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## The diffusive interaction of microtubule binding proteins

**Jeremy R. Cooper** and **Linda Wordeman**

*Dept. of Physiology and Biophysics, University of Washington School of Medicine, Seattle, WA 98195 USA*

### Summary

Microtubule based motility is often thought of as specifically referring to the directed stepping of microtubule based motors such as kinesin or dynein. However microtubule lattice diffusion (also known as diffusional motility) provides a second mode of transport that is shared by a much broader class of microtubule binding proteins. Microtubule lattice diffusion offers distinct advantages as a transport mechanism including speed, bi-directional microtubule end targeting, and no requirement for direct chemical energy (ie. ATP). It remains to be seen whether a universal binding mechanism for this interaction will be identified but electrostatic interactions appear to play a significant role. In the meantime, the well-studied subject of DNA binding proteins that diffuse along the DNA backbone provides an insightful analogue for understanding the nature of microtubule based diffusional motility.

### Introduction

The microtubule cytoskeleton provides the scaffold for a multitude of protein-protein interactions. Proteins that are able to bind directly to microtubules form a diverse ensemble referred to as microtubule binding proteins. Microtubule binding proteins, when examined at the level of single molecules, tend not to remain statically bound to the microtubule, but instead travel along the surface of the microtubule. Perhaps the most familiar example of such translocation is the directed motility exhibited by kinesin molecules, which step in a constant direction along the microtubule lattice. However a second mechanism, referred to as diffusional motility (Figure 1), has generated a significant amount of interest as an increasing number of microtubule binding proteins have been found to exhibit this behavior. Diffusional motility may even contribute to the stepping mechanism in kinesins by means of a diffusional search process of the free motor domain head [1], (also see companion review in this issue from Gennerich and Vale).

Diffusional motility is defined as a one-dimensional random walk along the microtubule lattice driven solely by thermal energy. The equations characterizing these motions are identical to that of classical Brownian motion, although the diffusion coefficients tend to be significantly lower [2-5]. Interestingly, such a reduction in diffusional speed has been predicted by models of DNA binding proteins [6]. This model assumes that 1-D diffusion is limited not only by the binding protein's 3-dimensional translational diffusion coefficient, but also by its rotational diffusion coefficient. Similar to a key sliding into a lock, the protein must adopt the correct

Correspondence to Linda Wordeman: E-mail: worde@u.washington.edu.

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rotational orientation before advancing along the polymeric filament. Any rotation away from the correct orientation will stall its progress.

## Survey of microtubule binding proteins that exhibit 1-D lattice diffusion

Some of the earliest single molecule observations of diffusional motion on the microtubule lattice were made while studying the directed motility of kinesin molecules [7,8]. Kinesin motor domains can bind strongly to the microtubule, which allows for the directed “hand-over-hand” motility characteristic of kinesin superfamily members. However, this process requires that the kinesin molecule’s trailing head disengage (calling for a weakly bound state) while the leading head remains strongly bound. Then the weakly bound head swivels around to become the new leading head. This weakly bound state characteristically occurs with ADP in the kinesin motor domain’s nucleotide binding pocket, while strongly bound states occur with either ATP or no nucleotide at all (Figure 1b). Typically, kinesin motility experiments are carried out with ATP in solution, which makes it likely for at least one of the kinesin molecule’s two heads to be strongly bound at any given time. However, kinesin single molecule motility experiments sometimes exhibit a non-directional diffusive component superimposed upon the otherwise directed path. This so-called “biased diffusion” has been observed in a variety of kinesins including conventional kinesin [8], KIF1A [7], CENP-E [9], Eg5 [10], and Ncd [11]. The diffusional component has been hypothesized to occur during brief interludes in which both motor domain heads are disengaged [9], resulting in relatively free movement of the molecule along the microtubule axis. Here the word “disengaged” is not meant to imply that the kinesin molecule is unbound (in which case it would be free to diffuse in 3 dimensions), but rather that the classical “lock-and-key” strong binding motif is not engaged. This hypothesis is supported by experiments in which ADP is substituted for ATP [10,12]. The kinesin molecules imaged in these experiments exhibit pure unbiased diffusion, presumably because the weakly bound motor domains spend a significant amount of time disengaged from the microtubule lattice.

Cytoplasmic dynein, like motile kinesins, also exhibits a combination of directed motility and 1-dimensional diffusion [13,14]. There are also a number of microtubule binding proteins that exhibit pure diffusional motility (ie. lacking the directed motility component) along the microtubule lattice including the kinesin-13 protein (MCAK) [3], the Dam1 complex [5], XMAP215 [15], the Ndc80 complex [16], and tau [17]. Finally, even one of the myosin family members, Myosin Va, has been shown to exhibit rapid unbiased diffusion along the microtubule lattice [4].

## Advantages of microtubule lattice diffusion as a transport mechanism

Clearly, the phenomenon of microtubule lattice diffusion is a ubiquitous mechanism occurring across a diverse set of microtubule binding proteins. Importantly, diffusional motility holds several key advantages over classical directed motility. First, as pure diffusion is by definition unbiased, it allows the binding protein to reach either end of the microtubule [18]. Significantly many of the microtubule binding proteins that exhibit pure diffusion have a function that requires localization to microtubule ends and diffusional motility provides a mechanism for delivery to the ends. The potential to target either the –end or the +end (or both simultaneously) greatly enhances the utility of diffusion as a targeting mechanism. Second, it is likely that diffusion allows the microtubule binding protein to more effectively skirt around obstacles on the microtubule surface. This is based on the assumption that the weakly bound state corresponding to diffusive motion [7] permits more frequent transitions between protofilaments while the strongly bound state of directed motility does not [19-21]. Such a feature may be relevant not only to those proteins that exhibit pure diffusion but also to the directed motility of kinesins and dynein [21]. As a specific example, the microtubules in neuronal axons are heavily decorated with the protein tau [22]. In order to navigate through

this “forest-like” layer of tau, it seems likely that a motor protein such as kinesin, which normally travels axially along the microtubule [20], would periodically need to make diffusive side-steps. In addition, diffusion of the tau itself might make the process more akin to moving through a forest of seaweed than a forest of pine trees. Third, over short distances ( $< 1 \mu\text{m}$ ), the speed of diffusive motion allows binding proteins to reach microtubule ends more rapidly than the directed motility mechanism of delivery. This is potentially advantageous because such rapid delivery permits the microtubule lattice to remain minimally cluttered. However, since diffusion is unbiased, directed motility is the clear winner when longer distances must be traversed, e.g. in larger cells and axons. Fourth, diffusional motility demands no external chemical energy (ie ATP) to propel the binding protein, making it an economical mode of transport.

## DNA binding proteins - an analogue for diffusion of microtubule binding proteins

The concept of microtubule binding proteins diffusing along the microtubule lattice has only recently evolved into a significant area of research. However, the similar phenomenon of DNA binding proteins diffusing along DNA strands has been intensely studied for decades [23-26]. Although the structures of DNA and microtubule biopolymers are radically different, the overall 1-D morphology and strong negative surface charge that they have in common makes this a potentially enlightening comparison. Interestingly, 1-D diffusion coefficients measured for both DNA binding proteins [27] and microtubule binding proteins [3-5] tend to be roughly in line with one another, ranging between  $0.1 \mu\text{m}^2/\text{sec}$  and  $0.4 \mu\text{m}^2/\text{sec}$ . Surprisingly, a recent study has shown that hOgg1 (a DNA binding protein involved in base excision repair) was found to have a 1-D diffusion coefficient that approaches the theoretical maximum [27]. The theoretical maximum diffusion coefficient is calculated assuming that the diffusing molecule encounters no barriers as it slides along the polymer surface [6]. In the case of hOgg1, the energy barrier (referred to as the activation energy) was calculated to be an almost nonexistent  $0.9 k_B T$ . Typically the mechanism of 1-D diffusive motion is modeled as if the binding protein makes discrete, albeit directionally un-biased, steps between binding positions (nucleotides in the case of DNA or tubulin subunits in the case of microtubules). In this model, the activation energy refers to the energy required for the binding protein to release from one binding position such that it can move to an adjacent position. The fact that hOgg1 exhibits such a minimal activation energy suggests that the entire concept of “steps” in diffusive motion may provide an inaccurate representation of the mechanism. Perhaps a more relevant concept is that of the binding protein associating with an elongated space around the DNA polymer, coined the “DNA domain” [28]. In this model, as the binding protein diffuses throughout the DNA domain, local variations in electrostatic potential would likely generate roughness in the energy profile, thus accounting for the observed non-zero activation energy. However, the barriers between local minima corresponding to this activation energy would be too small to legitimately refer to the minima as distinct chemical species. Instead, according to this model, the entire diffusive interaction should be thought of as a single chemical species [28].

A theoretical estimation for the maximum diffusion coefficient of barrierless diffusion on microtubules (equivalent to the theoretical treatment for DNA binding proteins [6]) is currently lacking. Thus, activation energies for the diffusing microtubule binding proteins have not yet been calculated. Consequently, it is unknown if the diffusion exhibited by these proteins is better described by an unbiased stepping behavior (from one tubulin subunit to the next) or instead by a concept in which it moves relatively freely throughout an extended “microtubule domain”, as has been described for DNA binding proteins (above) [28]. Indeed, since the 1-D diffusion coefficients measured for several microtubule binding proteins [3,4,15] approach that measured for hOgg1 [27], it is a strong possibility that these molecules may also diffuse with very little activation energy throughout a roughly isoenergetic microtubule domain. Future

theoretical work is needed to estimate the barrier-free diffusion coefficients for microtubule binding proteins.

### The diffusive binding interaction: flexible and non-specific

A question remains as to the nature of the interaction that would allow such freedom of motion without complete detachment from the microtubule lattice. Commonly, the binding interaction is thought to be purely electrostatic in nature due to the observation that the microtubule surface carries a strong negative surface charge and binding proteins tend to hold a net positive charge. This is additionally supported by studies that have shown that proteolytic removal of the microtubule's negative surface charges [3,4] or mutational neutralization of a binding protein's positive charges [29] can greatly reduce the interaction probability and duration. In addition, homology modeling suggests that there is little energy difference between the negatively charged C-terminal tails that project outward from the microtubule versus those that lie along the lattice surface, suggesting that the flexible C-termini are considerably unconstrained [30]. This feature is likely to be essential for microtubule function as deletion of either the c-terminus of alpha- or beta tubulin is lethal. Yet the c-termini are interchangeable in that cells are viable with either tail on both tubulins [31]. Such flexibility and interchangeability supports the idea that the tails promote the formation of an extended "microtubule domain" along the entire polymer. In addition, microtubule binding proteins often include highly disordered regions which might enhance the flexibility and non-specificity of the interaction. For example, crystallographic studies suggest that the distal two-thirds of MCAK's positively charged neck region is quite flexible and disordered [32]. Importantly the neck is thought to be a critical component of MCAK's ability to interact with microtubules [3,32,33].

Structural studies of the binding interaction are unfortunately limited by the fact that microtubules have not been able to be crystallized to date. Fortunately, small DNA oligomers can be crystallized [34-36] and we can once again use the DNA binding proteins as a model of what might be expected for the microtubule binding system. Curiously, a feature that is often found in the vicinity of DNA strands is structured hydration shells that surround the molecule, particularly in the minor groove and encircling the strongly negatively charged phosphate backbone [37-39]. Unlike stereotypical "lock-and-key" type protein-protein interactions, the non-specific, electrostatically-driven interactions of DNA binding proteins are greatly facilitated by semi-structured water molecules that often line the interfaces between the DNA polymer and binding-protein [37,38,40]. Conveniently, this provides an explanation for the extremely small activation energies observed for one-dimensional sliding. The interfacial water molecules can be thought of as a lubricant that mediates the electrostatic interaction while still allowing the binding molecule to slide almost freely along the axis of the polymer (Figure 2). Furthermore, the displacement of structured water (that had previously resided in the hydration shells) upon protein binding has been hypothesized to add an entropic component to the binding free energy [37,38].

The lack of atomic level structural data for microtubules prevents us from being able to directly observe the potential role of water molecules in the weak interactions corresponding to 1-dimensional diffusion of microtubule binding proteins. However, the common electrostatic nature of the interactions and the fact that very similar diffusion dynamics are observed between the microtubule binding and DNA binding proteins, presents a compelling case that a very similar phenomenon might be occurring. Perhaps future studies exploring the thermodynamics (entropic vs. enthalpic) of the microtubule binding interactions may provide more evidence of such a mechanism.

## Conclusions

Microtubule lattice diffusion is a widespread and effective mode of protein transport along microtubules. The fact that such a diversity of microtubule binding proteins exhibit this behavior has hindered attempts to develop a unifying description of the mechanism. However, microtubule-based diffusional motility shares important similarities with the well-studied subject of DNA-based diffusion (including comparable diffusion coefficients and a common electrostatic component of the interaction). This suggests that the latter may provide valuable insight into understanding the nature of this interaction. Based upon this analogy, we would expect extremely low activation energies for the diffusive motion of microtubule binding proteins (thus casting doubt on the idea that diffusive motion consists of discrete “steps”). Furthermore, we anticipate that the proximal water structure plays a significant role, both as a mediator of electrostatic interactions and as an entropic contributor to binding.

Finally, the diversity of molecules that diffuse on the surface of microtubules strongly implies that no obvious molecular structure (such as “two-headedness”) is required for robust diffusive motility. While motors and other microtubule binding proteins may have undergone small evolutionary refinements to capitalize on these properties, considerable responsibility also lies with the microtubule. Post-translational modifications of tubulin have the potential to define both subpopulations of microtubules and also heterogenous regions on the microtubule domain. Here, lessons can be inferred from the DNA methyltransferases whose diffusive motility and methyltransferase activity is enhanced upon encountering hemi-methylated DNA [41]. In the near future, we expect that increasingly sensitive methods to measure the activity and diffusive behavior of microtubule binding proteins will make it likely that the numerous post-translational modifications of the microtubule substrate will have a measurable and functionally relevant effect on these molecules both in vitro and in cells.

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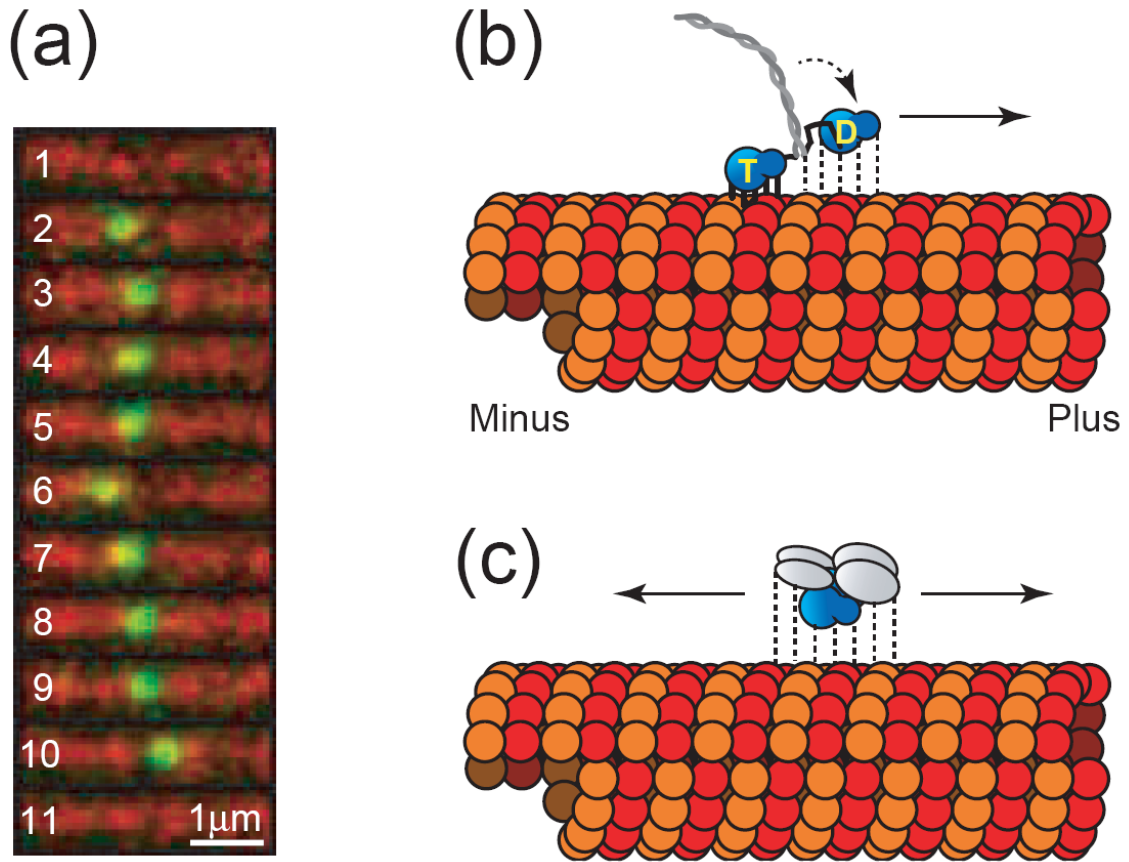
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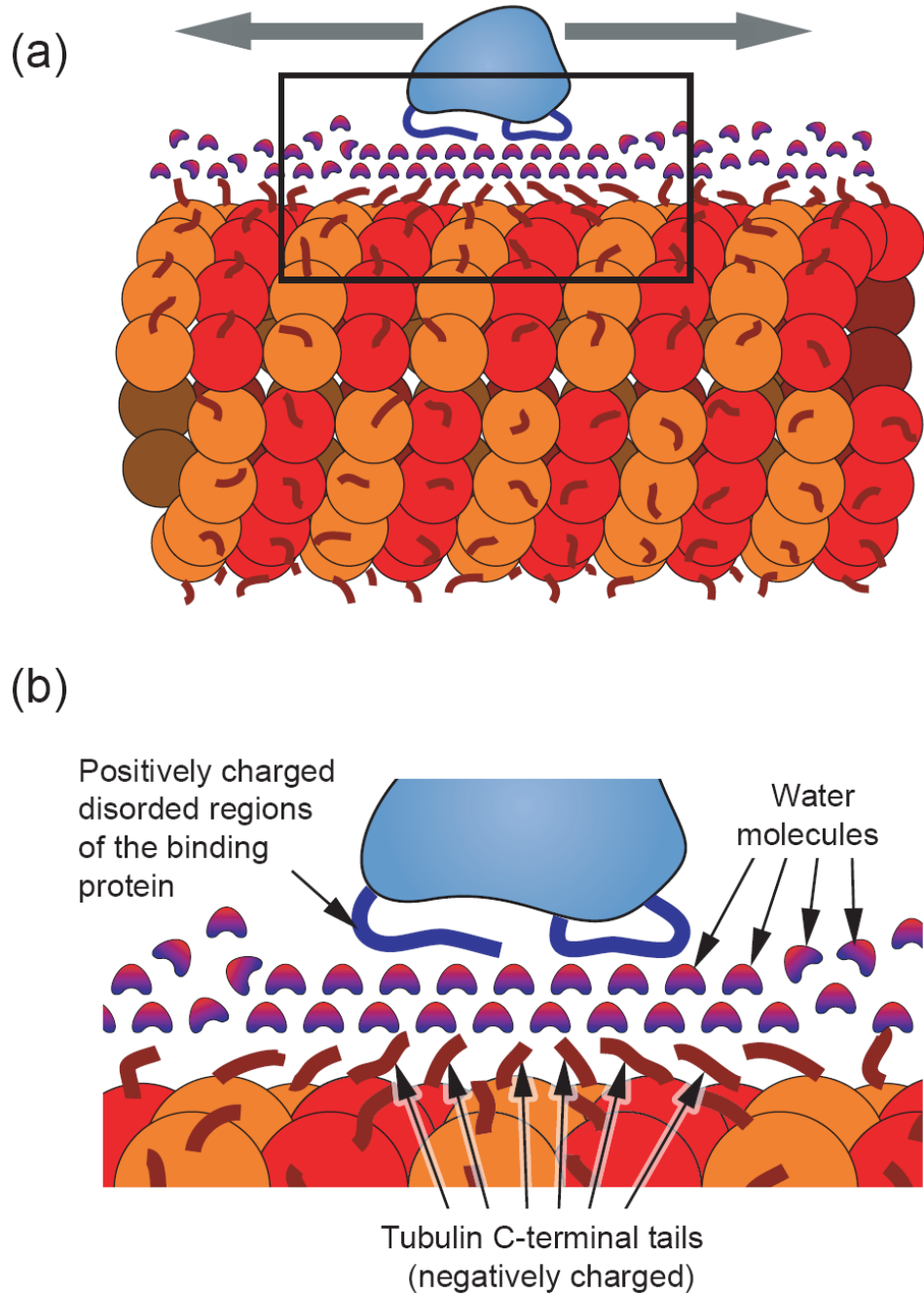
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**Figure 1. Diffusive and directed modes of microtubule based motility**

**(a)** Still frame series exhibiting unbiased diffusional motility of a single MCAK molecule. The molecule binds to the microtubule lattice (frame 2), diffuses 1-dimensionally along the microtubule lattice (frames 2-10), and then detaches (frame 10). Frames 1 and 11 show the microtubule immediately before and immediately after the binding event, respectively. Frame rate is 5 frames per second. GFP-labeled MCAK is shown in green and Cy5-labeled microtubules are shown in red (unpublished data). **(b)** Model of hand-over-hand directed motility of a kinesin motor. The two motor domain heads alternate between strongly bound (solid lines) and weakly bound states (dashed lines) while hydrolyzing ATP to advance toward the microtubule plus end. The strongly bound head possesses ATP (labeled “T”) in its nucleotide-binding pocket, while the weakly bound head has hydrolyzed its nucleotide to ADP (labeled “D”). During interludes where both motor domain heads possess ADP (not shown), the entire molecule would be weakly bound which can explain the diffusional component sometimes observed superimposed on top of directed motility [9]. **(c)** Model of unbiased diffusional motility of MCAK. The motor domain is shown in blue while the n-terminal and c-terminal dimerization domains are shown in gray. With either ADP or ATP in its nucleotide-binding pocket, MCAK maintains relatively weak binding to the microtubule lattice [3]. This allows for the molecule to slide randomly in either direction along the microtubule lattice while maintaining its attachment.





**Figure 2. Mechanistic model of microtubule based 1-dimensional diffusion**

**(a)** Model of a microtubule binding protein (light blue) diffusing axially along the length of a microtubule (red). The microtubule surface is covered with flexible negatively-charged c-terminal tubulin ends (dark red) [30]. Complementing this, the microtubule binding protein is shown with flexible positively-charged regions (blue) that interact with the microtubule. **(b)** An expanded view of the boxed area from part (a) depicting a hypothetical interaction scheme in which a layer (or possibly multiple layers) of structured water molecules form an intermediary for the electrostatic interaction. This concept is drawn from the analogous situation of DNA binding proteins in which structural data indicates the existence of water “lubricating” the interfacial surface [40] [37] [38]. This scenario could provide low energy

barriers for relatively free sliding along the length of the polymer. An alternative model would be one in which the charged disordered regions of both the microtubule and binding protein interact directly with very little interfacial water. In either scenario, the non-specificity of the interaction is a key feature for providing low energy barriers and the potential for fast sliding.