Initial conformation of kinesin's neck linker*

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How ATP binding initiates the docking process of kinesin's neck linker is a key question in understanding kinesin mechanisms. By exploiting a molecular dynamics method, we investigate the initial conformation of kinesin's neck linker in its docking process. We find that, in the initial conformation, the neck linker has interactions with $\beta 0$ and forms a 'cover-neck bundle' structure with $\beta 0$. From this initial structure, the formation of extra turns and the docking of the cover-neck bundle structure can be achieved. The motor head provides a forward force on the initial cover-neck bundle structure through ATP-induced rotation. This force, together with the hydrophobic interaction of ILE327 with the hydrophobic pocket on the motor head, drives the formation of the extra turn and initiates the neck linker docking process. Based on these findings, a pathway from ATP binding-induced motor head rotation to neck linker docking is proposed.

Keywords: kinesin, neck linker, molecular dynamics simulation

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1. Introduction

Conventional kinesin (kinesin-1)[1] is an ATP-powered microtubule-based dimeric motor protein responsible for organelle transportation in cells.[2–5] Kinesin's two identical motor heads are connected by a coiled-coil stalk and two neck linkers (NLs). The two heads switch their positions on a microtubule in a hand-over-hand manner to walk processively along the microtubule's surface.[2,6–8] In a walking cycle, each motor head can be in four nucleotide states, i.e., a nucleotide-free state, an ATP state, an ADP + Pi state (before Pi release), and an ADP state (after Pi release). The motor heads in the ATP and ADP + Pi states have similar conformations and bind to the microtubule surface strongly. The motor heads in the nucleotide-free and ADP states have similar conformations but the motor head in the ADP state binds to the microtubule weakly.[9–11] During the processive motion, kinesin's two NLs have large conformational changes and the docking process of the leading head's NL to the motor domain, which is induced by ATP binding, constitutes a crucial part of kinesin's force generation step (the power stroke).[12–17] In one of our previous papers,[18] we have shown that the force generated during the NL docking process arises from the contributions of various interactions among amino acids (AAs) of NL and the motor head. However, how the NL docking process is initiated remains unclear. To address the initiation mechanism of NL docking, the key question is to find out the right initial conformation of NL in its docking process.

Kinesin's NL, which locates between $\alpha 6$ of the motor domain and $\alpha 7$ of the coiled coil, is a short peptide ($\sim$ 14 AAs, LYS325-THR338 in 2KIN) consisting of three parts, including 1) the first three AAs which form an extra turn in the docked conformation of NL, 2) $\beta 9$, and 3) $\beta 10$.[2,12,14,19] It has been revealed that the NL of the leading head has a backward positioning as its initial conformation in its docking process and the NL of the trailing head has a forward extension along the length of the motor domain as depicted in, for example, Fig. 2(a) of Ref. [16]. The docking process of the leading head's NL is crucial for force generation and the replacement of NL with a designed random coil results in the decrease of microtubule velocity in motor gliding assays.[13]

NL docking is accomplished in three steps: (i) the first three AAs forming an extra turn structure, (ii) $\beta 9$ docking to the motor domain and then (iii) $\beta 10$ docking to the motor domain.[14,20,21] When kinesin's leading head is in a nucleotide-free state, this head's $\alpha 4$ is in an occluding conformation which prevents the formation of an extra turn and thus prevents the docking of $\beta 9$ and $\beta 10$.[14,20–22] ATP's binding to this nucleotide-free leading head induces a set of conformational changes which eliminate the obstruction of $\alpha 4$ to extra turn formation. In this way, the extra turn can form and initiate the subsequent NL docking.[2,14] Therefore, the extra turn formation is a crucial step of the entire NL docking process. Hwang *et al.*[20] and Khalil *et al.*[21] achieved an important progress in understanding NL's docking mecha-

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nism. Through molecular dynamics (MD) simulations, Hwang *et al.*[20] found that the second step of NL docking, i.e., $\beta 9$ docking to the motor domain, is achieved by forming a 'cover-neck bundle' (CNB) which is a special β-sheet formed by the N-terminal $\beta 0$ and NL's $\beta 9$. In Ref. [20], they started their simulation from a conformation in which the extra turn and the CNB have already formed and found that the CNB structure has a spontaneous docking motion. This provides a force generation mechanism for the second step of NL docking. In Refs. [20] and [21], like in some other references,[16,23] NL and $\beta 0$ were assumed to be free in the initial state of NL docking. Then there remain several questions, such as: What is the initial conformation of $\beta 0$ and NL before the formation of extra turn? What is the mechanical pathway for the formation of extra turn? How is the CNB structure formed and at what time is it formed? etc.

To address these questions, we perform a series of MD simulations. We find that, in NL's initial undocked state, NL and $\beta 0$ are not completely 'free'. Instead, there is a group of hydrogen bonds between them, i.e., the CNB structure exists already at the beginning of NL's docking process. The formation of an extra turn and the docking of CNB (i.e., the first and second steps of NL docking) can be achieved from this initial CNB structure. With the aid of the ATP- and ADP-state crystal structures of kinesin, we also find that the motor head rotation induced by ATP binding provides a forward force on the initial CNB structure. This force, together with the hydrophobic interaction between ILE327 and the hydrophobic pocket on the motor domain, drives the formation of the extra turn. Based on these understandings, a pathway from ATP binding-induced motor head rotation to extra turn formation is proposed.

2. Model and method

The structures we used for modeling are 2KIN[19] and 1BG2.[24] 2KIN is the X-ray structure of rat kinesin, which has a docked NL and is suggested to be in an ATP-like state. 1BG2 is human kinesin in the ADP state and does not have coordinates for NL. To save computational resources, we delete 2KIN's $\alpha 7$ (the residues after ALA339), ADP, and SO4. The motor domain is surrounded by explicit water and the spherical boundary condition is used. 2KIN does not have coordinates for the main part of L11 loop. We connect the two ends of L11 (VAL239 and ASN256 in 2KIN) directly. Because our model does not include the microtubule, we fix the C_α atoms of GLU158 at $\beta 5$, SER260 and GLU271 at $\alpha 4$ (AA numbering of 2KIN) to mimic the microtubule-bound state. The MD simulations are performed by using NAMD (version 2.9)[25] with force field CHARMM[26] at $T = 310$ K. The integration time step is 2 fs. The software we use for modeling is VMD (version 1.9.1).[27] Na^+ and Cl^- are added to ensure an ionic concentration of 150 mM and zero net charge.[28] The non-bonded Coulomb and van der Waals interactions are calculated with a cutoff using a switching function starting at a distance of 13 Å and reaching zero at 15 Å. TIP3P[29] is adopted to model water molecules. In the simulations, the protein–water system is minimized for 3×10^4 steps to remove bad contacts. Molecular drawings in this paper are produced by using Discovery studio 3.5 visualizer.

3. Results and discussion

In order to understand the mechanisms of the extra turn formation and the NL docking process, in the following subsections, we construct three different initial conformations for NL and $\beta 0$ of the nucleotide-free leading head and then run MD simulations to check which one reasonably reflects the realistic situation. The construction is based on two crystal structures of kinesin, 2KIN[19] and 1BG2.[24] 2KIN is of the ATP-bound state conformation and 1BG2 is of the ADP-bound state conformation.[10,11] Because the nucleotide-free state conformation is similar to that of the ADP-bound state,[10,11] we use the crystal structure of 1BG2 as the nucleotide-free state conformation in this investigation.

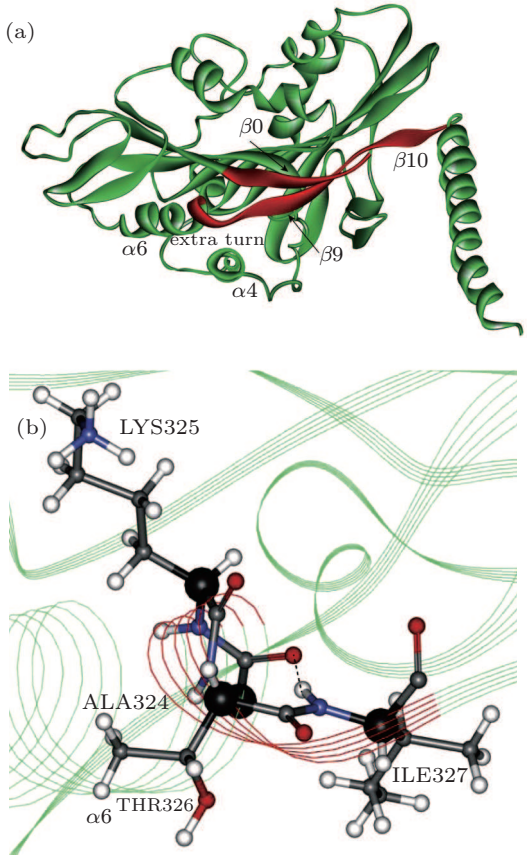


Fig. 1. (color online) The extra turn structure in the NL-docked state. (a) The motor head structure (2KIN) in the ATP state conformation. NL and $\beta 0$, which form a CNB structure, are depicted in red. (b) The extra turn structure in the ATP state conformation. The alpha carbons are depicted by bigger spheres and the hydrogen bond by a dotted line. The side chain of ALA324 is omitted with only the alpha carbon and the peptide plane remaining.

For convenience, we first give a description of the extra turn structure in the NL-docked conformation. The docking of NL starts from the formation of an extra turn. In the crystal structure of 2KIN, NL's first three AAs, LYS325, THR326, and ILE327, have formed an extra turn structure (Fig. 1). In the kinesin superfamily, LYS325 and ILE327 are two of the most conservative AAs. THR326 can be replaced by a serine, both of which have a hydroxide group.[30] The signature of the extra turn formation is the formation of a hydrogen bond between the backbone hydrogen of ILE327 and the backbone oxygen of ALA324, as depicted in Fig. 1(b). The condition for the formation of this hydrogen bond is that ILE327 is docked in the hydrophobic pocket formed by six hydrophobic AAs (including ILE9, ILE266, LEU269,ALA270, LEU292, and ALA324) of the motor domain. When ILE327 is out of the hydrophobic pocket, the extra turn is open.

3.1. CNB structure cannot be achieved from a conformation with free $\beta 0$

In the ATP-waiting state of kinesin, the leading head is in a nucleotide-free state (i.e., the initial state of NL docking) and the trailing head, which is located 8 nm behind the leading head, is in the ADP state.[31–33] The leading head's NL points to the minus end of microtubule in the nucleotide-free state. However, the conformation of leading head's $\beta 0$ in this initial state is still unclear. In some references, $\beta 0$ is assumed to be free.[16,20,21,23] Suppose the leading head's initial conformation has a backward pointing NL with an open extra turn and a free $\beta 0$, we must check whether the extra turn and the CNB could be achieved from this initial conformation since these two structures have already formed in the beginning conformation of the second step of NL docking. To this end, we construct an initial structure for the leading head from the crystal structure of 2KIN with a backward pointing NL and a free $\beta 0$. Taking this conformation as the initial state of NL docking, we perform an MD simulation.

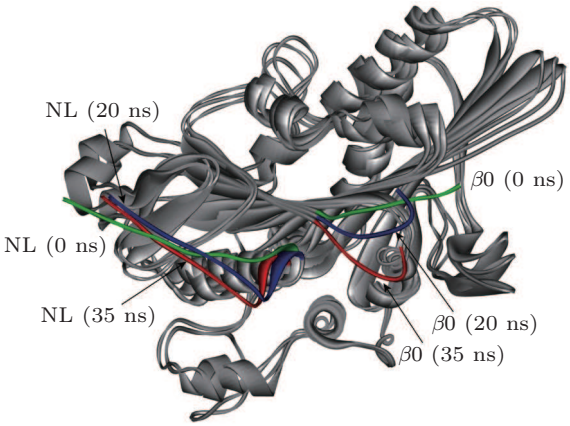


Fig. 2. (color online) Overlaid snapshots of conformations in the MD simulation with free $\beta 0$. NL and $\beta 0$ of the initial state conformation are shown in green and that of the conformations at 20 ns and 35 ns are shown in blue and red, respectively.

The result of a 35-ns simulation shows that NL has no tendency of docking to the motor domain and $\beta 0$ has no tendency of forming a CNB structure with NL (Fig. 2). As seen from Fig. 2, $\beta 0$ remains at the right side of NL in the simulation. To form CNB with NL, $\beta 0$ must bend to the left side of NL, which is a large conformational change and needs a backward force acting on $\beta 0$ to achieve it. From the structures of a free $\beta 0$ and the surrounding parts of the motor head, no such force can be found. Therefore, the CNB structure cannot be achieved from a structure with a free $\beta 0$ and such a structure cannot be the correct initial conformation of NL docking. Evidently this situation cannot be changed by prolonging the simulation time since it is a structure-based conclusion.

3.2. Without extra turn formation a backward CNB structure cannot properly dock to the motor domain

To obtain the initial conformation of NL and $\beta 0$ of the leading head, we unbind the docked NL of 2KIN by exerting a backward force at the C-terminus of NL in MD simulation. This method was also used in the simulation of Ref. [20], although the directions of the unbinding forces are different. The direction of the force used in our simulation is depicted in Fig. 3(a). This direction is set based on the following considerations. In the hand-over-hand movement of kinesin, the two motor heads walk along the same protofilament on the microtubule surface.[34] The force acting on the leading head's NL arises from the inter-head tension between the two motor heads.[33,35–38] Therefore, this force should point to the minus end of the microtubule as depicted in Fig. 3(a). In the simulation of Ref. [20], a very strong force ($>$ 400 pN) was used to unbind the docked NL. This force might be much stronger than the inter-head tension.[30] In our simulation, in order to approach the realistic situation and to save computational resources, we use a weaker force (160 pN) to unbind the docked NL. After 13-ns simulation, NL arrives at a backward pointing position similar to the NL position of the motor head in Fig. 2. In the meantime, $\beta 0$ keeps a CNB structure with NL (Fig. 3(b)). This is the second initial structure we constructed, which has a backward-pointing CNB structure and the motor domain has an ATP state conformation. In the whole unbinding process, four hydrogen bonds are maintained between $\beta 0$ and NL (two hydrogen bonds between VAL331 and ALA5 and another two between CYS7 and ASN329). This is a very interesting result beyond expectation because $\beta 0$ and NL are exposed to the surrounding water during the whole unbinding process. We propose that the side chains of AAs in CNB region effectively protect these hydrogen bonds from water attack. In this backward-pointing CNB structure, $\beta 0$ locates between NL and the β-domain ($\beta 1$a, $\beta 1$b, and $\beta 1$c) and the extra turn is open (Fig. 3(b)).

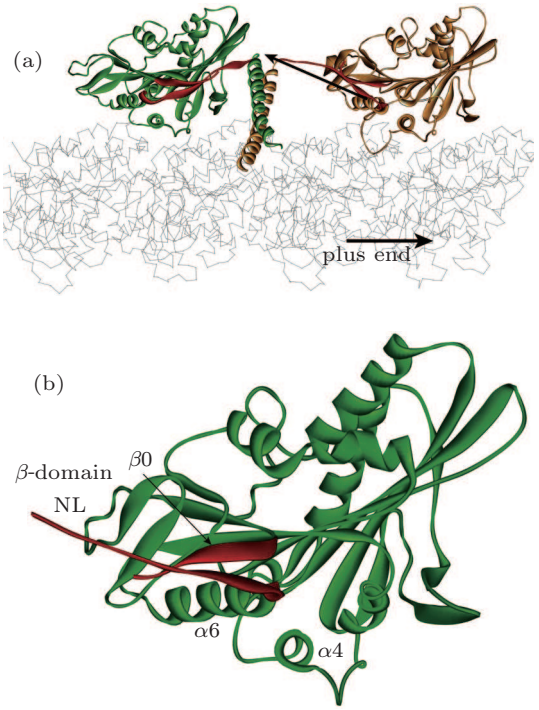


Fig. 3. (color online) NL unbinding simulation. (a) Two heads of kinesin bind to the microtubule simultaneously. The direction of the unbinding force is indicated by the arrow. The magnitude of the force is 160 pN in the NL unbinding simulation. (b) The resultant backward conformation of NL and $\beta 0$, which is also a CNB structure. $\beta 0$ locates between NL and β-domain.

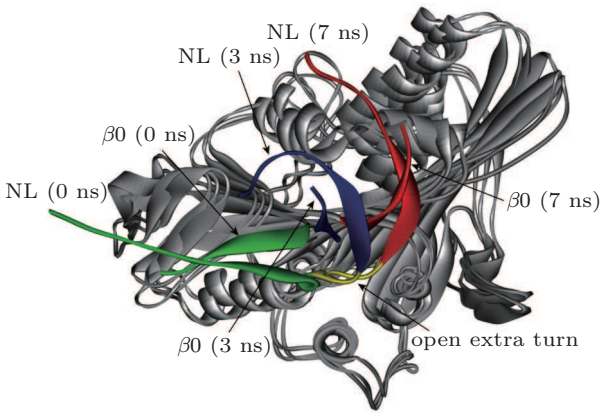


Fig. 4. (color online) Overlaid snapshots of the trajectory in the MD simulation with backward-pointing CNB and ATP-state motor head. NL and $\beta 0$ of the initial state are shown in green and that of the conformations at 3 ns and 7 ns are shown in blue and red, respectively. The open extra turns of the conformations at 3 ns and 7 ns are shown in yellow.

A series of MD simulations (the simulation time is 10 ns) is run with this structure as the initial state. We never, however, get a conformation with a correctly formed extra turn. In these simulations, the CNB structure has an obvious docking tendency which shows that the CNB structure has the mechanical property of bending forward. This is consistent with the simulation results of Ref. [20]. However, the first three AAs of NL never form an extra turn structure, i.e., the extra turn keeps open (Fig. 4). Because the AAs of extra turn form a loop but a helical turn in these simulations, the resultant NL is longer than that with formed extra turn in realistic situation. Though the CNB has an obvious docking tendency, the residues of NL cannot be in register with their docking sites in the motor head and the formation of the 'ASN latch' and the hydrogen bonds between NL and the motor domain proposed by Ref. [20] seems unlikely in these simulations. Therefore, without an extra turn formation, a backward CNB structure cannot dock to the motor domain properly.

In this initial structure, the motor domain is obtained based on the crystal structure of 2KIN which has an ATP-state conformation. However, as explained in the next section, the extra turn forms in the process of the motor head's conformational change from the nucleotide-free state to the ATP state due to ATP binding. Therefore, the structure with backward-pointing CNB and ATP-state motor domain is not the reasonable initial conformation of NL docking.

3.3. Initial conformation of NL and $\beta 0$ should be a backward pointing CNB based on a nucleotide-free state structure

As is well known, NL docking is induced by ATP binding.[12] Therefore, the correct initial conformation of NL and $\beta 0$ in the NL docking process should belong to the nucleotide-free state conformation rather than the ATP-state conformation of kinesin. This should be the reason why the extra turn cannot form correctly in the simulations of the above section. We thus graft the CNB structure of the above-obtained conformation from 2KIN (Fig. 3(b)) onto the ADP-state structure of 1BG2, which has no NL. The grafted initial CNB structure is optimized while the C-terminal end of NL is fixed to keep NL pointing backward. The resultant conformation is shown in Fig. 5(a). As seen, this conformation has an opened extra turn and $\beta 0$ locates between the β-domain and NL. We propose that this conformation should be the right initial structure of NL and $\beta 0$ in the ATP-waiting state. In this structure of kinesin's leading head, NL and $\beta 0$ still maintain a CNB structure.

As pointed out by Vale and Milligan,[2] one of the most important conformational differences between ATP and ADP states of kinesin is the relative position of $\alpha 6$ with respect to $\alpha 4$. In the ADP and nucleotide-free states, the C-terminal end of $\alpha 6$ is close to the C-terminal end of $\alpha 4$, whereas in the ATP state, $\alpha 6$ locates above $\alpha 4$. This conformational difference can be seen in Fig. 5(b), where the related secondary structures of both crystal structures are superposed and $\alpha 4$s of these two crystal structures are kept coincided.[11] With the two fixed $\alpha 4$s, the ATP-state conformation of $\alpha 6$ has an inward rotation relative to the nucleotide-free state conformation.[2,11,39–41] This inward rotation of $\alpha 6$ is very important because it removes the obstruction to the formation of extra turns and initiates the extra turn formation process. This rotation of $\alpha 6$ is caused by ATP binding-induced motor head rotation. From

structural analysis, we find that the rotation of the motor domain can cause the inward rotation of $\alpha 6$ through the CNB structure. The large conformational change of $\alpha 6$ is directly induced by the displacement of the N-terminal end of NL which forms the CNB structure with $\beta 0$. $\beta 0$ is connected with $\beta 1$ of the central β-sheet through the peptide bond between LYS10 ($\beta 1$) and ILE9 ($\beta 0$). Through these structural connections, the motor head rotation induced by ATP binding can transmit through the CNB structure to $\alpha 6$ and induce the rotation of $\alpha 6$. We should mention that the amplifying function of the β-domain, as shown in our previous work,[15] plays an important role in this process. The β-domain effectively amplifies the motor head rotation to the large movement of CNB. During the motor head rotation, LYS10 of the central β-sheet has a forward displacement and the CNB structure thus gets a forward force. It is this forward force that induces the rotation of $\alpha 6$. We propose that this is the mechanical pathway from motor head rotation to extra turn formation. To check this proposal and the third initial structure we obtained, we perform the following MD simulations to simulate the extra turn formation process.

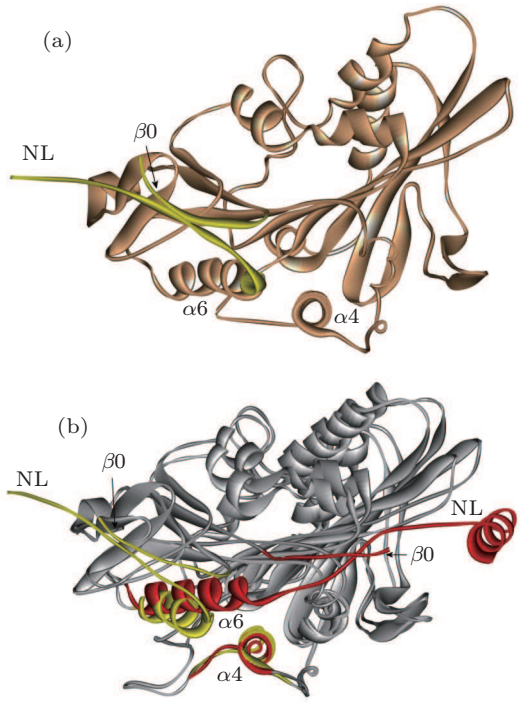


Fig. 5. (color online) Motor head rotation induced by ATP binding. (a) Kinesin's nucleotide-free state initial conformation for NL docking with NL and $\beta 0$ constituting a backward-pointing CNB structure. (b) Superposition of kinesin's ATP state structure (2KIN) and nucleotide-free state initial structure (based on 1BG2). $\alpha 4$s of the two structures are kept coincided. $\alpha 6$ of the ATP state structure (shown in red) has an inward rotation relative to its initial conformation (shown in yellow).

To simulate the action of the motor domain on CNB, we exert a 200-pN forward force on the C_α of LYS10 of the grafted structure. The direction of this force is along the connection line of the two C_αs of LYS10s in the ATP and nucleotide-free state conformations (the black arrow in Fig. 6(a)). With this forward force exerting on the C_α of LYS10, the right conformational change is achieved after 7-ns simulation, i.e., the extra turn rightly forms, $\alpha 6$ rotates to the position above $\alpha 4$ and CNB docks to the motor head (snapshots of the simulation trajectory are shown in Fig. 6(b)). In this simulation, the NL docking initiation step ($\alpha 6$ rotation and extra turn formation) is finished at ~ 4 ns and then CNB has a conformation similar to the initial conformation of CNB in the work of Ref. [20]. In the next 3 ns, CNB docks to the motor domain by itself and the applied force has no contribution at all. After the docking of CNB structure, we keep on simulating the docking process of $\beta 10$ for tens of nanoseconds. However, $\beta 10$ does not form specific non-bonding interactions with the motor head within the simulation time. This is consistent with the simulation of Ref. [20] and the docking mechanism of $\beta 10$ is still an open question.

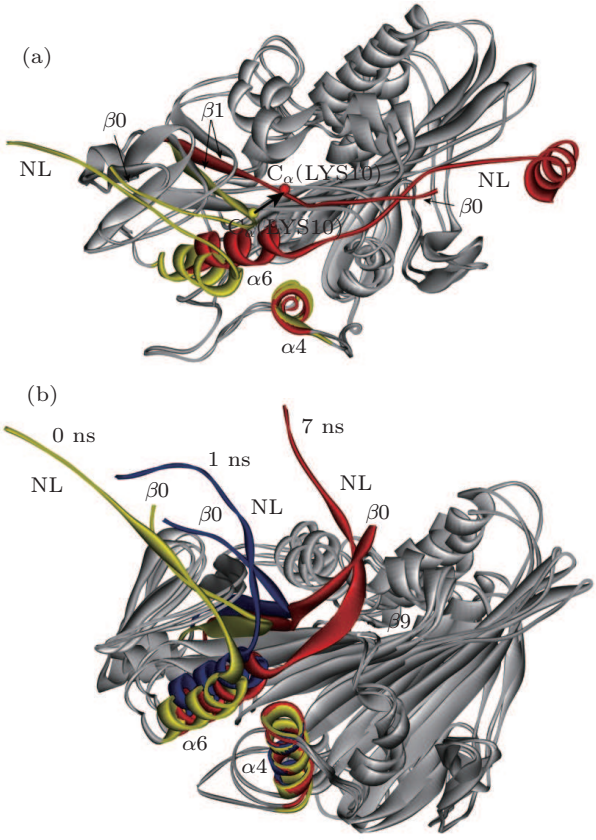


Fig. 6. (color online) NL docking simulation. (a) The force (black arrow) exerted on C_α (yellow sphere) of LYS10 of $\beta 1$ of the nucleotide-free state initial structure. The direction of the force is determined by the line connecting C_αs of LYS10s of the ATP- and nucleotide-free state structures. The force is produced by the rotation of the motor domain which can only be transmitted to CNB through the peptide bond between ILE9 of $\beta 0$ and LYS10 of $\beta 1$. Therefore we exert the force on C_α of LYS10 in the NL docking simulation. (b) Overlaid snapshots of the simulation trajectory. The NL at 7 ns has formed an extra turn and $\beta 9$ of NL docks to the motor domain correctly.

The above simulation results show that the initial structure with a backward-pointing CNB structure and a

nucleotide-free state motor domain reasonably reflects the realistic situation. From this initial structure, the extra turn formation and $\beta 9$ docking (i.e., the first and second steps of NL docking) are achieved and the mechanical pathway from motor head rotation to extra turn formation we proposed is proved to be reasonable.

We should emphasize that this mechanical pathway must be accomplished through the initial CNB structure. Rotation of the central β-sheet provides a forward force on $\beta 0$ through LYS10. Under the action of this force, $\beta 0$ moves forward. Because $\beta 0$ and NL form a CNB structure, NL also moves forward and the first three AAs of NL have a conformational change accordingly to form the extra turn structure. In this process, $\beta 0$ plays the role of the force transfer element from the motor head to NL by forming CNB with NL. Without forming CNB with $\beta 0$, NL (and $\alpha 6$) cannot obtain a forward force. Through analysis of the MD trajectories, we also find that the hydrophobic interaction between ILE327 and the hydrophobic pocket on the motor domain plays an important role in the formation of extra turns. ILE327's movement to the hydrophobic pocket facilitates the formation of the hydrogen bond between ALA324 and ILE327, which ensures the formation of an extra turn.

In our simulation, the magnitude of the forward force on the C_α of LYS10 is 200 pN. The magnitude of the force transmitted from the central β-sheet to CNB may be weaker than 200 pN in the wild-type kinesin. We make this choice for the force mainly according to the consideration of the computational cost.[42] However, in our understanding, the result from such a choice of the forward force is acceptable because this force cannot break more than one hydrogen bond simultaneously and we do not find any abnormal damage for the protein molecule. The simulation results show that this 200 pN forward force does induce the reasonable conformational changes of $\alpha 6$ and extra-turn AAs within tens of nanoseconds, and the CNB docking process is achieved in the right way.

In our MD simulations, the direction of the force which unbinds the docked NL (Fig. 3(a)) is different from that in the simulations of Ref. [20]. Due to this difference, the structure in the simulations of Ref. [20] has a formed extra turn and that in our simulations has an open extra turn. From the structure with a formed extra turn, the second step of NL docking ($\beta 9$ docking to the motor domain) is achieved in the simulations of Ref. [20]. Reference [20] also proposed a mechanism in which $\beta 0$ is free and the CNB does not form until ATP binding, whereas we propose here that the CNB is essentially stable throughout the power stroke, which is a major revision to the CNB hypothesis by Ref. [20]. A CNB structure cannot be achieved from a conformation with free $\beta 0$. $\beta 0$ is a crucial force transfer element. Without forming an initial CNB structure, the force produced by ATP-binding-induced motor head rotation cannot be transferred to NL and to $\alpha 6$.

4. Conclusion

The initial conformation of NL in its docking process is crucial for understanding the extra turn formation mechanism of NL docking and the kinesin force generation mechanism. By using an MD method and structural analysis, we obtain an initial structure of NL and $\beta 0$, which is a backward-pointing CNB structure. Through analysis of the structural and mechanical connections between the central β-sheet and CNB, we use a force acting on the root of $\beta 0$ to simulate the force produced by the rotation of motor head. Under the action of this force, $\alpha 6$ rotates from its position in the nucleotide-free state to that in the ATP state, an extra turn forms and $\beta 9$ docks to the motor domain successfully. These results show that the ATP binding-induced rotation of the motor head is transmitted through the initial CNB structure to NL and $\alpha 6$. This is the mechanical pathway from the motor head rotation to NL docking.

With the initial structure proposed in this paper, the CNB docking process shown by Ref. [20] can be self-consistently achieved after the rotation movement of $\alpha 6$ and the extra turn formation. The NL's initial conformation and the pathway from motor head rotation to NL docking proposed in this paper, together with the CNB docking mechanism proposed in Ref. [20], provide a consistent insight into the mechanisms underlying the first and second steps of NL docking. More work must be done on the mechanism of the third step of the NL docking process (i.e., $\beta 10$ docking to the motor head). We leave it for our further investigation.

Acknowledgments

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