

X-ray structure of functional full-length dynein motor domain

Dyneins are large motor complexes of 1–2 MDa that use ATP as an energy source to move toward the minus ends of microtubules [1,2]. This motor activity is crucial for a variety of cellular processes within eukaryotic cells, including the beating of cilia and flagella, cell division, cell migration, and the intracellular trafficking of various vesicles and organelles along microtubules. Dyneins power a wide range of cellular motility through the coordinated action of a number of subunits of the dynein complex together with various associated cellular components. Among them, the heavy chain (molecular mass ≥ 500 kDa), belonging to the AAA+ superfamily of mechanochemical enzymes, is solely responsible for dynein’s fundamental motor activities, such as ATP-hydrolysis, ATP-sensitive microtubule-binding and microtubule-based motile activities. Truncation studies have shown that the C-terminal 380-kDa portion of the heavy chain alone is sufficient to exert the motor activities, thus defining this portion as the dynein motor domain.

Here, we report an X-ray crystallographic analysis of a functional full-length motor domain of cytoplasmic dynein from *Dictyostelium discoideum* at 4.5 Å resolution, which revealed the detailed architecture of the functional units required for dynein’s motor activity [3]. Structural analysis was carried out using beamline BL44XU. Our analysis also provides structural insight into how dynein coordinates microtubule-binding and ATPase activities to produce force and movement along microtubules.

On the basis of good-quality maps at 4.5 Å resolution that clearly show α -helices and β -sheets, we assigned the helices to the functional units in the dynein motor domain (Fig. 1). The central AAA+ ring was identified to be composed of six AAA+ modules (AAA1–AAA6) arranged in a ring-shaped structure with pseudo-six-fold symmetry by referring to the structures of typical AAA+ proteins that are usually composed of an N-terminal domain with an α/β Rossmann fold and a C-terminal α -helical domain. In each of the six AAA+ modules, the α domain stretches outward from the α/β domain (Fig. 2). Two prominent coiled coils (CCs) protrude from the AAA+ ring. One is the stalk CC, which includes the small microtubule-binding domain (MTBD) at the tip (Fig. 2, yellow arrowhead). The other is a novel CC that interacts with the side of the stalk CC as if it works as a strut (Fig. 2, orange arrowhead). Therefore, we named it the “strut CC”. A series of helices were found above the front face of the AAA+ ring, (Fig. 3, magenta), which we identified as the core of the linker unit,

corresponding to the ~550 amino acid residues N-terminal to AAA1. On the back face of the AAA+ ring, two groups of helices were found (Fig. 3, gray), both of which were assigned as the C-terminal non-AAA+ sequence (C-sequence: ~400 residues).

The linker unit, which has been supposed to act as a mechanical lever or winch during dynein’s force generation [4], is mainly composed of a series of helices running parallel to the long axis of the unit. This rodlike structure bridges AAA1 and AAA4 by lying over the hole of the AAA+ ring. Notably, the linker is only in contact with AAA1 and AAA4 at the terminal portions, without any significant contacts with the other AAA+ modules. This architecture may allow the linker to swing as a rigid body around the linker-AAA1 junction (Fig. 3, magenta arrowhead) during the ATPase-dependent powerstroke [5].

The stalk and strut CCs are the most striking features of the dynein structure outside the AAA+ ring. One of the two helices of the stalk CC runs to the MTBD directly from one of the helices in the AAA4 α domain, while the other helix of the stalk CC returns from the MTBD to another helix in the same α domain

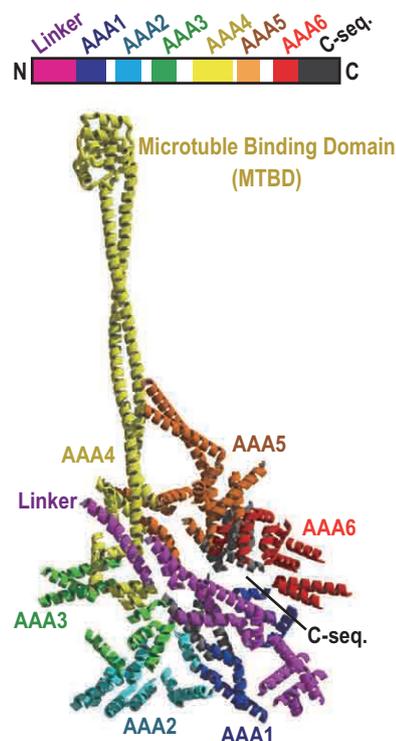


Fig. 1. Structure and sequence diagram of the dynein motor domain, showing the functional units: the linker, the ring containing six AAA+ modules, the C-sequence, and the microtubule-binding domain (MTBD).

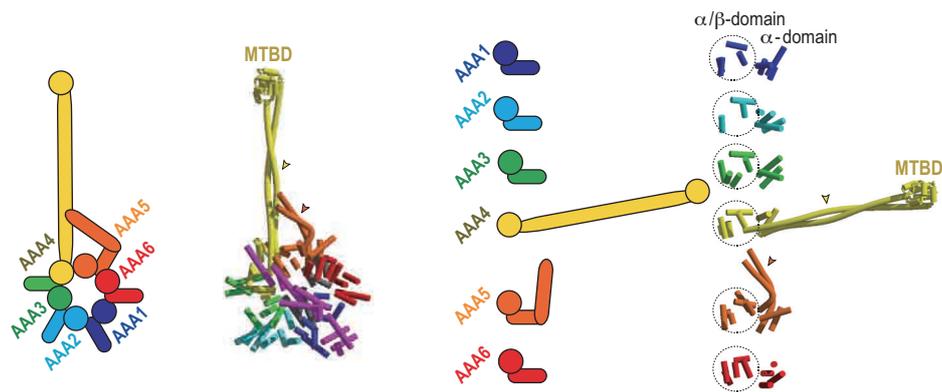


Fig. 2. AAA+ modules of the dynein motor domain. (left) Hexameric organization of the AAA+ modules. (right) Schematic presentation of the α and α/β domains. The yellow and orange arrowheads indicate the stalk and strut coiled coils, respectively.

(Fig. 2). Thus, the stalk CC can be regarded as a huge extension of the two helices in the AAA4 α domain, suggesting its evolutionary origin. Likewise, the strut CC can be described as a long extension of the corresponding helices in the AAA5 α domain (Fig. 2). This second long CC bends sharply at its middle and comes into direct contact with the middle region of the stalk CC, forming a Y-shaped structure together with the stalk CC. Collectively, our structure shows that the stalk unit is not an independent structure located between AAA4 and AAA5 as previously assumed, but is instead a component of a more complex structure composed of the stalk and strut CCs, as extensions of AAA4 and AAA5.

A widely held assumption about the two-way communication is that the structural information would be propagated to the base of the stalk CC through AAA1, 2, 3 and 4 in an ATPase-related manner analogous to other AAA+ proteins, because the first

four modules, but not AAA5 or 6, have ATP/ADP-binding or ATP-hydrolyzing activity. However, mutagenesis of the ATP/ADP binding sites in AAA2–4 does not completely block the communication between AAA1 and MTBD, and thus does not support this hypothesis. Instead, we favor the idea that the C-sequence–AAA5–strut CC serves as a two-way communication pathway between the AAA1 ATPase site and the MTBD at the tip of the stalk CC, because of the structural feature that the C-sequence lies on the AAA+ ring, and directly linking the AAA1 and AAA5 modules. This model is also compatible with previous biochemical studies providing evidence that the C-sequence regulates the AAA1 ATPase activity [6].

Even though the resolutions of the crystal structure are not yet high enough to map the motor domains at the atomic level, the α -helical model of the full-length motor domain presented here provides new insights into the mechanism of action of dynein, particularly the long-range two-way communication mechanism between the ATPase site and the MTBD.

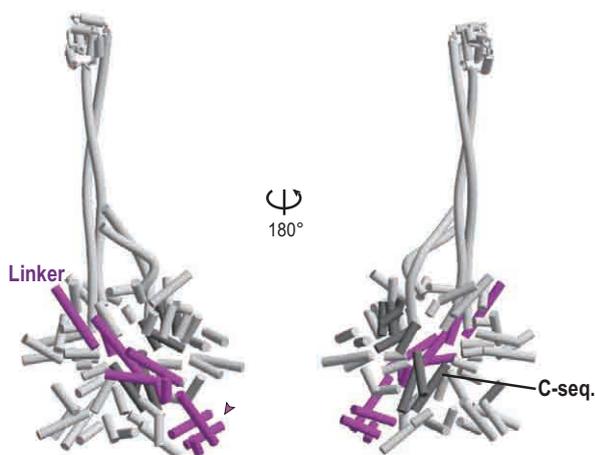


Fig. 3. Front and back views of the cylinder model of the dynein motor domain. The linker and C-sequence units are shown as magenta and gray helices, respectively.

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References

- [1] I.R. Gibbons, A.J. Rowe: *Science* **149** (1965) 424.
- [2] B. M. Paschal, R.B. Vallee: *Nature* **330** (1987) 181.
- [3] T. Kon, K. Sutoh, G. Kurisu: *Nature Struct. Mol. Biol.* **18** (2011) 638.
- [4] S.A. Burges *et al.*: *Nature* **421** (2003) 715.
- [5] A.J. Roberts *et al.*: *Cell* **136** (2009) 485.
- [6] N. Numata *et al.*: *Biochem. Soc. Trans.* **36** (2008) 131.