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Mutation Based Structural Modelling and Dynamics Study of Alpha Fetoprotein: An Insight to Inhibitory Mechanism in Breast Cancer

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Abstract

The incidence of breast cancer is amassed in the current era due to the advent in urbanization, increase in sophisticated vicissitude living, and espousal of western lifestyles. Alpha- fetoprotein, a serum glycoprotein produced during embryonic development tends to act as the curative mediator and has anti-estrotrophic properties to inhibit the growth of estrogen- dependent tumor's in breast cancer and metastasis. This oncofetal protein exhibits pharmaceutical activity during en route cancer metastasis and pathways lead to tumor cell progression and proliferation. In this work, the maximal inhibitory action of the peptide derived from the active site segment, which was previously suggested in experimental works against mice xenografts (8-mer Peptide), was derived from the structural model generated by homology modelling that retains the inhibitory activity exhibited by the derived AFPep P489- P496 (EMTPVNPG). A comparable mutation study has been undertaken in the derived peptide region to maximize the inhibitory action of the above-said activities. Comparative molecular dynamics study of each mutation has been carried out to know the stability of the octapeptide 489-496 to ensure the curative perspective that is indulged in inhibiting the progression and proliferation of oncofetal proteins in breast cancer. Another modification to the derived peptide was done by addition of hydroxyproline group to the region selected that was previously suggested with the combined effect of tamoxifen and hydroxyproline associated peptide. Molecular docking studies have also been carried out for the octapeptide against Hsp70 which might help in stabilising the anti tumour associated peptide AFPep for better binding efficacy for maximal inhibitory action and treatment of breast cancer.

Keywords: Breast cancer; Alpha fetoprotein peptide; Anti-estrotrophic; Cluspro2.0; Molecular dynamics; Hydroxyproline

Introduction

The incidence of breast cancer is amassed in the current era due to the advent in urbanization, increase in sophisticated living style, and espousal of western lifestyles as reported by CDC and WHO in Hispanic woman [1]. It is estimated that worldwide over 508000 women died in 2011 due to breast cancer [2]. Although breast cancer is thought to be a disease of the developed world, almost 50% of breast cancer cases and 58% of deaths occur in less urban localities (GLOBOCAN 2008). Breast cancer endurance rates fluctuate greatly worldwide, ranging from 80% or over in North America, Sweden and Japan to around 60% in middle- income countries and below 40% in low-income countries [3]. The low persistence frequencies in under developed countries can be illuminated mainly by the lack of Proper awareness, early detection programs, consequential in a high ratio of women exhibiting with late-stage disease, as well as by the privation of ample diagnosis and treatment. The increase in the risk of breast cancer in women is due to the indiscretion in menarche and menopause before or after certain age respectively, which may have an effect in life style, revelation to hormones who conceive their first child after the age of 30 or who has never conceived are also at an increased risk. Deceptively, hormonal imbalance in reproductive organs of female accelerates cell division in breast tissue, in which it imposes an enhancement of risk of mutations. As reported earlier in several cases studies the continuous usage of oral contraceptives may gradually increase the risk of breast cancer, but women who have used oral contraceptives for less than 10 years have the same risk as women who never used them [4]. Many studies have looked at the risk of estrogen replacement therapy (ERT), while there may be a slightly increased risk with ERT; this risk is usually offset by the benefits [5]. Sophisticated vicissitude living and exposure to ionizing radiation are also the risk factors for developing breast cancer [6]. Alpha-fetoprotein (AFP) is synthesized by the human fetal liver and yolk sac during 12-14 intrauterine weeks of which it is being the highest in terms of production [7,8]. AFP gene encodes alpha-fetoprotein in humans. After birth, circulating levels of AFP drop sharply, almost disappearing from the blood of normal adults. The AFP uptake stops when embryonic cells and tissue structures approach a high degree of differentiation, even if the blood concentration of AFP is still high or increasing. The alpha-fetoprotein (AFP) receptor is an oncofetal antigen found in most types of cancer. Experimental findings showed that malignant tumor cells regain the ability to take up AFP via a receptor that would be present in undifferentiated cells of either embryonic or tumour origin, but mostly absent in normal adult cells. *In vivo* studies depicts that the existence of such a receptor for AFP was characterized in several cell lines because AFP is found in a variety of cancers and in fetal cells, the receptor falls within the definition of a wide-spectrum oncofetal antigen with potential for cancer diagnosis, screening [9]. It is an embryo related protein and is mostly pragmatic in the embryonic developmental stage when transferrin and albumin are in lesser in content [10]. The appearance of AFP is significantly higher in adults during the developmental stage and clinical findings have also shown

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the results during liver regeneration, which is also known as restorative process and malignant growth. Mammalian alpha-fetoprotein (AFP) is classified as a member of the albuminoidal gene superfamily consisting of albumin, AFP, vitamin D (Gc) protein, and alpha-albumin [11]. During developmental stages of allied biological processes discussed above, the functional aspects of AFP are inadequately comprehended. The current findings deal with the antiestrotrophic activity of AFP peptide, which can act as the therapeutic agent to treat existing estrogen-dependent breast cancer to prevent malignant tissues in breast cancer. A previous finding suggests that the antiestrotrophic activity of AFP in mouse reported at amino acids position at p447 in sequence i.e. 472-479 octamer peptide (EMTPVNPG). The 8-mer peptide has the ability to inhibit estradiol stimulated uterine growth and shows the inhibitory action which opposes the action of estrogen including promotional effects of estradiol in a development of breast cancer.

Material and Methods

Sequence retrieval and analysis

As per the literature review, the FASTA sequence of Alpha-fetoprotein has been retrieved from NCBI bearing Accession No. NP_001125.1 Human mRNA encoding alpha-fetoprotein (AFP) length 609aa. The sequence has been derived from rat and experimentally sequenced on the basis of prediction and interaction with the heat shock proteins. The sequence analysis was done using ProtParam [12]. ProtParam is a tool that allows the computation of various physical and chemical parameters for a given protein stored in Swiss-Prot or TrEMBL or for a user entered protein sequence. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY).

Structural modeling

The sequence was subjected to BlastP [13] analysis and structural homologues templates were found when it was predicted against PDB database with default parameters. 4BKE, 2I2Z, 1BKE and 1AO6 templates were selected on the basis of their score identity and E-value. The prediction of the structure is required to observe in-silico based conformation and interaction mechanism of the protein. Since no structure has been deposited in PDB, the prediction was done using MODELLER 9.14 [14] from the homologous templates data retrieved from BlastP analysis. MODELLER is used for homology or comparative modelling of protein three-dimensional structures. The user provides an alignment of a sequence to be modelled with known related structures and MODELLER automatically calculates a model containing all non-hydrogen atoms. MODELLER implements comparative protein structure modeling by satisfaction of spatial restraints, and can perform many additional tasks, including *de novo* modeling of loops in protein structures, optimization of various models of protein structure with respect to a flexibly defined objective function, multiple alignment of protein sequences and/or structures, clustering, searching of sequence databases, comparison of protein structures, etc. Basic modelling approach (Single template modelling) is taken into consideration and the listed templates were subjected to comparison method in MODELLER9.14 (compare.py) to find the best template according to R-value.

Active site prediction

The peptide sequence was in the region 489-496. The active site prediction was done using CASTp [15] and DoGSiteScorer [16]. Active site was observed in the region of interest. CASTp provides identification and measurements of surface accessible pockets as well as interior inaccessible cavities, for proteins and other molecules. It measures analytically the area and volume of each pocket and cavity, both in a solvent accessible surface (SA, Richards' surface) and molecular surface (MS, Connolly's surface). It also measures the number of mouth openings, an area of the openings and circumference of mouth lips, in both SA and MS surfaces for each pocket. DoGSiteScorer is an automated pocket detection and analysis tool that can be used for protein druggability assessment. Predictions with DoGSiteScorer are based on calculated size, shape and chemical features of automatically predicted pockets, incorporated into a support vector machine for druggability estimation.

Mutational analysis

The mutation analysis was done using I-Mutant 2.0 [17], SIFT [18] and PolyPhen-2 [19]. I- Mutant2.0 is a Support Vector Machine -based web server for the automatic prediction of protein stability changes upon single-site mutations. SIFT predicts whether an amino acid substitution affects protein function. SIFT prediction is based on the degree of conservation of amino acid residues in sequence alignments derived from closely related sequences, collected through PSI-BLAST. PolyPhen-2 (Polymorphism Phenotyping v2) is a tool that predicts possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations.

Peptide building and geometry optimization

Argus Lab is a molecular modelling, graphics, and drug design program for Windows operating systems. The peptides were built using the structure-building tool with default psi and phi values.

optimized using Argus Lab using steepest descent method with UFF force fields in which the maximum geometry cycle was set to 500000.

Addition of hydroxyproline to the octapeptide

As studied experimentally the effect of the octapeptide was proven effective against the breast cancer cell lines in rat and showed maximum inhibitory action. But according toprevious experimental study, the octapeptide was modified by the addition of hydroxyproline to the position where proline is present. The structure was sketched using COOT and addition to the previously designed peptide was incorporated and optimized.

MD simulation

Molecular Dynamics has been carried out for the mutated peptides to check the conformation stability at intervals of time. Molecular Dynamics Simulation has emerged as a vital aspect to interpret the molecular level of several peptides. The state of experimental approaches has been simplified by simulation approach as it reveals the mechanism involved in an atomistic level of the peptides. Here in our study, the molecular dynamics simulation approach has been applied to study the functional and stability aspect of peptides. The peptides were imperiled to dynamics study under solvent environment used in GROMACS 4.5.5 [20] version. OPLS force field was chosen for the minimization of the peptides. Four cl- ions was added as counter ions to neutralize the charges in the system. The peptides with added ions were centered in a cubic box of default dimension with spc water molecules. Then the energy minimization was performed for 25000 steps with steepest descent algorithm until the potential energy was converged and

stabilized. PME for a treatment of long-range electrostatic interactions were applied. The equilibrium state of the system was then achieved with temperature and pressure kept constant for 100ps (50000 steps). Finally, the Molecular Dynamics was performed for the minimized protein keeping the parameters as default. The LEAP FROG algorithm was used for integrating Newton's equation in MD Simulation. Linear Constant Solver algorithm was used on protein covalent bonds to maintain constant bond lengths. Grid type neighbor searching was done and long-range electrostatics was handled using PME. Finally, the equilibrated system was subjected to MD simulations for 2 ns at 300K and all atoms were permitted to move freely for the purpose to study the stability and its conformations after simulation when compared to the wild-type modelled protein structure.

Protein-protein interaction study

To understand the binding of the designed peptides to Hsp70, HEX 8.0.0 [21] and ClusPro 2.0 [22] were used. Hex is an interactive protein docking and molecular superposition program. The designed peptides were docked to Hsp70 using Hex to analyze the best binding site and evaluate the binding affinity. The simulated peptides were docked to Hsp70 using ClusPro2.0. Cluspro is a fully automated web-based program used for rigid protein– protein docking. Users have the choice of uploading the PDB files from local machine or directly from PDB server (http://www.rcsb.org/pdb/) database. The algorithm used in Cluspro 2.0 is based on fast Fourier transform using continuum electrostatics and geometric fit (DOT) or a novel shape complementarity scoring function for protein– protein docking (Z-DOCK) to perform rigid body docking. After processing the files, the docking algorithm evaluates multiple number of conformations retaining the most favorable interactions provides a choice to choose from balanced, electrostatic-favoured, hydrophobic- favoured and VdW electrostatic- favoured interactions. The working of the Cluspro server is based on rotation of the ligand (protein) with 70,000 rotations relative to the receptor grid, and greedy clustering of the filtered 1000 interacted structures out of 70,000 rotations is based on positioning of ligand (protein) with 9 A℃-alpha RMSD radius until final 30 models were generated.

Results and Discussions

Computation of Physiochemical Parameters

As a result of the physical and chemical computation, alpha-fetoprotein has a molecular weight of 68677.5 and its theoretical pI is 5.48 (Table 1). The total number of atoms is 9580. The estimated half-life is 30 hours (mammalian reticulocytes, *in vitro*), >20 hours (yeast, *in vivo*) and >10 hours (*Escherichia coli*, *in vivo*). The protein has the aliphatic index of 82.69. A total number of negatively charged residues (Asp + Glu) is 82. A total number of positively charged residues (Arg + Lys) is 64. The atomic composition of Alpha-Fetoprotein was also computed using Protparam (Table 2).

Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu
50	22	20	23	32	40	59	26	16	34	60
8.20%	3.60%	3.30%	3.80%	5.30%	6.60%	9.70%	4.30%	2.60%	5.60%	9.90%
Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	Pyl	Sec
42	9	32	21	38	36	2	17	30	0	0
6.90%	1.50%	5.30%	3.40%	6.20%	5.90%	0.30%	2.80%	4.90%	0.00%	0.00%

Table 1: Amino acid composition of alpha-fetoprotein.

Protein Structure Modeling and Validation

The tertiary structure of alpha-fetoprotein was not present in PDB. So the structure was modelled using MODELLER tool and hundred different structures were obtained using MODELLER. Based on the DOPE score the best negative DOPE score was selected out of hundred structures obtained from MODELLER (Figure 1). The structure has been validated using ProSA and RAMPAGE (Figures 2-4). ProSA calculates an overall quality score for a specific input structure. If this score is outside a range characteristic for native proteins the structure probably contains errors. A plot of local quality scores points to problematic parts of the model that are also highlighted in a 3D molecule viewer to facilitate their detection. The overall model quality obtained was -10.83 (Z-Score). The protein was visualized in Chimera which is visualization software for nucleic acids and proteins. The location of the peptide was observed. The peptide was located at 489-496 (Figure 2).

Mutational study using SIFT, PolyPhen-2 and I-Mutant 2.0

The mutation analysis was done using a combined analysis of results obtained using 3 different tools. The stability, damaging property and tolerance factor can be seen in the (Table 3). The mutation was based on a specific threshold value, which covers all the predicted results from servers, and best-suited mutants have been selected (Table 4). SIFT (Sorting Intolerant From Tolerant) is a program that predicts whether an amino acid substitution affects protein function so that users can prioritise substitutions for further study.

Carbon	Hydrogen	Nitrogen	Oxygen	Sulfur
3030	4773	811	925	41

Table 2: Atomic composition of alpha-fetoprotein.

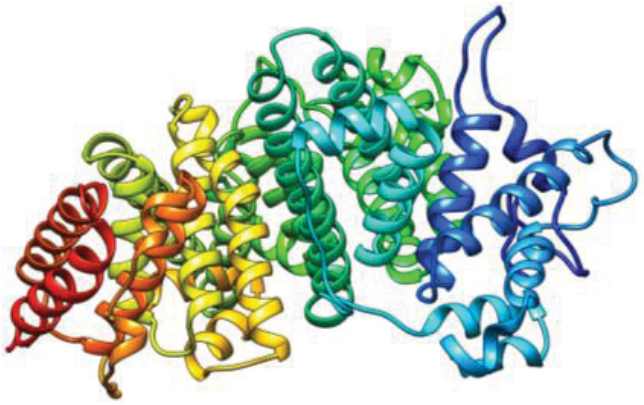


Figure 1: Modeled structure of alpha-fetoprotein by Modeller.

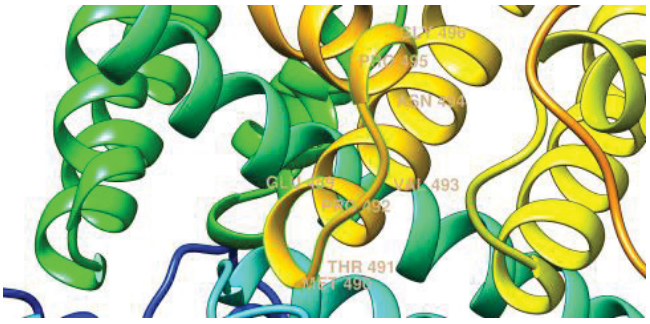


Figure 2: Location of peptide 489-496 as visualized in Chimera.

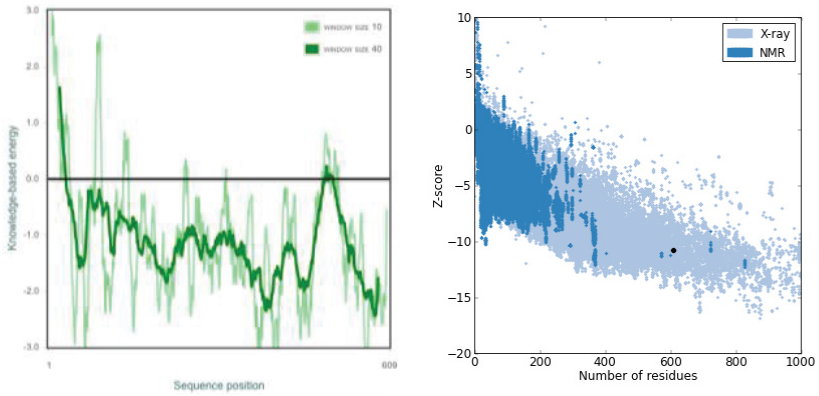
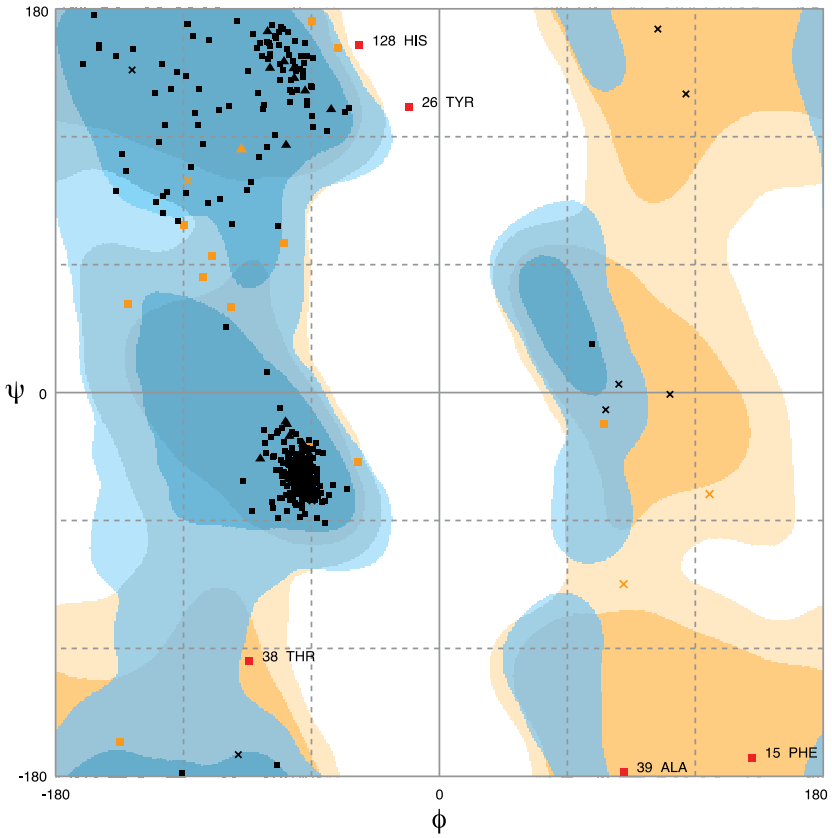


Figure 3: Model Quality obtained using ProSa.



Number of residues in favoured region (~98.0% expected) : 586 (96.5%)
Number of residues in allowed region (~2.0% expected) : 16 (2.6%)
Number of residues in outlier region : 5 (0.8%)

RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/

Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002) Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. Proteins: Structure, Function & Genetics. 50: 437-450

Figure 4: Ramachandran plot of the modelled structure.

Position	Substitution	Characteristic	SIFT Tolerated	SIFT Score	I-Mutant Stability	RI**	DDG*
489	E -> V	Nonpolar	Yes	0.06	Increase	3	-0.08
	E -> L	Nonpolar	Yes	0.21	Increase	3	0.29
	E -> I	Nonpolar	Yes	0.05	Increase	1	0.43
	E -> M	Nonpolar	No	0.03	Increase	4	0.54
	E -> Y	Uncharged Polar	No	0.02	Increase	1	-0.31
	E -> P	Nonpolar	Yes	0.05	Increase	4	-0.27
	E -> S	Uncharged Polar	Yes	0.06	Increase	0	-0.47
	E -> F	Nonpolar	No	0.03	Decrease	0	0.80
	E -> T	Uncharged Polar	Yes	0.06	Decrease	4	0.26
490	M -> D	Acidic	Yes	0.52	Increase	0	0.23
	M -> E	Acidic	Yes	1.00	Increase	2	-0.32
	M -> F	Nonpolar	Yes	0.06	Decrease	3	0.12
491	T -> M	Nonpolar	Yes	0.12	Decrease	1	0.34
	T -> F	Nonpolar	Yes	0.07	Decrease	2	0.62
	T -> W	Nonpolar	No	0.03	Decrease	2	0.13
	T -> Y	Uncharged Polar	Yes	0.09	Decrease	1	0.91
	T -> R	Basic	Yes	0.44	Decrease	1	0.17
	T -> D	Acidic	Yes	0.37	Decrease	3	0.26
	T -> E	Acidic	Yes	0.54	Decrease	5	0.08
492	P -> A	Nonpolar	Yes	0.08	Decrease	8	-1.72
	P -> S	Uncharged Polar	Yes	0.10	Decrease	6	-1.43
	P -> T	Uncharged Polar	Yes	0.30	Decrease	8	-1.50
493	V -> L	Nonpolar	Yes	0.05	Decrease	6	-0.39
	V -> I	Nonpolar	Yes	1.00	Decrease	7	-0.10
494	N ->N	-	-	-	-	-	-
495	P -> Q	Uncharged Polar	Yes	0.06	Decrease	9	-2.05
	P -> K	Basic	Yes	0.08	Decrease	7	-1.25
	P -> T	Uncharged Polar	Yes	0.12	Decrease	8	-1.43
	P -> E	Acidic	Yes	0.14	Decrease	4	-1.00
	P -> A	Nonpolar	Yes	0.14	Decrease	8	-1.80
	P -> G	Nonpolar	Yes	0.14	Decrease	9	-2.45
	P -> N	Uncharged Polar	Yes	0.17	Decrease	8	-1.89
	P -> D	Acidic	Yes	0.42	Decrease	8	-1.36
	P -> S	Uncharged Polar	Yes	0.44	Decrease	8	-1.65
496	G -> D	Acidic	Yes	0.09	Decrease	7	-0.16
	G -> S	Uncharged Polar	Yes	0.11	Decrease	7	-0.54
	G -> N	Uncharged Polar	Yes	0.37	Decrease	7	-0.13
	G -> A	Nonpolar	Yes	0.62	Decrease	5	0.19

Table 3: Mutation analysis.

Position	Substitution	Characteristic	SIFT Tolerated	SIFT Score	I-Mutant Stability	RI**	DDG*
489	E -> L	Nonpolar	Yes	0.21	Increase	3	0.29
490	M -> E	Acidic	Yes	1.00	Increase	2	-0.32
491	T -> E	Acidic	Yes	0.54	Decrease	5	0.08
492	P -> T	Uncharged	Yes	0.30	Decrease	8	-1.50
		Polar					
493	V -> I	Nonpolar	Yes	1.00	Decrease	7	-0.10
494	-	-	-	-	-	-	-
495	P -> S	Uncharged Polar	Yes	0.44	Decrease	8	-1.65
496	G -> A	Nonpolar	Yes	0.62	Decrease	5	0.19

Table 4: Selected mutants.

Peptide	Wild Type	All mutated	E489L	G496A	M490E	P492T	P495S	T491E	V493I	EKTOVNOGN
Energy	-365.64	-448.12	-393.47	-382.25	-388.67	-412.41	-398.13	-411.22	-365.8	-417.73

Table 5: Comparison binding energy of different docked complexes.

Protein peptide interaction using Hex 8.0.0

The peptides were docked to Hsp70 using Hex. The peptides after their optimization were docked against Hsp70 to study the binding affinity and its conformational positions. And all mutated peptide showed the least energy when docked with HSP70 and have the greater affinity towards HSP70. The best binding affinity is shown by All Mutated and is higher than EKTOVNOGN and wild type (Table 5).

Structural variations through MD simulation

Molecular dynamics studies of the peptides were performed in the solvent environment to ensure adequate testing. To characterize the structural variation during the course of the simulation, the RMSD of the entire protein for all simulations was calculated (Table 6). It is observed that all the peptides including the wild type peptide also shown decreased stability in comparison to hydroxyproline added peptide (EKTOVNOGN). The hydroxyproline gives a kink to the peptide which in turn gives stability to the peptide The source of its role as a helix breaker comes rather from side chain constraints and steric: the proline side chain is basically jammed into the space that should be occupied by the backbone of the alpha helix, a methylene group is in the space that would normally be occupied by a hydrogen-bonding amide proton, thus disrupting the H-bond network and steric of the helix (Figure 5.1 to 5.10).

Peptide	RMSD (Å)
Wildtype	1.80
E489L	2.14
M490E	16.83
T491E	3.21
P492T	2.15
V493I	3.02
P495S	1.92
G496A	1.99
All Mutated	11.60
EKTOVNOGN	1.54

Table 6: RMSD values of peptides.

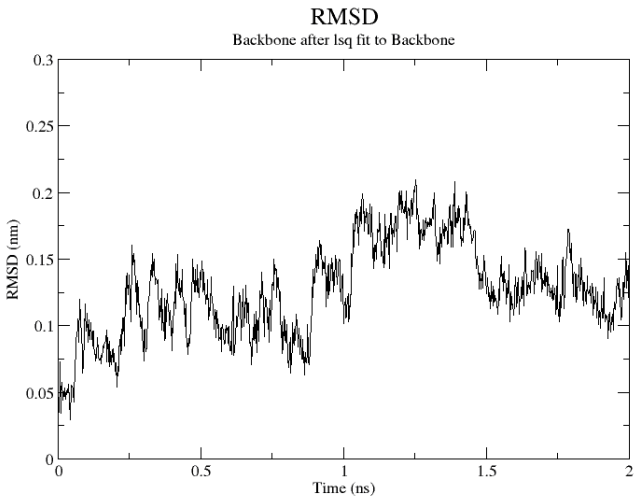


Figure 5.1: RMSD plot of wildtype peptide.

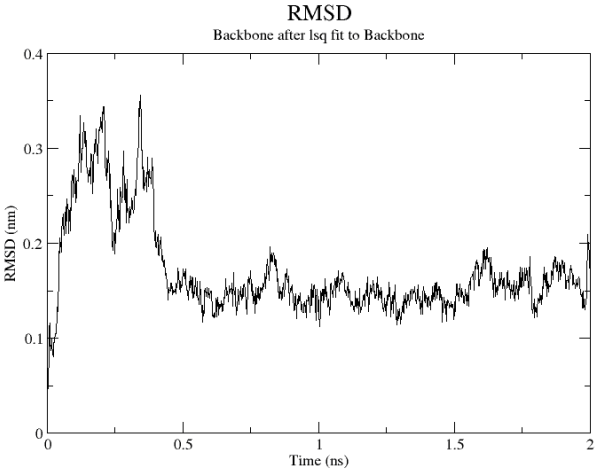


Figure 5.2: RMSD plot of E489L.

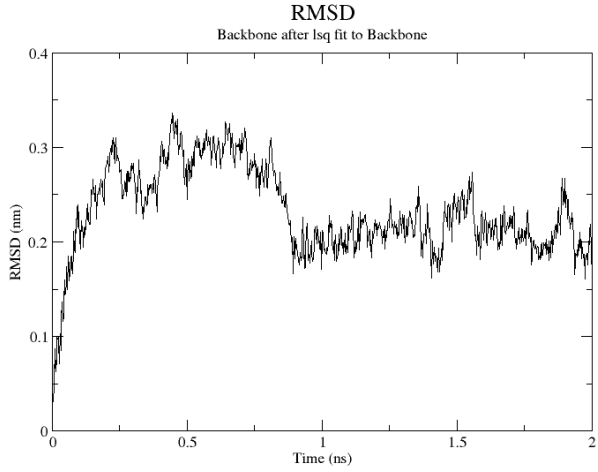


Figure 5.3: RMSD plot of M490E.

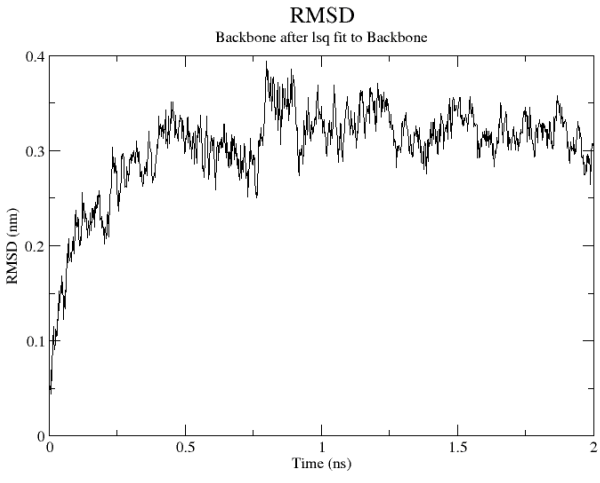


Figure 5.4: RMSD plot of T491E.

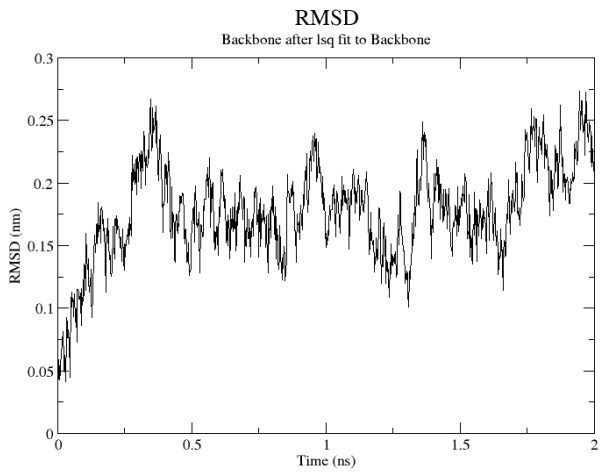


Figure 5.5: RMSD plot of P492T.

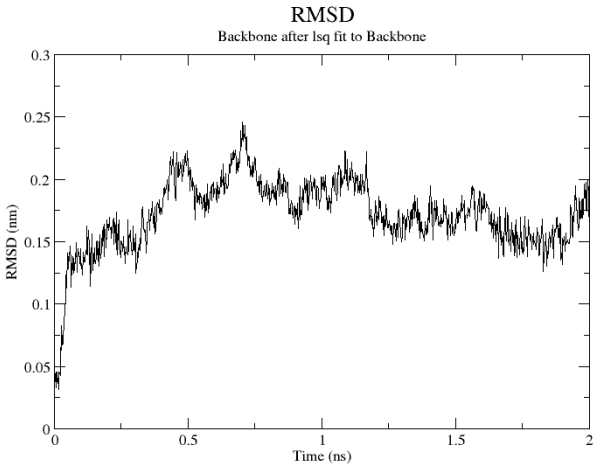


Figure 5.8: RMSD plot of G496A.

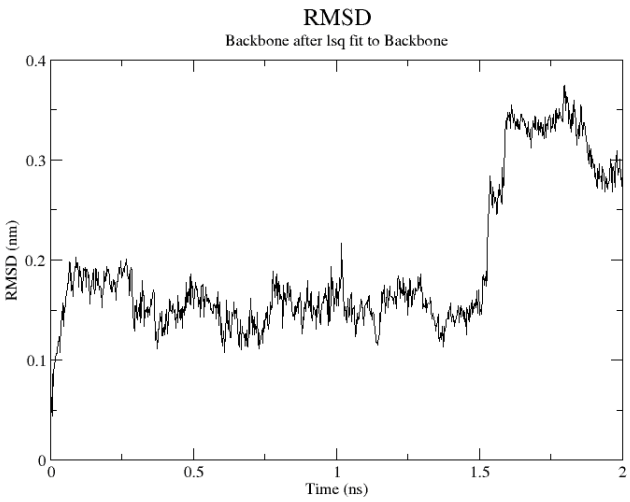


Figure 5.6: RMSD plot of V493I.

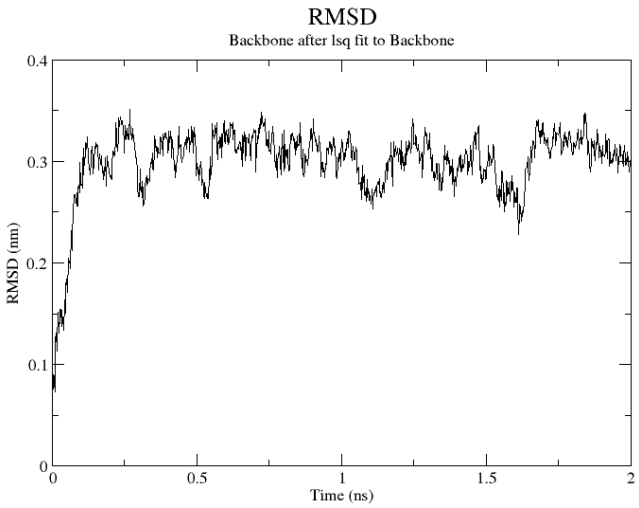


Figure 5.9: RMSD plot of all mutated.

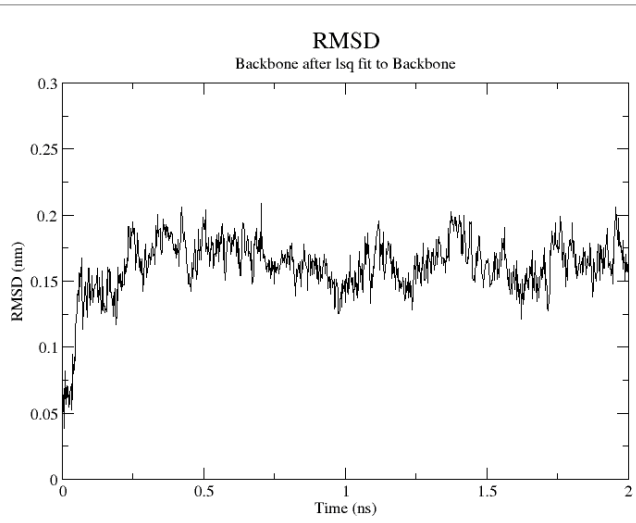


Figure 5.7: RMSD plot of P495S.

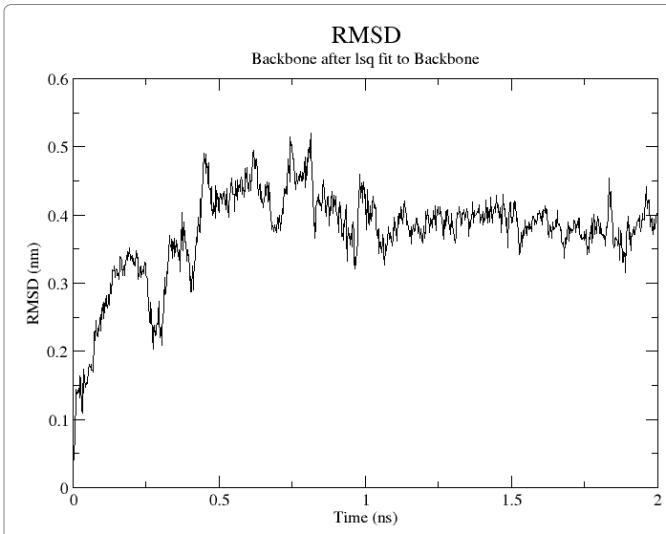


Figure 5.10: RMSD plot of EKTOVNOGN.

Simulated docking

After studying the dynamics of all the peptide structures, they were again docked to Hsp70 and their binding position as well as their affinity was studied using ClusPro. We used the Cluspro server for docking the interacting proteins to predict the protein complex. Cluspro is the first fully automated web-based program for docking proteins and was one of the top performers at CAPRI (Critical Assessment of Predicted Interactions) rounds 1-12, the community-wide experiment devoted to protein docking. The Cluspro server is based on a Fast Fourier Transform correlation approach, which makes it feasible to generate and evaluate billions of docked conformations by simple scoring functions. It is an implementation of a multistage protocol: rigid body docking, an energy based filtering, ranking the retained structures based on clustering properties, and finally, the refinement of a limited number of structures by energy minimization. The server (http://cluspro.bu.edu/ website) returns the top models based on energy and cluster size. We select one of the returned models after considering the energy and the size of the cluster – preferring lower energies and larger cluster sizes. As the Cluspro server implements rigid body docking, when a partner protein in a complex is structurally flexible Cluspro is not so able to account for this flexibility. A rigid body protein docking method (Cluspro) is used to predict the protein- protein interaction complexes. Binding sites of the docked complexes are characterized by their buried surface areas in the docked complexes, as a measure of the strength of an interaction as shown in (Table 7). The H-bonds were studied in which the constraints were relaxed by 4 angstroms and 20 degrees. The data obtained for H-bonds using Chimera [23] is observed (Figure 6.1 to 6.6) [24].

Peptide	Score	
	Centre	Lowest Energy
Wildtype	-468.5	-563.7
E489L	-456.7	-498.2
M490E	-424.4	-451.0
T491E	-432.5	-529.5
P492T	-392.1	-484.4
V493I	-431.2	-507.6
P495S	-440.6	-520.0
G496A	-385.6	-478.7
All Mutated	-439.0	-486.8
EKTOVNOGN	-395.9	-417.73

Table 7: Protein protein interaction study showing the lowest energy using Cluspro 2.0.

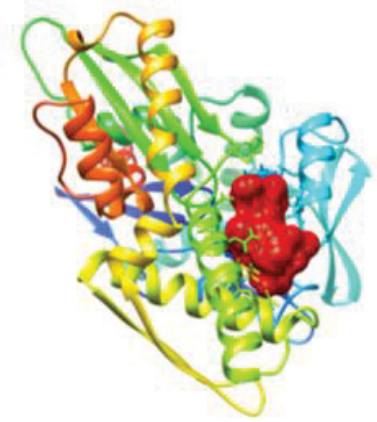


Figure 6.1: Docked structure of wildtype with Hsp70.

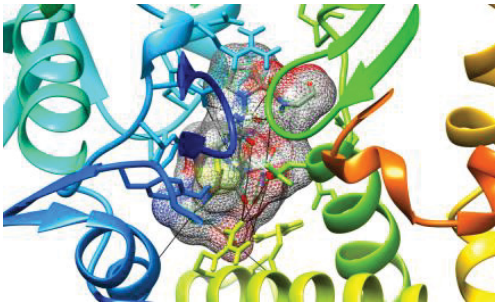


Figure 6.2: H-bonds (wildtype) as visualized in Chimera.

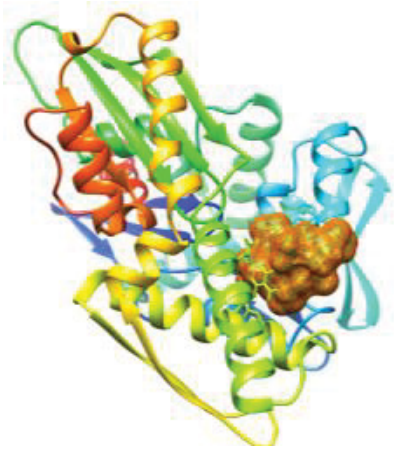


Figure 6.3: Docked structure of P495S with Hsp70.

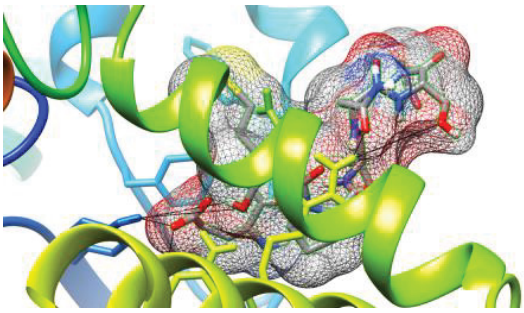


Figure 6.4: H-bonds (P495S) as visualized in Chimera.

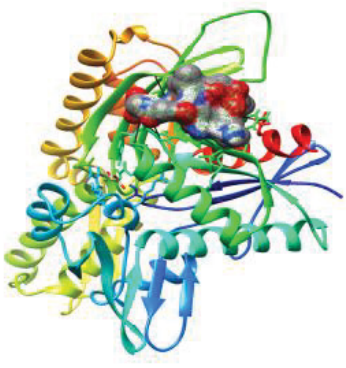


Figure 6.5: Docked structure of EKTOVNOGN with Hsp70.

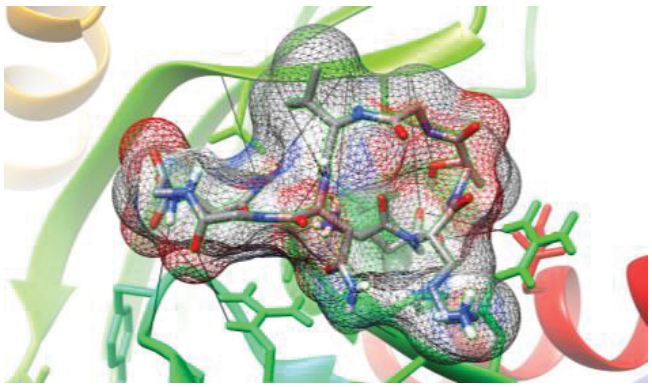


Figure 6.6: H-bonds (EKTOVNOGN) as visualized in Chimera.

Conclusion

The serum glycoprotein alpha-fetoprotein acts as a curative mediator and has anti estrotrophic activities. Studies were carried out based on the mutant variants considered from PloyPhen 2.0 and I-Mutant analysis. Further implications for this current research deal with the simulation of derived peptides for stability analysis which plays a vital role in the folding mechanism when docked against Hsp70. Previous studies showed that the pharmaceutical activities exhibited by the wild type octapeptide study in context with the maximal inhibitory properties towards breast cancer. Based on the above study, P495S (EMTPVNSG) and All Mutated (LEETINSA) peptides showed better stability and binding efficacy to Hsp70. Comparative molecular dynamics study of each mutation was carried out to understand the stability of the octapeptide (489-496) to ensure the curative perspective that is indulged in inhibiting the progression and proliferation of oncofetal proteins in breast cancer. Another modification to the derived peptide was done by the addition of hydroxyproline group to the region selected that was previously suggested with the combined effect of tamoxifen and hydroxyproline associated peptide. In the current study, we have tried to understand the role of AFPep and studied its interaction with Hsp70. AFPep regulates the estrogen levels after binding to Hsp70. We have carried out a mutation analysis at different positions in the octapeptide. All the mutated peptides were analyzed and their stability and binding affinity towards Hsp70 were studied. The mechanism by which AFPep regulates the estrogen levels after binding to Hsp70 still remains unknown. Our aim was to study the interactions. 100 ns, 200 ns, and 500 ns to get a better insight for their stability. Further, we can try to analyze mutation combinations of single as well as multiple mutations and the effect of simulation needs to be performed to have a better understanding of each set of mutations. Literature reviews suggest that the effect of tamoxifen is enhanced when used with AFPep. They both have the different site of actions still synergistically play a role in enhancing inhibitory activity. The site of action is not clearly understood and needs to be further investigated. Full-fledged understanding of the mechanism of AFPep will help to reveal the exact relation between AFPep and tamoxifen and the metabolic effects they converge into. In the current study, we have tried to understand the role of AFPep and studied its interaction with Hsp70. AFPep regulates the estrogen levels after binding to Hsp70. We have carried out a mutation analysis at different positions in the octapeptide. All the mutated peptides were analyzed and their stability and binding affinity towards Hsp70 was studied. The mechanism by which AFPep regulates the estrogen levels after binding to Hsp70 still remains unknown.

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