On complex, curved trajectories in microtubule gliding

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HIGHLIGHTS

- We model the dynamics of microtubules in gliding assays.
- We explain the circular and wavy trajectories observed experimentally.
- We show that they are due to the helical nature of microtubules.
- Circular and wavy trajectories alternate as a function of filament length.

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ABSTRACT

We study the dynamics of microtubules in gliding assays. These biofilaments are typically considered as purely semiflexible, hence their trajectories under the action of motors covering the substrate have been regarded so far as straight, modulo fluctuations. However, this is not always the case experimentally, where microtubules are known to move on large scale circles or spirals, or even display quite regular wavy trajectories and more complex dynamics. Incorporating recent experimental evidence for a (small) preferred curvature as well as the microtubules' well established lattice twist into a dynamic model for microtubule gliding, we could reproduce both types of trajectories. Interestingly, as a function of the microtubules' length we found length intervals of stable rings alternating with regions where wavy and more complex dynamics prevails. Finally, both types of dynamics (rings and waves) can be rationalized by considering simple limits of the full model.

1. Introduction

Microtubules (MTs) are one of the three major biopolymers of the cytoskeleton of eukaryotic cells (the others being the actin filaments, and the rather diverse group of intermediate filaments). They are involved in cellular mechanics and stability, but also serve very dynamic purposes like cellular transport and cell division, to mention but a few examples [1]. A large part of these 'active', i.e. non-equilibrium, processes is related to the action of molecular motors [2]. These small protein machines transform chemical energy delivered by the cell – typically in the form of adenosine-triphosphate (ATP) – into mechanical work, allowing them to move unidirectionally along the microtubules, and to exert forces and torques.

To visualize the combined action of microtubules and motors, a common and easy experimental setup is the so-called 'gliding assay' (also called 'motility assay'): microtubule-associated molecular motors – typically kinesins – are attached to a substrate, typically a glass slide. Adding microtubules into the ATP-rich solution, these are 'caught' by the motors and propelled by them along the surface. In fact, the most common microtubule motor, kinesin, was discovered this way [3]. Unfortunately, from the biophysics side there was only a brief period of interest in gliding motion, mostly concerned with the force exerted by and the fluctuations of motors [4–6]. More recently – first using the second major cytoskeletal filament, actin [7], and later on microtubules [8] – high-density gliding assays incited quite some interest as a model system for the collective motion of self-propelled particles. In spite of this, gliding assays rather eke out a living as a – though very important – tool to test the functioning of (e.g. freshly extracted) motors: if the filament motion is diffusive, the motors are not functional. If, in contrast, the filaments perform unidirectional motion – if they are

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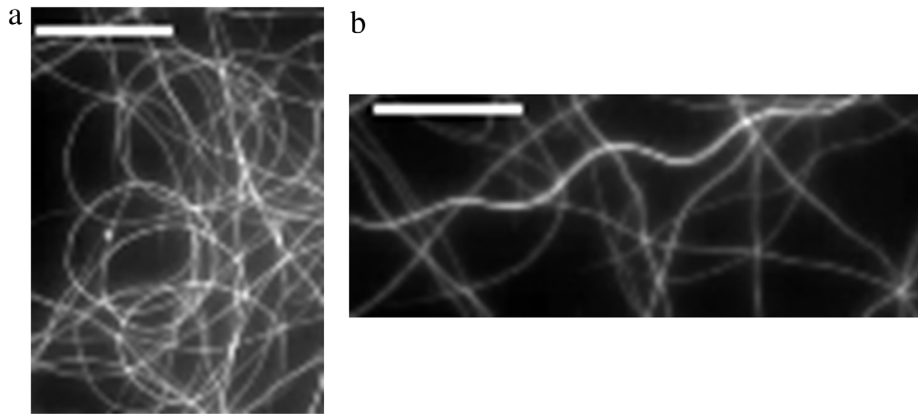E-mail address: falko.ziebert@physik.uni-freiburg.de (F. Ziebert).

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Fig. 1. Characteristic traces of various gliding microtubules over time spans of several minutes. They show distinctive patterns one would not expect for the propulsion of a semiflexible (WLC) filament: (a) circles and (b) waves. The scale bars are 10 μm. The underlying data are courtesy of Steven Koch's lab [13].

gliding along the substrate – this is a clear signature of active motor transport. In our opinion it has been mostly overlooked (with few exceptions, cf. [9,10]) that detailed observations of gliding dynamics can be used to study the mechanics and dynamics of *individual filaments*, and this in a well-controlled, non-equilibrium situation. Moreover, gliding assays are a very interesting dynamical system with underestimated complexity.

One expects gliding microtubules to be transported unidirectionally along straight trajectories—perturbed only by small thermal and motor-induced fluctuations. However, one consistently finds a fraction of the trajectories to be mysteriously curved, an observation in obvious contradiction with the MT being a simple, intrinsically straight, semiflexible filament: one can observe arc-shaped MTs running on circular trajectories [11,12] with radii of a few tens of microns, cf. Fig. 1(a). Other MTs move on quite regular wavy trajectories, cf. Fig. 1(b), with wavelengths of few tens of microns and somewhat smaller amplitude [13]. In addition, more complex trajectories like spirals can occur and circular gliding similar to the one described here has been recently also found in gliding assays at high filament density [14,8].

Obviously, complex trajectories of the kind shown in Fig. 1 can *not* be understood within the classical elastic beam or worm-like chain (WLC) model. On the other hand, they are too common – though often regarded as artifacts – and far too regular to be caused by motor-induced fluctuations. We here propose that the observed trajectories may well be a signature of the polymorphic structure of the MTs: in fact, MTs are known not to be simple semiflexible filaments, but rather to display both lattice twist and, as recently suggested [15–18], also intrinsic curvature.

2. Structure of microtubules and the elasticity of squeezed helices

Microtubules have a lattice twist. Microscopically, MTs are composed of a certain number of tubulin protofilaments (PFs), in vivo usually 13, that consist of head-to-tail polymerized tubulin dimers [1]. Together, the PFs build a hollow tube structure, the MT. MTs with 11–15 protofilaments are quite common, especially when polymerized in vitro. Due to the structure of the tubulin dimers and their arrangements within the MT's lattice, if their number differs from 13 the PFs are not straight but helically twisted along the axis of the MT's lattice. Consequently, in motility assays, as kinesin motors walk along the protofilaments [19], such MTs rotate around their axis while gliding. This fact was used to independently measure the lattice twist, in addition to structural analysis (cryo-TEM), and it is nowadays well accepted that the MT lattice twist repeat lengths (pitches) are about $P = +3$ μm (MT

with 12 PFs) and -6 μm (14 PFs), where $\pm$ signs refer to right-/left-handed twist [20,19]. Note that even 13 PF-microtubules are not perfectly straight, best fits to cryo-TEM data suggest a pitch of $+25$ μm, which is however often longer than the typical MT length.

Microtubules can have a preferred curvature. Apart from the structural property of lattice twist, it recently became increasingly evident that MTs have important internal degrees of freedom. Namely, experiments with isolated PFs not forming a MT lattice [11,21,15], and under taxol-stabilized conditions, suggest tubulins – and hence PFs and MTs – to be multistable, displaying several conformations: (i) a straight state; (ii) a weakly curved state with radius of curvature 250–300 nm; and (iii) a highly curved state with radius of curvature 20–30 nm (note that these curvatures correspond to a single PF). In fact, the curved states have slightly lower energy than the straight one and are hence preferred. However, when curved PFs are constrained in the MT's lattice, they cannot all bend as they would intrinsically prefer: the MT lattice is a frustrated, mechanically prestressed system. Developing models for the MT comparing all the relevant energies, we recently have shown [16–18] that states where few neighboring PFs are curved in their preferred direction, while the others have to accommodate and pay a bending energy penalty, can indeed have lower energy than the completely straight state. This allowed us already to explain the measured apparent length-dependent persistence length of MTs [22,16], as well as MT ring formation under gliding-induced buckling [18]. The typical radius of curvature for the whole MT lattice induced by PFs switching to their curved conformations is of order 10 μm [16] for the weakly curved state and $\simeq 1$ μm for the highly curved state [18]. The highly curved state typically has to be excited by larger forces, e.g. by motor-induced buckling as discussed in [18], than the weakly curved one.

For MTs gliding freely in a motility assay, only the weakly curved state will be accessible by the prevalent forces. We will neglect here also the possibility of a switching dynamics of the PFs (see [18] for a discussion of force/torque-induced switching) and, for simplicity, only consider a given static, i.e. quenched, state with fixed twist and intrinsic curvature. Obviously, twist and intrinsic curvature taken together give rise to an *overall helical structure* of the MT (i.e. not just lattice twist but a helix in space). As MTs in gliding assays are – for not too low motor coverage – effectively confined to two dimensions (2D), this leads us to the consideration of confined 'squeezed' helices and their self-propulsion dynamics.

Squeelices: three-dimensional helices confined to two dimensions. Let us first estimate the relevant energies in the problem. The bending energy of a worm-like chain is given by $\frac{B}{2} \int_0^L \kappa(s) ds$, where $B = 10^{-23}$ N m^2 is the bending stiffness of the MT, κ is the curvature and L the filament length. Assuming that a