İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

A SIMPLIFIED MODELLING OF PROTEIN-PROTEIN INTERACTIONS: BARNASE-BARSTAR INTERACTION BY COMPUTATIONAL METHODS

M.S. Thesis by

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Department : Advanced Technologies in Engineering

Programme: Molecular Biology-Genetics and Biotechnology

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PROTEİN-PROTEİN ETKİLEŞİMİNİN SADELEŞTİRİLMİŞ BİR MODELİ: HESAPSAL METOTLAR KULLANILARAK BARNASE-BARSTAR ETKİLEŞİMİNİN İNCELENMESİ

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HAZİRAN 2008

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May 2008
Banu BURUK

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ABBREVIATIONS

B. amyloliquefaciens	**:** Basillus amyloliquefaciens
ITC	**:** Isotermal Calorimetry
SPR	**:** Surface Plasmon Resonance
H_2O	**:** Water
NMR	**:** Nuclear Magmetic Resonance
MM	**:** Molecular Mechanics
MD	**:** Molecular Dynamics
QM	**:** Quantum Mechanics
Glu	**:** Gutamic Acid
Ala	**:** Alanine
Lys	**:** Lysine
Gln	**:** Glutamine
Asp	**:** Aspartic Acid
R-group	**:** Side chain

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ÖZET

Metabolik aktivite regülasyonu, biyokimyasal reaksiyon katalizlenmesi, hücrelerin yapısal bütünlüğünün muhafaza edilmesi gibi çok önemli yaşamsal faliyetlerin sorumlusu proteinlerdir. Bu sorumluluklarını birbirleri arasındaki sinyal mekanizmaları ile yerine getirdikleri için aralarındaki etkileşimler, üzerinde en çok düşünülen konulardan biri olmuştur. Proteinlerin sinyal mekanizmaları ile birbirleri arasında kurdukları iletişim ağı ile ilgili edinilen bilgiler; protein kompleksleri için etkili inhibitör üretimi ,hastalık tanımlama ve tedavisinde önemli yok katetme ve yeni terapötik yaklaşımların oluşturulmasında oldukça faydalı olmaktadırlar.

Bu çalışmada, barnase ve barstar proteinlerinin etkileşimi moleküler mekanik yaklaşımı ile incelenmiştir. Protein kompleksinin kristal yapısı baz alınarak, ara yüzey; geometri optimizasyonu ile çözümlenmeye çalışıldı. Ara yüzeydeki beşi barnase proteinine, üçü de barstar proteinine ait olmak üzere toplam sekiz amino asit ile optimizasyonlar gerçekleştirildi. Bu sekiz amino asitten barnase proteinin aktif bölgesinde bulunan Glu73 (glutamik asit) amino asiti üç farklı ortamda, dört farklı amino asit ile değiştirilmiştir. Radikal grupları birbirinden farklılık gösteren alanin, lizin, gulutamin ve aspartic asit; glutamik asit yerine denenen amino asitlerdir. Sistemde su moleküllerinin varlığı ile birlikte zvitteriyonik (çift kutuplu) ortam, gaz ortamı ve protein sekanslarının uçlarına CH_3 eklenerek üç farklı ortam yaratıldı. Elde edilen enerjiler, amino asitlerin R-grupları ile H_2O molekülleri arasındaki etkileşimlerin ve farklı olabilecek pH değerlerinin etkisinin önemini gösterdi. Karara vardırıcı sonuç ise protein sekanslarının uçlarına CH_3 eklenerek yaratılan ortamda orjinal barnase-barstar kompleksini en gerçekçi sonuçlarla taklit edebilmemiz olmuştur.

A SIMPLIFIED MODELLING OF PROTEIN-PROTEIN INTERACTIONS: BARNASE-BARSTAR INTERACTION BY COMPUTATIONAL METHODS

SUMMARY

Proteins are responsible for regulation of metabolic activity, catalyzing biochemical reactions, maintaining structural integrity of cells/organisms. All these vital and extremely important responsibilities are maintaining by the signaling mechanisms between proteins. Therefore, the information about protein-protein interactions enables searchers understand protein–protein contacts for to produce successful inhibitors for protein complexes and improves our understanding of diseases and can provide the basis for new therapeutic approaches. Consequently, protein interactions are that thought over the most.

In the current study, interaction of barnase-barstar proteins is analyzed by molecular mechanics. Having an X-ray crystallographic data of the protein complex, binding interface has been examined by geometry optimization processes. Totally eight amino acids in the interface of barnase-barstar complex; consisting of five amino acids from barnase and three amino acids from barstar have been used for optimization studies. Glu73; the main amino acid at the active site of barnase has been mutated into four different amino acids in three different conditions; zwitterionic form of proteins, form of proteins in gaseous surroundings and with CH_3 addition to the terminal groups of protein sequences, with water molecules. The obtained energies indicated the effect of molecular interactions between H_2O molecules and R-groups of amino acids and the pH of the system. The concluding result was that in the condition with CH_3 addition to the terminal groups of protein sequences gave the most realistic energies mimicking the original structure of the barnase-barstar.

1. INTRODUCTION

In 1838, the word protein was first needed to be used. The origin of the word is Grek; from the word proteios which means "the most important".

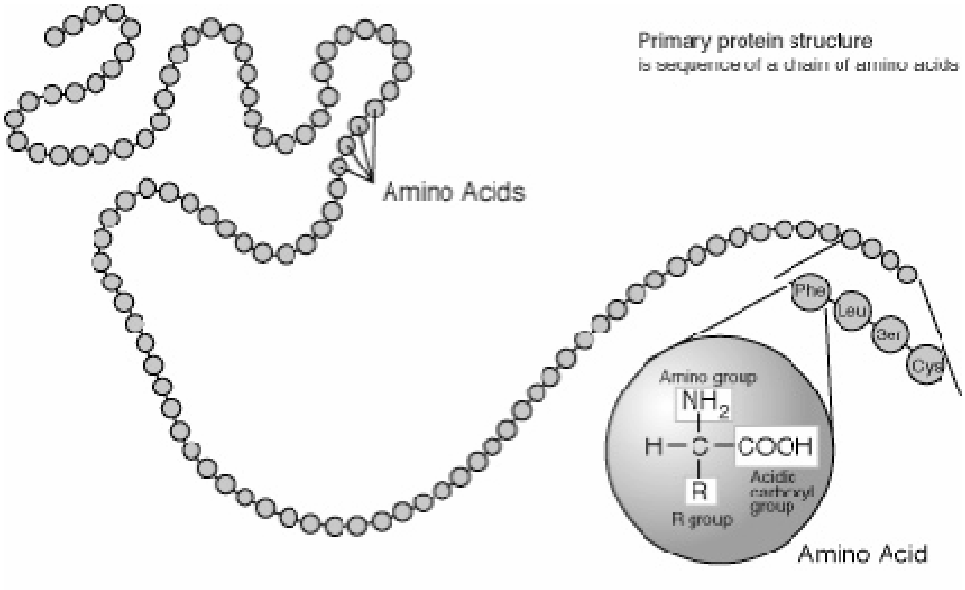


Figure 1.1: Primary structure of proteins [1].

While DNA is the first performer and responsible for the information supply during the process; proteins are the major performers of living organisms. It is because that proteins do the work of cells; all the same even if they are microbial, plant or animal cells. Regulation of metabolic activity, catalyzing biochemical reactions and maintaining structural integrity of cells and organisms are the principle responsibilities of proteins.

Here below, Table 1.1 summarizes functions of proteins and the classification of them according to these functions. The unique structure and chemical composition of each protein is important for its function. That’s why, the structure of amino acids and their role in forming the protein properties are need to be examined at the first rank [1].

Table 1.1: Classification of Proteins According to biological function [1].

Type:	Example:
Enzymes- Catalyze biological reactions	ß-galactosidase
Transport and Storage	Hemoglobin
Movement	Actin-Myosin
Immune Protection	Immunoglobulins (antibodies)
Regulatory Function within cells	Transcription Factors
Hormones	Estrogen
Structural	Collagen

All the biological functions proteins are responsible for, are being carried out by signaling through proteins. Signaling through different proteins or sites in proteins is of central importance for virtually every process in a living cell. First of all, signals from the exterior of a cell are mediated to the inside of that cell by protein-protein interactions of the signaling molecules, which is called signal transduction. For more, allosteric regulation of enzymes and folding events, cytoskeletal remodeling, transcription, cell cycle regulation and immune response are also examples of signalling mechanisms. Every system in cell, between cells and finally in all living organisms are carried out by protein-protein interactions. These protein associations are studied from the perspective of biochemistry, signal transduction and networks. General principles of cell communication are going even for unicellular organisms which can communicate and influence one another's behaviour in preparation for sexual mating [2]. Proteins are the main products those are responsible for collecting information, commenting on that information and letting biological pathways go on in their continuous way by building new products; in other words proteins. Biological pathways find their way by functionally important changes which are the results of ligand binding, phosphorylation or point mutations. To be able to understand the communication in proteins, conformational changes resulting from association of proteins are need to be examined [3]. Communication process between proteins starts with an entry to a region of electrostatic steering as they approach each other. If the attraction of these electrostatic interactions is well enough, the proteins enter the formation step of the encounter complex. The formation of the

transition-state, second intermediate and the bound complex are the following steps of the process. Electrostatic attraction between proteins is indispensible for one of the protein just to modify the other one. Focusing on interface models is optimal for molecular modeling purposes.

The chemical nature of protein–protein interfaces varies among different families of protein complexes and hence is not helpful for fully understanding protein–protein associations. Still the information about protein-protein interactions enables searchers understand protein–protein contacts for to produce successful inhibitors for protein complexes and improves our understanding of diseases and can provide the basis for new therapeutic approaches [4].

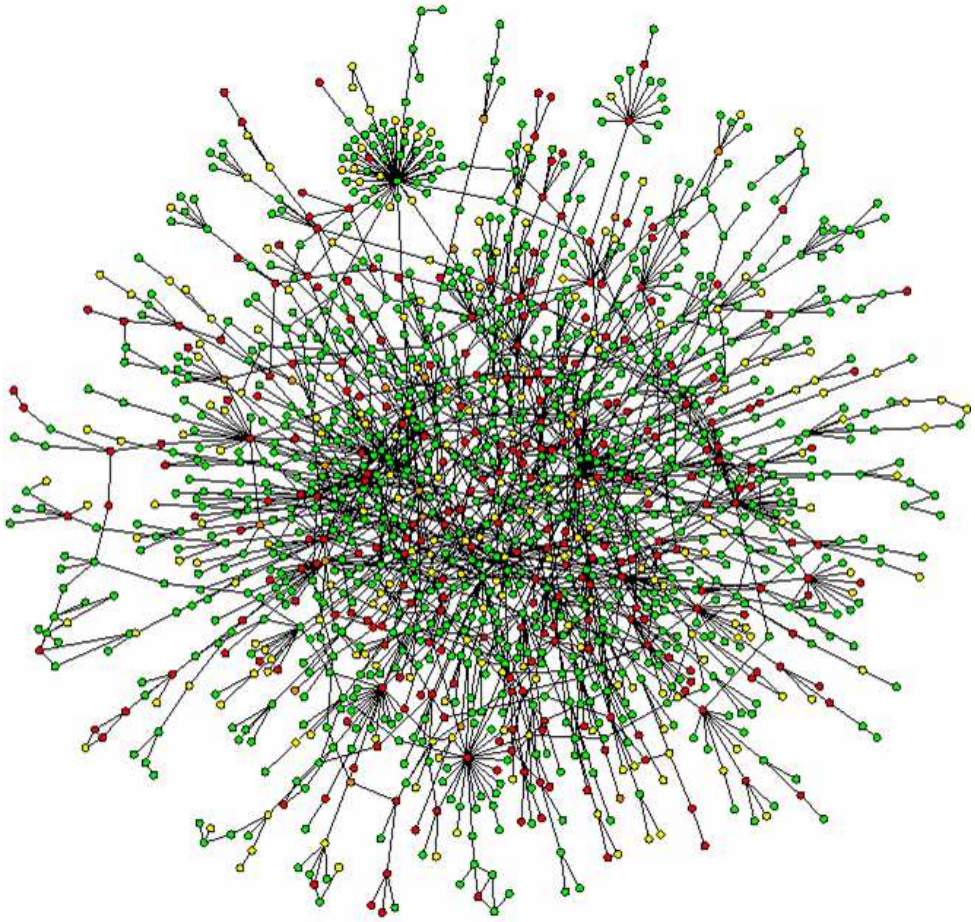
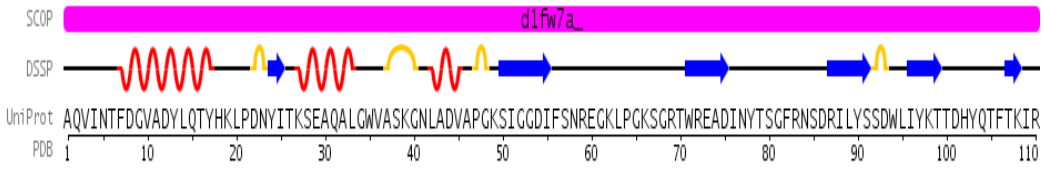


Figure 1.2: Map of protein-protein interactions. Red for lethal; green for non-lethal; orange for slow growth; yellow for unknown interactions [5].

The interaction between barstar and barnase is one of the most strongest protein-protein interactions, with a very fast association rate of 10^8–10^9 $M^{-1}s^{-1}$ at an ionic

strength of 50 mM. Barnase is a small (110 residues) extracellular ribonuclease from *Bacillus amyloliquefaciens* which is a bacterial species and a member of guanine-specific microbial ribonucleases. *B. amyloliquefaciens* is also known as the source of BamH1 restriction enzyme and a source of subtilisin, an enzyme that catalyzes the breakdown of proteins as the way trypsin does. Barstar; intracellular inhibitor of barnase is a 90-residue polypeptide; which has co-evolved to bind tightly and rapidly to barnase through salt bridges and hydrogen bonds. Barstar is a natural antibiotic that is synthesized by *B. amyloliquefaciens* [6]. Barnase and barstar are rather small proteins, with diameters of about 21.8Å and 28Å, respectively [7]. Barstar binds tightly to barnase inhibiting its RNase activity (potentially lethal functions). Once barstar bound to barnase, barstar sterically blocks the active site of barnase with an alpha helix and the loop segment connecting it to the adjacent helix. The presence of more than 35 water molecules within 4.5 angstroms of both protein molecules is the evidence for formation of the barstar-barnase complex is dependent on pH [8].

(a)



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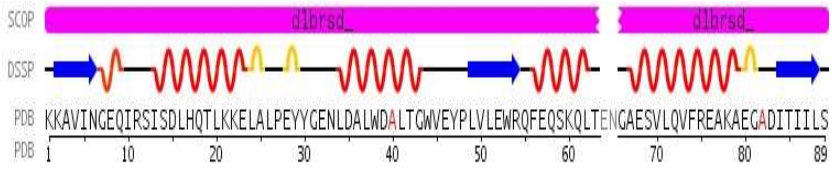


Figure1.3: (a) Primary structure of barnase and **(b)** Primary sturcture of barstar [9].

The barstar-mediated inhibition of any barnase activity in vivo is necessary for survival of barnase producing cells. Their binding interface consists of mainly polar and charged residues, and shows a high electrostatic complementarity [10, 11]. Barnase is found to be catalytically active. The active site of barnase an its binding site for barstar has the same subset of amino acids, whereas the only exception is Glu73 (the main base in catalysis). Glu73 is located at the centre of the binding site

and is seperated by three water molecules from barstar [11]. What is more is that, barstar functions by blocking the active site rather than affecting conformational changes [8]. Fig. 1.4 shows the interacting active sites of barnase and barstar.

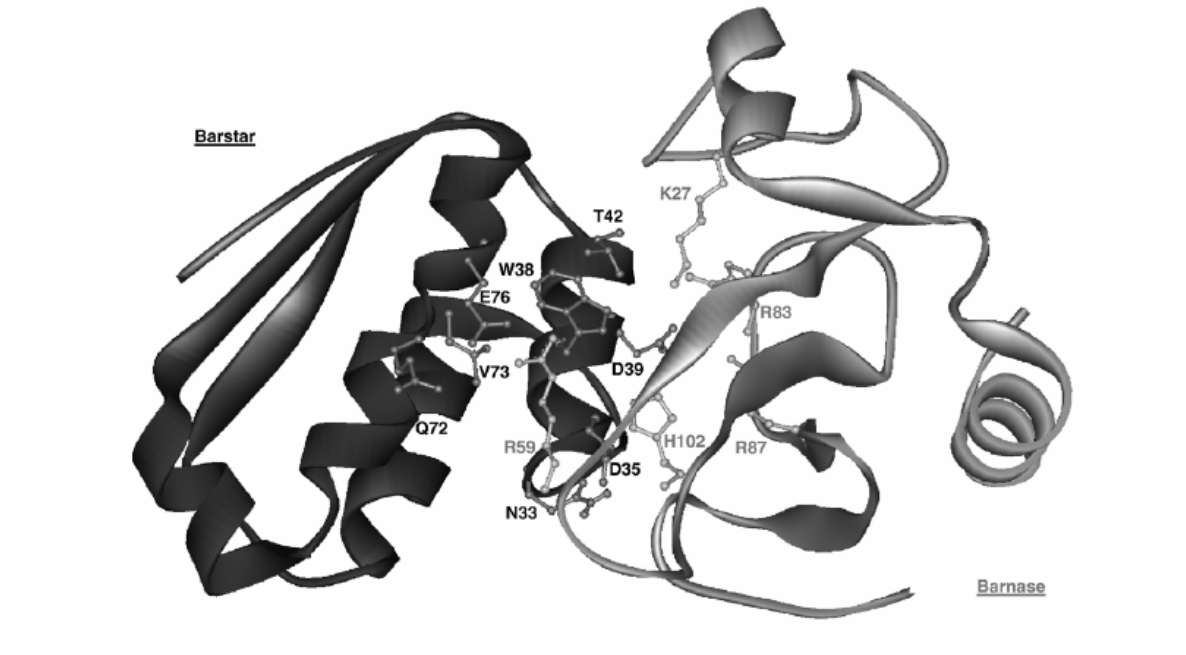


Figure 1.4: Representation of barnase-barstar interaction and important residues in this interaction [4].

Barnase-barstar system might serve as a model for use in other toxin-inhibitor recognition studies, in general protein-protein interaction studies [12]. During these studies the focus is on interactions between two different proteins. Oligomerization that can be explained as one protein interacting with other copies of itself, or three or more different proteins interacting should be in interest. How many of each protein involved are present in a given reaction, the stoichiometry of the interaction, the affinity of the interaction and the energy changes during binding are the min important aspects of protein interactions.

Computational studies those are focusing on protein interactions are the first tempting method for scientist in recent years. Results from computational structure or interaction studies help men of science predict pathways in cells, potential drugs, antibiotics and protein functions. On the other hand; proteins are large molecules and binding between them mostly involves many atoms and many interaction types

which are hydrogen bonds, hydrophobic interactions, salt bridges and etc [13]. Examining these interacitons, by making small changes, gives ideas about the natural and desired forms of protein couples. Site-specific mutagenesis is one of the methods in order to change the environment of interaction proteins and comment on the different results.

2. METHODS AND THEORY

While experimental techniques, such as isothermal calorimetry (ITC) or surface plasmon resonance (SPR) are very powerful in supplying researchers macroscopic aspects, they do not provide an adequate rationalization for protein–protein interaction energies in terms of separate energetic contributions, such as electrostatic and/or van der Waals contributions. Still, computational methods have the advantage of evaluating each energetic contribution involved in protein–protein association, which allows us to explore these contributions for the entire interface or for individual residues. Many of the computational tools that predict interactions are based on the energy of interactions [13]. Computational methods used are based on molecular mechanics (MM) calculations, applying usual MM force fields such as AMBER and CHARMM.

2.1 Software

The molecular modeling software HyperChem (Hypercube, Inc.) has been used for molecular visualization of conformers and geometry optimizations. Besides molecular mechanics (MM); HyperChem is also capable of performing molecular dynamics (MD), semi-emprical and ab-initio molecular orbital calculations.

HyperChem software is good at recognizing Hydrogen bonds. The algorithm which explains “hydrogen bond is formed if the distance between hydrogen and donor is less than 3.2 Å and the angle made by covalent bonds to the donor and acceptor atoms is less than 120°” is included by HyperChem software algorithms.

2.2 Hardware

All geometry optimizations were done with Intel P4 3.0 Gz processor with 1GB of RAM.

2.3 Molecular Mechanics

As well as classical atomistic MD simulations molecular mechanics (MM) calculation model is the energy of a molecule that can be described in terms of a function called the *force field* that depends only on the atomic positions, to a great extend a simplifying assumption. Clearly this function should provide a good description of the forces acting within the molecule. By the way, the energy of the molecule may be determined in terms of the internal co-ordinates, bond lengths, bond angles, dihedral angles of the atoms those diagramed in Figure 2.1 [14].

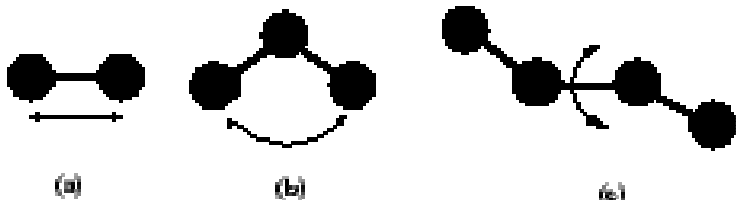


Figure 2.1: Representation of force field terms. a) Bond stretching, b) bond angle bending, and c) dihedral rotation [14].

In addition, Figure 2.2 below also summarizes the rotations and stretching of bonds.

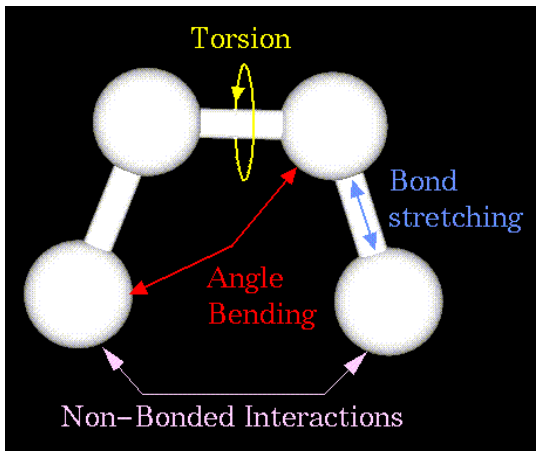


Figure 2.2: Bending, stretching and rotation of bonds.

Geometry optimizations have been carried out by Molecular Mechanics (MM) force fields because of their speed in calculation of large molecules. As in quantum

mechanics, electronic distribution in a molecule is not considered in MM calculations; obtaining quick calculations are the result of atomic distribution considerations. Atomic distributions includes electrons in a system, and dealing with electrons cause loss of huge time due to complex calculations. On the other hand; MM force field calculations deal with nuclear positions which are opening-closing of angles, rotations about single bonds and stretching of bonds. During MM calculation running, selected bonds are rotated and the system tries to detect the interactions of non-bonded parts of the protein complex as in this study.

The only laborious part of MM calculations is that the force fields are emprical. Every different group of molecule is favorable for different functional form of force field. For generalization main classes of molecules; those should be proteins, nucleic acids etc., are being calculated by AMBER and CHARMM force fields. Differences between AMBER and CHARMM force fields result from the different energy functions. Different function of the degrees of freedom in a molecule (bonds, angles, dihedrals) gives diversity to energy function calculations. In this study, CHARMM22 force field which is most suitable for proteins is used.

MM calculations deal with nuclear properties and for that reason atomic properties used in molecular mechanics come from previous experimental data or quantum mechanical calculations; what makes MM an empirical method. Instead of calculation of everything from the beginning; MM serves favorable time for examining nucleic acids, proteins and all other macromoleculer; in other words MM is more adaptable for larger systems.

Just like quantum mechanical or semi-empirical calculations, molecular mechanics deals with potential energy of a molecular system which is the sum of the energy stemming from both covalent and non-covalent interactions as shown below [15]:

$$v\left(r^N\right)=\sum_{bonds}\frac{k_i}{2}\left(l_i-l_{i,0}\right)^2+\sum_{angles}\frac{k_i}{2}\left(\theta_i-\theta_{i,0}\right)^2+\sum_{torsions}\frac{V_n}{2}\left(1+\cos\left(n\omega-\gamma\right)\right)$$

$$+\sum_{i=1}^{N}\sum_{j=i+1}^{N}\left(4\varepsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12}-\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right]+\frac{q_iq_i}{4\pi\varepsilon_0 r_{ij}}\right) \qquad \textbf{(2.1)}$$

$\nu(r^N)$ is the potential energy depending on the positions (r) of N particles. The first two terms (sum of bonds and angles), namely bond stretching and angle bending are both modeled by a simple harmonic potential [15]. The third term (sum of torsions) describes how the energy changes when a bond rotates. The fourth term represents non-bonded terms between all pairs of atoms which are separated by at least three bonds [15]. Here below, Figure 2.3 summurizes how the potential energy of a molecular system is changed with respect to changes in six most common parameters of MM calculations.

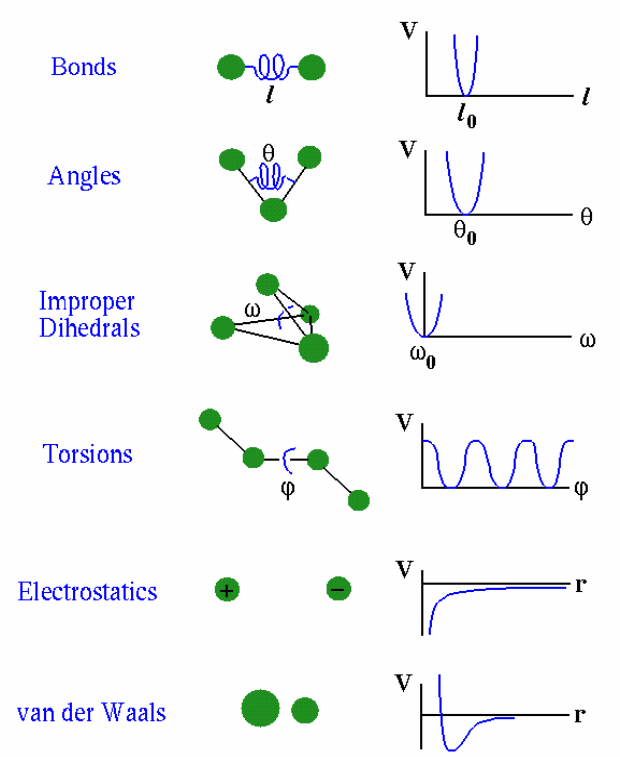


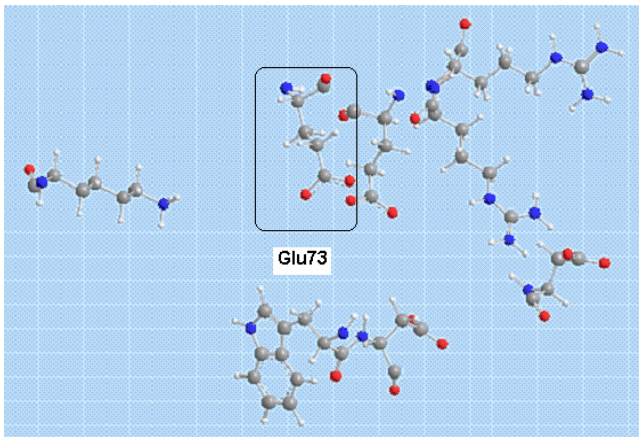
Figure 2.3: Explanation of MM representation of potential energy function.

Molecular mechanics is capable of producing worthless data on macromolecular structures. While X-ray crystallographic studies are limited to the sequences that are able to form crystals and need good resolution, NMR techniques experience some problems as they lack long-range distance restraints; MM calculations is therefore stand in the breach [16].

2.4 Mutations of Glu73

Interaction of barnase-barstar interface is firstly examined with the original form of the complex.

(a)



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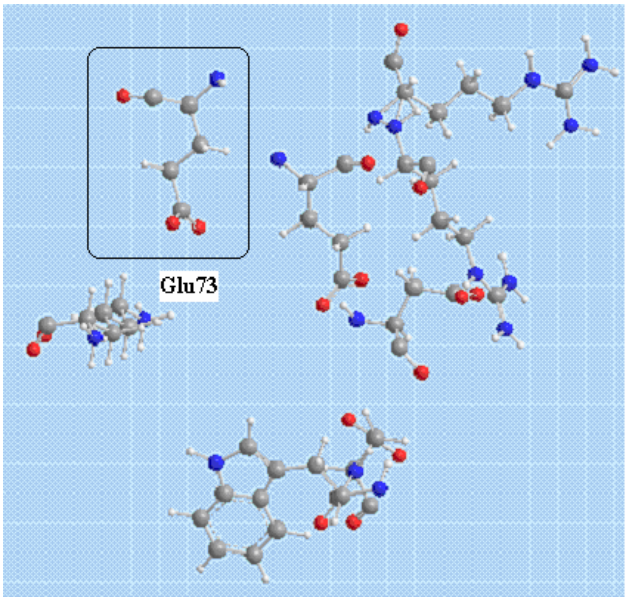

Figure 2.4 (a) Interface of barnase-barstar complex. Amino acids of barnase from left to right are; Lys27 Glu73 Glu75 Arg83 Arg87, and amino acids of barstar from left to right are Trp38 Asp39 Asp35. **(b)**Interface of barnase-barstar complex, from upright angle of view. Glu73 in circle is the critical residue for recognition.

It is known from the literature that five amino acids from the active site of barnase and three amino acids from the binding site of barstar play important role in binding. The important residues for inhibition of barnase by barstar is shown in Figure 2.4. In normal conditions, more than 35 water molecules within 4.5 angstroms of barnase and barstar molecules are present. To simulate the interface; we used three water molecules between the residues of interest. The first calculations, those are the geometry optimization results obtained gave the energies of the system with different amount of water molecules.

Subsequently, Glu73 residue has been changed with four different amino acids; alanine (Ala), lysine (Lys), aspartic acid (Asp), and glutamine (Gln). All these mutations have been carried out in three diffent conditions; with zwitterionic form of proteins, with the form of proteins in gaseous surroundings and with CH_3 addition to the terminal groups of protein sequences.

2.5 Structural and Enviromental Conditions

First, zwitterionic forms of terminal residues of the sequences have been examined. When an amino acid dissolved is in water, it exists in solution as the dipolar ion, or zwitterion (hybrid ion) form.

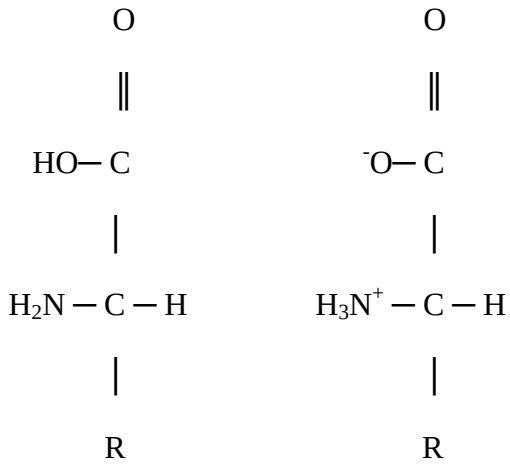

Figure 2.5 Nonionic and zwitterionic form of amino acids. The nonionic form does not notably occur in aqueous solutions, it is the form found in gaseous environments; whereas the zwitterion predominates at neutral pH.

In the gaseous environment, as we examined in the second order; amino acids are found to be in their nonionic form. Figure 2.5 shows the nonionic and zwitterionic forms of amino acids.

Lastly, geometry optimizations have been concluded with CH_3 addition to the terminal groups of protein sequences. CH_3 addition to nitrogen and oxygen of amino acid instead of H atoms is demonstrated in Figure 2.6.

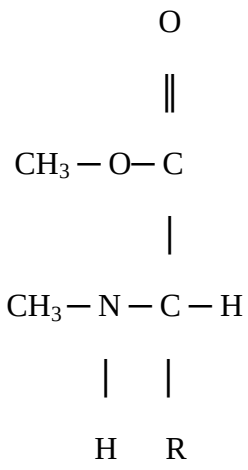

Figure 2.6 CH_3 addition instead of H atoms to Nitrogen and Oxygen of amino acid.

3. RESULTS AND DISCUSSION

We choosed the residue Glu73 as the center of interest because Glu73 is placed at a very critical point for the recognition of barnase by barstar. It is located at the centre of the binding site and separated by three water molecules from barstar. Although Glu73 does not interact directly with barstar, it is known from the literature that there is an electrostatic repulsion between Glu73 on barnase and the negatively charged binding surface of barstar. Besides there is an organizing role of the carboxylate of Glu73. It coordinates neighbouring positively charged groups in barnase, Lys27, Arg83, and Arg87 to interact with Asp39 in barstar. All the important functions of Glu73 mentioned above canalized us to focus on Glu73 mutations. In the cause of comment the role of Glu73 and the role of enviromental conditions, we used three different surroundings. In addition, the significance of presence of water molecules has been tested.

3.1 Significance of Water Molecules

Firstly, we reduced the number of water molecules located in the interface of barnase and barstar. As shown in Figure 3.1, in the original form of the complex there are three water molecules. We examined the system with three, two and one water molecules. In the case of one water molecule between barnase and barstar, three different combinations according to the location of water molecule have been tested. The acquired energy of formations from the geometry optimization calculations, it was demonstrated that number of three water molecules is necessary and favorable for the recognition of barnase by barstar. To state the matter differently, the results obtained showed in the case of presence of three water molecules the system gave the lowest energy; that is the most stable conformation.

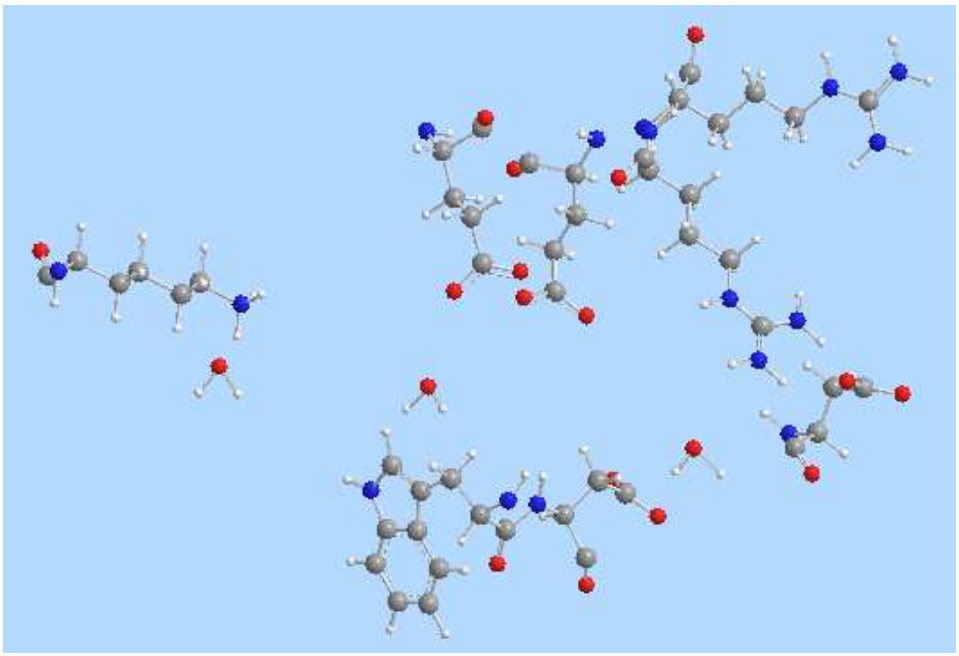

Figure 3.1: Barnase and Barstar binding sites including three water molecules between them.

In the case of two water molecules, we omitted the water molecule in the middle. It is seen in the Figure 3.2.

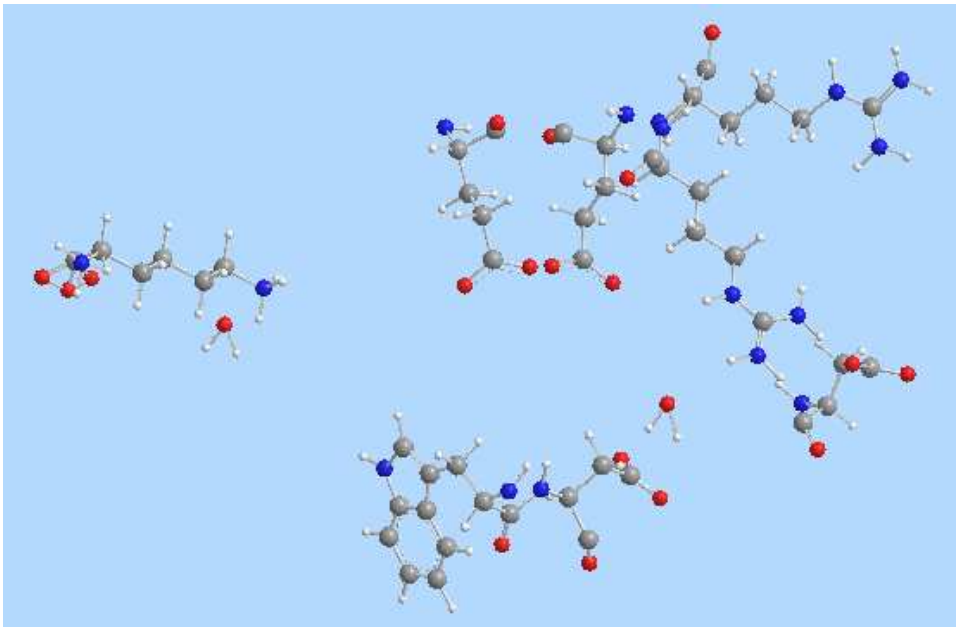

Figure 3.2: Barnase and Barstar binding sites including two water molecules between them.

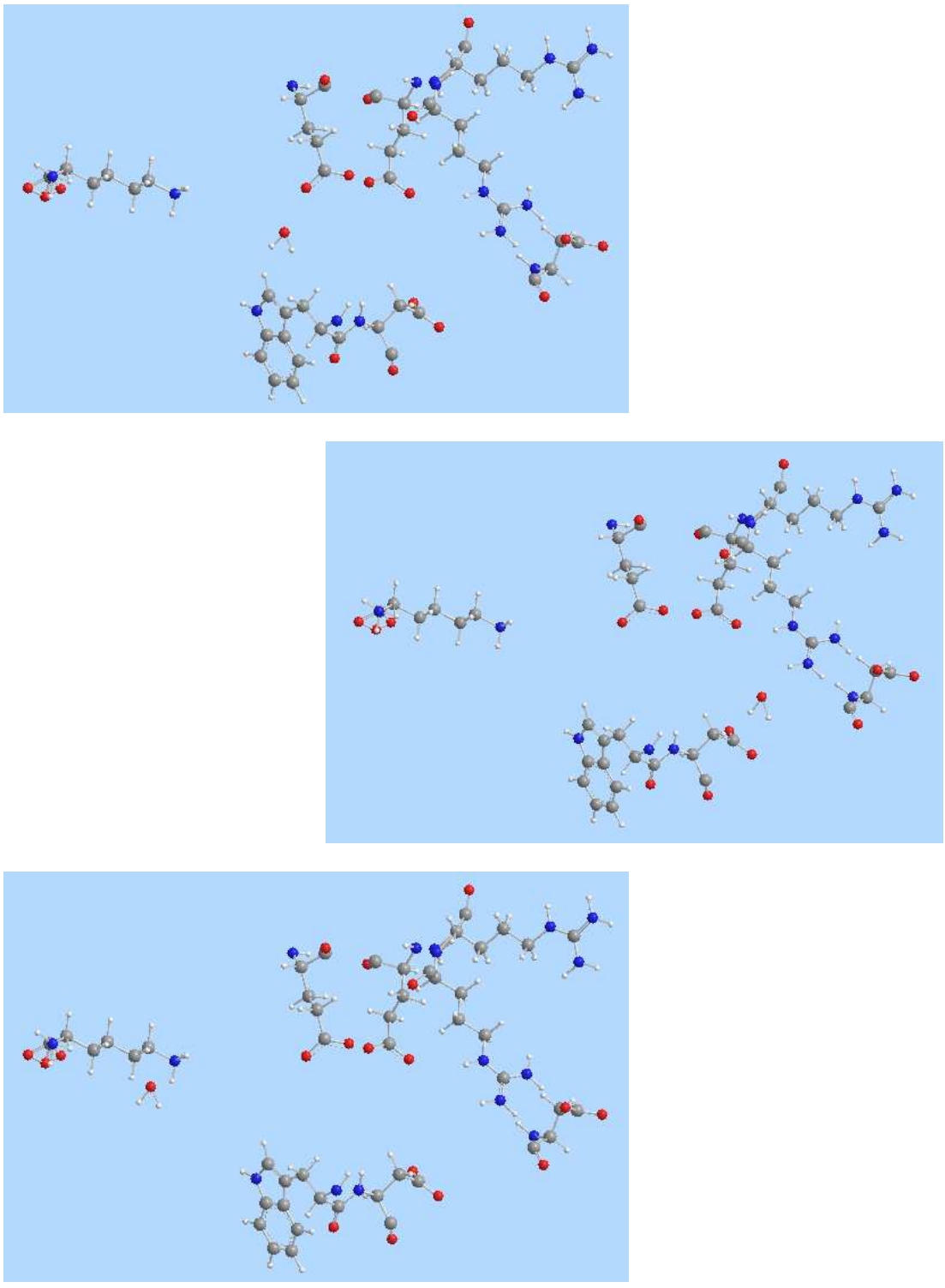

Figure 3.3: Barnase and Barstar binding sites including one water molecule between them, in three different combinations.

Figure 3.3 shows the water molecules located at different points in the interface. The first water molecule is in the middle of the between, the second water molecule is

located in front of the Arg87 of barnase and the third water molecule in the third representation is located in front of Lys27 of barnase. Table 3.1 summurizes the energies obtained with different number and different combination of water molecules.

Table 3.1 : Energies of barnase-barstar complex in the case of different number of water molecules (the lowest energy is written in red).

Number of H_2O molecules	Energy (kcal/mol)
1 H_2O on the left	-405.33
1 H_2O on the rigth	-383.55
1 H_2O in the middle	-383.51
2 H_2O	-375.60
3 H_2O	-408.40

3.2 Glu73 → Ala73 Mutation

Firstly, Glu73 residue has been mutated to alanine which is an hydrophobic amino acid and has the second ordered simplest chemical structure in all amino acids. The side chain (R-group) of alanine is one of the smallest ones among the other amino acids. Its R-group only consists of a CH_3 group.

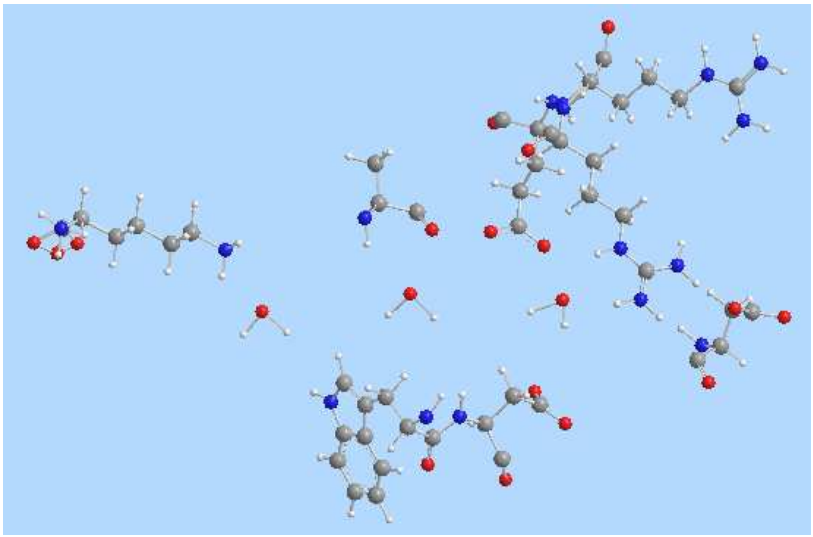

Figure 3.4: Glu73 → Ala73 mutation in the case of zwitterionic form.

Since the R-group of alanine is nonpolar and hydrophobic, it tends to cluster within proteins, stabilizing protein structure by means of hydrophobic interactions.

In all three different enviromental conditions, three water molecules have been used as in the natural form of barnase-barstar complex. Figure 3.4 represents the zwitterionic form of terminal group in the case of alanine mutation instead of glutamic acid. Zwitterionic form of an amino acid shows its dissolved form in water. In our study the water molecules we have within the system may enforce the amino acid terminals obtain their zwitterionic forms. Just the opposite, as shown in Figure 3.5, we examined the form of barnase- barstar complex as if it is in a gaseous environment. In this case, the terminal residues were in their non-ionic forms; and because of that we obtained different results with different energies.

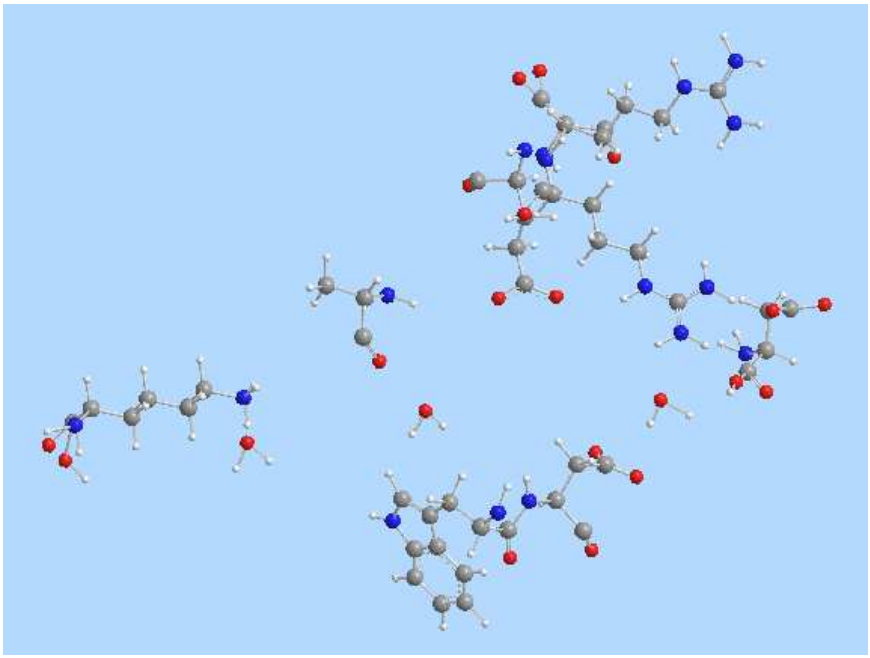

Figure 3.5: Glu73 → Ala73 mutation pretended to be in a gaseous environment.

We also examined Glu73 → Ala73 mutation in the case of CH_3 additions to carboxyl and amino ends of terminal residues. In Figure 3.6, alanine mutation is presented in the case of CH_3 additions. Addition of CH_3 groups to carboxyl and amino ends of terminal residues was also included to the study because the residues we were examining were not standing alone; in other words the terminal residues were bounded to the adjacent amino acids in the structure of barnase composed of 110

amino acids and barstar composed of 90 amino acids. By the help of this approach, we tried to mimic the whole barnase-barstar complex.

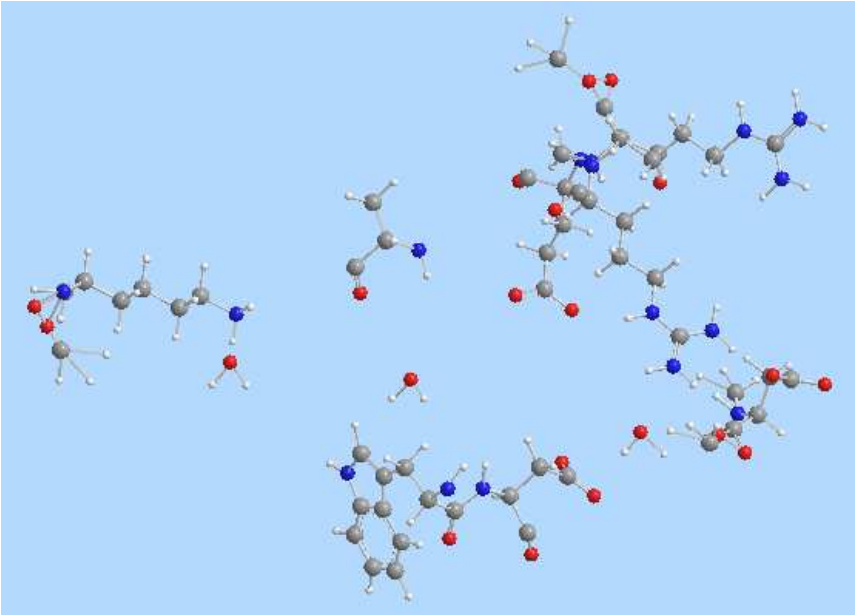

Figure 3.6: Glu73 → Ala73 mutation in the case of CH_3 additions to carboxyl and amino ends of terminal residues.

Below the Table 3.2 shows the energies of Gu73 → Ala73 mutation in three different conditions.

Table 3.2 : Energies of Glu73 → Ala73 mutation in the cases of zwitterionic form, nonionic form (in gaseous environment) and the form when CH_3 additions to carboxyl and amino ends of terminal residues.

Enviromental conditions	Energy (kcal/mol)
Zwitterionic form	-341.05
Nonionic form	-276.60
CH_3 addition	-227.95

3.3 Glu73 → Lys73 Mutation

As the second set of the study, Glu73 residue has been mutated to lysine whose side chain is positively charged. Lysine is one of the most hydrophilic amino acids and has a significant positive charge at neutral pH. Since the R-group of lysine is

positively charged and hydrophilic, it still has a different chemical structure from glutamic acid. Thereof, lysine gives different energy and conformations when located instead of Glu73 in barnase structure. As applied in alanine mutation, three water molecules have been used also in lysine mutation.

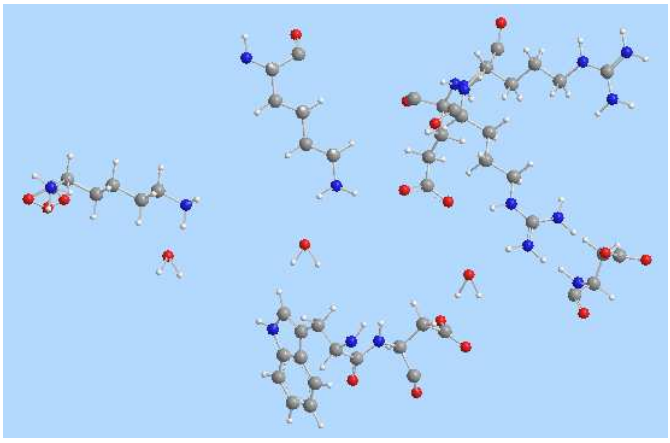

Figure 3.7: Glu73 → Lys73 mutation in the case of zwitterionic form.

As completed with alanine mutation, the same order of calculations were performed. Figure 3.7 represents the zwitterionic form of terminal group in the case of lysine mutation instead of glutamic acid.

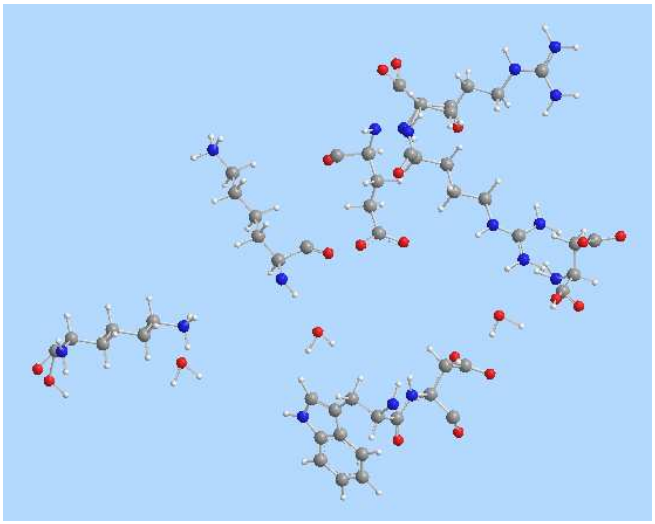

Figure 3.8: Glu73 → Lys73 mutation pretended to be in a gaseous environment.

Finally, we also examined Glu73 → Lys73 mutation in the case of CH_3 additions to carboxyl and amino ends of terminal residues. In Figure 3.9, lysine mutation is presented in the case of CH_3 additions.

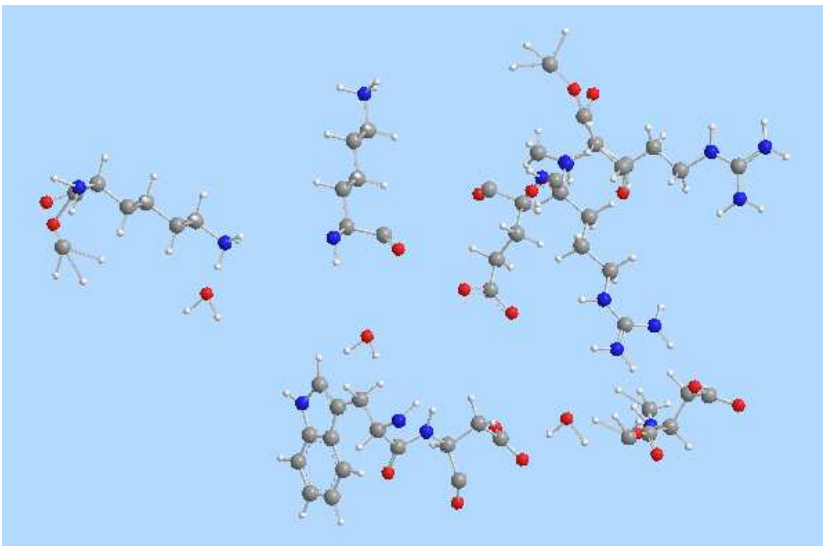

Figure 3.9: Glu73 → Lys73 mutation in the case of CH_3 additions to carboxyl and amino ends of terminal residues.

Here Table 3.3 below shows the energies of Gu73 → Ala73 mutation in three different conditions.

Table 3.3 : Energies of Glu73 → Lys73 mutation in the cases of zwitterionic form, nonionic form (in gaseous environment) and the form when CH_3 additions to carboxyl and amino ends of terminal residues.

Enviromental conditions	Energy (kcal/mol)
Zwitterionic form	-356.23
Nonionic form	-278.431
CH_3 addition	-234.504

3.4 Glu73 → Gln73 Mutation

In the third set of the study, Glu73 residue has been mutated to glutamine which is polar and uncharged. The polarity of glutamine is contributed by its amide group. Having a functional group that can form hydrogen bonds with water, glutamine

should also be regarded as a hyrophilic amino acid. As applied in previous two mutations, three water molecules have been used also in glutamine mutation.

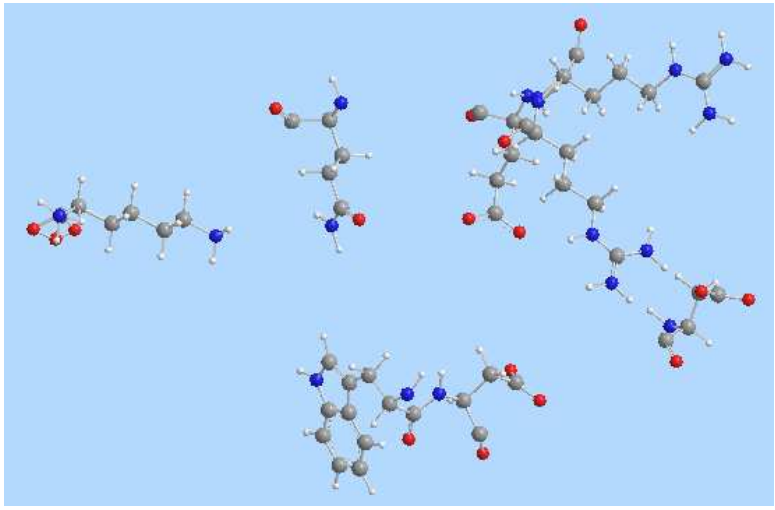

Figure 3.10: Glu73 → Gln73 mutation in the case of zwitterionic form.

Subsequent to the condition of zwitterionic form, nonionic form (in gaseous environment) and the form when CH_3 additions to carboxyl and amino ends of terminal residues have also been examined in the case of glutamine mutation. Figure 3.10 represents the zwitterionic form of terminal group in the case of glutamine mutation instead of glutamic acid.

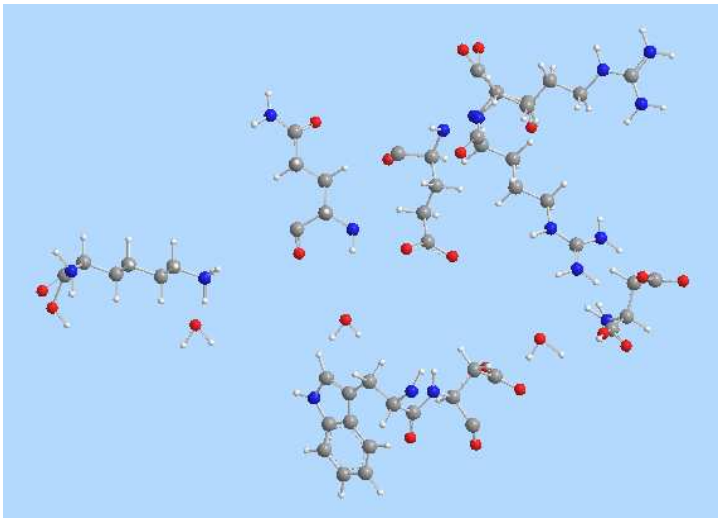

Figure 3.11: Glu73 → Gln73 mutation pretended to be in a gaseous environment.

Figure 3.11 and Figure 3.12 respectively show nonionic and CH_3 added forms.

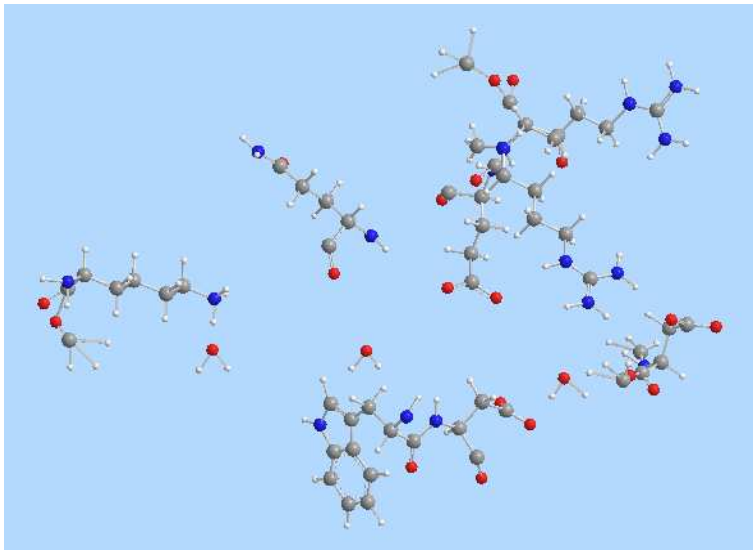

Figure 3.12: Glu73 → Gln73 mutation in the case of CH_3 additions to carboxyl and amino ends of terminal residues.

Table 3.4 below gives the energies of Gu73 → Gln73 mutation in three different conditions.

Table 3.4 : Energies Glu73 → Gln73 mutation in the cases of zwitterionic form, nonionic form (in gaseous environment) and the form when CH_3 additions to carboxyl and amino ends of terminal residues.

Enviromental conditions	Energy (kcal/mol)
Zwitterionic form	-355.55
Nonionic form	-322.38
CH_3 addition	-227.39

3.5 Glu73 → Asp73 Mutation

In the third set of the study, glutamic acid at the 73rd residue has been mutated to aspartic acid whose side chain is negatively charged. As glutamic acid, aspartic acid has a second carboxyl group in its R-group. Indifferent to prior mutations, three water molecules with three different conditions have been used also in aspartic acid mutation.

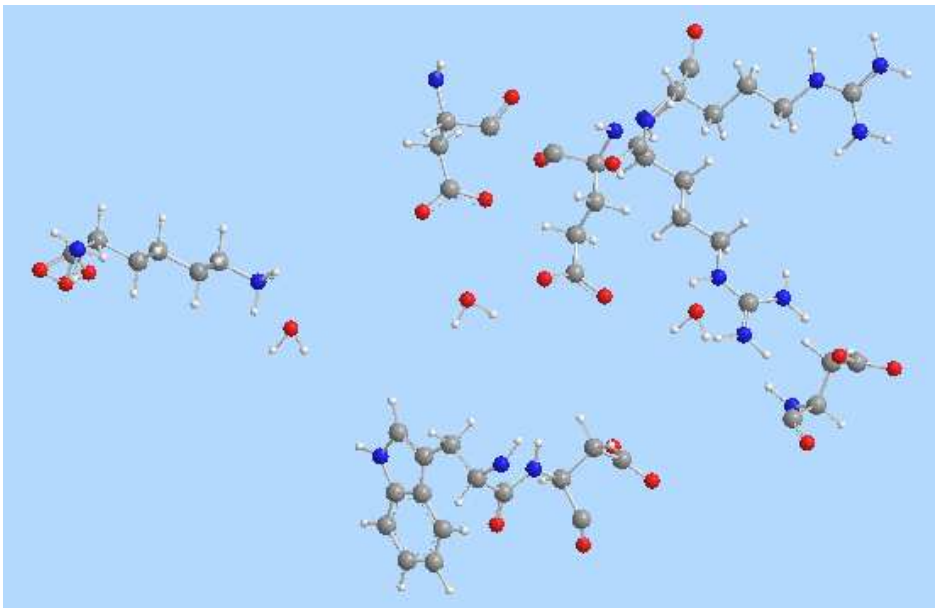

Figure 3.13: Glu73 → Asp73 mutation in the case of zwitterionic form.

Following the condition of zwitterionic form, nonionic form (in gaseous environment) and the form when CH_3 additions to carboxyl and amino ends of terminal residues have also been examined in the case of aspartic acid mutation as shown in Figure 3.14 and Figure 3.15.

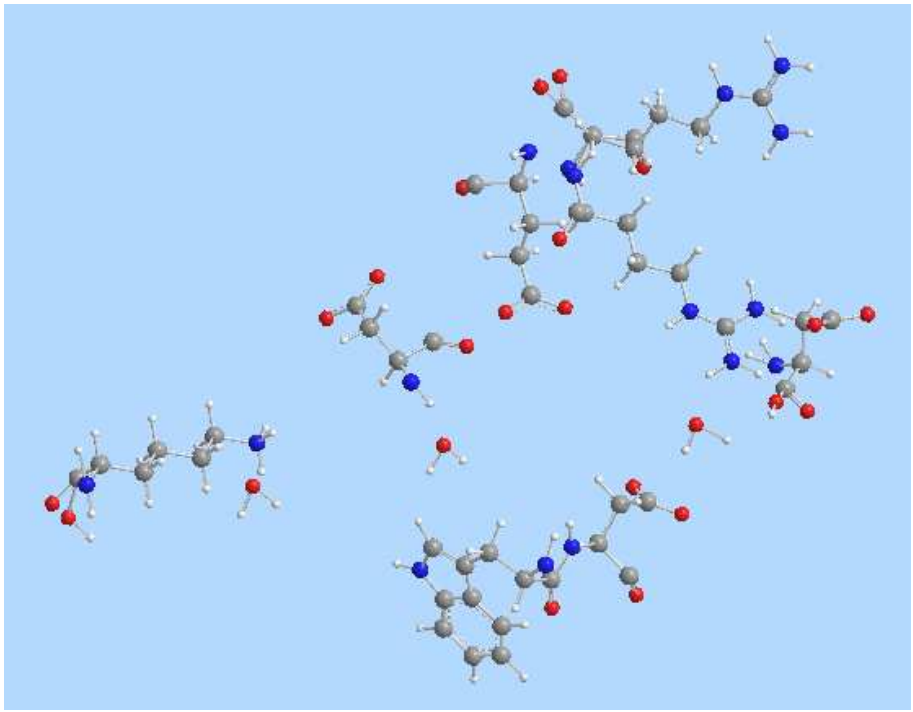

Figure 3.14: Glu73 → Asp73 mutation pretended to be in a gaseous environment.

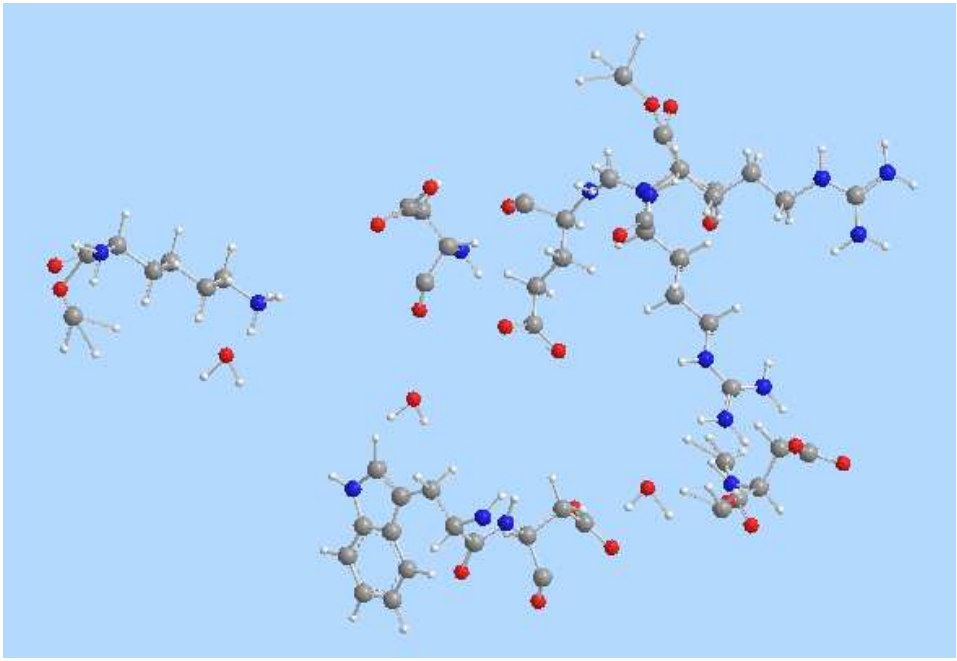

Figure 3.15: Glu73 → Asp73 mutation in the case of CH_3 additions to carboxyl and amino ends of terminal residues.

Finally Table 3.5 gives the energies of the interaction region of barnase-barstar complex with aspartic acid instead of glutamic acid at the 73rd position of barnase primary structure.

Table 3.5 : Energies Glu73 → Asp73 mutation in the cases of zwitterionic form, nonionic form (in gaseous environment) and the form when CH_3 additions to carboxyl and amino ends of terminal residues.

Enviromental conditions	Energy (kcal/mol)
Zwitterionic form	-432.08
Nonionic form	-362.02
CH_3 addition	-226.21

3.6 Glu73 at Its Original Location Without Any Mutations

In order to understant the role of Glu73 in the recognition process, we experimented all the different enviromental conditions without any mutations.

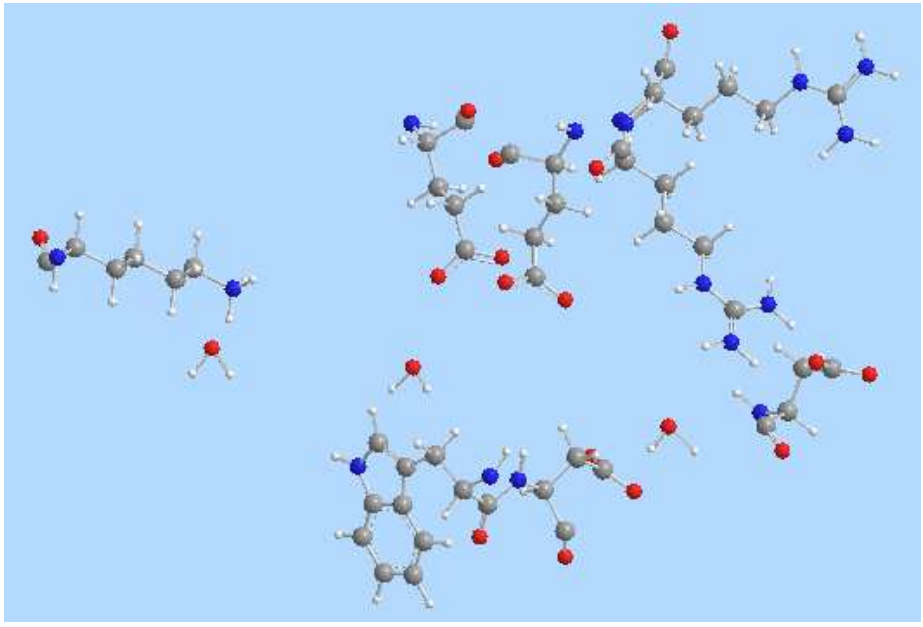

Figure 3.16: Zwitterionic form of barnase-barstar complex.

Here three figures named as Figure 3.16, Figure 3.17 and Figure 3.18 respectively show the zwitterionic form of terminal groups, nonionic form of terminal groups and CH_3 added form of the non-mutated barnase-barstar complex.

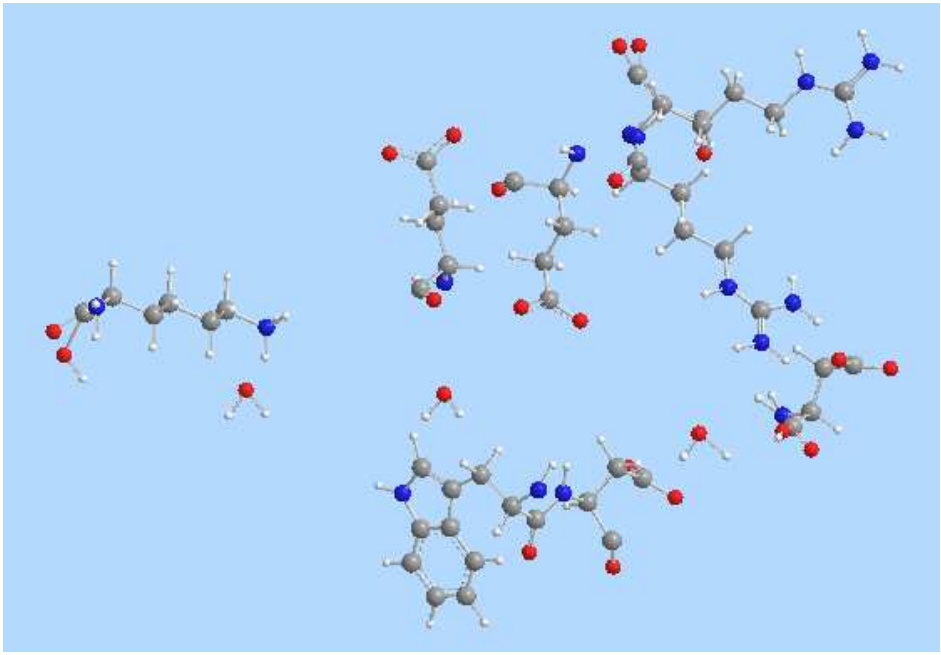

Figure 3.17: Nonionic form of barnase-barstar complex.

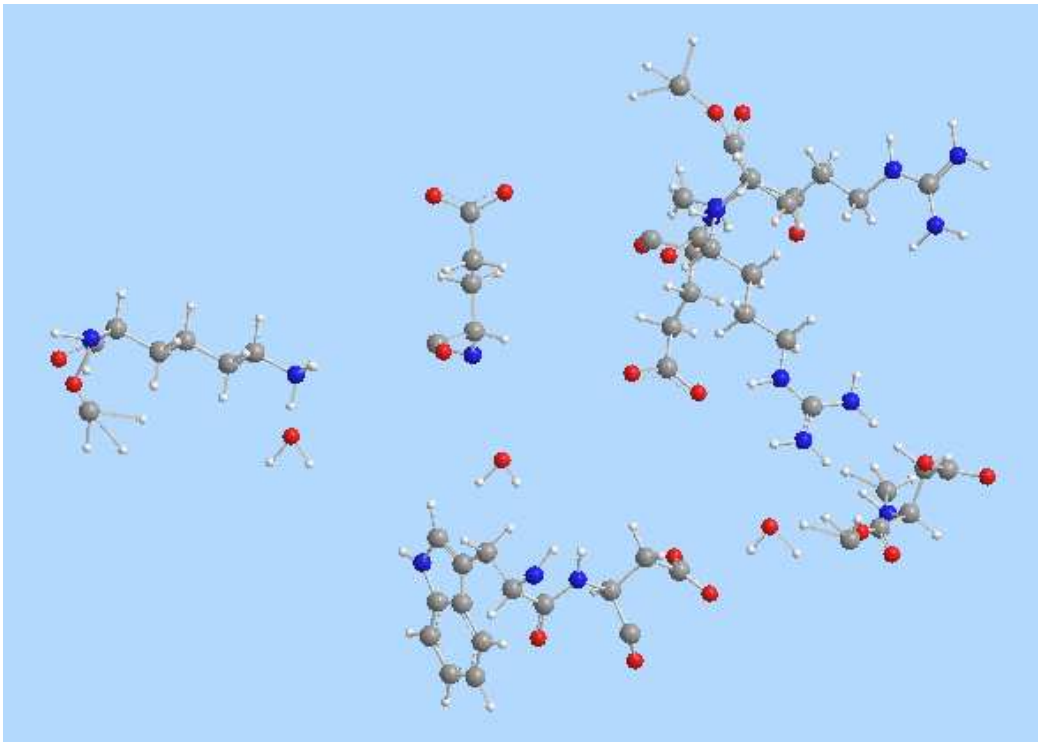

Figure 3.18: CH_3 additions to carboxyl and amino ends of terminal residues of barnase-barstar complex.

At last, Table 3.6 summarizes the energies of the interface in three different cases we applied all previously as the same.

Table 3.6 : Energies of barnase-barstar interface in the cases of zwitterionic form, nonionic form (in gaseous environment) and the form when CH_3 additions to carboxyl and amino ends of terminal residues.

Enviromental conditions	Energy (kcal/mol)
Zwitterionic form	-408.40
Nonionic form	-309.50
CH_3 addition	-260.80

All the energy of formations we obtained from the study should be listed as shown in Table 3.7. These results enable us to comment on. The mutations done gave different results according to the chemical structure of amino acids and environmental conditions. The four different amino acid; alanine, lysine, glutamine and aspartic

acid have been choosen according to their different side chains showing different properties within aqueous solutions. Alanine replacement instead of Glu73 gave the highest energy of formations as expected because alanine behaves showing hydrophobic properties and so that in aqueous solutions it is not very suitable for the system. Alanine has been choosen instead of glycine. Gyline is also a hydrophobic amino acid and has small R-group only consist of an hydrogen atom. What we wanted to test was the power of hydrophobic interactions enough to disorder the system, but only an H atom was not sufficient for that. The R-group of alanine which is CH_3 did its work well.

Table 3.7 : Energies of non-mutated and mutated forms in all three different conditions applied.

Mutations	Enviromental conditions	Energy (kcal/mol)
Glu73	Zwitterionic form	-408.40
	Nonionic form	-309.50
	CH_3 addition	-260.80
Ala73	Zwitterionic form	-341.05
	Nonionic form	-276.60
	CH_3 addition	-227.95
Lys73	Zwitterionic form	-356.23
	Nonionic form	-278.43
	CH_3 addition	-234.50
Gln73	Zwitterionic form	-355.55
	Nonionic form	-322.38
	CH_3 addition	-227.39
Asp73	Zwitterionic form	-432.08
	Nonionic form	-362.02
	CH_3 addition	-226.21

Second, lysine mutation has been concluded. Lysine is one of the most hydrophilic amino acids and has a significant positive charge at neutral pH, however its R-group has a long chain consists of four CH_2 groups those should be interacting with water molecules. With the intention that, the energies obtained from Lys73 mutation were not favorable enough in all conditions.

Third, glutamine mutation has been carried out. Glutamine is a polar and uncharged amino acid. Altough it can be regarded as a hydrophilic amino acid, in all three different conditions the results were not satisfactory. Glutamine gave the best result in gaseous, non-charged environment, but great attention should not be paid to this outcome, cause the results obtained from zwitterionic and CH_3 added forms were not pointed out as the same.

Terminally, glutamic acid has been changed with aspartic acid, which is very similar in structure; is negatively charged and has a second carboxyl group in its side chain. In zwitterionic form and gaseous environment, aspartic acid gave the best energies, but in the case of CH_3 addition; the lowest energy we obtained from the Asp73 mutation. The condition when we added CH_3 groups to the terminal residues; we could imitated the entire barnase-barstar complex in the best way. The CH_3 additions represented the other amino acids, away from or adjacent to the residues in the interface region. Thus, aspartic acid replacement again could not function as glutamic acid. Since, CH_3 added form reflects the original system most excellent, the result obtained from this condition were more realistic. In our study as in the natural form, glutamic acid at its original 73^{rd} position gave the lowest energy, means the most favorable result. By these results, it was proven that Glu73 is indispensable for its location and recognition. Indirect interactions are more important between Glu73 and residues of barstar (especially Asp39) than direct electrostatic ones; the carboxylate of Glu73 organizes neighbouring positively charged groups in barnase, Lys27, Arg83, and Arg87 to interact with Asp39 in barstar.

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RESUME

Banu Buruk was born in Izmit in 1982. She was graduated from Karsiyaka Anatolian High School in 2001. She had her Bachelor's Degree from Istanbul Technical University, Department of Molecular Biology and Genetics in 2005. Since December 2006 she has been working as an assistant expert of scientific programs in TUBITAK, Ankara.