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Binding of Testosterone Hormone with Anti-testosterone Fab Fragment Antibody

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Abstract

Testosterone is a steroid sex hormone that regulates sex differentiation, male sex characteristics, spermatogenesis and fertility. It also functions in carbohydrate, lipid, and protein metabolism. It is largely produced by the male gonad and a variety of female organs. A lack of testosterone hormone can cause in increased fat mass, reduced insulin action, and undesirable cholesterol deposition in the body. The concentration of this hormone might vary even within healthy human. A protein, anti-testosterone Fab fragment antibody, has been proposed as the binding molecule that can maintain the hormone appropriately in our body. In this study, we investigated the binding of testosterone hormone to light and heavy chains of antibody and compared the binding strength by utilizing various binding methods, hydrogen bonding, non-bonded contact, and binding free energy estimation. The results of molecular dynamics simulation demonstrate that testosterone hormone binds more strongly to the heavy chain than the light chain. The strongest hydrogen bonding in atom pairs has been found to be between the O2 atom of testosterone and the O atom of GLY104 in heavy chain of antibody molecule, while the others were substantially weaker. Non-bonded interactions (electrostatic and van der Waals) play a crucial role in the formation of the protein-hormone complex. The MM/GBSA approach reveals that the binding energy of testosterone and heavy chain pairs is - 18.27 kcal/mol, which is almost three times greater than that of testosterone and light chain pairs - 6.60 kcal/mol.

Keywords: Hormone, anti-testosterone, antibody, receptor, drug-efficacy

1. Introduction

Testosterone is a steroid hormone that regulates fertility in males and females [1,2]. This hormone is utilized to treat the primary hypogonadism and hypogonadotropic hypogonadism. It inhibits the androgen receptor, causing gene expression that promotes the growth and development of male sex organs and secondary sexual traits. The hormone plays a key role in carbohydrate, fat and protein metabolism. Testosterone deficiency is associated with an increased fat mass, reduced insulin sensitivity, impaired glucose tolerance, elevated triglycerides and cholesterol and low HDL-cholesterol [3].

Testosterone hormone is produced primarily from gonad in male and multiple organs in female. In female, the ovaries and adrenal glands create many precursors [4]. The molecular formula of testosterone is

$C_{19}H_{28}O_2$, containing 21 heavy atoms (19 carbon atoms and 2 oxygen atoms). The molecular structure of the testosterone is shown in the Figure 1(a) and binding of this molecule in the anti-testosterone Fab fragment antibody is shown in Figure 1(b). Its molecular weight is 288.4 g/mol, with net zero charge. It is physically crystalline power and is white or slightly cream-white structure. Its melting point is 153-157°C [4]. Testosterone molecule is insoluble in water and soluble in ethanol, ethyl ether and acetone [5,6].

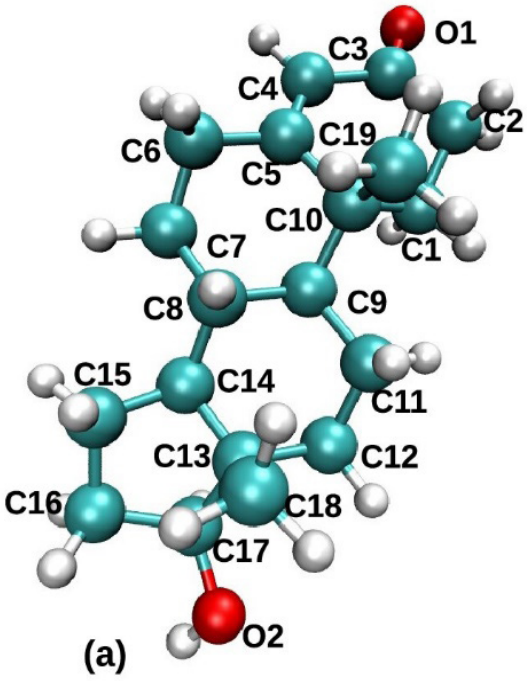
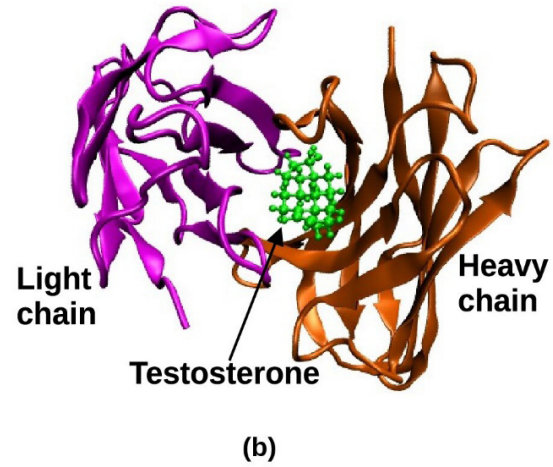


Figure 1: (a) A testosterone molecule showing the name of heavy atoms (19 carbon atoms and 2 oxygen atoms), small gray spheres represent the hydrogen atoms



(b) A testosterone molecule lies within the cavity of anti-testosterone Fab fragment antibody containing the light and heavy chains.

Human serum contains a vast number of steroid hormones, including testosterone, and can fluctuate even in healthy, normal people [7]. The fluctuation of serum levels produces a variety of health issues in the human body. Serum testosterone levels need to be measured for the diagnosis of hypogonadism, impotence, spermatogenesis and puberty development in males, as well as hyperandrogenism-related diseases in women [8,9]. To maintain an optimal level of testosterone, its binding protein is required. The anti-testosterone Fab fragment antibody has been proposed as the testosterone-binding protein. The antibody can basically be fulfilled through immunization. So, understanding the binding mechanism of testosterone hormone with its complimentary anti-testosterone Fab fragment is important for avoiding health issues caused by

testosterone hormone disorders in the body [7,10].

The testosterone hormone-anti-testosterone Fab fragment complex has two anti-testosterone chains (heavy chain and light chain). The testosterone hormone is located in the cavity between two chains [7]. The binding affinity of different chains is determined by many sorts of interaction processes. Hydrogen bonding, electrostatic contact, and van der Waals interaction all play important roles in the development of the molecular complex between testosterone hormone and anti-testosterone Fab fragments [11-14]. In this study, we investigate and compare the contributions of such interactions to form the stable complex. Many additional interactions, including hydrophobic and salt bridge interactions, have been investigated. Solvent accessible surface area (SASA) has been evaluated to study the hydrophobic nature of the complex.

We use molecular dynamics simulations to study the atomic-level interaction between the hormone and the anti-testosterone antibody. To the best of our knowledge, no molecular dynamics studies have been conducted on this issue. We hope that this work will contribute to evaluate the effectiveness of such antibodies in maintaining serum testosterone levels in the human body.

2. Methods and Methodology

2.1 System setup:

The molecular system for testosterone binding with anti-testosterone Fab fragment, an antibody, was obtained from the protein data bank under PDB ID 1I9J [15]. It contains a testosterone molecule that binds to the light chain of Fab fragment (219 residues) and a heavy chain fab of fragment (220 residues). Considering the suggestions of Valjakka and coworkers' that testosterone is primarily bound to the close residues, we removed the residues towards the remote side of Fab fragments from the testosterone, resulting in a light chain of 113 residues and a heavy chain of 117 residues with the complete structure of testosterone [7]. The online software "CHARMM-GUI" webserver [16] has been utilized for the molecular system set up. The new pdb and psf files were generated for the molecular complex and was solvated in a cubical simulation box having dimensions 72 × 72 × 72 $Å^3$ with TIP3P water. Entire solvated system was electrically neutralized by adding Na^+ and Cl^- ions with 0.15 M ion concentration.

2.2 Molecular Dynamics Simulation

All-atom molecular dynamics (MD) simulations were performed by using the NAnoscale Molecular Dynamics (NAMD) simulation program [17]. The CHARMM36m force field [18] was used throughout the simulation. The long-range interactions were treated using Particle Mesh Ewald (PME) with a non-bonded cutoff of 12.0 Å. The conjugate gradient algorithm was used to minimize energy over 10,000 steps. Then, equilibration run was executed to maintain the temperature and pressure at 300 K (27^{o}C) and 1 bar respectively, by using harmonically constrained heavy atoms with a 1 fs time step. Production simulation run was propagated for 100 ns under NPT condition. used to propagate

MD simulation runs, with a damping constant of 1 ps^{-1} [19,20].

2.3 Analysis Techniques

To investigate the interaction mechanism, we examined various procedures such as structural variation, interaction energy, and free energy calculation of the molecular complex. NAMD plugin packages in Visual Molecular Dynamics (VMD) [21] were employed to investigate the structural properties and interaction mechanism of testosterone hormones with their binding protein. The main interacting residues were identified by using "pycontact" software [22,23]. The "Molecular Mechanics with Generalized Born and Surface Area Solvation (MM/GBSA)" approach [24] was used to estimate the binding free energy of the hormone molecule to the light and heavy chains of the anti-testosterone Fab fragment. During the simulation output analysis, graphs and bar diagrams were created using Xmgrace and Microsoft Excel.

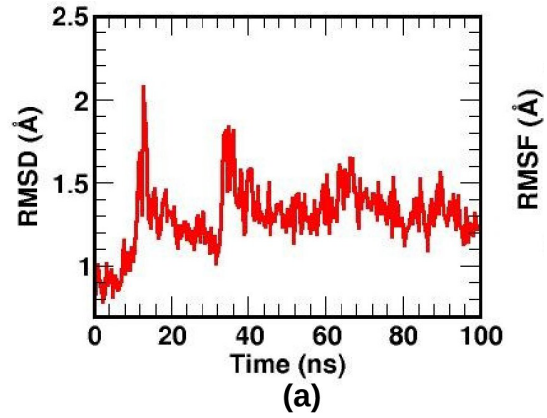
3. Results and Discussion

We have performed the molecular dynamics simulations to study the binding mechanism of a molecular complex, testosterone hormone molecule interaction with light and heavy chains of anti-testosterone antibody. We basically studied the structural properties, interaction energy calculations and the free energy estimation of the molecular complex.

3.1 Root Mean Square Deviation (RMSD)

RMSD is a thermodynamic measure that estimates the structural stability of an entire molecular system in aqueous medium. The estimation of RMSD is an appropriate method for determining whether the system is stable or not. We used the RMSD of the backbone of the system to evaluate stability. For the structural stable state, the RMSD of the system should be almost constant; otherwise, the MD analysis would be erroneous.

In this work, RMSD of entire molecular complex was plotted over time function during the simulation run as shown in Figure 2(a). We have taken the coordinates at the first frame as the reference coordinates and the average RMSD was estimated. Figure 2(a) shows the RMSD of the entire molecular complex of testosterone and its binding protein molecule. The RMSD was found fluctuating till the 40 ns simulation run. Afterwards, the plot became smooth showing the stable structure of the complex in the aqueous environment. This shows that molecular system would be suitable for the further estimation of structural and interaction energy estimation.



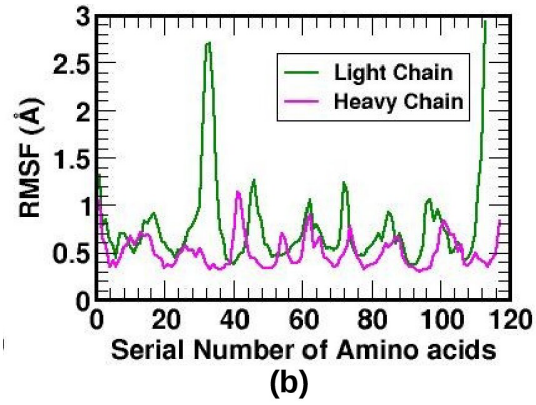


Figure 2: (a) Root Mean Square Deviation (RMSD) of entire complex containing testosterone hormone and anti-testosterone Fab fragment antibody (b) Root Mean Square Fluctuation (RMSF) of the residues in light chain and heavy chain of antibody molecule.

3.2 Root Mean Square Fluctuation (RMSF)

RMSF is the average deviation of individual residue of a molecule over time from a reference position. It is commonly used to represent the time-averaged position of targeted residue. RMSF, thus, evaluates the structural components that deviate the most or the least from their mean position. The larger RMSF represents the greater fluctuating residues and are basically the unstable in the structure, whereas the small RMSF shows the stable residue.

We have compared the RMSF of small and heavy chains of the antibody molecule during the 100 ns simulation run. The outcomes of the simulation run show that the fluctuations of some residues (basically the 28 to 38) in light chain are relatively higher as shown in Figure 2(b). In comparison of two chains, the residues in light chains are more fluctuating than that of heavy chain. This shows that the hormone molecule has the stronger binding to heavy chain than that of the light chain. In both molecules, most of the residues have the low fluctuation showing the stable structure of the molecules in the aqueous environment. At the terminal regions (N-terminal and C-terminal), the fluctuations of the residues are relatively higher because of the free from the atomic interaction at these regions.

3.3 Solvent Accessible Surface Area (SASA)

SASA of a molecule is the measure of portion of exposed surface that is available to the solvent. During the molecular dynamics simulation, an increase in SASA indicates that the amino acids on the surface of molecule are hydrophilic, whereas a reduction in SASA indicates that the residues on the surface are hydrophobic. In the molecular complex, if the SASA is decreased, there would be favorable binding between the components of the complex.

In this study, we examined the SASA during the 100 ns MD simulation run. Even though the SASA fluctuates during the simulation, the overall trend of plot is decreasing. The best fitted line exhibits a negative slope, which is shown from the linear fit equation, $y = 11580 - 1.0732\ x$, where y represents the SASA ($Å^2$) and x represents the time function (ns) during the simulation. The negative slope of SASA demonstrates that the general nature of the complex is hydrophobic, it means the residues reside

on the surface are somewhat arranged and hide within the surface molecule reducing overall surface. The trend of variation of SASA during the simulation and its best fitted line is shown in Figure 3.

3.4 Hydrogen Bonding

Hydrogen bonding is a sort of dipole-dipole interaction between molecules. It is a special type of weak non-covalent bonding. This bonding is the interaction between a hydrogen atom and a pair of other atoms with a strong affinity for electrons, like N, O and F. Its strength falls somewhere between covalent bonding and van der Waals forces [25].

We estimated the hydrogen bond occupancy percentage of testosterone molecule to the heavy and light chains of the anti-testosterone Fab fraction antibody. The hydrogen bonding of testosterone has been found to be greater in the heavy chain than with the light chain. The strongest hydrogen bond was identified between the O2 atom of testosterone and the O atom of GLY104 in the protein, while the others were found much weaker. Following the O atom in GLY104, backbone C atom of this residue has relatively stronger hydrogen bonding to the testosterone hormone. Table 1 shows the hydrogen bond occupancy percentage of testosterone hormone with the heavy and light chains of the antibody.

During the simulations, we detected 13 hydrogen bonds between testosterone and the heavy chain, but only 4 weak hydrogen bonds were observed between the hormone and the light chain. Out of the 13 hydrogen bonds between the hormone and the heavy chain is powerful, with a hydrogen bond occupancy percentage of 90%. The

Residue Pairs of		Occupancy %		Residue pairs of		Occupancy %
Testosterone	Heavy Chain			Testosterone	Light Chain	
TES1010-O2	SER35-OG	0.80		TES1010-C6	GLY96-O	0.40
TES1010-O2	GLY104-O	90.00		TES1010-C6	SER97-O	0.40
TES1010-O2	GLY104-C	6.40		TES1010-C7	GLY96-O	1.20
TES1010-C14	TYR102-O	3.20		TES1010-C15	GLY96-O	2.40
TES1010-C11	SER50-OG	0.80				
TES1010-C16	GLY104-N	0.80				
TES1010-C16	TYR102-O	0.40				
TES1010-C18	SER35-OG	2.80				
TES1010-C18	SER50-OG	0.80				
TES1010-C17	TYR102-O	1.60				
TES1010-C2	TYR58-OH	0.40				
TES1010-O2	LEU105-CD2	0.40				
TES1010-C12	TYR102-O	0.40				

Table 1: Occupancy percentage testosterone hormone with the heavy chain and light chain of anti-testosterone Fab fraction antibody.

hydrogen bond occupancy percentage the hormone with its binding protein is shown in bar diagram in Figure 4. The hydrogen bonding having occupancy percentage greater than 1 are shown in the bar diagram. In the figure, first 5 pairs are the hydrogen bonding pairs of testosterone with heavy chains, whereas the last 2 are the bonding with the light chain.

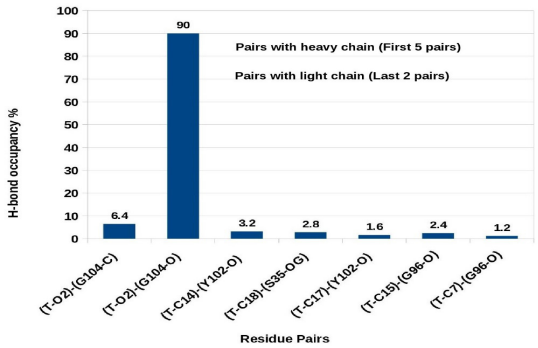


Fig. 4: The bar diagram showing the hydrogen bond occupancy of atom pairs between testosterone hormone and the atoms in the chains of anti-testosterone Fab fragment antibody.

3.5 Distance of Highest Binding Affinity Atoms

From the estimation of hydrogen bond occupancy percentage, we found that only one bonding pair, (TES1010-O2) – (GLY104-O), is the strongest. To examine the variation of distance between the strong pairing, we evaluated the distance over time during the simulation. Figure 5 shows that the pairing of these atoms and the plot of distance during the simulation. We found that the distance between these atoms lie within 3 Å distance forming the strong hydrogen bonding. This binding partner has the major role in biniding of hormone molecule with its binding protein chain. It also depicts that hormone is fabourably bound within the protein cavity.

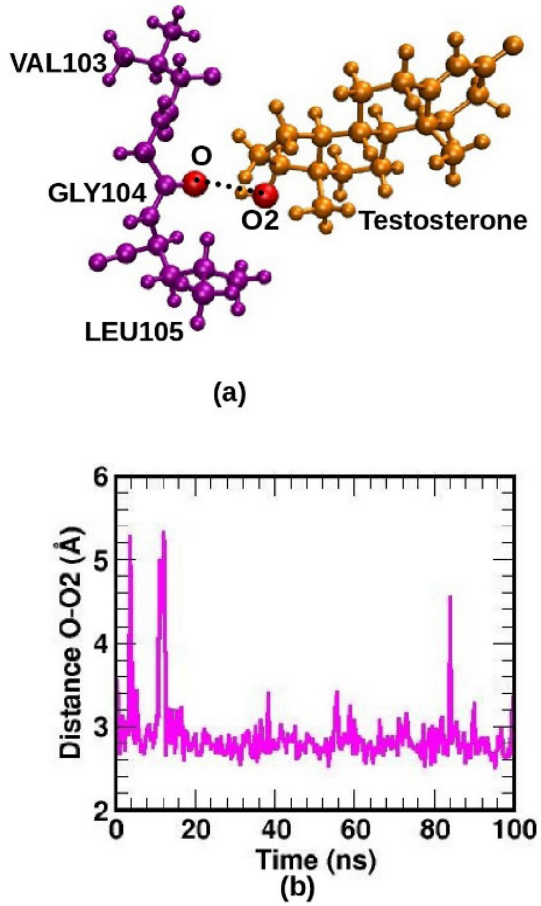


Figure 5: The atom pairs between the hormone molecule and antibody molecule and variation of distance between these atoms during the MD simulation.

3.6 Electrostatic and van der Waals interactions

Electrostatic interaction largely determined the structure, behaviour, and function of biomolecules. They are especially important for protein folding and stability, conformational changes, receptor

recognition of substrates, enzymatic catalysis, and other processes. On the other hand, van der Waals interaction is the induced electrical interactions between two or more atoms or molecules that are very close to one another. It is considered as the weakest type of intermolecular attraction between molecules. Nonetheless, the interaction can be very powerful if a lot of van der Waals interactions are involved between the atoms or molecules.

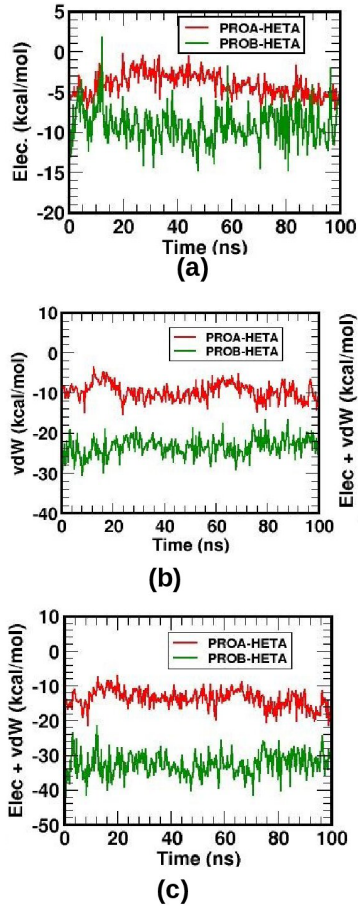


Figure 6: The variation of non-bonded interactions (electrostatics and van der Waals) between the hormone molecule and chains of the anti-testosterone antibody molecule.

The non-bonded energies were evaluated and compared for testosterone-light chain (PROA-HETA) and testosterone-heavy chain (PROB-HETA) complex. The quantitative value of electrostatic, van der Waals and total energies were estimated from the NAMD plugin packages in VMD. The variation of corresponding energies is plotted as shown in Figure 6. In the given interaction, van der Waals energy is stronger than that of the electrostatic energy, as predicted by Valjakka and coworkers [7]. The comparison of electrostatic, van der Waals and total energy are shown in Table 2.

Molecule pairs Types of energy	Testoster-one-light chain (PROA-HETA)	Testoster-one-Heavy chain (PROB-HETA)
Electrostatics (Elec.)	- 4.13 kcal/ mol	9.20 kcal/ mol
van der Waals (vdW)	- 9.20 kcal/ mol	23.59 kcal/ mol
Total (Elec. + vdW)	13.90 kcal/ mol	32.79 kcal/ mol

Table 2: Contribution of electrostatics and van der Waals interactions during the binding of testosterone hormone with light and heavy chain of anti-testosterone Fab fragment antibody. 3.7 Free Energy Estimation

Binding free energy is utilized primarily to estimate the binding affinity of biomolecular interactions and the efficacy of drugs. It is particularly significant in the computational drug design process. Many computational methods have been developed to evaluate the binding free energy of molecular

complexes. We used Molecular Mechanics with Generalized Born and Surface Area Solvation (MM/GBSA) approach to estimate the free energy of the hormone and binding proteins.

We estimated the binding free energy in three pairs, testosterone-light chain, testosterone-heavy chain and light-heavy chains. The pairing scheme of testosterone with the fractions is shown in the Figure 7.

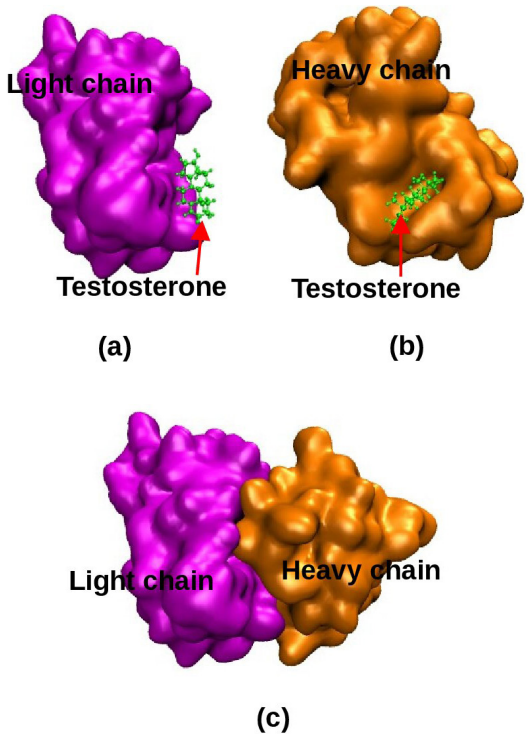


Figure 7: The binding scheme of testosterone with the chains of anti-testosterone Fab fragments and the fragments themselves (a) testosterone-light chain (b) testosterone-heavy chain (c) light-heavy chains.

The binding free energy between aforementioned pairs were estimated from MM/GBSA method. The free energy for the corresponding pairs is shown in Table 3. Our primary focus of the investigation is the binding of testosterone with the light and heavy chains. It shows that the binding of heavy chain with the testosterone is stronger (- 18.27 kcal/mol) than the light chain (- 6.60 kcal/mol) as predicted in hydrogen bonding and non-bonded interactions. The free energy of bonding between light chain and heavy chain of the antibody is – 51. 13 kcal/mol, and is obviously the greater than that of other two pairs, since they are the chains of large residues and relatively bigger in size and also the larger contact surface.

Molecule pairs	Energy
Testosterone – Light chain (HETA – PROA)	- 6.60 (kcal/mol)
Testosterone – Heavy chain (HETA – PROB)	- 18.27 (kcal/mol)
Light chain – Heavy chain (PROA – PROB)	- 51.13 (kcal/mol)

Table 3: Free energy estimation of testosterone-light chain, testosterone-heavy chain and light-heavy chains.

4. Conclusion

Testosterone hormone is a steroid hormone found mostly in sex differentiation, male sex characteristics, spermatogenesis and fertility in male sex organs and several feminine organs. This hormone is also essential in regulating carbohydrate, lipid, and protein metabolism. A lack of testosterone can create a variety of health problems in the human body. An antibody

protein, anti-testosterone Fab fraction, is a vital molecule capable of binding with testosterone. In this study, molecular dynamics simulation has been utilized to investigate the binding mechanism of testosterone hormone with the anti-testosterone Fab fraction antibody. The anti-testosterone antibody has two chains: light and heavy. The hormone is located in the cavity between these chains and is bound to the protein chains. Our investigation demonstrates that the hormone molecule binds more strongly to the heavy chain than that of light chain. Even though many hydrogen bonds act to bind the hormone and antibody protein, the strongest is the one between the O2 atom of testosterone and the O atom of GLY104 of heavy chain of antibody; the others are much weaker. Non-bonded interactions (electrostatics and van der Waals) are essential for the formation of the hormone-protein complex. The SASA during the MD simulation indicates that the compound is hydrophobic. The binding energy estimated from MM/GBSA technique demonstrates that testosterone hormone binds to the heavy chain about 3 times more strongly than that of light chain of binding protein.

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Conflict of Interest: All authors declare no conflict of interest.

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