NCD (Nonclaret Disjunctional): Kinesin Motor Protein

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Kinesins are eukaryotic microtubule-associated motor proteins. These proteins transport intracellular cargo along the microtubules via a force produced by ATP hydrolysis. Also, kinesins facilitate movement of the chromosomes and spindle in meiosis and mitosis.

Conventional kinesins are referred to as N-type kinesins because the motor domain is at the N terminus. NCD, on the other hand, is a C-type kinesin with its motor domain at the C terminus as shown in Figure 1. Another difference between the two is the neck region. NCD has a shorter neck linker compared to conventional kinesins.

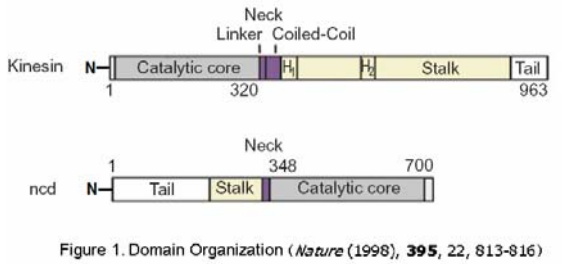

Figure 1. Domain Organization (*Nature* (1998), **395**, 22, 813-816)

The direction of motion of NCD differs from the N-type kinesins. As shown in Figure 2, the conventional kinesins move toward the plus end of the microtubules. Their unbound head is directed toward the plus end. In NCD, the unbound head and motion is directed towards the minus-end.

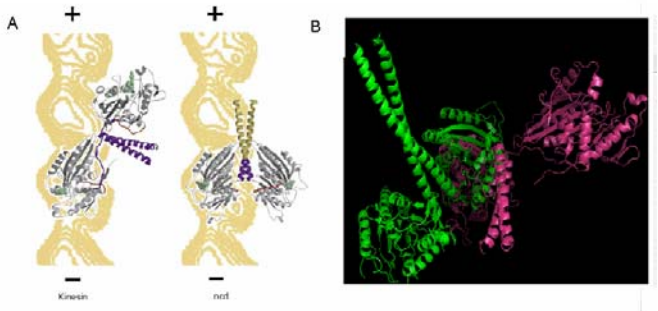

Figure 2. Direction of Motion. (A) *Nature* (1998), 395, 22, 813-816 (B) Refer to ncd-I F3

It has been shown that the NCD motor domain has ~40% sequence identity with conventional kinesins and that it has the same overall fold and same interaction site on microtubules as N-type kinesins. This led to the idea that the neck region is determines the different conformations and directionality of the kinesins. Unfortunately, no ATP-bound and nucleotide-free structures have been reported. Park and Endow's group approach was to understand the motor mechanism of the NCD and obtained a new crystal structure wherein the ADP state is weakened by mutating a residue in the motor domain required for the stabilization of nucleotide binding. Residue N60 is present in the microtubule-binding region of the motor domain and is highly conserved in kinesins. Mutation to K causes the motor to bind tightly to microtubules and bind ADP less stably. The new crystal structure obtained was deposited in the Protein Databank with accession number 1N6M. A summary of the data for the 1N6M is shown in Figure 3.

PDB ID: 1N6M
Source: *Drosophila melanogaster*
Resolution: 2.5 Å
R factor: 0.260
R_{free} : 0.302
Average b-factors: 61.5
Water molecules: 281
Residues: 818
Homodimer
4 domains
α helix content: 34%
β content: 19%
Residues in favored regions: 82.8%

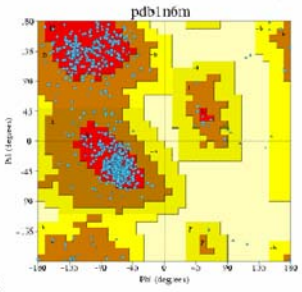

Figure 3. Summary of Data

The NCD has 4 domains as shown in Figure 4. The catalytic motor (pink) has two binding sites, one for microtubules and one for ATP. The neck (orange) domain connects the motor domain and the stalk (green). The tail, which is not seen in this crystal structure, binds the cellular cargo. The coiled coil which is responsible for dimerisation is formed by the neck and stalk regions. It has a heptad repeat with hydrophobic residues in a and d positions (L296, V300, L303, L310, L321, L328, L338, V342, L345). It is also stabilized by interactions between oppositely charged side chains from both helices. The motor core consists of an 8-stranded antiparallel β sheet flanked by 3 major helices on each side. There are also additional 3 small β strands and 3 small helices.

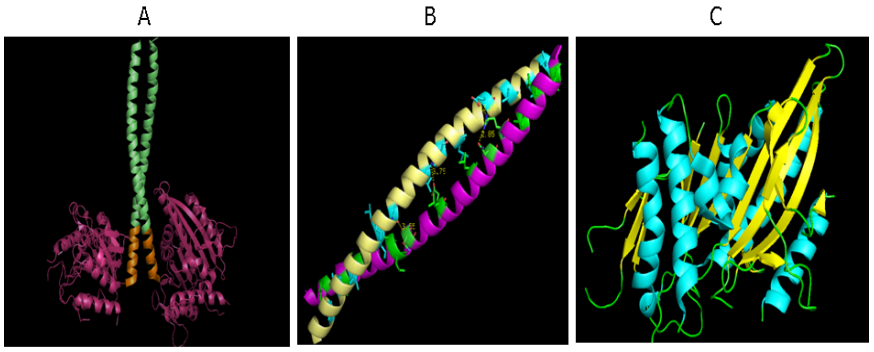

Figure 4. (A) NCD Domains. Refer to ncd-I F4. (B) Coiled-coil. Refer to ncd-I F5. (C) Motor domain. Refer to ncd-I F6.

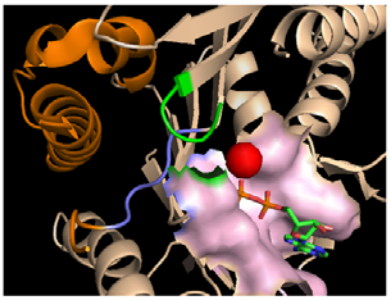

Figure 5. Motor Domain (Refer to ncd-I F8)

The catalytic motor domain has 3 major sites (see Figure 5). The microtubule binding site (orange) consists of loop11, helix4, loop a2 and helix 5. The binding of ATP is coordinated by a Mg ion (red sphere). In addition, switch I and switch II (green and blue, respectively) change conformations upon binding and release of ATP.

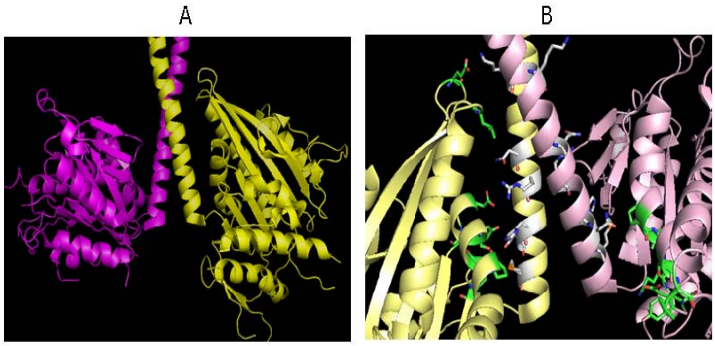

Figure 6. Orientation of Motor Domains 1 and 2. (Refer to ncd-I F9 & F10)

As shown in Figure 6, the two motor heads are asymmetric – they are oriented differently relative to the coiled-coil. The core in chain 1 (yellow) interacts with the coiled-coil through an extensive network of interactions. The interacting motor residues cluster mainly in helix 1, loop6, loop10 and loop 13. In chain 2 (pink), these residues are pointing away from the coiled-coil.

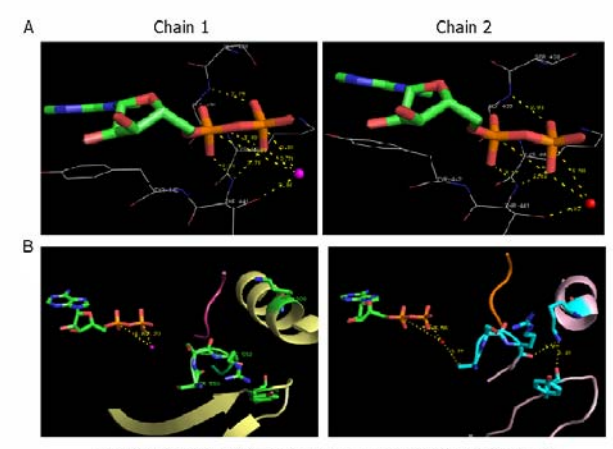

Figure 7. Comparison of ADP binding in Chains 1 and 2

This asymmetric orientation is due to the different strengths of interaction between the ADP and motor residues in chains 1 and 2. In chain 1, ADP is more stably bound as shown by the shorter distances between the motor residues and the ADP (see Figure 7). In chain 2, these distances are longer. Also, in chain 2 the N600K mutated residue interacts with the residues Y485 and R552. This interaction moves the switch I in such a way that the S550 can now coordinate with the Mg ion. This weakens the coordination of ADP by the Mg ion and results in instability of the ADP bound to the head of chain 2.

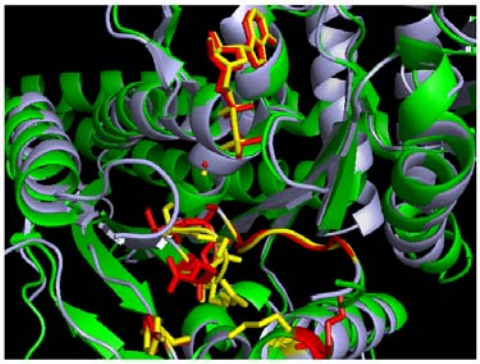

Figure 8. Alignment of heads 1 and 2.

The network of interactions between the motor domain and coiled-coil is weakened in chain 2 (pink) due to the movement of switch I and the unstable binding of ADP (see Figure 8). One of the important interactions between the motor head and the coiled-coil is between N340 of coiled-coil (green sphere) and K640 of the motor head (light blue). In chain 1 (yellow), these interactions are stable so H1 rotates with the coiled-coil whereas disrupting these interactions in chain 2 allows the coiled-coil to move or rotate. The point of rotation is at G437 (blue) which connects the neck to the motor domain. The Gly has hydrogen as a side chain, thus, it can adapt a wider range of phi/psi angles.

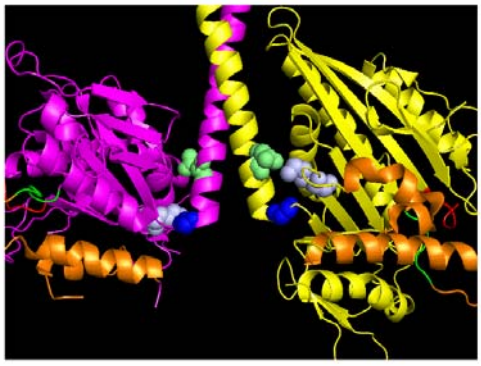

Figure 9. Rotation at G347 in Chain2 (Refer to ncd-II F2)

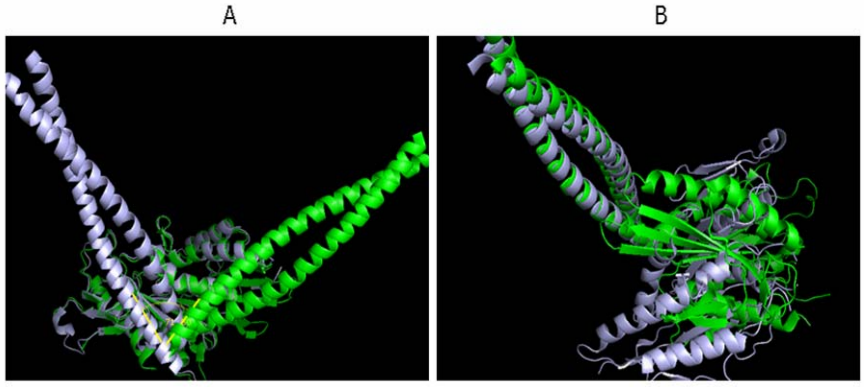

Figure 10. Alignment of motor heads and stalks (Refer to ncd-II- F4 and F5).

The asymmetric orientation of the motor heads of chains 1 and 2 is again shown in Figure 9. In A, the two motor heads are aligned and it is clearly seen that the stalk of chain 2 (light blue) is oriented about 75^{o} relative to chain 1 (green). In B, the stalks are aligned and the motor heads do not align at all.