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**Supporting Material**

# Deconstructed helicase

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# Supporting information
## Hexameric helicase deconstructed: interplay of conformational changes and substrate coupling

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Table S1: Crystallographic pair-wise distances between the key residues in the active site.

| | | Walker A (K432) | Walker B (D474) | Sensor I (N529) |
|---|---|---|---|---|
| Sensor II (K418) | Apo, $r_{ij}^{E}$ | 18.51±0.08 | 19.75±0.33 | 21.45±0.25 |
| | ADP, $r_{ij}^{D}$ | 15.85±1.72 | 17.19±2.41 | 18.74±1.90 |
| | ATP, $r_{ij}^{A}$ | 12.62±0.03 | 13.36±0.06 | 15.92±0.09 |
| Sensor III (R498) | Apo, $r_{ij}^{E}$ | 16.43±0.16 | 13.00±0.36 | 15.80±0.22 |
| | ADP, $r_{ij}^{D}$ | 14.07±0.53 | 10.59±0.95 | 13.63±0.17 |
| | ATP, $r_{ij}^{A}$ | 12.75±0.05 | 7.04±0.06 | 11.82±0.10 |
| Arginine Finger (R540) | Apo, $r_{ij}^{E}$ | 20.39±0.02 | 19.30±0.21 | 20.51±0.10 |
| | ADP, $r_{ij}^{D}$ | 18.09±0.78 | 16.88±1.65 | 17.88±1.07 |
| | ATP, $r_{ij}^{A}$ | 14.69±0.03 | 12.90±0.09 | 14.59±0.15 |

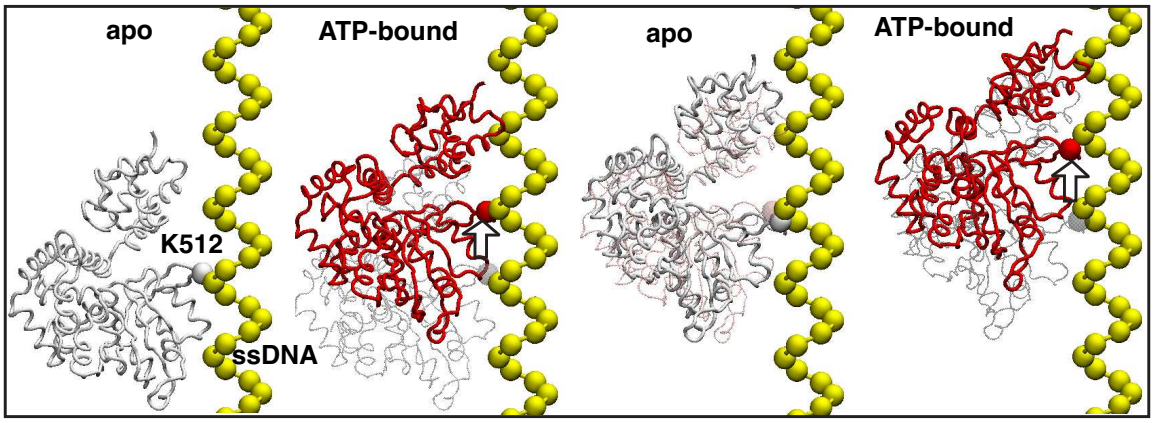


Figure S1: Snapshots from a trajectory undergoing unidirectional motion ($\epsilon_{DNA}$ = 4.5 kcal/mol). The MD snapshots of the same protein subunits were obtained at 0, 2, 6 and 8 million time steps (*left* to *right*) representing one complete cycle of the ATP binding and the following step. For clarity, the subunits in the front and back are not shown.

## Scheme S1: Limitations and assumptions of our coarse-grained model

Since the translocation of ssDNA by hexameric helicases most likely occurs on the order of milliseconds to microseconds time scales and involves many subunits of proteins, detailed all-atom approaches are not suitable to investigate this process. Instead, we employ a coarse-grained model that adequately mimics the hexameric helicase system and yet is simplified enough to permit the simulation of large system over relatively long time scales. However, several careful assumptions are built into the model and combined with previously known experimental measurements which allow us to explore the translocation mechanism of the helicase and test different ATP binding mechanisms. Some of these assumptions and limitations are: (i) As the result of the coarse grained representation of the system the time-scale information is lost and thus our simulations only represent the conformational change order, not necessarily the biological time needed for the translocation to occur. Most likely the simulations are much faster than the biologically relevant time scale for the translocation process. Also, the choice of the time-scale of the ATP binding and relaxation is somewhat arbitrary and only reflects the time steps needed to equilibrate the system properly. (ii) During the simulations the ssDNA is kept fixed based on the assumption that the length of the ssDNA bound to the hexameric helicase (i.e., 6 bases) is shorter than or equal to the persistence length (a parameter which characterizes the flexibility of the linear macromolecules) of ssDNA in the solution and hence displays only limited flexibility. Indeed a recent experimental study shows that a persistence length of the ssDNA is about 4 nm at $10^{-2}$M ionic strength which is approximately equal to the 12 nucleotide bases in the DNA length (1). However, the value of the persistence length may vary depending upon the ionic strength and the flexibility of ssDNA may in turn affect the step-size of the helicase translocation. (iii) In our simulations we explore only the strictly sequential and concerted ATP binding mechanisms but other ATP binding mechanisms involving ATP binding in a partially sequential (2, 3) or probabilistic manner (4) are also possible as suggested in analysis of some experiments. Nonetheless, for the hexamer motion to be extremely robust and processive, the key molecular features of the helicase revealed in this study will most likely remain unchanged.

## References


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# Description text of movies

**Supplementary movie 1:** Translocation of the hexameric helicase along ssDNA. To clarify the conformational change of monomers and the DNA-hexamer interactions, four monomers at the front and back are removed. The monomer colour represents its state during the ATP cycle, which is consistent with the colour definitions in Fig. 2c. When a monomer binds ATP, corresponding to colors pink and red, it undergoes a large conformational change and the DNA-binding lysine residue (shown as a non-transparent sphere, located in the vicinity of the helical strand) moves up. Then the lysine residue sticks to the DNA at this higher position and stays there until the next ATP binding event occurs. The sequential occurrence of ATP binding results in a unidirectional upward motion of the hexamer. During the course of 40 ATP binding events, the hexamer translocates by $\sim$13 nm (i.e., $\sim$40 bases). The details of this hexamer's trajectory are shown in Fig. 3b.

**Supplementary movie 2:** Sequential ATP-binding mechanism during the hexamer's translocation along ssDNA. In addition to the two monomers shown in supplementary movie 1, the other four monomers are also represented in this movie. Each monomer undergoes the same ATP cycle, but starts in a different state (see Fig. 3a). Because of the initial offset, ATP binding events progress around the ring-shaped hexamer. Note that the colours pink and red (i.e., ATP-bound state) rotate but the monomers rarely move around the helical strand.