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Investigation of MK-5172 resistance against R155K NS3/4A protease by molecular simulation approach

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ABSTRACT

Several million individuals around the world were found to be infected by Hepatitis C Virus (HCV). Many efforts have been taken to find more potent and efficient drug. After several clinical analyses the drugs namely telaprevir, danoprevir, vaniprevir & MK-5172 were evolved to inhibit the viral NS3/4A protease. However, the efficiency of the drug mainly relies on resistance factor. For instance single-site mutation at protease residue R155 confers resistance to most of the inhibitors in clinical trials. Thus knowledge about molecular basis of drug resistance is important to find perfect drug that can retain activity against resistant viral variant (R155K). Hence in the present study, we employed molecular docking and normal mode analysis to infer the mechanism of drug resistance. Furthermore, we examined the stability of the structures with the aid of salt bridge and stabilizing residues (SRide) analysis. We hope that the results obtained in the study certainly helpful for the better understanding of NS3/4A protease-MK 5172 interacting pattern.

Keywords: Molecular Dynamics (MD); Stabilizing Residues (SRide); Salt Bridge, Normal Mode Analysis.

INTRODUCTION

Hepatitis C Virus (HCV or sometimes HVC) is a small (55–65 nm in size), enveloped, positive-sense single-stranded RNA virus of the family *Flaviviridae* infecting an estimated 170 million people worldwide [1, 2]. Hepatitis C virus is mainly due to the cause of hepatitis C that is presented in the humans. Based on genetic diversity, HCV is divided into six major genotypes (genotypes 1–6) and numerous subtypes with different geographic distributions; genotypes 1 and 3 are the most common worldwide [3]. HCV NS3/4A protease is a trypsin-like serine protease which is essential for the generation of components of the viral RNA replication complex. It is a prime and most extensively studied anti-HCV therapeutic target [4]. Pharmaceutical companies have invested significant effort in developing NS3/4A protease inhibitors. Danoprevir [5], vaniprevir and telaprevir [6, 7] are three NS3/4A protease inhibitors recently approved by the Food and Drug Administration, marking an important milestone in anti-HCV research and drug development over the past two decades. However, in vitro or in vivo, six major position mutations in the NS3/4A protease (36, 54, 155, 156, 168 and 170) have now been reported associated with different levels of resistance. Clarifying the underlying molecular mechanisms of NS3/4A protease inhibitor resistance is therefore essential for developing new drugs that are less at risk to the resistance factor. In the present study, we analysed the impact of R155K mutation for the binding of MK-5172. Hence, a (Normal Mode Analysis) NMA has been carried out alongside docking studies to provide the detailed information on the primary source of MK-5172 resistance due to R155K mutation. It is probable that this present computational strategy provides valid information on exactly how point mutations influence structural, molecular properties, and drug–protein interactions.

MATERIALS AND METHODS

Data Set:
The three-dimensional (3D) structures of wild and mutant NS3/4A protease were taken from the crystal structures of the Brookhaven Protein Data Bank (PDB) [9] for our computational analysis. The corresponding PDB codes were 3SUD and 3SUE for the wild and mutant type, respectively [10, 11]. Both structures were solved with >2.0 Å resolution. MK-5172 was used as the small molecule/inhibitor for our investigation. All the water molecules and the heteroatoms were removed and the three-dimensional structure of target proteins (3SUD and 3SUE) and drug molecule (MK-5172) was also energy-minimized using GROMACS package 4.5.3 adopting the GROMOS43a1 force field parameters before performing the computational analysis.

Identification of Binding Site Residues of NS3/4A protease:
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 the NS3/4A protease with MK-5172, complex structures (PDB code: 3SUD and 3SUE) into the ligand contact tool (LCT) program [12]. This program calculates contacts between the binding residues of NS3/4A protease receptor with MK-5172 by using default parameters.

Stabilizing Residues:
We thought it would be useful to identify any patterns of correlation between the mutation and stabilizing residues of the protein structures. Therefore we examined the stabilizing residues of wild and mutant type structures using the computational technique. Stabilizing residues were computed using the parameters such as surrounding hydrophobicity, long-range order, stabilization centre and conservation score. We used the program stabilizing residues (SRide) for this purpose [13].

Salt bridge analysis:
The stability of the protein structure can be clearly determined by the salt bridge interactions that are presented in them. We performed the salt bridge analysis with the help of an online tool, Visual molecular dynamics (VMD) [14]. VMD is a molecular modelling and visualization computer program, it was primarily developed as a tool for viewing and analyzing the results of molecular dynamics simulations [15], but it also includes tools for working with volumetric data, sequence data, and arbitrary graphics objects. Here set of all the salt-bridge pairs are observed in a trajectory. These interactions are crucial in many areas of modern chemistry, especially in the field of molecular recognition and for structural stability [16, 17].

Computation of docking score between the ligand and the enzyme:
Docking was performed with the help of the Patch- Dock [18]. It is geometry based molecular docking algorithm. The PatchDock algorithm divides the Connolly dot surface representation [19] 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 [20]. 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 PatchDock services are available at http://bioinfo3d.cs.tau.ac.il/PatchDock/. The results obtained from the PatchDock program were also refined from the FireDock algorithm to improve the prediction accuracy.

Normal Mode Analysis:
A quantitative measure of the atomic motions in proteins can be obtained from the mean square fluctuations of the atoms relative to their average positions. These can be related to the B-factor [21, 22]. Analysis of B-factors, therefore, is likely to provide newer insights into protein dynamics, flexibility of amino acids, and protein stability [23]. It is to be noted that protein flexibility is important for protein function and for rational drug design [24]. 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 [25] and vice versa [26]. 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 [27].

It is also known that the motion of a domain or binding residue can often be captured by normal mode analysis (NMA). NMA, particularly with a simple elastic network model, can be helpful for the simulation of target protein. The NMA generates 11 possible conformations between DQMIN of -100 and DQMAX of 100 with DQSTEP step size of 20. It is to be noted that understanding the binding affinity between the target and the drug based on relevant normal modes will authorize the strength of docking process. Hence, entire trajectory files from the lowest frequency mode were used as the input for docking analysis.

RESULTS AND DISCUSSION

Binding Residues Analysis:

The binding site residues in the structures of NS3/4A protease were obtained from the LCT program by using the complex structure of NS3/4A protease with MK-5172 (PDB ID: 3SUD and 3SUE). Both wild and mutant structures have shown similar results. The results indicate that a total of 17 amino acid residues, viz., R-123, L-135, A-156, F-154, A-139, K-136, Q-41, G-58, F-43, Y-56, V-78, H-57, D-81, I-132, G-137, R-155, and A-157 act as binding residues in the NS3/4A protease. The LIGPLOT [28] tool was used to illustrate the contacts between NS3/4A protease binding residues and MK-5172. These results were shown in Figure 1 and Figure 2.

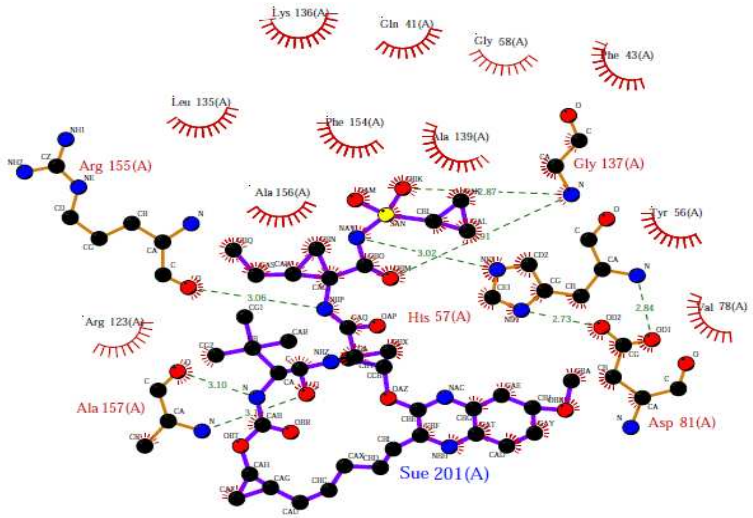

Figure 1: MK 5172 bound to the binding site of NS3/4A protease (native type). The figure was rendered using the program LIGPLOT

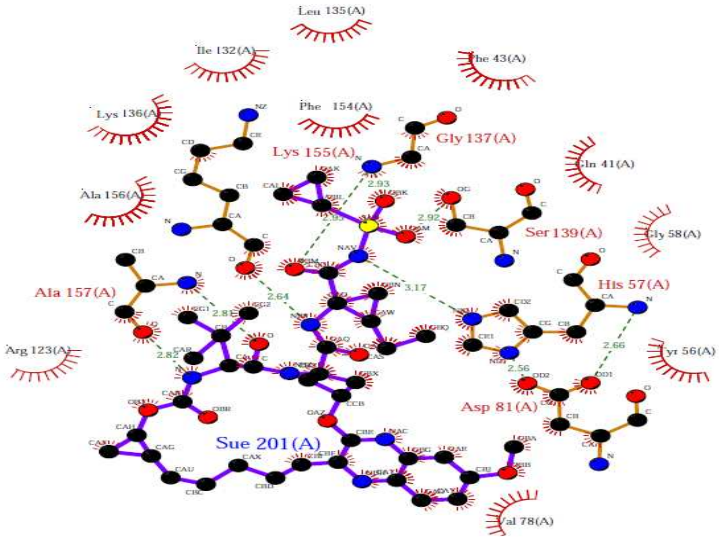

Figure 2: MK 5172 bound to the binding site of NS3/4A protease (R155K). The figure was rendered using the program LIGPLOT

Computation Stabilizing Residues:

Stabilizing residues were computed using the parameters such as surrounding hydrophobicity, long-range order, stabilization centre and conservation score. Conservation score of 6 is the cut off value used to identify the stabilizing residues. Residues are classified into nine categories according to their real conservation score. A score of 1 represents the most variable residues and a score of 9 represents the most conservative ones. The result is shown

in Table 1. It could be seen from the table that 12 residues act as stabilizing residues in the wild type structures. On the other hand, only 9 residues were identified as stabilizing residues in the mutant structures (R155K). The conservation score is same for both wild and mutant type stabilizing residues. This brings in the conclusion that substitution of Lysine instead of Arginine at position 155 leads to the change in conformation of the structure. Hence, this conformational change alters the function of a protein thus it affects the binding of MK-5172.

Table 1: Stabilizing residues of wild type and mutant type (R155K) NS3/4A protease structures.

S. No	Stabilizing residues in wild structure	Conservation score	Stabilizing residues in mutant Structure	Conservation Score
1	THR54	9	THR54	9
2	LEU82	6	LEU82	6
3	VAL83	8	VAL83	8
4	TRP85	6	TRP85	6
5	LEU104	7	LEU104	7
6	TYR105	9	TYR105	9
7	LEU106	9	-	-
8	GLY141	8	-	-
9	PRO142	9	-	-
10	CYS145	9	CYS145	9
11	GLY152	9	GLY152	9
12	PHE154	9	-	-
13	-	-	ALA164	9

Salt bridge analysis:

We have also examined the stability of the protein by means of salt bridge analysis. The salt bridge interactions in the protein structure were computed by Visual Molecular Dynamics (VMD) [29]. The salt bridge mainly relies on the protein structure stability factor. A salt bridge is generally considered to exist when the centres of charge are 4 Å or less apart. The resulting salt bridge pairs are collectively depicted in the Table 2, The wild type (PDB code: 3SUD) was found to maintain five salt bridges (Asp 81-His 57, Glu 30-Asp 3, Asp 168-Arg 155, Asp 168-Arg 123, Asp 25-Arg 11) and mutant type (PDB code: 3SUE) was found to maintain only three salt bridges (Asp 81-His 57, Asp 168-Arg 123, Asp 25-Arg 11).Then the distance between the salt bridges were also examined using SPDBV. It was interesting to observe that mutation at position 155 alters the conformation of other conserved residues in the structure. Hence, it cannot maintain the interaction with the partner molecule. Thus, we conclude that Lysine substitution at position 155 will provide destabilizing effect to the whole structure.

Table 2: Salt bridge computation in wild type (3SUD) and mutant type (3SUE) structures.

S. No	Salt bridge in native			Salt bridge in mutant		
	Donor	Acceptor	Distance	Donar	Acceptor	Distance
1	Asp 81	His 57	7.56	Asp 81	His 57	8.32
2	Glu 30	Asp 3	16.79	-	-	-
3	Asp 168	Arg 155	5.41	-	-	-
4	Asp 168	Arg 123	4.69	Asp 168	Arg 123	5.73
5	Asp 25	Arg 11	10.54	Asp 25	Arg 11	10.6

Computation of docking score between the ligand and the enzyme:

Furthermore, the functional impact of mutation was examined by docking analysis. 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 tool. Subsequently, the results obtained from the PatchDock algorithm refined by FireDock methodology. The result is shown in Figure 3. It was interesting to note that the affinity for MK-5172 was found to be -47.09 and -30.84 kcal/mol for the wild type and mutant type respectively. The PyMOL view of docked complex is shown in Figure 4 and Figure 5. The lesser binding affinity with mutant structures clearly signifies that the improper binding of MK-5172 with R155K structure. Thus causes resistance to the drug molecule.

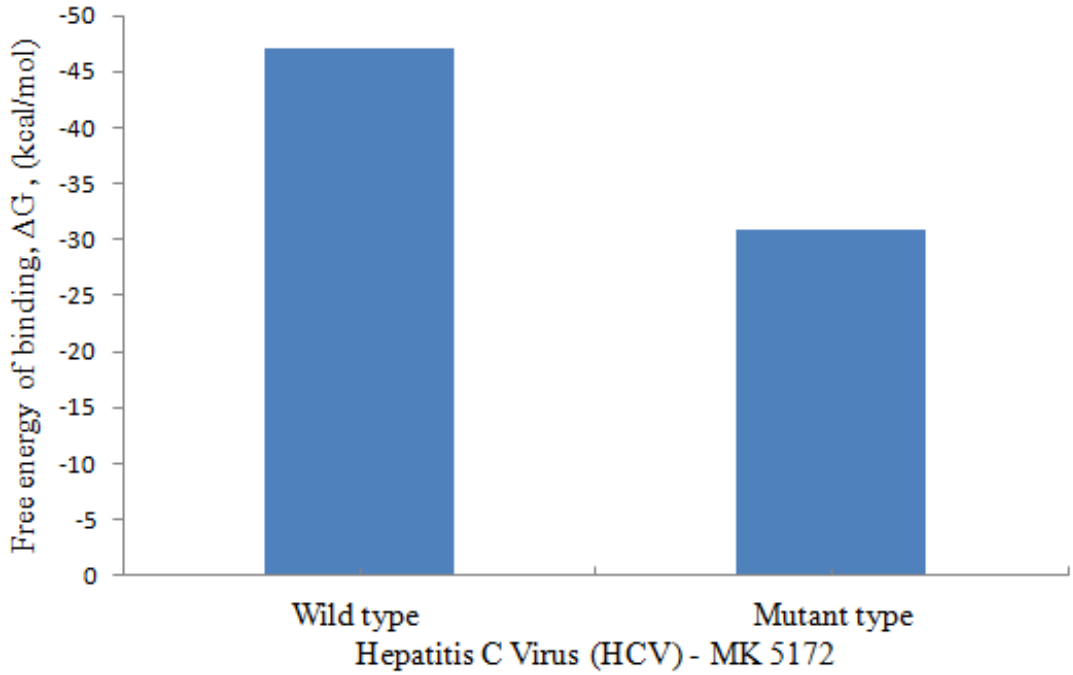

Figure 3: The binding free energies of the wild type (3SUD) and mutant (R155K) type (3SUE) NS3/4A protease – inhibitor complex

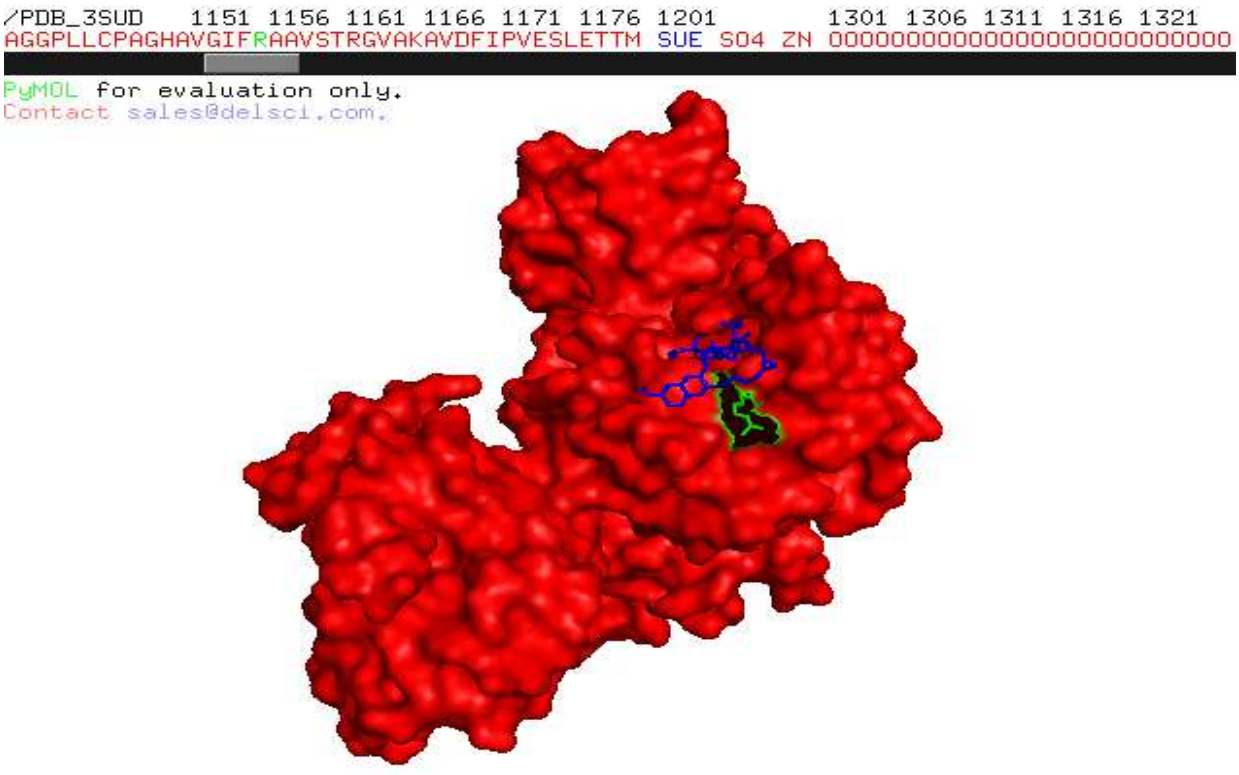

Figure 4: PyMOL representation of NS3/4A protease (Native type) docked with MK 5172

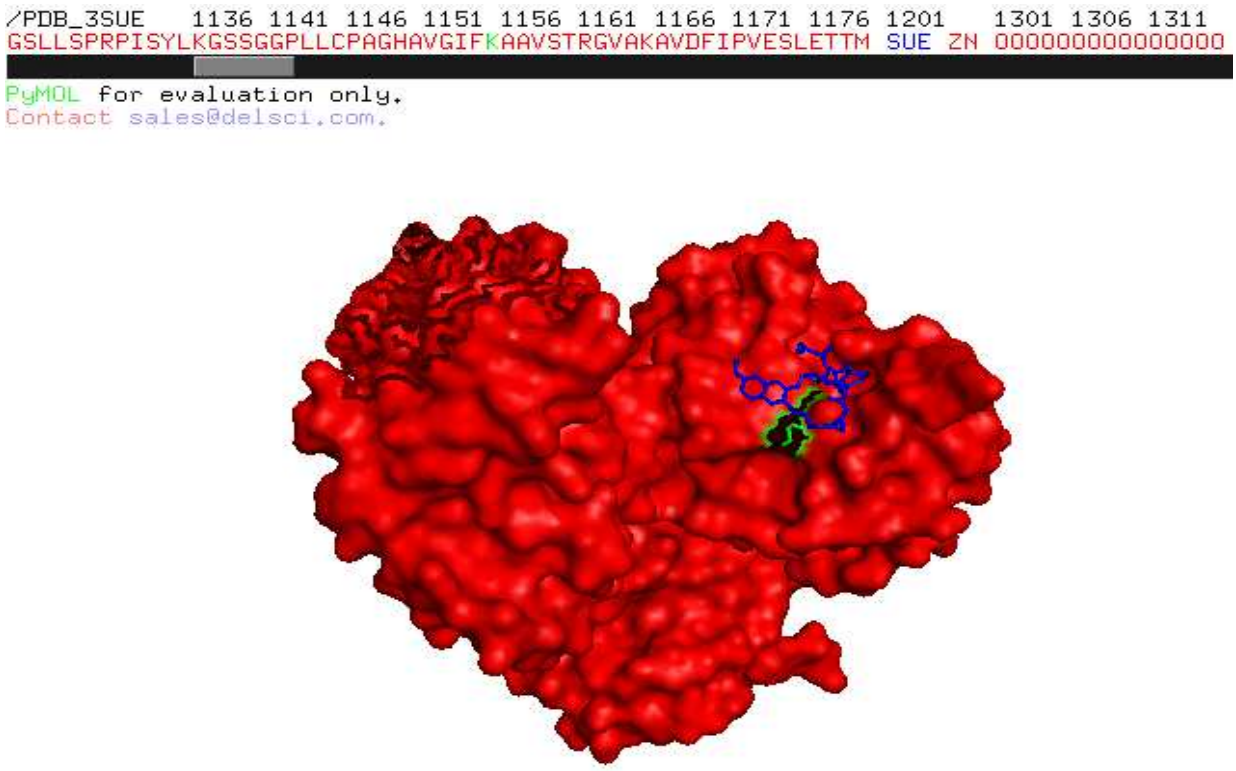

Figure 5: PyMOL representation of NS3/4A protease (R155K) docked with MK 5172

Normal Mode docking Analysis:

The collective motion in the energy-minimized structure was generated by means of normal mode analysis. It has been recently shown that half of the known protein movements can be modelled by using at most two low-frequency normal modes. Hence, we have chosen the seventh (the lowest frequency mode) mode for our docking study. The normal mode analysis generates 11 possible confirmations between DQMIN of −100 and DQMAX of 100 with DQSTEP step size of 20 [30]. It is to be noted that understanding the binding affinity between the target and the drug based on relevant normal modes will authorize the strength of docking process [31]. Hence, entire trajectory files from the lowest frequency mode were used as the input for docking analysis. The three-dimensional structure of MK-5172 was generated by using the tool CORINA [32]. The docking result is shown in Figure 6. We observed that free energy of binding for MK-5172 with wild type was substantially higher than mutant enzyme in most of the conformations. This observation indicates that this mutation (R155K) affects the structure of NS3/4A protease in such a way that decreases the binding affinity of MK-5172.

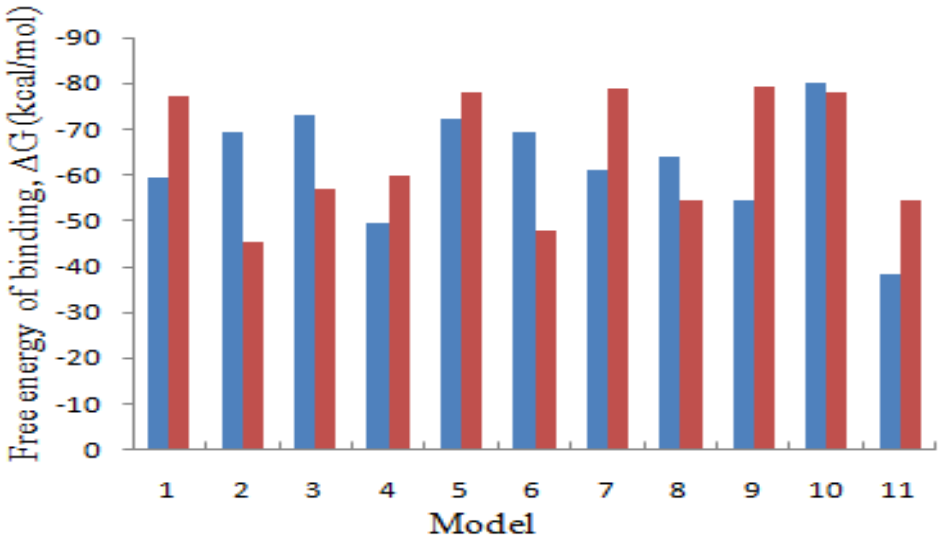

Figure 6: Comparison of free energies of binding for the wild (blue) and mutant (red) type NS3/4A protease by using normal mode analysis

Binding Residues Flexibility by Means of Normal Mode Analysis:

In order to understand the mechanism of drug resistances, we have analysed the flexibility of binding residues by R^2 analysis. Binding residue flexibility was fundamental to understand 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 R155K mutation, we used the program ElNemo to compare the flexibility of amino acids of both wild and mutants, which are involved in binding with MK-5172. Table 3 depicts the flexibility of amino acids in the drug-binding pocket of both wild 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 drug binding pocket of mutant which is exactly the same as <R2> of the amino acids in drug binding pocket of wild named as 'identical flexibility'. The second is the <R2> of amino acids in drug binding pocket of mutant, which is higher than <R2> of the amino acids in drug binding pocket of wild named as 'increased flexibility'. And the last is the <R2> of amino acids in the drug binding pocket of mutant, which is lesser than <R2> of amino acids in the drug binding pocket of wild named as 'decreased flexibility'. From the above classification, we understand that 82 % of Drug-binding amino acids were in the range of decreased flexibility and 18 % of drug binding amino acids were in the range of increased flexibility. On the other hand, none of the drug-binding amino acids were in the range of identical 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, MK-5172.

Table3: Comparison of normalized mean square displacement of drug-binding amino acids of wild and mutant types of NS3/4A protease structures.

S. No	Binding residues	Normalized mean square displacement, <R2> in wild type	Normalized mean square displacement, <R2> in mutant type
1	ARG 123	0.0163	**0.0062**
2	LEU 135	0.0176	**0.0078**
3	ALA 156	0.0107	**0.007**
4	PHE 154	0.0033	0.0061
5	ALA 139	0.0065	**0.0064**
6	LYS 136	0.0172	**0.0086**
7	GLN 41	0.019	0.0087
8	GLY 58	0.0153	**0.0077**
9	PHE 43	0.0109	**0.0058**
10	TYR 56	0.0159	**0.0073**
11	VAL 78	0.0214	**0.0092**
12	HIS 57	0.0173	**0.0083**
13	ASP 81	0.0162	**0.0069**
14	ILE 132	0.0309	**0.0094**
15	GLY 137	0.0104	**0.0082**
16	ARG 155	0.0063	0.0064
17	ALA 157	0.0197	**0.008**

Bold number indicates amino acids with decreased flexibility of mutant (R155K) compared to wild structure.

CONCLUSION

Several years of clinical trials was taken for the development of efficient drugs because of the increasing resistance of dangerous viruses that includes Hepatitis C virus. The time and cost associated with the drug development process can be greatly minimized by using the computational techniques. In this work, we used the NMA and molecular docking approach to gain an insight into MK-5172-resistant R155K mutation in the structure of NS3/4A protease. This approach provides the detailed information of the molecular and structural properties of the wild and mutant type structures. Furthermore, the <R2> data obtained in the NMA approach reveal that fluctuation behavior of binding residues in the mutant structure is slightly different from the wild structure. Finally, normal mode docking analysis undoubtedly indicates the improper binding of MK-5172 with the mutant NS3/4A protease structure. Hence, we conclude that this information should be taken into account for the design of inhibitors of NS3/4A protease. Thus, our findings suggest strategies for developing protease inhibitors that retain activity against a wider spectrum of drug-resistant HCV variants.

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REFERENCES

[1] D Lavanchy. *Liver Int*, **2009**, 29, 74–81.

[2] CW Shepard; L Finelli; MJ Alter. *Lancet Infect Dis*, **2005**, 5, 558–567.
[3] P Simmonds; J Bukh; C Combet; G Dele´age; N Enomoto. *Hepatology*, **2005**, 42, 962– 973.
[4] R Bartenschlager. *J. Viral Hepat*, **1999**, 6, 165–181.
[5] SD Seiwert; SW Andrews; Y Jiang; V Serebryany; H Tan. *Antimicrobe Agents Chemother*, **2008**, 52, 4432–4441.
[6] E Foy; K Li; C Wang; R Sumpter; M Ikeda. *Science*, **2003,** 300, 1145–1148.
[7] RB Perni; SJ Almquist; RA Byrn; G Chandorkar; PR Chaturvedi. *Antimicrob Agents Chemother*, **2006**, 50, 899–909.
[8] Philippe Halfon; Stephen Locarnini. *Journal of Hepatology*, **2011**, 55, 192–206.
[9] HM Berman; J Westbrook; Z Feng; G Gilliland; TN Bhat; H Weissig; IN Shindyalov; PE Bourne. *Nucleic Acids Research*, **2000**, 28, 235–242.
[10] RJ Russell; LF Haire; DJ Stevens; PJ Collins; YP Lin; GM Blackburn; AJ Hay; SJ Gamblin; JJ Skehel. *Nature*, **2006**, 443, 45–49.
[11] PJ Collins; LF Haire; YP Lin; J Liu; RJ Russell; PA Walker; JJ Skehel; SR Martin; AJ Hay; SJ Gamblin. *Nature*, **2008**, 453, 1258–1261.
[12] G Lopez; A Valencia; ML Tress. *Nucleic Acids Research*, **2007,** 35, 573–577.
[13] MM Gromiha; G Pujadas; C Magyar; S Selvaraj; I Simon. *Proteins***, 2004,** 55, 316-329.
[14] W Humphrey; A Dalke; K Schulten. *Journal of Molecular Graphics*, **1996**, 14, 33–8.
[15] IA Balabin; X Hu; DN Beratan. *J Comput Chem*, **2012,** 33, 906-10.
[16] CA Hunter; JKM Sanders. *J Am Chem Soc*, **1990,** 112, 5525-5534.
[17] R Wintjens; J LieAvin; M Rooman; E Buisine. *J Mol Biol*, **2000,** 302, 393-408.
[18] D Schneidman; Y Inbar; R Nussinov; HJ Wolfson. *Nucleic Acids Res*, **2005,** 33, 363– 367.
[19] ML Connolly. *Science*, **1983,** 221, 709–713.
[20] C Zhang; G Vasmatzis; JL Cornette; C DeLisi. *J Mol Biol*, **1997,** 267, 707–726.
[21] Z Yuan; TL Bailey; RD Teasdale. *Proteins*, **2005,** 58, 905–912.
[22] D Ringe; GA Petsko. Methods in *Enzymology*, **1986**, 131, 389–433.
[23] S Parthasarathy; M Murthy. r. *Protein Engineering*, **2000,** 13, 9–13.
[24] HA Carlson; JA McCammon. *Molecular Pharmacology*, **2000,** 57, 213–218.
[25] A Hinkle; LS Tobacman. *Journal of Biological Chemistry*, **2003,** 278, 506–513.
[26] R Rajasekaran; C George Priya Doss; C Sudandiradoss; K Ramanathan; R Purohit; R Sethumadhavan. *Comptes Rendus Biologies*, **2008**, 331, 409–417.
[27] K Suhre; YH Sanejouand. *Nucleic Acids Research*, 32, 610–614.
[28] AC Wallace; RA Laskowski; JM Thornton. *Protein Engineering*, **1995,** 8, 127-134.
[29] W Humphrey; A Dalke; K Schulten. *J Mol Graph*, ***1996***, *14*, *33-8*, *27-8*.
[30] K Suhre; YH Sanejouand. *Nucleic Acids Research*, **2004**, 32, 610–614.
[31] CN Cavasotto; JA Kovacs; RA Abagyan. *Journal of the American Chemical Society*, **2005,** 26, 9632–9640.
[32] J Gasteiger; C Rudolph; J Sadowski. *Compututer Method*, **1990,** 3, 537–547.