Initiation Mechanism of Kinesin's Neck Linker Docking Process *

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(Received 7 November 2016)

The neck linker (NL) docking to the motor domain is the key force generation process of a kinesin motor. In the initiation step of NL docking the first three residues (LYS325, THR326 and ILE327 in 2KIN) of the NL must form an 'extra turn', thus the other parts of the NL could dock to the motor domain. How the extra turn is formed remains elusive. We investigate the extra turn formation mechanism using structure-based mechanical analysis via molecular dynamics simulation. We find that the motor head rotation induced by ATP binding first drives ILE327 to move towards a hydrophobic pocket on the motor domain. The driving force, together with the hydrophobic interaction of ILE327 with the hydrophobic pocket, then causes a clockwise rotation of THR326, breaks the locking of LYS325, and finally drives the extra turn formation. This extra turn formation mechanism provides a clear pathway from ATP binding to NL docking of kinesin.

PACS: 87.16.Nn, 87.10.Tf, 87.15.hp DOI: 10.1088/0256-307X/34/2/028701

Conventional kinesin (kinesin-1, herein referred to as kinesin[1]) is a dimeric motor protein that walks processively along a microtubule to transport membranous organelles against resistance in cells.[2–9] In each walking cycle, the kinesin dimer's two motor heads (motor domains) switch their positions on the microtubule (i.e., move in a hand-over-hand manner) and the neck linker (NL), a short peptide (∼14 reisues) which connects the motor head and the coiled-coil stalk, has a large conformational change from an undocked conformation to a docked conformation on the motor domain (Fig. 1).[5,10–12] NL docking to the motor domain is proved to be one of the key force-generation steps of kinesin and this docking process is trigged by ATP binding.[6,10,12] Finding out the mechanical pathway from ATP binding to NL docking remains a central open question in the investigations of kinesin.

The kinesin's NL locates between the α6 helix of the motor domain and the α7 helix of the coiled coil. The NL consists of three parts (from LYS325 to THR338 in 2KIN,[13] herein we use the amino-acid sequence of 2KIN), i.e., (1) the initial part that consists of the first three residues (LYS325, THR326 and ILE327), (2) β9 (LYS328-VAL333) and (3) β10 (ASN334-THR338), as shown in Fig. 1.[11,14–17] Correspondingly, NL docking to the motor domain is accomplished in three sequential steps, each of which operates with a different mechanism.[18] In the docked state, the NL's first three residues form an extra turn structure along the C-terminal end of α6, whereas they form a loop structure in the undocked state. Therefore, the extra turn formation is the first step (the initiation step) of NL docking, which controls the entire docking process of the NL. However, the extra turn formation mechanism remains elusive.

The mechanism for the second step of NL docking, i.e., β9 docking to the motor domain, has been made clear through the important work of Hwang *et al.*[16,17] Using the molecular dynamics (MD) method, they found that β9 docks to the motor domain through forming a cover-neck bundle (CNB) structure with the N-terminal β0. This CNB structure has a strong tendency of bending from the undocked conformation to the docked one. In one of our simulation works on kinesin, we found that this CNB structure is able to resist a backward force of tens of pN.[19] This CNB mechanism for NL docking stands for a substantial progress in the understanding of kinesin's force-generation mechanism. However, this mechanism cannot be applied to the first and third steps of NL docking. In the investigation on the CNB mechanism by Hwang *et al.*,[16] they started their simulation from a conformation where the extra turn has already been formed, i.e., the first step of NL docking has been finished. Therefore, the initiation mechanism (extra turn formation mechanism) is still unclear.

During the extra turn forming process, α6, which is the N-terminal to the extra turn, changes from its nucleotide-free conformation to the ATP-state confor-

*Supported by the National Natural Science Foundation of China under Grant Nos 11545014 and 11605038, and the Open Project Program of State Key Laboratory of Theoretical Physics of Institute of Theoretical Physics of Chinese Academy of Science under Grant No Y5KF211CJ1.

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mation. Such a conformational change of a secondary structure needs a large energy input, i.e., it needs a strong driving force. Therefore, unlike the second step, the first step of NL docking (i.e., the extra turn formation) is not a force-generation step. In one of our previous works,[15] we proved that the strong driving force for extra turn formation and $\alpha 6$ movement is provided by the ATP-binding induced motor domain rotation. This force is conveyed to the NL through an initial CNB structure formed by $\beta 0$ and $\beta 9$. However, in Ref. [15], we focused on the identification of the proper initial conformation of kinesin's NL and the formation mechanism of an extra turn was not mentioned. In this work, we investigate, on the atomic level, the mechanical processes of the extra turn formation based on the results from MD simulations.

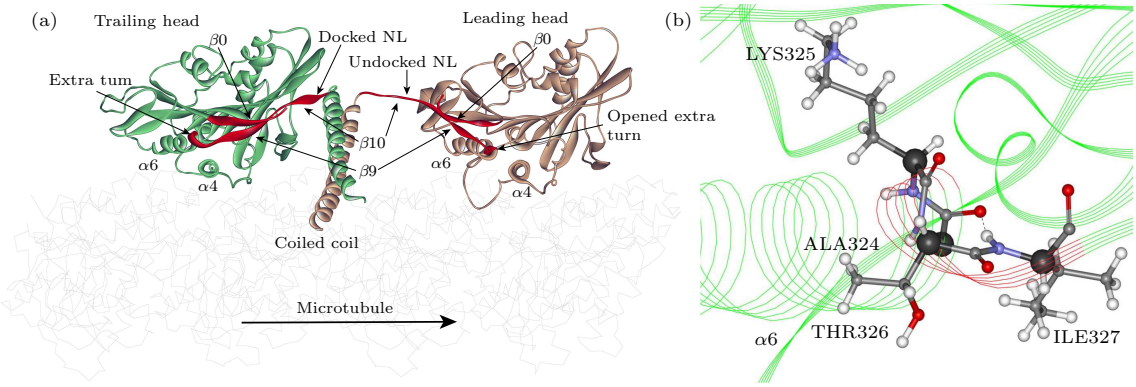

Fig. 1. Structure of kinesin's motor heads and the extra turn. (a) Structure of the two motor heads and the neck coiled coil. The two neck linkers and $\beta 0$s are shown in red. (b) Structure of the extra turn (red) formed by the first three amino acids of the NL in the trailing head (green). The alpha carbons are shown in larger balls. The sidechain of ALA324 is omitted.

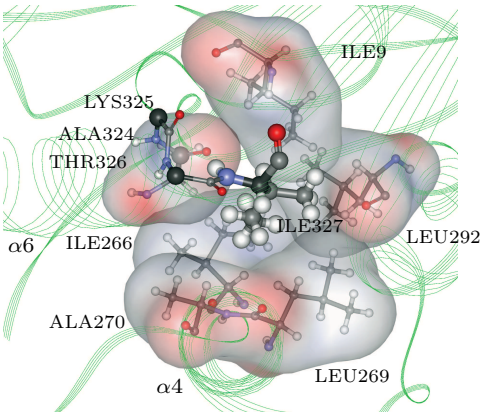

Fig. 2. Structure of the hydrophobic pocket for ILE327. ILE327 is shown in larger balls and thicker sticks. The sidechains of LYS325 and THR326 are omitted.

We performed a docking simulation for the NL using the MD method as in Ref. [15]. The docking simulation is based on the structure of 1BG2, which has an ADP state conformation.[20] Because the nucleotide-free conformation is similar to that of the ADP state conformation,[21] we take 1BG2 as the nucleotide-free structure. We use 2KIN as the ATP state structure for kinesin.[10] The initial CNB structure for the docking simulation is obtained from the result of our previous NL unbinding simulation.[19] The MD simulations are performed by using NAMD (version 2.9)[22] with force field CHARMM.[23] The software used for modeling is VMD (version 1.9.1).[24] Molecular drawings in this study are produced using Discovery Studio 3.5 Visualizer and Origin 8.5. Please refer to Ref. [15] for the simulation details.

Extra turn formation is the initiation step of the NL docking process, during which the three extra turn amino acids (LYS325, THR326 and ILE327) have a dramatic conformational change. We first describe the conformations of the three amino acids in the two states and compare their differences. Then we will give a detailed analysis of the mechanism for the extra turn formation process.

The first three extra turn amino acids are of three different types. LYS325 is the most typical charged amino acid with a positively charged head, THR326 is the most typical polar amino acid with a hydroxide group, and ILE327 is the most typical nonpolar (hydrophobic) amino acid with a totally aliphatic sidechain. As shown in Fig. 2, in the extra turn formed conformation, the sidechain of ILE327 is deeply buried in a hydrophobic pocket formed by ILE9, ILE266, LEU269, ALA270, LEU292 and ALA324 on the motor domain. The hydrophobic interaction between ILE327 and its hydrophobic pocket plays an important role in extra turn formation as discussed in the following. 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. When ILE327 is outside of the hydrophobic pocket, the hydrogen bond is broken and the extra turn is opened. In the extra turn formed conformation, the sidechains of THR326 and LYS325 have no significant interaction with the motor domain.

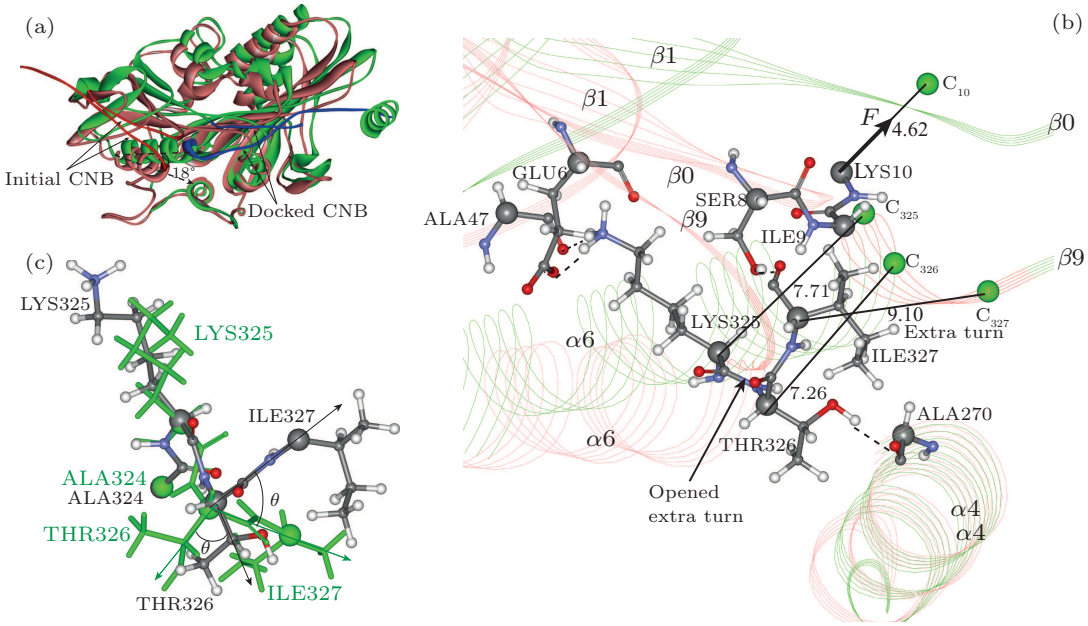

Fig. 3. Structures of the undocked and docked CNBs and extra turns, and the extra turn formation mechanics. (a) Superposition of the initial nucleotide-free conformation (pink) and the ATP-state conformation (green) of kinesin with two α4s coincided. The undocked CNB is shown in red and the docked CNB is shown in blue. The angle between the two arrows illustrates the orientation difference of α6 in the two states. (b) The atomic details of the opened extra turn and its position relative to the formed extra turn. The force acting on the initial CNB is indicated by $\boldsymbol{F}$. The hydrogen bonds are indicated by dotted lines. The residues of the formed extra turn and LYS10 are indicated by their C_α's (green balls) with the sidechains omitted. The sidechains of the residues ILE9, LYS10, ALA47 and ALA270 of the opened extra turn structure are also omitted. The distances between C_α's in the process of the conformational change. (c) Relative rotation of the opened and formed extra turns. The peptide planes between LYS325 and ALA324 of the two structures are kept to be coincided. The rotation of the three chemical bonds of C_{326} around the fourth bond, i.e., the bond connecting C_{326} and the peptide carbon, is indicated by θ, which stands for both the rotation angle of the peptide plane between THR326 and ILE327 and that of the sidechain of THR326.

In one of our previous works,[15] we have shown that the initial structure of NL and β0 for the NL docking process is a CNB structure, of which the first three amino acids of the NL have an opened extra turn structure, see Fig. 4 in Ref. [15]. In Fig. 3(a), we superpose the initial nucleotide-free conformation and the ATP-state conformation of kinesin with α4s kept coincided.[21] As is seen, the ATP-state conformation has a docked CNB, whereas the nucleotide-free conformation has an undocked backward pointing CNB. Another significant difference between the two conformations is that the two α6's span an angle of $\sim$18°.[25] Figure 3(b) gives the atomic details of the two extra turns in the two conformations. It is seen that ILE327 of the opened extra turn is totally out of the hydrophobic pocket on the motor domain. The distance between C_{327}'s of ILE327 (herein we use $C_{\text{residuenumber}}$ to represent the alpha carbon of the corresponding residue) of the opened and formed extra turn conformations is $\sim$9.10 Å. In addition, the backbone oxygen of ILE327 forms a hydrogen bond with the sidechain hydroxide hydrogen of SER8 on β0, which does not exist in the formed extra turn conformation.

In the opened extra turn, the conformation of THR326 significantly differs from that in the formed extra turn (Figs. 3(b) and 3(c)). First, the distance between the two C_{326}'s in the two states is $\sim$7.26 Å. Secondly, the orientations of the sidechains in the two states have a large difference. The relative rotation of the opened extra turn with respect to the formed one is illustrated in Fig. 3(c) with the peptide planes between LYS325 and ALA324 coincided. The rotation angle of the sidechain of THR326 is indicated by θ ($\sim$55°). Thirdly, the hydroxide group of THR326 forms a hydrogen bond with the backbone oxygen of ALA270 on α4 in the opened extra turn conformation (Fig. 3(b)). This hydrogen bond helps to lock α6 in the nucleotide-free state conformation. THR326 is not quite conservative in conventional kinesins, and it can be replaced by a serine.[16] However, the hydrogen bonding donor site is absolutely conservative at this extra turn position.

As the three extra turn residues have large conformational differences in the two states, the two extra turn peptide planes also have large conformational change. In the formed extra turn structure, the ϕ and ψ angles of the two peptide planes are 84.5° and 8.4°, respectively. In the opened extra turn structure, they are 46.5° and 38.5°, respectively. In Fig. 3(c), the relative rotation angle of the peptide plane between ILE327 and THR326 of the opened extra turn with respect to that of the formed extra turn is indicated

by θ ($\sim$55°), the angle between the two long arrows. The peptide plane between LYS325 and THR326 of the opened extra turn has a rotation of $\sim$49° relative to its position in the formed extra turn.

Extra turn formation takes place in the dynamical process when kinesin changes from the nucleotide-free conformation to the ATP-bound conformation.[5,14] In this process, the extra turn together with α6 have a large conformational change as described above. We realize this process through MD simulations. Based on the simulation trajectories, we analyze the extra turn formation process and conclude the initiation mechanism of the kinesin's NL docking process. As a mechanical process, the extra turn formation depends on the mechanical properties of the components and the related interactions.

Kinesin is a protein motor. As a protein mechanical device, it has three types of elementary mechanical components, i.e., alpha carbons, peptide planes and sidechains. An alpha carbon has four chemical bonds pointing to the four corners of a tetrahedron. The peptide plane and the sidechain can rotate around the bonds which connect the peptide plane or the sidechain with the alpha carbon. The planar shape of the peptide plane and the bond angles of the four bonds of alpha carbon are approximately kept to be unchanged in the conformational changes of protein, which constrains the possible conformations and conformational changes of protein.[26] The related interactions include hydrogen bonds, hydrophobic interactions, salt bridges, etc.

The extra turn formation must be accomplished together with the rotation of α6, which is the N-terminal to the extra turn. As a secondary structure, the movement of α6 needs a large energy input. In addition, the interaction between α6 and the microtubule surface must be broken during the movement. Therefore, the formation of the extra turn needs a strong driving force. In Ref. [15], we have shown that this strong driving force is provided by the motor head rotation induced by ATP binding. The NL locates at the C-terminus of the motor domain. In the initial state of NL docking, the NL is assumed to have a backward pointing conformation (see the leading head in Fig. 1(a)). The force produced by the motor head rotation can only be delivered to the NL through the initial CNB structure formed by the N-terminal β0 and β9 of the NL. The acting point of this force $\boldsymbol{F}$ is the C_{10} of LYS10, which locates between β0 of CNB and β1 of the motor domain (Fig. 3(b)). In Fig. 3, the two conformations of kinesin in the ATP-state and nucleotide-free state are superposed with two α4's coincided. The force $\boldsymbol{F}$ points from the C_{10} of LYS10 of the nucleotide-free state conformation to the C_{10} of LYS10 of the ATP-state conformation.

In the initial nucleotide-free state conformation, the CNB structure is held by the hydrogen bonds formed between ALA5, CYS7 in β0 and VAL331, ASN329 in β9, akin to the CNB structure in the ATP-state conformation. In addition, the backbone oxygen of ILE327 forms a hydrogen bond with the sidechain hydroxide hydrogen of SER8 in β0, which is broken when the NL is completely docked in the ATP-state conformation. In the first part of the extra turn formation process, this hydrogen bond is stable because the surrounding residues can effectively protect it from water attack. The conservation analysis shows that SER8 can be replaced by an asparagine, which also has a hydrogen bonding donor site. Under the action of the force $\boldsymbol{F}$, β0 moves forward and, via the interstrand hydrogen bonds and the hydrogen bond between ILE327 and SER8, drives C_{327} to move towards its docking site along the line connecting its initial and final positions (Fig. 3(b)). Through the relatively rigid peptide bond between ILE327 and THR326, the forward movement of C_{327} drives the clockwise rotation of the three chemical bonds of C_{326} around the fourth chemical bond between the peptide plane carbon and the alpha carbon of THR326. The mechanical property of the alpha carbon chemical bonds ensures that the angles between four bonds are relatively fixed, therefore the bond between C_{326} and C_β of THR326 also rotates θ clockwise, which then induces the clockwise rotation of the sidechain of THR326 (Fig. 3(c)). This sidechain rotation breaks the hydrogen bond between THR326 and ALA270 in α4, indicating that the C-terminal end of α6 is unlocked from its nucleotide-free state conformation. The clockwise rotation of THR326 is an important feature and a key step for the formation of an extra turn. Without this rotation of THR326, the extra turn formation cannot be accomplished and the NL could never dock to the motor domain in the right way.

In the nucleotide-free conformation, LYS325 forms a hydrogen bond with ALA47 in the β-domain (formed by β1a, β1b and β1c) and a salt bridge with GLU6 in β0, as shown in Fig. 3(b). These interactions have a special function as to lock the initial CNB structure in the backward pointing conformation.[27] When β0 moves forward under the action of the forward force, the positions of GLU6 relative to LYS325 are changed and the locking function of LYS325 is broken due to exposure to the surrounding water. This is another important step for the formation of an extra turn.

With the accomplishment of the first two steps, C_{327} can move close to the correct docking site and its hydrophobic sidechain can obtain an effective interaction with the hydrophobic pocket on the motor domain. This hydrophobic interaction, as mentioned above, provides a strong effective force for the final formation of an extra turn. The importance of this hydrophobic interaction to the initiation of NL docking has been pointed out in other references,

e.g., Refs. [14,15,18]. The contribution from another hydrophobic residue ILE9 should also be important, which, akin to ILE327, is highly conservative. In the ATP-state conformation, ILE9 and the other 5 hydrophobic residues form a typical hydrophobic pocket for ILE327 (Fig. 2). Therefore, ILE9 itself also locates in a hydrophobic environment. In the initial nucleotide-free conformation of kinesin, ILE9 positions completely outside the hydrophobic pocket and locates between ILE327 and the hydrophobic pocket. Because the sidechains of the two hydrophobic residues are in contact, ILE327's docking should be partially induced by ILE9's docking. Under the action of the hydrophobic interactions of ILE327 and ILE9 with the motor domain, the hydrogen bond between SER8 and ILE327 is broken and the extra turn formation is accomplished.

Kinesin is an exquisitely designed molecular force-generation device. It makes full use of the mechanical properties of its constructive element and the aqueous environment to accomplish its mechanical function. The first three amino acids of the NL forming an extra turn structure is the key step from ATP binding to NL docking. ATP binding induced motor domain rotation provides a strong forward force that drives the extra turn amino acids to achieve a large conformational change together with the rotation of $\alpha 6$. Therefore, the extra turn formation is an energy-consuming step.

The mechanical pathway from ATP binding to extra turn formation for kinesin as revealed here exemplifies how this motor machine accomplishes its complicated mechanical functions and brings deeper insight into the exquisite design of kinesin.

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