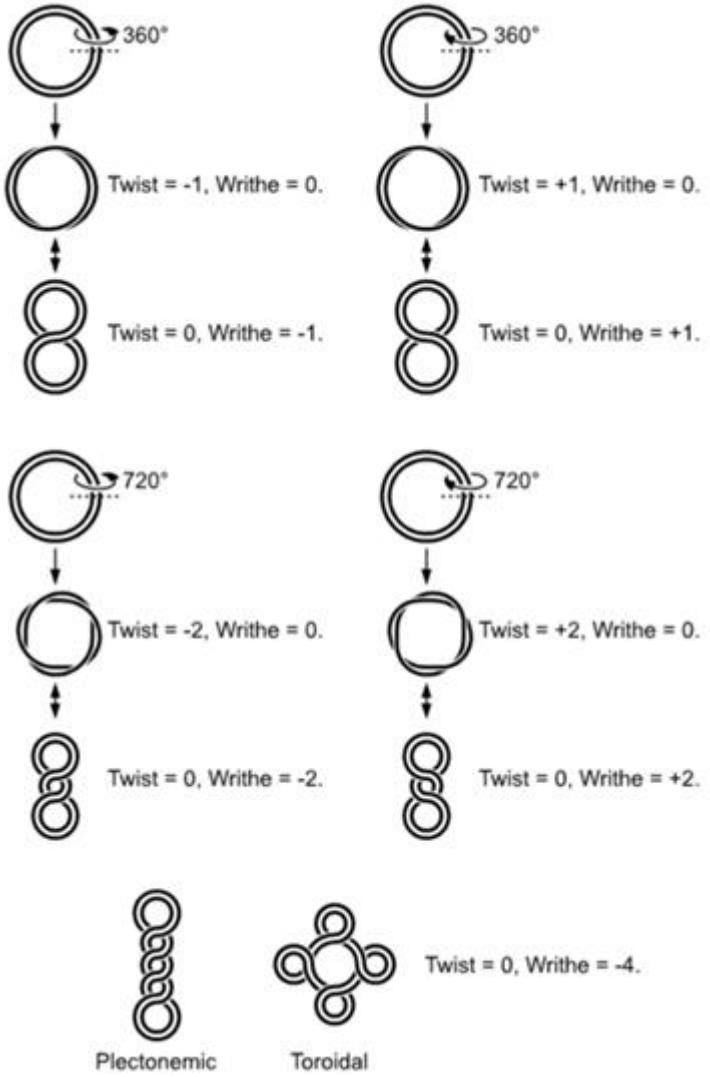

Figure 1.2 Twist and writhe values for supercoiled circular DNA [6]

1.2 TOPOISOMERASES

Topoisomerases are essential enzymes to solve topological problems due to transcription, replication, recombination etc. by forming a phosphodiester bond between tyrosine residue and one end of the cleaved strand(s) of DNA temporarily. They can cut one or both strands of DNA temporarily and allow to pass one or both strands through this break Hence supercoiled DNA relaxes and extends [7].

There are two types of topoisomerases; type I and type II. They have different mechanisms and cellular functions [8]. Type I enzymes are monomeric and remove torsional stress by breaking one strand of DNA molecule temporarily. They are divided into two subfamilies according to their sequence and reaction mechanisms. Type IA subfamily enzymes cleave the DNA`s strand by attaching 5' end, type IB enzymes do that by attaching 3' end. Human topoisomerase I is an example of the type IB subfamily.

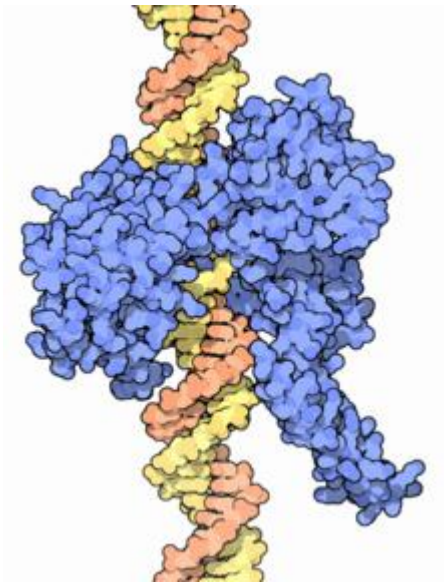

Figure 1.4 Structure of type 1 topoisomerases [9]

Figure 1.5 Structure of type II topoisomerases [10]

Type II topoisomerases are dimeric and they break both strands of DNA molecule simultaneously. ATP energy is required to pass DNA strand through the break. They have been divided into two subfamilies. Type IIA topoisomerases are essential to separate daughter strands at the end of replication.

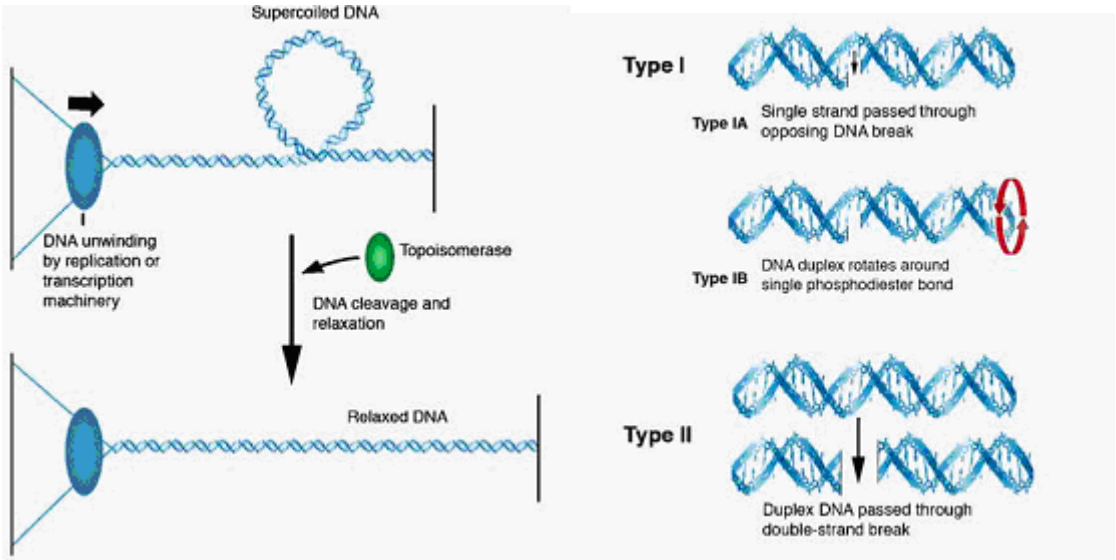

Figure 1.6 Working mechanisms of topoisomerases [11]

There are six topoisomerases in humans. Two of each IA, IB and IIA. Each of them has nuclear and mitochondrial forms and they resolve topological problems of DNA due to transcription, recombination, chromosome segregation and remodeling [12].

Table 1 Human DNA topoisomerases [11]

Enzyme	Type	Cellular roles
Topoisomerase I	IB	Replication, transcription, chromatin remodeling
mtTopoisomerase I	IB	Mitochondrial replication, transcription
Topoisomerase II α, β	IIA	Replication, chromosome segregation
Topoisomerase III α, β	IA	Recombination, repair

1.3 HUMAN TOPOISOMERASE I

Human topoisomerase I also called DNA topoisomerase I is one of the best studied human topoisomerase [11]. Because it is target of anti-cancer drugs.

Human topoisomrase I is a type IB topoisomerase that breaks one strand of double-stranded superhelical DNA molecule transiently. Type I topoisomerases are monomeric and break one strand of DNA to make transient enzyme –DNA intermediate. This enzyme can relax both positive and negative supercoils. It is essential for resolving torsional stress due to DNA replication, transcription and chromatin condensation [11].

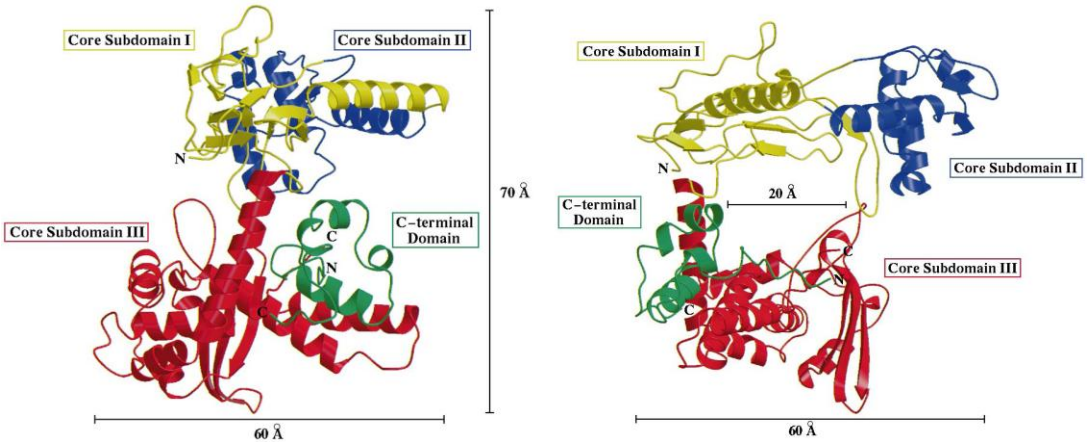

Figure 1.7 Human topoisomerase I a) Side view b) Front view [8]

1.3.1 Structure of the enzyme

Human topoisomerase I is a monomeric protein. It consists of 765 amino acids. It composed of four domains: NH2 terminal region which is highly charged, the conserved core domain, linker domain (positively charged) and the highly conserved COOH terminal domain [13]. ~215 amino acids are in NH2 terminal region. Amino acids between 215 and 635 are in core domain, 636-712 are in linker domain and ~ 713-765 amino acids are in COOH terminal domain, last part of the enzyme contains essential catalytic Tyr 723 amino acid, which forms a phosphotyrosine bond with the enzyme and DNA molecule [11].

The core domain is divided into three subdomains; I, II and III. Subdomains I and II form the upper lobe (cap) of the enzyme [8]. The lower lobe consists of subdomain III and the C terminal. There is a long helix within the subdomain III which attaches the lower lobe to the upper. At the top of the helix there is a hinge which allows the clamp to open or close around DNA. Across from the hinge there are two loops called “lips” which come together to bring the cap close to the base of the protein [11].

At the active site of the enzyme there are five conserved catalytic residues which are Arg-488, Lys-532, Arg-590, His-632, Tyr-723 [11].

Consequently, human topoisomerase I is a multidomain enzyme. Two of the domains are highly conserved and very important for catalytic activity. The others are not required for its activity.

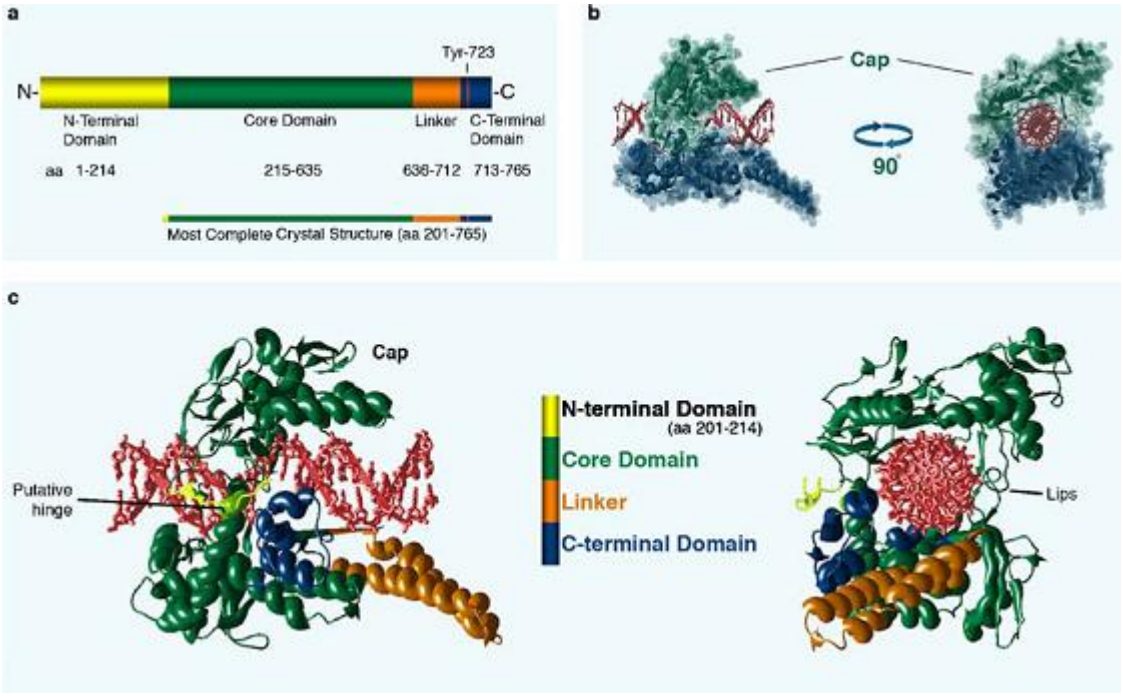

Figure 1.8 Structure of the Human topoisomerase I [11]

1.3.2 Working Mechanism of the Enzyme

There are five steps in the catalytic cycle of the enzyme; these steps are DNA association, cleavage, strand rotation, religation and dissociation. Substantial domain movements are required for the enzyme in order to bind the DNA molecule [13] then enzyme opens up its lips widely to bind the DNA duplex then closes them around the DNA strand. After that, cleavage step comes and one of the DNA strands cleaves through nucleophilic attack by the active site tyrosine(Tyr723), then covalent attachment of the enzyme to the DNA molecule occurs at the 3` phosphate on the nicked site [14].

Now strand rotation step occurs; the nicked strand rotates around the other one. It is thought that the torque which required for DNA rotation comes from supercoiling energy. DNA supercoiling direction defines the direction of rotation. The nick is

sutured back in religation step and the enzyme opens up its lips to release the DNA molecule [15]. Enzyme and DNA molecule then said to be dissociated.

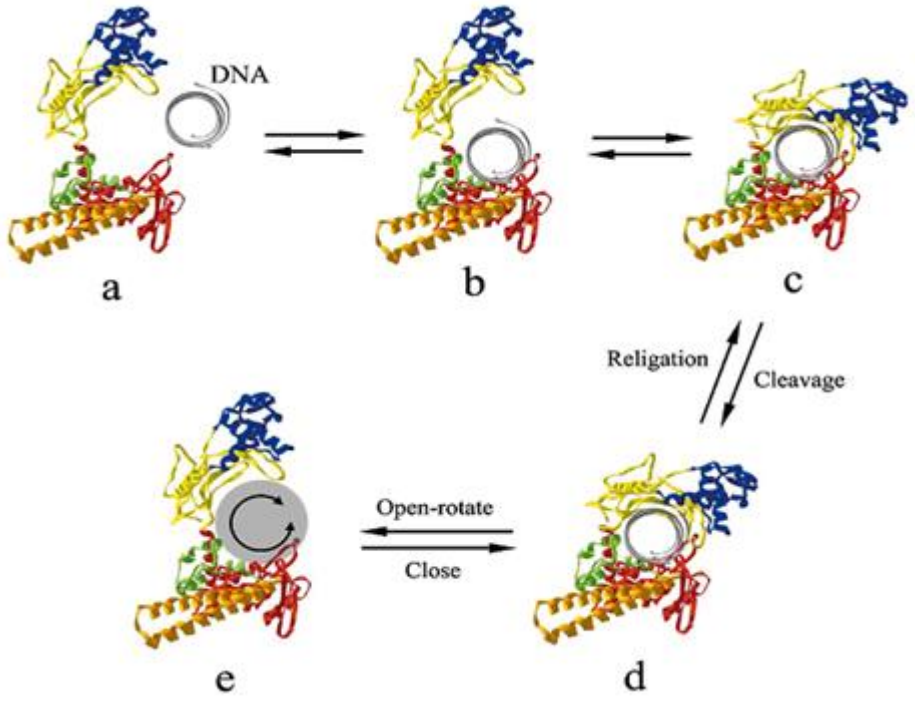

Figure 1.9 Working mechanism of human topoisomerase I [16]

1.4 ANTI-CANCER DRUGS

Although human topoisomerase I is very important for continuity and propagation of cell, it could also be harmful for the cell. It's structure is very important for cancer research, because cancer treatment drugs, which are camptothecin (CPT) and it's derivatives bind to covalent enzyme-DNA complex and prevent the enzyme's activity by stabilizing the complex [17]. Drug molecule joins enzyme-DNA complex after cleavage and binds both to enzyme and DNA. It behaves as a base pair of DNA and locates to the cleaved site. This causes DNA damage and therefore cell death.

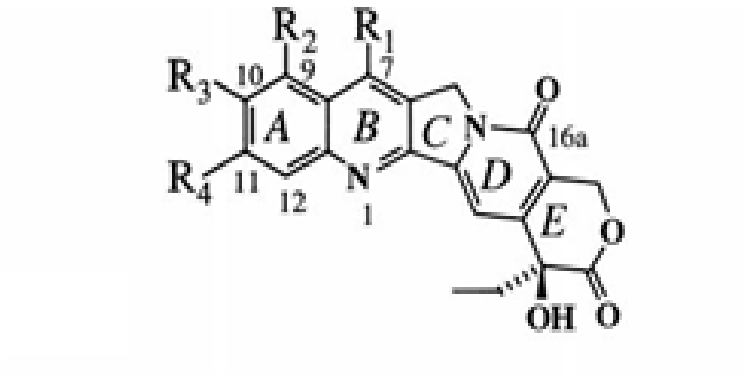

Figure 1.10 Structure of CPT molecule [18]

CPT is a natural product. It was discovered in 1966 [18].It was isolated from a tree native in China.

Structure of the drug molecule is important to bind the complex and to stabilize it. It is planar and has five rings. Its planar structure is very important to be used as topoisomerase I inhibitor. The most important part is E ring. This part interacts with the enzyme in three different positions (one hydrogen bond with Asp533 and two hydrogen bonds with Arg364). D ring interacts with +1 cytosine on intact strand, hence stabilizing the complex.

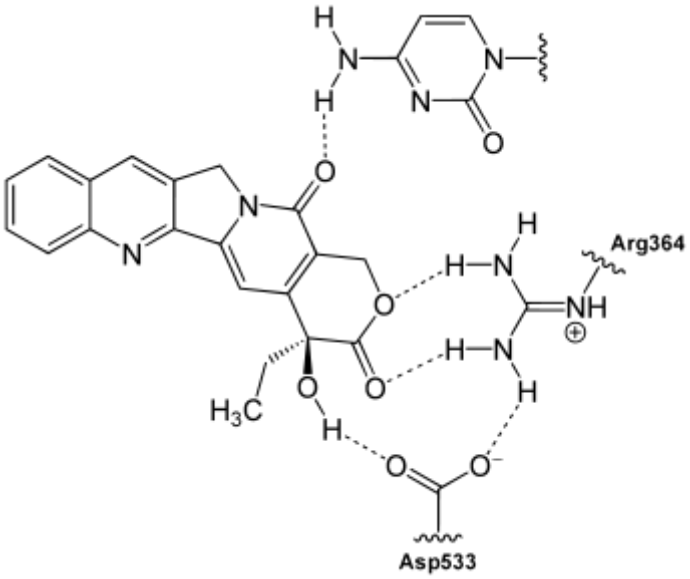

Figure 1.11 Binding of CPT to the topo1-DNA complex

Topotecan (TPT) is a derivative of camptothecin; it is being used in chemotherapy. In our study, we used TPT molecule to investigate its working mechanism as human topoiomerase I inhibitor. We used this molecule in ternary complex (TPT-DNA-

enzyme).

CHAPTER 2

THEORETICAL METHODS

2.1 MOLECULAR DYNAMICS ON THE FORCE FIELD POTENTIAL ENERGY

It is sometimes very difficult to perform some experiments in microscopic level such as studies about very small biomolecules and assemblies of several atoms. Furthermore, studying about these tiny biomolecules requires more than experimental setup. To search what is going on inside these, how they are behaving, which kind of reactions are taking place, what are the atoms doing and questions like these caused computer simulations to be conceived.

Simulations are like bridges between experiment and theory [18]. We can get information about the structure and mechanism at microscopic level and compare this information with the experimental data we got. Consequently, some hidden details could be revealed with this comparison and computer simulations provide us to study on very tiny molecules even a few atom(s). Some simulation methods are Molecular Dynamics (MD), Monte Carlo (MC) [19].

Molecular Dynamics method is introduced in 1950 by Alder and Wainwright [20]. To imitate the simulation to real atomic interactions Rahman applied a smooth, continuous potential [21]. MD simulations were developed for more complex molecules in 1970s and first protein simulation took place in 1976[22], [23]. Now this method is being applied in a wide range of science such as biophysics, biochemistry, enzymology, molecular biology [19].

Molecular dynamics (MD) is one of the most popular computer simulation methods that is used to visualize atoms movements in molecules and to make structural and dynamic analysis of atom assemblies. In MD, atoms and molecules are allowed to interact each other for a period of time by approximations of physics. In order to

observe fluctuations with respect to time MD solves Newton's equations of motion numerically, which is for a simple atomic system [19]:

$$m_i \bullet \ddot{r}_i = f_{i,} \qquad f_i = -\frac{\partial}{\partial r_i} U_{total} \tag{2.1}$$

Where m_i is mass and $\ddot{r}$ is acceleration of ith particle, r_i is the atomic coordinate, is the force acting on ith particle that is first derivative of the potential energy.

The most important part is choosing the appropriate energy function for simulation to be successful. Because potential describes the way by which the particles in the simulation will interact each other [19].

There are usually a large number of parameterized terms, which obtained from experimental and/or quantum mechanical studies, in energy functions. These parameterized terms form a set of function called force field [19].

Total potential energy is sum of non-bonded and bonded energies.

$$U_{total} = U_{non-bonded} + U_{bonded} \tag{2.2}$$

Lennard-Jones and Coulomb potentials are used for non-bonded interactions and we can write this like:

$$U_{non-bonded} = \varepsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] + \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}} \tag{2.3}$$

Where ε_{ij} is depth of potential well, σ_{ij} is distance where Lennard-Jones potential is zero, r_{ij} is distance between atoms i and j, ε_0 is the dielectric constant, q_i and q_j are charges of the particles.

For bonded interactions there are several potential functions for covalent bonds, bond angles, dihedral angles, simple two-body, three-body and four-body terms. We can write this as:

$$U_{bonded} = U_{bonds} + U_{angle} + U_{dihedral} + U_{improper} + U_{Urey-Bradley} \quad 2.4$$

We can summarize the equations above like this

$$\begin{aligned} U_{total} = & \sum_{bonds} k_b \left(b - b_0\right)^2 + \sum_{angles} k_\theta \left(\theta - \theta_0\right)^2 \\ & + \sum_{dihedral} k_\phi \left[1 + \cos\left(n\phi - \delta\right)\right] \\ & + \sum_{improper} k_\omega \left(\omega - \omega_0\right)^2 + \sum_{Urey-Bradley} k_u \left(u - u_0\right)^2 \\ & + \varepsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] + \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}} \end{aligned} \quad 2.5$$

If two atoms are one-bonded to each other then the term U_{bonds} is used to calculate the potential between them, when three atoms bonded together with an angle, then the term U_{angles} is used.

When four atoms bonded, then $U_{dihedral}$ refers to the motion of rotation of the atoms around the middle bond. It is assumed to be periodic and expressed in cosine function. $U_{improper}$ refers to the motion that is out of plane and used to maintain planarity. Urey-Bradley potential is an interaction between two atoms separated by two bonds

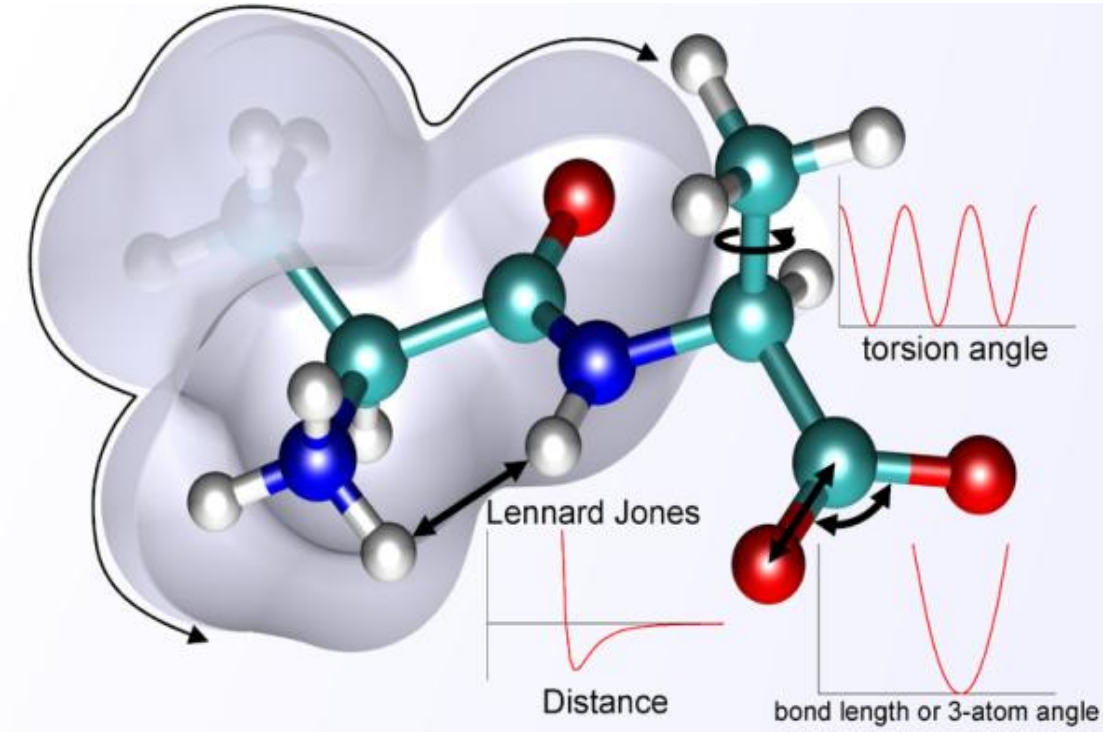

Figure 2.1 Bonded and Non-bonded Potential Energies [24]

Molecular Dynamic simulation method solves Newton's second law which can be written like:

$$F_i = m_i a_i \tag{2.6}$$

Where F_i is the force acting on an atom, m is mass of the same atom and a is acceleration of it. Force F is the gradient of the potential energy with respect to internal coordinates that can be written as:

$$F_i = -\frac{dU(r)}{dr_i} \tag{2.7}$$

If we combine these two equations

$$-\frac{dU(r)}{dr_i} = m_i \frac{d^2 r}{dt^2} \tag{2.8}$$

Potential energy is a function of positions of the atoms in the system. Hence this equation becomes very complicated and can not be solved analytically, it must be solved numerically [19]. To solve equations of motion, there are many numerical algorithms. Some of them are Verlet Algorithm, Velocity-Verlet Algorithm, and Leap-Frog Algorithm. In many algorithms integration is partitioned into small steps, each of these steps separated in specific time period Δt .

All integration algorithms calculate velocities, positions and accelerations of each particle approximately by using Taylor expansion. We can write Taylor expansion like:

$$f(x) = f(x_0) + \frac{f'(x_0)}{1}(x - x_0) + \frac{f''(x_0)}{1.2.}(x - x_0)^2 + \frac{f'''(x_0)}{1.2.3}(x - x_0)^3 + \ldots \qquad 2.9$$

More compactly

$$f(x) = \sum_{n=0}^{\infty} \frac{f^{(n)}(x_0)}{n!}(x - x_0)^n \qquad 2.10$$

In numerical integrations position, velocity and acceleration derived from equations:

$$r(t + \Delta t) = r(t) + r'(t)\Delta t + \frac{1}{2} r''(t)\Delta t^2 + \ldots \qquad 2.11$$

$$\mathrm{v}(t + \Delta t) = v(t) + v'(t)\Delta t + \frac{1}{2} v''(t)\Delta t^2 + \ldots \qquad 2.12$$

$$\mathrm{a}(t + \Delta t) = a(t) + a'(t)\Delta t + \ldots \qquad 2.13$$

Verlet algorithm is one of the most commonly used time integration method. It calculates the positions at time by using the position and acceleration at time and the positions from [27].We can derive Verlet algorithm by using equations above;

$$r(t + \Delta t) = r(t) + v(t)\Delta t + \frac{1}{2} a(t)\Delta t^2 \qquad 2.14$$

$$\mathrm{r}(t-\Delta t)=r(t)-v(t)\Delta t+\frac{1}{2}a(t)\Delta t^2 \qquad 2.15$$

If we add these two equations

$$r(t+\Delta t)=2r(t)-r(t-\Delta t)+a(t)\Delta t^2 \qquad 2.16$$

The other algorithm called Leap-Frog is also popular [19]. In this method, firstly velocities are calculated at time $t+\frac{\Delta t}{2}$ and using these velocities, positions at time $t+\Delta t$ calculated, as we can write this;

$$v\left(t+\frac{1}{2}\Delta t\right)=v\left(t-\frac{1}{2}\Delta t\right)+a(t)\Delta t \qquad 2.17$$

$$\mathrm{r}(t+\Delta t)=r(t)+v\left(t+\frac{1}{2}\Delta t\right)\Delta t \qquad 2.18$$

These and other commonly used integration methods are time reversible. That means direction of simulation is arbitrary. If velocities of all atoms are changed into opposite sign, then simulation runs the reverse direction [19].

MD simulation purposes to derive kinetic and thermodynamic data about the model system. Experiments are often carried out at constant temperature and volume or constant pressure and temperature. To keep the pressure constant during simulation, the volume needs to fluctuate. There are many methods to run MD simulations at a constant pressure [19]. Also there are many methods to keep the temperature constant without changing the energy such as Berendsen method[25] and Anderson method [26].

2.1 FORCE FIELD PARAMETRIZATION

Paratool is a plug-in for the molecule visualization program (VMD) and provides graphical interface for force field parametrization of molecules that are not in the force

field. It helps us to generate the molecule or a part of the molecule that should be parameterized and to produce the Quantum chemical calculations. Paratool computes force fields for bonds, angles, improper and dihedral [27].

(DFT)Density functional theory is a quantum-mechanical theory of electronic ground state structure. It is a reformulation of many body quantum mechanics in terms of probability density. Functionals (function of another function, in this method function of electron density) are used in this method and that's why it is called Density Functional Theory. It is successful to describe ground state properties of metals, insulators, semiconductors as well as proteins and carbon nano tubes.

This theory uses electron density distribution $n(r)$ instead of wave function [28]. The basic variable in DFT is electron density- a measure of the probability of an electron occupying very small place in space surrounding any given point-can be written as:

$$n(\vec{r}) = N\int d^3r_2 \int d^3r_3 ... \int d^3r_N \psi^*(\vec{r},\vec{r_2},...,\vec{r_N})\psi(\vec{r},\vec{r_2},...,\vec{r_N}) \qquad 2.19$$

Where N is the number of electrons ψ is the ground state wave function.

Also energy can be written like:

$$E[n(r)] = \langle \psi(n(r)) \ |T+U+V| \ \psi(n(r)) \rangle =$$

$$\langle \psi(n(r)) \ |T+U| \ \psi(n(r)) \rangle + \langle \psi(n(r)) \ |V| \ \psi(n(r)) \rangle \qquad 2.20$$

T is kinetic energy, U is electron-electron interaction energy, V is the external potential energy.

$$E[n(r)] = T[n(r)] + U[n(r)] + \int V(r)n(r)dr \qquad 2.21$$

If we assume

$$F\left[n(r)\right]=T\left[n(r)\right]+U\left[n(r)\right] \tag{2.22}$$

Then energy is

$$E\left[n(r)\right]=F\left[n(r)\right]+\int V(r)n(r)dr \tag{2.23}$$

Energy can be obtained from here as a functional of electron density.

We obtained the harmonic vibration frequencies by diagonalizing the Hessian matrix which is given by:

$$H(U)=\begin{vmatrix} \frac{\partial^2 U}{\partial x_1^2} & \frac{\partial^2 U}{\partial x_1 \partial x_2} & \cdots & \frac{\partial^2 U}{\partial x_1 \partial x_N} \\ \frac{\partial^2 U}{\partial x_2 \partial x_1} & \frac{\partial^2 U}{\partial x_2^2} & \cdots & \frac{\partial^2 U}{\partial x_2 \partial x_N} \\ . & . & .... & . \\ . & . & .... & . \\ . & . & .... & . \\ \frac{\partial^2 U}{\partial x_N \partial x_1} & \frac{\partial^2 U}{\partial x_N \partial x_2} & \cdots & \frac{\partial^2 U}{\partial x_N^2} \end{vmatrix} \tag{2.24}$$

2.2 COMPUTATIONAL TOOLS

In our study, we used CHARMM (Chemistry at HARvard Macromolecular Mechanics) program. It is a widely used molecular dynamics and analysis package. The

main purpose of this package is to evaluate and to manipulate the potential energy of a macromolecular system.

For graphical plotting we used gnu plot program.

Data structure files are required for CHARMM to initiate the molecular dynamics simulations.

These files are parameter file, protein structure file (PSF), residue topology file (RTF), and coordinate file

Parameter file contains the information necessary for calculating energies etc. combining with the information from PSF file. RTF contains properties such as name of the atom types, mass of the atom, hydrogen bonds etc. PSF is generated internally by CHARMM and is necessary to generate Hydrogen bonds and non-bonded list [29]. The Cartesian coordinates of all atoms in the system are in coordinate file.

We have used a computer cluster called “ulubatli” with seventy three cores and 116 GB RAM in this research. CPUs are Xeon 5365 and Xeon 5462 types that are connected with 10 Gbps infinitiband network. Load Sharing Facility (LSF) and Cluster Management Utility (CMU) are used for job and cluster management, respectively. The system has 4.2 TB disk storage. In addition, nine workstations with a total of 50 GB RAM memory and 7.5 TB disk storage

2.3 SIMULATION SET UP

In this study, the crystal structure with the pdb code 1K4T.pdb was used to get the t=0 coordinates of the atoms. Missing atoms and residues were built, and hydrogen atoms are included. The system is immersed into a 60 A radius of TIP3 water molecules, and stochastic boundary forces are applied. The system was neutralized by 22 sodium atoms, as the coordinates of the sodium atoms are chosen to be the highest potential energy points. The systems were first minimized by 1000 SD (Steepest Descent) and 1500 ABNR (Adapted Basis Newton Raphson) methods. Then, it was heated by 20 K increments to 300 K, and equilibrated for about 1 ns at this temperature. Subsequent production level calculations are carried out at constant temperature, number of particle, and volume, that is, under canonical conditions. The external forces (on the order of magnitude pN) are applied to bring large scale conformational changes in the protein.

2.4 POST SIMULATION ANALYSIS

Root mean square deviation (RMSD) and interaction energies were used to make analysis after simulation. Interaction energies referred to Van Der Waals and Coulomb energies. RMSD is used to make comparison between two structures. We used it to measure the differences between the initial and final positions of atom(s) and we can write it as:

$$d_{if} = \sqrt{\frac{1}{N}\sum_{k}\left(r_k^i - r_k^f\right)^2} \qquad 2.25$$

Where r^i is initial position, r^f is final position of an atom, N is number of atoms and k is the index of the atoms in the molecule.

CHAPTER 3

ANALYSES OF INTERACTION ENERGIES

The non-bonded interaction energies, which are cloumb and Van Der Walls, are analyzed between TPT and protein. First of all, to see which residues are interacting with TPT, we have calculated interaction energies between TPT and all protein residues. In figure 3.1, we present these interaction energies versus residue numbers.

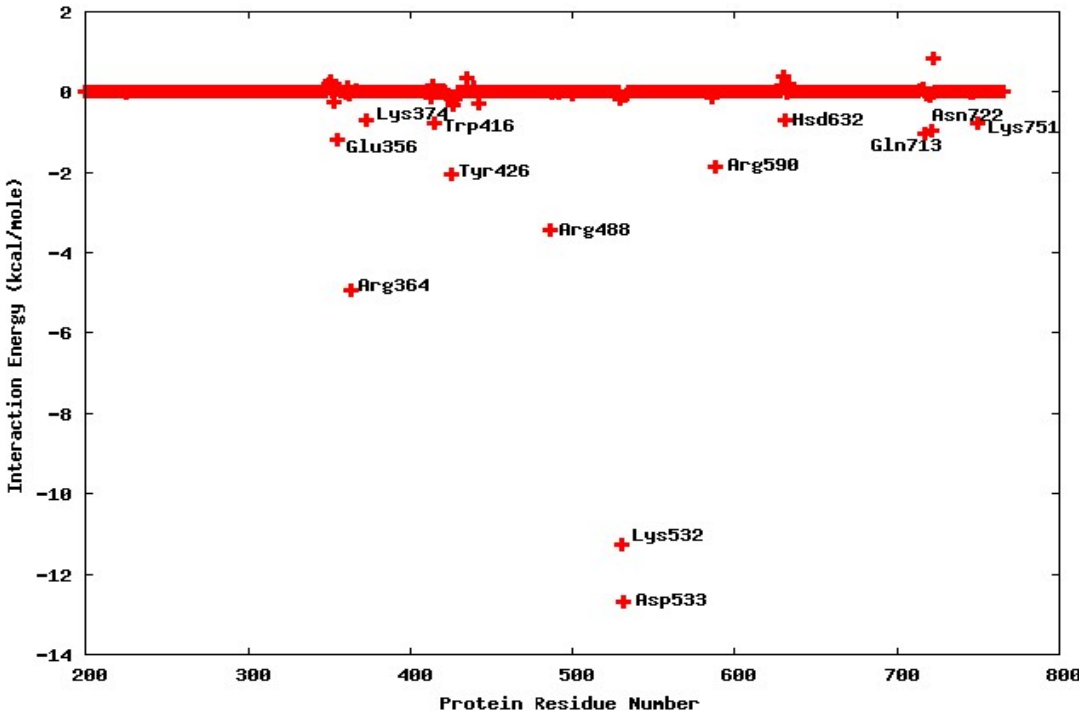

Figure 3.1 Interaction Energy Values between TPT Molecule and Residues of Enzyme

The non-bonded interaction energies between TPT and protein residues for the system where we apply 100 pN force to proximal separation is given in figure 3.2.

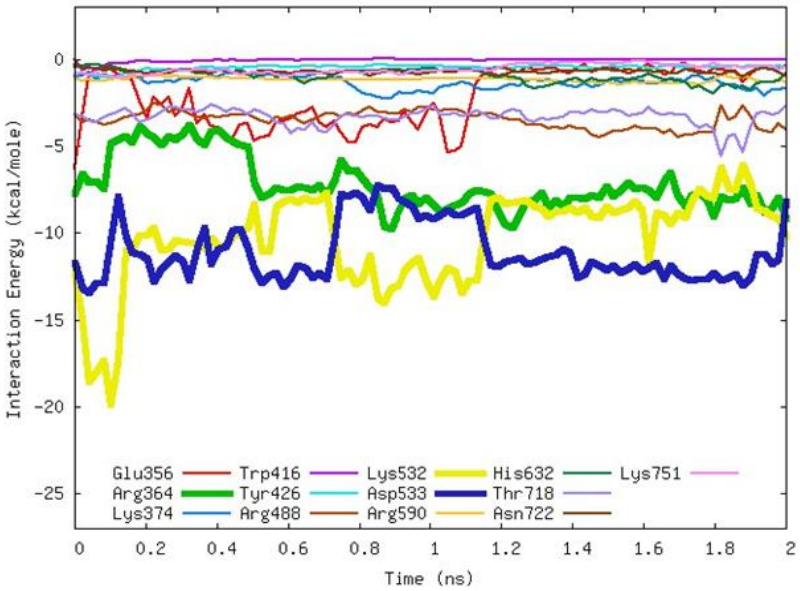

Figure 3.2 Interaction energy values between drug molecule and residues of enzyme in proximal with 100 pN force

The non-bonded interaction energies between TPT and protein residues for the system where we apply 200 pN force to proximal separation is given in figure 3.3.

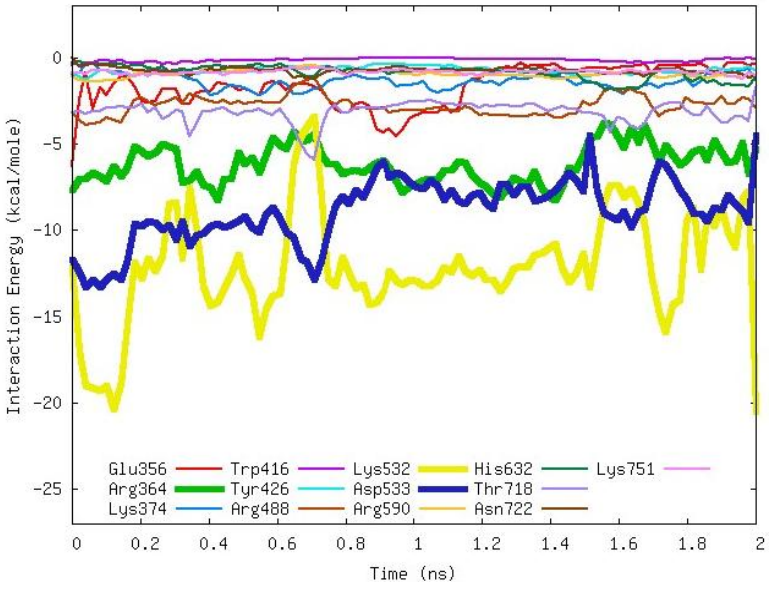

Figure 3.3 Interaction energy values between drug molecule and residues of enzyme in proximal region with 200 pN force

The non-bonded interaction energies between TPT and protein residues for the system where we apply 300 pN force to proximal separation is given in figure 3.4

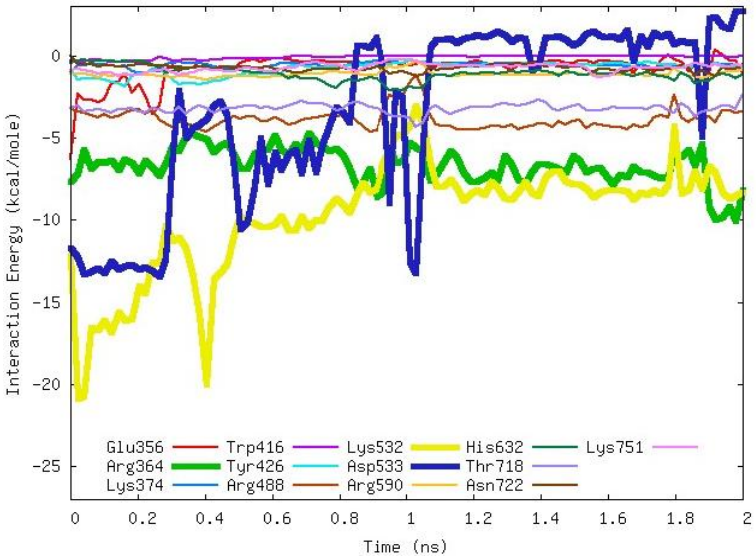

Figure 3.4 Interaction energy values between drug molecule and residues of enzyme in proximal region with 300 pN ($3x10^{-10}$ N) force

The non-bonded interaction energies between TPT and protein residues for the system where we apply 400 pN force to proximal separation is given in figure 3.5

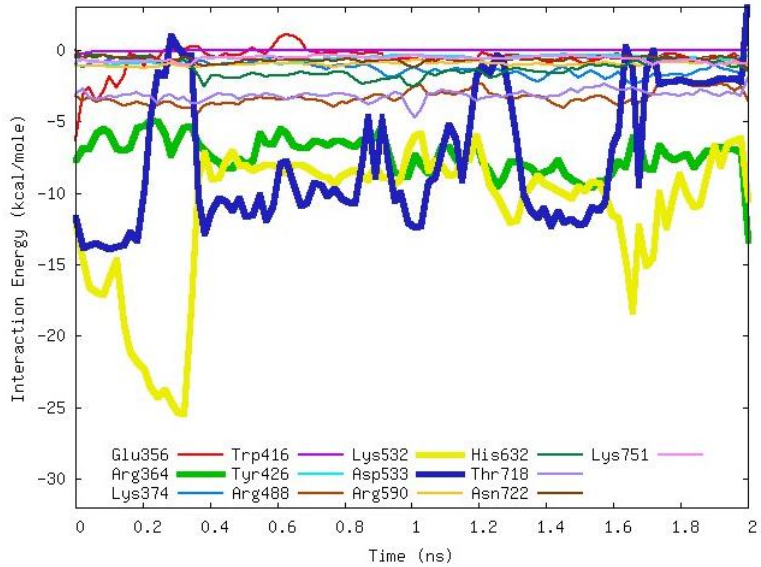

Figure 3.5 Interaction energy values between drug molecule and residues of enzyme in proximal region with 400 pN ($4x10^{-10}$ N) force.

The non-bonded interaction energies between TPT and protein residues for the system where we apply 100 pN force to distal separation is given in figure 3.6

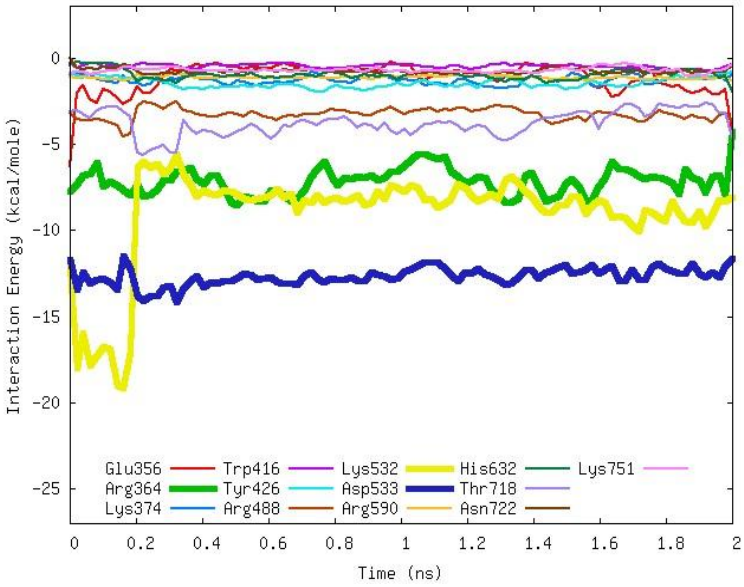

Figure 3.6 Interaction energy values between drug molecule and residues of enzyme in distal region with 100 pN ($1x10^{-10}$ N) force

The non-bonded interaction energies between TPT and protein residues for the system where we apply 200 pN force to distal separation is given in figure 3.7

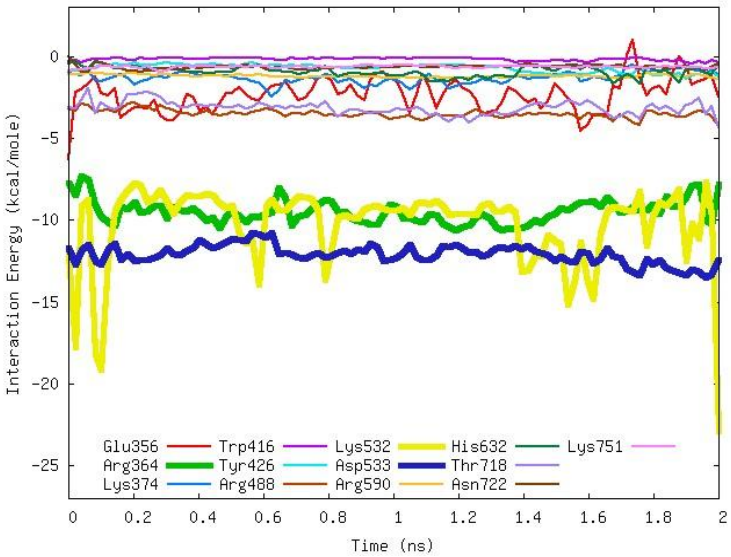

Figure 3.7 Interaction energy values between drug molecule and residues of enzyme in distal region with 200 pN ($2x10^{-10}$ N) force

The non-bonded interaction energies between TPT and protein residues for the system where we apply 300 pN force to proximal separation is given in figure 3.8

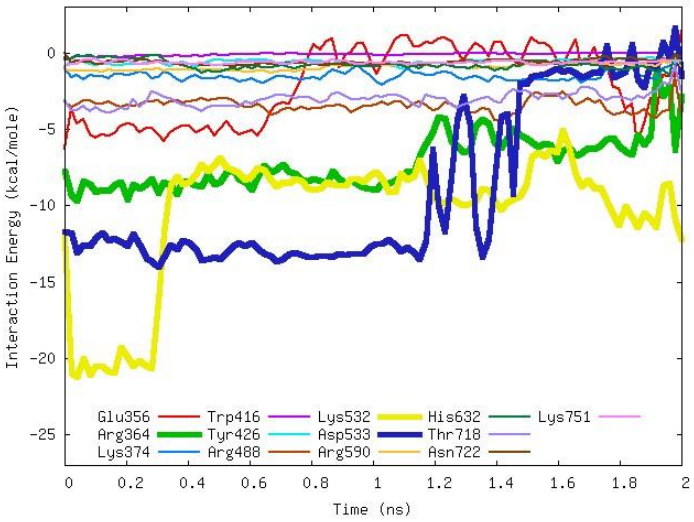

Figure 3.8 Interaction energy values between drug molecule and residues of enzyme in distal region with 300 pN ($3x10^{-10}$ N) force

The non-bonded interaction energies between TPT and protein residues for the system where we apply 400 pN force to proximal separation is given in figure 3.9

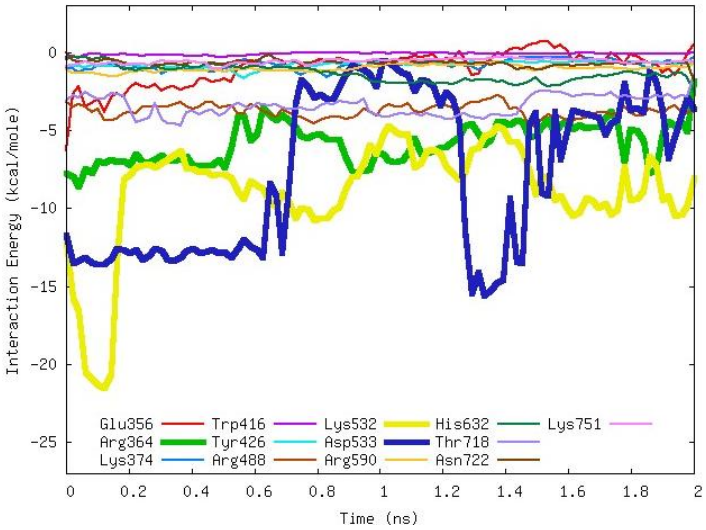

Figure 3.9 Interaction energy values between drug molecule and residues of enzyme in distal region with 400 pN ($4x10^{-10}$ N) force

CHAPTER 4

RESULTS AND DISCUSSION

The main purpose of this project [31] is to investigate the effects of anti-cancer drug molecule “topotecan” on the mechanism of the human topoisomerase I enzyme. In order to get new information about inhibition mechanism of this drug molecule, we have made structural and energetic analysis by using molecular dynamics simulation method. To get the large scale conformational changes, we have applied constant forces to the lips region of the protein. These forces are on the order of pico-newton, as the four different forces are applied. The forces are 100 pN, 200m pN, 300 pN, and 400 pN. The simulation time is 2 nanosecond, which is equal to 500.000 steps as each step is 2 femtosecond. All the simulations are repeated with and without the TPT molecule, and the results are analyzed in terms of both energetic and structure. In regards to energetic analyses, we have calculated nonbonded interactions, which are Van Der Walls and cloumb interactions. The hydrogen bonding is treated as the cloumb interactions.

We used the DNA-protein-TPT system shown in figure 4.1 which was generated before [32]. In this set-up, we have a total of 114.618 atoms, as the 9453 protein atoms, 1403 DNA atoms, 20 Na atoms (to neutralize the system), and the rest is water molecules. The system is pre-equilibrated at T=300 K for about 1 ns. During simulations, we consider the NVT canonical conditions that are; number of atoms in the system, volume, and temperature are constant. Also, Stochastic Boundary Forces were applied to water molecules in order to keep them around our system. The equilibrated initial system is shown in Fig 3.1.

As the structural analysis, we have monitored the distance between the two sides of lips of the protein in time. This is most important part of the study, as we are trying to understand amount of mouth opening with and without the anti-cancer drug molecule.

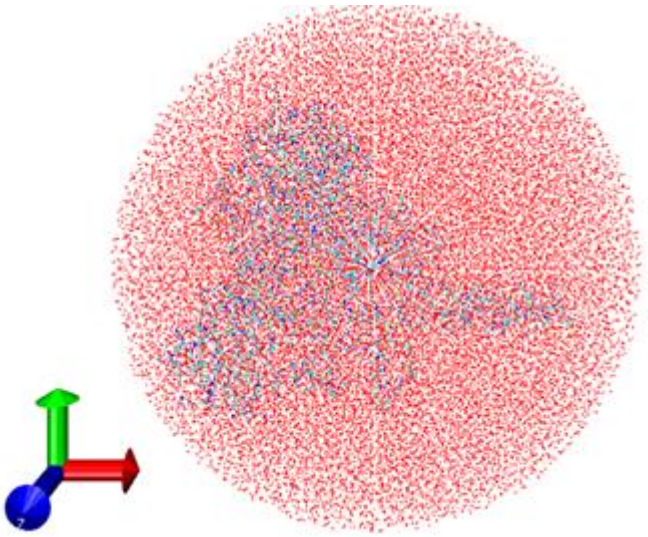

Figure 4.1 DNA-protein-TPT system in a water box [19]

The mouth opening is crucial here as the TPT stabilizes enzyme DNA ternary complex, and it is known that the DNA is unable to replicate due to collision between the replication fork and the stable complex. In this study, we investigate atomic resolution mechanism of inhibition, and try to understand the fundamental interactions that cause stability to the ternary complex. A recent study published in Nature [17] shows that within the ternary complex, angular velocity of the DNA rotations becomes 8-fold slower with the anti-cancer molecule TPT. Therefore, we think that this reduction of the DNA rotation might be due to fact that protein may stay in closed-form, and unable to open up its mouth when TPT is intercalated into the system. That is why; we first monitor the ability of mouth opening as we apply external forces.

The lips region of the protein is located between the upper and lower caps of the enzyme. The region where the external forces are applied is on the right side of the protein, when we look at from the linker side. The protein, DNA, TPT, and the region where forces are applied are shown in Fig 3.2.

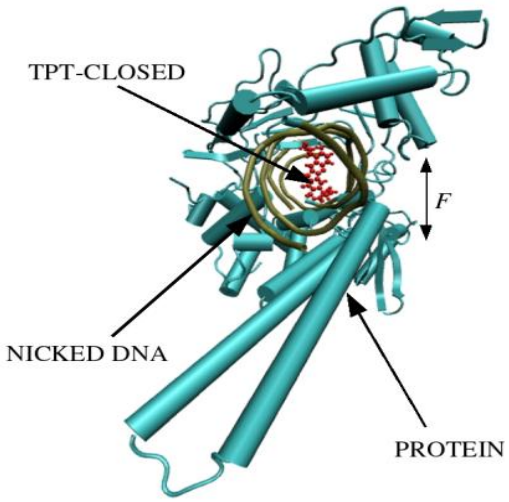

Figure 4.2 Force that acts on the lips region of the enzyme

It is important to elaborate the lips region in detail. The external forces are applied to the two different places within the lips region of the protein. These are, first, the so called ‘proximal’ point, in reference to proximity to the active side, and secondly ‘distal’ point. In the proximal side, we have chosen residues Gly365 and Ser534 which are exactly the same residues that are mutated by Woo et. Al. [14], and for the distal side His367 and Ala499 as they the same residues mutated by Carey et. Al. [29]. In fig. 3.3, TPT molecule, upper and lower parts of the lips, distal and proximal points, and protein residues that are interacting with TPT are given. In the figure, the initial distances of both distal and proximal separations are also shown. The only direct hydrogen bond between TPT and protein is the one via Asp533, and the initial distance in the crystal structure is labeled in the figure, as being 1.78 A.

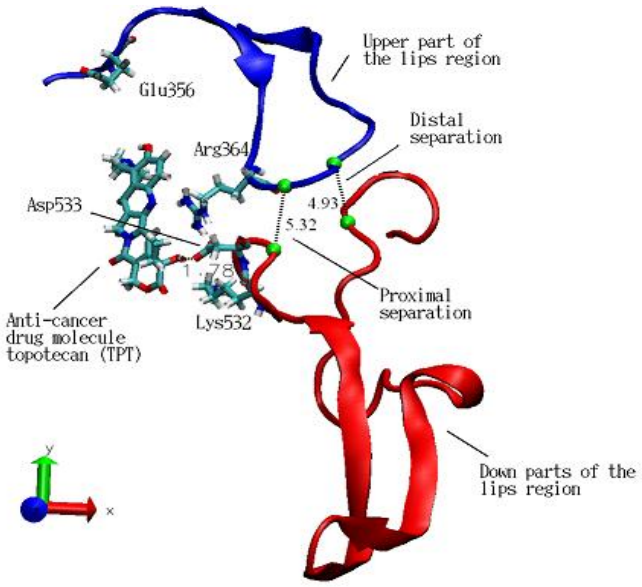

Figure 4.3 Distal and Proximal Positions of Lips of Human Topoisomerase I

First of all, when we look at the reactions given by the proximal separation to the external forces, we see an exciting finding that the protein does not open up its mouth when TPT is present, while a gradual opening is observed when TPT is removed from the system. This is the case when the applied force is 100 pN and 200 pN, however, when the force is increased to 300 pN and 400 pN, the proximal site gets separated. This behavior is clear in Fig. 3.4, where we plot the separations versus simulation time for each force we applied. If we closely analyzed the figure, we see that the separation remains around 5.5 A with around 1 A fluctuations when TPT is inside the protein, while the same separation increases to around 8 A when TPT is removed from the system. In the case of 300 pN, we see a sudden increase in the separation at around 0.6 ns, and the same behavior is also seen in the case of 400 pN, but a little bit earlier, at around 0.3 ns.

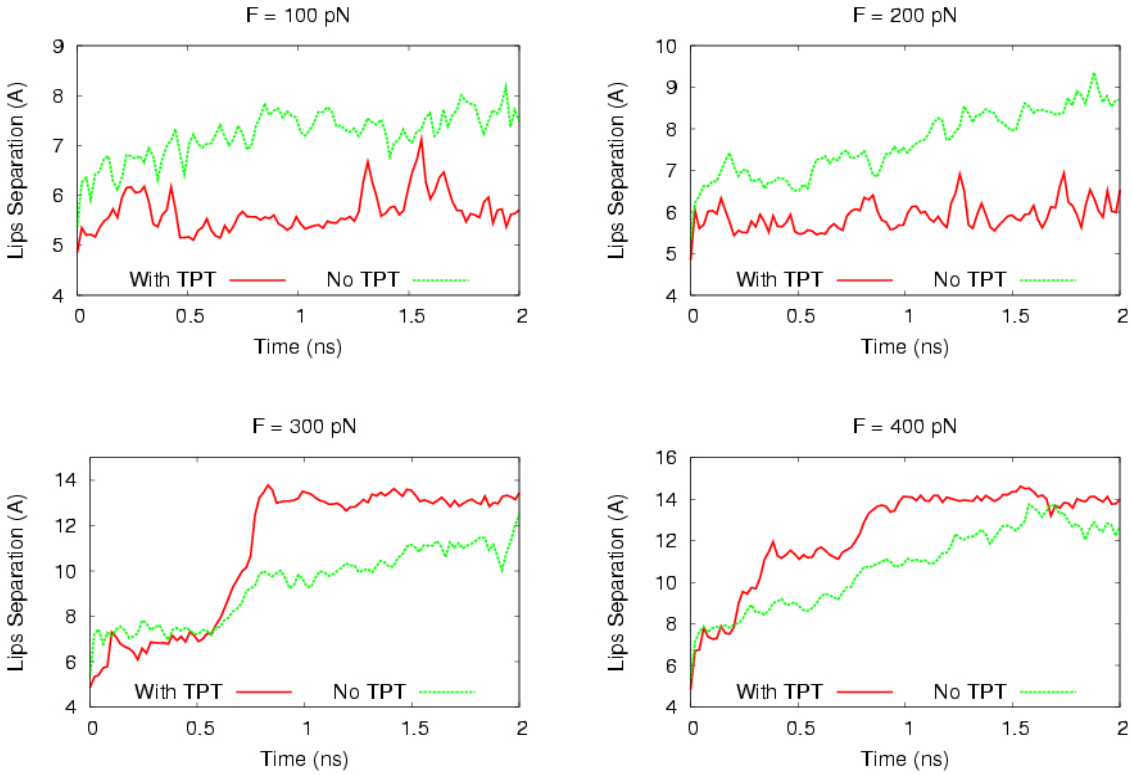

Figure 4.4 Separation between lips of the enzyme when we applied the force on proximal position

The reason for this surprising observation is thought to be the interaction of TPT with *both* side of the lips region. This is because; TPT keeps the lips together, as it interacts with Asp533 and Lys532 in the lower part and Arg364 in the upper part. However, if the applied force is increased to 300 pN and 400 pN, TPT can no longer keeps them together. The support this hypothesis, we have analyzed hydrogen bonding durations between TPT and surrounding protein residues. In Fig. 4.5, the durations of each hydrogen bond between TPT and surrounding protein residues are presented. Hydrogen bonds appearing less than 20 ps within the 2 ns time scale are omitted in the graphs. These graphs excitingly support our aforementioned hypothesis. As seen in the graphs, the direct hydrogen bond between Asp533 and TPT remains solid for about 1.8 ps within the 2 ns simulations, when the applied force is 100 pN. The situation is very similar for the 200 pN case, where this direct interaction remains unbroken for about 1.7 ps. Other than these interactions, Arg364, Lys532, and Thr718 are interacting with TPT with the durations shown in the figure. However, when the applied force is increased to 300 pN, we see that the direct hydrogen bond via Asp533 is reduced to

only about 0.5 ns, and to around 1 ns in the case of 400 pN, as a result of the break in the bond. Therefore, there is a quite close correlation between hydrogen bonding durations seen in Fig 3.5. and lips openings seen in Fig. 4.4.

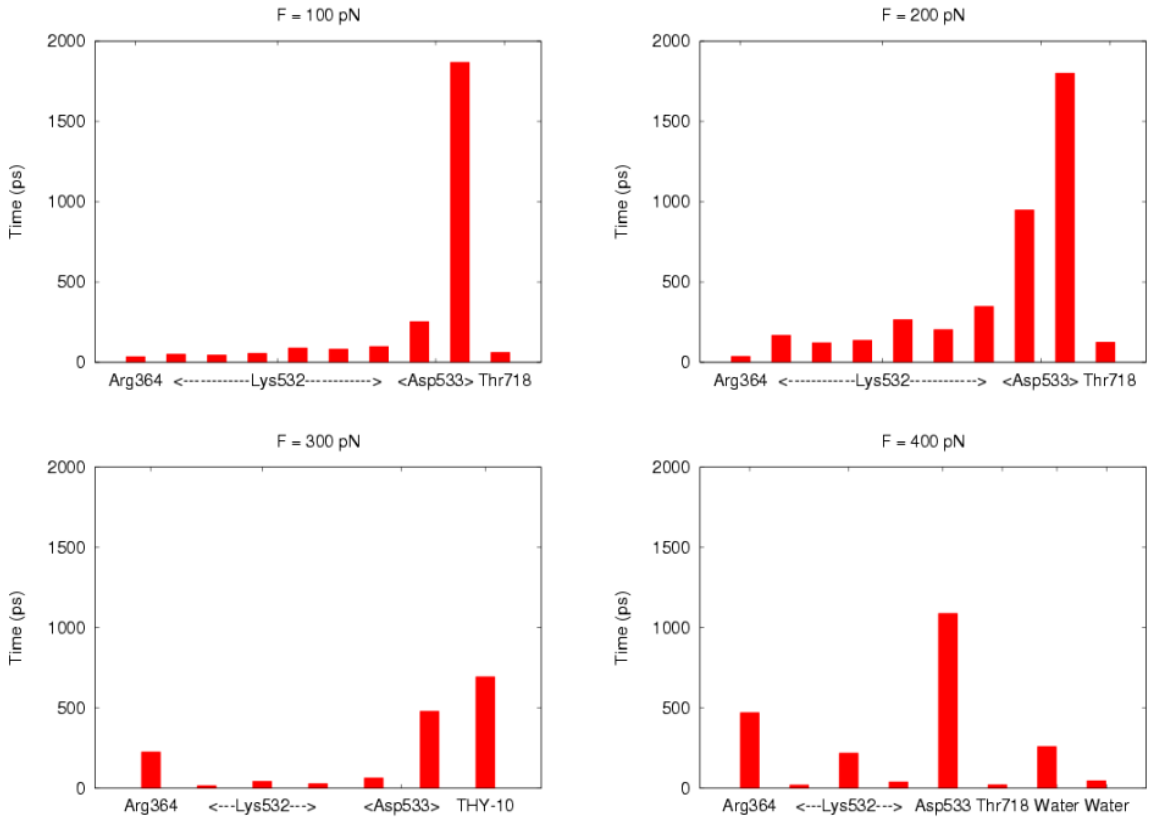

Figure 4.5 Hydrogen bonds between TPT molecule and residues at proximal position in lips region

To get a more concrete understanding, we have calculated interaction energies between TPT and protein. These interactions are given in the previous chapter in derail. As seen in Fig. 3.1. the most interacting residues are seen to be Arg364, Lys532, and Asp533. The interaction energies between these three residues and TPT are given in Fig. 4.6 for each force we applied. Such energies are calculated at each MD step, by reading our trajectories obtained under pulling forces.

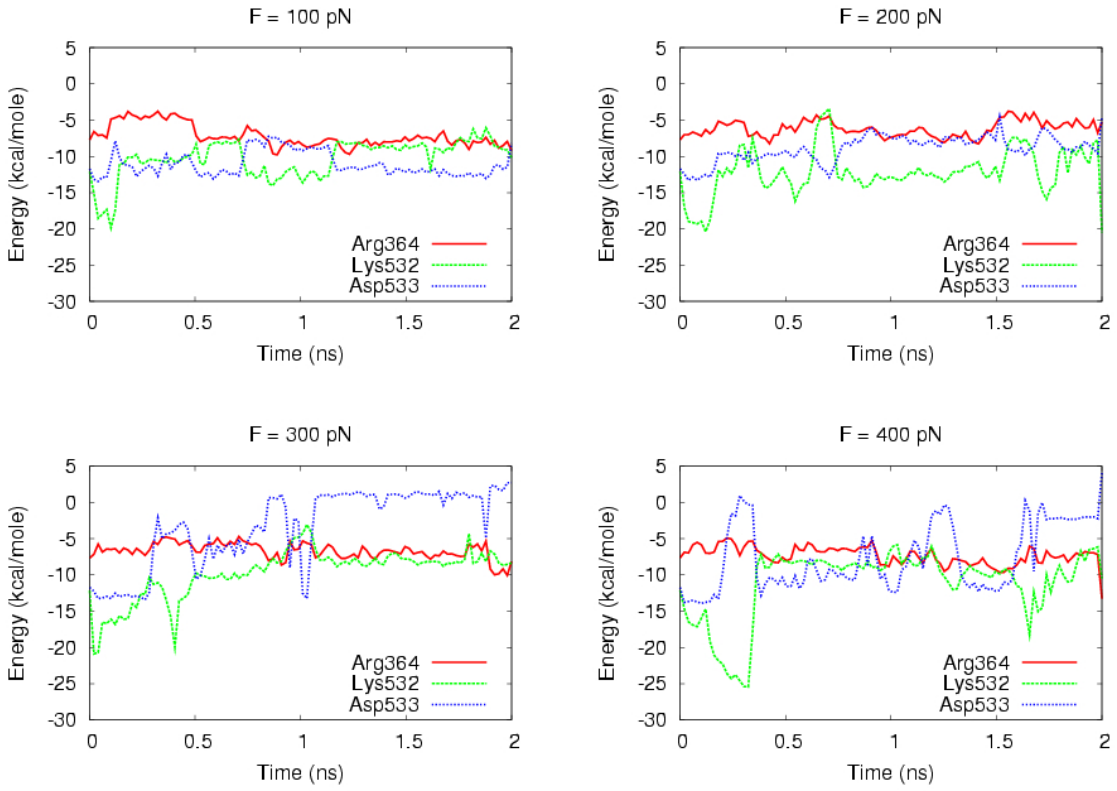

Figure 4.6 Energy values of residues of proximal positions which interact with drug molecule

As seen in the figure 4.6, interaction energies Arg364-TPT and Lys532-TPT are preserved very well under all different forces. However, the same interaction between Asp533 and TPT is preserved only in the case of 100 pN and 200 pN forces, while the interaction energy shifts to near zero values for 300 pN and 400 pN forces, at about 0.4 ns later. The correlation between this interaction and lips separation is quite clear, if we compare Fig 4.6 with Fig 4.4 where we see that lips separation starts to open up at exactly the same time (around 0.5 ns) with the destroy of direct hydrogen bond between Asp533 and TPT.

In the case of distal openings, we have carried out the same calculations, and performed the same analyses. When we look at the opening of the distal region under different forces, we see the same behavior that the forces of 100 pN and 200 pN are not enough to break the interaction between TPT and protein while under higher forces the distal separation opens up as in the case of proximal separation. As seen in figure 4.7, the distal separation remains un-separated for 100 pN and 200 pN when TPT is in the

system, while the same forces open the lips for about 2.5 A under 100 pN and 4.5 A under 400 pN when TPT is removed.

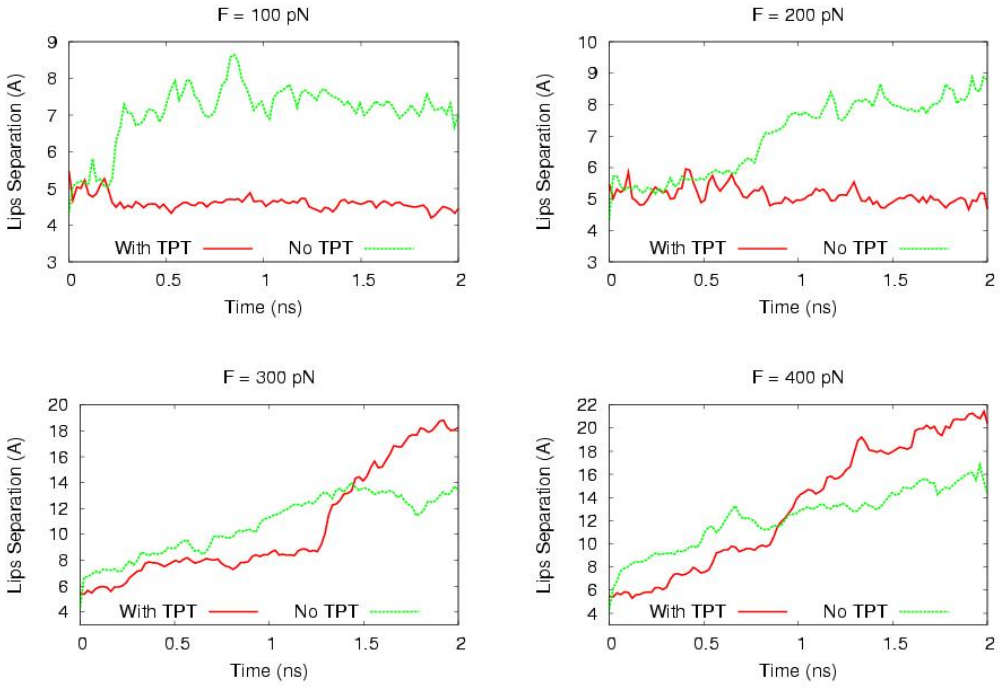

Figure 4.7 Separation between lips of the enzyme when we applied the force on distal position

As in the case of proximal separation, the distal separation opens till around 20 A under forces of 300 pN and 400 pN. The break in the TPT-protein interactions starts at around 1.3 ns for the forces of 300 pN, while the same break occurs at around 0.9 ns for the forces of 400 pN, as we expect.

When we carry out the same analysis of hydrogen bonding for the distal region, we see very similar result of what we get for the proximal separation. As seen in Figure 4.8, the direct hydrogen bonding between Asp533 and TPT stays almost all time during the 2 ns simulation, under the forces of 100 pN and 200 pN. However, when we increased the force to 300 pN, we see that the same bonding occurs only about 1.2 ns, and as the force is increased to 400 pN, it occurs only about 0.8 ns. This observation clearly supports the previous discussion on the opening amounts, where opening starts for the forces of 300 pN and 400 pN.

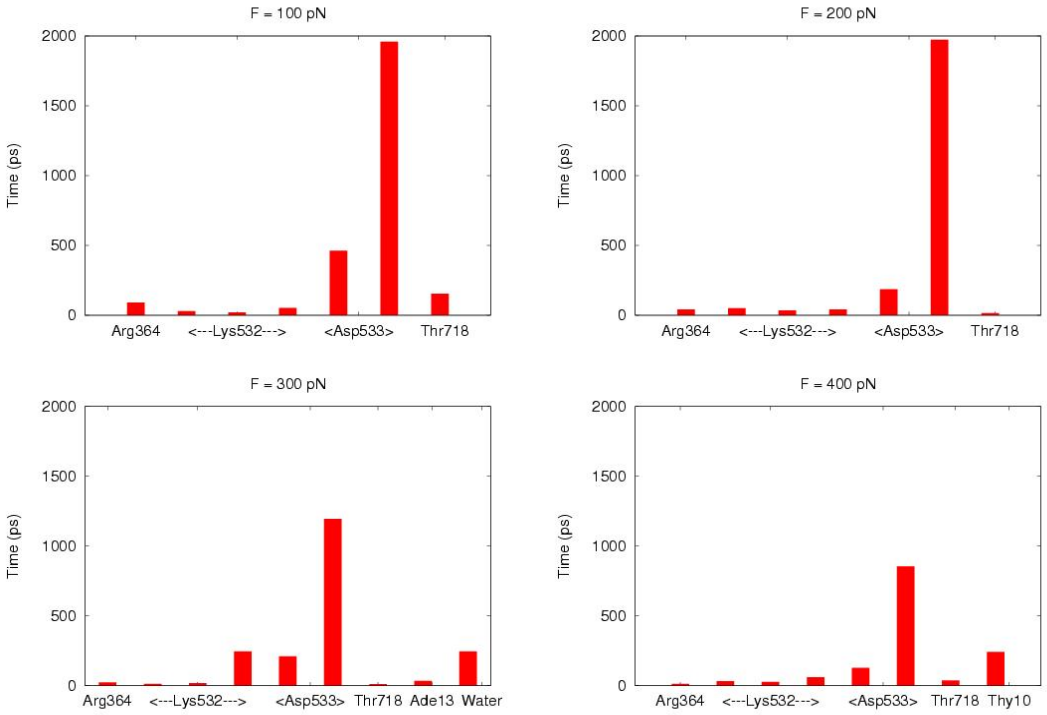

Figure 4.8 Hydrogen bonds between TPT molecule and residues at distal position in lips region.

The interaction energies between the TPT and protein amino acids are also performed as in the case of proximal separation. Here again, we calculated interaction energies between Arg364, Lys532, and Asp533 and TPT are calculated, and graphed in Figure 4.9. As seen in figure, the interaction energies remains constant (with small fluctuations) for 100 pN and 200 pN forces. This is a clear reason for the aforementioned fact that the lips separation does not open up under these forces. However, as the force is increased to 300 pN, the direct hydrogen bond between Asp533 and TPT collapses at exactly the same time when opening starts that is around 1.2 ns. Similarly, the same break in the interaction energy starts around 0.8 ns under the force of 400 pN, again very well in agreement with the hydrogen bond analysis

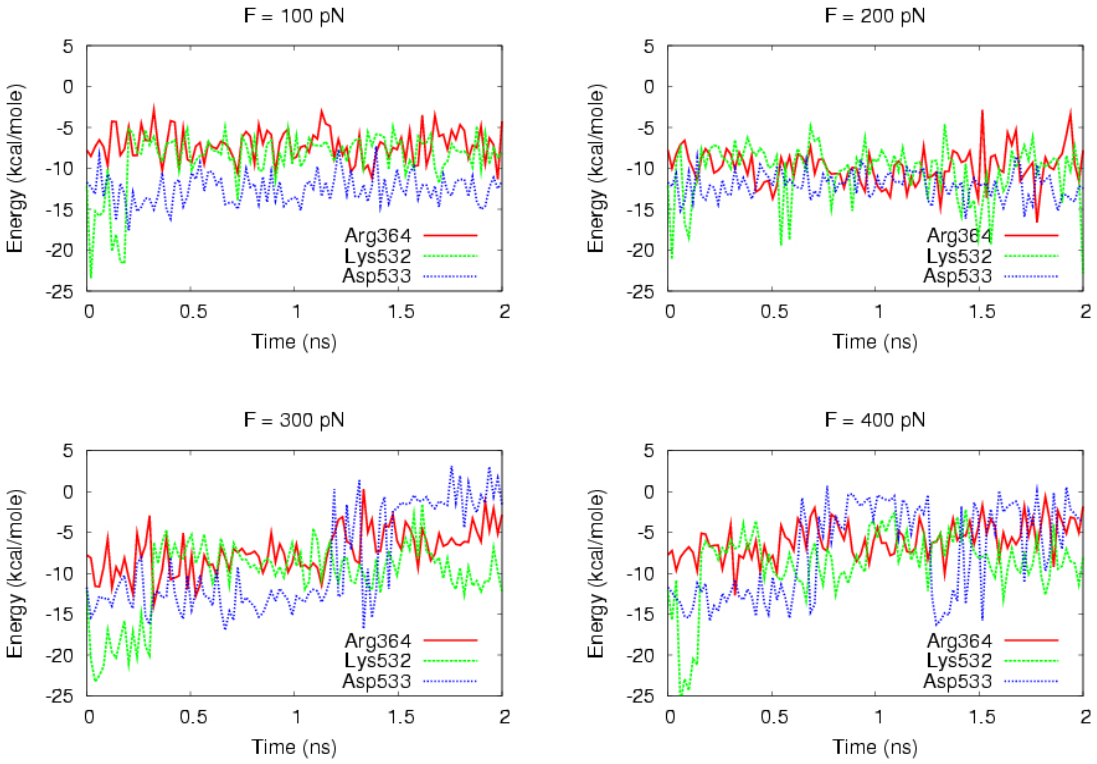

Figure 4.9 Energy values of residues of distal positions which interact with drug molecule

In brief, four different forces are applied to both proximal and distal regions in the lips domain, and both structural and energetic analyses were carried out. The results are discussed above in detail, and main conclusions are given in the next chapter. The findings that we mentioned in this study are quite crucial for the understanding of the inhibition mechanism of TPT. All of our results show that the cytotoxic effect of TPT starts by preventing the opening of the enzyme, and then impeding the DNA rotations. These findings are very well support the study of Koster et al. [17] published in Nature.

CHAPTER 5

CONCLUSIONS

Our current theoretical study on DNA-Human Topoisomerase I-TPT ternary complex brought quite unanticipated results that are very important for the cancer research. These findings are at the atomic resolution, and shed light on the inhibition mechanism of anti-cancer molecule TPT. We can summarize the main findings that are explained in the previous chapter in detail, as:

a-) The most important result of this study is the observation that the mouth of the enzyme does not open up in the presence of anti-cancer drug molecule TPT, while it opens up in the wild type mechanism.

b-) The reason for the observation mentioned above is found to be the interaction of TPT with the protein. These interactions are such that, TPT makes direct hydrogen bond with the lower part of the lips and also it interacts with the upper part. In this way, TPT clamps the lips.

c-) For both proximal and distal separations, forces of 100 pN and 200 pN are not enough to break the interactions between TPT and the enzyme, while forces of 300 pN and 400 pN are enough to break it, and let the mouth to open.

d-) The detail analyses of the TPT-protein interactions showed that the direct hydrogen bond between Asp533 and E-ring hydroxyl of TPT is the most crucial interaction, and when this interaction breaks, the lips of the enzyme starts to open up. Under low forces, this direct hydrogen bond is preserved for almost all simulation time of 2 ns.

e-) Exactly the same behavior in opening amounts, TPT-protein interaction energies, and hydrogen bonding is observed for both distal and proximal separations.

f-) Based on our current study, we claim that the cytotoxic effect of TPT starts by preventing mouth opening of the enzyme, which is needed normally to relax supercoiled DNA. As the mouth opening is prevented by TPT, DNA rotations within the closed clamped protein become harder, as directly shown by Koster et al. [17].

e-) Based on the results of our study, we can suggest that the anti-cancer effect may be achieved by a drug-free method if the lips of the enzyme can be clamped somehow.

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CHAPTER 1

INTRODUCTION

1.1 A MIRACLE MOLECULE: DNA

A DNA (deoxyribonucleic acid) is the most important molecule of life. It contains the genetic information of the cell and transfers this information to daughter cells by replicating itself before cell division. It stores information for a long time and this is the main role of DNA molecule. All cells except some viruses have this molecule.

DNA has double helical structure that was introduced by Watson and Crick in 1953 [1]. It has two strands which are running in opposite directions. The repeating units are called nucleotide (Adenine, Thymine, Guanine and Cytosine) and they form complementary base pairs. If there is A on one strand then there is T on the other side and same for G and C. Complementary base pairing is the most important property of DNA molecule. Because of this complemantarity, each preexisting strand acts as a template for the new strand. Hence genetic information can be passed to the daughter cells without changing. These nucleotides bind to each other with hydrogen bonds inside and the sugar-phosphate backbone outside. Double helical structure repeats on every about 10 base pairs. This structure is called B-DNA and also there are A-DNA, Z-DNA and closed circular DNA (ccDNA) [2].

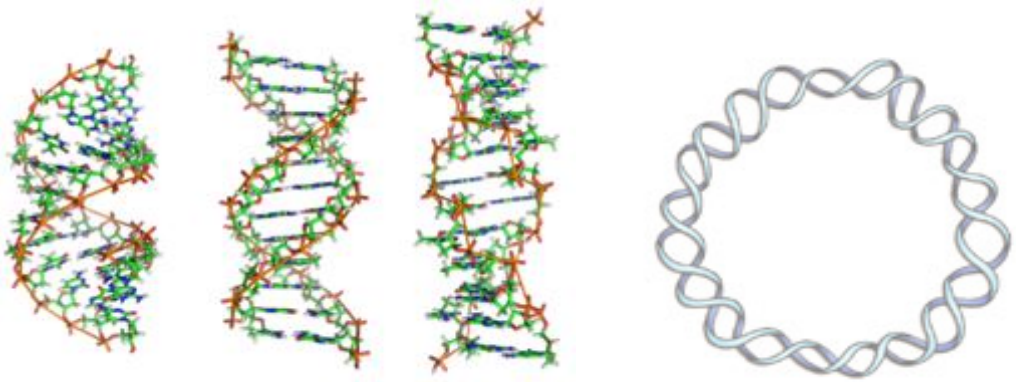

Figure 1.1 Structures of A-DNA, B-DNA, Z-DNA [3] and ccDNA [4]

CHAPTER 2

THEORETICAL METHODS

2.1 MOLECULAR DYNAMICS ON THE FORCE FIELD POTENTIAL ENERGY

It is sometimes very difficult to perform some experiments in microscopic level such as studies about very small biomolecules and assemblies of several atoms. Furthermore, studying about these tiny biomolecules requires more than experimental setup. To search what is going on inside these, how they are behaving, which kind of reactions are taking place, what are the atoms doing and questions like these caused computer simulations to be conceived.

Simulations are like bridges between experiment and theory [18]. We can get information about the structure and mechanism at microscopic level and compare this information with the experimental data we got. Consequently, some hidden details could be revealed with this comparison and computer simulations provide us to study on very tiny molecules even a few atom(s). Some simulation methods are Molecular Dynamics (MD), Monte Carlo (MC) [19].

Molecular Dynamics method is introduced in 1950 by Alder and Wainwright [20]. To imitate the simulation to real atomic interactions Rahman applied a smooth, continuous potential [21]. MD simulations were developed for more complex molecules in 1970s and first protein simulation took place in 1976[22], [23]. Now this method is being applied in a wide range of science such as biophysics, biochemistry, enzymology, molecular biology [19].

Molecular dynamics (MD) is one of the most popular computer simulation methods that is used to visualize atoms movements in molecules and to make structural and dynamic analysis of atom assemblies. In MD, atoms and molecules are allowed to interact each other for a period of time by approximations of physics. In order to

CHAPTER 3

ANALYSES OF INTERACTION ENERGIES

The non-bonded interaction energies, which are cloumb and Van Der Walls, are analyzed between TPT and protein. First of all, to see which residues are interacting with TPT, we have calculated interaction energies between TPT and all protein residues. In figure 3.1, we present these interaction energies versus residue numbers.

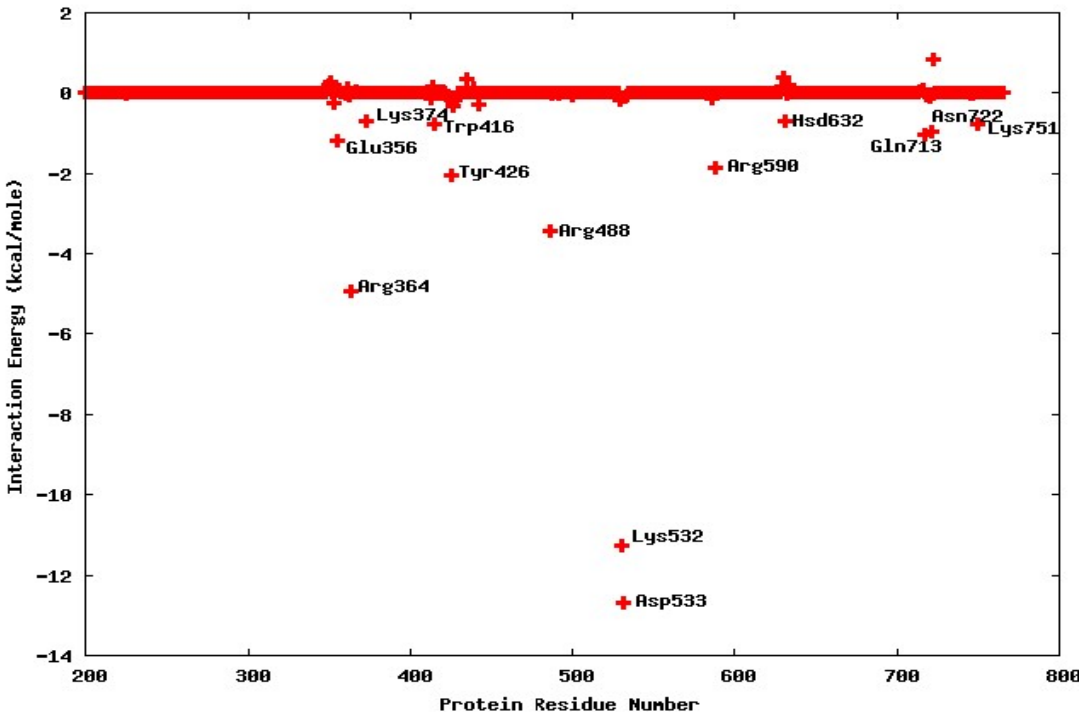

Figure 3.1 Interaction Energy Values between TPT Molecule and Residues of Enzyme

CHAPTER 4

RESULTS AND DISCUSSION

The main purpose of this project [30] is to investigate the effects of anti-cancer drug molecule “topotecan” on the mechanism of the human topoisomerase I enzyme. In order to get new information about inhibition mechanism of this drug molecule, we have made structural and energetic analysis by using molecular dynamics simulation method. To get the large scale conformational changes, we have applied constant forces to the lips region of the protein. These forces are on the order of pico-newton, as the four different forces are applied. The forces are 100 pN, 200m pN, 300 pN, and 400 pN. The simulation time is 2 nanosecond, which is equal to 500.000 steps as each step is 2 femtosecond. All the simulations are repeated with and without the PT molecule, and the results are analyzed in terms of both energetic and structure. In regards to energetic analyses, we have calculated nonbonded interactions, which are Van Der Walls and cloumb interactions. The hydrogen bonding is treated as the cloumb interactions.

We used the DNA-protein-TPT system shown in figure 3.1 which was generated before [neslihan]. In this set-up, we have a total of 114.618 atoms, as the 9453 protein atoms, 1403 DNA atoms, 20 Na atoms (to neutralize the system), and the rest is water molecules. The system is pre-equilibrated at T=300 K for about 1 ns. During simulations, we consider the NVT canonical conditions that are; number of atoms in the system, volume, and temperature are constant. Also, Stochastic Boundary Forces were applied to water molecules in order to keep them around our system. The equilibrated initial system is shown in Fig 3.1.

As the structural analysis, we have monitored the distance between the two sides of lips of the protein in time. This is most important part of the study, as we are trying to understand amount of mouth opening with and without the anti-cancer drug molecule.

CHAPTER 5

CONCLUSIONS

Our current theoretical study on DNA-Human Topoisomerase I-TPT ternary complex brought quite unanticipated results that are very important for the cancer research. These findings are at the atomic resolution, and shed light on the inhibition mechanism of anti-cancer molecule TPT. We can summarize the main findings that are explained in the previous chapter in detail, as:

a-) The most important result of this study is the observation that the mouth of the enzyme does not open up in the presence of anti-cancer drug molecule TPT, while it opens up in the wild type mechanism.

b-) The reason for the observation mentioned above is found to be the interaction of TPT with the protein. These interactions are such that, TPT makes direct hydrogen bond with the lower part of the lips and also it interacts with the upper part. In this way, TPT clamps the lips.

c-) For both proximal and distal separations, forces of 100 pN and 200 pN are not enough to break the interactions between TPT and the enzyme, while forces of 300 pN and 400 pN are enough to break it, and let the mouth to open.

d-) The detail analyses of the TPT-protein interactions showed that the direct hydrogen bond between Asp533 and E-ring hydroxyl of TPT is the most crucial interaction, and when this interaction breaks, the lips of the enzyme starts to open up. Under low forces, this direct hydrogen bond is preserved for almost all simulation time of 2 ns.