CY 305

Molecular Architecture and Evolution of Functions

Seminar 1

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Motor Protein Kinesin

Submitted by-

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Introduction

Cell is the basic unit of life. A typical cell measures few microns in diameter. Most molecules in the cell are few nanometers. There is an immense need to transport materials from one part of the cell to another. The need is all the more pronounced in cells like neurons which can be many centimeters long.

The task of transportation is carried out by a complex machinery of proteins. Tubulin molecules in cells assemble themselves into structures known as *microtubules* which provide highways for this transport to take place. A kinesin attaches to microtubules and moves along the microtubule in order to transport cellular cargo, such as vesicles.

Nature, through millions of years of evolution, has taken these molecular motors to near perfection; in the process, making them very complex. Various mathematical models have been proposed to account for the observed properties of kinesins. Understanding the structure and functioning of these molecules will help us mimic their efficiency and functionality and design systems which will have applications ranging from medical sciences to space exploration.

1. Molecular motors

Eukaryotic cells are organized into membrane bound organelles *viz.* the nucleus, the Golgi complex, the endoplasmic reticulum etc. The products of these have to be transported to other parts of the cell. The distribution system is complex, and uses three sets of molecular transporters: myosin, kinesin and dynein motors. Dozens of different motor proteins coexist in every eukaryotic cell.

Intracellular transport occurs along two sets of paths: the more or less randomly oriented actin filaments, used by myosin; and the often radially organized microtubules, used by both kinesin and dynein. Transport occurs along each of these when the appropriate motor binds to a cargo through its 'tail' and simultaneously binds to its filament through one of its 'heads'. The motor then moves along the filament by using repeated cycles of coordinated binding and unbinding of its two heads, powered by energy derived from hydrolysis of ATP.

The identity of the track and the direction of movement along it are determined by the motor domain (head), while the identity of the cargo is determined by the tail of the motor protein. Some of them also cause cytoskeletal filaments to slide against each other, generating the force that drives phenomena such as muscle contraction, ciliary beating, and cell division.

2. Microtubules

2.1. Structure

Microtubules are long hollow cylindrical structures found in the cytoplasm of almost all eukaryotic cells. They are used as molecular 'highways' for transport of materials from one part of the cell to another. Microtubules are made of tubulin dimers. Tubulins are 50 kDa, globular, slightly acidic proteins which occur in the cell as α-tubulin, β-tubulin and γ-tubulin. α-tubulin and β-tubulin both have a molecule of GTP attached to them and are capable of forming dimers. These dimers attach to each other with the hydrolysis of the GTP in the β-tubulin to form chains of alternating α and β-tubulin called *protofilament* (Fig 1). Thirteen of these protofilaments come together to form a tube like structure called *microtubule*. This tube has a discontinuity called the *seam* of the microtubule. γ-tubulin is ring shaped and found in *microtubule organizing centres.*

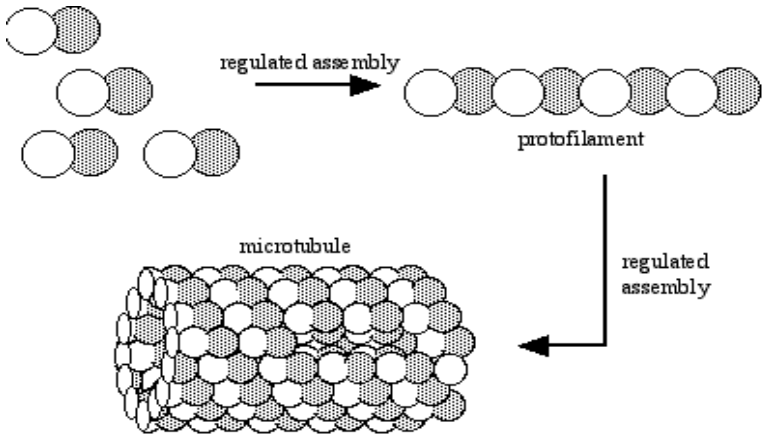


Fig 1: Microtubule

Each dimer in protofilament has directionality as the β-tubulin and α-tubulin are not identical. The protofilaments arrange themselves in such a way that the directionality is retained in the microtubule. Thus one end of the microtubule is called the plus end and the other end is called the minus end. In a free microtubule, the tubulin dimers keep adding at the plus end and keep falling off at the minus end. This is called *treadmilling.*

Most kinesin-related proteins move toward the plus end of the microtubule, as does kinesin itself, but some move toward the minus end.

2.2. Dynamic Instability

While formation of the protofilament of the microtubule, the GTP attached to the β-tubulin undergoes hydrolysis to form GDP. β-tubulin is more stable when it is associated with a GTP molecule than when it is associated with a GDP molecule. (Gelfand et al, 1991) So the formation of microtubule which is driven by GTP hydrolysis leads to destabilization of its own self. This makes microtubule capable of undergoing catastrophic destruction by converting GDP to GTP and stabilizing β-tubulin monomers. Thus Microtubules in the cell keep undergoing random cycles of polymerization and de-polymerization. (Erickson et al, 1992)

2.3. Functions

Microtubule is part of the cytoskeleton of the cell. It helps maintain the structure of the cell by providing support. It is especially important for transport of organelles and vesicles in the cells as motor proteins use them as tracks to move from one part of the cell to another. As microtubules are radially aligned in the cell they play an important role in *pigment translocation*. The flagella in many bacterial cells are made of microtubules. Microtubule also plays an important role in cell division.

2.4. Microtubule Associated Proteins

There are proteins which can bind to microtubules and affect their length, formation, rate of treadmilling, and stability. These proteins, which may widely differ in function and structure, are collectively called *microtubule-associated proteins.* Examples of such proteins are *tau* and MAP1.

3. Kinesin: Microtubule associated motor protein

Kinesins constitute a large motor-protein super-family that transports cargoes within a cell by moving on microtubule filaments (Miki, H. et al, 2001). There are at least ten families of *kinesin-related proteins*, or KRPs, in the kinesin super-family. It was first identified in the giant axon of the squid, where it carries membrane-enclosed organelles away from the neuronal cell body toward the axon terminal by walking toward the plus end of microtubules.

It is classified as one among the microtubule-binding proteins, owing to its attachment to microtubules for carrying out the transportation of cargo in cells. They are called motor proteins because they convert chemical energy into mechanical energy.

Kinesins are separated into three major classes according to the position of the motor domain on the peptide sequence. It can be on the N terminus, C terminus or in the middle of the amino acid sequence (Hirokawa, N. 1998). N-terminal kinesins are found to move towards the microtubule plus ends, whereas C-terminal kinesins move towards the minus ends (Hirokawa, N. 1998).

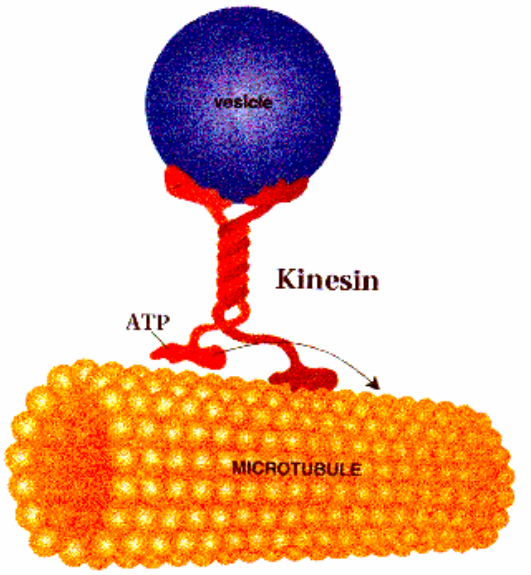


Fig 2: Typical kinesin bound to a microtubule.

In recent years, it has been found that microtubule-based molecular motors including a number of kinesin motors are capable of organizing two separate microtubule asters into metastable structures independent of any external positional cues. This self-organization is in turn dependent on the directionalities of these molecular motors as well as its processivity. The Kinesin 13 family acts as regulators of microtubule dynamics. A member of this family is MCAK which acts at the ends of microtubule polymers to depolymerize them.

3.1. Structure

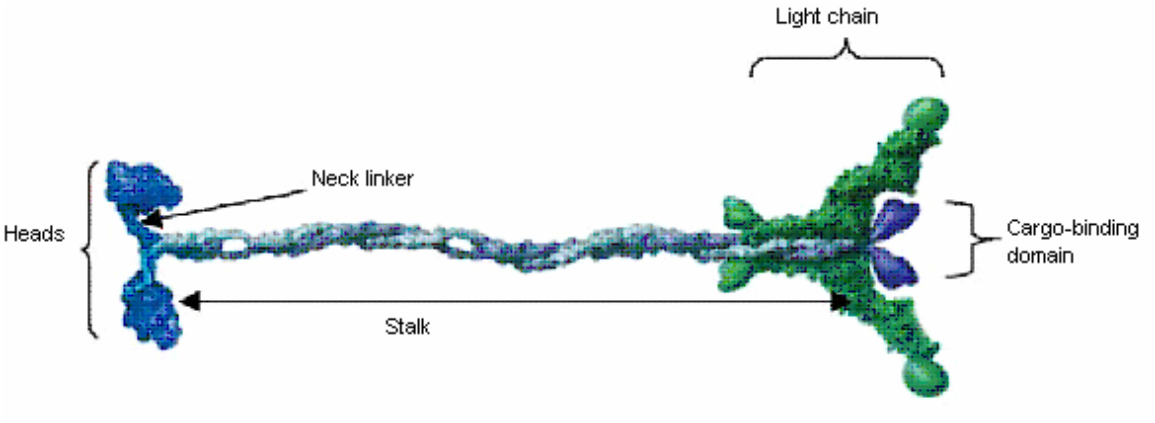


Fig 3: Structure of conventional kinesin. (Vale, R.D, 2003)

Conventional kinesin, which is prominent member of kinesin family, is a homodimeric motor protein. Each monomer consists of an N-terminal motor head, a neck linker, a long coiled-coil dimerization region and a globular tail domain. The motor heads bind to microtubules and ATP. Each head is connected to a flexible neck linker that enables motor stepping. The neck linker is connected to a 70-nm long coiled-coil stalk (body) that holds two heads together. At the end of the stalk, kinesin has a cargo-binding domain that recognizes membranous organelles and vesicles. The distance between the heads is 8.3 nm which is approximately the distance between adjacent tubulins. Conventional kinesin has two heavy chains and two light chains per active motor. Motor domain is the only common among all members of kinesin superfamily

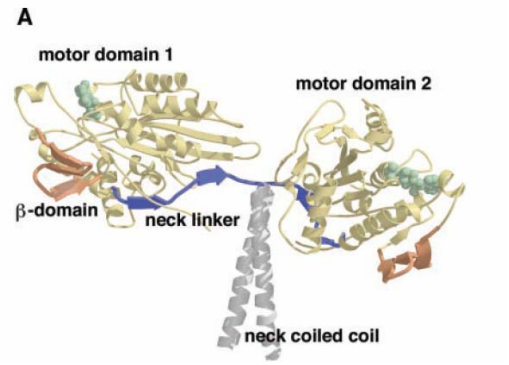


Fig 4: structural organization of conventional kinesin

All kinesins share the conserved catalytic core, which consists of a central ß-sheet sandwiched between six alpha-helices and a topologically conserved smaller lobe (called the ß-domain here) (Fig. 4) with three additional alpha-strands which have both the nucleotide- (Fig. 4) and microtubule-binding sites. Each head is connected to a neck linker, which is a mechanical element that undergoes nucleotide-dependent conformational changes. Neck-linker docking and undocking creates the powerstroke and determines the directionality of the motor movement. The neck linker is, in-turn, connected to a common stalk that leads to the globular tail domain (Vale.R et al,2003). Kinesin tail domains are highly diverse and not so well conserved even within a sub-family (Krishanu Ray,2006)**.** They are responsible for cargo association in certain motors. The kinesin may self assemble to form dimer and a coiled coil structure etc. One of the most striking features of the molecular self-assembly is the asymmetric nature of the association between the klc and khc subunits. Two coiled coil domains of khc always form a parallel or head-to head assembly with each other and two additional klc subunits associated with the dimeric coiled coil stalk of khc in yet another parallel assembly (A.Rehman et al, 1998). All these suggest that the helical interaction plays a key role in motor subunit assembly in vivo. It is also evident that almost all the kinesin complexes contain at least two motor subunits, which is essential for proper kinesin functions in vivo.

3.2. Function

The function of kinesin in a cell is to carry out organelle and vesicle transport to various parts of the cell. This is clearly visible in the fast axonal transport

mediated by kinesin-1 sub-family in which it transports mitochondria, secretory vesicles and various types of synaptic components to the axons. During interphase, kinesin translating along the microtubules organizes the position of different organelles in the daughter cells. This is best exemplified by the organization of endoplasmic reticulum and Golgi apparatus, whose orientation in the cells helps in their proper functioning.

Vesicular transport is best demonstrated in highly polarized and differentiated cells such as epithelial cells and neurons, this transport is very important for the proper targeting of proteins, packed into vesicles, to distinct parts of cells (Hirokawa, N et al. 1996). Kinesin 2 sub-family also play role in dispersion of pigment carrying granules known as melanosomes.

They are also involved in intraflagellar transport, which is responsible for formation and maintenance of cilia and flagella. Some types of kinesins are tightly associated with the microtubular central pair apparatus of motile 9+2 flagella, though the function of the central pair is unknown.

They play important role in mitosis and meiosis also. They are proposed to generate force required for assembly and maintenance of spindles, attachment of chromosomes to the spindles and movement of chromosomes to opposite ends. They have also been shown to play an important role in establishing spindle bipolarity i.e. separation of spindle pole body and also arrangement of chromosomes on the metaphase plate. Discovery of kinesin involved in depolymerizing microtubules suggest their role in microtubule kinetics during mitosis

3.3. Regulation

The kinesin–cargo interaction is likely to be controlled by altering the phosphorylation state of the protein, i.e., addition/removal of covalently attached phosphates ($–PO_4$) form certain regions of the protein. Some observations suggest that the same motor complex could independently interact with two different cargoes at the same time and space inside the cell, and deliver them to different destinations controlled by the molar

concentrations of the cargoes. The cargo association may also regulate the speed of the motor. A recent study using kinesins attached to the quantum dots showed that though a single kinesin molecule is capable of processive movement on the MT, the run lengths of the dot were proportionally increased with increasing number of motors attached. Certain experimental evidence from cultured neurons indicates that organization and structure of MT could influence the process to select their direction of transport within the cell. Processivity of the walk and run lengths are controlled by the sequences in the hinge neck region of the motor subunits. For the kinesin-1 family members, the motor activity is regulated by cargo interactions. Free khc dimers assumes a folded and compact form as a part of their tail domain binds the motor domain, thus, preventing the motor from interacting with MT . The light chain subunit (klc) associates with the C-terminal half of the coiled coil stalk and this could prevent the khc tail from interacting with the motor domain.

4. How does kinesin move?

The kinesins are processive motors having a duty ratio=0.5. The *duty ratio* indicates the relative time a motor domain remains bound on the filament as fraction of the entire ATP hydrolysis cycle. The conformational change in the motor region caused by ATP hydrolysis is converted to a net displacement. In the past decade, single-molecule studies have revealed that kinesin takes 8.3-nm steps per ATP hydrolyzed, which is equal to the distance between adjacent tubulins .The motor can complete approx 100 ATP turnovers and walk 800 nm per sec. A single powerstroke of the motor can generate a force of around 6 pN. Biochemical studies show that the two heads are strongly coordinated so that binding of the second head accelerates dissociation of the already bound head. The kinesin head binds to a microtubule in its ATP-bound state and detaches in the ADP-bound state. ATP binding causes the neck linker to be docked pointing towards the plus end of microtubules.

To transport cargos for a sufficiently long distance, kinesins would need to do the following: (i) associate with the cargo at the beginning of the run, (ii) maintain a sufficiently long run length while associated with the cargo, and (iii) release the cargo at an appropriate site at the end of the run or transfer it to another motor complex. The mechanism of initial docking and dissociation is still unclear. However a lot of work has been done on kinesin propogation.

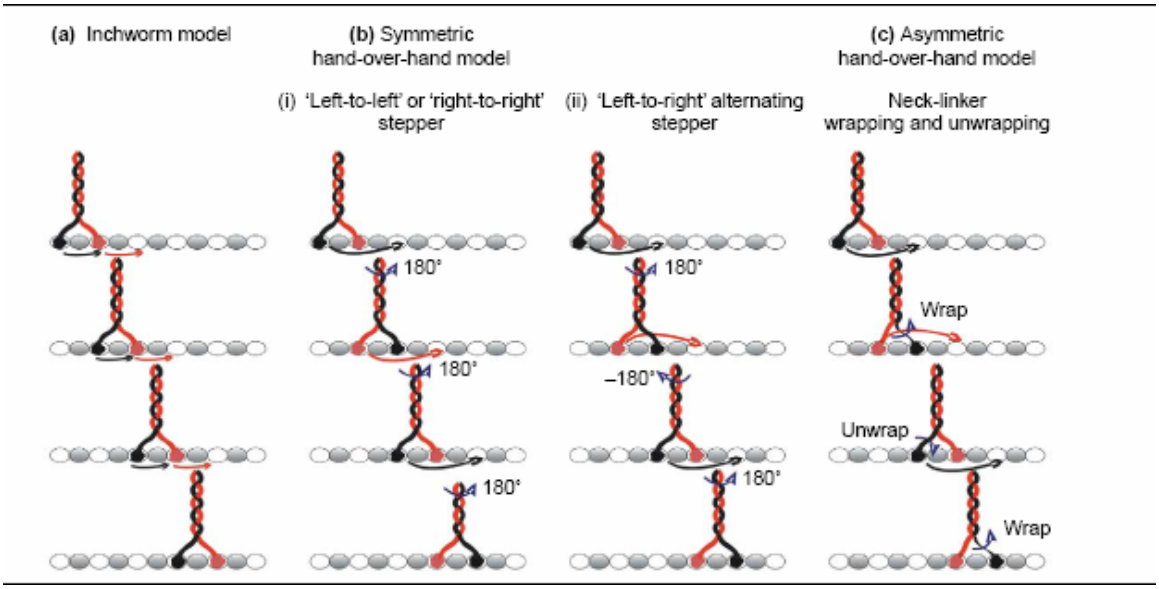


Fig 5: The above models have been proposed for the motion of kinesin**.**

4.1. In the **hand-over-hand model**, kinesin moves in much the same way as humans walk. The rear head (head 1) takes a step while the front head (head 2) remains stationary on the microtubule (Fig 5b). Head 1 attaches to the next tubulin-binding site and becomes the leading head. Thus, kinesin moves along the microtubule by alternating the positions of the heads. This means that, if the centroid position of the motor moves 8.3 nm, the rear head must move 16.6 nm and the front head must not move at all In the next step, head 2 moves forward and becomes the leading head again while head 1 stays fixed to the track. Therefore, each of the heads alternately takes 0-nm (i.e. it is stationary) and 16.6-nm steps, and the cargo is moved 8.3 nm in each case. In the first, the rear head always passes the stalk from the same side, presuming that the stalk rotates 180º along the same direction every step. Because the tail region is fixed when kinesin is attached to the cargo, rotation along one direction would overwind the stalk. After several steps, the torsional barrier would prevent kinesin from walking. In the second, the rear head alternately passes the stalk from the right side and then the left side, presuming that the stalk is rotated 180º back and forth every other step**.** (Ahmet Yildiz et al,2005; Hua .W et al,2002)

4.2. The inchworm mechanism suggests that only one head is catalytically active. In this model, one head always leads and the other head follows (Figure 5a). It is a symmetric model in which the motor can revert to the same state after each step without rotating the stalk.

(i) Kaseda et al. performed a motility experiment with a heterodimeric construct in which one head catalyzed ATP 18 times slower than the other head did. The experiment was designed to test whether both of the heads catalyze ATP, as in the hand-over-hand model, or whether only a single head does so, as in the inchworm model. The authors observed alternating short and long dwell periods during which the kinesin stalk took 8.3-nm steps. The overall speed of the motor decreased nine times compared with the speed of wild-type kinesin. The data are completely consistent with the hand-over-hand model (Kaseda, K. et al ,2003)

(ii) Asbury et al. performed an optical-trap assay with homodimeric kinesins that had stalks truncated at different positions . Under high load (around4.5

pN), kinesins truncated near the motor domain limped; the motor alternated between fast and slow motion, much like the observations that Kaseda et al. made. The limping behavior shows that kinesin uses its heads alternately, which is in agreement with the hand-over-hand model. (Asbury, C.L. et al.2003)

According to the Hoenger model (Hoenger et al. 2000)**,** the neck linker first wraps around the stalk and then unwraps during the second step (Figure 5c). When the neck linker wraps around the stalk, it is harder for the trailing head to move forward because of the torsional barrier. As the neck linker unwraps, this barrier is removed and the motor can take a faster step. Consequently, switching between wrapped and unwrapped states implies that kinesin, despite having two identical heads, moves by alternating slow and fast steps The experiment indicates that the limping is caused by the difference in geometry between the even and odd steps .

Surprisingly, kinesins with a full-length tail did not limp to a significant degree. It is more difficult and, hence, slower to take a step when the stalk is over wound. For this reason, the bead can bias the motor asymmetrically, particularly when the tail is too short. In full-length kinesin, however, the rotation of the stalk can average out on the long coiled coil region. Therefore, no limping should be observed. Such asymmetric models are attractive because the motor does not need to rotate its cargo within the cytoplasm, and many motors can work together more easily using this mechanism. Many kinesins must carry the cargo cooperatively and prevent its detachment from the microtubule. However, many motors could also work together using the symmetric mechanism if the 180º rotation were averaged out by twisting the long stalk instead of rotating the cargo. (Yildiz, A. et al. (2003), Yildiz, A. et al. (2004), Higuchi, H. et al. (2004)).

4.3. Coordination between Motor Domains in Processive Kinesins

Stability of the neck coiled coil, which does not unwind during movement of kinesin, keeps the two motor domains in register. At the same time, flexibility of the neck linker allows the motor domains to propel over each other,

optimizing their positions while searching for the new binding site. (Elena,PS et al.2004)

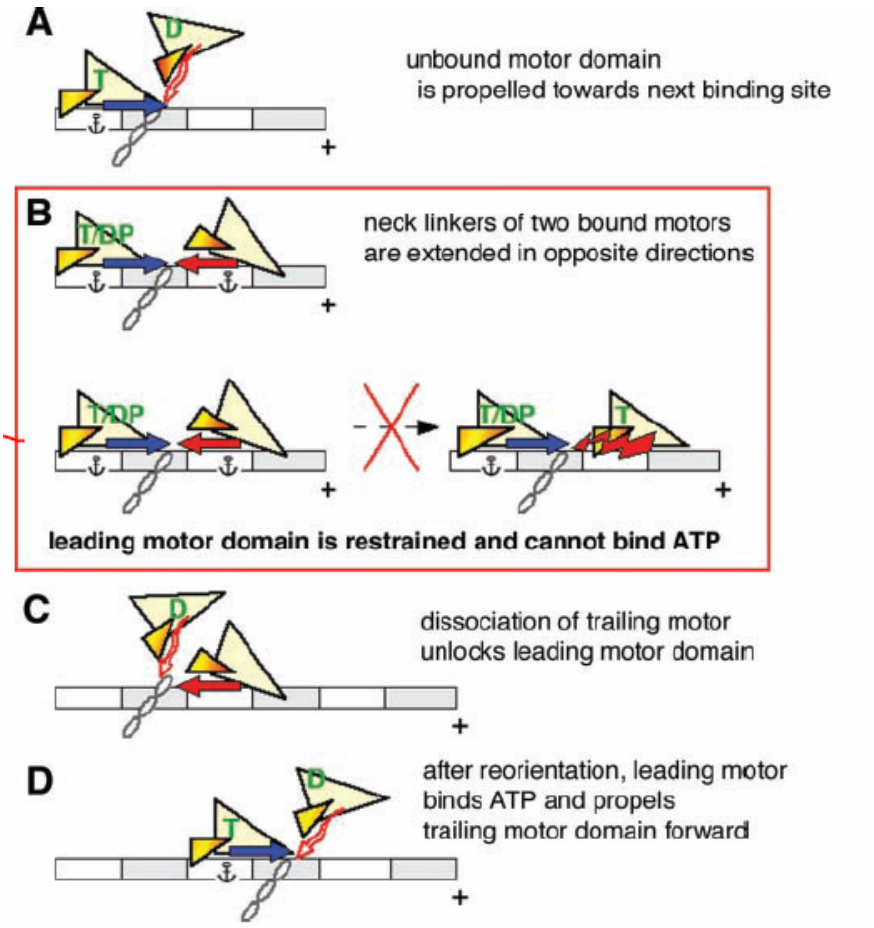


Fig 6: Coordination between motor domains in kinesin

Cooperatively between Motor Domains Drives Processive Movement of Kinesin that at a critical point of its movement conventional kinesin adopts a configuration on the microtubule with both motor domains bound to the track. In this “bridged” state , the trailing motor is firmly attached to the microtubule, has ATP/ADP-Pi in its nucleotide site, and adopts the ATP-like conformation with the neck linker docked alongside the core and pointing (from N to C termini) toward the plus end of the microtubule (Skiniotis,G et al.2003) (Fig. 6A, blue). This configuration allows the leading motor to attach to the next site on the protofilament, releasing ADP and adopting the nucleotide-free conformation with the neck linker pointing in the opposite direction leading motor cannot bind ATP until the trailing motor hydrolyzes ATP and releases

one of the hydrolysis products, Pi. This release weakens attachment of the motor core to the microtubule, and the trailing motor detaches from the track, loosing constraints placed onto the leading motor and allowing it to bind ATP and enter its nucleotide hydrolysis cycle. Note that, in the absence of bound nucleotide, the neck linker is distributed between forward (+125º) and backward (-30º) pointing states that are separated by 50A .On the basis of these two positions, it was predicted that the neck linker is able to pivot about a point that lies near the C terminus of a6. Subsequent binding of ATP analogues to the catalytic core locks the neck linker into a second forward pointing position at +50º. After phosphate release, however, the neck linker is again mobile and weakly interacts with two sites on the catalytic core (Sarah Rice et al. 1999**)** This dimer configuration is translated toward the minus end of the microtubule (by 3 Å) and rotated clockwise (by 10 degrees, Fig. 6B) relative to the position of the trailing core bound to the equivalent tubulin dimer. The mechanism underlying this changed orientation has been explained by the compensatory countermovement of the motor relative to its switch II cluster, which changes its position relative to the core between the nucleotide-free and ATP-bound states. In a sense, the extended leading neck linker in the constrained, bridged state of kinesin (Fig. 6B) acts as a structural sensor that coordinates work of the enzymatic active sites in two kinesin cores keeping them “out of phase.”

In its newly proposed role for conventional kinesin, ß-domain would function similar to the extended alpha-strands of the central core, stabilizing and pointing the neck linker either toward plus or minus end directions in the ATP/ADP-Pi and nucleotide-free states of the motor, respectively. The transition between two alternative docked conformations of the neck linker would not rely on Brownian motion only but would be forced by the countermovement of the core; placement of the detached trailing motor toward the new site on the track could be achieved more efficiently. Importantly, the countermovement of the leading motor upon binding ATP would also prevent the trailing motor from rebinding to the same site on the microtubule. The free-energy gain would come from favorable enthalpy changes balancing out unfavorable entropy changes associated with

straightening of the leading neck linker (Fig. 6, A and B) during forward stepping.

4.4. Kinesin steps back

A single kinesin molecule attached to a plastic bead was probed using optical tweezer. When it was pulled strongly enough, kinesin could be made to walk backwards in a sustained manner. This dramatic result could provide the basis for developing a motor to drive nanoscale molecular machines. (Carter, N. J. & Cross, R.A, .2005) it has been shown that the two kinesin heads move in 'hand-. over-hand' in a coordinated fashion and that movement stalls when the backward load exceeds about 7 piconewtons. But still two scenarios arose. First is about the presence of substeps or a single rapid step powered by the articulations of the molecule. Second suggests that kinesin can be made to step backwards.

Carter and Cross(Carter, N. J. & Cross, R.A,.2005) reported that sudden application of super stall force causes the motion to revert, whereby phasing of the ATP hydrolysis cycles on the lead and trailing heads seems to be swapped during backwards movement. If true, kinesin would use the same amount of ATP fuel when it walks forwards as it does staggering backwards under load. Carter and Cross suggested an underlying mechanism in which, once ATP has bound to the microtubule-attached head, the other head undergoes a diffusional search for its next site, the outcome of which can be biased by an applied load.

They did not observe sub-steps, so kinesin movements must be completed within the (excellent) time resolution of their measurements (less than 50 μs). The absence of substeps within the 8-nm kinesin step indicates that load transfers from one head to the other in a single mechanical event.

Backwards mechanical strain counteracts the intrinsic forward bias in the diffusional search, thus increasing the probability that the tethered head will bind behind the holdfast head. Probability of a forward step decreases exponentially with increasing load, whereas the probability of a backward step is constant. At stall (7.2 pN) (Nicholas et al 2006), the probability of forward stepping is equal to that of backward stepping. Above stall, Carter and Cross' model predicts that efficiency goes negative as backward steps

begin to predominate. There are previous reports of substeps, and several current models predict substeps. But their data also rule out substeps that take longer than 30 ms. This indicates that the appearance of substantial substeps in previous analyses might have been an artefact. However according to the recent experiments it is shown that the 8-nm step can be resolved into fast and slow substeps, each corresponding to a displacement of approximately 4 nm. The substeps are most probably generated by structural changes in one head of kinesin, leading to rectified forward thermal motions of the partner head. It is also possible that the kinesin steps along the 4-nm repeat of tubulin monomers. (Nishivama et al. 2001) Thus Processive stepping (walking) can usefully be thought of as the outcome of a race between lead head attachment and trail head detachment: if lead head attachment is faster than trail head detachment, then the motor is processive.

Finally the data indicate that stepping by kinesin-1 absolutely requires a functional (dockable) neck linker, and it was originally proposed that neck linker docking is the force-generating engine of kinesin. However, this view was revised in light of calculations showing that the free energy change on docking is too small to account for the ability of kinesin to step 8 nm against a 7 pN load. Strong possibility is that it biases the attachment position of the leading head.

The neck plays a very important role in the function. (Johann Jaud et al, 2006) Our findings indicate that a stiff Kinesin-1 neck coiled-coil impedes force generation. First, the average velocity is reduced under load and second, the run length of the stable neck mutant is severely affected by external forces. After the kinesin neck linker has docked to the rear head upon the binding of ATP, the leading head faces in the forward direction (Fig. 6 D, left). Driven by Brownian motion it undergoes a diffusive search for the next microtubule binding site. In a loaded state the head has to ‘‘borrow’’ a part of the necessary coverage to reach this binding site from a flexible element in the molecule, presumably the kinesin neck coiled-coil .They found no difference in velocities of the cross-linked and non-crosslinked motor under load, and only a subtle effect on the run length.

5. Probing

The most common techniques employed in probing kinesin for modeling purposes include optical trapping and video imaging. Since Optical trapping is often used to manipulate and study single molecules by interacting with a bead that has been attached to that molecule, it finds heavy application in the quantitative mathematical derivations of kinesin models while video imaging exploits the potential of a fluorophor in fluorescence microscopy.

The basic principle in optical trapping is the momentum transfer associated with the bending light.

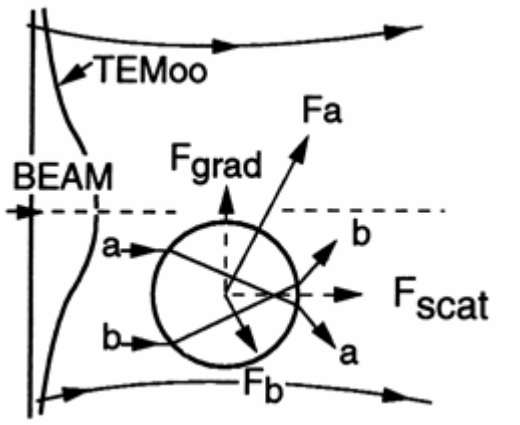


Fig 7: Optical trap (Ashkin A, 1997)

In a typical 2D *optical force clamp setup* the incoming light comes from a laser which has a Gaussian intensity profile. Because the intensity of ray "a" is higher than that of ray "b," the force F_a is greater than F_b. Adding all such symmetrical pairs of rays striking the sphere, one sees that the net force can be resolved into two components, F_{scat}, called the scattering force component pointing in the direction of the incident light, and F_{grad}, a gradient component arising from the gradient in light intensity and pointing transversely toward the high intensity region of the beam.

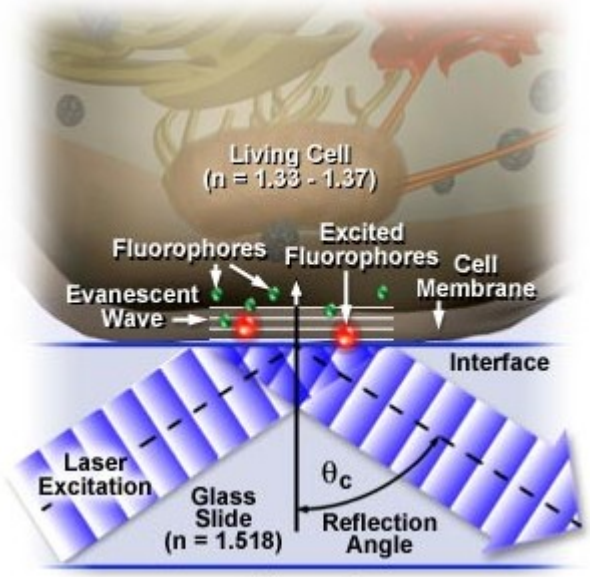
The restoring force of the optical trap works like an optical spring: the force is proportional to the displacement out of the trap. In practice, the bead is constantly moving with Brownian motion. But whenever it leaves the center of the optical trap the restoring force pulls it back to the center. If the kinesin attached to the bead were to pull the bead away from the center of the trap, a

restoring force would be imparted to the bead and thus to the kinesin. Advanced sensitive position detectors, based on interferometry or quadrant photodiodes (QPDs), are used to track bead motion and the interaction forces of the kinesin with the microtubule, with subnanometer accuracy and high bandwidth (Matthew J. Llang et al, 2002).

A drawback of optical trapping has been the damage induced by the intense trapping light, resulting in opticution-death by light (Keir C. Neuman et al, 1999). This was avoided by changing from the previous green (5145 Å) argon laser to an infrared yttrium/aluminum garnet laser at 1.06 μm (A. Ashkin et al, 1987).

The tweezers technique in combination with the so-called "microbeam" technique of pulsed laser cutting (sometimes called ‘laser scissors’ or ‘scalpel’) is employed for cutting and moving cells, organelles and manipulation of pieces of chromosomes for gene isolation (S. Seeger et al, 1991)

Imaging can be simply done using a fluorophore attached to kinesin to determine its mechanochemical properties. However, when these molecules are excited and detected with a conventional fluorescence microscope, the resulting fluorescence from those fluorophores bound to the surface is often overwhelmed by the background fluorescence due to the much larger population of non-bound molecules (Arne Seitz et al, 2006). Hence, Total Internal Reflection Fluoroscence Microcscopy (TIRFM) is employed.



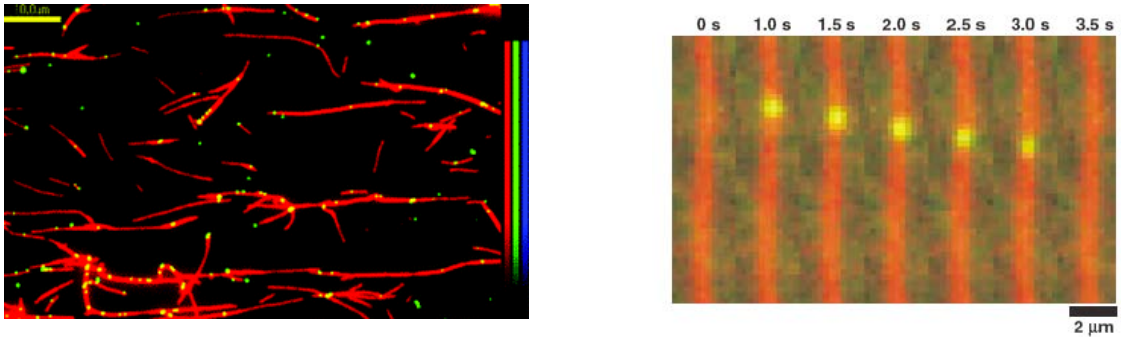
TIRFM, also called evanescent wave microscopy, selectively excites the fluorophores by impeding the beam at a high angle causing it to toally internally reflect (TIR). TIR generates a very thin electromagnetic field in the liquid

with the same frequency as the incident light, exponentially decaying in intensity with distance from the surface (Daniel Axeirod, 2001).

This field is capable of exciting fluorophores near the surface while avoiding excitation of a possibly much larger number of fluorophores farther out in the liquid possibly much larger number of fluorophores farther out in the liquid. More importantly, the fluorescence excitation energy of the evanescent wave is the same as the energy of the wavelength of the light that was totally internally reflected.

Some of the most commonly used fluorophores for biological systems include the Green fluorescent protein (GFP), luciferin and quantum dots (2-10 nm dia; 100-100,000 atoms).

Quantum dots are widely preferred because they reduce the effect of photobleaching, are photostable fluorophores and highly bright to facilitate effective tracking down of individual single molecules(Arne Seitz et al, 2006). Kinesin molecules labeled with GFP and streptavidin-coated quantum dots (585nm), and the microbtubules labeled with Texas red have been used to study its mechanochemical properties (Fig. 8). Qdots-Kinesin is linked through streptavidin-biotin linkage.



(a) (b)

Fig 8: (Arne Seitz et al, 2006) : (a) GFP-Kinesin on MTs shown in green colour (b) Quantum dots labeled kinesin on red coloured microtubules.

6. Kinetics of Kinesin

The kinetic mechanism of Kinesin evolved from results obtained from various experiments. These results includes: microtubules increase the rate of ATP turnover; the non-hydrolysable analogue AMPPNP (5'-adenylylimido-diphosphate) which is presumed to mimic ATP, induces a tightly bound (stable) microtubule–kinesin complex; and ADP release is the rate-limiting step of ATP turnover in the absence of microtubules (G.S. Bloom et al, 1995). Kinesin dimers moving at a low load take approx. 50-200 steps per second, requires one molecule of ATP in each step (M.J. Schnitzer et al, 1997) and the two heads step alternatively. Mg●ADP acts as a competitive inhibitor of Mg●ATP binding to Kinesin(S.A. Cohn et al, 1989) with an inhibition constant of 150 μM. In the absence of microtubules, hydrolysis of ATP by single heads is 7 s^{-1} (K.A. Foster et al, 1998). Microtubule binding accelerates this to 200–300 s^{-1}.One of the possible reasons is the allosteric stabilization by microtubules of the catalytically active Kinesin conformation (M. Kikkawa et al, 2001). Another important observation is that the rate of phosphate release from kinesin monomers is not greatly affected by microtubule binding (Robert A. Cross, 2004). This leads to the conclusion that K●ADP●Pi is strongly bound and therefore that phosphate release occurs from a microtubule-attached closed state.

The first major breakthrough in this respect came when it was discovered that ADP is released sequentially from Kinesin dimers (D.D. Hackney et.al, 1994). When K●ADP dimers are mixed with microtubules, only one of the two trapped ATP's is released. Release of the second ADP depends on the binding of an ATP to the first head. That is ATP dependent conformational change in the trail head works as a trigger for microtubule-activated release of ADP from the lead head(S.P. Gilbert et al, 1998). The key aspects of kinesin's forward (plus-end) cycle have been elucidated through a varied multitude of experiments, including cryo-EM, x-ray structural, force bead, and others .This can be explained by the following diagram:

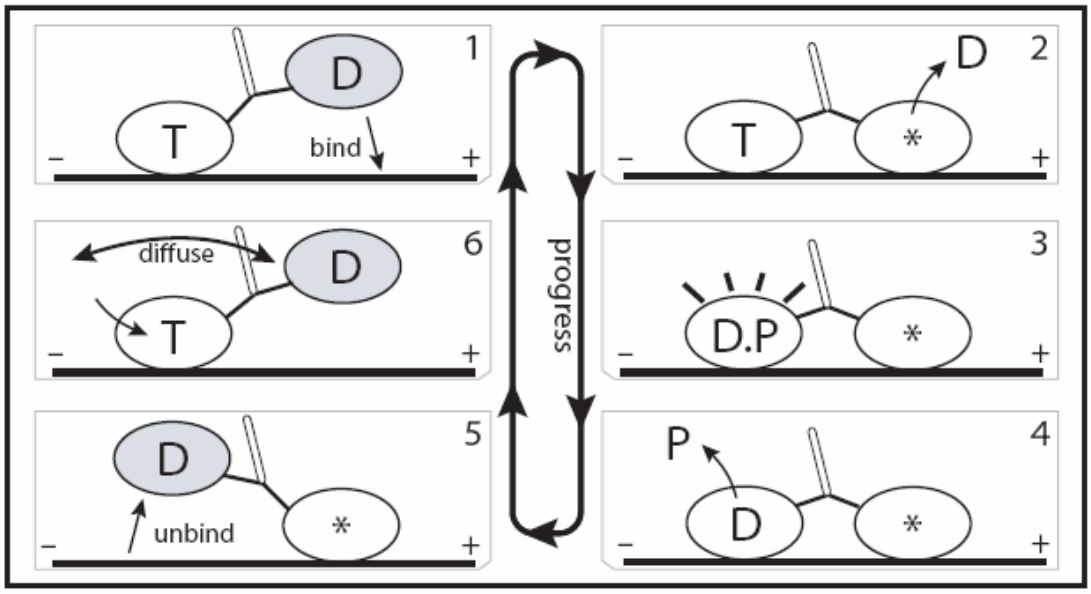


Fig 9: The diagram above depicts the six stages in Kinesin.

In the diagram “T” labels the ATP nucleotide state, “D” the ADP nucleotide state, “∗” the no-nucleotide state, and “P” the phosphate after ATP hydrolysis.

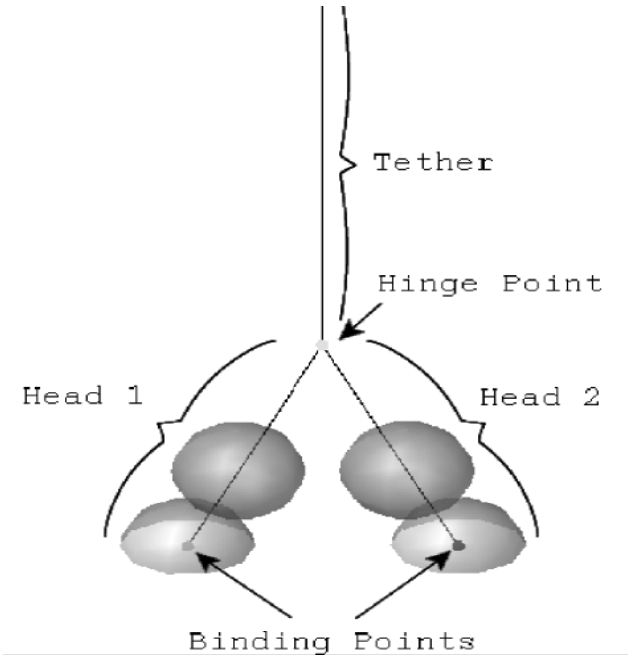
Frames 1, 2: The free head weakly binds to the plus-end binding site, leading to strong binding once ADP is released.

Frames 3–5**:** hydrolysis of ATP in the minus-end head leads to an intermediate ADP-phosphate state, “D.P,” and phosphate release alters the binding of the minus-end head into weak binding, which allows rapid release of the minus-end head from tubulin.

Frame 6**:** the free head tends not to strongly bind until ATP binds to the microtubule-bound head. ATP binding initiates zippering of the microtubule-bound head’s neck linker, coinciding with a large acceleration of the rate for the free head to bind onto microtubule. This entire forward cycle consumes one ATP and moves the center of mass of the system by approximately 8 nm.

7. Brownian Dynamics model of Kinesin

The basic aim of any model made on Kinesin is to understand mathematically how the protein converts chemical energy to mechanical work. Among the various models, which have been proposed for Kinesin movement, one has been discussed here. It is a three dimensional Brownian Dynamics model that takes into account excluded volume interactions. The two homologous globular domains, referred to as “heads” are joined together by a long coiled-coil alpha-helix structure, which extends to attach like a tether to cargo transported by the motor (Goldstein, 2001; Kamal and Goldstein, 2002). The motor moves along the track laid by the microtubule by binding and unbinding its heads from interaction sites on the microtubule surface (Gilbert and Johnson, 1995). The binding sites are spaced at approximately 8-nm increments (Svoboda et al., 1993).The main aim of this model is to calculate the force that the tether provides and the potential energy of the entire system.



A diagram for Kinesin has been shown alongside(Fig 10). The heads have special binding site for ATP hydrolysis and separate sites for interaction with the microtubules. The microtubule binding sites are modeled by hemispherical regions of radius 2 nm as shown in the diagram. The internal geometry of the two heads is neglected in the model and is represented by two spherical excluded volumes. The neck linker is a sequence of 15 amino acid residues joining the heads to the hinge point. The coiled-coil tether transmits the forces acting on the cargo to the motor and hence when the

cargo is transmitted subject to a load force the tether elasticity plays an important role. The tether is actually modeled as a nonlinear spring with a force-extension relationship derived from experimental data (optical trap data).

It was shown that depending on the model of the motor protein different tether stiffness were optimal in the velocity attained by the motor. A nomenclature used in this model is that when both the heads of the Kinesin are bound to the microtubule, the one closer to plus end will be referred to as the “leading head” and the one away from it will be referred to as the “trailing head”.

The detailed geometry of the stage-mounted microtubule is also neglected since it is too small to make significant contribution to bead-diffusion dynamics. The following figure drawn to scale will illustrate the point further.

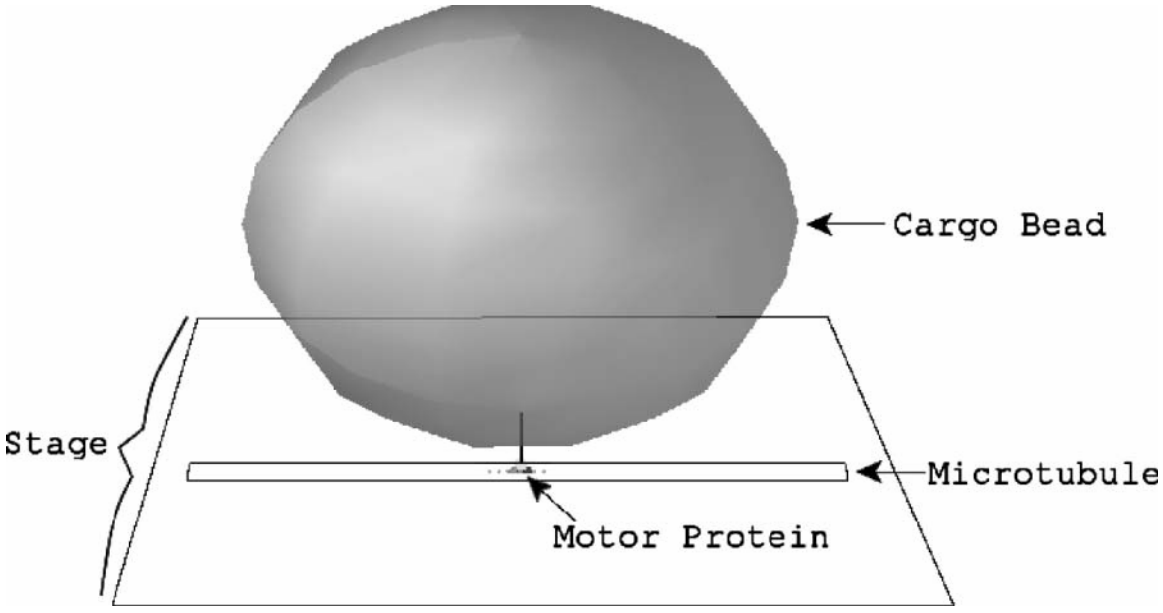


Fig 11: Illustration of the model plotted according to scale.

Optical traps are capable of manipulating nanometer and micrometer sized dielectric particles by exerting extremely small forces by a highly focused

laser beam. The narrowest point on the beam is known as “beam waist” (as shown in the diagram below) which contains a very strong electric field gradient and hence particles are attracted to the centre of the beam (region of strongest electric field). The particle to be observed experiences a force even when it moves slightly away from the centre of the trap. This force is linear with respect to its displacement from the center of the trap as long as the displacement is small. So for small displacements an optical trap turns out to be a simple linear spring following Hooke’s law.

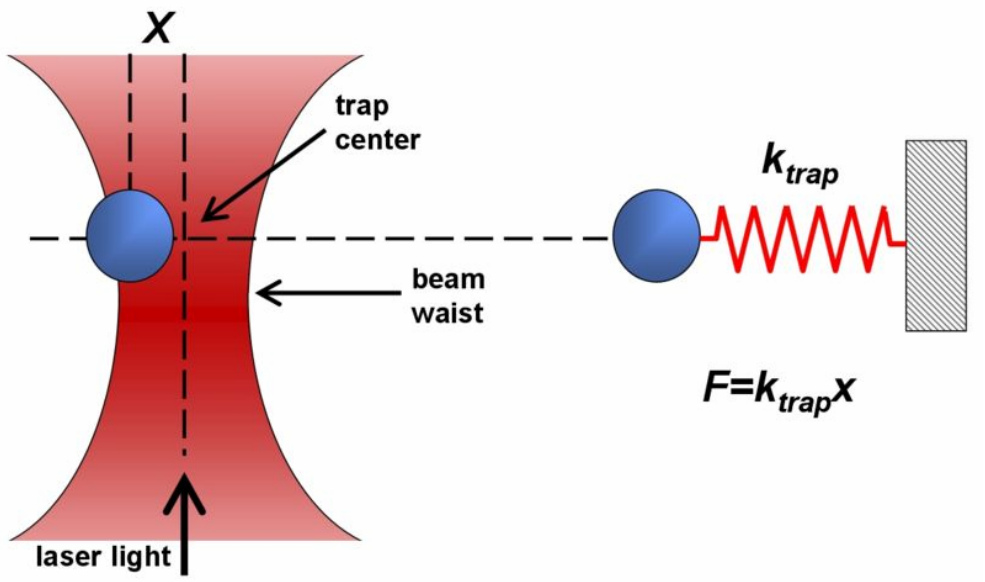


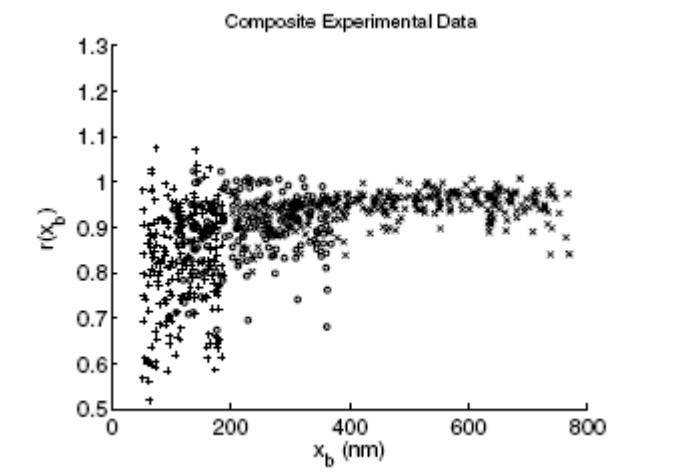
Fig 12: Diagram of optical trap showing its equivalence to a linear spring

7.1. Model based on Svoboda and Block experiments

Svoboda and Block (1994) carried out optical trap experiments where they used a bead as the cargo. The bead was attached to an inactive Kinesin protein which was bound to a microtubule. The microtubule was mounted to a microscopic stage which was moved at a fixed speed $V_{m.}$ As the stage moves the tether pulls the bead from the centre of the trap which then exerts an opposing optical restoring force. They experimentally plotted a graph between r and x(b) where

r= (Velocity of the bead/Velocity of the stage) =V_b/ V_m

x(b) denotes the one dimensional position of the bead.



The experiment was repeated for three different values of the laser beam and the combined graph is as shown alongside. In the plot (+) points were obtained with 15mW laser power, (◦) with 30mW, and (×) 62mW laser power.

Fig 13: Experimental Plot from Svoboda and block optical trap experiments.

The same experiment has been used for developing the model. One important point in the model is in nanometer scale region the effect of thermal fluctuations of the environment cannot be neglected. These will lead to unwanted movement of beads in all possible directions. In the model it has been assumed that the stage velocity is slow relative to the time scale for the bead diffusion to reach its equilibrium distribution. Hence thermal fluctuations can be neglected.

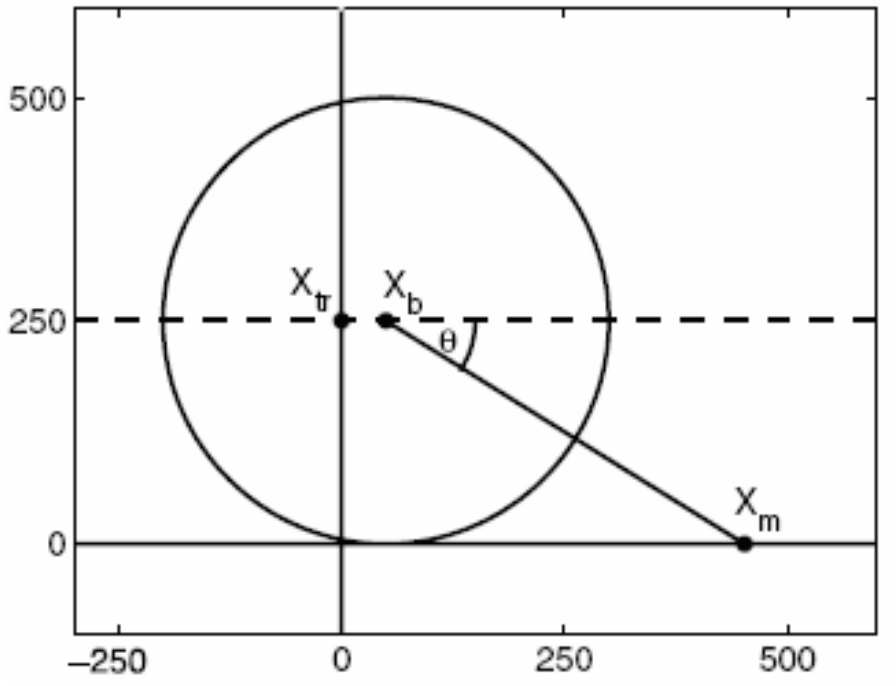


Fig 14: Diagram of the optical trap experimental setup for the bead-motor tether.

As shown in the diagram above a 250-nm latex bead was attached to the motor protein and the center of the optical trap was located 250 nm above the microscope stage Because of this arrangement the bead to the stage at all times during the experiment. The geometry of the experiment and the fact that thermal fluctuations are neglected leads to the consideration of the model as a one dimensional system. Also Force balance is possible along the microtubule axis.

Conventions used:

X_b→bead position in one dimension

X_{tr}→trap position in one direction

X_m→motor position along the microtubule.

The force in the direction of the microtubule because of the optical trap is

$$K_{tr}(X_b - X_{tr})$$

The assumption of balance of forces for each configuration X_b of the bead and motor X_m requires that the tether force F_{tether} balance the force of the optical trap in the direction of the microtubule axis. This requires that the tether force satisfy

$$F_{\text{tether}} \cos(\theta) = K_{\text{tr}}(x_{\text{b}} - x_{\text{tr}})$$

where θ is the angle the tether makes with the direction of microtubule axis.

K_{tr} has been estimated for each laser power by calibration measurements.So to find out the tether force only value of cos(θ) is required. From the trigonometry of the figure

$$\cos(\theta) = \frac{x_{\text{m}} - x_{\text{b}}}{L + R_{\text{bead}}}.$$

where L is the length of the tether. The extra quantity R_{bead} is taken into account because of the fact that the tether attaches to the surface of the bead and not its center of mass. Substituting the above equation in the force balance, the final expression of the tether force comes out to be

$$F_{\text{tether}} = \frac{L + R_{\text{bead}}}{x_{\text{m}} - x_{\text{b}}} K_{\text{tr}}(x_{\text{b}} - x_{\text{tr}})$$

where by the application of Pythagoras theorem

$$L = \sqrt{(x_{\text{b}} - x_{\text{m}})^2 + R_{\text{bead}}^2} - R_{\text{bead}}.$$

Reconstruction of force-extension profile is carried out for various powers of laser beam used and the graph obtained is shown below.

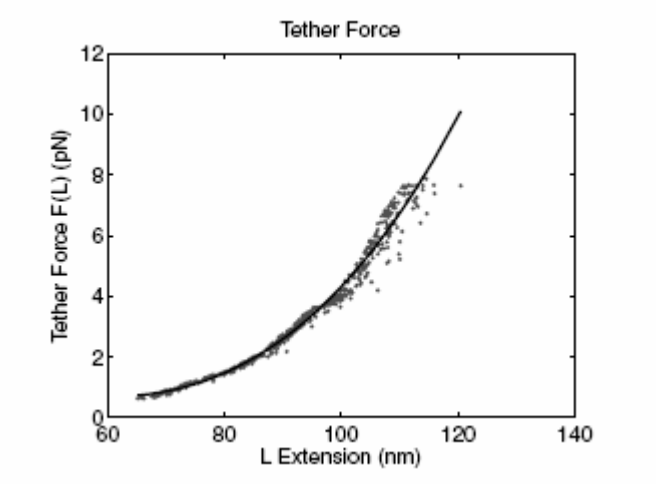


Fig 15: Tether Force-Extension Profile

The solid curve is a cubic polynomial with coefficients fit by the method of least squares to the reconstructed force-extension profile of the tether.

7.2. Potential Energy of the System

The potential energy for the entire system consists of four individual potential energies. These are the energies due to the tether, hinge, motor and trap. Hence the entire potential energy of the system will be

$$V(\vec{X}) = V_{\text{tether}} + V_{\text{hinge}} + V_{\text{motor}} + V_{\text{trap}}.$$

$\vec{X}$ refers to the degrees of freedom. There are four degrees of freedom describing the system: one due to the bead, one due to hinge one due to each head. Hence

$$\vec{X} = [\vec{X}_{\text{bead}}, \vec{X}_{\text{hinge}}, \vec{X}_{\text{h1}}, \vec{X}_{\text{h2}}]$$

In all cases one degree of freedom refers to the three dimensional position of that part. Hence In the model, the energy is taken to be a function of only

these four degrees of freedom and consists of contributions from the stretching of the tether, preferred hinge orientation, elasticity of the heads, and the optical trap. The procedure by which each of these energies can be modeled is shown below.

Tether

As explained earlier the force was found out to be a cubic polynomial. The tether potential energy is modeled according to this. Hence

$$V_{\text{tether}} = E_{\text{tether}}(|\vec{X}_{\text{b}} - \vec{X}_{\text{hinge}}|)$$
$$E_{\text{tether}}(s) = a_0 s + \frac{a_1}{2}s^2 + \frac{a_2}{3}s^3 + \frac{a_4}{4}s^4.$$

Trap

The force exerted by the optical trap is modeled by a constant load force reflecting the small spatial range over which we are interested in the experimental setup. This potential is given by

$$V_{\text{trap}} = -\vec{F} \cdot \vec{X}_{\text{bead}}.$$

Hinge

It has been assumed in the model that the free head is biased towards the plus end of the microtubule and hence there must be a force which has the tendency to keep the bound head at a fixed angle with the microtubule. Hence arises the potential energy of the hinge.

This is modeled by linear springs in all three dimensions. This force referred to as the “biasing force” is modeled by springs with different force constants. Hence the potential energy becomes

$$V_{\text{hinge}} = \frac{K_{\text{bias}}^{(1)}}{2}\left|\vec{X}_{\text{hinge}}^{(1)} - \left(\vec{X}_{\text{bound}}^{(1)} + \vec{x}_0^{(1)}\right)\right|^2 + \frac{K_{\text{bias}}^{(2)}}{2}\left|\vec{X}_{\text{hinge}}^{(2)}\right.$$
$$\left. - \left(\vec{X}_{\text{bound}}^{(2)} + \vec{x}_0^{(2)}\right)\right|^2 + \frac{K_{\text{bias}}^{(3)}}{2}\left|\vec{X}_{\text{hinge}}^{(3)} - \left(\vec{X}_{\text{bound}}^{(3)} + \vec{x}_0^{(3)}\right)\right|^2.$$

Motor

The elasticity of the globular domains of the motor protein that mediate the force between the microtubule interaction sites of the head and the hinge point is modeled by two linear springs that contribute the following energy to the potential of the system.

$$V_{\text{motor}} = \frac{K_m}{2}(|\vec{X}_{\text{hinge}} - \vec{X}_{\text{h1}}| - L)^2 + \frac{K_m}{2}(|\vec{X}_{\text{hinge}} - \vec{X}_{\text{h2}}| - L)^2.$$

So this is the final expression obtained for the total potential energy from this model.

8. Future Applications

Designing of nanomachines based on the mechanism of Kinesin still proves to be a challenge in this field of Kinesin research. These nanomachines will help us in scanning surfaces and studying microscopic objects in greater detail than is possible with microscopes. Another major advantage of these proposed nanorobots is their specificity in action. For example they might be used for precise drug delivery to specific cells in need of repair. Some researchers believe this might even cause a shift from treatment to prevention in medical sciences (Requicha, 2003).The backward movement of Kinesin is also an increasingly growing research interest in this field. On increasing the force on the bead in opposite direction Kinesin can be made to move backwards. This dramatic result could provide the basis for developing a motor to drive nanoscale molecular machines. The mechanism of interaction of more than two heads in Kinesin movement also remains to be elucidated (Cross et.al, 2004).

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