SUPPLEMENTARY MATERIAL

Mechanism of Flexibility Control for ATP Access of Hepatitis C Virus NS3 Helicase

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Choosing Hinge Residue T324 for Study

A. We started the study with the intent of elucidating the detailed atomistic mechanism of ATP binding and hydrolysis to polynucleotide unwinding coupling. Based on an experimental mutagenesis study (Tai et al, (2001) *J Virol* **75**, 8289-97) and X-ray crystal structures (Gu et al, (2010) *Proc Natl Acad Sci U S A* **107**, 521-8) of NS3 helicase, three critical residues (T324, V432 and Q460) were identified, which were potentially major players in the microenvironment rearrangement involved in ATP binding and strand-separation mechanisms. The main conclusions from these studies were:

I. T324: T324A mutation drastically reduced ATPase activity, which resulted most likely from its special hinge location and its role in modulating the opening and closing of the ATP binding cleft.

II. V432: Two key hydrophobic interactions between the nucleic acid bases and residues W501 and V432 are involved in the strand-separation mechanism.

III. Q460: DNA splitting functionality is tightly coupled with the ATPase binding and hydrolysis. Q460 is a key residue in the active-site rearrangement for catalysis.

Two wild type HCV NS3 helicase structures (PDB: 3KQH and 3KQL) and two computationally mutated structures were used for MD simulation similarly to the 20 ns simulation presented in the manuscript. In both structures hydrophobic residue V432, Q460 and T324 were computationally mutated to alanine giving eight distinct structures – including the two wild-type configurations – for MD simulation (see table next page).

Wild-type	3KQH	3KQL
1. V432A	3KQH-A432	3KQL-A432
2. Q460A	3KQH-A460	3KQL-A460
3. T324A	3KQH-A324	3KQL-A324

For each construct, 2 x 1 ns pilot MD simulations (two different seeds) were carried out using GROMACS with the same parameters as in the 20 ns simulation. For all eight simulations, the minimum distance was calculated between a hotspot residue group and another strategically selected group of interest, such that the distance data could shed light on the dynamic behavior of that particular region (see table next page). For all eight cases, the mean and standard deviation of residue pair distances were calculated using histogram analysis (S-Figure 1-7). The most interesting finding was related to hinge residue mutation T324A. In the ATP bound helicase structure the minimal distance between D290-R467 drastically decreased, which implies that domain 1 and 2 are more compacted when ATP is present (S-Figure 7). T324A mutation did not show a significant difference in the ATP-bound cases, but when no ATP was introduced to the structures it greatly influenced the regional stability of domain 1 and 2 with a two-fold increase in rigidity maintaining a characteristic separation of about 20 Å (S-Figure 6).

Hotspot residue	Distance measurement		Dynamic function
	START	END	
V432	three backbone atoms of residue V432	first three atoms of NA chain (DA5) closest to V432	probes structural transition to propel helicase motion
Q460	three backbone atoms of residue Q460	three backbone atoms of residue E291	probes the top pocket of ATP binding groove 3D structural dynamics
		oxygen atom of ATP closest to Q460 (O2B)	probes hotspot residue and ATP interaction
T324	three backbone atoms of residue D290	three backbone atoms of R467	probes hinge modulation

Thus, we choose T324A mutation of the unbound ATP structure (3KQH) as the target of our analysis, since this showed the most promising dynamic change related to the atomistic mechanism of ATP binding and hydrolysis.

Supplementary Figures and Tables

Supplementary Figures

S-Figure 1. Minimum distance histograms of V432A residue mutation with DA5. (A) Minimum distance frequency of wild-type HCV NS3 structure without bound ATP. (B) Minimum distance frequency of mutant HCV NS3 structure without bound ATP. Note almost same population mean and exact matching standard deviation.

S-Figure 2. Minimum distance histograms of V432A residue mutation with DA5. (A) Minimum distance frequency of wild-type HCV NS3 structure with bound ATP. (B) Minimum distance frequency of mutant HCV NS3 structure with bound ATP. Note almost 20% increase in population mean and similar increase in standard deviation.

S-Figure 3. Minimum distance histograms of Q460A residue mutation with E291. (A) Minimum distance frequency of wild-type HCV NS3 structure without bound ATP. (B) Minimum distance frequency of mutant HCV NS3 structure without bound ATP. Note that there is no significant difference between population mean and standard deviation values for each case.

S-Figure 4. Minimum distance histograms of Q460A residue mutation with E291. (A) Minimum distance frequency of wild-type HCV NS3 structure with bound ATP. (B) Minimum distance frequency of mutant HCV NS3 structure with bound ATP. Note that there is no significant difference between population mean and standard deviation values for each case.

S-Figure 5. Minimum distance histograms of Q460A residue mutation with ATP2. (A) Minimum distance frequency of wild-type HCV NS3 structure with bound ATP. (B) Minimum distance frequency of mutant HCV NS3 structure with bound ATP. Note that there is a small difference between population mean and very similar standard deviation values for each case.

S-Figure 6. Minimum distance histograms of T324A residue mutation with D290-R467. (A) Minimum distance frequency of wild-type HCV NS3 structure without bound ATP. (B) Minimum distance frequency of mutant HCV NS3 structure without bound ATP. Note drastic difference in standard deviation of (A) and (B), but steady value for population mean.

S-Figure 7. Minimum distance histograms of T324A residue mutation with D290-R467. (A) Minimum distance frequency of wild-type HCV NS3 structure with bound ATP. (B) Minimum distance frequency of mutant HCV NS3 structure with bound ATP. Note that there is a small difference between population mean and very similar standard deviation values for each case.

Supplementary Tables

TABLE S-I

Hydrogen Bond Formation and Residence Time Analysis in “Water Bridge”

Atom from D454	Atom from H293	Atom from W1	Atom from W2	Total Time (ps)	Number of Events
OD1	-	HW1	-	478.3	47
OD2	-	-	HW2	265.1	46
-	HB1	OW	-	125.5	58
-	HB2	-	OW	127.9	34
-	-	OW	HW1	51.6	24

H-bonds with duration less than 1 ps were not counted.