# Structure-Based Simulations of the Translocation Mechanism of the Hepatitis C Virus NS3 Helicase along Single-Stranded Nucleic Acid

Wenjun Zheng and Mustafa Tekpinar

Physics Department, University at Buffalo, Buffalo, New York

# Supporting Information

## Methods

We can expand the ENM potential energy (see Eq 1 of main text) to second order:

$$E \approx \frac{1}{2} X^T H X , \quad (1)$$

where $X$ is a 3$N$-dimension vector representing the 3D displacement of $N$ $C_\alpha$ atoms away from their equilibrium positions, $H$ is the Hessian matrix which is obtained by calculating the second derivatives of ENM potential energy with respect to the 3D coordinates of $C_\alpha$ atoms.

NMA solves $HW_m = \lambda_m W_m$, where $\lambda_m$ and $W_m$ represent the eigenvalue and eigenvector of mode $m$. After excluding 6 zero modes corresponding to 3 rotations and 3 translations, we keep 3$N$-6 non-zero modes, which are numbered from 1 to 3$N$-6 in order of ascending eigenvalue.

To validate NMA, we compare each mode (mode $m$) with the observed structural changes between two crystal structures (represented by a 3$N$-dimension vector $X_{obs}$) by calculating the following overlap:

$$I_m = \frac{\left| X_{obs}^T W_m \right|}{\left| X_{obs} \right| \cdot \left| W_m \right|}, \quad (2)$$

where $X_{obs}^T W_m$ is the dot product between vectors $X_{obs}$ and $W_m$, $\left| X_{obs} \right|$ and $\left| W_m \right|$ represent their magnitudes.

In addition, the following cumulative overlap is calculated to assess how well the lowest 10 modes describe $X_{obs}$:

$$C_{10} = \sqrt{\sum_{1 \le m \le 10} I_m^2} . \quad (3)$$

Because $\sum_{1 \le m \le 3N-6} I_m^2 = 1$, $C_{10}^2$ gives the percentage of the observed structural changes captured by the lowest 10 modes.

## Discussion

Unlike linear-interpolation-based methods like the Yale Morph server, iENM is more physically based because it searches for saddle points (SP) corresponding to minimal energy barrier of a transition. Therefore, iENM can predict a distinct order of structural motions involving different protein parts rather than highly synchronized motions as predicted by linear interpolation. Compared with mixed-ENM, which is an early approximate version of iENM, iENM solves the SP equation more accurately. Compared with another ENM-based method --- MinActionPath, iENM predicts a more robust pathway independent of the mathematic form of double-well potential

function and the energy offset between two end states. For a more detailed comparison between iENM and alternative methods, see ref 45.

# Tables

**Table S1**. Comparison between the lowest 10 normal modes and the crystallographically observed conformational changes in HCV NS3hel

| PDB_chain id of two NS3hel structures | RMSD (Å) | Mode# | Overlap |
|---|---|---|---|
| 3kqk_AD → 3kqu_B | 3.26 | #3<br>#4<br>#1~10 | 0.49<br>0.57<br>0.92 |
| 3kqu_BM → 3kql_A | 1.03 | #1<br>#1~10 | 0.61<br>0.72 |
| 3kql_AE → 3kqk_A | 3.37 | #5<br>#6<br>#1~10 | 0.45<br>0.50<br>0.82 |
| 3o8c_AC → 3o8r_A | 1.58 | #6<br>#1~10 | 0.49<br>0.77 |

# Figures

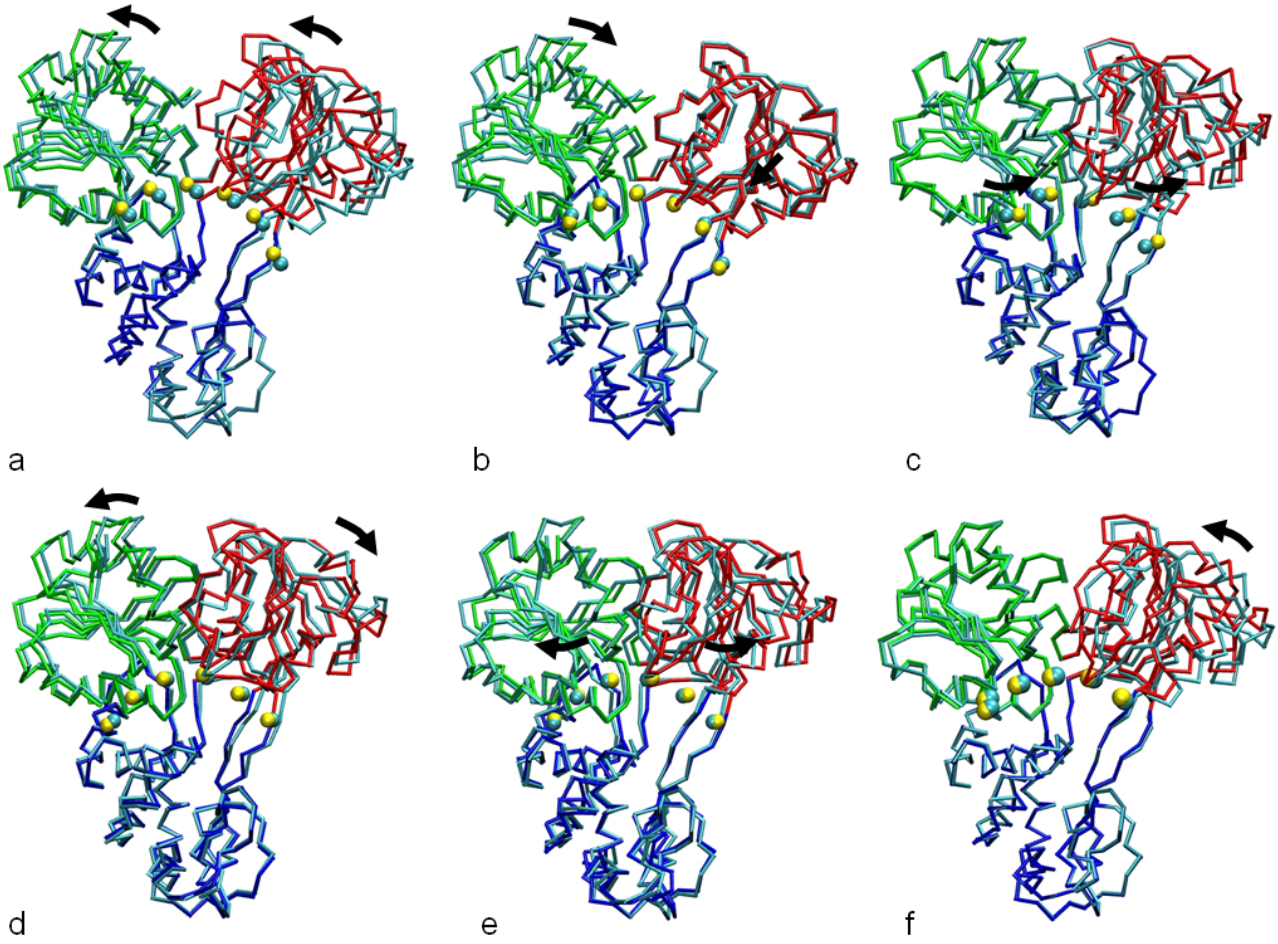
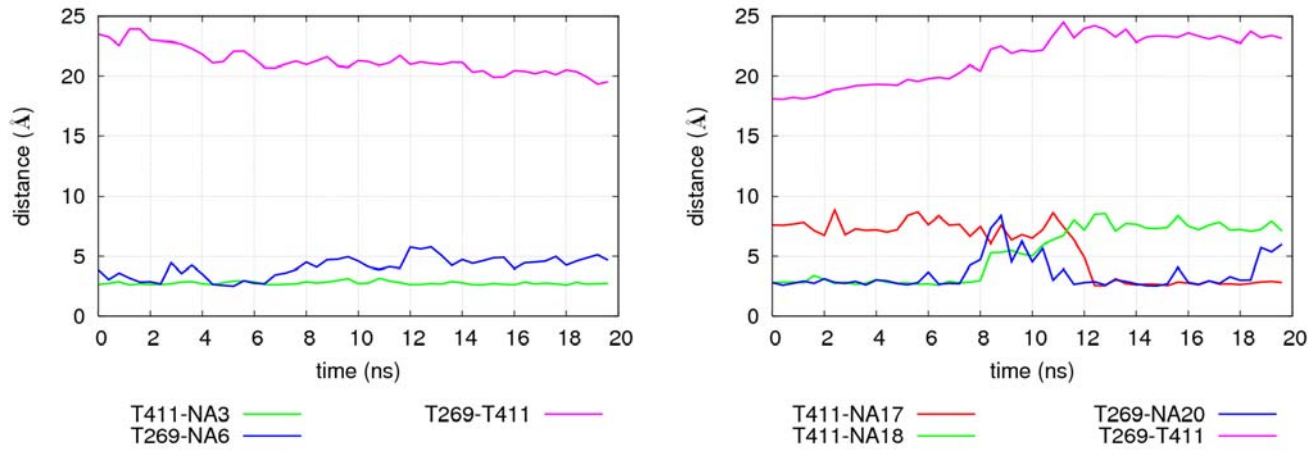



**Figure S1**. Conformational changes in NS3hel as predicted by ENM-based normal modes as follows: (a) mode #3, (b) mode #4, which are solved from the apo-state NS3hel-ssDNA structure (PDB id: 3kqk); (c) mode #1, which is solved from the ATP-state NS3hel-ssDNA structure (PDB id: 3kqu); (d) mode #5, (e) mode #6, which are solved from the ADP-Pi-state NS3hel-ssDNA structure (PDB id: 3kql); (f) mode #6, which is solved from the apo-state full-length NS3-ssDNA structure (PDB id: 3o8c).

Two structures of NS3hel (before and after the conformational changes predicted by each mode) are superimposed along domain 3. The starting NS3hel-ssDNA structure is colored cyan. The NS3hel-ssDNA structure after the conformational changes is colored as follows: domain 1, 2 and 3 are colored green, red and blue, respectively, and ssDNA is shown as a chain of yellow beads located at C4’ atoms. The domain rotations are shown by arrows.




a b

**Figure S2.** Time evolution of selected pairwise distances during targeted MD simulations for the following transitions in NS3hel: (a) apo state → ATP state; (b) ADP-Pi-state → apo state.

In panel (a), the curves for the T269:CA – T411:CA distance (measuring the closing motion between domain 1 and 2), the T411:OG1 – NA3:OP1 hydrogen bond distance, the T269:OG1 – NA6:OP1 hydrogen bond distance are colored purple, green, blue, respectively.

In panel (b), the curves for the T269:CA – T411:CA distance (measuring the opening motion between domain 1 and 2), the T411:OG1 – NA18:OP1 hydrogen bond distance, the T411:OG1 – NA17:OP1 hydrogen bond distance, the T269:OG1 – NA20:OP1 hydrogen bond distance are colored purple, green, red, blue, respectively.

**Movie S1**. Conformational changes in NS3hel as predicted by ENM-based normal modes as follows:
(a) superposition of the lowest 10 modes, (b) mode #3, (c) mode #4, which are solved from the apo-state NS3hel-ssDNA structure (PDB id: 3kqk);
(d) superposition of the lowest 10 modes, (e) mode #1, which are solved from the ATP-state NS3hel-ssDNA structure (PDB id: 3kqu);
(f) superposition of the lowest 10 modes, (g) mode #5, (h) mode #6, which are solved from the ADP-Pi-state NS3hel-ssDNA structure (PDB id: 3kql);
(i) superposition of the lowest 10 modes, (j) mode #6, which are solved from the apo-state full-length NS3-ssDNA structure (PDB id: 3o8c);
Domain 1, 2 and 3 are colored green, red and blue, respectively. ssDNA is shown as a chain of yellow beads located at C4' atoms. 10 snapshots are generated for each movie, with NS3hel superimposed along domain 3.

**Movie S2:** Transition pathways predicted by iENM for the following transitions in NS3hel:
(a) apo state $\rightarrow$ ATP state;
(b) ATP state $\rightarrow$ ADP-Pi-state;
(c) ADP-Pi-state $\rightarrow$ apo state;
Domain 1, 2 and 3 are colored green, red and blue, respectively. ssDNA is shown as a chain of yellow beads located at C4' atoms. NS3hel structure is superimposed along domain 3.

**Movie S3:** CG simulations by iENM for the 3-state ATP cycle in NS3hel (ATP $\rightarrow$ ADP-Pi $\rightarrow$ apo $\rightarrow$ ATP):
(a) all protein-DNA interactions are kept;
(b) protein-DNA interactions involving residue V432 are turned off;
(c) protein-DNA interactions involving residue W501 are turned off;
Domain 1, 2 and 3 are colored green, red and blue, respectively. ssDNA is shown as a chain of yellow beads located at C4' atoms. NS3hel structure is superimposed along domain 3.

**Movie S4:** targeted MD simulations for the following transitions in NS3hel:
(a) apo state $\rightarrow$ ATP state;
(b) ADP-Pi-state $\rightarrow$ apo state;
NS3hel, ssDNA and selected ssDNA-binding residues are represented by cartoon, lines and bonds, respectively. Key protein-ssDNA hydrogen bonds are marked by dotted lines with donor-acceptor distance labeled. NS3hel structure is superimposed along domain 3.