Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2013, 5 (3):39-47
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Impact of drug resistance mutation in the occurrence of non canonical interactions: A computational study

Vineeta Singh[1], Surbhi Bhandari[1] and K. Ramanathan[2]*

[1]*Industrial Biotechnology Division, School of Bio Sciences and Technology, VIT University, Vellore, Tamil Nadu, India*
[2]*Bioinformatics Division, School of Bio Sciences and Technology, VIT University, Vellore, Tamil Nadu, India*

ABSTRACT

Stability of NS3/4A protease structure (native and mutant) contributes significantly towards the discovery of new drug molecules. In the present study, we have investigated the role of non canonical interactions (CH/π, NH/π, C-H…O) on the structural stability of NS3/4A protease structure by means of analyzing the existence of these interactions in the native and mutant form of the structures. The study initiated by analyzing effect of the mutation in the binding of the drug molecule by molecular docking analysis. Subsequently the results were validated by means of PEARLS program and Flexibility analysis. Finally, the prevalence of non canonical interaction in the native type structure examined by using HBAT algorithms and compared with ASP168ALA structure. The obtained results certainly indicate that the non canonical interactions contribute significantly to the overall stability of protein structure.

Keywords: CH/π interaction, NH/π interaction, C-H…O interaction, Molecular docking, PEARLS.

INTRODUCTION

The importance of conventional interactions such as, hydrogen bonds, salt bridges hydrophobicity and other standard interactions in the stabilization of secondary structures [1], protein folding and stability [2, 3] are well established [4-6]. With the recent advances in computational biology one can assess the effect of non-standard interactions on the stability of protein tertiary structure. Among the nonconventional interactions, there is little data on the contribution of CH/π, NH/π, C-H…O interactions to protein stability. The exothermic dissolution of benzene and similar compounds (π -electron system: proton acceptor) in chloroform (C–H group: proton donor) was perhaps the origin of an interaction, now known as CH/π interactions, a form of weak hydrogen bond [7]. In 1957, Reeves and Schneider showed by NMR that this interaction was a type of H-bond [8]. Since then, CH/π interactions have been described in a vast number of small molecule systems from simple olefinic and aromatic compounds to complicated clathrates and inclusion complexes. In 1998 Nishio *et al.* published excellent treatise of these observations [9]. In this way CH/π interactions are gradually gaining a lot of importance. Positively charged or δ(+) amino groups of lysine, arginine, asparagine, glutamine and histidine are preferentially located within 6Å of the ring centroids of phenylalanine, tyrosine and tryptophan, where they make van der Waals contact with the δ(-) π-electrons and avoid the δ (+) ring edge. This geometric pattern is recognized as NH/ π interaction [10]. This kind of non-covalent interaction involving the π ring system as hydrogen bond acceptor were first described by Wulf et al. [11] through spectroscopic analysis of small molecules and subsequently in peptides by McPhail and Sim [12],

but their importance was not immediately appreciated. Much later, the N-H/ π interactions in proteins attracted the greater attention, following the observation of the stabilizing effect of such interactions in beta sheets [13], helix termini [14].

Another type of hydrogen bond that has undergone a resurgence of attention is that characterized by a CH donor, in place of the more common OH or NH groups. When first proposed many years ago [15, 16] there was some resistance due to the low electro negativity of C which was presumed to make it a weak proton donor. However, Support was later added to this idea on the basis of IR data [17-19] and the geometry of molecular complexes in the gas phase [20-22] and in crystalline environment [23, 24]. This type of interaction called C-H…O interactions. This C-H...O hydrogen bond systems share numerous features with the more traditional hydrogen bonds, such as geometric preference, NMR chemical shifts, and electron density patterns. It is only now gaining wide acceptance as a genuine hydrogen bond [25, 26]. Recently, we published our results on the cation/π and C-H/π interactions in the structural stability of proteins [27, 28]. However, till today there has been no comparative analysis of these interactions between native and mutant structures. It is the objective of the present paper to carry out just this sort of comparative analysis between native and mutant NS3/4A protease structures. It is noteworthy to mention here that mutant structure still maintaining the similar number of non canonical interactions like native type structure. Hence we postulate that the incorporation of the entirely of these interactions could provide new perspectives and possibly new answers for structural biologists.

MATERIALS AND METHODS

Data set

The three-dimensional (3D) structures of native and mutant NS3/4A protease were taken from the crystal structures of the Brookhaven Protein Data Bank (PDB) [29] to carry out computational analysis. The corresponding PDB codes were 3SV6 and 3SV8 [30, 31]. Both structures were solved with >2.0 Å resolution. Telaprevir was used as the small molecule/inhibitor for our investigation. The SMILES strings were collected from PubChem, a database maintained in NCBI [32] and was submitted to CORINA for constructing the 3D structure of small molecule [33].

Identification of binding site residues

It was a challenging task to extrapolate a mechanism of action from the view of three dimensional structures. Detailed biochemical information about the enzyme can be used to design substrate or transition state analogues, which can then be bound into the enzyme for structure determination. These can reveal binding site locations and identify residues, which are likely to take part in the receptor–ligand interaction. From this, a catalytic mechanism can be proposed. In order to identify the binding residues in the structure of NS3/4A protease, we submitted NS3/4A protease complexed with Telaprevir (PDB code: 3sv8) into the PDBsum program. PDBsum provides summary information about intermolecular contacts existing in the complex structure. These informations were used to determine the binding site residues in the NS3/4A protease.

Conservation score

We computed the conservation score of binding site residues interacting with amino acid residues in each protein using the ConSurf server [34]. This server computes the conservation based on the comparison of the sequence of a PDB chain with the proteins deposited in Swiss-Prot [35] and finds the ones that are homologous to the PDB sequence. The number of PSI-BLAST iterations and the *E*-value cutoff used in all similarity searches were 1 and 0.001. All the sequences that are evolutionarily related with each one of the proteins in the data set were used in the subsequent multiple alignments. Based on these protein sequence alignments the residues are classified into nine categories from highly variable to highly conserved. Residues with a score of 1 are considered highly variable and residues with a score of 9 are considered highly conserved residues.

Stabilizing residues

Stabilizing residues were computed using the parameters such as surrounding hydrophobicity, long-range order, stabilization center and conservation score as described by Gromiha [36]. We used the server SRide [36] for this purpose. Conservation score of ≥ 6 is the cutoff value used to identify the stabilizing residues.

Computation of docking score between the ligand and the enzyme

Docking was performed with the help of the Patch-Dock [37]. It is geometry based molecular docking algorithm. The PatchDock algorithm divides the Connolly dot surface representation of the molecules into concave, convex and

flat patches. Then, complementary patches are matched in order to generate candidate transformations. Each candidate transformation is further evaluated by a scoring function that considers both geometric fit and atomic desolvation energy. Finally, RMSD (root mean square deviation) clustering was applied to the candidate solutions to discard redundant solutions. The input parameters for the docking were the PDB coordinate file for the protein and the ligand molecule. This algorithm has three major stages (i) Molecular Shape Representation (ii) Surface Patch Matching and (iii) Filtering and Scoring. The services are available at http://bioinfo3d.cs.tau.ac.il/ PatchDock/

Energetic analysis by PEARLS

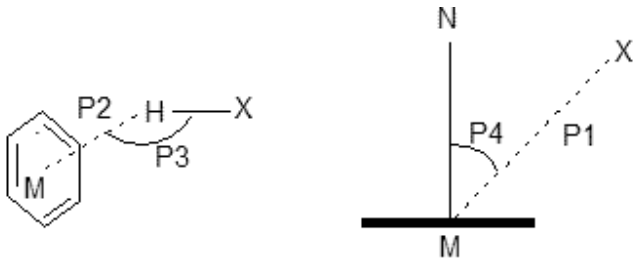
Analysis of the energetic of ligand-protein, ligand-nucleic acid, and protein-nucleic acid interactions facilitates the quantitative understanding of molecular interaction that regulate the function and conformations of proteins. It has also been extensively used for ranking potential new ligands in virtual drug screening. There is Web based software, PEARLS (program for energetic analysis of ligand receptor systems), for computing interaction energies of ligand-protein, ligand-nucleic acid, protein-nucleic acid, and ligand-protein-nucleic acid complex from their 3D structures. In the present study, we examined the total receptor-ligand interaction energy by means of the PEARLS program [38].

Flexibility of Binding Residue by Normal Mode Analysis

A quantitative measure of the atomic motions in proteins can obtained from the mean square fluctuations of the atoms relative to their average positions. These can be related to the B-factor [39, 40]. Analysis of B-factors, therefore, is likely to provide newer insights into protein dynamics, flexibility of amino acids, and protein stability [41]. It is to be noted that protein flexibility is important for protein function and for rational drug design [42]. Also, flexibility of certain amino acids in protein is useful for various types of interactions. Moreover, flexibility of amino acids in drug binding pocket is considered to be a significant parameter to understand the binding efficiency. In fact, loss of flexibility impairs the binding effect [43, 44]. Hence, this can be analyzed by the B-factor, which is computed from the mean square displacement, <R2> of the lowest frequency normal mode using the ElNemo program [45].

Non canonical interactions

XH/ π interactions were calculated using the program available for this purpose called HBAT [46]. The positions and geometry of donor and acceptor atom are shown in figure **1**. The donor group is represented as X-H and the acceptor is the π system. The distances are usually measured from the centroid (M) i.e, centre of the π ring. P1 and P2 are distances from X and H, respectively, to M. P3 is the angle between vectors X-H and H-M while P4 is the angle between the XM and MN. Here N is a normal to the centre of the π ring. The geometry is adapted from earlier work of babu [47].



(a) **(b)**

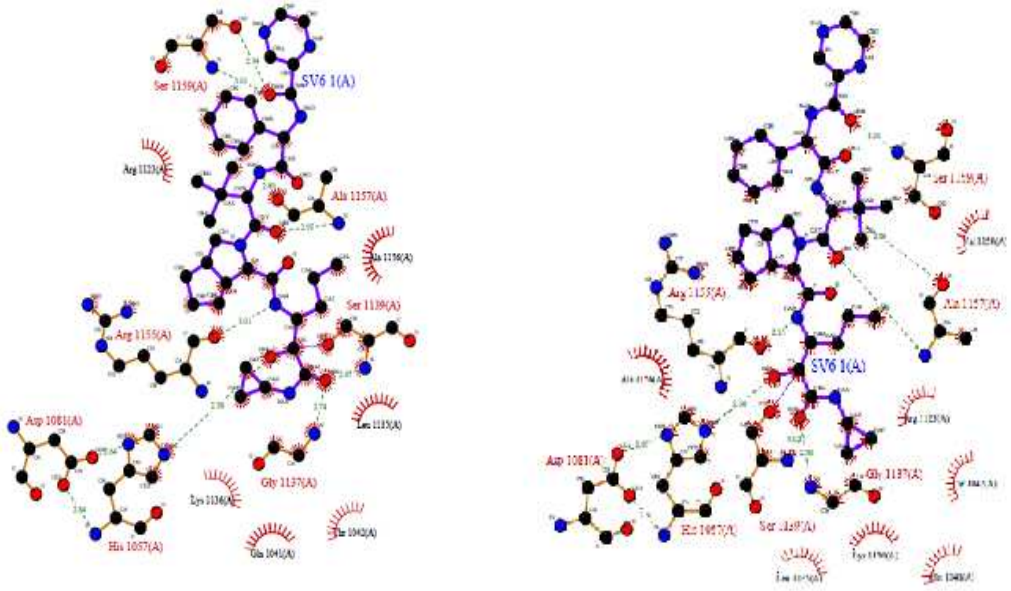
Figure 1: Parameters for X-H…. π interaction (X: C and N)

C-H...O interactions were identified using the program available for this purpose called HBAT [46]. The C-H...O interactions considered here were between all possible donor C-H groups in the therapeutic proteins structures (Cα-H, Cali-H and Caro-H) and oxygen containing proton acceptor molecule. The oxygen atoms in proteins are of the hydroxyl, carbonyl and carboxyl type. In terms of their electronegativity, this increases in the order O-H<C=O<C-O−. The position and geometry is adapted from earlier work of Babu [47].

RESULTS AND DISCUSSION

Binding Residues Analysis

The binding site residues in the structure of NS3/4A protease were obtained from the PDBsum by using the complex structure. Both native and mutant structures were used for the analysis. Almost similar numbers of contacting residue were observed in both structures. The result is shown in figure 2. The results indicate that a total of 15 amino acid residues act as a binding site residues in NS3/4A protease. It was interesting to note that, the residues such as SER(1158), ALA(1157), SER(1139), GLY(1137), HIS(1057), ASP(1081) and ARG(1155) makes Hydrogen bond with Telaprevir and the other residues namely ARG(1123), ASP(1168), ALA(1156), THR(1042), GLN(1041), LYS(1136), LEU(1135) and VAL(1158) makes hydrophobic interaction with the Telaprevir. Both native and mutant structure shows the total of 12 hydrogen bond interactions. Similarly the total of 8 and 6 hydophobic interactions observed in the native and mutant structure respectively.



(a) (b)

Figure 2: Telaprevir bound to the binding site of NS3/4A protease (a) and ASP168ALA (b) structures. The figure was rendered using the program LIGPLOT

Conservation score

The binding site residue further screened by means of conservation score**.** We used the ConSurf server to compute the conservation score of amino acid residues involved in binding site residues of NS3/4A protease. The result is shown in table 1. 40% of the amino acid residues had the highest conservation score of 9, 45% of the amino acid residues had a conservation score in the range of 6-8 and 15% of the amino acid residues had the conservation score less than 5. It is believed that most of the amino acid residues involved in binding site interactions had a high conservation scores. Hence the residues having the score 9 will be consider as binding site residue in our analysis.

Table 1 Computation of Binding site residues and its Conservation score.

S. No.	Binding site residues in native structure (PDB code: 3sv6)	Conservation score	Binding site residues in mutant structure (PDB code: 3sv8)	Conservation score
1	SER(1159)	5	SER(1159)	5
2	ARG(1155)	8	ARG(1155)	8
3	ALA(1157)	9	ALA(1157)	9
4	SER(1139)	9	SER(1139)	9
5	GLY(1137)	8	GLY(1137)	8
6	HIS(1057)	9	HIS(1057)	9
7	ASP(1081)	9	ASP(1081)	9
8	ARG(1123)	7	ARG(1123)	7
9	ASP(1168)	6	-	-
10	ALA(1156)	8	ALA(1156)	8
11	THR(1042)	4	THR(1042)	4
12	GLN(1041)	9	GLN(1041)	9
13	LYS(1136)	9	LYS(1136)	9
14	LEU(1135)	8	LEU(1135)	8
15	VAL(1158)	8	-	-

Stabilizing Residues

Initially, the stability of the structures were examined by using number of stabilizing residues [37]. Stabilizing residues were computed using the parameters such as surrounding hydrophobicity, long-range order, stabilization center and conservation score. We used the server SRide for this purpose. The result is shown in table 2. It was interesting to note that the number of stabilizing residues in the mutant structure was significantly higher than the native structure. It certainly indicates the increase in stability of mutant (ASP168ALA) structure than native type.

Table 2 Comparison of stabilizing residues between native and mutant structures

S.No.	Stabilizing residues in the native structure (PDB code: 3sv6)	Conservation score	Stabilizing residues in the mutant structure (PDB code: 3sv8)	Conservation score
1	THR(1054)	9	THR(1054)	9
2	VAL(1083)	8	VAL(1083)	8
3	TRP(1085)	6	TRP(1085)	6
4	LEU(1104)	7	LEU(1104)	7
5	LEU(1106)	9	LEU(1106)	9
6	GLY(1141)	8	GLY(1141)	8
7	PHE(1154)	9	PHE(1154)	9
8	ALA(1164)	9	-	-
9	-	-	TYR(1105)	9
10	-	-	CYS(1145)	9
11	-	-	GLY(1152)	9

Computation of docking score between the ligand and the enzyme

Proteins are the basis of the life process at the molecular level. The protein interaction is either with other protein or with small molecules. Many biological studies, both in academia and in industry, may benefit from credible high-accuracy interaction predictions. Here, we used PatchDock, a very efficient algorithm for protein-ligand docking for our analysis. The PDB format of the two molecules and the receptor binding sites were uploaded in to the server. It was interesting to note that docking score of native structure is higher than the mutant structure. This result is shown in figure 3. It is likely that the higher number of stabilizing residues makes the mutant (ASP168ALA) structure highly stable and rigid. Hence, Telaprevir is not able to bind properly with the mutant structure. This result was further validated by using PEARLS figure 4. It could be seen from figure that total ligand receptor interaction energy was -5.01 kcal/mol in native structure whereas in mutant structure it was -3.72 kcal/mol only. These results certainly confirm that ASP168ALA mutation alters the conformation of binding pocket residue thus cause drug resistance.

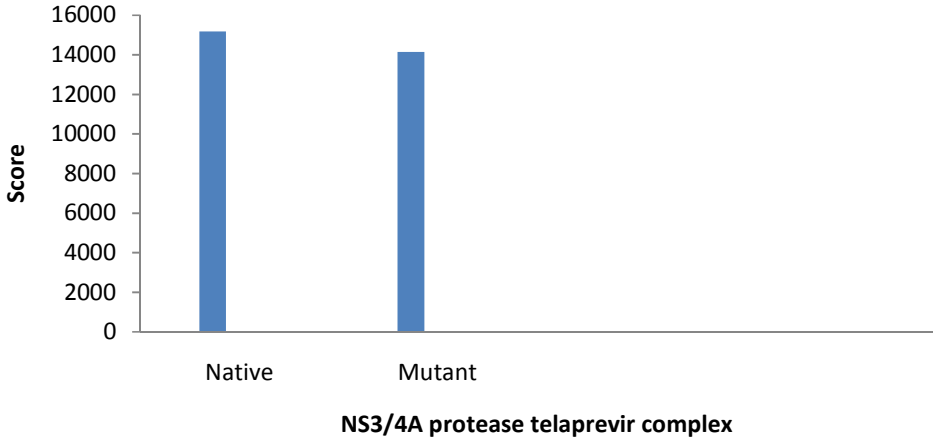


Figure 3: Comparison of scores in native and mutant structures using molecular docking.

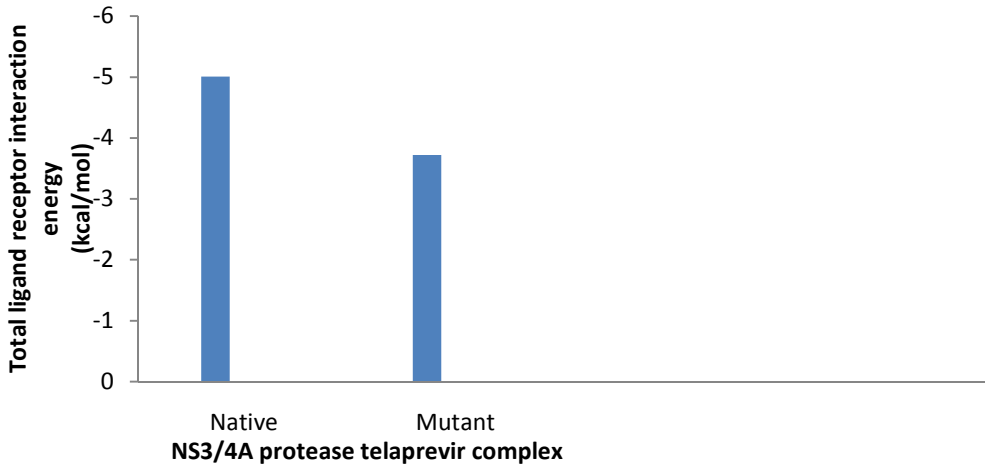


Figure 4: Comparison of total ligand receptor interaction energy between native and mutant structures using PEARLS.

Table 3: Comparison of normalized mean square displacement and conservation score of drug- binding amino acids of native and mutant type.

S.No.	Binding residues in native structure (PDB code: 3sv6)	Normalized mean square displacement <R2>in native type (PDB code: 3sv6)	Conservation Score	Binding residues in mutant structure (PDB code: 3sv8)	Normalized mean square displacement <R2>in mutant type (PDB code: 3sv8)	Conservation Score
1	GLN (1041)	0.0168	9	GLN (1041)	0.0149	9
2	PHE (1043)	0.0101	9	PHE (1043)	0.0089	9
3	HIS (1057)	0.0156	9	HIS (1057)	0.0100	9
4	ASP (1081)	0.0143	9	ASP (1081)	0.0078	9
5	LYS (1136)	0.0120	9	LYS (1136)	0.0124	9
6	SER (1138)	0.0071	9	SER (1138)	0.0097	9
7	SER (1139)	0.0074	9	SER (1139)	0.0085	9
8	ALA (1157)	0.0070	9	ALA (1157)	0.0105	9

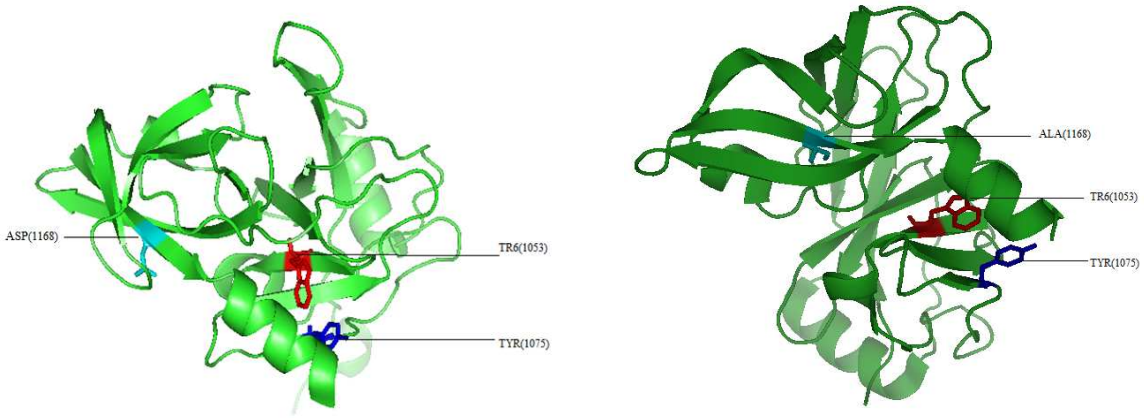
Binding Residues Flexibility by Means of Normal Mode Analysis

The conservation score criteria were employed to screen the binding site residues. Binding residue flexibility was fundamental to understanding the ways in which drug exerts biological effects. This flexibility allows increased affinity to be achieved between a drug and its target enzyme. In order to understand the cause of drug insensitivity by D168A mutation, we used the program ElNemo [45] to compare the flexibility of amino acids of both native and mutants, which are involved in binding with Telaprevir. Table 3 depicts the flexibility of amino acids in the drug-binding pocket of both native and mutants by means of normalized mean square displacement, <R2>.

We further sorted out these data into three different ranges of flexibility. One is the<R2>of amino acids in the drug-binding pocket of mutants which is exactly the same as<R2>of the amino acids in the drug-binding pocket of natives named as "identical flexibility." The second was the<R2>of amino acids in the drug-binding pocket of mutants which is higher than<R2> of the amino acids in the drug-binding pocket of natives named as "increased flexibility." And the last is the<R2>of amino acids in the drug-binding pocket of mutants which is lesser than<R2>of amino acids in the drug-binding pocket of native named as "decreased flexibility." From the above classification, we understand that 60 % of drug-binding amino acids were in the range of decreased flexibility and 40 % of drug binding amino acids were in the range of increased flexibility (Table 3). This evidently exemplified that majority of amino acids participated in the drug-binding pocket of these mutants lost their flexibility due to their occurrence in the range of "decreased flexibility" which signifies the loss of binding efficiency with the inhibitor, Telaprevir.

Non Canonical Interactions

Non canonical interactions were analyzed in native and mutant structures by using HBAT program. In particular we have analyzed the CH/π, NH/π, and C-H…O interactions in the structural stability of native and mutant protease structure. The result is shown in table 4. It could be seen from the table that 2 CH/π interactions, 1 NH/π interaction and 17 C-H…O interactions were contributed the stability of the native structure. It is interesting to note that mutant structure was also maintained 1 CH/π interactions, 3 NH/π interactions and 14 C-H…O interactions. The PyMol view of CH/π interactions in the native and mutant structure were shown in figure 5.



(A) **(B)**

Figure 5: Pymol view of (A) CH/ π interaction in 3sv6 (B) NH/ π interactions in 3sv8

Furthermore we classified these interactions into three category namely short range, medium range and long range contacts. The residues that are within a distance of two residues are considered to contribute to short-range interactions, whereas, those within a distance of ±3 or ±4 residues contribute to medium-range and those more than four residues away contribute to long-range interactions [48-50]. About 60% of the non canonical interactions were observed as long-range interactions, 26% and 14% of non canonical interactions were found to be short-range and medium-range interactions respectively. Long-range non canonical interactions are the predominant type of interactions in set of HCV studied. Hence, we concluded that although the structure is mutated but it could still stabilized by significant number of the weak interactions particularly long range contacts.

Table 4: Analysis of Non Conventional Interactions in the HCV protein structures

Interaction types	Donor residue in Native type (PDB code: 3sv6)	C.S	Acceptor residue in native type (PDB code: 3sv6)	C.S	D_{seq}	Donor residue in mutant type (PDB code: 3sv8)	C.S	Acceptor residue in mutant type (PDB code: 3sv8)	C.S	D_{seq}
CH/ π	TRP(1053)	9	TYR(1075)	8	22	TRP(1053)	9	TYR(1075)	8	22
	TYR(1075)	8	TR6 (1053)	9	22	-	-	-	-	-
NH/ π	ALA(984)	6	HIS(982)	6	2	ALA(984)	6	HIS(982)	6	2
	-	-	-	-	-	ILE(1064)	1	TR5(1085)	7	21
	-	-	-	-	-	SER(985)	6	HIS(982)	6	3
C-H…O	TYR(1006)	7	ALA(1007)	1	1	TYR(1006)	7	ALA(1007)	1	1
	TYR(1006)	7	THR(1004)	8	2	TYR(1006)	7	THR(1004)	8	2
	PHE(1043)	9	LEU(1044)	8	1	PHE(1043)	9	LEU(1044)	8	1
	PHE(1043)	9	SER(1139)	2	4	PHE(1043)	9	SER(1139)	2	4
	PHE(1043)	9	THR(1054)	8	11	PHE(1043)	9	THR(1054)	8	11
	-	-	-	-	-	PHE(1043)	9	THR(1054)	8	11
	TRP(1053)	9	THR(1046)	7	7	TRP(1053)	9	THR(1046)	7	7
	TRP(1053)	9	VAL(1083)	9	30	TRP(1053)	9	VAL(1083)	9	30
	TYR(1075)	8	THR(1178)	1	3	TYR(1075)	8	THR(1178)	1	3
	TRP(1085)	7	VAL(1071)	1	14	TRP(1085)	7	VAL(1071)	1	14
	TRP(1085)	7	THR(1072)	1	13	TRP(1085)	7	THR(1072)	1	13
	TRP(1085)	7	LEU(1144)	1	59	TRP(1085)	7	LEU(1144)	1	59
	TRP(1085)	7	ARG(1062)	4	23	TRP(1085)	7	ARG(1062)	4	23
	TYR(1105)	3	LEU(1144)	1	39	-	-	-	-	-
	PHE(1154)	8	SER(1138)	9	16	-	-	-	-	-
	PHE(1154)	8	ALA(1156)	8	2	-	-	-	-	-
	PHE(1154)	8	LEU(1135)	8	19	-	-	-	-	-
	PHE1169)	9	ILE(1170)	1	1	-	-	-	-	-
	-	-	-	-	-	PHE(1169)	9	ARG(1123)	7	46

C.S: Conservation score; D_{seq}: sequential distance.

CONCLUSION

All cohesive inter atomic interactions, whatever their specific nature, contribute to the overall stability of any macromolecule. Hydrogen bonds, however, are known to play a key part in many other phenomena, including enzymatic catalysis. Here we have investigated the role of non canonical interactions in the structural stability of proteins. Initially molecular docking and PEARLS program were used to examine the affinity of Telaprevir on NS3/4A protease structure. Lesser binding affinity confirmed the conformational change in the protein structure because of mutation. Subsequently we have analyzed the weaker interactions contribute in the structural stability by means of HBAT program. We understand that mutant structure can still maintain significant number of non canonical contacts. Thus, we conclude that ASP168ALA mutation gives resistance to drug not for the non canonical interaction. Even though, the non canonical interactions are not only comparable in strength to a traditional hydrogen bond but cumulatively can make a quantitatively greater energetic contribution to folding and stability. The frequency of occurrence obtained in the present study enev in the ASP168ALA structure unequivocally shows that the non canonical interactions cannot and must not be neglected. Hence, without ambiguity, we can confirm that non canonical interactions play an important role in the structural stability of proteins.

Acknowledgments
The authors thank the management of Vellore Institute of Technology, for providing the facilities to carry out this work.

REFERENCES

[1] D Bordo; P Argos. *J Mol Biol*, **1994**, 243, 504.
[2] KA Dill. *Biochemistry*, **1990**, 29, 7133.
[3] AR Fersht; L Serrano. *Curr Opin Struct Biol*, **1993**, 3, 75.
[4] EN Baker; RE Hubbard. *Prog Biophys Mol Biol*, **1984**, 44, 97.
[5] GA Jeffery; W Saenger. *Springer-Verlag, New York*, **1991**.
[6] IK McDonald; JM Thornton. *J Mol Biol*, **1994**, 238, 777.
[7] M Tamres. *J Am Chem Soc*, **1952**, 74, 3375.

[8] LW Reeves; WG Schneider. *Can J Chem*, **1957**, 35, 251.
[9] M Nishio; M Hirota; Y Umezawa. *Wiley-VCH, New York*, **1998.**
[10] SK Burley; GA Petsko. *FEBS Letters*, **1986**, 203, 139.
[11] OR Wulf; U Liddel; SB Hendricks. *J Amer Chem Socie*, **1936**, 56, 2287.
[12] AT McPhail; GA Sim. *Chemical Communications*, **1965**, 00, 124.
[13] GF Fabiola; S Krishnaswamy; V Nagarajan; V Pattabhi. *Acta Crystallog Sect*, **1997**, 53, 316.
[14] MM Babu; SK Singh; P Balaram. *J Mol Biol*, **2002**, 322, 871.
[15] S Glasstone. *Trans Faraday Soc*, **1937**, 33, 200.
[16] JFJ Dippy. *Chem Rev*, **1939**, 25, 151.
[17] A Allerhand; PVR Schleyer. *J Am Chem Soc*, **1963**, 85, 1715.
[18] AM DeLaat; BS Ault. *J Am Chem Soc*, **1987**, 109, 4232.
[19] BM Kariuki; KDM Harris; D Philp; JMA Robinson. *J Am Chem Soc*, **1997**, 110, 12679.
[20] EE Astrup; AM Aomar. *Acta Chem Scand*, **1975**, A29, 794.
[21] KI Peterson; W Klemperer. *J Chem Phys*, **1984**, 81, 3842.
[22] A Fujii; E Fujimaki; T Ebata; N Mikamiet. *J Am Chem Soc*, **1998**, 120, 13256.
[23] DJ Sutor. *Nature*, **1962**, 195, 68.
[24] R Kaufmann; A Knochel; J Kopf; J Oehler; G Rudolph. *Chemische Berichte*, **1977**, 110, 2249.
[25] MC Wahl; M Sundaralingam. *Trends Biochem Sci*, **1997**, 22, 97.
[26] GR Desiraju; T Steiner. *Oxford University Press, New York*, **1999**, 29.
[27] V Shanthi; K Ramanathan; Sethumadhavan Rao. *Journal of Computer Science & Systems Biology*, **2009**, 2, 051.
[28] K Ramanathan; V Shanthi; Sethumadhavan Rao. *The Open Structural Biology Journal*, **2009**, 3, 1.
[29] HM Berman; J Westbrook; Z Feng; G Gilliland; TN Bhat; H Weissig; IN Shindyalov; PE Bourne. *Nucleic Acids Research*, **2000**, 28, 235.
[30] RJ Russell; LF Haire; DJ Stevens; PJ Collins; YP Lin; GM Blackburn; AJ Hay; SJ Gamblin; JJ Skehel. *Nature*, **2006,** 443, 45.
[31] PJ Collins; LF Haire; YP Lin; J Liu; RJ Russell, PA Walker; JJ Skehel; SR Martin; AJ Hay; SJ Gamblin. *Nature*, **2008**, 453, 1258.
[32] J Feldman; KA Snyder; A Ticol; G Pintilie; CW Hogue. *FEBS Letters*, **2006**, 580, 1649.
[33] J Gasteiger; C Rudolph; J Sadowski. *Tetrahedron. Compututer, Method*,**1990**, 3, 537.
[34] F Glaser; T Pupko; I Paz. *Bioinformatics*, **2003**, 19, 163.
[35] B Boeckman; A Bairoch; R Apweiler. *Nucleic Acids Res*, ***2003***, 31,365.
[36] MM Gromiha; G Pujadas; C Magya; S Selvaraj; I Simon. *Proteins*, **2004**, 55, 316.
[37] D Schneidman; Y Inbar; R Nussinov; HJ Wolfson. *Nucleic Acids*, **2005**, 33, 363.
[38] LY Han; HH Lin; ZR Li; CJ Zhena; B Xie; YZ Chen. *J Chem lnf Model*, **2006,** 16, 445.
[39] Z Yuan; TL Bailey; RD Teasdale. *Proteins*, **2005**, 58, 905.
[40] D Ringe; GA Petsko. *Methods in Enzymology*, **1986**, 131, 389.
[41] S Parthasarathy; M Murthy. *Protein Engineering*, **2000**, 13, 9.
[42] HA Carlson; JA McCammon. *Molecular Pharmacology*, **2000**, 57, 213.
[43] A Hinkle; LS Tobacman. *Journal of Biological Chemistry*, **2003**, 278, 506.
[44] R Rajasekaran; C George Priya Doss; C Sudandiradoss; K Ramanathan; R Purohit; R Sethumadhavan. *Comptes Rendus Biologies*, **2008**, 331, 409.
[45] K Suhre; YH Sanejouand. *Nucleic Acids Research*, **2004**, 32, 610.
[46] A Tiwari; SK Panigrahi. *In Silico Biol*, **2007**, 7, 651.
[47] MM Babu. *Nucleic Acid Res*, **2003**, 31, 3345.
[48] MM Gromiha; S Selvaraj. *J Biol Phys*, **1997**, 23, 151.
[49] MM Gromiha; S Selvaraj. *Prog Biophys Mol Biol*, **2004,** 86, 235.
[50] MM Gromiha; C Santhosh; S Ahmed. *Int J Biol Macromol*, **2004**, 34, 203.