

# Structural Basis for the ATP-induced Isomerization of Kinesin

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## 1 Introduction

We report the first crystal structure of KIF4 complexed with the non-hydrolyzable ATP analog, AMPPNP (adenylyl imidodiphosphate), at 1.7 Å resolution. By combining our structure with previously solved KIF1A structures complexed with two ATP analogs, molecular snapshots during ATP binding reveal that the closure of the nucleotide-binding pocket during ATP binding is achieved by closure of the backdoor. Closure of the backdoor stabilizes two mobile regions, switch I and switch II, to generate the phosphate tube from which hydrolyzed phosphate is released. Through the stabilization of switch II, the local conformational change at the catalytic center is further relayed to the neck-linker element that fully docks to the catalytic core to produce the power stroke. Because the neck-linker is a sole element that connects the partner heads in dimeric KIFs, this tight structural coordination between the catalytic center and neck-linker enables inter-domain communication between the partner heads.

## 2 Experiment

A KIF4 motor domain construct, KIF4-344 (residues 1-344 of KIF4 followed by a (His)<sub>7</sub> tag), was cloned into the pET21b vector (Novagen) and transformed into BL21(DE3) cells (Novagen) for expression. Cells were grown in YT media with 0.5% glycerol, 0.05% glucose and 0.2% β-lactose at 30°C for 10 h, resuspended in lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5% glycerol, 5 mM imidazole and protease inhibitors), and lysed using a French press (Ohtake). The soluble protein fraction was loaded onto a His-select nickel affinity column (Sigma) equilibrated with the lysis buffer, and 500 mM imidazole was used to elute the bound protein. The pooled fractions were dialyzed against CIEX buffer (50 mM HEPES, pH 7.0 and 50 mM NaCl), loaded onto a RESOURCE S column (GE Healthcare) equilibrated with CIEX buffer, and a linear gradient of NaCl was applied to elute the protein. The pooled protein fractions were then dialyzed against SEC buffer (10 mM Tris-HCl, pH 8.0, 500 mM NaCl and 1 mM DTT), and loaded onto a Superdex 200 10/300 GL column (GE Healthcare)

equilibrated with SEC buffer. The final protein fractions were dialyzed against the final buffer (10 mM Tris-HCl, pH 8.0, 100 mM NaCl and 1 mM DTT), concentrated to 15 mg ml<sup>-1</sup> and used for crystallization with the vapor diffusion method (sitting drop). Crystals of KIF4-AMPPNP were obtained using the reservoir buffer (28% w/v PEG4000, 100 mM Tris-HCl, pH 8.5 and 200 mM sodium acetate) in the presence of 5 mM AMPPNP. X-ray diffraction data of KIF4-AMPPNP were collected at -180°C to 1.80 Å on the beam line NW12A (wavelength λ = 1.00 Å) at the Photon Factory (PF). All data were integrated using XDS and scaled using Pointless and Scala. The structures of KIF4-AMPPNP were determined by molecular replacement with MOLREP using the Eg5 structure (3HQD) as a starting model. After subsequent rounds of model building and refinement using Refmac5 and Coot, the electron density maps were improved, resulting in R and R<sub>free</sub> values of 16.4 and 19.7% for KIF4-AMPPNP at the PF.

## 3 Results and Discussion

In the electron density map of the KIF4 motor domain, the AMPPNP moiety is clearly observed in the nucleotide-binding pocket (Fig. 1). The KIF4-AMPPNP structure possesses both features necessary for the pre-hydrolysis state. The γ-phosphate of AMPPNP tightly interacts with both S211 in switch I and G244 in switch II (Fig. 1). The backdoor salt-bridge is also tightly formed.

In the KIF4-AMPPNP structure, the backdoor further recruits another water molecule next to the first water molecule, such that a tight hydrogen-bond network is formed in the narrow space surrounding the γ-phosphate, two γ-phosphate sensors and the backdoor residues (Fig. 1).

Backdoor closure also stabilizes two mobile loops, loop L9 (switch I) or loop L11 (switch II), which precede or follow the backdoor residues, respectively. In the KIF1A-AMPPNP structure, these two loops are flexible and invisible in the crystal structure<sup>2</sup>. In the KIF4-AMPPNP structure, which possesses the totally closed backdoor, these two loops are stabilized and are clearly visible (Fig. 1, 2).

Our structural result indicates that the backdoor closure stabilizes loop L11 by it being wound up into the long helix  $\alpha 4$ , and this mechanism might be common at least between N-kinesins. As such, KIFs exhibit a shorter helix  $\alpha 4$  in the nucleotide free state and ATP binding stabilizes loop L11 to give the longest helix  $\alpha 4$ , information that is further relayed to the neck-linker. This structural model fits very well with the previous structural report of the kar3-microtubule complex<sup>3</sup> that indicates the shortest helix  $\alpha 4$  in the nucleotide free state, and a similar structure was also reported with KIF1A<sup>2</sup>.

Backdoor formation further affects the conformation of L11- $\alpha 4$ -L12- $\alpha 5$ -L13 in switch II. Stabilization of loop L11, which is located close to the catalytic center (left side when on the microtubule), induces the clockwise rotation of helices  $\alpha 4$  and  $\alpha 5$  up to 5° during the isomerization step. This rotation relays the conformational change to loop L13 located on the other side of the catalytic core (right side when on the microtubule), which provides the stable docking site for the neck-linker, resulting in the full-docking of the neck-linker to the catalytic core (Fig. 1).

Considering that the neck-linker connects two motor heads in the dimeric KIFs, and that its length is optimized to be strained if both heads are bound to the microtubule, this tight regulation of the neck-linker conformation by ATP binding to the catalytic center of the rigor head serves as the structural basis for placing the tethered head to the next-binding site. This results in the power stroke in the asymmetric hand-over-hand motility by achieving the tight coupling of the ATPase cycle between two motor heads necessary for the processive movement of dimeric kinesins (ATP gating)<sup>2</sup>.

#### References

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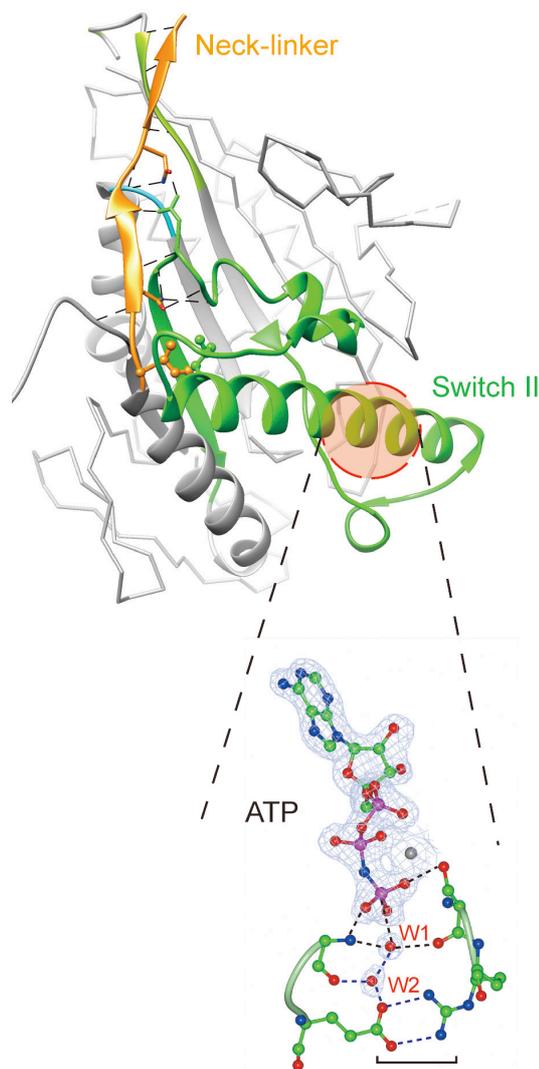


Fig.1

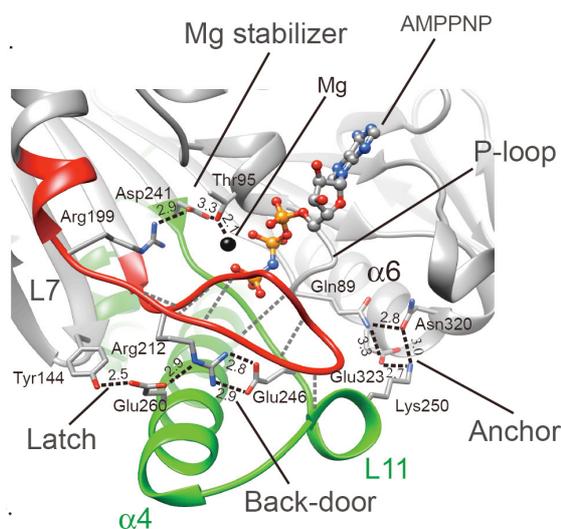


Fig.2