Structures of Kinesin Motor Proteins

Alexander Marx[1,*], **Andreas Hoenger**[2], and **Eckhard Mandelkow**[1]

[1] Max-Planck-Unit for Structural Molecular Biology, c/o DESY, Notkestrasse 85, 22607 Hamburg, Germany

[2] Dept. Molecular, Cellular and Developmental Biology, University of Colorado, Porter Science Bldg, B031A, Colorado Ave., Boulder CO, 80302, U.S.A.

Abstract

Almost 25 years of kinesin research have led to the accumulation of a large body of knowledge about this widespread superfamily of motor and non-motor proteins present in all eukaryotic cells. This review covers developments in kinesin research with an emphasis on structural aspects obtained by X-ray crystallography and cryo-electron microscopy 3-D analysis on kinesin motor domains complexed to microtubules.

Keywords

kinesin; motor protein; microtubules; cytoskeleton; X-ray crystallography; cryo-electron microscopy; image reconstruction

Introduction

In 1985, kinesin was identified and partially purified as an novel, microtubule-based motor protein with an ATPase activity characteristic of fast axonal transport (Brady 1985; Vale et al. 1985). About 10 years later, the structure of the enzymatically active domain was determined by x-ray crystallography of truncated constructs derived from human and rat conventional kinesin (Kozielski et al. 1997; Kull et al. 1996; Sack et al. 1997). Since the first structures became available in 1997, the number of kinesin structures deposited in the PDB has increased substantially (Fig. 1). In the meantime kinesins have grown to a superfamily of 14 classes whose nomenclature has been redefined recently (Dagenbach and Endow 2004; Lawrence et al. 2004), including kinesin-related proteins of various functions. At present more than 60 structures of kinesin constructs containing the catalytic motor domain are known from 9 classes of the kinesin superfamily, and from sources as diverse as plants, fungi, and mammals. Now, after another decade of kinesin research that has benefited from the structural details elucidated by x-ray crystallography and high-resolution cryo-electron microscopy, an overall understanding has emerged of the mechanisms used by kinesins to fulfill their tasks. A number of reviews on kinesin have been published recently (e.g. Block 2007; Hirokawa and Noda 2008; Kikkawa 2008; Marx et al. 2005; Valentine and Gilbert 2007). The aim of this particular contribution is to recapitulate the main structural aspects obtained by X-ray crystallography and high resolution electron microscopy.

*Correspondence to Alexander Marx; marx@mpasmb.desy.de.

Classification of the kinesin superfamily

Members of the kinesin superfamily are characterized by a common, high-homology ATP-binding domain rich in both α and β secondary structure. Since most members of the kinesin superfamily (i.e. conventional kinesin or Kinesin-1 as well as many kinesin-related proteins of the other kinesin classes) are involved in active transport and movement, this domain is usually called motor domain (MD), sometimes even in the case of non-motor kinesins. Other domains are mostly variable and class specific. The same holds for the sequential position of the motor domain. This allows a classification of the kinesin family into N-type kinesins with the motor domain at or near to the N-terminus (Kinesin-1 to 12), M-type kinesins with the motor domain flanked on both sides by other domains (Kinesin-13), and C-type kinesins with the motor domain close to the C-terminus (Kinesin-14). This domain structure-based classification corresponds surprisingly well to a functional classification in plus-end directed motors (N-type), minus-end directed motors (C-type), and non-motor kinesins (I-type).

Most kinesins have two motor domains that work in tandem. In the biologically active state they form dimeric or even higher-order complexes of motor proteins and possibly other accessory proteins. Conventional kinesin, the first kinesin identified and the founding member of the kinesin superfamily, is a tetramer consisting of a homodimer of two Kinesin-1 heavy chains (KHC) responsible for motor activity and two identical light chains (KLC) which control the activity of the complex and connect it to the cargo. The typical Kinesin-2 motor seems to be a heterotrimeric complex composed of two different but complementary motor protein chains, complemented with a third, accessory molecule. Kinesin-5 (BimC “bipolar” kinesin, Eg5) motors are homotetramers that may be considered an anti-parallel assembly of two dimeric motors. In the mitotic spindle, Eg5 interacts with two adjacent microtubules of antiparallel orientation and makes them slide along each other. A remarkable exception is KIF1A (Kinesin-3), which is monomeric in solution and employs a helper domain (K-loop) that prevents diffusion from the microtubule surface.

Early phase of structure analysis

Most of the early structural work was focussed on plus- or minus-end directed, dimeric kinesins, and on monomeric kinesin. These motor proteins promised the most direct approach to an understanding of the force generation mechanism of kinesin. At that time, the list of known kinesin variants was far from complete, and it was reasonable to search for a common mechanism applicable to all kinesins, possibly displaying only slight variations. In fact, this turned out to be approximately correct, even with regard to the broader basis of today’s knowledge. Structures of the motor domain of human KHC (Kull et al. 1996) or rat KHC (including part of the adjacent “neck linker” and “neck” (Kozielski et al. 1997; Sack et al. 1997)) defined a common motif that still serves as the reference for new MD structures. So far, not a single MD structure exists (not even that of microtubule-depolymerizers, Kinesin-13), that does not display a general fold similar to kinesin-1 (in fact, even the motor homology domain Vik1 (Allingham et al. 2007) that form heterodimers with Kar3 strongly resembles that of kinesin-1). Class-specific differences are mostly limited (and attributed) to special loops between the common structural elements of the motor domain (e.g. K-loop in monomeric kinesins, loop L5 in Eg5, KVD finger in M-type kinesins), or to the effect of interactions with other, non-MD domains (e.g. FHA domain in Unc-104 (Al-Bassam et al. 2003; Lee et al. 2004)). Moreover, the dimeric structure of rat KHC (Kozielski et al. 1997) combined with EM image reconstructions (Hoenger et al. 1998) and the assignment of microtubule binding sites by alanine mutations (Woehlke et al. 1997) provided a first structural basis for intuitive models of kinesin’s movement, which could account for the cooperative behaviour of processive kinesins (Fig. 2). Many more details about the structure and kinetics of the kinesin-microtubule interaction have been collected since then [Hirose et

al. 2006; Sindelar and Downing 2007], see also example of Fig. 3, and Auerbach and Johnson [2005]; Hackney and Stock [2008]; Hackney et al. [2003]; Valentine and Gilbert [2007]. Interestingly, the structure of dimeric rat kinesin from 1997 is still the only one that shows conventional, processive kinesin in a conformation that may be representative of a functional dimer.

The fold of the kinesin MD exhibits an unforeseen similarity with that of myosin, suggesting that both motor proteins originate from a common ancestor (Kull et al. 1998; Vale and Milligan 2000). This prompted speculations that myosin and kinesin could use the same mechanism. More specifically, the coiled coil of the neck helices could serve as a rigid arm in analogy to the "swinging lever" or "power stroke" mechanism proposed for myosin (Holmes 1997; Rayment 1996). However, the conclusions drawn from the comparison with myosin were less significant than expected. In the case of dimeric, but unprocessive Ncd (Endres et al. 2006; Wendt et al. 2002) and possibly in NcKin3 (an unusual member of the Kinesin-3 family (Marx et al. 2008)), a power stroke induced by ATP hydrolysis has been invoked. Here, the stalk domain, potentially strengthened by the interaction with the second, catalytically inactive motor domain, could act as a rigid lever. Overall, the similarities between myosin and kinesin seem to be restricted to the mode of nucleotide binding and to the local rearrangements caused by ATP binding and processing. How the local changes are translated and amplified into large-scale movements seems to vary between different kinds of kinesins, and certainly between myosins and kinesins.

General architecture of the kinesin motor domain

The ATP binding site in the kinesin motor domain consists of four motifs that are common to P-loop containing proteins with a Walker fold (Walker et al. 1982) such as also found in myosin, and G-proteins. The Walker A motif (GxxxxGKT/S) forms a phosphate binding loop (P-loop) between β-strand 3 of the central β-sheet and helix α2. This loop tightly binds the βphosphate of the nucleotide. Two other motifs, the switch-1 (NxxSSR) and the switch-2 motif (DxxGxE), change their conformation and interaction in response to the presence or absence of γ-phosphate. These local changes are transmitted and amplified by adjacent structural elements, and eventually result in large-scale effects. How exactly this is achieved is still subject of ongoing research.

Using a combination of different methods including cryo-EM of gold-labeled kinesin motor domains attached to microtubules, Vale, Milligan and coworkers showed that the neck linker, a stretch of about 15 amino acids at the C-terminal end of the motor domain, adopts an immobilized, extended ("docked") conformation when the MD is bound to microtubules in the presence of ATP (Rice et al. 1999). In the presence of ADP or the absence of nucleotide, the neck linker is rather flexible. Thus, ATP induces a conformational change of the microtubule-attached MD that could affect other regions of the kinesin molecule (or the kinesin motor-cargo complex) by pushing or pulling towards the plus end of the microtubule.

Most important for the reversible docking of the neck linker is a subdomain called switch-2 cluster that constitutes the major microtubule binding surface. It consists of helices α4 (switch-2 or "relay" helix) and α5 and the intervening loop L12. The switch-2 cluster is connected to switch-2 by loop L11, and it slides like a rigid body over the core β-sheet, in a nucleotide-dependent manner (Fig. 4). In the ATP bound state (with the MD attached to the microtubule), the switch-2 cluster prefers a permissive position that allows the neck linker to dock to the MD core (Vale and Milligan 2000). When ADP is bound to kinesin, the switch-2 cluster tends to obstruct the binding site of the neck linker. In the absence of microtubules, the nucleotide in the active site is not sufficient to determine the conformation of the motor

domain. This proved to be a source of confusion for the interpretation of high-resolution structures obtained by x-ray crystallography of isolated MD constructs (i.e. in the absence of microtubules). With respect to the conformation of the switch-2 cluster some of the kinesin crystal structures with ADP in the nucleotide pocket represent the "ATP state" rather than the "ADP state". This cluster is linked to the active site (the switch-2 motif) via the flexible loop L11, which is disordered in most crystal structures. According to a common model, loop L11 needs the interaction with a microtubule to become rigid and to act as a mechanical transducer that is able to transmit conformational signals from the nucleotide binding pocket to the switch-2 cluster and further to the neck linker (e.g. K-loop in KIF1A).

For similar reasons, the role of the switch-1 motif, the directly linked loop L9 and helix α3 ("switch-1 region") was even less clear from crystal structures. Comparisons between known crystal structures show a high variability in this region (Kikkawa et al. 2001; Song et al. 2001). Loop L9 may assume various conformations, ranging from predominantly helical to hairpin-like. Concomitantly, helix α3 moves and tilts considerably. Recent results of enhanced cryo-EM data analysis with sub-nanometer resolution confirmed the idea that switch-1 and switch-2 regions are indeed significantly affected when conventional kinesin binds to microtubules (Sindelar and Downing 2007). According to these data, switch-1 of conventional kinesin seems to play a key role in microtubule-induced ADP release. The response of switch-1 to microtubule binding is explained by a direct communication with switch-2/switch-2 cluster, involving a substantial rearrangement of L11 upon interaction with the microtubule.

Modes of movement

Conventional kinesin (Kinesin-1) uses two identical motor domains in alternation to move along the microtubule, taking on the order of 100 steps per run, each of about 8 nm in size (corresponding to the length of an α,β-tubulin dimer), and hydrolyzing one ATP molecule per step (Hackney 1994; Hackney 1995; Hua et al. 1997; Schnitzer and Block 1997). Initially, various types of models had been proposed to describe this type of motion, and the exact details are still under discussion. However, it seems to be widely accepted that the processive motion of conventional kinesin's is most naturally explained by some kind of "hand-overhand" walking model (Asbury et al. 2003; Kaseda et al. 2003; Yildiz et al. 2004). This requires a tight coordination of ATP hydrolysis, microtubule binding, and force generation within the two kinesin heavy chains. The assumption of an "alternating head" movement was strongly supported by kinetics measurements of ADP release (Hackney 1994). Monomeric KHC from *Drosophila* lost ADP rapidly and quantitatively when incubated with microtubules, whereas a dimeric construct displayed "half-site reactivity": it releases only 50% of the bound ADP in a fast, initial phase; exchange of the remaining ADP is slow, but can be accelerated by addition of ATP (Gilbert et al. 1998).

The neck linker and the neck coiled coil play an important role for the communication between the nucleotide and microtubule binding sites of both molecules (Thorn et al. 2000). According to the hand-over-hand walking model, at any time point (during a processive run) at least one motor domain must stay attached to the microtubule while the other one is moving to the next binding site. This implies that the two motor domains can bind simultaneously to adjacent tubulin dimers, at least temporarily. Two this end, the two "heads" of conventional kinesin, as seen in the crystal structure of the dimeric construct from rat kinesin, must somehow untangle and separate from each other (Fig. 2). As demonstrated by EM of microtubules decorated with SH3-labeled kinesin constructs (Skiniotis et al. 2003) and confirmed by FRET experiments (Tomishige et al. 2006), the neck linker of the "leading head" undocks from the motor core and flips by about 180°, thus allowing the two heads to bind at a distance of ~8 nm in essentially identical orientations

(Fig. 5; see also (Krzysiak et al. 2006) where the conformation of Eg5 dimers on microtubules are imaged directly by high-resolution shadowing). The necessary communication between the active sites can be explained by the strain that must build up during simultaneous binding of the motor domains, which always stay connected to each other via the neck coiled-coil (Hyeon and Onuchic 2007).

However, not all kinesin motors are processive and use the same gait to advance along the microtubule surface. As conventional kinesin, the minus end-directed Ncd, a C-type motor, has two motor domains. Crystal structures of dimeric Ncd constructs show that the stalk forms a continuous coiled coil with the class-specific neck-linker/neck at the N-terminus of the motor domain. The earlier structures revealed a symmetric (Sablin et al. 1998) or almost symmetric (Kozielski et al. 1999) conformation of the Ncd dimer. But a more recent structure of a similar construct showed that the neck and stalk coiled coil together with one of the motor domains can rotate by 75° relative to the previous structure (assuming one head to be fixed), suggesting a power stroke type of force production with the stalk/neck acting as rigid lever (Yun et al. 2003). This leads to a the assumption of a “hopping” model in agreement with cryo-EM image reconstructions of Ncd-decorated microtubules (Endres et al. 2006; Wendt et al. 2002).

Another finding that challenged the universal hand-over-hand walking model for all kinesins was the discovery of the Unc104/KIF1 family of monomeric kinesins (Kinesin-3). In biochemical characterization of mouse KIF1A, it appeared as a monomeric, globular protein able to sustain fast anterograde transport of membrane organelles (Okada et al. 1995). Later on, atomic coordinates of the KIF1A MD and image reconstructions of the KIF1A-microtubule complex have been determined for various nucleotide states, providing insight into the structural dynamics of KIF1A and kinesins in general (Kikkawa 2008; Kikkawa and Hirokawa 2006; Kikkawa et al. 2000; Kikkawa et al. 2001; Nitta et al. 2004; Nitta et al. 2008). In single-molecule motility assays, KIF1A was shown to move processively, but with the characteristics of biased diffusion, suggesting a Brownian ratchet type of mechanism (Okada and Hirokawa 1999; Okada and Hirokawa 2000). An extension of loop L12 (part of switch-2, “K-loop”), which is specific to Kinesin-3 family members, is essential for single-headed processivity of KIF1A (Okada and Hirokawa 2000). The K-loop contains multiple lysine residues which are positively charged and can electrostatically interact with the negatively charged tails of the tubulin subunits. This interaction could prevent complete dissociation from the microtubule during weak binding phases. Thus, single-headed processive movement of KIF1A can be explained by the presence of the K-loop which partially compensates for the lack of a second motor domain. In the meantime, however, strong evidence emerged that biased diffusion is not the only type of motion, and probably not even the most important one for KIF1A function (Al-Bassam et al. 2003; Rashid et al. 2005; Shimizu et al. 2005; Tomishige et al. 2002). Under crowding conditions (e.g. at the high local concentrations expected at the surface of membrane-bounded organelles), KIF1A can dimerize by coiled coil formation of the neck linker or stalk domains. Hence, it appears as if a deliberate and controlled induction of dimerization between KIF1A chains is just another way of regulating motor activity, a mechanism used predominantely by “monomeric” kinesins of the Kinesin-3 family.

Conclusions and perspectives

The results of kinesin research described so far revealed a picture that is characterized by certain ambivalence between uniformity and diversity. On one hand, there are general traits that are common to most, if not all, kinesins: the overall fold of the MD, the similarity in nucleotide binding and processing with common motifs and switches that are responsible for the coordination of ATP hydrolysis and its transformation into local conformational effects.

Consequently, many concepts derived form early investigations are still valid and useful, if only as a reference to manage the upcoming diversity of kinesins. On the other hand, an amazing variability emerged, not only in the details of kinesin structures and the basic mechanisms it can use, but also in the diversity of molecular machines that have evolved from a common motor domain.

An extreme example for functional diversification is the Kinesin-13 family of microtubule depolymerizing kinesins. MCAK and MKCM1, two members of the Kinesin-13 family, use the energy of ATP for the disassembly of microtubules rather than for directed motion along a microtubule (Desai et al. 1999; Maney et al. 2001; Wordeman and Mitchison 1995). Nevertheless, crystal structure analysis of Kinesin-13 members KIF2C and pKinI (MCAK homologues from mouse and from *P. falciparum*) showed that the general architecture of their catalytic domain is similar to the well-known MD of conventional kinesin (Ogawa et al. 2004; Shipley et al. 2004). The N-terminal, helical neck and class-specific modifications of the MD (especially an extension of loop L2, the “KVD finger”) are supposed to be responsible for targeting of the microtubule ends and for weak binding to the microtubule wall, allowing one-dimensional diffusion along the microtubule (Helenius et al. 2006). This is consistent with cryo-EM data demonstrating that Kinesin-13 preferentially binds to tubulin rings and may induce outward bending of protofilaments at the end of a depolymerizing microtubule (Moores et al. 2002, 2006; Tan et al. 2008).

During the past five years, the number of high-resolution structures from various kinesin-subfamilies increased considerably (Fig. 1). Kinesin-13 is only one example. Another, even more prominent example is Kinesin-5 (Eg5), which is an essential part of the mitotic apparatus. Today, the “new subfamily” structures contribute about 50% to the total number of known structures. This could mark a major change in perspective: in the early phase, kinesin research was largely motivated by the aim to understand the physical principles by which kinesin works, i.e. to understand how kinesin converts the free energy of ATP into mechanical work. This attitude was combined with a rather simplistic view of kinesin as a molecular machine that plays a role in many different cellular processes. Now, the main driving forces have changed. The high number of Kinesin-5 structures recently deposited to the Protein Data Bank is explained by the fact that Eg5 is a target for anti-cancer drug development (e.g. monastrol: see Krzysiak et al. [2006],Yan et al. [2004]). Thus, most of the Kinesin-5 structures are MD-ligand complexes with potential drug targets. Most of the rare structures of kinesin families 7, 9, 2, and 10 (in total 6 structures, since 2004) have been solved within the context of structural proteomics projects (SPINE, SGC). It seems that the previous main incentive for studying crystal structures of kinesin MD constructs has been superseded by the more practical aim of finding new therapeutically active substances and by a systematic exploration of the whole inventory of the kinesin superfamily.

If this change of perspective is real, it could be taken as an indication that our understanding of the mechanistic principles is approaching completeness. Most experts of the field would fiercely dispute this opinion. Steven Block expressed this in the Epilogue to his recent review on kinesin motor mechanics: “… it astonishes me how often some of my colleagues have seemed ready to declare victory based on a latest insight, experimental discovery, or model, only to be humbled—or at least transiently silenced!—by the next set of experiments to be published. A great deal more remains to be discovered about motor proteins” (Block 2007). This latter statement is indeed undisputable.

Cryo-EM and X-ray crystal structure analysis have both proved invaluable in the past, and will certainly continue to make significant contributions to future progress for structure-function investigations in macromolecular assemblies of all kinds. High-resolution cryo-electron tomography will open new avenues for structural analyses into macromolecular

assemblies that are not necessarily suitable for average-based image reconstruction as the examples presented here (Hoenger and McIntosh 2009). Due to technical circumstances cryo-EM and protein crystallography are both restricted to static structures, and high-resolution NMR-spectroscopy is not well suited for the structural analysis of large complexes. The limitation to static views is certainly an issue, especially for proteins that are designed to move. However, both methods allow capturing multiple distinct conformational (nucleotide) states which then can be assembled in silico to functional models at high resolution. Here cryo-EM and X-ray crystallography are complementary and the combination of their particular strengths is tremendously valuable. Protein crystallography delivers atomic-resolution detail while cryo-EM/image reconstruction trades resolution for its ability to resolve large complexes under near-physiologic conditions. In addition, present developments such as X-ray microscopy and in particular x-ray laser technology with applications to single molecule analysis at an ultra-fast time scale may add a new perspective to the field and, potentially, lead to a new boost in high-resolution structure analysis of kinesin and other motor proteins in a few years from now

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Abbreviations

PDB
: Protein Data Bank (http//www.wwpdb.org/)

MD
: motor domain

SPINE
: Structural Proteomics in Europe

SGC
: Structural Genomics Consortium

EM
: electron microscopy

KHC
: kinesin heavy chain

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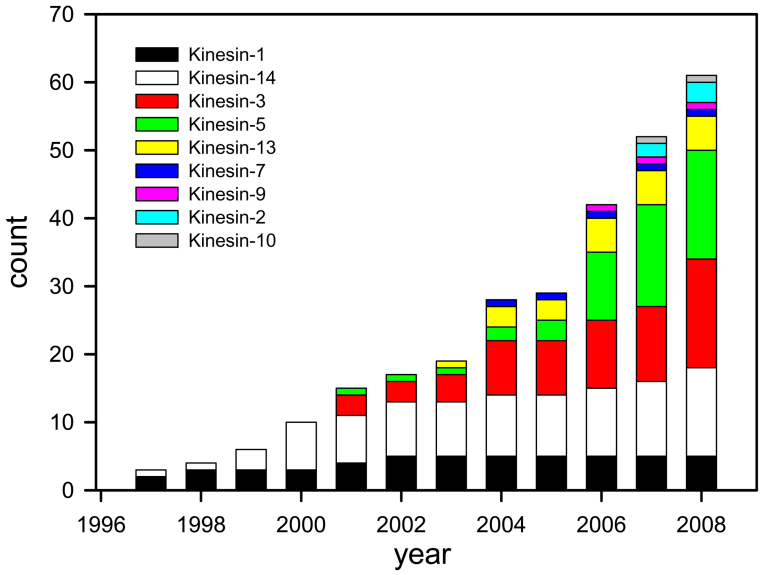

Figure 1.
Number of kinesin motor domain structures in the PDB. The bars represent cumulative numbers of structures, and the corresponding years refer to the date of deposition.

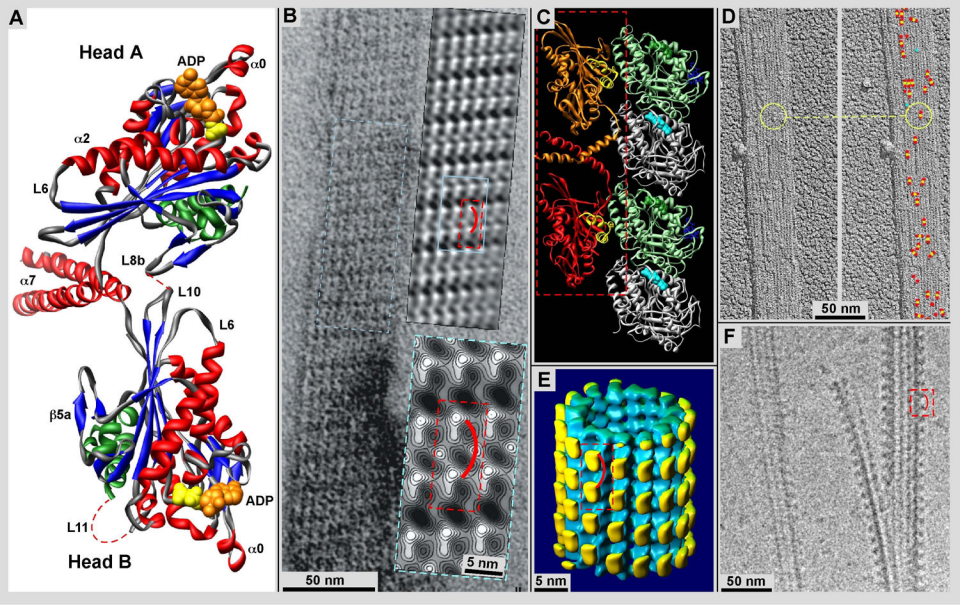

Figure 2.

Kinesin-1 in x-ray structure analysis and cryo-EM image reconstruction. Red frames outline the dimer structure in each panel (A) Ribbon diagram of the structure of RnK379, a dimeric construct from rat conventional kinesin (PDB-ID 3kin). The construct comprises the motor domain with neck linker and the first half of the neck helix that forms a coiled coil (Kozielski et al. 1997). (B) Negatively stained image of a tubulin sheet decorated with dimeric RnK379 in the presence of AMP-PNP. Both heads touch the tubulin surface along one protofilament as modelled in panel C. In lucky cases these dimers bind in register and form a regular 16-nm repeat. (D) The same binding pattern is also directly visualized by high-resolution shadowing on sparsely decorated tubulin sheets. Left and right panel are identical images with dimers marked in the right panel (Eg5 dimers: Krzysiak et al., 2006). (E) Typically kinesin-1 dimers bind stochastically and therefore helically averaged 3-D reconstructions loose the connection between trailing and leading head (Hoenger et al., 1998 Hoenger et al., 2000). The density of the bridging coiled-coil neck is very low and usually not directly visible in cryo-electron micrographs.

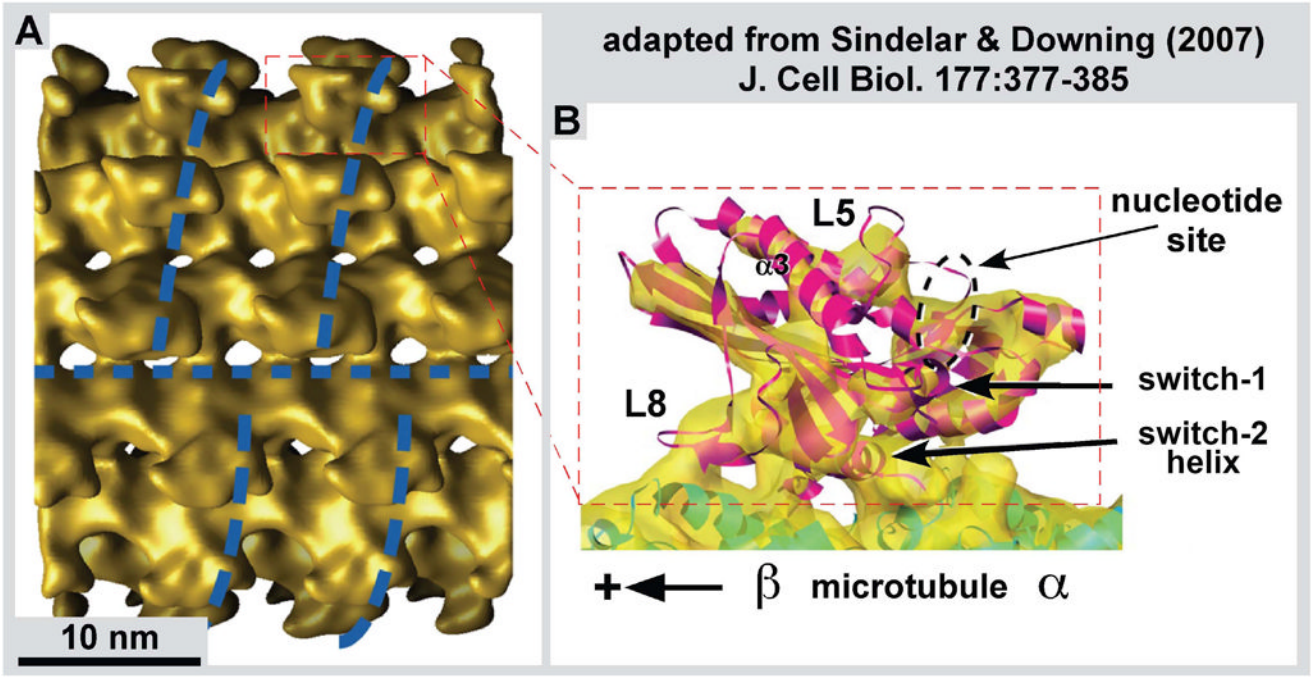

Figure 3.
Kinesin-microtubule complex derived from 9Å resolution cryo-EM analysis of microtubules decorated with a monomeric construct (K349) from human conventional kinesin (Figure adapted from Sindelar and Downing [2007]).

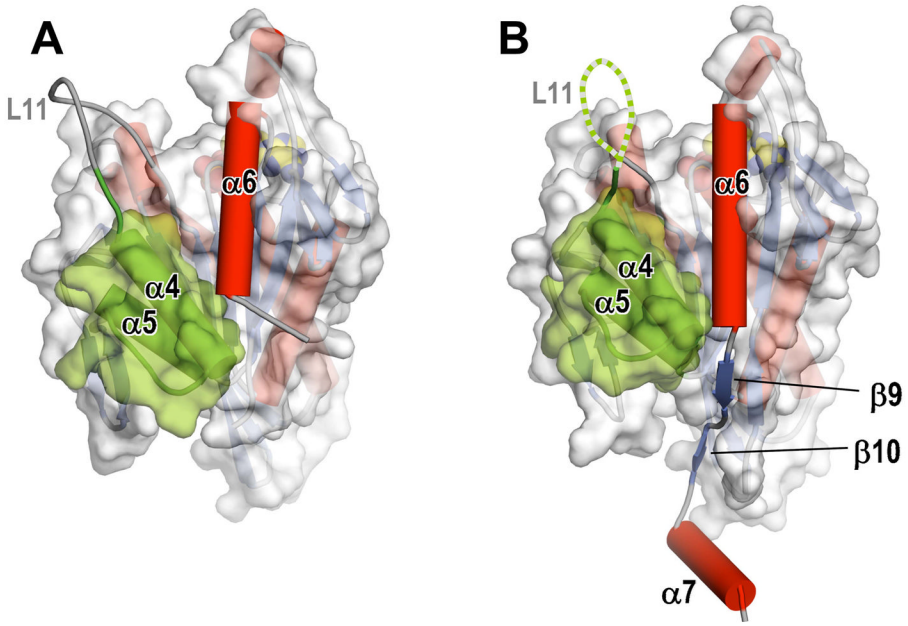

Figure 4.

Docking and undocking of the neck linker controlled by the switch-2 cluster. Kinesin-1 motor domain structures representing the "ADP state" (A, human KHC, PDB-ID 1bg2) and the "ATP state" (B, rat KHC, PDB-ID 2kin) are shown as cartoon models with semitransparent surface representations of the switch-2 cluster (green) and the "core" domain (motor domain without switch-2 cluster and helix α6; grey). In the "ADP state" (A), the switch-2 cluster prevents binding of the neck linker to the core domain; neck linker and neck are mainly disordered and invisible. In transition to the "ATP state" (B), the switch-2 cluster moves up and opens a binding groove for the neck linker (β9, β10). Note that the rat KHC structure (B) represents the "ATP state" although it has ADP bound, demonstrating that without microtubules the nucleotide is not sufficient to determine the conformation of the motor domain.

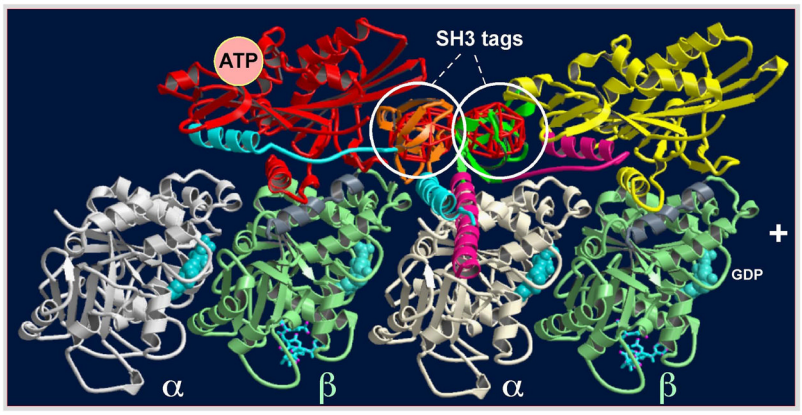

Figure 5.

Sideview of a microtubule protofilament complexed with a dimeric kinesin rKS379 MD construct from rat conventional kinesin with an SH3 domain inserted between the neck linker and the neck helix of each head (white circles). The SH3 domains serve as a label to identify the position of the neck linkers in cryo-EM image reconstructions (Skiniotis et al. 2003). The trailing head is in an ATP configuration with the neck-linker locked into a plus-end directing position. The leading head shows a nucleotide free configuration with a flexible neck-linker that is pulled backwards by the coiled-coil connection to the trailing head. Nucleotides are displayed as spheres and are GDP in β-tubulin, and GTP in α-tubulin. Taxol is seen as ball-&-stick at the inner tube face of the protofilament.