Exploiting and exploring microtubules and kinesin motor proteins in nanofabricated devices

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Contents

1 Introduction ................................................................. 7
  1.1 Okay..., but what can you do with it? .......................... 8
    1.1.1 1676, April 24, Delft, The Netherlands .................. 8
    1.1.2 1947, Christmas Eve, Murray Hill NJ, USA ............ 9
    1.1.3 2007, a time for new discoveries ......................... 10
  1.2 Nanotechnology ...................................................... 11
    1.2.1 Applications of nanotechnology ......................... 11
    1.2.2 Nanoscale motion ............................................ 12
  1.3 Biological motors .................................................. 15
    1.3.1 Microtubules .................................................. 16
    1.3.2 Kinesin motor proteins .................................... 18
  1.4 Bionanotechnology .................................................. 21
    1.4.1 Exploiting motor proteins for nanotechnology .......... 21
    1.4.2 Exploring nanotechnology for biophysics ............... 22
  1.5 Outline of this thesis .............................................. 23
  References ................................................................... 25

2 Motor Proteins at Work for Nanotechnology ....................... 29
  2.1 Introduction ............................................................ 30
  2.2 Nature’s workhorses in the cell .................................. 30
  2.3 Muscle power for nanotechnology ............................... 32
  2.4 Kinesin- and myosin-driven transport on chips .............. 34
  2.5 Outlook: will bio-motors make their way? ..................... 37
  References ................................................................... 39

3 High Rectifying Efficiencies of Microtubule Motility on Kinesin- Coated Gold Nanostructures .................... 43
  3.1 Introduction ............................................................ 44
3.2 Fabrication process .............................................. 45
3.3 Device characterization ...................................... 46
3.4 Motility Characterization ............................... 46
3.5 Rectification of motility ................................ 50
3.6 Conclusions ......................................................... 54
References ............................................................. 54

4 Electrical Docking of Microtubules for Kinesin-Driven Motility in Nanostructures 57
4.1 Introduction .................................................... 58
4.2 Results and discussion .................................. 58
4.3 Conclusions ......................................................... 68
References ............................................................. 69
Supplementary Information .................................. 72

5 Molecular Sorting by Electrical Steering of Microtubules in Kinesin-Coated Channels 77
5.1 Introduction .................................................... 78
5.2 Results and discussion .................................. 78
5.3 Conclusions ......................................................... 86
References ............................................................. 87
Supplementary Information .................................. 90

6 Electrophoresis of Individual Microtubules in Microchannels 97
6.1 Introduction .................................................... 98
6.2 Theoretical framework .................................. 99
6.2.1 Fluid motion around a sphere .................. 99
6.2.2 Hydrodynamic versus electrophoretic motion .... 100
6.2.3 Electrophoresis of a cylinder .................. 102
6.3 Experimental Results ................................ 103
6.3.1 Electrophoresis of microtubules .............. 104
6.3.2 Electro-osmotic flow ........................ 106
6.4 Discussion ........................................................ 107
6.4.1 Electrophoretic motion is not described by Stokes drag .. 108
6.4.2 Effective surface-charge density $\sigma_{\text{eff}}$ .... 108
6.4.3 Effective tubulin-dimer charge $Q_{\text{eff}}$ .......... 109
6.4.4 Charge modification by digestion .......... 112
6.5 Conclusions ......................................................... 113
References ............................................................. 114
## Contents

Supplementary Information ........................................... 116  
Drag coefficients for cylinders near a surface ....................... 122

### 7 Microtubule Curvatures under Perpendicular Electric Forces Reveal a Low Persistence Length

7.1 Introduction ....................................................... 132  
7.2 Results and discussion .......................................... 133  
7.3 Conclusions ...................................................... 143  
7.4 Materials and Methods ........................................... 145  
    References ....................................................... 145  
Supplementary Information ........................................... 147

### 8 Persistence Length Measurements from Stochastic Single-Microtubule Trajectories

8.1 Introduction ....................................................... 154  
8.2 Stochastic microtubule trajectories ............................... 155  
8.3 Single microtubule trajectories .................................. 156  
8.4 Averaging over multiple trajectories ............................. 159  
8.5 Simulations of stochastic trajectories ............................ 161  
8.6 Discussion ....................................................... 164  
8.7 Conclusions ...................................................... 165  
8.8 Materials and methods .......................................... 165  
    References ....................................................... 166

### 9 Single-Molecule Observation of Hydrodynamic Deformation and Anomalous Orientation of Microtubules in Electrophoresis

9.1 Introduction ....................................................... 170  
9.2 Results and discussion .......................................... 171  
9.3 Conclusions ...................................................... 180  
    References ....................................................... 181

Summary ........................................................................... 183

Samenvatting .................................................................. 187

Acknowledgement .......................................................... 191

Curriculum Vitae ............................................................ 195

List of Publications .......................................................... 197
Chapter 1

Introduction

This chapter introduces the field of bionanotechnology, a young and still evolving interdisciplinary area of research at the interface between nanotechnology and biology. We first sketch two examples that stood at the beginning of these two fields. Then, we give a short introduction to nanotechnology, which is a highly multidisciplinary field with elements from chemistry, physics, material science, electronics, and mechanical engineering. Subsequently, we introduce biological motors, which are nanometer-sized proteins that can be considered as natural nanotechnological machines. These biological motors are the cellular solution to the challenge of directing nanoscale motion and assembly and have resulted from billions of years of evolution. The combination of nanotechnology and biology, bionanotechnology, is the subject of the third section of this chapter and of this thesis. Finally, we discuss the outline of this thesis.
1.1 Okay..., but what can you do with it?

When friends ask me what my thesis is about, I give them either a long or a short answer. The long answer often results in semi-comprehending nods and silence. My short answer, which is that I try to exploit biological motors in nanofabricated devices, is more prone to invoke further questions, of which “Why would you do that?”, and “For what will this be useful?” are on top of the list (perhaps because most of my friends studied economics).

These good questions are easily asked, but less easily answered. As for motivation, it was fascinating for me to learn about the existence of biological motors, that constantly move things around within your cells. The bare fact that these miniature machines exist is in my opinion already reason to explore if and, if so, to what extent we can exploit these motor proteins for technological purposes.

The second question, is it useful, is even harder to answer and I think that we do not have a fully satisfactory answer, yet. But I also think that it is a little too early to expect an answer. In fact, it is often hard to foresee the possibilities and wider implications of discoveries. I illustrate this with two examples, that show how an initial discovery or technological achievement has led to applications that could or were not foreseen, but which had a profound impact on our world.

1.1.1 1676, April 24, Delft, The Netherlands

It was about 350 years ago that the young tradesman Antonie van Leeuwenhoek (1632 – 1723) set up business in Delft as a fabric merchant [1]. It was important for him to judge the quality of his fabric, for which magnifying glasses were used to count the density of the threads. At that time, magnifying glasses and microscopes were not particularly good, and not practical for magnifications of more than twenty or thirty times. Van Leeuwenhoek trained himself in glass processing, which allowed him to produce lenses of significantly better quality than of those that were around. Together with improvements on the illumination, he was able to reach at least ten times higher magnifications than other microscopes at that time. His technique for making better lenses was relatively simple, but, being a tradesman, he kept the details for himself.

Van Leeuwenhoek’s skills in lens production gave him an advantage with respect to other colleagues and enabled him to perform a thorough study of the microscopic world. Although he was not a scientist by education, Van Leeuwenhoek possessed great curiosity and studied almost everything that he could place under his microscope. He is most known for his discovery of microscopic, single-cellular life. In April 1676, Van Leeuwenhoek intended to investigate whether the
spiciness of pepper was due to microscopic spikes. He had placed some pepper in water three weeks before, and when he studied this water under his microscope on April 24th, instead of pepper spikes he observed an enormous amount of tiny little creatures, which he referred to as little animals (animalcules or 'kleijne diertgens' in Dutch) [1]. Leeuwenhoek was amazed at the small size of these organisms:

\[\text{They were incredibly small, nay so small, in my sight, that I judged that even if 100 of these very wee animals lay stretched out one against another, they could not reach to the length of a grain of coarse sand.}\]

Nowadays, we know that Van Leeuwenhoek made the first observations of bacteria, spermatozoa, and protozoa, organisms that measure a few micrometers in size. Moreover, revolutionary new insight in the 20th century has revealed that inside these bacteria, processes and mechanisms are occurring on a scale that is yet a staggering thousand times smaller. The curiosity of a tradesman, together with his experience in glass polishing, has unintentionally led to the discovery of microscopic life, which has revolutionized the way in which we think, for example about hygiene, and has led to many important developments such as production of antibiotics.

1.1.2 1947, Christmas Eve, Murray Hill NJ, USA

Let me give a second example of a discovery that significantly shaped the world in which we now live, but in a different way than that was initially foreseen. In November 1947, Shockley, Brattain and Bardeen assembled the first transistor, which was a rather crude device to our current standards [2, 3]. They gradually improved upon the design, and on Christmas Eve 1947, they demonstrated transistor action in an oscillatory circuit to management of their employer, Bell labs. It is interesting to consider the events that led to the discovery of the transistor, of which nice descriptions can be found in literature [2, 3]. Around world war II, all amplification and logic inside electronics was performed by vacuum-tube technology, which seemed capable to meet all conceivable requirements [2]. However, one of the shortcomings of vacuum tubes appeared during the second world war, when the resolution of radar systems was limited by the poor high-frequency performance of vacuum tubes [3]. After the war, the team at Bell labs set out to develop a solid-state device that could replace the vacuum tube, which led to the first transistor in 1947. Interestingly, it was only after the discovery of the transistor and during its development that it dawned upon the scientists that the significance of the transistor was not in replacing the vacuum tube, but instead, in the new possibilities that it offered [2]. Eventually the transistor did
replace the tube, but it took almost two decades. More important, the integration of the transistor into integrated circuit (IC) technology, and the subsequent trend of miniaturization very much changed the world, with personal computers, telecommunication, and internet.

1.1.3 2007, a time for new discoveries

Above, we described two examples in which an initial invention led to totally unexpected developments and opened up new worlds and possibilities, triggering further development. Three hundred years after Van Leeuwenhoek, we are now able to look inside his *animalcules* and we find that these cells are reminiscent of little factories, full with protein machines that are converting energy, manufacturing other proteins, and transporting products through the cells. Recently, it has become possible to manipulate and study single molecules inside these cells, and other structures at the size of nanometers. For example, we are able to take a single molecule out of the cell, and see how it responds to an applied force.

About 50 years after the first, centimeter-sized, transistor, miniaturization has progressed in a tremendous pace. Currently, transistors have reached a size of less than 100 nm and using the techniques that were developed for the semiconductor industry, we can now routinely fabricate a range of structures and devices with nanometer dimensions, which have led to exciting developments, for example nanoelectronics, nanooptics, or nanofluidics.

Would it not be fascinating to combine the best of these two worlds? What if we mix the feats of nanotechnology, with top-down fabrication techniques reaching nanometer resolution and exquisite control of material properties, with the bottom-up, autonomously operating protein machines from the biological cell? Imagine the possibilities that would arise if we were able to control and direct motion with nanometer precision. Could we consider devising and building nanometer-sized factories or self-repairing machines, or perhaps miniature devices circulating through our system and repairing damage to our body?

We have only just begun exploring the possibilities of this new field of bionanotechnology. In the future, people that look back at our efforts may find these naive, and probably primitive, but it may well be that these will lead to unforeseen developments. The experimental work described in this thesis is on this interface of nanotechnology and biology. In particular, we investigate whether biological motors can be exploited for nanotechnology, and whether we can use tools from nanotechnology to explore fundamental questions about biological systems. In the following, we first shortly sketch the fields of nanotechnology and biological motors, and then discuss the merging of these fields.
1.2 Nanotechnology

Although nanotechnology is a thriving research field which is actively pursued by thousands of researchers around the world, it is not easy to give a simple definition for nanotechnology. Roughly, nanotechnology involves structures, devices or systems that have at least one dimension between 1 to 100 nm. In addition to this, for something to fall within the realm of nanotechnology, it requires a certain level of control at the level of a single entity. Bulk chemistry, for example, is the construction of molecules, but the major part of chemistry is not generally considered as nanotechnology. Another aspect that is usually associated with nanotechnology, is that new phenomena occur due to the scaling down of materials. For example, when materials decrease in size down to the nanometer regime, surface effects become more important than volume effects. Perhaps, nanotechnology can be defined as the art and science of manipulating matter at the nanoscale, where unique phenomena enable novel applications.

1.2.1 Applications of nanotechnology

Nanotechnology research cuts across a large variety of subjects and this section intends to give a flavor of the diversity of subjects. For example, in the ongoing effort for miniaturization of electronic devices, nanoelectronics is aimed at the assembly of electronic circuits on nanometer scales. Carbon nanotubes \[4, 5\], rolled-up sheets of graphene with a diameter of 1 nm, and silicon nanowires \[6, 7\] are actively researched in this area as both passive and active elements. On an even smaller scale, the transport through individual organic molecules has been investigated \[8\].

When materials are scaled down to nanometer-size proportions, their properties can deviate significantly from the bulk material. For example, in commercial products such as cosmetics and sunscreens nanoparticles made of titanium dioxide and zinc oxide are added for their efficient blocking of ultraviolet radiation, without the white appearance that emulsions of larger particles would display. Another example includes carbon nanotubes that have extraordinary mechanical properties, even stronger than steel, and one commercial application of them is as a reinforcement for composite materials.

The small size of nanomaterials also leads to new physical phenomena due to quantum effects, such as quantum confinement of electrons within semiconductor particles \[10\], or enhanced surface-plasmon-resonance (SPR) scattering of light on small metallic clusters \[11, 12\]. Semiconductor nanocrystals with their bright and tunable fluorescent optical properties \[13\] are used for biological detection \[14\].
1.2.2 Nanoscale motion

Richard Feynman was one of the first to envision nanotechnology and promulgate the opportunities that would arise from being able to control nanoscale motion and assembly. In a famous speech addressing the American Physical Society in 1959 [21] he articulated a vision of nanoscale machines, and, ultimately, control of individual atoms:

So I want to build a billion tiny factories, models of each other, which are manufacturing simultaneously, drilling holes, stamping parts, and so on. [...] The principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom.

It was 20 years later that nanotechnology really got started with the invention of novel tools that opened up a new window on the nanoscale world. Scanning
probes techniques such as atomic force microscopy (AFM) and scanning tunneling microscopy (STM) made it possible to image and manipulate individual atoms on a surface. It was in 1990 that Feynman’s suggestion of moving things atom by atom were realized by Don Eigler, who used an STM tip to manipulate individual xenon atoms on a nickel surface, thereby spelling out the name of his employer IBM with atomic resolution (Fig. 1.2) [22]. Fabrication techniques, such as electron-beam (e-beam) lithography, and deposition techniques such as molecular-beam epitaxy and atomic-layer deposition have enabled the fabrication of nanometer sized structures.

Despite all progress, the concept of tiny factories or, more general, controlled motion on the nanoscale has not been realized to the point that Feynman imagined. Figure 1.3 illustrates the current state of synthetic nanofabricated motors. Zettl and coworkers [23] assembled a rotational actuator, which was based on a multi-walled carbon nanotube (MWNT) that acted as a shaft around which a metal plate of about 300 nm size could rotate.

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**Figure 1.2:** STM image of xenon atoms on a nickel surface. The STM was used to arrange individual nickel atoms in the letters IBM. Image reproduced from [22].

**Figure 1.3:** A synthetic nanorotor. The artist impression shows a freestanding gold rotor (approximately 300 nm size) connected to a multi-walled nanotube (MWNT). Rotation of the rotor can be controlled by applying voltages to the nearby electrodes. SEM images on the right show a complete rotation of the device. Image adapted from [23].
Figure 1.4: Schematic of a cell illustrating different structures and compartments. The central compartment is the nucleus containing the genetic information encoded in the DNA. This information can be transcribed onto messenger RNA (mRNA) and exit the nucleus through nuclear pores. Ribosomes are protein factories, that use the information that is encoded in mRNA to assemble proteins. Ribosomes are mostly found in the endoplasmic reticulum, which is an organelle that is involved in fabrication of proteins and other molecules. The Golgi apparatus is reminiscent of a hub inside the cell and is central to packaging, labeling and directing molecules to different parts of the cell. Transport of cargo takes places over microtubules and actin filaments that constitute the cytoskeleton, together with intermediate filaments. Mitochondria are the energy factories inside cells. They are central to metabolism, using oxygen to convert high-energy molecules from food into ATP, the cell’s energy currency. Peroxisomes and lysosomes are the cell’s cleaning force, being involved in digestion and detoxification of cells. Image adapted from [24].

One problem with fabricating nanometer-sized motors is that on these small scales qualitatively different forces become important. Mass and inertia, phenomena that rule at macroscopic dimensions, become negligible at the nanoscale, whereas other forces such as friction, Van der Waals forces and surface tension become increasingly dominant. Thermal forces, which are negligible for large objects, have profound effects on the motion of nanoscale objects. Thus, one cannot just simply scale down macroscopic machines and motors to nanometer proportions without taking into account these concepts.
1.3 Biological motors

For inspiration on how to solve the challenge of controlled nanoscale motion, we just have to look at biology, because in a cell, motion, actuation and assembly all originate at the nanoscale. In fact, the biological cell contains the ultimate of nanomachines (Fig. 1.4), with, for example, the ribosome as an assembly line for proteins, different protein complexes that replicate the DNA, voltage-gated ion channels, rotary motors such as the bacterial flagellar motor, or the photosynthetic reaction center that converts light into high-energy electrons. Besides the many opportunities that the biological cell has to offer to us, it could also learn us a great deal about design principles for molecular motors. The latter has been well formulated by George Whitesides [25]:

*The fact that biological structures—although functioning in familiar ways—operate using principles that are entirely unfamiliar based on everyday experience suggests that would-be designers of nanomachines have much to learn from biology.*

Most of these biological machines are made of protein. Proteins are long polymers consisting of a linear chain of amino acids that fold in a defined structure. There are 20 different amino acids that each have a specific chemical side group, which can give the amino acid hydrophobic or hydrophilic properties, or a positive or negative charge. The specific sequence of amino acids (their primary structure) leads to a stable folding of the amino-acid chain into certain secondary structures, such as \(\alpha\)-helixes or \(\beta\)-sheets. Those are mechanically stable elements and are further folded into protein domains, constituting the functionally active tertiary structure of proteins. Finally, individual proteins can combine into larger structures to form assemblies or polymers. In this hierarchical organization, the linear sequence of amino acids—encoded in a protein’s gene—determines the three-dimensional configuration of a protein complex, which is stabilized by different interactions within the structure.

Motor proteins are responsible for almost all movement and force generation within cells [26]. Most notably, for the intracellular transport of materials cells employ motor proteins that carry cargo along linear protein filaments that extend throughout the cell (Fig. 1.5a). Myosin motors move along actin filaments, while kinesin and dynein motors move along microtubules. Microtubules and actin filaments, together with intermediate filaments, constitute the cytoskeleton. The cytoskeleton is not only important for supporting motor-powered transport, but it also defines cell morphology and it provides mechanical stiffness to the cell.
Besides intracellular transport, cytoskeletal motors are also involved in cell division and cell motility. In this thesis we describe experimental work involving kinesin motor proteins in nanofabricated devices and in the following sections we shortly describe kinesin and the track along which it moves, the microtubule.

1.3.1 Microtubules

Microtubules are stiff cylindrical protein filaments that extend in a roughly radial organization throughout the cell (Fig. 1.5). They usually originate at one end from the centrosome, which is located close to the cell’s nucleus, and extend in all directions towards the periphery of the cell, establishing an efficient track system for molecular motors. In this organization, microtubules can also resist external forces that are applied to the cell. Moreover, growing and shrinking microtubules can exert pushing and pulling forces [29, 30]. One special example is cell division, where microtubules form the mitotic spindle that orients the cleavage plane and separates the chromosomes into the two daughter cells [31]. Microtubules also form the structural elements inside cilia and flagella, for example, the beating tail of sperm cells.

Structurally, microtubules are hollow cylinders with an outer diameter of 25 nm and with lengths ranging from several micrometers to hundreds of micrometers in axons. They are formed from polymerization of $\alpha\beta$-tubulin dimers [32]. These dimers associate head-to-tail with other tubulin dimers, thereby forming
Figure 1.6: Schematic of a microtubule and its structure. A 13-protofilament microtubule can be described as a 3-start helix with $\alpha$ and $\beta$ tubulin laterally connected in different protofilaments. As a result of the $\sim0.92$ nm displacement between protofilaments, the microtubule contains a seam, where $\alpha$ and $\beta$ tubulins are connected laterally. The ribbon diagram (top) is derived from electron crystallography [33] and was made using the Molecular Biology Toolkit (MBT) [34].

a protofilament with a 8.2 nm repeat distance (Fig. 1.6). Polymerizing tubulin dimers need to bind a molecule of guanine triphosphate (GTP) in the $\beta$ subunit, which is hydrolyzed upon incorporation of the dimer in the microtubule. Multiple protofilaments can bind laterally into a sheet, which can subsequently close and form the cylindrical microtubule structure. Most microtubules contain 13 protofilaments, although this number can vary in different cell types.

Within the microtubule, the lateral interaction between protofilaments are through monomers of the same kind. That is, $\alpha$ and $\beta$ monomers in one protofilament associate with the $\alpha$ and $\beta$ monomers, respectively, in the next protofilament. However, tubulin dimers in neighboring protofilaments have a slight offset of $\sim0.92$ nm, such that when adjacent monomers of the same kind are followed, a helical path around the microtubule is traced (Fig. 1.6). Following this path for one full rotation around the microtubule (13 protofilaments), one arrives 3 monomers above it starting position. Therefore, the microtubule is described with a so-called 3-start helix structure with the protofilaments running parallel to the microtubule axis. For a 13 filament microtubule this also implies that a
seam exists, where $\alpha$ tubulin must associate with $\beta$ tubulin, and visa versa. The atomic structure of the tubulin dimer and its orientation inside the microtubule has been solved by electron crystallography [33, 35].

Because $\alpha$ and $\beta$ tubulin are not identical, the tubulin dimer and thus the microtubule have a structural polarity. The microtubule end which terminates with $\beta$ tubulin is denoted the plus end—polymerization occurs faster at this end—whereas the other side, ending with $\alpha$ tubulin, is denoted the minus end, where microtubule growth is much slower. The structural polarity of microtubules is central to cell organization and directed intracellular transport. Microtubules in cells are usually oriented with their plus ends located toward the cell’s periphery and the minus ends connected to the centrosome. Motor proteins that move along microtubules ‘sense’ the polarity of their track and are either plus-end or minus-end directed. Most dyneins, for example, are minus-end directed and transport cargo toward the cell’s nucleus, while most kinesins are plus-end directed and move toward the cell’s periphery.

Microtubules are highly dynamical structures that display dynamic instability, which is the phenomenon that a period of slow microtubule growth is suddenly alternated with a period of fast microtubule depolymerization [32, 36, 37]. The sudden change from polymerization to depolymerization is called catastrophe, while the reverse process is referred to as rescue. It is believed that catastrophe and rescue are associated with the loss and gain of a stabilizing cap at the end of the microtubule consisting of GTP tubulin. When microtubules are in the growing phase, tubulin subunits that bind to the plus end have a GTP molecule bound, which is hydrolyzed soon thereafter. If this hydrolysis takes place before a new GTP-tubulin has been added, depolymerisation starts (catastrophe), and when the GTP cap is regained, a rescue event results. In vivo, microtubule structure can be stabilized and regulated by various microtubule-associated proteins (MAPs). Microtubules can also be stabilized by certain cross-linking chemical or drugs. For example, the anti-cancer drug paclitaxel (Taxol) binds to the $\beta$ subunit on the inside of the microtubule, strengthening the lateral contacts between adjacent protofilaments [35]. This prevents the depolymerization of microtubules and is used in the experiments described in this thesis.

1.3.2 Kinesin motor proteins

Since the discovery of the first kinesin motor in 1985 [38], it has become clear that many variants of kinesins exist. Kinesins are involved in transport of different cargo, such as vesicles or organelles, which is of particular importance in neurons, that can become very large. Kinesins are also involved in cell division,
and the organization of the cytoskeleton and of cilia and flagella. All kinesins are microtubule-based motors, and although many are plus-end directed, some kinesins move toward the minus end of microtubules. The kinesin that is used in this thesis is kinesin-1, also known as conventional kinesin.

Single-molecule experiments have generated a wealth of knowledge about kinesin. A single kinesin molecules moves in a large number of discrete 8.2 nm steps along a microtubule [40], which it does while following a single protofilament [41]. When moving along a microtubule, a single kinesin molecule can withstand an opposing force of up to about 6 pN [40, 42], which is the force at which the kinesin’s velocity has decreased to zero (the stall force). While moving against a force, the kinesin molecule performs work, for which it uses the energy from hydrolysis of adenosine triphosphate (ATP). It has been established that there is a tight coupling between hydrolysis of a single molecule of ATP and a single 8 nm step [43–45]. The maximum work that kinesin can perform (∼6 pN×8 nm) is about 50% of the energy that it can derive from ATP hydrolysis (∼80 pN·nm), which makes it a fairly efficient motor.

Figure 1.7: Structure of the kinesin molecule. The two motor domain complexes (bottom) contain ATP- and microtubule-binding domains. The motor domains are connected to a stalk. At the end of the stalk, cargo can bind to the tail, possibly through accessory proteins, including the kinesin light chain. A ribbon diagram of the motor domains, neck, and dimerization domains, derived from X-ray crystallography, is shown on the right [39] and was made using the Molecular Biology Toolkit (MBT) [34].
Kinesin-1 is a dimeric motor, formed from two identical subunits (Fig. 1.7). These subunits each consist of head, neck, stalk and tail domains, where dimerization occurs upon the formation of a coiled coil structure in the stalk. The head domains have ATP- and microtubule-binding domains and exhibit catalytic activity. The neck connects the two motors to the common stalk which links to the tail domain that is involved in cargo binding.

The fact that kinesin has two heads allows it to walk processively along a microtubule [46], typically for about 100 steps without detaching. It is now established that kinesin walks in an asymmetric hand-over-hand model [47, 48], which means that, similar to a walking person, each motor domain alternately advances in 16 nm steps, displacing the center-of-mass of the kinesin molecule with 8 nm per step.

The working principle of motor proteins is that chemical changes (in the state of the bound nucleotide) are coupled to mechanical motion of certain elements in the motor protein, the so-called mechanochemical coupling. Central to this principle is that a chemical change (hydrolysis of ATP, binding or unbinding of a nucleotide) leads to a deformation in the kinesin molecule, changing kinesin’s binding affinity for the microtubule. Alternately, binding to the microtubule can induce deformations in the kinesin, catalyzing chemical changes in the bound nucleotide.

Although many details still need to be resolved, a basic understanding of the mechanochemical cycle has emerged after much biochemical and single-molecule research [49], which is depicted in Fig. 1.8. Starting (arbitrarily) at the top of the figure, the leading head of kinesin is bound to the microtubule and has no nucleotide bound. The rear head is detached from the microtubule and contains adenosine diphosphate (ADP). Upon binding of ATP in the leading head, a structural change occurs in the kinesin molecule, which involves docking of kinesin’s neck linker onto the motor domain [50]. It is believed that this neck linker docking drives a small displacement of the trailing head toward the plus end of the microtubule, but the mechanism is still under debate for a number of reason [49]. The remainder of the kinesin step is thought to result from a biased diffusional search for the next binding site. After reaching the next site, the kinesin has progressed over 8 nm. The ADP-containing head is in a weak binding state with the microtubule, which changes to a strong binding state upon ADP release, the latter being catalyzed by the microtubule. It is thought that the strong binding of the new leading head induces strain within the kinesin, which acts to coordinate the cycles of the two heads (either through preventing ATP binding to the front head [51], or through accelerating detachment of the rear head [52]). The hydrolysis of ATP in the trailing head and the subsequent release of phosphate...
1.4 Bionanotechnology

1.4.1 Exploiting motor proteins for nanotechnology

The experimental work in this thesis is conducted in the area of bionanotechnology, which is a young field at the interface of nanotechnology and biology [53]. One aim of this field is to try to exploit biological components for technological purposes [54]. Examples include the integration of neuronal networks with

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**Figure 1.8:** Although further details still need to be resolved, there is a fair consensus about the mechanochemical cycle of kinesin [49]. (I) ATP binding to the leading head is thought to induce a small conformational change which drags the trailing head away from the microtubule minus end. (II) After a diffusional search, the trailing head reaches the next binding site on the microtubule. (III) The microtubule catalyzes ADP release which induces a strong binding of the leading head. This results in intramolecular strain (red neck) which is believed to be used as a gating mechanism keeping the two motor domains in different states. (IV) Hydrolysis of ATP and (V) subsequent release of phosphate releases the internal strain. (VI) The trailing head can release from the microtubule and the front head can bind ATP. Note that the coloring of the two heads has switched now, and in this cycle the kinesin has moved forward by 8.2 nm.

(P) releases the strain and leaves the trailing head in a weak binding state with the microtubule. The leading head is now free to bind ATP for a new step.
microelectronics for neuronal computers [55], the use of bacterial surface layers (S-layers) for structuring and patterning molecules or nanoparticles [56], biological nanopores that are employed as sensors [57], and bacteriorhodopsin, a bacterial light-driven protein pump that is explored for technical applications such as data storage, energy conversion, or photoelectric applications [58].

One particularly active field of research is the use of motor proteins for actuation and transport in artificial nanofabricated environments [59, 60]. Given the difficulties in constructing artificial nanoscale motors, the availability of a wide range of biological motors makes it attractive to explore their application for technological purposes. In the first part of this thesis (Chapters 2 – 5), we describe experimental work on the exploitation of kinesin and microtubules in nanofabricated environments. In chapter 2, we give a fairly extensive overview of the field.

1.4.2 Exploring nanotechnology for biophysics

Another aim of bionanotechnology is to use tools from nanotechnology for the exploration of fundamental questions about biomolecules [61]. For example, tools such as AFM and tweezers have allowed to learn a great deal about individual biomolecules. Products of nanotechnology such as quantum dots and metallic nanoparticles have allowed tracking of individual molecules [15]. Electric forces in nano- and microfabricated structures have recently emerged as a versatile tool for the manipulation and study of individual biomolecules. Examples include the use of electrokinetic forces to compensate Brownian motion of individual proteins, allowing for extended periods of optical observations [62], or the use of electric forces in solid-state nanopores to measure the effective charge of an individual DNA molecule [63]. Microfabricated structures have been used for electric-field-driven DNA separation [64, 65], or for probing force-velocity diagrams of myosin-driven actin filaments using electric forces [66].

The second part of this thesis (Chapters 6 – 9) focusses on experimental work that explores more fundamental aspects of biomolecules and physics using nanotechnology. In particular, we investigate the electrophoretic motion of individual microtubules, the stiffness of short microtubule ends and hydrodynamic coupling effects.
1.5 Outline of this thesis

This thesis reports experimental work on microtubules and kinesin motor proteins in nanofabricated devices. In the first part of this thesis we describe the possibilities of exploiting kinesin motor proteins in nanotechnology. We investigate rectifier geometries to influence the direction of motility. We also explore the use of electrical forces as a control of the docking of microtubules inside nanofabricated structures. Finally, we show that we can use electric forces to direct the motion of individual microtubules inside Y-junctions and that this can be used in molecular sorting experiments.

The second part of this thesis describes the use of nanotechnology as a tool for the exploration of biomolecules. We use micron-sized fluidic channels to confine and measure the electrophoretic motion of individual microtubules. We also use electric forces to induce a bending of the trajectories of individual microtubules, which is a new tool to study the stiffness of microtubule ends.

The outline of this thesis is as follows.

Chapter 2 serves as an introduction to the field of motor proteins operating in nanofabricated environments. We give an overview of several prominent examples of motor proteins and the functions that they perform inside cells. We then describe a number of examples were biological motors have been exploited for powering nanoscale components with an emphasis on kinesin and myosin motors.

Chapter 3 describes a novel fabrication process for the creation of gold tracks recessed in silicon oxide for kinesin-driven microtubule motility. The process combines topographical and chemical patterning of kinesin motors and results in an excellent confinement of microtubules to the tracks. We also introduce different design for rectifier geometries that are all very effective in creating unidirectional motion. The most efficient geometry achieves 92% rectification.

Chapter 4 introduces electrical control of the docking of microtubules onto nanofabricated kinesin-coated structures. We can control the density of microtubules moving on the kinesin by applying a voltage to the gold tracks. The increase and decrease in density follow an exponential time dependence which we understand by a kinetic model of microtubule transport. This model allows to determine the binding affinities of microtubules onto kinesin-coated glass and gold surfaces.

Chapter 5 describes the opportunities that arise from the integration of kinesin-driven microtubule motility within microfluidic channels. By the use of enclosed fluidic channels we achieved full confinement of the microtubules to their tracks, but it also allowed for a full electrical control of the direction of individual microtubules. We demonstrate that microtubules approaching a Y
junction can be steered into the desired direction using electric forces. Employing this technique, we demonstrate a molecular-sorting experiment, using differently labeled microtubules.

**Chapter 6** introduces the use of microfluidic channels as an excellent tool to study the electrophoresis of individual microtubules. The stiffness of microtubules makes them a good model system for rod-like particles, for which an anisotropic electrophoretic mobility has been predicted. We provide the first single-molecule measurements of the electrophoretic mobility of rod-like particles, and provide an experimental verification of the anisotropy. We discuss the difference between electrophoretic motion and purely hydrodynamic motion due to the presence of counterions and the implications that this has for interpreting mobility measurements. We infer a value of the effective charge of the tubulin dimer. We also perform measurements on microtubules that have their C-termini removed and show that this corresponds to a strong reduction in charge.

**Chapter 7** introduces electric forces as a new tool to measure the stiffness of very short microtubule ends. The anisotropic mechanical structure of microtubules was reported in previous studies to result in a reduced bending rigidity for short lengths of microtubules. In this chapter we report measurements on length scales that are even an order of magnitude smaller, using a novel method, viz., by studying the curvature of trajectories of kinesin-propelled microtubules under perpendicular electric forces. These experiments illustrate that electric forces are an excellent tool for biophysics experiments and that the magnitude of these forces can be surprisingly well quantified.

**Chapter 8** demonstrates that we can also obtain a measurement of the persistence length of microtubule ends by tracking of stochastic microtubule trajectories in gliding assays. The tangent angle of a microtubule trajectory is similar to a random walk, that is solely determined by the stiffness of the leading tip and the velocity of the microtubule. We show that even a single microtubule trajectory can yield a measurement of the persistence length. By averaging the data of a large number of single microtubule trajectories we find a value of the persistence length that is in reasonable agreement with the value that we report in Chapter 7.

**Chapter 9** describes new insight into hydrodynamic-coupling effects as measured on individual microtubules. We observe an unexpected deformation and anomalous orientation of microtubules in electrophoresis, which can be explained by hydrodynamic interactions between different parts of a microtubule. We show data of this phenomenon for a large range of electric fields. These results are evidence for the hypothesis that hydrodynamic coupling is responsible for the anomalous signals reported in electrical birefringence experiments.
References


References

Chapter 2

Motor Proteins at Work for Nanotechnology

The biological cell is equipped with a variety of molecular machines that perform complex mechanical tasks such as cell division or intracellular transport. One can envision employing these biological motors in artificial environments. We review the progress that has been made in using motor proteins for powering or manipulating nanoscale components. In particular, kinesin and myosin biomotors that move along linear biofilaments have been widely explored as active components. Currently realized applications are merely proof-of-principle demonstrations. Yet, the sheer availability of an entire ready-to-use toolbox of nanosized biological motors is a great opportunity that calls for exploration.

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2.1 Introduction

A huge amount of biological research in recent decades has spurred the realization that the living cell can be viewed as a miniature factory that contains a large collection of dedicated protein machines [1]. Consider the complicated tasks that a single cell can perform: It can create a full copy of itself in less than an hour; it can proofread and repair errors in its own DNA, sense its environment and respond to it, change its shape and morphology, and obtain energy from photosynthesis or metabolism, using principles that are similar to solar cells or batteries. All this functionality derives from thousands of sophisticated proteins, optimized by billions of years of evolution. At the moment, we can only dream of constructing machines of similar size that possess just a fraction of the functionality of these natural wonders.

One particular class of proteins is formed by molecular motor enzymes, which are catalytic proteins that contain moving parts and use a source of free energy to direct their motion. Upon studying these motors, their resemblance to machines becomes more and more clear. We find rotary motors that comprise shafts and bearings, as well as linear motors that move along tracks in a step-by-step fashion. We find motors that are powered by chemical energy, derived from hydrolyzing adenosine triphosphate (ATP) molecules (the cell’s major energy currency), and motors that employ a gradient of ions, using both electric and entropic forces.

It is of interest to ponder whether we can employ these biological nanomachines in artificial environments outside the cell to perform tasks that we design to our benefit [2, 3]. Or, at the very least, can these proteins provide us with the inspiration to mimic biocomponents or design artificial motors on comparable scales?

2.2 Nature’s workhorses in the cell

In contrast to macroscopic machines, motor proteins operate in a world where Brownian motion and viscous forces dominate. The relevant energy scale here is $k_B T$, the product of Boltzmann’s constant and temperature, which amounts to 4 pN-nm. This may be compared to the $\sim 80$ pN-nm of energy derived from hydrolysis of a single ATP molecule at physiological conditions. Thermal, non-deterministic motion is thus an important aspect of the dynamics of motor proteins.

Let’s briefly consider some examples of biomotors. The rotary engine $\text{F}_0\text{F}_1$-ATP synthase (Fig. 2.1A) synthesizes ATP from adenosine diphosphate (ADP) and phosphate [8]. The flow of protons along an electrochemical gradient through the membrane-bound $\text{F}_0$ motor drives rotation of the $\text{F}_0$ ring and the central
2.2 Nature’s workhorses in the cell

Figure 2.1: Motor proteins in the cell. (A) Representation of F$_0$F$_1$-ATPase [Reprinted with permission from [4]; ©2006 Wiley-VCH]. (B) Representation of the bacterial flagellar motor (Image courtesy of Keiichi Namba, Osaka University). Inset shows an electron-microscopy image of the motor [Reprinted with permission from [5]; ©2001 Elsevier]. (C) Conventional kinesin and dynein transport cargo in opposite directions along microtubules [Adapted from [6]]. (D) Kinesin is a processive motor, consisting of two heads, that walks in alternate steps of 8 nm along the microtubule [Adapted from [7]]. (E) Muscle contraction is caused by the sliding of interdigitated actin and myosin filaments in a sarcomere unit. The non-processive myosin II motor detaches after each power stroke, so as not to impede the further sliding of the actin filament caused by other myosins [Adapted from [7]]. (F) RNA polymerase transcribes a RNA copy of the double-stranded DNA template (Image courtesy of David S. Goodsell, Scripps Research Institute)
stalk connecting the F₀ and F₁ motors. This induces conformational changes of the F₁ motor that drives the catalytic formation of ATP. Remarkably, the complex can also work in reverse, using the energy of ATP hydrolysis to drive the reverse rotation of the F₁ motor and subsequently pump protons against their electrochemical gradient.

The rotary bacterial flagellar motor (Fig. 2.1B) is used by bacteria such as Escherichia coli as a propulsion mechanism by spinning a helical flagellum [9]. This powerful motor, assembled from more than 20 different proteins, is driven by an inward proton flux that is converted by several torque-generating stators into a rotary motion of the cylindrical rings and central shaft. The motor generates torques of more than $10^3 \text{ pN} \cdot \text{nm} \ (250 k_B T)$ and rotates at speeds of over 100 Hz [9].

Linear-motion motors are found among the members of the super families of kinesin, dynein, and myosin proteins [7] (Fig. 2.1C to E). These motors move in discrete steps along tracks made of long protein polymers (actin filaments for myosin, microtubules for kinesin and dynein) that form the cytoskeleton that extends throughout the cell. The structural polarity of these filaments (denoted by a plus and minus end) allows unidirectional movement of motors along their tracks. Cytoskeletal motors are involved in almost every aspect of controlled motion and force generation within cells, such as intracellular transport of materials (Fig. 2.1C) [6], cell division, or powering eukaryotic flagella and cilia. The contraction of a muscle is driven by the orchestrated sliding of series of actin filaments with respect to arrays of myosin motors (Fig. 2.1E). Typically, a linear motor can generate forces of up to $\sim 10 \text{ pN}$.

Many other proteins exist that can use energy to perform work, such as ion channels, DNA- or RNA-processing enzymes (Fig. 2.1F), ribosomes, or light-powered electron pumps, but these fall outside the scope of this review.

### 2.3 Muscle power for nanotechnology

One striking demonstration of a biomolecule-powered nanostructure is the construction of a nickel nanopropeller that rotates through the action of an engineered F₁-ATPase motor [10] (Fig. 2.2A). The directed assembly of the devices was controlled through genetic engineering of histidine tags that stuck the F₁-ATPase onto nickel posts, with its central stalk protruding upwards. This connected to a nickel propeller of $\sim 1 \mu m$ length through biotin-streptavidin bonds. Addition of ATP caused rotation of the propeller. A metal-binding site was engineered into the motor and acted as a reversible on-off switch by obstructing the rotation upon binding of a zinc ion [11], similar to the action of putting a stick between two cogwheels.
2.3 Muscle power for nanotechnology

On a larger scale, gliding bacteria have been used to power a micromechanical device comprising a cogwheel-shaped rotor of 20 \( \mu \)m diameter rotating in a silicon track [12]. Bacteria adhered to the rotor, turning it with \( \sim 2 \) rpm (Fig. 2.2B). The increase in size (cells compared with individual proteins) is accompanied with larger torques, together with self-repairing properties. Cardiomyocytes (heart muscle) have been used to drive a self-assembled microwalker [13]. The coordinated contraction of muscle bundles, which were assembled onto a \( \sim 0.1 \) mm-large two-legged SiO\(_2\) structure, drove its stepwise movement with a speed of 38 \( \mu \)m/s.

Linear cytoskeletal kinesin and myosin motors have dominated the emerging field of protein-powered devices because they are relatively robust and readily available. Actin and tubulin can be commercially purchased, whereas the motor proteins can be purified from cells or expressed in recombinant bacterial systems and harvested in large quantities. In their most basic geometry, these motor
systems are employed in a so-called gliding assay, in which the cytoskeletal filaments (usually about 1 to 20 µm in length) are propelled by surface-bound motors (Fig. 2.2C). The rotational flexibility of the motor stalks is high enough to rotate the randomly bound motors into the correct orientation for binding onto the microtubule or actin filament. Plus-end-directed motors will then propel the filaments with their minus end leading. Like nanoscale trucks, the microtubules or actin filaments can act as shuttles that transport an attached cargo such as nanoparticles or DNA [15] (Fig. 2.2C).

In an alternative geometry, motor-coated cargo can move along cytoskeletal filaments that are adsorbed onto a substrate. This requires the controlled placement of filaments onto a substrate and precoating of the cargo with motors. The inverted gliding geometry offers better opportunities, however, for actuation, functionalization, assembly, and control and is thus preferred. In general, multiple motors attach to a single filament shuttle, so that large forces (≫ 10 pN) can be generated. Another advantage is that the shuttles can routinely be interfaced to a variety of cargo using the biotin-streptavidin linkage or through antibodies.

2.4 Kinesin- and myosin-driven transport on chips

One vision is that motor proteins will be used for controlled cargo manipulation on a chip, with applications in sorting, separation, purification, or assembly of materials [2, 17]. To reach this goal, one needs to develop controlled motion along specific routes, directionality, coupling to cargo, external control, and steering.

A prerequisite of any useful transport system is that motion and transport can be (uni-) directionally guided along predesigned pathways. When filaments are absorbed randomly onto a substrate, the direction of cargo transport is random as well (Fig. 2.3A-I). Therefore, considerable effort has been directed at creating confined motility by employing either chemical patterning of active motor proteins [18] or fabricated topographical patterns (Fig. 2.3A-II) [16, 19]. A disadvantage of purely chemical patterns is that filaments easily derail from their tracks, which occurs when the leading end of the filament cannot find a new motor to bind to, whereas in purely topographically structured surfaces the selectivity of functional motor absorption is lost.

A combination of topographical and chemical patterning [20], with the motor proteins only at the bottom of the trenches (Fig. 2.3A-III), has proven to combine the best of both approaches with respect to guiding and confinement of microtubules [21] and actin [22]. The recent use of enclosed fluidic channels [23, 24]
can be considered as a logical final step in the development toward confinement, offering much better perspectives for packaging [23], and for the addressability of individual filaments through electric fields or flows [24].

For sorting applications, it is desirable that motion occur unidirectionally. Because the motors bound to a surface are rotationally flexible, unidirectional motion in gliding geometries can only be achieved through reorientation of the filaments. One method exploits asymmetrical arrow-shaped structures [20] (Fig. 2.3B) that rely on the principle that the probability to traverse the rectifier structure depends on the direction from which the filament enters. This hands-off method can achieve up to 92% efficient rectification per arrow [25]. A different, active-control method is to use external force fields that bend and align the leading end of the motor-propelled filaments parallel to the field, which can be electric [19], magnetic [26], or flow fields [27]. By subsequent fixation of the filaments to the underlying motors (using a chemical such as gluteraldehyde), the carpet can serve as a directionally aligned surface for motor-protein coated cargoes [28, 29].

The coupling of cargo to protein shuttles is relatively straightforward. The simplest configuration relies on the nonspecific electrostatic or hydrophobic adsorption of cargo onto kinesin, which was used for unidirectional transport of materials such as gold, polystyrene, and glass [28]. Biotin-functionalized microtubules and actin filaments can be interfaced to any cargo with streptavidin groups. Using the inverted assay, transport of polystyrene beads [16], DNA molecules [15, 30] (Fig. 2.3C), and quantum dots [31, 32] has been demonstrated.

A disadvantage of these methods is that the cargo has to be prefunctionalized. Therefore, a promising and versatile method is the use of microtubules that are coated with antibodies to the cargo that needs to be transported [34, 35]. This technique was used to pick up tobacco mosaic virus particles [34] and specific proteins [35] from solution, which can be advantageous, for example, in sensing applications. A similar example is the use of microtubules coated with single-stranded DNA oligonucleotides, which could hybridize very specifically with its target DNA in solution, with sensitivity for a single-basepair mismatch [36]. Another interesting approach is the report on myosin-driven transport of gold nanowires [37]. The nanowires were created by catalytic enlargement of gold nanoparticles bound to actin filaments, while leaving some actin free to interact with the myosin-coated surface. This method could offer a way of assembling small electrical circuits.

One way to achieve reversible starting and stopping of the motility is to control the concentration of ATP or other necessary cofactors in solution. Light-controlled switching of the motility was achieved using caged ATP, an inactive form of ATP, in conjunction with hexokinase, an ATP-consuming enzyme.
Figure 2.3: Biomotor-driven transport. (A) Evolution in the confinement of motility. (I) On flat surfaces, the motion of filaments is in random directions (fluorescence image at bottom). (II) To confine the motion, people initially used chemical patterning of motors (as indicated by red x’s), or (middle) topographical structuring of the substrates. (Bottom) Scanning electron microscopy (SEM) image shows microfabricated channels in SiO₂. (III) A combination of both methods proved more effective. Bottom image shows time-integrated fluorescence of actin filaments which are mobile exclusively in the letter-shaped tracks [reprinted with permission from [22]; ©2004, Institute of Physics Publishing]. (IV) The use of sub-micrometer fluidic channels offers three-dimensional confinement. (Bottom) SEM image of a closed channel. (B) Arrow-shaped structures rectify the motility. Initially, the amount of microtubules is equal in both reservoirs, but after 18 minutes most microtubules have collected in the left reservoir [reprinted with permission from [20]; ©2001 Biophysical Society]. (C) A kinesin-propelled microtubule binds to and stretches a DNA molecule attached to a gold post [Reprinted with permission from [30]; ©2006, Wiley-VCH]. (D) Thermoresponsive polymers form a clever way of switching the motility on and off [Reprinted with permission from [33]; ©2006, ACS]. (E) An electric force is used to steer individual kinesin-propelled microtubules within an enclosed fluidic channel.

Flashes of ultraviolet (UV) light liberated the ATP, which was concurrently depleted by the hexokinase, creating spikes in the motility that lasted several minutes [16]. A faster time response of about 10 s was obtained through simply flushing hexokinase or ATP into the flow cell [38], but this requires more elaborate handling.

Temperature modulation in a flow cell, as through the fabrication of an electrical heater on a cover slip, allowed for reversible control of the velocity of actin filaments [39], although the motion could not be entirely stopped. Another method exploits thermo-responsive polymers on a surface to control the motility of mi-
2.5 Outlook: will bio-motors make their way?

When people think of molecular motors and areas for their applications, they initially come up with scaled-down extensions of macroscopic systems: rotary motors to drive a propeller, and linear motors as locomotives pulling cargo. A recurring theme is the building of a molecular transport system or assembly line using kinesin or myosin motors (Fig. 2.4A). Indeed, applications can be imagined along these lines, where antibody-functionalized shuttles capture and separate target molecules that are present in otherwise undetectably low quantities in an
analyte. Such a motor-assisted nanotechnology can be used for concentration of molecules and more sensitive detection.

Other areas where a role for motor proteins is envisioned include the use of motors to drive and accelerate self-assembly processes of nanostructures [17], to power nanoscale mechanical elements [e.g., a nanoscale version of the bacteriorhodopsin-powered cogwheel (Fig. 2.2B)], or to drive small fluidic pumps [45]. Ideas for applications that employ the massively parallel nature of autonomous molecular motors include the use of kinesin-propelled microtubules as a probe for surface topography [46] or for biocomputational maze-solving, where a large number of motile probes find different ways through a microfabricated maze [47].

Many of these applications are little more than proof-of-principle examples, for which more developed alternative technologies exist. For example, a biomolecular transport system should be gauged against lab-on-a-chip or micro-total-analysis systems, which are fairly well established technologies. Perhaps, though, the applicability of biomotors is merely limited by the imagination and creativity of researchers (including us). Progress in the field will likely come from integration
of achievements of the past few years into more complete and functional devices. Promising in this respect is the sol-gel packaging of vesicles containing bacteriorhodopsin, a light-driven proton pump, and $F_0F_1$-ATP synthase [48] (Fig. 2.4B). Upon illumination of these sol gels, protons are pumped into the vesicles and ATP is created outside the vesicle by the ATP synthase. Besides the excellent stability of these gels (bacteriorhodopsin continued functioning for a month), this technology provides a convenient packaging method and a way to use light energy for fueling devices. Another interesting development is the engineering of polypeptides that can specifically bind to inorganic materials [49]. When engineered into motor proteins, this technology could provide new opportunities for motor-driven nanoscale assembly of different materials.

A related but even more futuristic field is the development of artificial molecular machines by bottom-up organic chemistry [50]. Artificial molecular machines are synthesized molecules that can switch between different shapes upon illumination with light or through electrochemical reactions. An illustrative example is shown in Fig. 2.4C. The molecular motor molecules, embedded within the surface of a textured liquid crystal film, induce the rotation of a macroscopic glass particle through a continuous reorganization of the film texture [44]. Although the stability of these molecular machines is probably superior to proteins, their control, directionality, and interface to the outside world are yet far less developed [50].

The small size and force-exerting capabilities of motor proteins and the range of opportunities for specific engineering give them unique advantages over current human-made motors. Upon studying and using biomotors, we will gather a lot of knowledge that is of interest to biology, material science, and chemistry, and it is reasonable to expect spin-offs for medicine, sensors, electronics, or engineering. The exploration of biomotors in technology will thus remain an interdisciplinary playground for many years to come.

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References


Chapter 3

High Rectifying Efficiencies of Microtubule Motility on Kinesin-Coated Gold Nanostructures

We demonstrate highly efficient rectification of microtubule motility on gold nanofabricated structures. First, we present a novel nanofabrication process for the creation of gold tracks for microtubule motility recessed in silicon oxide. This approach is particularly useful because it enables the use of the well-understood PEG-silane chemistry on SiO$_2$ for the blocking of kinesin, whereas the gold tracks allow possible electrical control. We demonstrate excellent confinement of microtubule motility to the gold nanostructures and that microtubules move on the gold with speeds comparable to that on glass. Secondly, we present designs of three advanced rectifier geometries. We analyze the microtubule pathways through the geometries, and we demonstrate highly efficient rectification with up to 92% efficiency. As a result, we find that up to 97% of the microtubules move unidirectionally.

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3.1 Introduction

Biomolecular motors are complex nanosized protein assemblies that have the ability to convert chemical energy into mechanical work. The motor protein kinesin uses the energy from ATP hydrolysis to step along microtubule filaments [1]. A microtubule is a protein assembly, consisting of tubulin subunits polymerized into a tubular structure with an outer diameter of 25 nm and lengths up to tens of microns. Integration of biomolecular motors in nanoengineered environments may lead to new technologies, in which parallel and automated control of molecular matter may become possible [2]. In the inverted gliding assay, kinesin motor proteins are adsorbed onto a substrate, and microtubules are propelled in random directions over the surface. If the kinesin–microtubule system is to be used in a reliable transportation system, then the movement of microtubules has to be effectively guided, confined and directed. In particular, the rectification of microtubule movement will be crucial for molecular sorting applications, where it is envisioned that a large number of microtubules approaches a Y-junction unidirectionally from one side. Efforts have been made to create micro- and nanofabricated channels in a combination with chemical patterning to confine and guide the motion of microtubules [3–6]. Unidirectional motion of microtubules in fabricated structures has been obtained by using arrow-shaped rectifier designs that redirected microtubule motion with up to 70% efficiency [3]. In all previous work, contrast in active motor density was obtained by using polymer material and absorbed detergents or block copolymers as the inactive region and glass substrates as the motility-supporting material. However, the mechanism behind the blocking of kinesin adsorption on the detergent-coated polymers is generally not well understood, and conflicting results have been reported [3, 4, 7]. Moreover, we previously demonstrated that the integration of metal electrodes into the structures can be advantageous, for example, to use electric fields to control microtubule docking [8].

In this work, we describe a new fabrication process for the creation of gold tracks for microtubule motility recessed in silicon oxide (SiO$_2$). We show that motility is possible on gold surfaces, and we demonstrate a high contrast in microtubule motility on gold versus PEG-coated SiO$_2$. We present designs of three advanced rectifier geometries aimed at high rectification efficiencies. We demonstrate and analyze the highly efficient rectification in all three rectifier geometries.
3.2 Fabrication process

To achieve a high contrast in active motor density, we use nanofabrication techniques combined with chemical adsorption (Fig. 3.1a). As substrates, we used silicon wafers, on which a 1-µm-thick layer of SiO$_2$ was thermally grown. The Si/SiO$_2$ substrates were cleaned by subsequent sonications during 5 min in acetone, fuming nitric acid, and 2-propanol (IPA). Then, a 1.25-µm-thick layer of electron-beam (e-beam)-sensitive resist poly(methyl methacrylate) (PMMA, molecular weight 950K, 7% solution of PMMA dissolved in chlorobenzene) was spin-coated (2250 rpm for 1 min) onto the substrate. The samples were baked on a hot plate for 15 – 40 min at 175 °C. Structures were written with a Leica electron-beam pattern-generator with a dose of 1600 µC/cm$^2$. The samples were developed by a 75-s immersion in a mixture (1:3) of methyl-isobutyl ketone and IPA and a subsequent immersion of 25 s in IPA to stop the development. We then used a reactive-ion etching step in CHF$_3$/O$_2$ plasma (flow rates of 100 sccm and 2.5 sccm) to transfer the pattern $\sim$800 nm into the SiO$_2$. The dry etching creates a highly anisotropic trench profile with steep sidewalls. The pressure of the plasma was set to 50 µbar to achieve an anisotropic etch and keep the etch selectivity between the SiO$_2$ and PMMA reasonable. Etch rates for SiO$_2$ and PMMA were $\sim$30 nm min$^{-1}$ and 23 nm min$^{-1}$, respectively. E-beam evaporation was used to deposit first a 5-nm sticking layer of chromium (Cr) and then a 30-nm-thick layer of gold onto the substrates. Lift-off was performed to remove the resist such that only the metal on the bottom of the trench was left. To enhance the lift-off, we exposed the samples for 7.5 min to a high-pressure (200 µbar) O$_2$ (50 sccm) plasma etch. The lift-off was done by sonicating the samples for 2 min in heated (90 °C) positive-resist stripper (PRS3000), sonicating for 10 s in ddH$_2$O, and sonicating for 10 s in IPA.

To block motility on the trench sidewalls and elevated SiO$_2$ regions, we used a poly(ethylene glycol) (PEG) self-assembled monolayer (SAM) that is known to be protein repellant [9, 10]. The grafting of the PEG-SAM was based on the protocol described in ref. [9]. The trimethoxysilane headgroup of the SAM covalently binds to the SiO$_2$ such that the PEG-tails stick out from the surface. The samples were first sonicated for 5 min in a mixture of ethanol and water (1:1). Then, the samples were submerged for 10 min in Piranha solution, a strongly oxidating mixture of hydrogen peroxide (31%) and sulfuric acid (96%) in a 3:1 mixture, heated to 75 °C. Then the samples were rinsed three times in ddH$_2$O and once in ethanol and finally sonicated for 10 min in ddH$_2$O. After sonication, the samples were blown dry with nitrogen and directly immersed in the PEG solution. The PEG solution consisted of 5 mM 2-[methoxypoly(ethyleneoxy)propyl]-trimethoxysilane
(90%, ABCR Karlsruhe, molecules about 3 nm long) and 0.08% hydrochloric acid (36%) in toluene. After 24 h, the samples were taken out of the grafting solution, rinsed in toluene, rinsed in ethanol twice, rinsed in ddH₂O twice, sonicated in ddH₂O for 2 min, and finally blown dry with nitrogen.

3.3 Device characterization

In Fig. 3.1b, we show a scanning electron microscope (SEM) image of a cross section of a nanofabricated structure for microtubule motility. The arrowlike structure was designed to rectify microtubule motility as discussed below. The picture clearly shows recessed areas covered with gold and elevated SiO₂ plateaus. The inset shows a close-up of the cross section, where the boundary between the Si and the thermally grown SiO₂ layer is clearly visible. The trenches have a rectangular profile as a result of the dry etching. Because of the rectangular profile and the fact that e-beam evaporation is a very directional process, the gold is evaporated only on the trench bottoms and not on the trench sidewalls. In combination with the PEG grafting on the SiO₂, this prevents kinesin molecules from adsorbing onto the trench sidewalls and accordingly helps to confine the microtubules to the trenches.

3.4 Motility Characterization

We tested microtubule motility [11] in our nanofabricated structures using a flow cell constructed from the substrate, spacers of double-sided tape, and a microscope glass cover slip. The microtubules were imaged by fluorescence microscopy on an inverted Olympus IX81 microscope using a Zeiss 100× oil-immersion objective (1.30 NA) and a Hamamatsu Orca CCD camera.

Figure 3.2 shows images of a high contrast in microtubule motility in three different structures. Figure 3.2a shows a rectifier structure intended to redirect the downward motion of microtubules into the upward direction [3]. Figure 3.2b shows a circular track with four straight tangential connections, which causes microtubules to move in the counterclockwise direction [12]. In Fig. 3.2c we show a track ending in a Y-junction. In each picture, we superimposed a bright-field reflection image of the gold–SiO₂ substrate (green) with a fluorescence image (red) of the microtubules.

We find a clear contrast in the density of microtubules between the gold and the PEG-coated SiO₂. Microtubules are observed to move only on the recessed gold areas, but never on the SiO₂ plateaus. In the same experiments, however,
3.4 Motility Characterization

Figure 3.1: (a) Cross-sectional outlines of the fabrication process. Substrates are 500-µm-thick Si wafers, on which a 1-µm-thick layer of SiO2 was thermally grown. In the first step, an e-beam-sensitive resist layer was spin-coated onto the substrates. Then, e-beam lithography was used to define the structures, and the pattern was developed. In the third step, an etching plasma transferred the patterns into the SiO2. Then, e-beam evaporation was used to deposit 5 nm chromium and 30 nm gold. Fifth, lift-off was done to remove the resist and metal layers outside of the trench bottoms. In the sixth step, a PEG monolayer (~3 nm tail size) was grafted onto the SiO2 to block motility outside the gold areas. Finally, kinesin was adsorbed onto the gold, propelling microtubules through the trenches. (b) SEM image of a rectifier structure. The substrate was broken to show the device in cross section. SEM imaging was done under an angle. The inset shows a 2× magnification of the cross section.
motility was always observed on the glass cover slip. In control experiments with the PEG−SAM omitted, microtubules moved over both the gold and the SiO$_2$ regions (data not shown). We attribute the absence of motility on the SiO$_2$ to a low concentration of kinesin molecules due to the protein repellant properties of the PEG [10]. We determined that microtubules moved on the gold with a speed of 0.73 ± 0.13 $\mu$m/s (mean ± standard deviation). This compares well with the speed on the glass cover slide (0.78 ± 0.08 $\mu$m/s).

We observe a distinct variation in the microtubule density on the gold nanostructures from different experiments. Under similar experimental conditions, some samples show a microtubule surface density that is comparable to that on the glass cover slip as well as good guiding within the channels, whereas other samples had almost no microtubules moving over the gold and a high chance of microtubule detachment upon colliding with sidewalls. These differences were not due to the fabrication process or chemical surface treatments because we observed the variations also between samples that were prepared in the same run. To quantify these observations, we examined the guiding probability for microtubules upon colliding with sidewalls in our structures. To this aim, we traced microtubules moving through a rectifier structure and we measured, for each collision with a sidewall, the approach angle ($\alpha$) and the outcome of the collision (guided along the wall and remaining on the surface or detached from the gold and lost to solution). Figure 3.3 shows the guiding probability as a function of approach angle (in 10° bins) for two typical assays. The assay with high microtubule surface density shows very effective guiding (100%) up to collision angles of 50 − 60°. Only for higher angles does the probability of staying within the

**Figure 3.2:** Overlay images of a bright field picture of the gold structures (green) and fluorescence images of microtubules (red). All images show a very clear contrast in microtubule density between the gold and SiO$_2$ areas (black). All scale bars are 10 $\mu$m. (a) Rectifier structure. (b) Circular structure used to generate motility in the counterclockwise direction. (c) Straight track ending in a Y-junction.
channel decrease to about 50% for orthogonal collisions. The assay with a low microtubule surface density already shows a decrease in guiding probability for angles higher than $20 - 30^\circ$, and the probability drops to about 20% for orthogonal collisions. The guiding performance for high collision angles in our devices is less than in some reports by other groups [4, 5], who used resist sidewalls on glass substrates. This may be due to a lower kinesin density on gold compared to glass surfaces [8]. The method presented in this work has the advantage of integrating metal electrodes into the tracks.

A variation in the active kinesin density is a possible explanation for the decreased guiding performance at high collision angles and the varying microtubule density among samples. The equilibrium surface density of microtubules is proportional to the bulk microtubule concentration and the binding affinity (defined as the on-rate divided by the off-rate) of microtubules [8]. The bulk concentration of microtubules is approximately constant between different experiments. Furthermore, possible differences in the microtubule concentration would not affect the guiding probability (Fig. 3.3). However, we expect that a lower motor density has a negative effect on both the binding affinity as well as on the guiding probability. We thus speculate that the kinesin density varies among different samples, for reasons that are so far not well understood.
3.5 Rectification of motility

To demonstrate and achieve unidirectional motion of randomly moving microtubules, we designed and tested three different rectifier geometries. The designs with relevant dimensions are shown in Fig. 3.5b–d. The rectifiers are all designed to rectify microtubule motility into the upward (as drawn) direction; that is, microtubules entering from the upper channel entrance should be reversed in direction and exit through the same upper channel exit. Microtubules that enter from the lower entrance should pass through the structure and exit through the upper channel exit. Design A (Fig. 3.5b) is the simplest form of a rectifier structure. Design B (Fig. 3.5c) has an entrance that is slightly offset with respect to the exit in order to minimize the chance of a microtubule passing straight from the upper to lower channel. Finally, design C (Fig. 3.5d) has been proposed before for a 'thermal-ratchet sorting' [12, 13] of the microtubules. A microtubule entering from the upper entrance has only a slight chance of making a sharp turn and passing through in the downward direction. Indeed, it has a high chance of being guided into the rounded region and being redirected towards the upper exit. The curved channels in designs B and C are meant to align the exit and entrance channels for an easy modular integration into larger designs.

Figure 3.4 shows examples of rectification and passing events of microtubules. Figure 3.4a displays snapshots with 20 s intervals of a microtubule entering from the lower entrance and exiting through the upper channel as intended. Figure 3.4b shows snapshots of the successful rectification of a microtubule that enters and exits through the upper entrance. At \( t = 60 \) s, the microtubule has collided with the sidewall at the bottom of the rounded region on the right and is being redirected by the wall \( (t = 80, 100 \) and \( 120 \) s) back toward the upper channel \( (t = 140 \) s). Figure 3.4c shows the superimposed traces of all microtubule paths passing through a structure of design B during 75 s. Most microtubules originating from the upper entrance (red traces) follow a more or less straight path down, are guided in the rounded region on the right, and are redirected by the rounded walls, following the sidewalls back to the original entrance. The set of microtubules that originates from the lower channel (green traces) mainly pass straight through the structure toward the funnel at the upper channel, as intended. The yellow traces result from overlap in green and red microtubule paths.

To quantify the performance of the three rectifier designs, we systematically traced and analyzed the traversal of microtubules through the structures according to their origin and destination. As indicated in Fig. 3.4d, microtubules can enter the structure through the lower ("passing") channel entrance \( (I_p) \) and then either be rectified \( (R_p) \), pass through the structure \( (P_p) \), or be lost to so-
3.5 Rectification of motility

![Figure 3.4](image)

**Figure 3.4:** Microtubule traversal through a rectifier structure. The structure is designed to rectify microtubule motility in the upward direction. Microtubules entering the structure from below should pass unhindered. (a) Demonstration of a passing event. A microtubule entering the structure from below traverses the structure in 60 s and exits through the upper channel. The picture on the right traces the traversed path. (b) Demonstration of a rectification event. A microtubule entering the structure from the upper channel \((t = 0 \text{ s})\) traverses the structure, collides with the round region at the right side \((t = 60 \text{ s})\) and is redirected along the sidewalls \((t = 80 \text{ s}, 100, \text{ and } 120 \text{ s})\) in the upward direction. Finally \((t = 140 \text{ s})\), it exits the structure through the same channel. As a result of the process, its velocity has been reversed. The picture on the right traces the traversed path. (c) Overlay image of all microtubule paths during 75 s in a rectifier of design B. Microtubule paths originating from the upper entrance are shown red, microtubules from the lower entrance are shown green. The yellow areas result from overlapping green and red traces. (d) Schematic classification of all possible pathways of microtubules traversing a rectifier structure, according to their origin and destination. Arrows denote the direction of motion. Symbols are defined in the text and represent numbers of microtubules.

Solution \((L_p)\). Microtubules that exit the structure through the lower passing exit \((O_p)\) have originated either from the passing entrance itself via a rectification event \((R_p)\), from the upper ("rectifying") entrance via a passing event \((P_r)\), or from solution via binding \((C_p)\). A similar classification can also be made for the upper rectifying entrance by interchanging the subscripts ‘\(r\)’ and ‘\(p\)’. 
Figure 3.5: Results of the analysis of microtubule traversal through three different rectifiers designs. (a) Legend for b–d. Desired pathways are shown in solid lines, and undesired pathways are shown by dotted lines. The rectifier geometries are designed such that microtubules entering the structure from the upper channel ($I_r$) should be rectified and exit through the same channel ($R_r$). Passing through and exiting through the other channel ($P_r$) or detachment and loss to solution ($L_r$) are undesired. Conversely, microtubules entering through the lower channel ($I_p$) should pass unhindered and exit through the opposite channel ($P_p$), whereas none should be lost ($L_p$) or reversed ($R_p$). (b–d) Results for three different designs. The arrow diagrams denote the fraction of microtubules and the absolute number (between brackets) that traverse the structure according to the legend in a. The width of the arrow scales with the fraction of microtubules. The designs with their relevant dimensions are shown on the bottom of each graph.

We demonstrate a clear asymmetry between both entrances in the number of microtubules traversing the different pathways, as shown in Fig. 3.5. Figure 3.5a depicts the desired traversals in solid lines, and undesired pathways in dotted lines. Microtubules entering the structure from the lower channel ought to pass through, whereas microtubules entering from the upper channel should be reversed. For the three rectifier designs, Fig. 3.5b–d shows, for each entrance, the redirected, passed, and lost fractions (and absolute numbers) of the total amount of microtubules that entered each structure. In all three designs, microtubules that enter the structure through the lower passing entrance ($I_p$) are mainly passed through ($P_p = 64$ to $75\%$) as desired, whereas only a negligible amount of microtubules is reversed ($R_p = 0$ to $2\%$). The remainder of the microtubules is lost to solution, mainly after collisions with sidewalls. Conversely, of the microtubules entering through the upper rectifying entrance ($I_r$) a large fraction reverses its direction ($R_r = 38$ and $53\%$) as intended, and only a small fraction passes through without being rectified ($P_r = 5$ to $12\%$). The remainder of the microtubules is lost to solution. The observed losses are roughly consistent
3.5 Rectification of motility

Table 3.1: Measured rectifying ($R_t$) and passing ($P_p$) fractions and rectifying ($\eta_r$) and passing ($\eta_p$) efficiencies for three rectifier designs. The standard errors are calculated as $\sqrt{p(1-p)/n}$ [14].

<table>
<thead>
<tr>
<th></th>
<th>$R_t$</th>
<th>$P_p$</th>
<th>$\eta_r$</th>
<th>$\eta_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>design A</td>
<td>0.47 ± 0.03</td>
<td>0.64 ± 0.04</td>
<td>0.79 ± 0.03</td>
<td>0.98 ± 0.01</td>
</tr>
<tr>
<td>design B</td>
<td>0.53 ± 0.04</td>
<td>0.75 ± 0.03</td>
<td>0.92 ± 0.03</td>
<td>1.00</td>
</tr>
<tr>
<td>design C</td>
<td>0.38 ± 0.04</td>
<td>0.66 ± 0.04</td>
<td>0.83 ± 0.04</td>
<td>0.97 ± 0.02</td>
</tr>
</tbody>
</table>

with the data presented in Fig. 3.3 for high collision angles. It is difficult to distinguish between the rectifying efficiencies of the three different designs by a straightforward comparison of the $R_t$ values because the variation in the fraction of microtubules that is lost to solution ($L_r$ varies between 41 and 55%) is about as large as the variation in $R_t$ (between 38 and 53%), obscuring possible differences in the rectifying efficiencies.

To enable a meaningful comparison between the three designs, we estimate the rectifying efficiency ($\eta_r$) and passing efficiency ($\eta_p$) with respect to the number of microtubules that remain surface-bound:

$$\eta_r = \frac{R_t}{I_t - L_r},$$

$$\eta_p = \frac{P_p}{I_p - L_p}.$$  (3.1)  (3.2)

Table 3.1 displays the measured fractions $R_t$ and $P_p$ and the corresponding rectifying and passing efficiencies $\eta_r$ and $\eta_p$. Excellent rectifying behavior is obtained. In all cases, the estimated rectifying efficiencies (between 0.79 and 0.92) are very high, and the passing efficiencies are close to unity. Hiratsuka et al. previously published a rectifying probability of 0.7 [3]. We do not find a significant difference in the performance of the three devices, although design B seems slightly more efficient with a rectifying efficiency of 0.92, and a passing efficiency of 1.00.

As a result of the efficient rectifier geometries, we observe that a very large fraction of the microtubules traversing the lower channel move unidirectionally into the upward direction. The number of microtubules that pass through the lower channel in the upward direction is $I_p$, whereas the number of microtubules traversing the same channel in the downward direction is $P_r + R_p$ (Fig. 3.5a). We thus find that in design B 97% of the microtubules in the lower channel move unidirectionally into the upward direction. In a future exploitation of rectifier geometries, the rectifying efficiencies can even be improved by placing multiple rectifiers in series.
3.6 Conclusions

We have described a novel fabrication process for the creation of gold nanotrails for microtubule motility. We used a combination of topographic patterning and chemical adsorption to create a very high contrast in active motor density, where active kinesin adsorbs only on gold patterns recessed in SiO$_2$ trenches. This fabrication approach offers the opportunity of using the well-understood PEG–silane chemistry to block kinesin adsorption on SiO$_2$, whereas the gold nanostructures can be advantageous for electrical control. We demonstrated microtubule motility in our gold nanostructures, and we observed that microtubules moved on the gold with speeds of $0.73 \pm 0.13 \mu m/s$, which is comparable to the speed on the glass cover slide ($0.78 \pm 0.08 \mu m/s$). Microtubules were never present on PEG-coated SiO$_2$. We characterized the guiding probability of microtubules upon sidewall collisions in our structures. Finally, we introduced three new designs for rectification structures for which we thoroughly analyzed the microtubule pathways. A rectifying efficiency as high as 92% was demonstrated, whereas the passing efficiency for microtubules moving in the desired direction was about 100%. As a result, we obtained unidirectional motion of 97% of the microtubules. The possibilities of using gold as a substrate and controlling the directionality of motility may be important for the use of biomolecular motors in novel applications such as a molecular transportation system.

References


[11] The protocol for the motility experiments was as follows. The flow cell was first incubated for 5 min with a casein solution containing 0.5 mg/mL casein in BRB80 buffer (80 mM Pipes, 1 mM MgCl$_2$, 1 mM EGTA, pH=6.9). Then, kinesin was added to the flow cell (6 µg/mL full-length *Drosophila* conventional kinesin in BRB80, 0.2 mg/mL casein, 1 mM ATP) and incubated for 5 min. Finally, the flow cell contents were exchanged with motility solution containing rhodamine-labeled paclitaxel-stabilized microtubules (∼30 nM tubulin, 1 mM ATP, 1 mM MgCl$_2$, 10 µM Taxol, and anti-bleaching cocktail (20 mM D-Glucose, 0.020 mg/mL glucose oxidase, 0.008 mg/mL catalase, 1% β-mercaptoethanol)) all in BRB80 buffer. Microtubules were polymerized from 10 µL of bovine brain tubulin (4 mg/mL, 1 rhodamine labeled unit, 3 unlabeled units, Cytoskeleton, Denver, CO) in the presence of 4 mM MgCl$_2$, 1 mM GTP and 5% DMSO in BRB80 buffer (37 °C for 60 min). Then the microtubules were stabilized and 100× diluted in BRB80 containing 10 µM Taxol.


[14] The standard error for binomial distributions is defined as $\sqrt{p(1-p)/n}$, where $p$ is the measured probability of success and $n$ is the number of observations. This definition underestimates the standard error for values of $p$ approaching 0 or 1.
We demonstrate localized electrical control of the docking of microtubules onto engineered kinesin-coated structures. After applying a voltage to a gold electrode, we observe an enhanced transport of microtubules from solution toward the surface and a subsequent increase of the amount of moving microtubule shuttles. Switching off the voltage leads to a partial detachment of microtubules from the surface. The surface coverage of microtubules, during both the docking and undocking events, follows an exponential time dependence. We provide a simple kinetic model, incorporating the equilibrium between free and surface-bound microtubules, that explains these data.

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4.1 Introduction

The biological cell possesses a variety of force generating elements to perform mechanical work. For example, kinesin motor proteins can bind some cargo and walk along microtubules, their associated protein filaments, thus providing a means of directed intracellular transport of material. Another well-known example of force generation is the contracting muscle system, caused by myosin motors that translocate actin filaments. Both kinesin and myosin derive energy from the hydrolysis of adenosine trisphosphate (ATP). Exploitation of biomolecular motors in a nanofabricated environment will open up ways of controlling matter on the nanoscale [1, 2], for example the transport of DNA molecules [3] or polystyrene beads [4]. Recent work has concentrated on using the filaments as transporting shuttles [3–5] that glide over motor-protein-coated surfaces. Micro- and nanofabrication techniques, chemical patterning, and combinations thereof have been used to create well-defined tracks for filament shuttles (microtubules [4, 6–9], or actin [10–12]), allowing spatial control over the motility. Electric fields that can be switched on and off in time have been used to influence velocity and orientation of microtubules [6, 13] and actin filaments [14, 15] in motility assays at a large (∼mm) macroscopic scale. Combined spatial and temporal control over microtubule motility has, however, not been reported until now.

In this paper we report the use of localized electric fields to control the transport of negatively charged microtubule shuttles from bulk solution into nanoengineered structures on a substrate. When scaling down the tracks for microtubule motility toward smaller dimensions, there is a need for a more efficient docking of microtubules into those structures. One way to achieve this would be to use large kinesin-coated areas that collect microtubules from solution and feed them into the network of tracks (Fig. 4.1a). The use of nanofabrication techniques allows for an easy coupling between the microscopic docking pads and the nanosized microtubule tracks. Another tool, which does allow additional control, is to use switchable electric fields to drive microtubules toward the substrate. Nanofabrication makes it possible to integrate the electrodes into the structures, thereby enabling the control of shuttle docking at specific places on a substrate.

4.2 Results and discussion

To demonstrate the electrical docking of microtubules, we used nanofabrication techniques to fabricate gold (Au) electrodes in recessed areas in silicon dioxide (SiO$_2$) substrates (Fig. 4.1a) and chemical patterning to block motility on the SiO$_2$. The details of the fabrication process involve the following. Onto a silicon
4.2 Results and discussion

Figure 4.1: (a) Scanning electron microscope (SEM) image of a nanofabricated structure serving as a track for microtubule motility. The large droplet-shaped area is a docking place for microtubule shuttles from solution. The inset shows the connection of the docking area into the circular structure. The tracks consist of recessed areas in the SiO$_2$ substrates (see inset, depth $\sim 860$ nm), with a gold-covered bottom. (b) Schematic layout of a flow cell (not to scale). Square test microstructures (yellow) are fabricated in the substrate, according to the process described in the text. Similar to the SEM pictures in (a), the structures consist of gold areas recessed into SiO$_2$. They are connected to bonding pads at the edge of the chip via small leads. Two 0.1 mm thick spacers cover the bonding pads and part of the connection leads. The top of the flow cell consists of a cover slide with an Au counter electrode at the inside. A transparent window is kept open for microscopy imaging. Voltages (V) were applied between the test structures and the counter electrode and the resulting current (I) was measured.

(Si) substrate with a 1 $\mu$m thick layer of thermally grown SiO$_2$, an electron-beam (e-beam) sensitive resist layer of 600 nm was spun. The patterns were defined by e-beam lithography, and a reactive ion-etching step then transferred the pattern into the SiO$_2$. Finally, e-beam evaporation was used to deposit a 5 nm thick chromium (Cr) sticking layer and a 30 nm thick Au layer. This was followed by a lift-off step to remove the resist. The samples were subsequently cleaned and a hydrophilic poly-ethylene-glycol (PEG) ([methoxy(poly-ethyleneoxy)-propyl]-trimethoxysilane, 90%, ABCR Karlsruhe) self-assembled monolayer (SAM) was grafted onto the SiO$_2$. Finally, a hydrophobic hexadecanethiol-SAM was grafted onto the gold. In the experiments, we used $9 \times 9$ mm$^2$ substrates on which square test structures with lengths of 50 and 100 $\mu$m were fabricated. Two small leads ($2000 \times 2 \mu$m$^2$) connected the test structures to bonding electrodes at the edge of the substrate. The bonding electrodes were connected to the outside electronics by wire bonding. A flow cell (Fig. 4.1b) was built by putting two spacers ($\sim 0.1$ mm thick Parafilm or Scotch double-sided tape) over the bonding electrodes and part of the leads and covering the chip with a microscope slide ($24 \times 24$ mm$^2$). A 0.1 $\mu$m thick gold layer was evaporated on the cover slide as the counter electrode. A transparent window of $\sim 3$mm width was kept free from gold to allow for imaging.
Microtubule motility was imaged by fluorescence microscopy with an inverted Olympus microscope (Zeiss 100× oil objective, 1.30 NA) and images were recorded with a CCD camera (Hamamatsu ORCA). The flow cell was first incubated for 5 min with a casein solution (0.5 mg/mL casein in BRB80 buffer (80 mM pipes, 1 mM MgCl$_2$, 1 mM EGTA, pH = 6.9)) to passivate the Au surface. Then a kinesin-containing solution was perfused into the flow cell (6 µg/mL full-length Drosophila conventional kinesin in BRB80, 0.2 mg/mL casein, 1 mM ATP) and incubated for 5 min. Finally, the flow cell contents were exchanged with motility solution containing rhodamine-labeled paclitaxel-stabilized microtubules [16] (~30 nM tubulin, 1 mM ATP, 1 mM MgCl$_2$, 10 µM Taxol), an anti-bleaching cocktail (20 mM D-glucose, 0.020 mg/mL glucose oxidase, 0.008 mg/mL catalase, 1% β-mercaptoethanol (BME)), and a redox mediator (3 mM ferrocenedimethanol (Fc(MeOH)$_2$)), all in BRB80 buffer. As intended, microtubules could be visualized moving on both the cover slide as well as on the gold, but not on the SiO$_2$. Voltage pulses were applied to the microelectrode, with a duration of 30 to 90 s and with amplitudes between +0.5 and +1.2 V (with respect to the counter electrode). Fluorescence images were taken each second. For both the application of the voltages and the monitoring of the resulting current, an electrochemical potentiostat (BAS CV50W) was used in a two-electrode setup.

Figure 4.2 shows the increasing density of microtubules upon applying a voltage to an electrode. A typical sequence of snapshots from a movie is displayed, as taken during and after the application of a voltage. Initially (Fig. 4.2a), the number of microtubules present at the gold surface inside the square structure is limited. Then, at $t = 0$ s, a voltage of 0.7 V is applied during 60 s and the number of microtubules at the electrode surface starts to increase with time ($t = 20$ s, $t = 60$ s). After switching off the voltage (at $t = 60$ s) the number of microtubules that are moving on the gold surface gradually decreases again. Microtubules were seen to detach from the gold either by spontaneous release or by collisions with the SiO$_2$ walls of the structure. At $t = 120$ s (60 s after switching off the voltage), the number of microtubules inside the structure has decreased considerably, although there are still more than at the beginning of the docking event (cf. Fig. 4.2a, $t = 0$ versus $t = 120$ s). In general, motility was observed to continue unimpeded during bias, although some microtubules got stuck to the gold after landing. In some rare cases, motility was slowed or halted during application of the voltage. Motility on the SiO$_2$ substrate (i.e., outside the square area in Fig. 4.2) was not observed due to the blocking by the PEG monolayer.

To analyze the docking efficiency of microtubules, we quantify the amount of microtubules present in each camera image. In an automated fashion we count
4.2 Results and discussion

The total number of pixels belonging to microtubule particles in each frame. The latter number serves to quantify the microtubule density. The image processing of the raw images consisted of a brightness-contrast-gamma correction and a convolution filter step to improve image quality (see Appendix 4.A). An automatic thresholding was then used to discriminate between microtubule particles and background. As a result, a binary version of each camera image was obtained with all microtubule pixels set to 1 and all background pixels set to 0. Finally, particles smaller than a critical size were removed. To determine the time evolution of the microtubule-covered area ($A$), this analysis was done for each camera image.

The result of this analysis is shown in Fig. 4.3b, where we plot the microtubule-covered area during the event depicted in Fig. 4.2. Upon application of the voltage at $t = 0$ s, the surface coverage of microtubules starts to increase, from an initial value of $\sim 10^3$ pixels to $20 \cdot 10^3$ pixels at $t = 60$ s. The microtubule density thus has increased more than an order of magnitude. After switching off the voltage ($t = 60$ s), the surface coverage decreases again, in accordance with Fig. 4.2, toward a value of $\sim 10^3$ pixels at $t = 125$ s. From the time trace it is clear that the growth rate of microtubules on the surface levels off with increasing time. The decrease in coverage after switching off the voltage follows a similar time dependence. We found that both the growth and decay traces can be well fit with exponentials (red lines in Fig. 4.3b).
Figure 4.3: Time traces of the microtubule-covered area ($A$) and current density ($J_q$). (a,b) Measurement data of $A$ (b) and $J_q$ (a) corresponding to the event shown in Fig. 4.2. The increase and decrease in area were fitted with exponentials (red lines, see text) with time constants $\tau$ equal to 37 and 27 s for the docking and undocking, respectively. (c,d) Series of docking and undocking events from a different experiment. A variety of voltage pulse durations (30 to 90 s) and amplitudes (0.55 to 0.75 V) was applied. The high noise in the $A(t)$ data present in the last event in Fig. 4.3d is due to the passing of cluster of fluorescent material. (e,f) Results from an analytical model [eqs 6-8] describing the qualitative response in surface coverage (f) upon application of a block-shaped $J_q$ pulse of 60 s duration (e). Time constants were chosen in accordance with the time constants in (b) to facilitate a comparison. (g,h) Control experiment on a glass surface. Plotted is the measured change in surface coverage of microtubules on a glass slide (h) after changing the bulk microtubule concentration $[M]$ (g). At $t = 0$ s, microtubules are added and at $t = 900$ s the flow cell was flushed with a motility solution without microtubules. The docking and undocking from the glass slide follow similar exponential time dependences (red lines). The plotted time dependence of $[M]$ (g) is an estimate based on the fits in (h). All images contained 672 x 512 pixels.

In Fig. 4.3a, we also plot the time evolution of the current density ($J_q$) during the same event. The values of $J_q$ were obtained by dividing the measured current by the area of the gold exposed to solution [17]. Upon application of the voltage, $J_q$ initially peaks and then a gradual decrease is observed. Within $\sim 10$ s the current density reaches an approximately constant value of about 35 A/m$^2$ (measured current $\sim 200$ nA).
As a further investigation, we show in Fig. 4.3c and d similar data ($J_q$ and $A$) for a series of docking events measured in another experiment. In this measurement series, we applied a variety of pulse durations and amplitudes. The time evolutions of both $J_q$ and $A$ show a similar behavior as described above. We observe a correlation between the peak value of $A$ and the magnitude of $J_q$ in each event.

We attribute the transport of microtubules into the structures to migration as a result of the electrochemical current \[18\]. Upon application of a positive bias to the electrode, both Fc(MeOH)$_2$ and BME are being oxidized at the electrode surface. Depletion of these redox species at the electrode surface leads to the development of an electric field in the solution, driving a migration current. A part of this migration current will be carried by negatively charged microtubules \[6, 13\] in solution, resulting in the transport toward the electrode. The decrease of $J_q$ with time can be associated with the formation of a depletion region of electroactive species close to the electrode \[19\]. Both the data of the docking events upon application of a voltage, and the subsequent undocking data can be explained by considering the transport and the binding/unbinding of microtubules at the electrode surface.

We now develop a simple model to describe these data. We consider, on one hand, the transport of microtubules between the bulk and the surface, and on the other hand, the binding and unbinding dynamics of microtubules at the surface (Fig. 4.4). We thus distinguish microtubules in three different states, viz., microtubules bound to kinesin molecules at the surface, free microtubules at the surface (but not bound to kinesin), and free microtubules in the bulk. Microtubules in the bulk (volume concentration $[M]$) can be transported toward the surface by both diffusion and migration. We denote the local volume concentration of microtubules in a small layer $dz$ above the surface as $[M_0]$. Microtubules within this distance $dz$ from the surface can bind to kinesin molecules with a rate, denoted $k'_b$, and start moving over the surface (surface concentration of bound microtubules $[MK]$). Bound microtubules can also detach from the surface with a rate $k_{\text{off}}$.

Considering first the transport of microtubules between the bulk and the surface, the rate of change in the total amount of microtubules (bound and free) at the surface is given as

$$\frac{\partial ([MK] + dz[M_0])}{\partial t} = J_{\text{electric}} + J_{\text{diff}}, \quad (4.1)$$

where $J_{\text{electric}}$ and $J_{\text{diff}}$ are the migration and diffusion-driven fluxes of micro-
Figure 4.4: Schematic of the transport of microtubules between 3 different states. Microtubules can be transported between the bulk solution (volume concentration $[M]$) and the surface (local volume concentration $[M_0]$) via diffusion and via migration transport in the case of an electric field. Free microtubules close to the surface are in equilibrium with microtubules bound to kinesin molecules (surface concentration of kinesin-bound microtubules $[MK]$). Only microtubules that are within a distance $dz$ from the surface can interact with and bind to kinesin. The microtubule fluxes at the surface are denoted $J_{\text{on}}$ and $J_{\text{off}}$, for binding and unbinding, respectively. We denote the diffusion flux $J_{\text{diff}}$ (driven by a concentration gradient over a characteristic length $h$) and the migration flux $J_{\text{electric}}$. We denote the height of the flow cell as $z$.

The microtubules, respectively. We approximate the magnitude of $J_{\text{diff}}$ using the result for linear diffusion [19] as

$$J_{\text{diff}} = D_{\text{mt}} \frac{([M] - [M_0])}{h} = k_{\text{diff}} ([M] - [M_0]).$$

(4.2)

Here, $k_{\text{diff}} = D_{\text{mt}}/h$, where $D_{\text{mt}}$ is the diffusion constant of a microtubule and $h$ is the length scale of the diffusion layer thickness. Upon application of a voltage to the electrode, an electrochemical current starts to flow and microtubules migrate toward the electrode as a result of the electric field in the solution. The additional flux of microtubules is proportional to the bulk concentration and mobility of microtubules ($\mu_{\text{mt}}$) and the electric field in the bulk. The latter quantity equals $J_{\text{q}}/\sigma$ (with $\sigma$ the conductivity), yielding [18]

$$J_{\text{electric}} = \frac{\mu_{\text{mt}} J_{\text{q}}}{\sigma} [M].$$

(4.3)
Finally, assuming equilibrium between $[MK]$ and $[M_0]$ at all times [20], $[MK]$ can be expressed as a fraction of the total amount of microtubules (bound and free) present at the surface:

\[ [MK](t) = \frac{k_b}{k_b + dzk_{\text{off}}} ([MK](t) + dz[M_0](t)), \]  

(4.4)

where $dz \cdot k'_b = k_b$. Combining Eqs. 4.1–4.4 yields for the rate of change in the concentration of microtubules bound to the surface:

\[ \frac{\partial [MK]}{\partial t} = \frac{k_b}{k_b + dzk_{\text{off}}} \left( \frac{k_{\text{diff}} + \mu_{\text{mt}}J_q}{\sigma} [M] - \frac{k_{\text{off}}k_{\text{diff}}[MK]}{k_b} \right). \]  

(4.5)

To convert the surface concentration of microtubules $[MK]$ into units of covered-area pixels ($A$), we use that $A = [MK]N_A S a_0$. Here, $N_A$ is Avogadro’s number, $S$ is the electrode area (in m²) visible within the camera image, and $a_0$ is the average area of a microtubule in an image (in pixels). Using this transform and solving Eq. 4.5 (boundary condition $A_{t=0} = A_0$) under the assumption that the microtubule concentration in the bulk is constant [21], we obtain for $A(t)$:

\[ A(t) = A_{ss} + (A_0 - A_{ss})e^{-t/\tau}, \]  

(4.6)

where the time constant $\tau = (k_{\text{off}}k_{\text{diff}}/k_b)^{-1}$ under the assumption that $k_b \gg dzk_{\text{off}}$ [20]. We will show later from the results that this assumption indeed holds. The steady-state coverage $A_{ss}$ is given by

\[ A_{ss} = k_{\text{on}}(J_q)[M] \tau AS a_0, \]  

(4.7)

where we defined an effective transport on-rate $k_{\text{on}}(J_q)$ as

\[ k_{\text{on}}(J_q) = \left( k_{\text{diff}} + \frac{\mu_{\text{mt}}}{\sigma} J_q \right). \]  

(4.8)

Figure 4.3 shows that Eq. 4.6 provides a good analytical description of the observed data. In Fig. 4.3f we plot the time evolution of the covered area upon application of a block-shaped $J_q$ pulse of 60 s duration (Fig. 4.3e). As a result of the $J_q$ there is an exponential growth in covered area from $A_0$ toward a steady-state coverage $A_{ss}$. The magnitude of $A_{ss}$ depends on $J_q$ according to Eqs. 4.7 and 4.8. Switching off $J_q$ leads to an exponential decay toward the initial $A_{ss}(J_q = 0)$.

We analyzed 45 docking and subsequent undocking events from 12 movies that were recorded in different experiments in two electrode sizes (50 and 100 µm lengths). First, all the undocking events were fitted with the model Eq. 4.6, with
Figure 4.5: Histogram of time constants derived from fitting the undocking events. Events in both electrode structures (45 events) were used. The arrow indicates the median value. The inverse of the time constant equals $k_b/(k_{off}k_{diff}) = \tau^{-1} = 4 \cdot 10^{-2} \text{ s}^{-1}$.

$A_0$, $A_{ss}$, and $\tau$ as free parameters. For each undocking event, we calculated the values of the binding affinity ratio $k_b/k_{off}$ and the diffusion transport rate $k_{diff}$ via Eqs. 4.7 and 4.8 [22], putting $J_q = 0$. The results of the fitted values for $\tau$, $k_b/k_{off}$, and $k_{diff}$ are displayed in Table 4.1. The distribution of time constants is given in Fig. 4.5. A clear peak between 10 and 30 s is present in the data, with the median value of the distribution $\tau = 25 \pm 3 \text{ s}$ (standard error of the mean).

We compare the derived average value of $k_{diff}$, viz., $(5.3 \pm 0.8) \cdot 10^{-7} \text{ m/s}$ (Table 4.1), with an estimate for $k_{diff}$ (Eq. 4.2). For the latter we use $D_{mt} = 4 \cdot 10^{-13} \text{ m}^2/\text{s}$, as calculated from the Einstein relation $D = k_B T/\gamma$. Here, $k_B$ is the Boltzmann constant, $T$ is the absolute temperature, and $\gamma$ is the drag coefficient. The value of $\gamma$ was analytically estimated to be $\gamma = 10^{-8} \text{ kg/s}$ for a cylinder with the dimensions of a microtubule [23] (Appendix 4.B). Using this order-of-magnitude estimate for $D_{mt}$ we can calculate that, on the time scale of the experiments, the diffusion layer thickness $h$ is on the order of a few $\mu\text{m}$ ($h = \sqrt{D_{mt} t}$). Thus we obtain a rough estimate for $k_{diff} = 10^{-7} \text{ m/s}$, which is in good agreement with the fitted value.

As a second step in the analysis, we used Eq. 4.6 to fit the data of the docking events in order to investigate the correlation between $A_{ss}$ and $J_q$. In the fitting

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Visible electrode area $S$ (µm²)</th>
<th>Time constant $\tau$ (s)</th>
<th>Diffusion rate $k_{diff}$ (m/s)</th>
<th>Binding affinity $k_b/k_{off}$ (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small ($N = 16$)</td>
<td>2500</td>
<td>24 ± 2</td>
<td>$(6.2 \pm 1.4) \cdot 10^{-7}$</td>
<td>$(9.8 \pm 1.3) \cdot 10^{-6}$</td>
</tr>
<tr>
<td>Large ($N = 29$)</td>
<td>4880</td>
<td>25 ± 4</td>
<td>$(4.8 \pm 1.0) \cdot 10^{-7}$</td>
<td>$(9.1 \pm 1.2) \cdot 10^{-6}$</td>
</tr>
<tr>
<td>All events ($N = 45$)</td>
<td>not applicable</td>
<td>25 ± 3</td>
<td>$(5.3 \pm 0.8) \cdot 10^{-7}$</td>
<td>$(9.3 \pm 0.9) \cdot 10^{-6}$</td>
</tr>
</tbody>
</table>
4.2 Results and discussion

**Figure 4.6:** Steady-state microtubule coverage as a function of current density at the electrode. Lines are least-squares fits through the data with the y-axis intercept fixed at the $k_{\text{diff}}$ values for each electrode size (Table 4.1). (a) Data recorded in a (50 µm)$^2$-sized electrode; data points corresponding to events shown in Fig. 4.3c and d. The fit has a slope $\partial k_{\text{on}}/\partial J_q = 1.4 \cdot 10^{-7}$ m$^3$/As ($\partial A_{ss}/\partial J_q = 5.6 \cdot 10^2$ pixels m$^2$/A). (b) Data for a movie recorded in a (100 µm)$^2$-sized electrode. Slope $\partial k_{\text{on}}/\partial J_q = 4.1 \cdot 10^{-8}$ m$^3$/As from the fit ($\partial A_{ss}/\partial J_q = 2.9 \cdot 10^2$ pixels m$^2$/A).

We confirm the validity of the equilibrium model for docking and undocking of microtubules to a surface with a control experiment on a glass surface (Fig. 4.3h). Here, we measured the time evolution of microtubules binding to a kinesin-coated
glass slide in a flow cell consisting of two glass cover slides separated by 100 µm thick spacers. At \( t = 20 \text{ s} \) we flushed the flow cell with motility solution containing 2 pM microtubules (Fig. 4.3g). As predicted from the model, the microtubule population on glass grows exponentially toward an equilibrium value. Then, at \( t = 920 \text{ s} \) the flow cell was flushed with a motility solution without microtubules, effectively setting \([M] = 0\) at that time (second arrow in Fig. 4.3g). As a result, the population of microtubules bound to the glass flow cell decreases exponentially toward its new equilibrium value. We can compare the rate constants for microtubule docking onto our gold microstructures with the docking observed on a macroscopic glass slide. In the case of a glass flow cell the value of \([M]\) cannot be taken constant during the experiment \[21\]. This results in a slight modification in the expressions for \( \tau \) and \( A_{ss} \), but the qualitative behavior for the surface coverage is similar. From the fits in Fig. 4.3h we calculate (Appendix 4.C) a binding affinity of microtubules on a glass surface of \( k_b/k_{off} = 2 \cdot 10^{-5} \text{ m} \). This is slightly higher than the value \( 0.9 \pm 10^{-5} \text{ m} \) found for the gold surfaces in our structures, in line with the observation that the microtubule density on glass (Fig. 4.3h, \( A_{ss} \approx 10^4 \text{ pixels in a 61 } \times \text{ 80 } \mu\text{m}^2 \text{ camera image} \)) is usually slightly higher than on gold surfaces without applied voltage (Fig. 4.3d, \( A_{ss}(J_q = 0) \approx 10^3 \text{ pixels in a 50 } \times \text{ 50 } \mu\text{m}^2 \text{ electrode area} \)).

### 4.3 Conclusions

In conclusion, we have observed the electrostatic docking of microtubule shuttles into microfabricated structures after application of a voltage. The microtubule coverage on the gold surface exponentially approaches a steady-state equilibrium value \( A_{ss} \). The value of \( A_{ss} \) is found to be linearly dependent on the current density flowing through the electrode. Switching off the applied voltage leads to the partial undocking of microtubules, again displaying an exponential dependence. We provided a simple kinetic model for the equilibrium between microtubules in solution and microtubules bound to kinesin at the surface. From this model, we estimated the binding affinity of microtubules for a gold surface to be \( k_b/k_{off} = 0.9 \cdot 10^{-5} \text{ m} \), which is lower than for a glass surface \( (k_b/k_{off} = 2 \cdot 10^{-5} \text{ m}) \). From the data we also derived that the diffusion-driven transport rate \( k_{diff} \) of microtubules into square gold electrodes was \( k_{diff} = (5.3 \pm 0.8) \cdot 10^{-7} \text{ m/s} \). We achieved a \( J_q \)-dependent increase of the microtubule-transport rate (up to a 10-fold increase compared to \( k_{diff} \)) by applying a voltage to the gold surface. We attribute this effect to migration of microtubules. We have shown that the same model also describes the binding and unbinding to a glass surface. The use of
localized electric fields in nanofabricated structures and the better understanding of the binding dynamics of microtubule shuttles to a surface will be useful for the future exploitation of biomolecular motors in a nanotransport system.

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References


[16] Microtubules were polymerized from 10 µL of bovine brain tubulin (4 mg/mL, 1 rhodamine labelled unit, 3 unlabelled units, Cytoskeleton, Denver USA) in presence of 4 mM MgCl$_2$, 1 mM GTP and 5% DMSO in BRB80 buffer (37 °C for 60 min.). Then the microtubules were stabilized and 100× diluted in BRB80 containing 10 µM Taxol (BRB80T). The final tubulin concentration would then be ∼32 nM. For the experiments in the 100 µm sized electrode, the 10 µL of polymerized microtubules were stabilized and diluted 40× in BRB80T. To remove the unpolymerized tubulin, the 400 µL was centrifuged at 178,000g in a Beckman airfuge for 5 min and the pellet was resuspended in 1000 µL of BRB80T, yielding again a tubulin concentration of ∼32 nM. In all experiments, we shortened the microtubules by shearing them in a 30g needle.

[17] The connection leads had ∼1500×2 µm$^2$ area ($A_{\text{lead}}$) exposed to the solution, which is significant compared to the areas of the square structures ($A_{\text{square}}$). To calculate the current density we therefore divided the measured current by the total exposed gold area ($A_{\text{lead}} + A_{\text{square}}$). In general the diffusion-limited current density is dependent on the geometry of the electrode. The observed $J_q$, therefore, should be a weighted average of the $J_q$ values for a square and rectangular microelectrode and $J_q$ as calculated in our procedure does not necessarily equal the exact current density at the square electrode. Also, because the ratio $A_{\text{lead}}/A_{\text{square}}$ is different for the 50 and 100 µm-sized structures, the calculated $J_q$ values can, strictly speaking, not be directly compared between both electrode sizes.


[20] The assumption of assuming equilibrium between [MK] and [M$_0$] is equivalent to assuming that $(k_b + dz \cdot k_{\text{off}}) \gg k_{\text{diff}}$. From the experiments we derive that $k_b \gg dz \cdot k_{\text{off}}$ (upon adopting a reasonable value for $dz = 10^{-8}$ m), so the surface-equilibrium assumption simplifies to assuming $k_b \gg k_{\text{diff}}$. Although the validity of this statement cannot be directly confirmed from the data (as we have no way to extract the value of $k_b$ from the data), we can show that the less strict condition $k_b \geq k_{\text{diff}}$ does hold. In the most pessimistic case $k_b \approx k_{\text{diff}}$, the fitted values for $k_{\text{diff}}$ and $\mu_m/\sigma$ then represent lower
bounds, while the presented value of $k_b/k_{\text{off}}$ is still exact. We also solved the model under the opposite assumption ($k_b \ll k_{\text{diff}}$) and made fits to the data. This yielded values for $k_b$ and $k_{\text{diff}}$ that were the same order of magnitude ($k_b \approx k_{\text{diff}}$), thereby invalidating the assumption that $k_b \ll k_{\text{diff}}$ and ruling out this possibility.

[21] The approximation that $[M]$ is a constant is valid as long as the amount of microtubules docking into a structure is insignificant compared to the total number of microtubules in solution. In the electrically controlled docking experiments, this assumption holds well since the electrode area is small. \(Viz.,\) the total number of microtubules inside the flow cell volume ($\sim 5 \mu L$) is estimated as $\sim 3 \cdot 10^6$, while the number of microtubules attracted inside an electrode structure is much smaller, $\sim 10^2 – 10^3$. For the control experiment in a glass flow cell (Fig. 4.3h), however, $[M]$ is changed significantly during the experiment, since microtubules dock over the entire flow cell area. From Fig. 4.3h we estimate $\sim 10^6$ microtubules moving on the glass, a significant part of the number of microtubules in solution (Fig. 4.3g). As a result, the expressions for $\tau$ and $A_{ss}$ are slightly modified in this control experiment. (See Appendix 4.C).

[22] We used the following values for the different variables: $\mu_{mt} = 3 \cdot 10^{-8}$ m$^2$/Vs (from Ref. [13]), $a_0 = 100$ pixels/microtubule (determined from camera images) and $\sigma = 0.7$ A/Vm (measured with a conductivity meter). The microtubule concentration in all experiments was estimated to be $[M] \approx 10^{-12}$ M. This value was calculated assuming complete polymerization from the tubulin concentration of $\sim 32$ nM, an average microtubule length of 5 $\mu$m and each microtubule containing $\sim 10^4$ tubulin dimers.

Supplementary Information

This appendix contains additional information that was published as supporting information to the main text.

4.A Image processing

To analyze the number of microtubules inside a camera image, we used home-written Labview software (National Instruments, with IMAQ Vision add-on) with standard built-in functions. The image processing consisted of the following steps: a brightness-contrast-gamma (BCG) correction, spatial filtering, automatic thresholding, removal of small particles and a particle erosion step. The total number of pixels belonging to particles was used as a measure of the number of particles. Figure S.1 shows the results of the processing in the different steps. In Fig. S.1a we show the original grayscale image (672 × 512 pixels), with microtubules inside a square 50 × 50 µm² test structure. There is a significant background signal from fluorescent microtubules in the bulk solution. First, a linear high-pass convolution-filtering step is applied (Fig. S.1b). The filtering step consist of a convolution of the original image with a convolution kernel matrix, in our case a 7 × 7 Laplacian matrix. The coefficients of a Laplacian matrix are all set to a value of −1, except for the central coefficient that was set to values between +49 and +60. The effect of the filtering step is to replace each pixel value by a weighted average of the values of the pixels itself and its neighbor pixels. This particular filter acts as a gradient operator, enhancing regions of high variations in light intensity. Secondly, we used built-in thresholding functionality to separate the distribution of grayscale values in two populations. Pixels with an intensity value below an automatically determined threshold were set to 0, pixels with higher intensity where set to 1. The result is a binary version of the image (Fig. S.1c). To remove the noise, all particles with an area below a critical value (usually 30 – 50 pixels) were removed. Finally, a particle erosion step was performed to eliminate isolated pixels, yielding the final binary image (Fig. S.1d). The total number of pixels set to 1 was determined from the final image.
Supplementary Information

Figure S.1: Image processing steps used in the determination of the microtubule coverage inside a camera image. The total number of pixels inside a frame belonging to microtubule particles was taken as a measure of the total number of particles. (a) Raw data grayscale (8-bit) image showing microtubules inside a square 50 x 50 µm² test structure. (b) Same 8-bit image as in (a) after a BCG-correction and spatial high-pass filtering step. The effect of the filtering step is to highlight details. Both microtubule particles and noisy pixels are enhanced. (c) Automatic thresholding of the image in (b) yields a binarized (1-bit) version of the image. All pixels considered to be part of the background are set to 0 (black), while all pixels considered to belong to particles are set to 1 (white). (d) The final binary image after a particle-removal and a particle-erosion step. All particles with areas smaller than 50 pixels were removed. The total number of pixels in this picture was determined to be 16196 pixels.

4.B Estimate of the diffusion constant of a microtubule

To estimate the value of $D_{mt}$ we use the Einstein relation $D_{mt} = k_B T / \gamma$. Here $k_B T$ is the thermal energy, equal to the product of Boltzmann's constant $k_B$ and the absolute temperature $T$, and $\gamma$ is the drag coefficient of a microtubule. Following reference [13] we estimate $\gamma$ as the drag coefficient for a cylinder, $\gamma = f L \eta$. Here $\eta$ is the dynamic fluid viscosity, $L$ the length of the cylinder, and $f$ a dimensionless factor incorporating the average orientation of the cylinder axis with respect to the velocity vector. Using $f = 1.9$ [13, 23], $\eta = 10^{-3}$ kg/ms for the viscosity of water, and $L = 5 \mu m$ for the microtubule length, we can calculate that $D_{mt} = 4 \cdot 10^{-13} \text{ m}^2/\text{s}$ at room temperature. A theoretical estimate from for the value of $k_{diff}$, describing the diffusion transport of microtubules then yields $4 \cdot 10^{-7} \text{ m/s}$. 
4.C Full solution for the growth rate of microtubule coverage on glass

In the main text we solved Eq. 4.5 under the assumption that the microtubule concentration \([M]\) in the bulk is constant. This assumption is valid as long as the number of microtubules attracted to the electrode surface is much smaller than the total amount of microtubules present in the bulk. For the small area electrodes in the experiments the assumption holds. However, in the experiment shown in Fig. 4.3h we showed a control experiment in which the microtubule coverage on a glass cover slide was measured. In this situation \([M]\) is not constant anymore, but instead it is a function of the density of microtubules bound to the surface \([MK]\). We now solve the complete model as depicted in Fig. 4.4 in the main text. In this general case, Eqs. 4.1 to 4.5 in the main text stay valid, the only difference with the treatment in the main text is that we now take the value of \([M]\) in Eq. 4.5 to be time dependent. We already defined the fluxes of microtubules between the bulk and surface due to diffusion and migration as \(J_{\text{diff}}\) (Eq. 4.1) and \(J_{\text{electric}}\) (Eq. 4.2), respectively. In the glass experiment, \(J_{\text{electric}}\) is non-existent. For completeness, we define here the fluxes due to binding and unbinding of microtubules to kinesin molecules at the surface:

\[
J_{\text{on}} = k'_b dz[M_0] = k_b dz[M_0],
\]

\[
J_{\text{off}} = k_{\text{off}}[MK].
\]

From assuming equilibrium at the surface and considering then the rate of change in the surface concentration of \([MK]\), we arrived at Eq. 4.5 in the main text. To solve this equation using a time dependent bulk concentration, one has to use the fact that the total number of microtubules summed over all 3 states is conserved:

\[
2[MK](t) + 2dz[M_0](t) + z[M](t) = 2[MK]_{t=0} + 2dz[M_0]_{t=0} + z[M]_{t=0}
\]

This equation can be simplified by substituting \([MK] + dz[M_0] = (k_b + dzk_{\text{off}})[MK]/k_b\) (Eq. 4.4), with the resulting expression for \([M](t)\):

\[
[M](t) = \frac{2(k_b + dzk_{\text{off}})}{z k_b} ([MK]_{t=0} - [MK](t)) + [M]_{t=0}.
\]

Finally, substituting this expression for \([M]\) into Eq. 4.5 and then solving for \([MK]\) yields similar exponential solutions as Eq. 4.6 in the main text for the
growth and decay of microtubules on the glass surface. The time constant $\tau$ of the exponential is, however, modified to:

$$\tau = \frac{1}{k_{\text{diff}}} \left( \frac{k_{\text{off}}}{k_b} + \frac{2}{z} \right)^{-1}. \tag{S.5}$$

The corresponding steady-state coverage of microtubules $A_{ss}$ is:

$$A_{ss} = k_{\text{diff}} \tau \left( [M]_{t=0} + \frac{2}{z} + [MK]_{t=0} \right) N_a S a_0. \tag{S.6}$$

Finally, we can calculate then the ratio $k_b/k_{\text{off}}$ from the fitted steady-state coverage (Eq. S.6) for the docking and undocking on the glass surfaces. Substituting $[MK]_{t=0} N_a S a_0 = A_0$, we calculate $k_b/k_{\text{off}}$ for the growth ($[MK]_{t=0} = 0$) and decay ($[M]_{t=0} = 0$) cases respectively as:

$$\frac{k_b}{k_{\text{off}}} = \frac{z A_{ss}}{[M]_{t=0} N_a S a_0 z - 2 A_{ss}} \tag{S.7}$$

$$\frac{k_b}{k_{\text{off}}} = \frac{z}{2 \left( \frac{A_0}{A_{ss}} - 1 \right)} \tag{S.8}$$

With the obtained ratio $k_b/k_{\text{off}}$ from Eqs. S.7 and S.8, and with the fitted time constant, we can finally calculate $k_{\text{diff}}$ from Eq. S.5. The fits in Fig. 4.3h in the main text yield fitted values $A_{ss} = 11 \cdot 10^3$ pixels, and $\tau = 186$ s for the growth curve and $A_{ss} = 2.0 \cdot 10^3$ pixels, $\tau = 125$ s and $A_0 = 11 \cdot 10^3$ pixels for the decay curve. Using Eqs. S.7, S.8, and S.5, we calculate that in the growth regime $k_b/k_{\text{off}} = 3 \cdot 10^{-5}$ m and $k_{\text{diff}} = 1 \cdot 10^{-7}$ m/s and in the decay regime $k_b/k_{\text{off}} = 1 \cdot 10^{-5}$ m and $k_{\text{diff}} = 0.7 \cdot 10^{-7}$ m/s. Since we performed this particular experiment only once, we take the average $k_b/k_{\text{off}} = 2 \cdot 10^{-5}$ m as an estimate for the binding affinity on a glass surface.
Chapter 5

Molecular Sorting by Electrical Steering of Microtubules in Kinesin-Coated Channels

Integration of biomolecular motors in nanoengineered structures raises the intriguing possibility of manipulating materials on nanometer scales. We have managed to integrate kinesin motor proteins in closed submicron channels and to realize active electrical control of the direction of individual kinesin-propelled microtubule filaments at Y junctions. Using this technique, we demonstrate molecular sorting of differently labeled microtubules. We attribute the steering of microtubules to electric field-induced bending of the leading tip. From measurements of the orientation-dependent electrophoretic motion of individual, freely suspended microtubules, we estimate the net applied force on the tip to be in the picoNewton range and we infer an effective charge of 12 e\(^{-1}\) per tubulin dimer under physiological conditions.

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5.1 Introduction

Recent years have witnessed a strong interest in the exploration of biomolecular motors in nanotechnology [1–4]. A molecular motor such as kinesin, which translocates in 8-nm steps along microtubule filaments through hydrolysis of adenosine triphosphate (ATP) [5], can potentially be used as the workhorse in miniaturized analytical systems or nanoelectromechanical systems [6, 7]. In particular, it is envisioned that microtubule shuttles translocating over kinesin-coated tracks can be used to carry a specific cargo to designated places on a chip [8]. First steps toward such applications have been realized, such as partial confinement of microtubules to micron-sized kinesin-coated trenches [9–11], docking of shuttles to their tracks [12], rectification of motility [13, 14], and coupling of cargo [15]. The much-sought-after goal of dynamic control of the direction of individual microtubules, a key requirement for molecular sorting applications, has not been achieved so far. Attempts to use electric fields to manipulate the negatively charged microtubules have only resulted in large-scale alignment [16] or bulk transport of the filaments [11, 12].

In this report, we reconstituted the kinesin-microtubule transport system in enclosed fluidic channels, which represents two major advances. First, fluidic channels achieved full confinement of the microtubules to their tracks, without the need for any surface modifications or selective patterning of kinesin molecules in open-trench structures. Second, the confined geometry of channels allowed the localized application of strong, directed electric fields, which could be exploited to steer individual microtubules, as well as to perform single-molecule biophysical experiments. By measuring the electrophoretic motion of individual microtubules, we determined the magnitude of the electric field-induced force on the microtubule tip, and we directly confirmed the predicted anisotropy in electrophoretic mobility.

5.2 Results and discussion

We fabricated fluidic channels 800 nm deep between entrance holes in fused-silica substrates and sealed them (Fig. 5.1, A and B). Microtubule motility was reconstituted in the channels by a pressure-driven flow to flush the necessary protein constituents (casein, kinesin, and fluorescently labeled paclitaxel-stabilized associated proteinfree microtubules) from the entrance reservoirs into the channels (Fig. 5.1C). Using epifluorescence microscopy, we could discern microtubules moving on either the top or bottom surface of a channel by adjusting the focus of the objective (Fig. 5.1D). The enclosed geometry of the device com-
5.2 Results and discussion

Figure 5.1: Enclosed submicron channels allow for full confinement of microtubules. (A) E-beam lithography and wet etching create 800-nm-deep trenches in SiO$_2$ substrates. A coverslip coated with a sodium-silicate solution (dark blue) seals the channel after annealing. (B) Scanning electron microscopy image of a cross section of a nanochannel. (C) Schematic cross section along a channel. Kinesin motor proteins (green) and microtubule filaments (red) are added to the channels by a pressure-driven flow. Electric fields can be induced in the channels by applying a potential difference between the reservoirs. (D) Fluorescence microscopy shows that microtubules (red) move on both sides of the channels (blue). Yellow ovals indicate microtubules in the focal plane; white ones denote microtubules just out of focus. (E) Traces of several microtubules moving through a confined channel. The figure is an overlay of microtubule traces traversing the image during a period of 7 min. No microtubules are observed outside of the tracks. (F) More complex geometries, such as the bend in a rectifying geometry, could also be well coated with kinesin molecules and could support microtubule motility.

 completamente confined the microtubules to their tracks (Fig. 5.1E). All regions could be coated with kinesin proteins, even less accessible regions in more complicated networks, such as bends (Fig. 5.1F). The speed of the microtubules in our channels ($0.75 \pm 0.02 \, \mu m/s$) was the same as on a glass coverslip in a standard flow cell ($0.74 \pm 0.04 \, \mu m/s$). By applying a voltage difference between platinum electrodes inserted in reservoirs at either end of a perpendicular cross-channel, we induced an electric field $E$ perpendicular to the direction of microtubule motion (Fig. 5.2A). In this way, the electrical force on the negatively charged microtubules was directed opposite to the electric field.

We demonstrated that microtubules can be directed with an electric field. The trajectory of a microtubule that was subjected to an electric field of strength $|E| = 35 \, kV/m$ (70 V over 2 mm) is shown in Fig. 5.2B. At the beginning of the path, the microtubule was oriented perpendicular to the electric field. As
the microtubule progressed, its leading end gradually oriented itself opposite to the applied field, until the microtubule finally changed course by 90° and moved parallel to the electric field and toward the positive electrode. A trace of the leading and trailing-end coordinates of the microtubule showed that they followed exactly the same path (Fig. 5.2B). This clearly indicates that there was no motion of the microtubule perpendicular to its long axis, which is expected if kinesin molecules hold onto the microtubule. Up to 110 kV/m, we did not observe a measurable increase or decrease of the microtubule velocity due to the electric field [17].

The electric force was used to actively steer individual microtubules into a desired channel of a Y junction, across and through which a perpendicular channel was fabricated in order to confine the electric field. As a microtubule approached the junction, it was steered into the right channel by adjustment of the perpendicular electric field, whose magnitude was between 0 and 50 kV/m (Fig. 5.2C). Figure 5.2D shows an experiment in which many microtubules were selectively steered into the right or the left channel.

We attribute the observed steering of the microtubules to a biased search of the free leading tip of the microtubule for the next kinesin motor (Fig. 5.2E) [16]. As a microtubule is propelled by kinesin molecules, its tip fluctuates freely by Brownian motion until it binds to a new kinesin motor [18]. In the absence of applied force, the equilibrium position of the free tip is collinear with the microtubule’s long axis, which gives an equal probability of finding the next

Figure 5.2: (Right page) Bending and steering of microtubules by an electric field. (A) An electric field \( E \) is induced in a horizontal channel perpendicular to the vertical direction of microtubule (red) motion. The electric force on the negatively charged microtubule is along \( E \), denoted by the blue arrow. Furthermore, the applied electric field will induce motion of the positive ions in the double layer at the channel walls (characteristic thickness \( \lambda_D \)), causing an electro-osmotic flow in the channel. (B) Overlapping coordinates of leading (red symbols) and trailing (black symbols) ends of a microtubule oriented in a field \( |E| = 50 \text{ kV/m} \). (Inset) The positions trace (dashed) in a fluorescence image. (C) A microtubule entering from the central channel at the top is steered into the right lead of the Y junction by application of a perpendicular electric field that varies in magnitude between 0 and 50 kV/m (indicated by length of arrow). (D) By using magnitude and direction of the electric field, many microtubules are steered to the right (top panel) and left (bottom panel). (E) Bending of a microtubule by a net force. The main part of the microtubule is held fixed by kinesin molecules; only the free leading tip (length \( \delta \)) is subject to a force density \( f_{\text{net}} \), which bends it with curvature \( r^{-1} \). (F) Measurements of the average trajectories that microtubules travel under three different applied fields \( E \). Black traces represent averages of many individual paths. Gray lines are the individual trajectories at \( |E| = 0 \). At \( |E| \neq 0 \), the spread in the individual trajectories is similar, but they are not shown for clarity. Initial curvatures of the trajectories are denoted by red circles. (Inset) The linear relation between electric field strength \( |E| \) and curvature \( R^{-1} \). The values of \( f_{\text{net}} \) were calculated from \( E \) (see text).
motor in either direction from this axis. The presence of a perpendicular force, however, will bias the equilibrium position toward the direction of higher potential and thereby increases the probability of finding the next binding site in this direction.

The field-dependent trajectories of microtubules were traced to quantify the microscopic bending in experiments analogous to Fig. 5.2B. For $|E| = 0$, the average path of several ($N = 18$) microtubules was indeed a straight line in the initial direction of motion (Fig. 5.2F). For $|E| = 35$ kV/m, the average microtubule path deviated from the straight path, ultimately orienting itself along the electric field ($N = 17$). The curvature of the trajectory upon entering the electric field...
field was fitted by a circle of radius $R = 15 \pm 2 \, \mu m$. At a higher $|E| = 70 \, \text{kV/m}$, the average bending of the path ($N = 10$) was more pronounced, and the curvature was described by a circle of $R = 8 \pm 1 \, \mu m$. The observed curvature of the microtubule path increased linearly with applied electric field (Fig. 5.2F, inset). The average bending radius did not depend on microtubule length.

The forces that act on the microtubules in the presence of an electric field have a nontrivial origin. The net force per unit length on the microtubule, $f_{\text{net}}$, stems from two components. First, the electric force was shown to equal the drag that moving counterions in the microtubule’s double layer experience from the surrounding fluid: $f_e = c \mu_e E$ [19–21]. Here, $c$ is the Stokes drag coefficient per unit length and $\mu_e$ the (negative) electrophoretic mobility of the microtubule. The second contribution to the force on the microtubule results from the fluid motion in the channel due to the field-driven motion of counterions at the glass channel walls. This electro-osmotic flow (EOF) (Fig. 5.2A) exerts a drag force on the microtubule opposite from the electric force, $f_{\text{EOF}} = c \mu_{\text{EOF}} E$. Here, $\mu_{\text{EOF}}$ is the (positive) electro-osmotic mobility. At the beginning of the microtubule path, the electric field is perpendicular to the microtubule, resulting in a net force:

$$f_{\text{net}} = c \mu_{e,\bot} + c \mu_{\text{EOF}} E.$$  \hspace{1cm} (5.1)

Electrokinetic measurements were performed on single microtubules in order to measure the magnitude of this force. The electrophoretic mobility of rod-like polymers, such as microtubules, has only been treated theoretically, and is predicted to be anisotropic for perpendicular ($\mu_{e,\bot}$) and parallel ($\mu_{e,\parallel}$) orientations of the rod to the electric field [19, 22]:

$$\mu_{e,\parallel} = \frac{\varepsilon \zeta}{\eta},$$  \hspace{1cm} (5.2)

$$\mu_{e,\bot} = \frac{2}{3} g \varepsilon \zeta.$$  \hspace{1cm} (5.3)

Here, $\eta$ and $\varepsilon$ are the viscosity and dielectric constant of the fluid, respectively, and $\zeta$ is the electrostatic potential at the no-slip plane of the microtubule. The anisotropy factor $g$ accounts for the perturbation of the ionic atmosphere around the rod [19, 22] and approaches a maximum value of $3/2$ for infinitely small Debye lengths [23]. Measurements of these anisotropic electrophoretic mobilities of colloidal cylinders have not, as far as we know, been reported. The small height of our channels allowed us to observe individual microtubules in a single focal plane, which enabled us to test the predicted anisotropy of the mobility. The anisotropic mobilities induced an observable orientation-dependent velocity...
of microtubules, with components perpendicular \((v_x)\) and parallel \((v_y)\) to the electric field (Fig. S.1):

\[
v_x = \frac{1}{2} (\mu_{e,\parallel} - \mu_{e,\perp}) \sin(2\theta) E, \tag{5.4}
\]

\[
v_y = \left[ (\mu_{e,\parallel} - \mu_{e,\perp}) \sin^2(\theta) + (\mu_{e,\perp} + \mu_{EOF}) \right] E. \tag{5.5}
\]

Here, \(\theta\) is the orientation of the filament with respect to the \(x\) axis (Fig. 5.3A). Note that the velocity is not necessarily collinear with the electric field. The EOF adds linearly as an orientation-independent velocity decrease in the \(y\) direction.

The electrophoretic motion of individual microtubules in channels without kinesin is shown in Fig. 5.3A. In accordance with the bending experiments, we observed that freely suspended microtubules move opposite to the applied electric field along the channel. Values of orientation-dependent \(v_x\) (Fig. 5.3B) and \(v_y\) (Fig. 5.3C) were measured for different \(E\). The red lines are fits of Eqs. 5.4 and 5.5 through the binned data and clearly describe the \(\theta\) dependence of the velocities very well. These data are direct evidence of the anisotropic electrophoretic microtubule mobility, and the amplitudes of the curves provide a straightforward measure of the anisotropy. As expected from Eqs. 5.2 and 5.3, microtubules move fastest if they are oriented parallel to the electric field and slowest for perpendicular orientations (Fig. 5.3C). To extract the values of the anisotropic microtubule mobilities, we measured \(\mu_{EOF} = (1.33 \pm 0.01) \cdot 10^{-8} \text{ m}^2/\text{Vs}\) in our kinesin-coated channels (Fig. 5.3D), yielding \(\mu_{e,\perp} = (2.30 \pm 0.04) \cdot 10^{-8} \text{ m}^2/\text{Vs}\) and \(\mu_{e,\parallel} = (2.93 \pm 0.02) \cdot 10^{-8} \text{ m}^2/\text{Vs}\). The anisotropy in electrophoretic mobility is not related to the well-known factor 2 anisotropy in Stokes drag coefficients [24], because, in electrophoresis, the retarding shear acts on the length scale of the Debye length \(\lambda_D\), whereas, in Stokes drag, this length scale is on the order of the size of the object. The values of \(\mu_{EOF}, \mu_{e,\perp}, \text{ and } \mu_{e,\parallel}\) are all constant over the probed range of electric fields (insets of Fig. 5.3, B to D).

These mobility measurements permit a calculation of the net force applied in the bending experiments (Eq. 5.1). From the values \(c_\perp = (7.4 \pm 2.0) \cdot 10^{-3} \text{ kg/ms} \) [25] and \((\mu_{e,\perp} + \mu_{EOF}) = (9.7 \pm 0.2) \cdot 10^{-9} \text{ m}^2/\text{Vs}\), we calculated the net electrokinetic applied force on the microtubule as \(f_{net} = [(7.2 \pm 2.0) \cdot 10^{-11} \text{ N/V}]E\). The forces applied in the bending experiments (Fig. 5.2F) thus ranged up to \(5.0 \pm 1.4 \text{ pN/mm} \) at \(|E| = 70 \text{ kV/m}\). Knowledge of the magnitude of this force allowed determination of both the persistence length and the surface charge of the microtubules.

The persistence length of the microtubules was determined from observations of the bending radius under a known perpendicular force. The bending of the microtubule tip can be expressed in terms of the free tip length \(\delta\) (inversely
Figure 5.3: Electrophoresis of single microtubules and measurement of the EOF velocity. All measurements were performed at neutral pH in the presence of 160 mM K\(^+\). (A) Electrophoresis in channels of individual microtubules. Shown is an overlay of images at 0 s (blue microtubules) and at 0.6 s (red microtubules). Intermediate positions of two selected microtubules are indicated by white lines. The selected microtubules not only move in the direction of the electric field, but also perpendicular to it. (B and C) Binned data (N = 1496) of orientation-dependent electrophoretic velocities of microtubules in the x direction [perpendicular to the electric field (B)] and in the y direction [parallel to the electric field (C)]. Error bars denote standard deviation of binned data. The amplitudes \(A\) in both curves equal \((\mu_{e,\parallel} - \mu_{e,\perp})E\). The offset of the \(v_y\) graph, \(B_y = (\mu_{e,\perp} + \mu_{EOF})E \propto f_{\text{net}}\), applied during steering as in Fig. 5.2A. (Insets) Linear relation between \(B_y\) and \(E\). There was no significant dependence of the velocity on microtubule length. (D) EOF velocity as a function of electric field, measured by monitoring the change in resistance of a channel that is replenished by a solution of a different resistivity \(\rho\) by an EOF. (Inset) Two typical measurements of the time \(\Delta t\) that it takes the EOF to replace a channel volume (length 5 mm) at \(|V| = 80\) V. Each data point in the main graph represents an average of 4 to 6 separate measurements. The slope of the graph equals \(\mu_{EOF}\).

proportional to motor density [18]) and its persistence length \(L_p\). For perpendicular forces \(f_{\text{net}}\), the induced curvature \(r^{-1}\) of the free tip, in the limit of small deflections, is as follows (Fig. 5.2E and Fig. S.2):

\[
r^{-1} = \frac{1}{4k_B T L_p} \frac{\delta^2}{f_{\text{net}}},
\]

(5.6)

where \(k_B\) is Boltzmann’s constant and \(T\) is temperature. We assert that the
5.2 Results and discussion

Microscopic tip curvature $r^{-1}$ equals the macroscopic path curvature $R^{-1}$. From the linear relation of $R^{-1}$ versus $f_{\text{net}}$ (Fig. 5.2F, inset, and Eq. 5.6), we determined $\delta^2/L_p = 0.41 \pm 0.12 \text{nm}$. We estimated $\delta = 0.26 \pm 0.06 \mu\text{m}$ for the free tip length from our observations of the length of the shortest microtubules $(0.52 \pm 0.13 \mu\text{m})$ that still moved in smooth trajectories in our channels. The persistence length of the microtubule's free tip was thus determined to be $L_p = 0.16 \pm 0.09 \text{mm}$. This value is on the low end of published values on paclitaxel-stabilized microtubules that find $L_p = 0.2$ to $5.2 \text{mm}$ [26, 27], but the persistence length of the tip does not necessarily represent the persistence length of the entire microtubule [28].

The electric charge of a microtubule is determined in two independent ways from the electrophoretic mobilities. First, the value of $\mu_{e,\parallel}$ is proportional to $\zeta$ (Eq. 5.3), which is a direct measure of the surface charge density $\sigma$ via the linear Grahame equation $\sigma = \varepsilon \zeta / \lambda_D$. From $\mu_{e,\parallel}$, we find that $\zeta = -37 \text{mV}$, which yields a surface charge density of $0.20 \text{e}^-/\text{nm}^2$. A second measure of the surface charge follows from the value of $g_{\perp}$ [19, 22]. The experimentally determined anisotropy factor $g_{\perp} = \frac{3 \mu_{e,\perp}}{2 \mu_{e,\parallel}} = 1.18$ yields a value of $\zeta = -50 \text{mV}$ via the inverse transformation [23] $\zeta = F^{-1}(g_{\perp}, a/\lambda_D)$. This $\zeta$ corresponds to $\sigma = 0.27 \text{e}^-/\text{nm}^2$. The average of both measurements gives $\zeta = 0.24 \pm 0.04 \text{e}^-/\text{nm}^2$, with the error estimated from the difference between the two values [29].

From the measured $\sigma$ and the known 25-nm diameter of a 13-protofilament microtubule, we calculated an effective charge of $12 \pm 2 \text{e}^-$ for the solution-exposed surface of the tubulin dimer. A rough estimate of the bare charge of the free dimer of $47 \text{e}^-$ at pH = 6.9 follows from the crystal structure of the tubulin dimer [30], if one assumes the isolated dissociation constants of the individual amino acids. The effective charge constituted only about 25% of the bare charge, which can be attributed mainly to screening by counterions that are tightly bound to the microtubule surface.

Having developed an understanding of the steering mechanism of individual microtubules, we demonstrated the applicability of the electrical steering of microtubules in nanostructures by sorting a population of two different molecules on a chip (Fig. 5.4A). To this end, we introduced a mixture of rhodamine- (red) and fluorescein-labeled (green) microtubules into our nanostructures. Using a colorsensitive camera, red microtubules approaching a Y junction were sent into the red-collecting reservoir and green microtubules into the green reservoir (Fig. 5.4B) by reversing the polarity of the electric field. After a series of successful single-molecule redirections, one reservoir contained predominantly red microtubules, whereas the other reservoir contained mainly green microtubules (Fig. 5.4A).

We quantified the sorting efficiency in Fig. 5.4C. A large fraction (72%) of the red microtubules approaching the junction were directed, as intended, into the
Figure 5.4: Demonstration of molecular sorting. (A) Color image of a mixture of red- and green-labeled microtubules approaching a Y junction. Electrical force is used to steer microtubules carrying green and red fluorophores into the right and left reservoirs, respectively. (B) Example of successful sorting events for a green- and a red-labeled microtubule. As a function of time, first a green microtubule is steered into the right reservoir ($t \leq 10$ s), and subsequently a red microtubule is sent into the left reservoir. (C) Steering efficiencies for 189 red and 110 green microtubules. Steering of microtubules into the wrong reservoir ($3$ and $12\%$) was mainly due to differences in stiffness between microtubules or due to the simultaneous arrival of microtubules of opposite color. The fractions $19$ and $25\%$ of microtubules in the perpendicular channels are artificially high because microtubules were sometimes purposely directed into the perpendicular steering channel so as to avoid such errors.

red reservoir. Only 3\% of the red microtubules incorrectly ended up in the green reservoir. The remainder of the red microtubules (25\%) was steered into the perpendicular channel. A similar analysis was made for the green microtubules (69, 12, and 19\%, respectively, Fig. 5.4C). The final result is that 91\% of the microtubules sent into the left reservoir are red (136 out of 149), and 94\% of the microtubules in the right reservoir are green (76 out of 81). Real use of this sorting method, e.g., for purification, will benefit from automation, which can be incorporated straightforwardly. The use of biomolecular motors for sorting forms an interesting alternative to existing pressure- or EOF-driven microfluidic devices [31–33] by which whole cells are sorted.

5.3 Conclusions

In conclusion, our experiments demonstrate the ability to electrically steer individual microtubules in enclosed submicron channels, as demonstrated by the single-molecule sorting of fluorescein- and rhodamine-labeled microtubules. The steering of microtubules is described in terms of force-induced bending of the free tip of the microtubule, which yields a persistence length $L_p = 0.16 \pm 0.09$ mm. Our single-microtubule electrophoresis experiments revealed an orientation-dependent
electrophoretic mobility and yield a charge of $12 \pm 2 \, e^-$ per tubulin dimer under physiological conditions. This value may be important to elucidate the effect of in vivo electric forces on microtubules. Endogenous physiological electric fields, with a typical value up to $10^3 \, V/m$, are shown to be involved in cell division, wound healing [34], and embryonic cell development [35], but their microscopic effect has so far not been understood. The application of biomotors in nanofabricated environments is an exciting development, offering novel possibilities for future developments in lab-on-chip sorting or purification applications.

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**References**


At large fields, higher than $\sim 110 \text{ kV/m}$, we observe an effect on the microtubule velocity along the electric field. Microtubules moving parallel to the electric field display higher and lower speeds depending on the direction of the field. For fields oriented perpendicular to the long axis, microtubules display sideward motion. We are currently investigating these effects.

The electrophoretic force on a stationary microtubule in the absence of a bulk EOF consists of a direct force on the negative microtubule charge, $-|\sigma|E$, and an opposing indirect friction, $\tau$, exerted by the microtubule’s counterions moving along the electric field. The velocity of the counterions increases from zero at the microtubule surface to $|\mu_e|E$. If $\lambda_D \ll R$, the magnitude of $\tau = +|\sigma|E - c|\mu_e|E$ and equals the total force density exerted on the double layer, plus the drag force exerted on the moving counter ions [20].

The value of $g_\perp$ only depends on the $\zeta$-potential of a microtubule and the relative thickness of the double layer ($\lambda_D$) with respect to the cylinder radius $a$, i.e. $g_\perp = F(\zeta, a/\lambda_D)$, and numerical values have been tabulated [19]. In the limit of infinitely small $\lambda_D$, $g_\perp$ reaches its maximum value of 1.5 and $\mu_{e,\perp} \rightarrow \mu_{e,\parallel}$. The experimentally determined value of $g_\perp$ is thus a measure of the $\zeta$-potential via $\zeta = F^{-1}(g_\perp, a/\lambda_D)$, using $a = 12.5 \text{ nm}$, $\lambda_D = 0.8 \text{ nm}$ for the Debye length in our 160 mM buffer.

We calculate $c_\perp$ using the analytical result from Hunt et al. [24] with the following numerical values: microtubule radius $a = 12.5 \pm 1 \text{ nm}$, viscosity $\eta = 0.89 \pm 0.09 \cdot 10^{-3} \text{ kg/ms}$, $h = 30 \pm 10 \text{ nm}$ for the distance of the microtubule axis to the surface.
References


[28] For long microtubules and high kinesin density, the persistence length of the microtubule trajectory $L_{tr}$ equals the persistence length of the tip $L_p$ [18]. This trajectory persistence length of microtubules in the absence of electric fields has been quantified to be 0.11 mm [36]. Our value of the tip persistence length is close to this value. The suggestion of Kis *et al.* [37] that protofilament sliding reduces the stiffness of short lengths of microtubules, could serve as a possible explanation of the low $L_p$, together with possible defects in the tip structure.

[29] The use of the linear Grahame equation is strictly speaking only valid for $\zeta \ll k_B T/e = 26$ mV. However, at $\zeta = 50$ mV, the use of the linearized Grahame equation introduces only an error of 14 % in $\sigma$. The use of the non-linear version of the Grahame would invoke an unknown source of error, since we would then have to assume a value for the double-layer capacitance of the microtubule.


Supplementary Information

This appendix contains additional information that was published as supporting information to the main text.

5.A Materials and Methods

5.A.1 Nanofabrication

Nanofluidic channels were fabricated in 500 $\mu$m thick fused-silica substrates of $13 \times 19$ mm, containing a $2 \times 3$ array of 1 mm diameter entrance holes separated by 5 mm. The chips with holes (created by powder blasting) were purchased from PlanOptik (Unter den Eichen, Germany). Substrates were cleaned by subsequent sonications of 5 min in acetone, fuming nitric acid ($\text{HNO}_3$), and iso-propanol alcohol (IPA). Then, a 35 nm thick layer of chromium (Cr) was deposited by sputter deposition, functioning as an electron sink during electron-beam (e-beam) lithography. A $\sim 800$ nm thick e-beam-sensitive resist polymethylmethacrylate (PMMA) was spincoated on the sample, followed by a 15 min bake on a hotplate at 170 °C. Channel structures were defined with a Leica electron-beam pattern-generator with a dose of 1200 $\mu$C/cm$^2$. The samples were developed by a 75 s immersion in a mixture (1:3) of methyl-isobutyl ketone and IPA and a subsequent immersion of 45 s in IPA to stop the development. A 1 min immersion in Cr-etchant (Merck) removed the Cr layer in the structures, exposing the fused silica. The channels were wet etched $\sim 800$ nm into the substrates using ammonium-fluorid etchant (Merck 7:1). After etching, the resist and chromium were removed by a 15 min sonication of the substrates in PRS3000 (Merck) to strip the PMMA, and then a 1 min submersion in Cr-etchant.

The channel structures were sealed by a 170 $\mu$m thick microscope cover slip using a silica bonding procedure [38]. To this end, the fused-silica substrates and the cover slips were thoroughly cleaned by 15 min sonication in PRS3000 and 10 min sonication in $\text{HNO}_3$. Then, the substrates were exposed to a 0.5 % solution of hydro-fluor acid for 1 min. A 2% sodium-silicate solution (Sigma) was spin-coated (6000 rpm, 1 min) onto the cover slip, immediately followed by pressing the sodium-silicate surface to the patterned channel surface. Finally, the devices were cured for 2 hours at 90 °C.
5.A.2 Polymerization of microtubules

Microtubules were polymerized from 5 µL of bovine brain tubulin (4 mg/mL, Cytoskeleton, Denver, CO). For the red-labeled microtubules we used a stoichiometry of one rhodamine-labeled unit and three unlabeled units of tubulin. For the green-labeled microtubules we used a stoichiometry of one fluorescein-labeled unit and two unlabeled units, to match the intensity of red and green fluorescence in our setup. Polymerization occurred in the presence of 4 mM MgCl$_2$, 1 mM GTP and 5% DMSO in BRB80 buffer (37 °C for 45 min). Then the microtubules were stabilized and 40× diluted in BRB80 containing 100 µM Taxol (BRB80T).

5.A.3 Motility protocol

Microtubule motility was reconstituted in fluidic channels by flushing in the protein constituents using a pressure-driven flow. First, channels were filled with BRB80 buffer solution (80 mM Pipes, 1 mM MgCl$_2$, 1 mM EGTA, pH=6.9), supplemented with 100 µM paclitaxel (Taxol), 5 mM ATP, and 2% beta-mercaptoethanol (BME) and flushed to remove air bubbles. The resistance of the channel was measured to check the integrity of a channel. Second, a 0.9 mg/mL casein solution in BRB80 was added to the entrance reservoir and flushed through the channel using a pressure difference of 5 kPa. This flow was maintained for a time sufficiently long to coat the entire channel structure with casein (~30 min, depending on geometry of the channel). Third, a kinesin solution (70 µg/mL histagged full-length Drosophila conventional kinesin [39], 0.9 mg/mL casein, 9 mM ATP in BRB80) was flown through the channels with the same pressure. After coating the channels with kinesin, the entrance reservoir was emptied and a motility solution was added. The motility solution contained microtubules (~200 nM tubulin) in BRB80, supplemented with 100 µM Taxol, 6 mM ATP and an antifade cocktail (120 mM D-Glucose, 0.12 mg/mL glucose-oxidase, 0.05 mg/µL catalase and 2% BME) to prevent photobleaching. Subsequently, microtubules are propelled by kinesin towards the entrance of the channel structures.

5.A.4 Experimental setup

Microtubule motility was observed using fluorescence microscopy on an inverted IX81-olympus microscope using a 100× oil-immersion objective (Olympus, NA = 1.35) and a Hamamatsu CCD-camera. For most observations we used an ORCA ER B/W camera. For the molecular sorting experiments, we used a Hamamatsu C7780-10 color camera. All experiments were performed at room temperature.
5.A.5 Electric field application

Electric fields in the nanochannels were induced by applying voltages to platinum electrodes in the entrance reservoirs using a Keithley 6517A electrometer. At all times, the current through the channel was simultaneously measured using the electrometer. During experiments, the entrance reservoirs were exposed to atmospheric pressure, thus preventing a Poiseuille backflow due to EOF-induced pressure build-up. The buffer levels in the reservoirs were equalized within an accuracy of ≤ 1 mm. The corresponding maximum hydrostatic pressure difference between the reservoirs of 10 Pa would induce a maximum fluid flux of \( \sim 10^{-17} \text{ m}^3/\text{s} \). Given the geometry of our channels, the average fluid velocity due to unequal buffer levels is thus limited to ≤ 1 \( \mu \text{m/s} \), which is negligible to EOF-velocities at the field strengths used.

The effect of electric field application on the buffer temperature can be estimated as small. The dissipation per unit volume in a channel due to the current can be estimated as \( P_{\text{diss}} = \sigma E^2 \), where \( \sigma = 1.25 \text{ S/m} \) is the conductivity of the buffer solution, and \( E \) is the electric field. In steady state, the dissipation is balanced by thermal conduction through the glass. The thermal flux \( \Phi \) equals \( \lambda \partial T/\partial z \), with \( \lambda \) the thermal conductivity of glass (\( \lambda = 1.38 \text{ W/Km} \)) and \( \partial T/\partial z \) the temperature gradient. In steady state the temperature gradient in the glass substrate is thus given as \( \partial T/\partial z = \sigma E^2 h/\lambda = 10^5 \text{ K/m} \), for a channel of height \( h = 1 \mu\text{m} \) at the highest \( E = 100 \text{ kV/m} \). Assuming linear gradients in the glass substrates of 170 \( \mu\text{m} \) and 500 \( \mu\text{m} \) thickness on both sides of the channel, the temperature increase in the channel due to the electric field is limited to 2 °C. An in-situ probe of the temperature is provided by the velocity of the motility in our channels. Motility speed is known to double for approximately every 10 °C increase in temperature [40]. The fact that we do not see any systematic increase in gliding velocity during field application can be taken as a indication that the temperature increase in our channels is much smaller than 10 °C.

The effect of electric field application on buffer composition is also negligible. The total buffer volume in each reservoir is \( \sim 20 \mu\text{L} \). Measured currents in our experiments are \( \sim 1 \mu\text{A} \), inducing an electron-transfer rate at each electrode of \( \sim 10^{13} \text{ e/s} \). Assuming hydrolysis of water in the reservoirs, these currents induce a local change in the ion-concentration of \( \sim 1 \mu\text{M/s} \). During the time span of experiments (\( \sim 1 \text{ hour} \)), the current application creates \( \sim \text{mM of mainly H}^+/\text{OH}^- \) ions, which is easily buffered in our 80 mM PIPES buffer.
5.A.6 Electrophoresis experiments

Observations of the electrophoretic velocity of microtubules were made at a rate of $15 - 20$ Hz. Within this time resolution, Brownian motion of the microtubules can be neglected. We estimate the center-of-mass diffusion of a microtubule of length $L$ as $\Delta r^2 = 2k_B T \left( c_\perp^{-1} + c_\parallel^{-1} \right) / L = 6k_B T / c_\perp L$. Here, we have used the Einstein relation and the longitudinal drag coefficient $c_\parallel = c_\perp / 2$. Using the value of $c_\perp$ mentioned in the main text, we find that for a $5 \mu m$ long microtubule the Brownian movement between successive frames is limited to $\Delta r \leq 0.2 \mu m$.

5.A.7 Electro-osmotic flow measurements

Values of the electro-osmotic flow (EOF) mobility $\nabla$ were measured by a current-monitoring method [41]. In these experiments we first coated the channels with casein and kinesin to create identical surface properties as in the motility experiments. The two reservoirs at both ends of the channels were filled with respectively 100% buffer and 90% buffer, diluted in ddH$_2$O. The reservoirs thus contain the same electrolyte, however, with a different conductivity. By applying a potential difference between the reservoirs, an EOF is induced. The polarity of the voltage determines the direction of EOF and thus which buffer solution will replace the channel volume. Depending on flow direction, the monitored channel resistance will linearly increase or decrease until the total channel is filled with solution from one of either reservoirs. This measurement was repeated 6 times by reversing polarity and the EOF velocity was obtained from dividing the channel length (5 mm) by the average time interval of the conductivity change.

5.B Supporting text

5.B.1 Orientation-dependent electrophoretic velocity

The anisotropic mobilities of cylindrical colloidal particles for electrophoretic motion parallel ($\mu_{e,\parallel}$) and perpendicular ($\mu_{e,\perp}$) to the axis lead to an orientation-dependent velocity. For a cylinder oriented under an angle $\theta$ with respect to the $x$ axis (Fig. S.1), and the electric field $E$ along the $y$ axis, the electric field can be decomposed into components parallel and perpendicular to the long axis. The resulting electrophoretic perpendicular ($v_\perp$) and parallel ($v_\parallel$) velocities are then:

$$v_\perp = \mu_{e,\perp} E \cos(\theta),$$  \hspace{1cm} (S.1)
$$v_\parallel = \mu_{e,\parallel} E \sin(\theta).$$  \hspace{1cm} (S.2)
Figure S.1: The electrophoretic velocity \( v \) of a cylinder under an externally applied field \( E \) depends on the orientation \( \theta \) of the filament with respect to \( E \). (A) The anisotropic mobilities for movement parallel \( (\mu_{e,\parallel}) \) and perpendicular \( (\mu_{e,\perp}) \) to the cylinder’s axis, with \( (\mu_{e,\parallel}) > (\mu_{e,\perp}) \), result in a larger speed parallel \( (v_\parallel) \) than perpendicular \( (v_\perp) \) to the axis. The velocity of the cylinder is thus not collinear with the electric field. (B) The velocity components \( v_\parallel \) and \( v_\perp \) can be decomposed into velocities along the \( x \) \( (v_x) \) and \( y \) \( (v_y) \) directions. The EOF-velocity \( (v_{EOF}) \) acts in the \( y \)-direction.

Both \( v_\perp \) and \( v_\parallel \) can be decomposed in their \( x \) and \( y \)-components in the coordinate system defined in Fig. S.1:

\[
v_x = v_\parallel \cos(\theta) - v_\perp \sin(\theta), \tag{S.3}
\]
\[
v_y = v_\parallel \sin(\theta) + v_\perp \cos(\theta). \tag{S.4}
\]

Combining Eqs. S.1-S.4 and adding the EOF-velocity along the electric field, we obtain Eqs. 5.4-5.5 in the main text.

### 5.B.2 Bending of microtubules under distributed load

The bending of a microtubule tip by the electric field induced force is equivalent to the deviation of a clamped beam with length \( \delta \) under a distributed load \( f_\perp \) (See Fig. S.2A). For small deflections \( y \) of the beam, the deflection at each position \( x \) is related to the bending moment \( M(x) \) and the flexural rigidity \( EI \) of the beam:

\[
\frac{\partial y^2}{\partial x^2} = -\frac{M(x)}{EI}. \tag{S.5}
\]

In the situation depicted in Fig. S.2A, the bending moment can be derived from equilibrium to be:

\[
M(x) = -\frac{f_\perp}{2} (\delta - x)^2. \tag{S.6}
\]
Figure S.2: Deflection and induced curvature of a clamped beam of length $\delta$ under a load $f_\perp$.

(A) The deflection of the beam $y(x)$ increases to a maximum value of $y_m = y(\delta)$. (B) For small deflections $y_m$ of the beam, the beam deflection can be approximated with an arc segment with a radius of curvature $r$.

Combining Eqs. S.5 and S.6 and integrating using the boundary condition $\partial y/\partial x = 0$ at $x = 0$ yields for the deflection as a function of position [42]:

$$y(x) = \frac{f_\perp}{24EI} \left( (\delta - x)^4 + 4\delta^3x - \delta^4 \right).$$

(S.7)

From this, we calculate the maximum deflection $y_m$ at $x = \delta$ to be:

$$y_m = \frac{f_\perp\delta^4}{8EI}.$$  

(S.8)

We approximate the deflected beam as part of an arc segment with radius of curvature $r$ (Fig S.2B). We relate the deflection $y_m$ to $r$ as $y_m = r \left(1 - \cos \left(\frac{\delta}{r}\right)\right)$. For small $\delta/r$, this yields $y_m \approx \delta^2/2r$. Substituting this relation in Eq. S.6 and using $EI = k_BT L_p$ results in Eq. 5.6 in the main text.
Chapter 6

Electrophoresis of Individual Microtubules in Microchannels

We use micron-sized fluidic channels to confine and measure electrophoresis of freely suspended individual microtubules. We measure orientation-dependent velocities of microtubules and the electro-osmotic flow mobility in our channels to infer the anisotropic electrophoretic mobility of microtubules under physiological conditions. We discuss the difference between electrophoresis and purely hydrodynamic motion and its implications for interpreting mobility measurements. We show that the mobility anisotropy is a factor 0.83, clearly different from the well-known anisotropy factor of 0.5 in Stokes drag coefficients for cylindrical objects. We also show that the velocity is independent of microtubule length, which would be different for hydrodynamic motion. We demonstrate that the electric force on the counterions has important consequences for the interpretation of electrophoresis experiments and that ignoring this can lead to an underestimation of the effective charge by orders of magnitude. From the electrophoresis measurements we calculate an effective surface-charge density of $-36.7 \pm 0.4 \text{ mC/m}^2$ for microtubules. Electrophoretic measurements of subtilisin-digested microtubules, which have the negatively charged C-termini on the outer surface removed, show a 24% decrease in mobility and, correspondingly, in surface charge, but no change in anisotropy.

6.1 Introduction

The electrophoretic mobility of molecules is a fundamental property, that relates a molecule’s velocity to an external electric field. In ensemble electrophoresis measurements, such as gel electrophoresis or dynamic light scattering, the differences between individual molecules are obscured. To overcome this limitation, individual molecules can be made visible by fluorescent labeling and their electrophoretic motion can be imaged using fluorescence microscopy [1], provided that the motion of the molecules is confined within the focal plane of the objective. Microfabricated slit-like fluidic channels form an excellent system to confine and observe the electrophoretic motion of individual fluorescently labeled biomolecules, such as microtubules [2], actin filaments or virus particles [3].

In this paper, we present measurements of the electrophoretic mobility of individual microtubules in micron-sized fluidic channels. Microtubules are stiff cylindrical biopolymers with a diameter of 25 nm and lengths of several micrometers. Their high persistence length (∼1-5 mm) makes them a good model system for other rod-like particles such as very short DNA molecules or tobacco mosaic viruses. The electrophoretic mobility of cylindrical colloidal particles was predicted long ago to be anisotropic [4], which was only recently confirmed experimentally [2].

Here, we present an extensive study of the microtubule anisotropic mobility. This is not only interesting from a fundamental colloid science point of view. The mobility also determines the force that is applied in, for example, bionanotechnological applications, where electric fields bend and steer microtubules [2] or actin filaments [5] that are propelled by molecular motors. Finally, the mobility of a biomolecule is a measure of its effective charge (by which we mean the bare charge plus absorbed charge).

This paper starts with a brief summary of the theoretical framework of electrophoresis. This reiterates some original work from 1933 [6], because electrophoretic experiments are often incompletely interpreted, neglecting the effect of the counter ions. We show that this has led to orders-of-magnitude underestimates for the effective charge. Combined with measurements of the electrophoretic mobility of individual microtubules, we aim to give a compact theoretical description and experimental demonstration of the differences between electrophoretic and purely hydrodynamic motion.
6.2 Theoretical framework

The charge of a colloidal particle in an electrolyte is screened by counter ions that are organized in a double layer structure. The first layer of ions is confined to the surface in the Stern layer, whereas the diffuse layer reflects a balance between electrostatic attraction and entropic repulsion and is described by Poisson-Boltzmann theory. As a result, the space charge density $\rho$ decays exponentially with the Debye length $\lambda_D = \sqrt{k_B T \varepsilon / \sum z_i^2 e^2 n_i}$, where $k_B$ is Boltzmann’s constant, $T$ is temperature, $\varepsilon$ is the solvent’s dielectric constant and $e$ is the electron charge. The summation runs over all ion species $i$ with valence $z_i$ and number density $n_i$.

The presence of the counter ions makes electrophoretic motion markedly different from purely hydrodynamic motion caused by nonelectric forces. The main difference is that an electric field exerts force both on the object and on the surrounding fluid via the counter ions, the so-called retardation effect, whereas in gravitational or magnetic sedimentation of colloids, the externally applied force acts only on the object. This has been pointed out by several authors [4, 6, 7], but despite this, force balance in electrophoresis is often incompletely stated in terms of the electric force on the particle and Stokes hydrodynamic friction [3, 8, 9], thereby neglecting the retardation effect.

In the following, we first demonstrate the importance of the retardation effect by comparing the fluid motion around a sphere both in electrophoretic and hydrodynamic motion [6]. Then, we describe the electrophoresis of cylinders while allowing for a deformation of the ionic double layer by the external field, the so-called relaxation effect.

6.2.1 Fluid motion around a sphere

Following Henry’s [6] key arguments, we solve the fluid velocity $\mathbf{u}$ around a spherical insulating particle of radius $R$, and uniformly distributed charge $Q$ (Detailed steps are given in Appendix 6.A). The particle moves with velocity $\mathbf{v}$ by an electric field $\mathbf{E}$ (Fig. 6.1b). It is assumed that inertial terms can be neglected and that the potential due to the external electric field, $V$, can be superimposed on the potential in the double layer $\psi$. Implicit in this assumption is that the ionic double layer is not distorted. By imposing a velocity $-\mathbf{v}$ on the system, the particle is at rest and the fluid at infinity moves with $-\mathbf{v}$. Under these conditions, the fluid motion is described by the Navier-Stokes equation:

$$\eta \nabla^2 \mathbf{u} + \nabla p + \rho \nabla (V + \psi) = 0,$$

(6.1)

with $\eta$ the viscosity of the fluid and $p$ the hydrostatic pressure.
Chapter 6: Electrophoresis of individual microtubules

For the boundary conditions, it is assumed that a no-slip plane exists for the particle. The fluid between the surface and the no-slip plane is stationary with respect to the particle and the charge contained within the no-slip plane is effectively part of the objects charge. The potential at the no-slip plane, \( \zeta \), is denoted the zeta potential.

The solution of Eq. 6.1 describes the fluid motion with respect to the sphere’s origin, in polar coordinates:

\[
   u_r = -\cos(\theta)\left[\left(1 - \frac{3R}{2r} + \frac{R^3}{2r^3}\right)v + \psi_r(\psi, r, E)\right],
\]

\[
   u_\theta = \sin(\theta)\left[\left(1 - \frac{3R}{4r} - \frac{R^3}{4r^3}\right)v + \psi_\theta(\psi, r, E)\right].
\]

The functionals \( \psi_r(\psi, r, E) \) and \( \psi_\theta(\psi, r, E) \) represent the long-range fluid disturbance due to the electric forces on the counter ions and they depend on the local electrostatic potential \( \psi \) (explicit form in Eqs. S.4 and S.5 in Appendix 6.A).

We calculate the resulting particle velocity \( v \) from the force balance on the sphere. The total force exerted by the fluid, found from integrating all the viscous and pressure forces around the sphere, is

\[
   F_{\text{fluid}} = -6\pi \eta Rv + 4\pi \varepsilon E \int_\infty^R \xi(\psi, r)dr - QE.
\]

Here, \( \xi(\psi, r) \) is a functional of the particle’s electrostatic potential (explicit form in Eq. S.6 in Appendix 6.A). Interestingly, the external force on the particle \( F_{\text{el}} = +QE \) exactly cancels the last term in Eq. 6.4, and force balance \( (F_{\text{fluid}} + F_{\text{el}} = 0) \) thus yields the velocity of the particle as

\[
   v = \frac{2\varepsilon E}{3\eta} \int_\infty^R \xi(\psi, r)dr,
\]

which does not depend explicitly on the object’s total charge \( Q \), but rather on \( \zeta \) potential and \( \lambda_D \) through the integral term. Eq. 6.5 allows to evaluate the velocity, provided the electrostatic potential \( \psi \) is known [10].

6.2.2 Hydrodynamic versus electrophoretic motion

We now present explicit solutions to the fluid flow problem so as to arrive at a simple physical picture of the differences between electrophoretic and hydrodynamic motion in the length scales of the fluid disturbance. For purely hydrodynamic motion of an uncharged particle \( (\psi = 0, \psi_r = \psi_\theta = 0) \), the fluid motion
6.2 Theoretical framework

Figure 6.1: Hydrodynamic versus electrophoretic motion. (a) Calculated flow lines around a particle that is moved by an external (non-electric) force. The fluid (moving with velocity $-v$ with respect to the particle) has a long-range perturbation. (b) Calculated flowlines around an electrophoretically-driven particle (with $R/\lambda_D = 10$ and velocity $v$). The fluid is only perturbed within a much smaller distance around the particle. (c) Velocity decay in front of the particle for electrophoretic and hydrodynamic motion. The fluid disturbance in electrophoretic motion decays approximately as $r^{-3}$ (dashed line), which is longer range than the exponential Debye-Hückel decay (dotted line). In hydrodynamic motion the fluid disturbance decays much slower, $\propto r^{-1}$ (dash-dotted line). (d) Velocity decay perpendicular to the particle motion (Lines are as in (c)). (e) Numerically calculated values (symbols) [4, 11] of Henry’s function $f_{\perp}$ for a cylinder (Eq. 6.7) as a function of $R/\lambda_D$ for 4 different values of $\zeta$. Dashed lines are guides to the eye. The solid line is the limit of Henry’s function for low $\zeta$ and no relaxation effect [12].
(Eqs. 6.2–6.3) reduces, as expected, to the long-range Stokes profile around a sphere. In Fig. 6.1a we display the flowlines around the particle, which are curved around the particle up to distances $> 4R$.

For the fluid motion around a sphere during electrophoresis, we evaluate Eqs. 6.2–6.3 for a Debye-Hückel ionic atmosphere [10]. Figure 6.1b visualizes the flow lines, which, in contrast to hydrodynamic motion, are almost undisturbed by the particle. In electrophoresis, the additional long-range fluid disturbance caused by the motion of the counterions exactly cancels the long-range fluid disturbance induced by the particle motion.

We quantify the length scale of the fluid perturbations in Figs. 6.1c and d, where we plot the velocity in front of the particle, $u_r(r, \theta = 0^\circ)$ (Fig. 6.1c), and perpendicular to the particle, $u_\theta(r, \theta = 90^\circ)$ (Fig. 6.1d) as a function of distance. In hydrodynamic motion the velocity decays to its undisturbed velocity, $-v$, much slower ($\propto r^{-1}$) than in electrophoresis, which decays approximately as $\propto r^{-3}$, in accordance with the argument presented by Long and Ajdari [13]. The fluid disturbance is different from the exponential Debye-Hückel decay of the charge density around the sphere (dotted lines).

The electric force on the counterions has consequences for the interpretation of electrophoresis experiments. In hydrodynamic motion under external force $F_{\text{ext}}$, the fluid is sheared over a distance of order $\sim R$ (Fig. 6.1a). Force balance (Eq. 6.4, with $\psi = 0$) gives the well-known result that velocity is inversely proportional to the Stokes drag coefficient, $6\pi \eta R$. On the other hand, in electrophoretic motion the long-range fluid motions of the sphere and the counterions exactly cancel and the fluid is sheared over a much shorter distance. The restraining force is thus significantly increased compared to Stokes drag. Conversely, to move an object with the same velocity $v$ in electrophoresis, the electrical force that is needed to compensate the fluid forces (Eq. 6.4) is much higher than in hydrodynamic motion (Fig. 6.1a vs. b). Stating force balance in electrophoresis merely in terms of the electric force ($Q E$) and the hydrodynamic stokes drag of the object ($6\pi \eta R$) will thus typically lead to an underestimate of the restraining force and thus of the object’s charge $Q$ that can be very significant as we will show.

### 6.2.3 Electrophoresis of a cylinder

Experimentally, we study electrophoresis of cylindrical geometries that are predicted to have an orientation-dependent mobility. Under the same assumptions as for the spherical particle (ignoring relaxation effects), the mobility of infinitely long cylinders oriented axially along the electric field is given by the Smoluchowski
6.3 Experimental Results

The electrophoretic mobility, \( \mu_{\perp} \), is given by \([6, 12]\)

\[ \mu_{\perp} = f_{\perp} \frac{\epsilon \zeta}{\eta}. \]  

(6.7)

The prefactor \( f_{\perp} \) is Henry’s function for a cylinder and depends on \( \zeta \) and \( R/\lambda_D \). For low \( \zeta \) potential (using the linearized Poisson-Boltzmann equation) and ignoring relaxation, \( f_{\perp} \) increases monotonically from \( \frac{1}{2} \) for \( R \ll \lambda_D \) toward 1 for \( R \gg \lambda_D \) (solid line in Fig. 6.1e).

At higher \( \zeta \) potential, two assumptions break down: (i) The linearisation of the Poisson-Boltzmann is invalidated, and (ii) the relaxation effect becomes important. During electrophoresis, the ionic double layer is continuously destroyed behind the migrating object, and rebuilt in front of it. The finite mobility of the ions leads to cylindrically asymmetric \( \rho(r, \theta) \) and \( \psi(r, \theta) \), which leads to a decrease in \( f_{\perp} \) for moderate values of \( R/\lambda_D \) \([4, 14]\). For \( \mu_{\perp} \), the effects of relaxation and high \( \zeta \) potential have been calculated by numerical methods for spherical \([14]\) and cylindrical \([4, 15]\) geometries, which are shown in Fig. 6.1e. The decrease in \( f_{\perp} \) with increasing \( \zeta \) reflects mainly the effect of relaxation, whereas the non-linearity of the Poisson-Boltzmann equation only has a small effect \([14]\). For \( \mu_{\parallel} \), Stigter \([15]\) argues that Eq. 6.6 is still a good approximation for finite-length cylinders, as long as the length of the cylinder is large compared to \( \lambda_D \) \([16]\).

Experimental values of the \( \zeta \) potentials presented below will be \(< 2k_BT/e\).
channel except within a distance $\lambda_D = 0.7$ nm of the channel walls. The EOF-mobility $\mu_{\text{EOF}}$ is given by the Schmoluchowski result (Eq. 6.6) with substitution of the $\zeta$-potential of the glass, $\zeta_{\text{SiO}_2}$. [Eq. 6.6 describes the relative motion between a charged surface and the fluid and therefore equivalently applies to electrophoresis and EOF.] We first present measurements of the combined EOF- and electrophoretically-driven motion of microtubules, followed by measurements of the EOF-velocity in our channels.

### 6.3.1 Electrophoresis of microtubules

Figure 6.2d and e show two representative series of time-lapse camera images of the electrophoretic motion of microtubules ($E = -4$ kV/m along the $y$ axis). Figure 6.2d shows the displacements of two microtubules in perpendicular and axial orientations to $E$. After 0.6 s, the perpendicular microtubule has clearly moved over a shorter distance (dashed arrow) than the axially oriented microtubule. Figure 6.2e shows the displacements of two microtubules that are not exclusively oriented axially or perpendicularly to the electric field, but under approximately equal, opposite angles to $E$. Both microtubules show a displacement that is not collinear with $E$. Instead, the velocity of each filament is oriented slightly toward its axis (dashed arrows). The displacements of both microtubules in the direction of the electric field are approximately equal in this case.

The direction and magnitude of the electrophoretic velocity of a microtubule are observed to depend on orientation with respect to the electric field. This orientation-dependent velocity is a hallmark of the predicted anisotropic mobility of a cylindrical particle (Eqs. 6.6 and 6.7). In Fig. 6.2f, we show a diagram of a microtubule oriented under an angle $\theta$ with the $x$ axis and the electric field along the $y$ axis. As a result of the different proportionality constants to $E_\parallel$ and $E_\perp$, the velocity of the microtubule, $v$, is not collinear with the driving field, but instead has a component perpendicular to $E$. The electro-osmotic flow also contributes a velocity to the microtubule in the $y$ direction. From Fig. 6.2f we thus express the velocity in the $x$ direction, $v_x$, and $y$ direction, $v_y$, in terms of the anisotropic and EOF mobilities:

$$v_x = \frac{1}{2}(\mu_\parallel - \mu_\perp)\sin(2\theta)E,$$

$$v_y = [(\mu_\parallel - \mu_\perp)\sin^2(\theta) + (\mu_\perp + \mu_{\text{EOF}})]E.$$

(6.8) (6.9)
6.3 Experimental Results

Figure 6.2: Experimental situation. (a) Electrophoretic motion of microtubules in channels is observed using fluorescence microscopy. (b) Scanning-electron microscope image of a part of the cross-section of a channel. (c) The velocity of microtubules is a superposition of their electrophoretic velocity and an EOF velocity. (d,e) Fluorescence images with 0.2 s intervals of electrophoresis ($E = 4 \text{kV/m}$) of individual microtubules. (d) A microtubule oriented axially along the electric field moves faster than a microtubule oriented perpendicular to the field. (e) Microtubules can have a velocity that is not collinear with $E$. (f) Diagram of the velocity components of a microtubule oriented under an angle $\theta$ with $E$ along the $y$ axis. The electric field has components axially ($E_\parallel$) and perpendicular ($E_\perp$) to the microtubule. The anisotropic mobility results in net velocity $v$ that is not collinear with $E$. (g) Overlay of 2 images taken at a 0.2 s interval. For each microtubule, the average orientation $\theta$ and center-of-mass displacements in the $x$ and $y$ directions, $\Delta x$ and $\Delta y$ respectively, are determined.
To determine the anisotropic mobilities, we measure the \( \theta \)-dependent displacements of a large number of microtubules in the \( x \) and \( y \) directions between consecutive frames (Fig. 6.2g). Figures 6.3a and b display experimental results for the orientation-dependent \( v_x \) and \( v_y \) at \( E = -4 \text{ kV/m} \). In accordance with Figs. 6.2d and e, microtubules oriented with \( 0 < \theta < 90^\circ \) have a positive velocity perpendicular to \( E \), whereas microtubules in the opposite orientation, \( 90^\circ < \theta < 180^\circ \), move in the negative \( x \)-direction. From Fig. 6.3b, we confirm that microtubules oriented perpendicular to \( E \) have lower \( v_y \) than microtubules with axial orientations to the field. The solid lines in Figs. 6.3a and b are fits of Eqs. 6.8-6.9 to the data. These equations clearly describe the observed orientation dependence very well. The fitted amplitude \( A = (\mu_\parallel - \mu_\perp)E \) and offset \( B = (\mu_\perp + \mu_{\text{EOF}})E \) contain information about the mobilities (Eqs. 6.8 and 6.9).

We measured orientation-dependent velocities for different electric fields ranging up to 24 kV/m. Figure 6.3c displays the values of \( A \) and \( B \) as a function of the electric field. From the linear \( v - E \) relation, we infer that we can safely use the linear superposition of parallel and axial components of the velocity (Fig. 6.2f). Any coupling between the relaxation effect in the perpendicular direction (\( \propto E \)) and the electric field in the axial direction would induce a response \( \propto E^2 \) as well as a torque on the microtubules. The absence of any alignment (Fig. 6.2d and e) and the linear response in \( E \) (Fig. 6.3c) indicate that coupling is negligible and justify the use of Eqs. 6.8 and 6.9 up to the field strengths used.

### 6.3.2 Electro-osmotic flow

To separate the electrophoretic and EOF contributions to the observed microtubule velocities, we measure the velocity of the EOF in our channels using a current-monitoring method [17]. The reservoirs at the ends of the channel are
6.4 Discussion

We now determine the electrophoretic mobilities of the microtubules by subtraction of the EOF mobility. From the data in Figs. 6.3c and 6.4c we cal-

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**Figure 6.4:** Electro-osmotic flow. (a) Schematic of current-monitoring method for measurement of the EOF velocity. (b) Typical measurement of the EOF velocity ($E = 20$ kV/m). The channel resistance (lower panel) changes over $\Delta t \sim 20$ s upon changes of voltage (upper panel). (c) Measured EOF velocities in a single channel with different surface coatings as function of $E$. From the fits we find $\mu_{\text{EOF}} = +(2.00 \pm 0.04) \cdot 10^{-8}$ m$^2$/Vs, $+(1.41 \pm 0.01) \cdot 10^{-8}$ m$^2$/Vs, and $+(1.29 \pm 0.01) \cdot 10^{-8}$ m$^2$/Vs for the untreated channel (bare SiO$_2$), the casein-coated, and the casein-kinesin coated channel, respectively. Inset shows measurements for the channel that was used in the electrophoresis experiments.
calculate the electrophoretic microtubule mobilities for axial and perpendicular orientations to the electric field as \( \mu_\parallel = -(2.59 \pm 0.02) \cdot 10^{-8} \text{ m}^2/\text{Vs} \) and \( \mu_\perp = -(2.15 \pm 0.01) \cdot 10^{-8} \text{ m}^2/\text{Vs} \), respectively. This finding is in good agreement with previous bulk measurements of the average mobility \([8, 18]\). We calculate that the anisotropy \( \frac{\mu_\perp}{\mu_\parallel} = 0.83 \pm 0.01 \).

### 6.4.1 Electrophoretic motion is not described by Stokes drag

Our experimental results illustrate that electrophoretic motion indeed differs significantly from purely hydrodynamic motion. First, we note that the measured velocity anisotropy \( 0.83 \pm 0.01 \) is clearly different from the well-known anisotropy 0.5 in Stokes-drag coefficients for long cylinders. Moreover, Fig. 6.5 displays the measured electrophoretic velocities of microtubules as a function of their length for an electric field \( E = -8 \text{ kV/m} \). We do not observe any significant length dependence of the measured \( v_y \), for neither perpendicular (\( \theta = 0^\circ \)) nor parallel (\( \theta = 90^\circ \)) orientations of microtubules to the electric field. The length-independent electrophoretic velocity of microtubules is in agreement with Eqs. 6.6 and 6.7, but in clear contrast with a Stokes description.

If electrophoretic motion of microtubules were a balance of electric (\( \propto QE \)) and Stokes-drag forces (\( \propto cL\nu \)), we would expect a length dependence, since the Stokes-drag coefficient \( c \) for a cylinder is itself dependent on \( L \) \([19]\) (Eqs. S.7 and S.8 in Appendix 6.B). This applies for cylinders far from a surface, which is a good approximation to our experimental situation (Fig. S.1 in Appendix 6.B). Figure 6.5 shows the expected length dependence for cylinder motion that would result from a balance between electric and Stokes drag forces (dashed lines). The clear deviation of the experimental data from the simulated data \([\sim \ln(L)]\) shows the inadequacy of the Stokes treatment of electrophoresis.

### 6.4.2 Effective surface-charge density \( \sigma_{\text{eff}} \)

From the EOF-mobility measurements (Fig. 6.4c), we deduce the \( \zeta \) potential (Eq. 6.6) of the channel walls. For the untreated channel wall we calculate \( \zeta = -25.1 \pm 0.05 \text{ mV} \). For the protein-coated channels, we find values \( \zeta = -19.0 \pm 0.1 \text{ mV} \), and \( \zeta = -17.3 \pm 0.1 \text{ mV} \) for the casein and casein-kinesin coated channels, respectively \([20]\). We determine the effective surface-charge density \( \sigma_{\text{eff}} \) of the channels from the Grahame equation, that relates the \( \zeta \) potential to \( \sigma_{\text{eff}} \) \([21]\):

\[
\sigma_{\text{eff}} = \frac{2k_B T \varepsilon}{e \lambda_D} \sinh\left( \frac{e \zeta}{2k_B T} \right)
\]
6.4 Discussion

Figure 6.5: Measured length dependence of the electrophoretic motion of microtubules for perpendicular orientation (open circles) and for axial orientations (solid squares) to the electric field \((E = -8 \text{kV/m})\). The solid lines are guides to the eye. The dotted lines show the expected length dependence of the purely hydrodynamic motion \(\sim \ln(L/R)\) for cylinders \((R = 12.5 \text{nm})\).

For the bare channel, we find \(\sigma_{\text{eff}} = -27.5 \pm 0.5 \text{mC/m}^2\), which corresponds well with values that were determined by streaming current measurements in similarly fabricated channels [22].

Similarly, for microtubules, we infer, directly from the value of \(\mu\parallel\) that \(\zeta = -32.6 \pm 0.3 \text{mV}\), corresponding to \(\sigma_{\text{eff}} = -36.7 \pm 0.4 \text{mC/m}^2\). Note that the measured mobility anisotropy provides a second independent estimate for the value of \(\zeta\). For a perfect cylinder, the anisotropy \(\frac{\mu_{\bot}}{\mu_{\parallel}}\) equals the Henry factor \(f_{\bot}\), from which we derive the \(\zeta\)-potential through Fig. 6.1e. In this way, we find a similar value \(\zeta = -39 \pm 6 \text{mV}\), but this method is less accurate because the uncertainty in the anisotropy amplifies the error in \(\zeta\). Nevertheless, it is interesting that the two different methods yield similar values of \(\zeta\). The measurement of \(\mu_{\parallel}\) reflects a measure of the \(\zeta\) potentials of both the inner- and outer surfaces of the cylindrical microtubule, whereas the value of \(\mu_{\bot}\) only reflects the \(\zeta\) potential of the outer surface. The agreement between the two methods for calculating \(\zeta\) thus indicates that the inner- and outer-surface \(\zeta\) potentials are comparable.

6.4.3 Effective tubulin-dimer charge \(Q_{\text{eff}}\)

To calculate the effective charge per tubulin dimer \(Q_{\text{eff}}\) from \(\sigma_{\text{eff}}\), we compute the solution-exposed surface of the tubulin dimer. Microtubules are composed of 13 protofilaments that associate laterally into a cylinder. The protofilaments consist of head-to-tail associated dimers of \(\alpha\)- and \(\beta\)-tubulin. The dimensions of the cross-section, derived from an axial projection of the electron-density map of the microtubule [23, 24], are shown in Fig. 6.6a. For a 13 protofilament micro-
tubule, we calculate that the solution-exposed outer surface of the tubulin dimer is 67 nm$^2$, whereas the inner surface is 33 nm$^2$. From the measured $\sigma_{\text{eff}}$, we then calculate $Q_{\text{eff}} = -23 \pm 0.2$ e/dimer, assuming homogeneous charge distributions on the microtubule’s inner and outer surfaces.

This value of the effective charge is higher than we previously calculated [2], because we use a more accurate model for the microtubule outer surface here (undulated surface instead of a smooth cylinder), and because we include the inner surface of the microtubule. The rationale for the latter is that the screening length is much smaller than the inner radius of the microtubules. The fluid can thus flow unhindered through the microtubule and according to the Smoluchowski result, the mobility is only dependent on the potential at the no-slip plane.

We compare the value of $Q_{\text{eff}}$ to the bare charge of the tubulin dimer as calculated from the amino acid sequence [25, 26]. Figure 6.6a displays the calculated pH-dependent bare dimer charge $Q_{\text{bare}}$ in a range of pH = 1 – 14, obtained from a summation over all charged amino acids (Eq. S.10 and Table S.1 in Appendix 6.C). Around physiological pH = 6.5–8, the bare charge is only slightly dependent on pH. At the pH = 6.9 of our experiments, we calculate $Q_{\text{bare}} = -47$ e per dimer. We find that the value of $Q_{\text{eff}}$ from our experiments constitutes about 50% of the calculated bare charge. We attribute this lower value to screening. $Q_{\text{eff}}$ is the sum of the bare microtubule charge and the counterion charge contained within the no-slip plane. Our result suggest that 50% of the counterion charges are strongly bound to, and move with the microtubule under the electric field strengths used.

For comparison, reports of $Q_{\text{eff}}$ that ignore the retardation effect yielded values varying from $-0.2$ [8] to $-3 \cdot 10^{-4}$ e/dimer [9]. Clearly, the restraining force of the counterions can be so large that, depending on the experimental conditions, ignoring it can lead to an underestimation of the effective charge up to 5 orders of magnitude.

The value of $Q_{\text{eff}}$ that we infer from the experimentally determined $\zeta$ potential relies on three assumptions. First, by speaking of the $\zeta$ potential, we implicitly assume the existence of a no-slip plane, which is a widely used but theoretical entity [27]. Second, by calculating $\sigma_{\text{eff}}$ from $\zeta$, we assume the validity of the mean-field Poisson-Boltzmann equation. However, under our experimental conditions, $\lambda_D$ is of the same order of magnitude as the hydrated-ion diameter and finite ion-size effects can be expected to play a role. Third, the value of $Q_{\text{eff}}$ is dependent on the solution-exposed surface area that we use for the tubulin dimer. In the extreme limit of modeling the microtubule as a solid cylinder the exposed area is reduced by 50%. We expect that the latter assumption forms the largest contribution to the uncertainty in our estimate for $Q_{\text{eff}}$. Despite the above
6.4 Discussion

Figure 6.6: Charge modification by digestion. (a) Calculated $Q_{\text{bare}}$ per tubulin dimer from sequence data. At a pH = 6.9, the calculated bare charge is $-47$ e per dimer. (Inset) Cross-section dimensions of a microtubule constructed from an axial projection of the microtubule electron-density map [24]. The 13 protofilaments are displayed as half-ellipsoids of 4.6 nm width and 3.0 nm height, placed around a 1.1 nm thick cylinder of inner radius of 8.4 nm. (b) Denaturing SDS-PAGE gel electrophoresis of digested microtubules. 'Control' shows untreated centrifuged microtubules in a single band. The subsequent lanes show the results for subtilisin-treated microtubules for different digestion times, obtained after centrifugation. Subtilisin is present at the 25 kDa band before centrifugation (first three lanes). (c) Amplitude of measured $v_\theta(\theta)$ and $v_y(\theta)$ of digested microtubules as function of $E$. 'Control' is from Fig. 6.3c for undigested microtubules. (d) Offset of measured $v_y(\theta)$ of digested microtubules for different electric fields.

considerations, we believe that our method provides a fair estimate of the protein charge, unlike the reports that make orders-of-magnitude systematic errors by ignoring the retardation effect.
6.4.4 Charge modification by digestion

We also probed the effect of charge reduction on the mobility of microtubules. The α and β subunits consist of 450 and 445 amino-acids respectively, each with a molecular weight of \( \sim 50 \) kDa [25, 26]. The subunits exhibit globular folding with their C-termini, which are relatively rich in acidic groups, exposed to the outer surface of the microtubule [28].

We modified the charge of the tubulin dimer by removing part of the subunits C-termini’s by proteolytic digestion using subtilisin [29]. Subtilisin cleavage occurs with very high efficiency (94%) at positions Asp-438, and Gln-433 for α and β-tubulin, respectively [30], thereby removing a fragment of 13 amino-acids, of which 8 are acidic, from each monomer. With Eq. S.10 we calculate \( Q_{\text{bare}} = -30 \) e for the dimer’s bare charge after subtilisin-digestion, i.e. a reduction of 36%.

We performed subtilisin digestion on taxol-stabilized microtubules of the same batch as we used before, for different periods of time. Fig. 6.6b shows the progression of the digestion reaction on denaturing gel electrophoresis. For undigested microtubules (control), the α and β show a single band at \( \sim 50 \) kDa. After subtilisin digestion, the original tubulin band disappears, and two bands start to appear of a slightly lower apparent molecular weight, indicative of tubulin digestion [31]. The digestion of α-tubulin (upper band) is faster (starting after 10 min digestion) than the digestion of β-tubulin (lower band, after 30 min) [31]. Within the resolution of the gel, digestion is complete after 60 min. Microtubules treated for 60 min with subtilisin were still intact and used in electrophoresis experiments.

We measured the orientation-dependent velocity of digested microtubules for different electric fields. The experiments are performed in the same channel as used for the undigested microtubules. Figures 6.6c and d present the fitted amplitudes and offsets of the \( v_x \) and \( v_y \) data as a function of electric field for digested microtubules. The subtilisin-digested microtubules have significantly reduced velocities compared to undigested microtubules, since the EOF velocity is the same for both experiments. From the linear fits we determine \( \mu_\parallel = -(2.00 \pm 0.02) \times 10^{-8} \text{ m}^2/\text{Vs} \), and \( \mu_\perp = -(1.64 \pm 0.01) \times 10^{-8} \text{ m}^2/\text{Vs} \) for digested microtubules.

We find that the mobility of the digested microtubules has been reduced significantly, as expected from the large reduction in charge residing on the outer surface of the microtubule. It is interesting that the values of \( \mu_\parallel \) and \( \mu_\perp \) have been reduced by the same amount, by 23 ± 2 % and 24 ± 1 %, respectively, and that the mobility anisotropy is thus not different for the digested microtubules. From Fig. 6.1e, we would expect an increase of \( f_\perp(\zeta) \) from 0.83 to 0.86 upon a 23% reduction in surface charge. However, these numerical results apply to
homogeneously charged cylinders and it remains a theoretical challenge to describe inhomogeneous charge distributions on open cylinders. Our experimental results can serve as input for further theoretical investigations in the mobility of composite cylindrical objects with different inner and outer surface potentials.

6.5 Conclusions

We used microfabricated fluidic channels to measure the electrophoretic motion of individual microtubules, which, with their cylindrical shape, constitute an important basic geometry. Together with measurements of the EOF velocity in our channels, we determined the electrophoretic mobility. These measurements experimentally demonstrated two important differences with purely hydrodynamic motion of cylindrical filaments. (i) The mobility is independent of microtubule length, in contrast to the length-dependent hydrodynamic drag. (ii) The electrophoretic mobility is anisotropic, with microtubules oriented perpendicular to the electric field moving a factor 0.83 slower than for axial orientations. This is markedly different from hydrodynamic motion, where the drag anisotropy is a factor 0.5. We have argued that these differences are due to the motion of the counterions, which makes the fluid perturbations much shorter-range than in hydrodynamic motion, and that this has important implications in the interpretation of electrophoresis measurements. Although understood long ago, these results are often neglected in recent electrophoresis experiments, which leads to order-of-magnitude underestimations of the effective charge.

From the mobility measurements we inferred $Q_{\text{eff}} = -23 \pm 0.2 \text{ e per tubulin dimer}$. We also measured the electrophoretic mobility of subtilisin-treated microtubules that had their highly negatively charged C-termini removed by digestion. We measured a 23% reduction in mobility, but no change in anisotropy.

Enclosed microfluidic channels form an excellent system to measure electrophoretic motion of individual biomolecules. From these experiments we gained valuable insights into fundamental electrophoretic properties of colloidal cylinders and obtained measurements of the effective charge of individual biomolecules.

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References


[10] For the illustrative example of a Debye-Hückel potential ($\psi(r) = \frac{\zeta R}{r} e^{-\frac{(r-R)}{\lambda_D}}$), Eq. 6.5 yields $v = f(R/\lambda_D) \frac{\zeta E}{\eta}$. Here, $f(R/\lambda_D)$ is Henry’s function (increasing from $\frac{2}{3}$ for $R \ll \lambda_D$ to 1 for $R \gg \lambda_D$). Indeed $v$ depends directly on $\zeta$ and $\lambda_D$.


[16] In fact, the Smoluchowski result is exact for objects of arbitrary shape as long as the objects’s curvature $1/R$ is small compared to $\lambda_D$, also at high $\zeta$-potential.


We use $\varepsilon = 7.08 \cdot 10^{-12}$ F/m, and $\eta = 0.89 \cdot 10^{-3}$ kg/ms. We assume that the viscosity of the fluid is unchanged by the protein absorption.


[27] It is conceivable that the no-slip plane is not a clear-cut boundary between free ions and ions absorbed to and travelling with the molecule, but instead a finite region where the mobility of the counterions changes from its free bulk value to zero over a finite distance. This would similarly blur the definition of $Q_{\text{eff}}$.


Supplementary Information

This appendix contains additional information that was published as supporting information to the main text.

6.A Detailed steps of the theoretical framework

In his original paper, Henry [6] solves the fluid velocity $u$ around a spherical particle from the hydrodynamic equations for an incompressible fluid (Eq. 6.1 in the main text), together with $\nabla \cdot u = 0$. His approach is to first solve the Navier-Stokes equations for the pressure distribution $p$ and then for the velocity distribution $u$, which together present the particular solution to the problem. Then he adds the homogeneous solution to the problem ($\eta \nabla^2 u + \nabla p = 0, \nabla \cdot u = 0$) to arrive at the full solution to the electro-hydrodynamical problem. Application of the boundary conditions, for $r = R$, $u_r = u_\theta = 0$, $\psi = \zeta$ and for $r = \infty$, $u_r = -v \cos(\theta)$, $u_\theta = v \sin(\theta)$, $\psi = 0$ yields for the pressure and velocity:

\[
p = \int_{\infty}^{R} \rho \frac{\partial \psi}{\partial r} + \cos(\theta) \left[ \frac{3\eta R}{2r^2} v - \varepsilon E \frac{R}{r^2} \int_{\infty}^{R} \xi dr - \varepsilon E(3 \frac{\partial \psi}{\partial r} - 2\xi) \right], \tag{S.1}
\]

\[
u_r = -\cos(\theta) \left[ (1 - \frac{3R}{2r} + \frac{R^3}{2r^3})v + U_r(\psi, r) \right], \tag{S.2}
\]

\[
u_\theta = \sin(\theta) \left[ (1 - \frac{3R}{4r} - \frac{R^3}{4r^3})v + U_\theta(\psi, r) \right]. \tag{S.3}
\]

The functionals $U_r(\psi, r)$ and $U_\theta(\psi, r)$ are the long-range fluid flows due to the electric forces on the counter ions, dependent on the local potential $\psi$:

\[
U_r(\psi, r) = \left( \frac{R}{r} - \frac{R^3}{3r^3} \right) \frac{\varepsilon E}{\eta} \int_{\infty}^{R} \xi dr - \frac{2\varepsilon E}{3\eta} \left( \int_{\infty}^{R} \xi dr - \frac{1}{r^3} \int_{R}^{r} r^3 \xi dr \right), \tag{S.4}
\]

\[
U_\theta(\psi, r) = \left( \frac{R}{2r} + \frac{R^3}{6r^3} \right) \frac{\varepsilon E}{\eta} \int_{\infty}^{R} \xi dr - \frac{2\varepsilon E}{3\eta} \left( \int_{\infty}^{R} \xi dr + \frac{1}{2r^3} \int_{R}^{r} r^3 \xi dr \right). \tag{S.5}
\]

Here, $\xi$ is a functional of the electrostatic potential of the particle, and is rapidly decaying throughout the ionic double layer:

\[
\xi = \frac{\partial \psi}{\partial r} + \frac{1}{2} \frac{R^3 \varepsilon}{r^4} \int_{\infty}^{r} \frac{1}{r^4} \nabla^2 \psi dr. \tag{S.6}
\]

Equations S.1 to S.3 (with substitution of Eqs. S.4 to S.6) provide a complete description of the fluid velocity profile and pressure distribution around the sphere during electrophoresis.
6.B Hydrodynamic drag coefficients for cylinders

In the main text we use the hydrodynamic drag coefficients for cylinders to plot the expected length dependence of hydrodynamic motion. The drag coefficients $c$ depend on axial ratio $p = L/2R$ and are, for axial movements,

$$c_{\parallel} = \frac{2\pi \eta}{\ln(p) - 0.114}.$$  

(S.7)

whereas for perpendicular motion:

$$c_{\perp} = \frac{4\pi \eta}{\ln(p) + 0.866}.$$  

(S.8)

These equations are the result of numerical calculations for cylinders far from a surface [19]. The presence of a nearby wall can increase the drag on a cylinder significantly. Hunt et al. [2] have calculated the drag coefficients for cylinders with their axis a height $h$ above a planar surface,

$$c_{\parallel} = \frac{2\pi \eta}{\text{arccosh}(h/R)}; \quad c_{\perp} = 2c_{\parallel}. $$  

(S.9)

The question arises which of the expressions (Eqs. S.7 and S.8 or Eq. S.9) applies to our experimental situation. We now show that the formulas for drag near a wall are only applicable when the cylinder is so close to the wall that $h \approx R$. Since the microtubule radius is only 12.5 nm, and the average distance to a channel wall in our 1 $\mu$m high devices is 250 nm, we argue that the drag coefficients for cylinders far from a wall are a better approximation to our experimental situation.

Figure S.1a shows the lines of constant viscous dissipation per unit volume around a cylinder at a distance $h = 2R$ above the surface [2]. Almost all the dissipation is taking place immediately in the gap between the cylinder and the surface. In the calculation of the drag coefficient (Eq. S.9), Hunt et al. integrate all the shear forces that are exerted at the planar surface ($Y = 0$) and then assume that this is equal to the total drag force on the cylinder. For a cylinder at $h = 2R$ this seems a good assumption. However, in Fig. S.1b and c we show the lines of dissipation of a cylinder at a distance $h = 5R$ and $h = 20R$ above the surface, respectively. In contrast to the cylinder at $h = 2R$, the dissipation is now almost entirely taking place in the immediate vicinity around the cylinder. For this situation the approximation used in the calculation of Eq. S.9 breaks down.
Figure S.1: (a) Lines of constant dissipation per unit volume (after Hunt et al. [2]) caused by a infinite cylinder, moving along its axis at a distance \( h = 2R \) above a surface. The maximum dissipation is at the bottom of the cylinder and relative to this maximum, contours are drawn at dissipation of 0.9, 0.8, ..., 0.1. Almost all dissipation takes place within the gap between the cylinder and the surface. (b) Similar plot for a cylinder located at a distance \( h = 5R \) above the surface. Contrary to (a), almost all dissipation takes place in the immediate vicinity around the cylinder, without significant influence of the surface. (c) The lines of constant dissipation of a cylinder at a distance \( h = 20R \) above the surface, corresponding to our experimental situation. The influence of the surface is absent even for the dissipation contour of 0.1 relative to the maximum. (d) Plots of the calculated drag coefficients for motion parallel to the cylinder axis. The red line shows a plot of Eq. S.9, where the strongly enhanced drag close to the surface is apparent. The black lines show plots of Eq. S.7 for two different lengths of microtubules. The labels a,b,c denote the distances corresponding to the panels.

To our knowledge, no analytical result is available for the drag coefficient of cylinders at distances \( h \gg R \). The limitation of the drag coefficients calculated by Hunt et al. for larger distances from the wall becomes also apparent from Fig. S.1d. At large distances the drag coefficient calculated from Eq. S.9 becomes smaller than the drag coefficient for a cylinder calculated from Eq. S.7 which is clearly an unphysical result. In this regime, the bulk description as used in the main text is more appropriate.
6.C Calculated bare charge of the tubulin dimer

From the pH-sensitive dissociation fraction of the amino acids we calculate the total bare charge $Q_{\text{bare}}$ on the tubulin dimer from a summation over all acidic and basic groups (Table S.1)

$$Q_{\text{bare}} = \sum_{\text{acid}} -e \frac{-\epsilon}{1 + 10^{(pK_a - pH)}} + \sum_{\text{base}} +e \frac{+\epsilon}{1 + 10^{-(pK_a - pH)}}.$$  

(S.10)

In this calculation we assumed that the displayed pK_a values of the amino acids still apply within the folded protein.

Table S.1: Number of acidic (D, E), and basic (K, R, H) amino-acids in $\alpha$- and $\beta$-tubulin [25, 26], together with pK_a values.

<table>
<thead>
<tr>
<th>Acidic amino acid</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>pK_a</th>
<th>Basic amino acid</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>pK_a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid (D)</td>
<td>27</td>
<td>26</td>
<td>3.9</td>
<td>Lysine (K)</td>
<td>19</td>
<td>15</td>
<td>10.5</td>
</tr>
<tr>
<td>Glutamic acid (E)</td>
<td>38</td>
<td>36</td>
<td>4.3</td>
<td>Arginine (R)</td>
<td>21</td>
<td>22</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Histidine (H)</td>
<td>13</td>
<td>10</td>
<td>6.0</td>
</tr>
<tr>
<td>Total negative</td>
<td>-127 e</td>
<td></td>
<td></td>
<td>Total positive</td>
<td>+100 e</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6.D Materials and methods

6.D.1 Microfabrication

Microfluidic channels were fabricated between two 1 mm diameter entrance holes in 500 $\mu$m thick fused-silica substrates of 13×19 mm. The entrance holes were separated by 5 mm, and created by powder-blasting. The substrates with prefabricated holes were purchased from PlanOptik (Unter den Eichen, Germany). Substrates were cleaned by subsequent sonications of 5 min in acetone, fuming nitric acid (HNO_3), and iso-propanol alcohol (IPA). Using sputter deposition, a 35 nm thick layer of chromium (Cr) was deposited on the samples acting as an electron sink during electron-beam (e-beam) lithography. A 800 nm thick e-beam-sensitive resist polymethylmethacrylate (PMMA) was spincoated on the sample, followed by a 15 min bake at 170 °C. Channels of 50 $\mu$m width were defined with a Leica electron-beam pattern-generator with a dose of 1200 $\mu$C/cm². The samples were developed by a 75 s immersion in a mixture (1:3) of methylisobutyl ketone and IPA and a subsequent immersion of 45 s in IPA to stop the development. A 1 min immersion in Cr etchant (Merck) removed the Cr layer in the structures, exposing the fused silica. The channels were wet etched 1 $\mu$m deep into the substrates using ammonium-fluorid etchant (Merck 7:1). After etching,
the remaining resist and chromium were removed by a 15 min sonication of the substrates in PRS3000 (Merck) to strip the PMMA, and then a 1 min submersion in Cr etchant.

The channel structures were sealed with a 170 µm thick fused silica substrate using a silica bonding procedure. To this end, the fused-silica substrates were thoroughly cleaned by 15 min sonication in PRS3000 and 10 min sonication in HNO$_3$. Then, the substrates were exposed to a 0.5% solution of hydro-fluoric acid for 1 min. A 2% sodium-silicate solution (Sigma) was spin-coated (6000 rpm, 1 min) onto the cover, immediately followed by pressing the sodium-silicate surface to the patterned channel surface. Finally, the devices were cured for 2 hours at 90 °C.

6.D.2 Materials

All experiments were conducted in BRB80 buffer (82 mM Pipes, 1 mM MgCl$_2$, 1 mM EGTA, pH = 6.9). At this pH, the buffer contained $\sim$46 mM PIPES$^{2-}$, $\sim$36 mM PIPES$^{-}$, $\sim$34 mM Cl$^{-}$, $\sim$160 mM K$^+$, and $\sim$1 mM Mg$^{2+}$. Under these conditions, the Debye screening length $\lambda_D$ = 0.7 nm.

Rhodamine-labeled fluorescent microtubules were polymerized from 35 µL of bovine brain tubulin (4 mg/mL, Cytoskeleton, Denver, CO) in a 1:3 stoichiometry of labeled:unlabelled tubulin. Polymerization occurred in the presence of 4 mM MgCl$_2$, 1 mM GTP and 5% DMSO in BRB80 buffer (37 °C for 45 min). Then the microtubules were stabilized and 25× diluted in BRB80 containing 10 µM paclitaxel (Taxol, BRB80T). We then centrifuged the microtubules at $\sim$140,000g for 10 min (Airfuge, Beckman Coulter) to remove unpolymerized tubulin. The pellet was resuspended in an equal amount of BRB80T, which yielding microtubules at a concentration of approximately 170 µg/mL.

Subtilisin-digested microtubules were created from the same batch of rhodamine-labelled microtubules. For the digestion we added subtilisin to taxol-stabilized microtubules in ratio of 0.6:1 (110 µg/mL). Digestion was performed at 37 °C for different times up to 1 hour. The digestion reaction was stopped by adding phenylmethylsulfonyl fluoride (PMSF) at a final concentration of 5 mM. To remove the C-termini fragments and the subtilisin, the subtilisin-treated microtubules were centrifuged over a cushion buffer (containing 40% glycerol in BRB80T) at 140,000g for 25 min. The pellet containing the digested microtubules was resuspended in BRB80T, yielding an estimated tubulin concentration of 130 µg/mL. Untreated microtubules and digested microtubules, before and after centrifugation, were examined by SDS-PAGE gel analysis.
6.D.3 Experimental methods

Pressure-driven flows (created using water columns) were used to fill and flush the channels via the reservoirs at either end. First, the channels were filled with BRB80 containing 1 mM adenosine-triphosphate (ATP) and 100 $\mu$M Taxol. A hydrostatic pressure of $\sim$5 kPa was applied for 30 min to flush the channels and remove any air bubbles. Then 20 $\mu$L of 0.9 mg/mL casein solution in BRB80 was added to an emptied reservoir and flushed through the channel. This flow was maintained for 2 hours and coated the entire channel with casein to prevent sticking of microtubules to the walls. Finally, 10 $\mu$L of a microtubule solution (20 nM tubulin) was added to the reservoir containing 80 $\mu$M taxol, 7 mM ATP and an antifade cocktail (0.15 M D-Glucose, 0.15 mg/mL glucose oxidase, 0.06 mg/mL Catalase, 2.5% beta-mercapthoetanol) to prevent photobleaching of the microtubules.

In separate experiments we tested that the entire channel was coated with casein after the 2 hours by confirming that microtubules were prevented from sticking to the channel walls. After shorter flushing times, microtubules would be prevented from sticking at the beginning of the channel, but not further downstream from the reservoir. With increasing flushing times, the channel length where microtubules would not stick increased until, after 2 hours, microtubules were prevented from sticking in the entire 5 mm long channel.

Electric fields in the channels were induced by applying voltages to platinum electrodes in the entrance reservoirs using a Keithley 6517A electrometer. At all times, the current through the channel was simultaneously measured using the electrometer. During experiments, the entrance reservoirs were exposed to atmospheric pressure, thus preventing a Poiseuille backflow due to EOF-induced pressure build-up. The buffer height levels in the reservoirs were equalized within an accuracy of $\leq$ 1 mm. The corresponding maximum hydrostatic pressure difference between the reservoirs of 10 Pa would induce a maximum fluid flux of $\sim$10 fL/s. Given the geometry of our channels, the average fluid velocity due to unequal buffer levels is thus limited to $\lesssim$ 0.2 $\mu$m/s, which is negligible to the observed electrophoretic or EOF-velocities.

The electrophoretic motion of microtubules was observed using fluorescence microscopy on an inverted IX81-olympus microscope with a 100× oil-immersion objective (Olympus, NA = 1.35) and a CCD-camera (Hamamatsu ORCA ER). All experiments were performed at room temperature.
Appendix: Additional calculations regarding the drag coefficients for cylinders near a surface

This appendix contains an additional theoretical study of approximations for the hydrodynamic drag coefficients of cylinders in the presence of a surface. This work was triggered by a discussion with Jonathan Howard following the publication of this chapter [1].

Below, we discuss the Stokes drag coefficients that describe purely hydrodynamic motion of finite-length cylinders in the presence of a wall. Approximations are known for motion that is either very close, or far away from the wall with respect to cylinder length, but we are specifically interested in motion at intermediate distances from the wall. We evaluate approximations found in literature that apply in this intermediate regime to calculate the velocity of cylinders of $1 - 15 \, \mu m$ length at a distance of 500 nm from a surface. For the parameter range relevant to our experiments, these approximations predict a length-dependent velocity for cylinders that move parallel to their axis, whereas there is only a slight length dependence for motion perpendicular to the axis.

6.E.1 Introduction

In our publication about electrophoresis of microtubules [1], we plot the electrophoretic velocity of microtubules in perpendicular and axial orientations to the electric field as a function of their length (Fig. 5 in [1] and Fig. 6.5 in this thesis). We find that this velocity is length independent. To illustrate that electrophoretic motion is qualitatively different from purely hydrodynamic motion, we also plotted the expected functional length dependence that follows from the Stokes drag coefficients for finite-length cylinders that are far away from any surface. However, it was subsequently argued by Jonathon Howard that, instead, we should use the drag coefficients that were derived by Hunt et al. [2] for cylinders close to a surface. Those drag coefficients were derived based on the assumption of infinitely long cylinder length. Jonathon Howard argued that these equation apply to our case because the lengths of the microtubules ($1 - 15 \, \mu m$) greatly exceed the height above the surface, which validates the assumption of infinite cylinder length.

We shortly summarize here our arguments about the choice of Stokes-drag coefficients for plotting the length dependence of hypothetical hydrodynamic motion of cylinders in our channels. We noted in Fig. S.1d in the supplementary information to this chapter that the Stokes drag per unit length that is predicted for a cylinder close to a surface (based on Hunt’s infinite-length assumption) can
become comparable to, or even smaller than, the Stokes drag per unit length that would be predicted for a cylinder far away from a surface, in particular when the distance between the cylinder and the surface becomes large. We illustrated this using the dimensions that correspond to our experimental geometry (with microtubules having a length-to-diameter ratio $p = L/2R$ that varies between 40 and 300, and assuming an average surface separation of 250 nm). We show that our situation apparently corresponds to a transition (Fig. S.1d): For the longest microtubules ($p = 300$) we find that, as expected, the predicted drag per unit length using the infinite length assumption of Hunt et al. is slightly higher than the values that are predicted for cylinders in a free solution. However, for the shortest microtubules ($p = 40$) we showed that the formula’s of Hunt actually predict a lower drag than the one that is calculated for cylinders without the presence of a surface. Clearly, caution is required in deciding which drag coefficients to choose, since our experimental situation certainly does not qualify as being far from a surface in the sense that the wall effect is weak, nor does it qualify as being so close to a surface that the drag per unit length as applicable to an infinite-length cylinder applies.

Clearly, for cylinder-wall separations of $h \approx L$ the cylinders are too far away for the infinite-length assumption to hold. The central question is thus: where does $h \approx L$ stop, and where does $h \ll L$ start? In Hunt et al. [2] this is motivated in Appendix A, and through Fig. 10 (here reproduced in Fig S.1a), where it is stated that:

“For the motion of an infinite cylinder, the viscous drag arises almost entirely from shear dissipation taking place in the immediate vicinity of the cylinder, primarily between the cylinder and the wall. [...] The localized dissipation implies that the total drag on a microtubule thousands of nanometers long, close to a wall, is essentially the same as if the length were infinite. Near the ends (tens of nanometers away), the drag might be less, but the length itself is unknown to this accuracy.”

In this context we now comment on Fig. S.1a-c in the supplementary information to this chapter. We note that all the force on the cylinder gets transmitted to the wall in the situation of a cylinder (either finite or infinitely long) that is close to a single wall in the low Reynolds number regime. We also note that the

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As we will show, hydrodynamic motion of cylinders in a parallel-plate geometry will only have a stable configuration midway between the plates, so a value of 500 nm for the wall-separation will be a more realistic value. In this case even a larger part of our microtubule lengths would not qualify for the infinite-length assumption.
wall is necessary to make the fluid stationary for the infinitely long cylinder in the low Reynolds number limit: there is no stationary solution for the fluid profile around a moving infinitely long cylinder in an unbounded solution possible, unless inertial terms are invoked (the Stokes paradox). The statement in our supporting information, regarding the drag around a cylinder at a height $h = 20R$ above the surface “For this situation the approximation used in the calculation of Eq. S.9 breaks down” is in this respect not accurately formulated. We realize it may raise the (wrong) impression that not all the force is transmitted to the surface. However, this statement, together with the Figs. S.1a-c, intend to make it reasonable that for finite length cylinders close to a surface, the assumptions regarding the applicability of infinite length cylinders breaks down. Inspired by Hunt et al. (“The localized dissipation implies that the total drag on a microtubule thousands of nanometers long, close to a wall, is essentially the same as if the length were infinite”, Fig. S.1a), we intend to show that for larger separation distances the dissipation is not anymore localized “..primarily between the cylinder and the wall” (Fig. S.1c). This is a qualitative statement. Basically, the argument is about deciding how close a cylinder must be for the assumption of infinite length to hold.

6.E.2 Approximations for the drag coefficients of cylinders in the intermediate regime

It seems that to describe the motion of cylinders of $1-15 \mu m$ length that move in purely hydrodynamic motion through a $1 \mu m$ high channel, caution is warranted in assuming limiting values to the distances-to-length ratios. The cylinders are neither close to, nor far away from a surface with regard to the applicability of various approximations that are in use for the Stokes drag coefficients. Here we summarize approximations that more closely resembles our situation at intermediate distances.

First, we note that a number of approximations that consider the drag of finite-length cylinders close to a wall are applicable only in either one of two limits: Either in a weak-boundary limit, with the distance from the surface considerable larger than the length of the cylinder ($h \gg L$), or in the strong-boundary limit, where the cylinders are much closer to the surface than their length ($h \ll L$). Only few approximations are applicable without requiring a limit of $h/L$. Moreover, a general not of caution is that most approximations for describing slender-body motion rely on the assumption that $\varepsilon^2$ is small with regard to $\varepsilon$, where the slenderness $\varepsilon$ is given by:

$$\varepsilon = \frac{1}{\ln(L/R)}.$$  \hspace{1cm} (S.11)
Appendix: Additional calculations

However, because of the logarithm, \( \varepsilon \) is only a slowly varying function; for the cylinders in our experiments, with \( L/R \) varying between 80 and 1200, the slenderness changes only from 0.22 to 0.14. It must be kept in mind, that many slender bodies in practice, despite having large length-to-diameter ratios of \( \sim 1 \times 10^3 \), do have that \( \varepsilon^2 \) is small, but not very much smaller than \( \varepsilon \). It is not immediately obvious how large the effect is of neglecting terms of order \( \varepsilon^2 \).

Second, we note that in our experiments the microtubules are in a parallel-plate geometry and there are two walls involved, not one, while most approximations are valid at only one wall. We first consider theoretical approximations that apply to finite-length cylinders at any height from a single surface (although \( h \gg R \) is necessary), but realizing our experimental situation, we also consider available experimental data involving cylinder motion between planes at comparable \( h/L \) ratios as in our experiments.

The well established values for the drag coefficients of finite-length cylinders in free solution are from Broersma et al. [3, 4]:

\[
c^*_{\parallel} = \frac{1}{\ln(p) - 0.114},
\]

(S.12)

for axial motion, whereas

\[
c^*_{\perp} = \frac{2}{\ln(p) + 0.866},
\]

(S.13)

for perpendicular motion. Here, we denote with \( c^* \) the normalized drag per unit length, that is, the drag coefficient per unit length divided by \( 2\pi \eta \). It is reasonable to require that approximations that claim to be valid at large \( h/L \) approach the Broersma results for large \( h \).

Similarly, for cylinders that are very close to a surface, at very small \( h/L \), the drag coefficients derived by Hunt et al. [2] for infinite-length cylinders should apply:

\[
c^*_{\parallel} = \frac{1}{\text{arccosh}(h/r)},
\]

(S.14)

\[
c^*_{\perp} = 2c^*_{\parallel}.
\]

(S.15)

We expect that any approximation that claims validity at intermediate distances from a surface approaches these results from Hunt for very small height.

Of interest to our experimental situation is the motion of finite-length cylinders in the presence of a wall. Approximations to this situation have been the subject of many papers in the limits of small and large \( h/L \). For example, the
drag force of slender bodies far from a single wall, in the limit of \( h \gg L \) has been treated by Brenner [5], and the drag force for cylinders close to a wall, in the limit of \( h \ll L \) was discussed by Katz et al. [6]. The drag coefficients of slender bodies that are moving midway between two planes have been discussed for the limit of \( h \gg L \) in refs. [5, 7, 8], and for the limit of \( h \ll L \) in ref. [6].

Only few papers discuss the intermediate regime, without any restriction on \( h/L \) [8–10], which is the case of interest to us. The work of Fulford et al. [10] considers the motion of cylinders near a interface of two immiscible fluids of different viscosity. Their results yield the motion in the presence of a solid plane wall by letting the viscosity of the neighboring fluid approach infinity (\( \theta \to \infty \) in Ref. [10]). The flow around the cylinder is solved by employing a distribution of force singularities (so-called Stokeslets) to represent the cylinder, where the magnitude of these singularities is chosen such that the flow obeys the boundary conditions. The normalized drag coefficients for cylinders moving parallel to a solid plane surface are:

\[
c^*_\parallel = \frac{1}{\ln(p) - 0.117 - \frac{1}{2} I_\parallel},
\]

(S.16)

for axial motion, whereas for transversal motion:

\[
c^*_\perp = \frac{2}{\ln(p) + 0.883 - \frac{1}{2} I_\perp}.
\]

(S.17)

Here, the effect of the wall is contained in the functionals \( I_\parallel \) and \( I_\perp \), which are:

\[
I_\parallel = 2 \sinh^{-1} \frac{L}{2h} - 3 \left( 1 + \frac{4h^2}{L^2} \right)^{1/2} + \frac{7h}{L} \left( 1 + \frac{4h^2}{L^2} \right)^{-1/2},
\]

(S.18)

\[
I_\perp = 2 \sinh^{-1} \frac{L}{2h} - \left( 1 + \frac{4h^2}{L^2} \right)^{1/2} + \frac{2h}{L}.
\]

(S.19)

These are the equations 11-14b in Ref. [10]. These equations serve to evaluate the drag coefficients in the presence of a single plane surface in the regime where \( h \gg R \). As expected, for large distances \( L/h \to 0 \) the interface terms \( I_\parallel \) and \( I_\perp \) approach to zero. The solutions depend on the assumptions that (i) \( R \ll L \), (ii) that \( R \ll h \), and (iii) that terms of order \( \varepsilon^3 \) can be neglected. All three assumptions seem reasonable for our situation. It must also be noted that these solutions describe the flow around the main part of the body, but not around the ends. The finite length of the cylinders is thus included in the approximation, whereas end-effects are not.
Before evaluating Eqs. S.16 and S.17 we like to point out that in hydrodynamic motion of cylinders between two parallel walls the most stable motion is midway between the planes. Cylinders that moving close to a single wall will experience a torque \[8, 11\], such that a cylinder that is moving parallel to a surface, in the direction along its long axis, will rotate its leading end away from the wall \[8\]. Similarly, a rod that is oriented with its long axis perpendicular to a wall will experience a torque that aligns the rod parallel to the wall \[8\]. For hydrodynamic motion of rods between two parallel planes, motion thus will only be stable when the cylinders are oriented midway between the channel. Thus, for the evaluation for hydrodynamic motion in our channel it seems reasonable to adapt a value of \(h = 500\ \text{nm}\).

Now, we use the drag coefficients (Eqs. S.16 and S.17) to evaluate the expected length dependence of the velocity of cylinders in purely hydrodynamic motion close to a single surface. For a driving force that is proportional to the cylinder length, we expect that the velocity of cylinders will be proportional to the inverse of the normalized drag coefficient. In Fig. S.2 we plot the values of \(c^\parallel\) and \(c^\perp\) as obtained from evaluating Eqs. S.16 and S.17 at a height of \(h = 500\ \text{nm}\) from the surface for lengths ranging from \(0.1 - 500\ \mu\text{m}\). As expected, both the velocity perpendicular and parallel to the cylinder axis become independent of length for cylinder lengths that are much longer than the height of the cylinder, in agreement with the prediction by Hunt \textit{et al}. When the cylinder length becomes shorter, the velocity decreases due to the decreased effect of the surface. The values of the inverse drag from Fulford \textit{et al}. (Eqs. S.16 and S.17) asymptotically approach the inverse drag coefficients from Hunt \textit{et al}. (Eqs. S.14 and S.15) for \(h \ll L\) and approach the free cylinder values of Broersma (Eqs. S.12 and S.13). The transition region in between ranges roughly from \(0.3 - 3\ \mu\text{m}\) for motion perpendicular to the cylinder axis, and from \(1 - 10\ \mu\text{m}\) for motion parallel to the axis. One could say that cylinders are very close to a surface when \(L > 20h\). The values in our experiments are shaded grey in the figure and largely overlap with the transition regions.

It is interesting to note that the axial motion of cylinders seems less affected by the surface than perpendicular motion. This is also noted by De Mestre \textit{et al}. \[9\], who note that the effect of the wall in axial motion becomes even less when the slenderness ratio \(\varepsilon\) increases.

For completeness, we reproduce here Fig. 6.5 using the approximations from Fulford \textit{et al}. for the drag coefficients of cylinders in the intermediate regime. In Fig. S.3 we show the experimental length-independent velocities of electrophoretic motion of microtubules, together with the expected velocities from evaluating Eqs. S.16 and S.17 (dashed lines). The latter curves were obtained by rescal-
Figure S.2: Inverse normalized drag coefficient (proportional to velocity) as a function of cylinder length for hydrodynamic motion at a distance $h = 500$ nm from a single plane surface. The red and black solid line are plots of Eqs. S.16 and S.17, respectively, from Fulford et al. [10] and approximate the velocity for cylinders near a surface in perpendicular and axial movement. For large cylinder lengths, $L \gg h$, the inverse drag (velocity) flattens and becomes independent of length, and exactly approaches the inverse drag coefficients (blue dash) as predicted by Hunt et al. [2] for cylinders close to a surface (Eqs. S.14 and S.15). For small lengths $L \ll h$, the free cylinder drag coefficients (green dots) of Broersma [3, 4] are asymptotically approached (Eqs. S.12 and S.13). The cylinder lengths of relevance to our experiments are shaded grey.

6.E.3 Conclusion

The effect of a wall on the drag on a finite-length cylinder is clear in the limit of a very close approach. If the distance is very much smaller than the length the drag per unit length is the same as for an infinite cylinder in the presence of a surface. If the cylinder is much more than a body length away from the surface, the drag coefficients for a free cylinder are a good approximation. We evaluated approximations for cylinders at the intermediate regime and find that the transition region roughly extends from $0.05 < h/L < 1$. In this regime, the
hypothetical hydrodynamic velocity would depend on length, in particular for axial motion and less for transverse motion. We did not find approximations for the motion of cylinders between two parallel walls that are valid in this intermediate regime.

References

Chapter 7

Microtubule Curvatures under Perpendicular Electric Forces Reveal a Low Persistence Length

The mechanics of microtubules, cylindrical protein filaments that constitute the cytoskeleton, have been well characterized on long length scales. Here, we investigate the persistence length of short (∼0.4 μm) microtubule ends by measuring the trajectories of kinesin-propelled microtubules under perpendicular electric forces. We relate the measured trajectory curvatures to the biased thermal fluctuations of the leading microtubule end, and upon including all electro-hydrodynamic forces, we find that the persistence length of the microtubule ends is only 0.55 ± 0.22 mm. This is significantly shorter than the well-established value of about 6 mm that is measured for long microtubules. Our data indicate an upper bound to the recently proposed shear-induced bending of microtubules, attributed to shearing of adjacent protofilaments. More generally, our experiments illustrate that electric forces are an excellent tool to apply forces of well-defined magnitude and direction on biomolecules for biophysics studies.

7.1 Introduction

Microtubules are stiff cylindrical protein filaments that constitute an important part of the cytoskeleton of eukaryotic cells. They have an outer diameter of about 25 nm and lengths of several micrometers and can serve as tracks along which motor proteins, such as kinesin, move. Microtubules are important for defining morphology and providing mechanical stiffness to cells. The mechanical properties of microtubules have therefore been studied intensively for over a decade [1–7]. The consensus was that microtubules can be characterized by a persistence length of 4 – 8 mm [1, 6]. However, recent experiments have shown a strongly reduced persistence length for short microtubules, which has been attributed to non-negligible shear deflections on short length scales [4, 7]. Most prominently, thermal fluctuations of taxol-stabilized microtubule ends indicated that the persistence length decreased from 5 mm to 0.11 mm upon decreasing the contour length from 48 µm down to 3 µm [7]. It remained an open question whether this trend will continue for shorter length scales, but the resolution of these measurements is limited.

In order to measure the persistence lengths of microtubule ends on even shorter length scales, we study the trajectories of kinesin-propelled microtubules subjected to perpendicular electric forces. The (macroscopic) curvature of the microtubule trajectories is an amplification of the microscopic bending of the ~0.4 µm short microtubule ends in our experiments and provides a convenient measure of their stiffness. These experiments provide the first measurements of the persistence length of very short microtubule ends. We find a value that is significantly lower than expected for entire microtubules, but higher than expected from extrapolating the previously reported decrease in stiffness [7] to our length scales. Additionally, our measurements provide a quantitative understanding of the steering mechanism of microtubules in bio-nanotechnological applications [8] which can help a rational design of structures for kinesin-powered devices.

Electric forces in microfabricated structures have proven to be a versatile tool for the manipulation and study of biomolecules. For example, electrokinetic forces have been used to suppress Brownian motion and trap individual proteins for optical observation [9], to probe force-velocity diagrams of myosin-driven actin filaments [10], and to study binding kinetics of microtubules onto kinesin-coated surfaces [11]. Electric forces are advantageous because they can exert a homogeneous force density of well-controlled magnitude and direction on biomolecules, which are naturally charged. In contrast, optical tweezers apply a localized force and require linkage of the molecule to a bead where the exact geometry of the linkage is unknown. Hydrodynamic shear flows require knowledge of the detailed
shear profile, and thermal forces are hard to control. We perform our experiments in micronsized channels because their large surface-to-volume ratio prevents Joule heating of the solution while the electric field is applied. Consequently, we can apply much higher electric fields than in regular flow cells [12].

7.2 Results and discussion

The experimental layout of our devices is depicted in Fig. 7.1. Channel structures were etched 1 \( \mu \text{m} \) deep in fused-silica substrates between entrance reservoirs separated by 5 mm and subsequently bonded using another fused-silica substrate (Fig. 7.1a,c). We reconstituted kinesin-driven microtubule motility in the channels using pressure-driven flows to flush the protein constituents (casein, kinesin, microtubules) from one of the entrance reservoirs through the channels. Motility of taxol-stabilized microtubules (polymerized for 45 min at 37 \(^\circ\)C) was observed using fluorescence microscopy through the fused silica. Electric fields were induced in the channels by application of a DC-voltage difference between platinum electrodes in the reservoirs, under constant monitoring of the current (Fig. 7.1b).

In order to study microtubule trajectories under perpendicular electric fields, we fabricated channels with a perpendicular crossing (Fig. 7.1d,e). The narrow channel (vertical channel at the bottom of the figure) serves to feed microtubules into the wide horizontal channel in which a homogeneous electric field is present. In Fig. 7.1d, we show representative fluorescence images of microtubules that move while an electric field is continuously applied. We highlighted three microtubules that enter from the small channel, in snapshots taken with 10 s intervals. All three microtubules traverse the structure with a pronounced curvature of their paths, in such a way as to become aligned against the field.

The electric field induces a constant force density on the homogeneously charged microtubule in the direction opposite to the electric field. Kinesin molecules, distributed along the length of the microtubule, exert the opposing forces and prevent the movement of the microtubule perpendicular to its axis. As was suggested and demonstrated previously [8, 10, 12, 13], the thermally fluctuating tip of the microtubule is biased into the direction of the force (Fig. 7.1e), thereby orienting the microtubule slowly, in a step-by-step fashion, into the direction of the electric field.

For a quantitative determination of the curvature of microtubule trajectories, we acquire fluorescence images with 1 s intervals of a large number of microtubules entering the electric field region. We used custom-written MATLAB routines to trace the coordinates of the leading and trailing ends of all microtubules that
traversed the structure during 15 min. In Fig. 7.2a, we show microtubule trajectories that were measured under an electric field of 18 kV/m. For later analysis, we excluded those parts of the trajectories that have touched one of the channel walls. As expected, most of the trajectories originate from the small injection channel below and display a pronounced bending to the right. Some trajectories originate from microtubules that were already present in the wide channel, and these trajectories also tend to align with the field. There is a large variance in the orientation with which microtubules enter the channel, and the magnitude of the perpendicular force decreases upon aligning with the field, thereby reducing the curvature [8].

In Fig. 7.2b we show a selection of paths under two different fields. The green and red trajectories are from microtubules that originate from the injection channel and that are being subjected to electric fields of 8.8 kV/m and 35 kV/m, respectively. It is clear that the bending of microtubule trajectories becomes more pronounced when we increase the electric field strength. Note that the spread
between individual paths under a single electric field is of the same order as the differences between trajectories under different electric fields.

To unambiguously show that the microtubules indeed align with the electric field by a bending of the leading tip, we display the coordinates of both leading and trailing ends of two microtubules in Fig. 7.2c. At both electric fields, even at the highest field strength used \(E = 44\, \text{kV/m}\), the coordinates of the front and rear ends of the microtubule overlap perfectly over the entire trajectory. This demonstrates that, within the resolution of our measurement, both ends of the microtubule trace the same path. In other words, they get propelled by the same kinesin molecules and there is no motion of the microtubule perpendicular to its long axis. The field-induced alignment of the microtubule must therefore be attributed to bending of the leading tip.

We now relate the macroscopically observable curvature of the trajectory to the microscopic properties of the microtubule tip. The microtubule is propelled by several bound kinesin molecules that are distributed stochastically along its length with average spacing \(<d>\). Upon progression of the microtubule, the leading tip will progressively increase in length from 0 to an average value \(<d>\), just before the moment of binding to the next kinesin molecule. In the absence of any forces, the tip will fluctuate under thermal forces, with a mean change in orientation of the tip \(<\theta'_m> = 0\). However, an externally applied force \(f_\perp\) that is oriented perpendicular to the microtubule, displaces the equilibrium position of the free tip (Fig. 7.2c). At the moment of attachment to the next motor, the mean increase in orientation of the tip, \(<\theta'_m>\), is equal to (Appendix 7.A):
\[ \langle \theta' \rangle_m = \frac{\langle d \rangle^3}{6k_B T p} f_{\perp}, \]  

(7.1)

where \( k_B \) is Boltzmann’s constant, \( T \) temperature, and \( p \) the persistence length of the microtubule tip.

We describe the microtubule trajectory using (macroscopic) coordinates \( \theta \) and \( s \), as defined in Fig. 7.2c. Because the trajectory of the microtubule is determined by the orientation of its leading end, we assert that \( \langle \frac{d\theta}{ds}(s) \rangle = \frac{\langle \theta'_m(s) \rangle}{\langle d \rangle} \), which holds as long as the curvature is determined on length scales larger than \( \langle d \rangle \). Substitution of Eq. 7.1 and using \( f_{\perp} = f_e \sin \theta \) yields for the trajectory curvature

\[ \langle \frac{d\theta}{ds} \rangle = \frac{\langle d \rangle^2}{6k_B T p} f_e \sin \theta, \]  

(7.2)

where \( f_e \) is the electric field-induced force. This description of the trajectory, which employs the angular deviation of the tip, is more appropriate than the modeling in terms of the spatial deflection of the tip, which we and others previously employed [8, 14]. It does not rely on the small-angle approximation, and it naturally captures the reduction of the perpendicular force upon alignment with the electric field.

We determine tangent angles \( \theta \) and curvatures \( \frac{d\theta}{ds} \) from the measured microtubule trajectories at every coordinate. In this way, we obtain a large number of orientation-dependent curvatures. Figure 7.3a displays measured trajectory curvatures as a function of tangent trajectory angle at \( E = 18 \text{kV/m} \). We confirm that curvature has a sinusoidal dependence on orientation, as expected from Eq. 7.2. Given the layout of our device, most trajectories start at \( \theta = \pi/2 \), and end at \( \theta = 0 \) and, consequently, the error bars are smallest in this range.

In order to eliminate the orientation dependence in our quantitative analysis, we divide the measured curvatures by the sine of the tangent angle (Eq. 7.2). The resulting orientation-invariant curvatures \( \frac{d\theta}{ds} (\sin \theta)^{-1} \) are plotted in Fig. 7.3b versus \( \theta \). We find that there is a strong divergence in the curvature around \( \theta = 0, \pm \pi \), which are those parts of the trajectories that are aligned with the electric field. In particular for these orientations we expect no appreciable influence of the electric field. The observed divergence is due to the division of finite, thermally driven, trajectory curvatures by very small (\( \sim 0 \)) values of \( \sin \theta \). For the determination of the field-induced curvature, we thus retain only data points for orientations where \( |\sin(\theta)| \geq 0.5 \), that is, those parts of the trajectories where there is an appreciable field-induced curvature (indicated between red lines in Fig. 7.3b).
The histogram in Fig. 7.3c displays the resulting distribution of measured orientation-invariant curvatures. We observe that the shape of the distribution is Gaussian, which we attribute to thermal fluctuations. Indeed, the orientation of the end of a one-sided clamped beam with length \( \langle d \rangle \) is expected to be Gaussian distributed with a standard deviation \( \sigma = \sqrt{\langle d \rangle} \) [15]. In our experiments, variations in the tip length \( d \) due to the random dispersion of kinesin motors on the surface, may cause an additional variation in the measured curvature. However, curvature is expected to scale \( \propto d^2 \) (Eq. 7.2), and a significant variation in \( d \) would thus appear as an asymmetric tail of the curvature distribution towards negative \( \frac{d\theta}{ds}(\sin \theta)^{-1} \). The absence of this asymmetry, even at high forces, indicates that the observed variance in measured curvatures is mainly due to thermal fluctuations. This can also be inferred from the following argument. The binding of the progressing microtubule tip to a new kinesin motor is a Poisson process.
and, consequently, values of $d$ are expected to be exponentially distributed, with a mean $\langle d \rangle$ and variance $\langle d \rangle^2$. Thus, the majority of tip lengths will be smaller than $\langle d \rangle$ and will not contribute significantly to the measured variance in curvature.

We take the center of a Gaussian fit (red line in Fig. 7.3c) as a measure of the mean orientation-invariant curvature at this particular field. We measured the orientation-invariant curvatures for a range of electric fields from 0 to 44 kV/m and Fig. 7.3d displays the results as a function of the electric field. The error bars denote errors in the mean of the distribution centers, not the standard deviation of each individual distribution which is much larger. As expected (Eq. 7.2 and $f_e \propto E$), the orientation-invariant curvature increases linearly with the electric field with a slope of $1.30 \pm 0.02 \text{ V}^{-1}$. We note that retaining only points of $|\sin(\theta)| \geq 0.5$ has no significant effect on this value (data not shown).

To determine the persistence length of the microtubule tip, we need to calibrate the magnitude of the electric field-induced force that we apply. The magnitude of this force in the experimental situation of so-called stationary electrophoresis equals [16]

$$f_\perp = c_\perp \mu_{\perp,e} E_\perp,$$

(7.3)

where $\mu_{\perp,e}$ is the mobility of a freely suspended microtubule inside the channel during free electrophoresis, and $c_\perp$ is the perpendicular Stokes drag coefficient per unit length of a microtubule tethered to the surface via kinesin molecules. We emphasize that this result for calculating the magnitude of the electric-field induced force is remarkably simple and exact, given the complicated boundary conditions due to the nearby walls. This simplicity stems from the linearity of the Navier-Stokes equations that allows to split the problem of stationary electrophoresis into two different problems with known solutions, being free electrophoretic motion and purely hydrodynamic motion of a microtubule close to a surface. We show this formally in Appendix 7.B.

The perpendicular hydrodynamic drag-coefficient $c_\perp$ (Eq. 7.3) for a microtubule tethered to kinesin on a casein-coated surface was measured as $c_\perp = 1.19 \pm 0.11 \cdot 10^{-2} \text{ Ns/m}^2$ by Hunt et al. [17]. This was done by observing the rotational diffusion of microtubules tethered to a single kinesin molecule. This value of the drag-coefficient corresponds to an elevation of the microtubule axis of $18 \pm 1 \text{ nm}$ above the casein surface, in good agreement with a direct measurement of this distance [18]. The value of $\mu_{\perp,e}$ in Eq. 7.3 was measured by electrophoresis of freely suspended microtubules in our channels (as described previously [19]), which yields $\mu_{\perp,e} = -(1.03 \pm 0.01) \cdot 10^{-8} \text{ m}^2/\text{Vs}$. From Eq. 7.3 we thus calculate that in our experiments we have applied force densities up to a maximum
$f_e = 5.4 \pm 0.6 \text{ pN/µm}$ at the highest electric field of 44 kV/m. With this knowledge we determine from the slope in Fig. 7.3d that $(\delta^2_p) = 2.61 \pm 0.31 \cdot 10^{-10} \text{ m}$ (Eq. 7.2).

We can obtain an estimate of the average distance between bound kinesin molecules $\langle d \rangle$ from observations of the movement of very short microtubules in the absence of applied forces. Microtubule filaments are usually bound to and propelled by several kinesin molecules distributed along their length and will therefore preserve their directionality. However, if the microtubule length becomes comparable to $\langle d \rangle$, occasionally the filament will be bound to only a single kinesin molecule and display diffusive rotational motion around the motor [17], thereby rapidly changing orientation. Fig. 7.4a shows traces of two short microtubules that move in markedly different manners. The longer microtubule of the two (black trace in Fig. 7.4a, fluorescence image in Fig. 7.4c) moves, as expected, in a smooth fashion over the surface without sudden changes of direction. In contrast, the shorter microtubule of the two (red trace in Fig. 7.4a, fluorescence image in Fig. 7.4b) moves much more irregularly, with many sharp turns in its trajectory. Given the sudden changes in direction, this shorter microtubule with length $L_s$ is, on several occasions in its trajectory, bound to only a single kinesin. This places a lower limit on $\langle d \rangle$ on the assertion that, on average, this microtubule is bound to less than two kinesins, thus $2\langle d \rangle \geq L_s$. Similarly, the longer microtubule with length $L_l$ must be propelled by at least two kinesin molecules. An upper limit on $\langle d \rangle$ follows from $2\langle d \rangle \leq L_l$.

We determine the lengths of short microtubules from a deconvolution of intensity line scans along their long axes. Intensity profiles along the length of the short (Fig. 7.4b) and longer (Fig. 7.4c) microtubule are shown in Figs. 7.4e and g, respectively. Due to the finite optical resolution of the microscope, the resulting profiles consist of convolutions of the objectives point-spread-function (PSF) with the microtubule lengths and, as a result, their lengths are overestimated. We approximate the PSF from the intensity profile perpendicular to the microtubule axis (Figs. 7.4d and f). These profiles have a full-width-at-half-maximum (FWHM) of 0.35 and 0.39 µm, which is much more than the 25 nm microtubule diameter, justifying their use as an approximation of the PSF. Deconvolution of the intensity length profiles shown in Figs. 7.4e and g yields values of $L = 0.62 \mu\text{m}$, and $L = 0.93 \mu\text{m}$, respectively. We find several short microtubules that move in smooth trajectories and the shortest microtubule of this set has a length $L_1 = 0.89 \pm 0.06 \mu\text{m}$ (mean ± standard deviation (STD) as determined from 6 different images). We also find several very short microtubules that display more random movement and the longest microtubule of those has a length $L_s = 0.63 \pm 0.05 \mu\text{m}$ (determined from 9 different images). From this we
Figure 7.4: (a) Trajectories of two short microtubules without electric field. The longer microtubule \( (L_1 = 0.89 \text{ µm}, \text{black trace}) \) moves smoothly over the surface without sudden changes of direction. This indicates that at all times, it must be bound by at least two kinesin molecules. The shortest microtubule \( (L_0 = 0.63 \text{ µm}, \text{red trace}) \) moves in a very irregular way with sudden changes in direction, presumably because at these moments it is bound to only a single kinesin molecule and rotationally free. On average, the filament is thus bound to less than two kinesin molecules. (b-c) Fluorescence images of the two microtubules. Intensity linescans are denoted. (d-g) Intensity linescans corresponding to denotations in panels b and c. The scans perpendicular to the microtubules (d,f) are taken as an approximation of the point-spread-function, with the full-width-at-half-maximum indicated. In the scans along the microtubules (e,g) we indicate the deconvoluted length.

determine that the distance \( \langle d \rangle \) between bound kinesin molecules is in the range 0.32 ± 0.03 − 0.45 ± 0.03 µm, thus \( \langle d \rangle = 0.38 ± 0.06 \text{ µm} \).

We find that the forces that are exerted on the leading tip are small compared to typical forces that can be withstand by a kinesin motor. At the highest electric field, the total force on the tip amounts to only 2 ± 1 pN. This force will yield a deflection of the microtubule end of a few nanometers at most, which is smaller than its 25 nm radius. It is remarkable that a succession of these small deviations can lead to a complete reorientation of the entire microtubule. Interestingly, the field-induced bending energies are thus comparable to \( k_B T \). This is also apparent
from the fact that the thermally induced variance in the measured curvatures (Fig. 7.3c) is comparable to the field-induced deviation of the mean curvature from zero. We can thus safely assume that the value that we determined for the tip length under unloaded conditions also applies in the situation when a perpendicular force is applied. Any possible effect of pulling the microtubule tip loose from the leading kinesin motors would be expected to increase with force and consequently, the measured curvature would increase more than linearly with the electric field. The fact that, instead, curvature is linearly proportional to $E$ over the entire range of fields (Fig. 7.3d) clearly shows that the measured value of $\langle d \rangle$ applies at all the forces that we have probed.

With our estimate for the average inter-kinesin distance, we are able to determine the persistence length of the leading microtubule end as $p = 0.55 \pm 0.22$ mm. This value is significantly shorter than the generally accepted value of $\sim 5$ mm [1, 6] for long microtubules. A shorter persistence length for the tube end is, however, in agreement with the reported reduced persistence length for short microtubules. Measurements by Pampaloni et al. [7] reported a systematic decrease in the persistence length of taxol-stabilized microtubule ends from 5.0 mm to 0.11 mm when the microtubule length decreased from 48 $\mu$m to 2.6 $\mu$m.

The reduced persistence length for short microtubules is attributed to the strongly anisotropic material properties of microtubules [4, 7]. Microtubules are hollow, cylindrical structures that are built from 13 protofilaments, arranged in parallel. Both refs. [4, 7] argue that the deflection of microtubule ends consists of both bending and shear deformations, where the shear deformations arise mainly due to sub-nanometer displacements of adjacent protofilaments. The deflection of a microtubule end of length $L$ scales $\propto L^3$ for pure bending, but $\propto L$ for shear deformations and the latter thus becomes dominant for short $L$. This model predicts a decrease of the persistence length $\propto L^{-2}$ for short microtubules, which describes the data of Pampaloni et al. well in their length range of 2.6 – 48 $\mu$m.

Interestingly, this model is expected to break down for much shorter lengths because then the required shear displacement between adjacent protofilaments becomes so large, in the order of nanometers, that the microtubule structure is expected to be compromised. The required displacement $dx$ of adjacent protofilaments can be estimated as $dx = 3\delta D/L$, where $D = 4$ nm is the diameter of a protofilament, and $\delta$ is the deflection of the microtubule tip, which is $\langle \delta^2 \rangle = L^3/3p$ for thermal fluctuations [7]. For example, for a microtubule bending over a length $L = 10$ $\mu$m the persistence length is about 1 mm [7], which requires a $dx = 0.6$ nm. This displacement is small compared to the 4 nm tubulin monomer size. However for a shorter $L = 0.4$ $\mu$m, comparable to our tip length, the persistence length predicted by the shear model is only about 0.002 mm. In this case the re-
quired sliding of protofilaments would amount to $dx \approx 3\text{ nm}$, which likely would cause the microtubule to lose its structure. Indeed, our measurements indicate that the persistence length for very short microtubule ends is about two orders of magnitude higher than predicted from the shear model.

Another measurement of the stiffness of short lengths of microtubules was performed by Kis et al., who use an atomic force microscope (AFM) to induce elastic deformations on gluteraldehyde-stabilized microtubules that were suspended over $0.08 - 0.17\text{ nm}$ gaps. They found that the persistence decreased from $0.11\text{ mm}$ to $0.02\text{ mm}$ in this size range. However, these results cannot straightforwardly be compared to ours, because in the AFM experiments the microtubules were supported at both ends, which increases the relative contribution of shear-induced deformations and will result in an enhanced compliance to bending. The gluteraldehyde cross-linker used for stabilization further complicates the comparison. An independent measure of the persistence length of microtubule ends can be obtained by measuring the persistence length of microtubule trajectories over kinesin-coated surfaces [20]. Although the length of the leading tip was not specified in these measurements, reported values of the persistence length of microtubule trajectories range from $0.1\text{ mm}$ [21] to $0.5\text{ mm}$ [22] which agrees well with the value that we determine here.

Our results do also provide input for bio-nanotechnological applications, in which kinesin-propelled microtubules can act as shuttles transporting an attached cargo through micro-fabricated devices [23, 24]. Our first estimate of the distance $\langle d \rangle$ between bound kinesin molecules serves to determine the surface density $\sigma$ of active kinesin molecules as $\sigma = 1/\langle d \rangle w$, where $w \approx 20\text{ nm}$ is the distance over which a kinesin molecule can reach to interact with a microtubule [20]. We find that $\sigma \approx 10^2\text{ nm}^{-2}$, which is at least an order of magnitude lower than values that are often assumed based on a close packing of kinesins. Furthermore, from the analysis of the distribution of trajectory curvatures we inferred that thermal fluctuations are the main cause of the dispersion in microtubule trajectories. This places a fundamental lower limit to control of the dispersion, which cannot be improved by a more controlled placement of kinesin motors on surfaces.
7.3 Conclusions

Microfabricated structures are a very useful tool for the study of biomolecules. We have used microfabricated channel structures for a controlled study of microtubule trajectories under perpendicular electric fields. We have measured the curvature of a large number of microtubule trajectories for different values of the electric field. We related the trajectory curvature to the bending of the microtubule tip, which allows us to infer the stiffness of the microtubule ends. For this purpose, we calibrated the magnitude of the electric field-induced force, and we determined the average maximum length of the leading tip from observations of the trajectories of very short microtubules. With this knowledge we were able to infer that the apparent persistence length of taxol-stabilized microtubule ends, with a length of $0.38 \pm 0.06 \, \mu m$, is $0.55 \pm 0.22 \, mm$.

The low value of the persistence length that we measure for our microtubule ends provides the first measurements on very short taxol-stabilized microtubules. The value that we find for the persistence length is significantly lower than the 4–8 mm values that have been reported for long microtubules. Our results supplement recent measurements of reduced persistence lengths for short microtubules. Our data demonstrate that the model of shear-induced microtubule deflections encounters its limits for very short lengths, which is expected if one considers the required amount of protofilament sliding. Our results also provide a quantitative understanding of the steering mechanism of microtubules on kinesin-coated surfaces, which is of interest for technological applications employing molecular motors [8].

7.4 Materials and Methods

Microfluidic channels were fabricated between two 1 mm diameter entrance holes in 500 μm thick fused-silica substrates. Substrates were cleaned in acetone, fuming nitric acid (HNO₃), and iso-propanol alcohol (IPA). The substrates were covered with 35 nm chromium (Cr) and 800 nm e-beam-sensitive resist polymethylmethacrylate (PMMA). Channels were defined in the resist with a Leica electron-beam pattern-generator and developed in a mixture (1:3) of methylisobutyl ketone and IPA. The remaining Cr layer in the structures was removed by a 1 min immersion in Cr etchant. Channels were wet-etched 1 μm deep into the substrates using ammonium-fluorid etchant. After etching, the remaining resist and chromium were removed using PRS3000 and Cr etchant. Channels were sealed using a silica-bonding procedure [25]. Both the channel and sealing substrates were cleaned in PRS3000 HNO₃. Then, the substrates were dipped
into a 0.5% solution of hydro-fluoric acid for 1 min. A 2% sodium-silicate solution (Sigma) was spin-coated onto the cover substrate, immediately followed by pressing the sodium-silicate coated surface onto the patterned channel surface. Finally, the devices were cured for 1 h at 90 °C.

Channel structures were coated with casein and kinesin using pressure-driven flows (~5 kPa), created by water columns on the entrance reservoirs. First, channels were filled with BRB80 buffer (82 mM Pipes [pH = 6.9], 1 mM MgCl$_2$, 1 mM EGTA) supplemented with 1 mM adenosine-triphosphate (ATP), 100 µM paclitaxel (Taxol), and 2% beta-mercaptoethanol (BME) and any air bubbles were removed with pressure. Then 20 µL of 0.9 mg/mL casein solution in BRB80 was added to an emptied reservoir and flushed through the channels during 1.5 hours. Finally, a kinesin solution (70 µg/mL his-tagged full-length Drosophila conventional kinesin [26], 0.9 mg/mL casein, 9 mM ATP in BR80) was flushed through the channels with the same pressure during 2 h. Because proteins can enter the structures only from the reservoir, the protein coating progresses from upstream towards downstream with time. In the last step, a motility solution containing microtubules (200 nM tubulin) in BRB80, supplemented with 100 µM Taxol, 6 mM ATP and an antifade cocktail (120 mM D-glucose, 0.12 mg/mL glucose-oxidase, 0.05 mg/µL catalase and 2% BME) was added to the entrance reservoir. Rhodamine-labeled microtubules were polymerized (37 °C for 45 min) from bovine brain tubulin (4 mg/mL, Cytoskeleton, Denver, CO) in a 1:3 stoichiometry of labeled:unlabeled tubulin in the presence of 4 mM MgCl$_2$, 1 mM GTP and 5% DMSO in BRB80 buffer. Microtubules were stabilized and 20× diluted in BRB80 containing 100 µM paclitaxel.

Electric fields were applied using a Keithley 6517A electrometer. Voltages up to 125 V were applied, where the electrical current would be 2 µA. During experiments, the entrance reservoirs were exposed to atmospheric pressure to prevent a Poiseuille backflow due to EOF-induced pressure build-up. Consequently, the velocity profile in the channel is homogeneous except within the Debye length scale of ~1 nm from the surface. Joule heating is limited due to the large surface-to-volume ratio of our channels. In steady-state, the electrical dissipation in the channel is balanced by thermal conduction through the glass substrates. For a slit-like channel of height $h$ (filled with a buffer solution of conductivity $\sigma = 1.3$ S/m), the temperature gradient over the glass substrates equals $\frac{dT}{dz} \simeq \frac{\sigma E^2 h}{\lambda}$, where $\lambda$ is the thermal conductivity of glass (1.4 W/Km). The temperature increase in our channels at the largest field strengths used is thus expected to be limited to ~1°C with respect to ambient room temperature. An in-situ probe of the temperature is provided by the velocity of the motility in our channels. Motility speed is known to double for approximately every 10 °C
increase in temperature [27]. The absence of a systematic increase in gliding veloc-
ity when an electric field is applied confirms that the temperature increase is
limited.

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Supplementary Information

This appendix contains additional information that was published as supporting information to the main text.

7.A  Bending of the microtubule tip and progression of the path

We calculate the average microtubule trajectory under a force density $f_e$ directed along the $x$ axis (Fig. S.1a). The microtubule’s path coordinates $s, \theta$ are defined within the cartesian reference frame as depicted in the figure. As explained in the main text, the average curvature of the microtubule path $\langle d\theta/ds \rangle$ is related to the microscopic bending of the microtubule tip $\langle \theta'_m(s) \rangle$ (Fig. S.1b) as

$$\langle \frac{d\theta}{ds}(s) \rangle \approx \langle \theta'_m(s) \rangle.$$

(S.1)

In order to determine the macroscopic $\theta(s)$ we need to calculate the microscopic bending $\theta'_m(s)$ due to an external perpendicular force $f_\perp = f_e \sin\theta(s)$.

7.A.1  Bending of the microtubule tip

We relate the microscopic bending of the microtubule tip to its material properties. We consider the bending of the free tip of length $\langle d \rangle$ (Fig. S.1c) that is fixed in position and orientation ($\theta' = 0$) at the origin. Using the microscopic coordinates $\theta', s'$ as defined in the figure, we express the microscopic tip curvature as a function of the bending momentum $M(s')$ [28]

$$\frac{d\theta'}{ds'} = -\frac{M(s')}{EI}.$$

(S.2)

Here $E$ is the bending modulus and $I$ is the second moment of inertia. We calculate the internal shear force $F(s')$ and internal bending momentum $M(s')$ on a cross section of the tip segment of length $(\langle d \rangle - s')$ (Fig. S.1d). From force balance we find $F(s') = f_\perp (\langle d \rangle - s')$ and from balance of momentum we find $M(s') = -\frac{f_\perp}{2} (\langle d \rangle - s')^2$. Substituting $M(s')$ into Eq. S.2 we obtain an expression for the shape of the bent tip,

$$\frac{d\theta'}{ds'} = \frac{f_\perp}{2EI} (\langle d \rangle - s')^2.$$

(S.3)
Solving this equation with the boundary condition $\theta'(0) = 0$ yields for the shape of the tip

$$\theta'(s') = \frac{f_\perp}{6EI} \left( s'^3 - 3\langle d \rangle s'^2 + 3\langle d \rangle^2 s' \right), \quad (S.4)$$

which defines the deviation at the end of the tip as $\theta'_m = \frac{f_\perp\langle d \rangle^3}{6EI}$. Substituting this in Eq. S.1 we find that the local curvature of the microtubule trajectory is

$$\langle \frac{d\theta}{ds} \rangle = \frac{f_e\langle d \rangle^2}{6EI} \sin\theta. \quad (S.5)$$

Substitution of $EI = k_B T p$ yields the result as stated in the main text.

### 7.A.2 Expression for the microtubule trajectory

Having obtained an expression for the local curvature (Eq. S.5), we now find the expression for the microtubule trajectory. For this purpose, we will express the path curvature in cartesian coordinates as

$$\frac{d\theta}{ds} = \frac{1}{\left[ 1 + \left( \frac{dy}{dx} \right)^2 \right]^{3/2}} \frac{d^2y}{dx^2}. \quad (S.6)$$
Supplementary Information

To circumvent an infinite boundary condition at the origin \( \frac{dy}{dx} \equiv \infty \) we will solve the trajectory for a microtubule starting at the origin oriented along the \( x \) axis, and subjected to a force density directed along the \( y \) axis. In the final solution we will then interchange the \( x \) and \( y \) coordinates to arrive at a description for the trajectory as depicted in Fig. S.1a. Thus, we combine Eqs. S.5 and S.6 where we replace \( \sin \theta \) with \( \cos \theta \) because of the axis interchange. Rewriting \( \cos \theta = \left[ 1 + \left( \frac{dy}{dx} \right)^2 \right]^{-1/2} \) we arrive at the differential equation describing the microtubule trajectory

\[
\frac{d^2 y}{dx^2} = \left[ 1 + \left( \frac{dy}{dx} \right)^2 \right] \frac{f_e \langle d \rangle^2}{6k_B T_p}.
\]  

(S.7)

Solving the equation with initial conditions \( \frac{dy}{dx} \bigg|_{x=0} = 0 \), and \( y(0) = 0 \) we find that \( y(x) = -R_0 \ln(\cos \frac{x}{R_0}) \), where \( R_0 = \frac{6k_B T_p}{f_e \langle d \rangle^2} \). Interchanging the \( x \) and \( y \) coordinates according to Fig. S.1a we obtain

\[
y(x) = R_0 \arccos(e^{-x/R_0}).
\]  

(S.8)

7.B Magnitude of the electric field-induced force

We calculate the expression for the electric field-induced force in the situation of stationary electrophoresis, that is, the force that is exerted on a charged object which is prevented from moving in an electric field. We show a cartoon of the experimental situation Fig. S.2a. A negatively charged microtubule is subjected to an electric field \( E_\perp \) which is directed perpendicular to its long axis. The field exerts a force per unit length of the microtubule which we denote \( f_q \). Moreover, the electric field also exerts force on the counter ions immediately around the microtubule. As a result of this, and additionally because of any electro-osmotic flow, the fluid will move around the microtubule and exert a force per unit length of the microtubule, \( f_{\text{fluid}} \), in the direction indicated. Thus, the effect of the electric field is to exert a net perpendicular force on the microtubule,

\[
f_\perp = f_q + f_{\text{fluid}},
\]  

(S.9)

which is balanced by the mechanical force \( f_{\text{mech}} \), that is exerted through the kinesin molecules.
Figure S.2: (a) Schematic of the experimental situation. A microtubule is subjected to a perpendicular electric field $E_\perp$. The electric field exerts a force density $f_q$ on the negatively charged microtubule. The moving fluid exerts a force density $f_{\text{fluid}}$ which we calculate. The microtubule is held taut by a mechanical force exerted by the kinesin $f_{\text{mech}}$. (b-d) To calculate $f_{\text{fluid}}$ we make use of the linearity of the Navier-Stokes equations. The schematic fluid velocity profile around the microtubule in stationary electrophoresis is shown in (b) as a function of distance from the microtubule surface. This profile is a superposition of the fluid velocity profiles shown in (c-d). (c) Fluid velocity around an object that moves in purely hydrodynamic motion to the left with a velocity $-\mu E_\perp$. (d) Fluid velocity profile around an object in free electrophoresis, that moves to the right with a velocity $\mu E_\perp$.

It remains to calculate the magnitude of the forces in Eq. S.9. The magnitude of the electrical force $f_q$ equals $\lambda E_\perp$, where $\lambda$ is the line-charge density of the microtubule. The magnitude of the fluid forces can be calculated from the fluid velocity profile $\mathbf{v}$ around the microtubule tip. This profile can be obtained from solving the Navier-Stokes equation,

$$\eta \nabla^2 \mathbf{v} + \nabla P = -\rho \nabla \psi.$$  \hspace{1cm} (S.10)

The first term represents the viscous forces, with $\eta$ the viscosity of the fluid, the second term represents the pressure forces, with $P$ the hydrostatic pressure, and the last term denotes the electrical forces on the fluid, through the charge density $\rho$ and potential $\psi$. In the situation of stationary electrophoresis, we need to solve Eq. S.10 with the boundary conditions $\mathbf{v} = 0$ at positions 0 (microtubule surface) and $X$, where $X$ denotes the surfaces of the channel walls (no-slip boundary condition). A schematic of the fluid velocity profile is depicted in Fig. S.2b.
The linearity of the Navier-Stokes equations allows us to state the solution to Eq. S.10 as \( \mathbf{v} = \mathbf{v}_h + \mathbf{v}_e \) [16], where \( \mathbf{v}_h \) is the solution to the homogeneous differential equation, and \( \mathbf{v}_e \) is a particular solution to Eq. S.10. The boundary conditions request that \( \mathbf{v}_h = \mathbf{v}_e = 0 \) at the no-slip surfaces \( X \) and that \( \mathbf{v}_h = -\mathbf{v}_e \) at the surface of the microtubule (Fig. S.2b-d). In other words, the solution to the problem of stationary electrophoresis can be found from a superposition of solutions to the following homogeneous and particular differential equations for \( \mathbf{v}_h \) and \( \mathbf{v}_e \), respectively (with \( P_h + P_e = P \)),

\[
\begin{align*}
\eta \nabla^2 \mathbf{v}_h + \nabla P_h &= 0 \quad \text{with } \mathbf{v}_h(X) = 0, \quad \mathbf{v}_h(0) = -\mathbf{v}_e(0), \\
\eta \nabla^2 \mathbf{v}_e + \nabla P_e &= -\rho \nabla \psi \quad \text{with } \mathbf{v}_e(X) = 0, \quad \mathbf{v}_e(0) = \mu \perp E \perp. 
\end{align*}
\] (S.11)

These differential equations describe the well-known situations of (1) the motion of an object with a certain velocity \( \mathbf{v}_h(0) \) by a non-electric force (Fig. S.2c), that is, purely hydrodynamic motion, and (2) the free electrophoresis of an object (Fig. S.2d) with velocity \( \mathbf{v}_e(0) \), due to its mobility \( \mu \perp \).

For both situations the magnitude of the resultant fluid forces are known. In the case of purely hydrodynamic motion of an object with velocity \( \mathbf{v}_h(0) \), the fluid forces have to balance the external force, and add up to \( f_{\text{fluid},h} = -c_\perp \mathbf{v}_h(0) \), where \( c_\perp \) is the Stokes-friction coefficient for the object. In the case of free electrophoresis, the fluid forces completely balance the electrical force on the object and thus \( f_{\text{fluid},e} = -\lambda E \perp \). Since \( \mathbf{v}_h(0) = -\mu \perp E \perp \) (Fig. S.2c, Eq. S.11), we calculate that \( f_{\text{fluid},h} = c_\perp \mu \perp E \perp \). Therefore, in the case of stationary electrophoresis, the total force exerted by the fluid profile \( \mathbf{v} = \mathbf{v}_h + \mathbf{v}_e \) around the stationary microtubule equals \( c_\perp \mu \perp E \perp - \lambda E \perp \). The total electric field-induced force (Eq. S.9) is thus equal to

\[ f_\perp = c_\perp \mu \perp E \perp. \] (S.12)

### 7.C Measurement of perpendicular mobility

We measure the value of \( \mu \perp,e \) in Eq. 7.3 by performing electrophoresis experiments on freely suspended microtubules in channels as described previously [19]. In short, we coat the inside of straight 1 \( \mu \)m high channels with casein to prevent sticking of microtubules, with omission of kinesin, and then add microtubules. Upon application of an electric field, we observe the electrophoresis of individual microtubules in various orientations (Fig. S.3). As expected, the electrophoretic motion of microtubules is in the direction opposite to the electric field. Because of their anisotropic mobility [19], microtubules that are oriented parallel to the
Figure S.3: (a) Overlay of snapshots with 0.12 s intervals of freely suspended microtubules in free electrophoresis ($E = 4 \text{ kV/m}$). The velocity is orientation dependent [19] as is obvious from these two microtubules in extreme orientations. (b) Binned data (5800 data points) of orientation-dependent velocities under the same electric field. The inset shows the value of $v_\perp$ for different electric fields.

electric field move faster than microtubules oriented perpendicular to the field (Fig. S.3a). Figure S.3b is a plot of binned values of a large number (~5800) of orientation-dependent velocities, measured with $E = 4 \text{ kV/m}$. For the calibration of the perpendicular force (Eq. 7.3) we are only interested in the velocity of microtubules oriented perpendicular to the electric field, $v_\perp = \mu_{\perp,e}E$. The inset in Fig. S.3b shows measured values of $v_\perp$ for different electric fields. From the linear relation between $v_\perp$ and $E$ we determine $\mu_{\perp,e} = -(1.03 \pm 0.01) \cdot 10^{-8} \text{ m}^2/\text{Vs}$. We note that this value represents both the electrical properties of the microtubules as well as the electro-osmotic flow (EOF) velocity in the channel. We confirmed in separate experiments [19] that the additional coating of kinesin molecules had no significant influence on the EOF.
Persistence Length Measurements from Stochastic Single-Microtubule Trajectories

We present a simple method to determine the persistence length of short sub-micrometer microtubule ends from their stochastic trajectories on kinesin-coated surfaces. The tangent angle of a microtubule trajectory is similar to a random walk, that is solely determined by the stiffness of the leading tip and the velocity of the microtubule. We demonstrate that even a single microtubule trajectory suffices to obtain a reliable value of the persistence length. We do this by calculating the variance in the tangent trajectory angle of an individual microtubule. By averaging over many individual microtubule trajectories, we find that the persistence length of microtubule tips is $0.24 \pm 0.03$ mm.

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8.1 Introduction

Cytoskeletal molecular motors such as dynein, kinesin or myosin use the energy of ATP hydrolysis to move unidirectionally along their associated protein filaments (microtubules for dynein and kinesin, actin filaments for myosin) [1]. Cytoskeletal motors are involved in cellular organization, force generation and directed intracellular transport of cargo [2], and the use of these motor proteins for nanotechnological tasks is being explored [3]. The motility of motor proteins can be reconstituted in vitro in an inverted gliding assay [4]. Here, purified motor proteins are adsorbed onto a glass slide and subsequently bind to their cytoskeletal filaments, propelling them over the surface. By observing the motion of the filaments, various properties of the motor proteins, such as velocity, directionality, and processivity, have been studied [5].

In this work we use the inverted gliding assay to examine properties of the biofilaments themselves, in particular the stiffness of microtubule ends. Microtubules gliding over a kinesin-coated surface follow a random trajectory, the properties of which are determined by the fluctuations of the leading end. By measuring the mean-square deviation of the trajectory tangent angle as a function of time, we are able to infer the persistence length of the leading microtubule ends that have only sub-micrometer lengths.

It is of interest to measure the persistence length of short microtubule ends because microtubules show material properties on short length scales that deviate from the long length behavior [6, 7]. Recently, it was measured that the persistence length of microtubules strongly decreased when the microtubule length was reduced [7]. This was inferred from observing thermal fluctuations of microtubule ends that were grafted on a substrate edge, and had a fluorescent reporter bead attached to it. This method was used on microtubule lengths down to 3 $\mu$m. In gliding assays, the length of the leading microtubule end that extends beyond the last kinesin motor protein that binds to it can be even much shorter, depending on the surface concentration of motors. Observation of stochastic microtubule trajectories thus provides a convenient way of characterizing the persistence length of these short ends.

We present measurements of microtubule trajectories on unstructured kinesin-coated glass surfaces. We demonstrate that a measure of the persistence length can already be obtained from a single microtubule trajectory. By averaging over multiple trajectories, we find that the persistence length of the taxol-stabilized microtubule ends is $0.24 \pm 0.03$ mm, which is indeed much shorter than expected for longer microtubules. This method provides a simple way to probe the magnitude of thermal fluctuations of microtubule ends that can be very short.
8.2 Stochastic microtubule trajectories

The trajectories of microtubules over a kinesin-coated surface obey statistics of a random walk [8]. As a microtubule is propelled, its leading end becomes progressively longer and is free to fluctuate through thermal agitation (Fig. 8.1a). By doing so, the microtubule end explores a certain area ahead of it, where it will find a new kinesin to bind to. The range of fluctuations of the leading tip depends on the length and the stiffness of the microtubule end. The stiffness of the microtubule tip determines the curvature of the microtubule between adjacent motors and thus the curvature of the trajectory. We assume that the fluctuations of the microtubule tip are determined by the properties of the tip, while the remaining and largest part of the microtubule is fixated by the many bound kinesin motors distributed along its length (Fig. 8.1a). The persistence length of the microtubule trajectory then equals the persistence length of the leading tip, provided the following two conditions are met [8]: (i) microtubules should be long enough to be attached to more than one kinesin at all times in order to prevent pivoting around a single motor, and (ii) the kinesin surface density should be high enough to prevent buckling of the microtubule tip when it is fluctuating.

Both conditions are met in our experiments. The first condition is fulfilled because we limit our experiments to microtubules that move without sudden changes of direction. In practice, this is the normal behavior for microtubules that are longer than one micrometer. This indicates that the distance between kinesin molecules bound to the microtubules is smaller than half this value. From this, we estimate that the surface density of active kinesin molecules is about $100 \ \mu m^{-2}$ [9]. This density is well above the critical limit at which the microtubule end is expected to buckle, thus fulfilling the second condition.

We now relate the curvature of microtubule trajectories to the persistence length of the leading end. The orientations of a thermally fluctuating microtubule end of length $d$ are Gaussian distributed around zero with a standard deviation $\sqrt{d/p}$, where $p$ is the persistence length of the fluctuating end [10]. The trajectory of the microtubule is mapped out by the orientation of its leading end, and thus the variance of the trajectory tangent angle $\theta$ will increase on each consecutive displacement $d_i$. The evolution of $\theta$ can be described as a random walk, and after $N$ steps, the variance in $\theta$ is given by $\text{Var}(\theta) = \sum_{i=1}^{N} \frac{d_i}{p}$. After time $t$, the $\sum_{i=1}^{N} d_i = vt$, with $v$ the speed of the microtubule, which yields a single expression for the time evolution of $\text{Var}(\theta)$:
Figure 8.1: (a) Microtubules in a gliding assay follow a random trajectory due to thermal fluctuations of the leading tip. (b) Overlay of fluorescence images (with 10 s intervals) of microtubule gliding on a kinesin-coated glass substrate. (c) Zoom of an microtubule trajectory. The image was made by overlaying 8 fluorescence images with 2 second intervals of a single microtubule with a length of 3 \( \mu \)m. One fluorescence image in the overlay has increased brightness to accentuate the microtubule. During the time of 14 s the microtubule has traversed 10 \( \mu \)m. Superimposed are the coordinates of the leading tip of the microtubule, which are determined with 1 s intervals, using a fully automated MatLab routine. From the coordinates of the leading tip, tangent trajectory angles are determined at each step from the coordinates of the two adjacent time points.

\[
\text{Var}(\theta) = \frac{vt}{p}. \quad (8.1)
\]

8.3 Single microtubule trajectories

We use fluorescence microscopy to record time-lapse images of microtubules gliding over a kinesin-coated surface (Materials and Methods). We take fluorescence images with one-second time resolution of an area that measures 87 \( \times \) 66 \( \mu \)m\(^2\). In Fig. 8.1b we show an overlay of fluorescence images with 10 second intervals that shows gliding motion of individual microtubules. The microtubules largely preserve their directionality during their trajectory, and only little curvature is present. From the image it is thus clear that the persistence length of the microtubule ends is larger than the length of their trajectories (\( \sim 30 \) \( \mu \)m in this image).
8.3 Single microtubule trajectories

Figure 8.2: Single microtubule trajectories were used to determine the variance of \( \theta \) as a function of time by internal averaging. (a) A microtubule trajectory (upper panel) containing 97 datapoints. The calculated \( \langle \theta^2(t) \rangle \) is shown in the lower panel. Red line is a weighed fits through points 1 – 9 s, that have expected relative errors less than 40 %. (b) Similar data for a microtubule trajectory of 64 points, with a fit through points 1 – 6 s (c) Microtubule trajectory containing 59 data points, with a fit through 1 – 6 s.

We use an automated tracing routine written in Matlab to determine the coordinates of the leading end of microtubules in consecutive frames. In this way, we obtain time-lapse trajectories of a large number of microtubules. In Fig. 8.1c we show an overlay of fluorescence images of an individual microtubule during 14 seconds, on which we superimposed the coordinates of its leading tip, as determined using our tracing routine. As expected, the coordinates of the leading tip overlap over the entire trajectory. From the coordinates of the leading microtubule tip, we calculate tangent angles \( \theta \) at each time point of the trajectory from the coordinates of the two adjacent time points. In this way, we obtain a collection of angles, \( \theta_1, \theta_2, \theta_3, ..., \theta_N \) at times \( t_1, t_2, t_3, ..., t_N \) from an individual microtubule trajectory measured over \( N + 2 \) images.

We note that there is some uncertainty in the determination of the precise coordinates of the microtubule tip. This can be due to the optical blurring of the microtubule, its movement during the image recording (\( \sim 70 \) nm during the 100 ms integration time of the camera), pixel discretization (65 nm), or the tracing routine. However, these errors are expected to be random and uncorrelated over time and will not have an effect on the measured variance of \( \theta \), except at zero time lag between the points for which the variance is calculated [11].

We now show that we can obtain an estimate of the variance of \( \theta \) of even a single microtubule trajectory. We do this using internal averaging of a microtubule trajectory, which is analogous to single-particle tracking experiments, in which the diffusional motion of a single particle is used to measure its diffusion.
coefficient \([11, 12]\). We consider the calculation of the variance of \(\theta\) as a function of time from a single trajectory of \(N + 2\) images, by averaging over all pairs of points in the trajectory with a certain time lag. Given the collection of tangent angles \(\theta(n \triangle t)\), with \(\triangle t\) the time between the images and \(n\) any value from 1 to \(N\), there are \(N - n\) pairs that have a time lag \(n \triangle t\), and the mean of the variance of \(\theta\) at that time interval is:

\[
\langle \theta^2(n \triangle t) \rangle = \frac{1}{N - n} \sum_{i=1}^{N-n} [\theta((n + i) \triangle t) - \theta(i \triangle t)]^2.
\]  

(8.2)

Figure 8.2 shows 3 representative traces of microtubules trajectories, together with plots of \(\langle \theta^2(t) \rangle\), obtained from these individual trajectories using Eq. 8.2. In Fig. 8.2a, we show that \(\langle \theta^2(t) \rangle\) increases linearly with time, as expected from Eq. 8.1. It should be noted that the error bars that are indicated on the data are much larger than the scatter in the data suggests. This is a result of two effects. First, the number of data points that is used to calculate \(\langle \theta^2 \rangle\) decreases with time and, second, the data points are not statistically independent \([11]\). As an illustration, the value of \(\langle \theta^2(1) \rangle\) in Fig. 8.2a is obtained from the average of \(N - 1 = 96\) statistically independent pairs (Eq. 8.2). On the contrary, the value \(\langle \theta^2(40) \rangle\) is obtained from only \(N - 40 = 57\) individual measurements (i.e. \([\theta(41) - \theta(1)], [\theta(42) - \theta(2)], \ldots, [\theta(97) - \theta(57)]\)), and these are highly correlated because they partially overlap, which results in the smooth variation of the variance as a function of time.

To estimate the error in \(\langle \theta^2 \rangle\) at each particular time, one needs to take into account this statistical correlation. An analysis in ref. \([11]\) estimated this error for a random walk in two dimensions, which can easily be extended to a one-dimensional random walk, as in our case for the trajectory angle \(\theta\). Consider that the value of \(\theta\) is Gaussian distributed and that its mean-square deviation \(\sigma_{\theta}^2\) increases with time as \(vt/p\) (Eq. 8.1). Then, the variance to be expected in the mean-square deviation of \(\theta^2\) \((\text{var}(\theta^2) = \langle(\theta^2 - \sigma_{\theta}^2)^2\rangle\) equals \(2(vt/p)^2\) \([11]\), and if \(\theta^2\) is calculated by averaging over different segments that are not statistically independent, then the expected variance in \(\theta^2\) was shown to be \(2(vt/p)^2 \cdot 2n/3(N - n)\). Thus, if we take the standard deviation of \(\theta^2\) as the expected error, the relative error on each particular value of \(\langle \theta^2(n \triangle t) \rangle\) follows as \(\sqrt{4n/3(N - n)}\). This value for the expected relative error is valid as long as \(n < N/2\) \([11]\). The errors shown in Fig. 8.2 where calculated using this formula.

The length of the microtubule trajectories varies significantly in our data. Obviously, it cannot exceed the size of the microscope field-of-view. In prac-
tice it is limited by our automated tracing routine that cannot reliably track a microtubule if it crosses another microtubule. This frequently happens at the moderate microtubule densities which we need to collect a statistically significant amount of data. Spontaneous detachment of microtubules from the surface was not a reason for limited trajectory lengths because this never happens at the high kinesin-densities that we use. Another reason for ending a microtubule trajectory is that a microtubule gets pinned at the surface, presumably by a defect or an inactive kinesin motor, but this occurred only very infrequently. The resulting length distribution of trajectories is reminiscent of an exponential distribution with approximately 70% of the trajectories having a length smaller than the mean trajectory length of 13 \( \mu m \).

We obtain a value of the persistence length from individual microtubule trajectories from the slope of a weighted linear least-squares fit of Eq. 8.1 to the data, where the least-square residues are weighted inversely proportional to the error bars squared. We (arbitrarily) chose to include only those data points of each trajectory for which the estimated relative error in \( \langle \theta^2(t) \rangle \) was less than 40%. Given the short lengths of our trajectories the relative error increases fast for increasing time lag \( n \Delta t \) and at the chosen error margin typically only 5 to 10 points were included in each single-trajectory fit. Thus, this error margin is also an indication of the accuracy of the persistence length that we expect to derive from the individual slopes. From the fit to the data in Fig. 8.2a (red line) we find a slope \((2.1 \pm 0.2) \cdot 10^{-3} \text{ s}^{-1}\). The velocity of this microtubule was \(0.75 \pm 0.01 \mu m/s\) (mean \(\pm\) standard error of the mean (SEM)), which results in a persistence length \(p = 0.36 \pm 0.03 \text{ mm}\) for this particular microtubule. Similarly, we obtain from the trajectories in Fig. 8.2b-c persistence length values of \(p = 0.18 \pm 0.01 \text{ mm}\), and \(p = 0.15 \pm 0.02 \text{ mm}\), respectively. Note that these errors represent only the least-squares errors of the linear fit, but that the actual error in \(p\) can be as large as 40%, given our choice to fit data points up to the time \(t\) were the expected standard deviation in \(\langle \theta^2(t) \rangle\) reaches 40%.

### 8.4 Averaging over multiple trajectories

Although we demonstrate that individual microtubule trajectories allow to determine the persistence length of the microtubule end, the statistical uncertainty in these values is relatively large because the length of the microtubule trajectories is experimentally limited. As we show in Fig. 8.2, the expected relative error on \(\langle \theta^2(t) \rangle\) can easily become larger than 40% for values of \(\langle \theta^2(t) \rangle\) that are calculated for points along the trajectory that are separated by several seconds. For larger
Figure 8.3: (a) The distribution of slopes obtained from 121 single microtubule trajectories. The red line indicates the value of the slope that is obtained from panel (b). (b) The mean variance of $\theta$ as a function of time, calculated from the average of single-microtubule $\theta^2(t)$’s using Eq. 8.3. The error on the values of $\langle \langle \theta^2(t) \rangle \rangle_{MT}$ is calculated as the standard error of the mean of the errors of the single-trajectory $\langle \theta^2(t) \rangle$. The red line is a weighed least-squares fit through data points 1 – 40 s. (c) The velocity of microtubules is normally distributed with $0.77 \pm 0.09 \mu m/s$ (mean ± standard deviation).

The statistical uncertainty in the determination of the persistence length from single-microtubule trajectories is apparent from the distribution of slopes that we determined from a large number of single-microtubule trajectories in a similar way as illustrated in Fig. 8.2. The slopes were obtained by linear fits through those points of single-microtubule-trajectories $\langle \theta^2(t) \rangle$ that have a relative error of less than 40%. In Fig. 8.3a we show the resulting distribution of slopes that was obtained from 121 trajectories. In accordance with the allowed error margin for the points included in the fitting procedure, the slopes are broadly distributed, with $(3.2 \pm 2.0) \cdot 10^{-3} \text{ s}^{-1}$ (mean ± standard deviation (STD)). We note that the standard deviation is larger (63% of the mean) than expected from the allowed relative error of 40% in the fits of the individual trajectories. This additional variance can be due to variations in the persistence length for different microtubules.

A more accurate estimate for the persistence length can be obtained by averaging the values of $\theta^2(t)$ from multiple microtubule trajectories. We construct a multi-trajectory $\langle \langle \theta^2(t) \rangle \rangle_{MT}$ from an average over $\theta^2(t)$’s:

$$\langle \langle \theta^2(n\Delta t) \rangle \rangle_{MT} = \left( \frac{1}{\sum_{j=1}^{M} N_j - n} \right) \sum_{j=1}^{M} \sum_{i=1}^{N_j-n} \left[ \theta((n+i)\Delta t) - \theta(i\Delta t) \right]^2,$$  (8.3)
8.5 Simulations of stochastic trajectories

where the sum $\sum_{j=1}^{M}$ runs over all $M$ microtubule trajectories $j$ that each contain $N_j$ data points. With respect to the expected error on the values of $\langle \langle \theta^2(t) \rangle \rangle_{MT}$ we note that a simple standard error of the mean will yield an underestimate, for the reason that we previously discussed; a large number of $\theta^2$’s that enter in the sum of Eq. 8.3 are not statistically independent. On the other hand, an upper bound of the expected error can be found from considering the mean of the variances of the individual microtubule trajectories at each time point. Thus, as an upper bound, we approximate the error on each value of the multi-trajectory average $\langle \langle \theta^2(t) \rangle \rangle_{MT}$ with the standard error of the mean of the absolute errors of the single-trajectory $\langle \theta^2(t) \rangle$.

In Fig. 8.3b we show the average $\langle \langle \theta^2(t) \rangle \rangle_{MT}$ as obtained from averaging data from 885 individual microtubule trajectories. The magnitude of the errors is not monotonic as a function of time, because not all trajectories that are used in the approximation of the errors have the same length. The red line is a weighted fit of Eq. 8.1 through the data which yields a slope of $(3.19 \pm 0.04) \cdot 10^{-3}$ s$^{-1}$. In agreement with the larger data set, this slope can be more accurately determined than from individual trajectories. As we indicate in Fig. 8.3a, this value of the slope is slightly higher than the value corresponding with the peak of the distribution of single-trajectory slopes. We attribute this discrepancy to the limited and finite length of the individual microtubule trajectories, as we will discuss below.

To obtain the persistence length of the microtubule population, we determine the velocity of microtubules from their displacement between frames. As expected, the velocity of the microtubules is Gaussian distributed (Fig. 8.3c) and has an average of $0.77 \pm 0.09$ µm/s (mean ± standard deviation). Using this value, we find an average persistence length $p = 0.24 \pm 0.03$ mm for the entire population.

8.5 Simulations of stochastic trajectories

We have determined a value of the persistence length of microtubule tips from individual microtubule trajectories, as well as from an average over multiple microtubule trajectories. Additionally, we confirm the validity of this method by simulating the trajectories of a number of microtubule of known stiffness and subjecting it to the same analysis. In particular we are interested to see if we can extract the value of the persistence length that we have entered in the simulations, and whether we can assess the accuracy of the distribution of individual slopes.
We simulate a number of stochastic microtubule trajectories by calculating the step-wise progression of microtubules with Gaussian-distributed tip orientations, that were taken from a distribution with standard deviation $\sqrt{d/p}$ [10]. We use values for the microtubule velocity, time resolution, persistence length, the number of microtubule trajectories, and for length distribution of trajectories that are close to the experimental data [13]. The simulated microtubule trajectories were subsequently analyzed using the same procedures as for the experimental data.
In Fig. 8.4a we show a selection of simulated microtubule traces. As expected the trajectories are relatively straight since they are short compared to the persistence length of the microtubule tips. In our experiments the trajectory lengths of microtubules are limited mainly by microtubule crossings, and for this reason we took a similar distribution of trajectory lengths for our simulations, of which Fig. 8.4a gives a representative impression. Most microtubule trajectories are relatively short with lengths of approximately 5 – 15 µm. We determine a distribution of slopes from individual microtubule trajectories as before, taking only those points with relative errors less than 40%. The distribution of slopes from 116 trajectories (Fig. 8.4b), normalized to the expected value of $v/p$, is qualitatively similar to what we experimentally found, with a rather broad distribution of $0.98 \pm 0.36$ (mean ± standard deviation (STD) in units of the expected value of $v/p$). In Fig. 8.4c we show the multi-trajectory average which is obtained from the simulated data using Eq. 8.3. The features of the experimental data in Fig. 8.3b are qualitatively reproduced, most notably the non-monotonic increase of the error bars, and a deviation from linear behavior for larger times. A weighted linear fit yield $0.98 \pm 0.01$ in units of $v/p$.

The values that we find from the distribution of single-trajectory slopes and from the slope of the multi-trajectory average are in good agreement with the value that we have entered into the simulation, which confirms the validity of our analysis. Moreover, we note that the standard deviation of the distribution of slopes 36% of the mean, which is close to 40% that is expected from the maximum relative error in the data that we allow in the fit from individual single-microtubule trajectories. From this, we confirm that the larger standard deviation in the experimental distribution of slopes (Fig. 8.3a) most likely reflects an additional variance due to differences between individual microtubules. The value that we have determined from the experimental multi-trajectory average (Fig. 8.3b) is expected to form an accurate population average.

Finally, we want to confirm that the asymmetric distribution of experimental single-trajectory slopes is indeed due to the finite-length of the trajectories as we previously surmised. Single-particle tracking applied to simulated two-dimensional Brownian motion has yielded similar results, with the distribution of slopes becoming increasingly wider and peaked towards lower values than expected upon increasing the number of points that were included in the fit [12]. To test this for the one-dimensional variable $\theta$ in our experiments, we simulate a large number of trajectories (5000) and we determine the distribution of single-trajectory slopes, upon varying the maximum relative error that we allow in the data that is used for the fits of the slopes. Figure 8.4d shows the resulting distribution of slopes determined from single trajectories where the fit included data.
points that had an expected relative error of less than 20%, 50%, and 100%, respectively. It is clear that all upon allowing a larger error, the distribution of slopes becomes increasingly wider and peaked toward lower values, in agreement with the Brownian-motion results in ref. [12].

8.6 Discussion

We have shown that we can measure a value of the persistence length of the tips of single microtubules by tracing the coordinates of their leading tip. Calculation of the variance of the tangent trajectory angles, together with internal averaging of the angles, provides a simple method to construct a $\langle \theta^2 \rangle$ versus time plot for a single microtubule trajectory. The correlation that is induced by internal averaging is estimated by attributing relative errors to the $\langle \theta^2 \rangle$ data of magnitude $\sqrt{4n/3(N-n)}$ [11]. By fitting the linear part of the $\langle \theta^2(t) \rangle$ data, and by measuring the velocity of the microtubule, the persistence length of its tip can be calculated.

This method yields satisfactory results as long as the attributed relative errors are small, for which a relatively long trajectory is needed. For example, if a trajectory consists of $N = 100$ points, and a relative error of 40% in the value of $\langle \theta^2(t) \rangle$ is accepted, only the first $n = 10$ points can be used. If the acceptable error is 20%, only the first two or three points can be used. This inaccuracy is reflected in the width of the distribution of slopes that we determined from a large number of experimental single-microtubule trajectories. However, we find a distribution that is wider than expected, which could indicate variations between individual microtubules. We showed that by averaging over multiple independent microtubule trajectories we could obtain more accurate results. The consistency of our method was checked with simulations.

The persistence length $p = 0.24 \pm 0.03$ mm that we find for the microtubule ends in our experiments is much smaller than the value of $4 - 8$ mm that is measured for long (5 - 50 µm) microtubules [14, 15], but in agreement with the reducing trend that was observed by Pampaloni et al. down to microtubule ends of 3 µm [7]. This effect was attributed to shear deformation of microtubules due to sliding of adjacent protofilaments, which gives an extra compliance and that becomes dominant if the bending occurs on short length scales. In our experiments, we have varied the kinesin density over a factor of ten (Materials and Method), and we did not find a significantly different value of the persistence length in this range. Interestingly, two previous reports mention a value of the persistence length of microtubule trajectories, but did not make a connection
to the persistence length of the microtubule tip. Values of 0.1 mm [16] and 0.5 mm [17] were reported that agree well with the value that we find.

We recently obtained an independent measurement of the persistence length of microtubule ends by a different method, viz., by measuring the trajectory curvature of microtubules that were subjected to perpendicular electric forces. We related the curvature to the magnitude of the electric force and the average length of the microtubule tip and we found that in these experiments $p = 0.55 \pm 0.22$ mm, which agrees reasonably with the value that we report here. However, our previous measurement relied on the measurement of the average tip length, which is hard to do very precisely, and on a calibration of the magnitude of the electric force. The method that we present here does not rely on other parameters.

8.7 Conclusions

We have demonstrated that we can obtain a measurement of the persistence length of microtubule ends by tracking of stochastic microtubule trajectories in gliding assays. The tangent angle of a microtubule trajectory can be described as a random walk, and the variance of the tangent angle increases linearly with time. We show that even a single microtubule trajectory can yield a measurement of the persistence length if it is long enough and when internal averaging of the trajectory is used. By averaging the data over a large number of microtubule trajectories, we find a persistence length of the microtubule tip that is $p = 0.24 \pm 0.03$ mm. The value that we find for our microtubule ends, that we estimate to be of submicrometer length, is in good agreement with other recent data that reports a reduction of the persistence length for short microtubules.

8.8 Materials and methods

Motility assays were performed as follows. A flow cell was assembled from two glass cover slips and double-sided tape, and first incubated for 5 min with a casein solution (0.5 mg/mL casein in BRB80 buffer (80 mM Pipes, 1 mM MgCl$_2$, 1mM EGTA, pH=6.9). Second, a kinesin solution was perfused into the flow cell and incubated for 5 min. The concentration of our kinesin stock is 200 µg/mL, and the kinesin concentration in the solution that was added to the flow cell varied between 10 – 100 µg/mL. The kinesin was diluted in BRB80, supported with 0.2 mg/mL casein and 1 mM ATP. The data presented in the text were taken in a flow cell that was incubated with 40 µg/mL kinesin. Finally, the flow cell contents were exchanged with motility solution containing rhodamine-labeled
paclitaxel-stabilized microtubules (~8 nM tubulin, 1 mM ATP, 1 mM MgCl₂, 10 µM Taxol, and anti-bleaching cocktail (20 mM D-Glucose, 0.020 mg/mL glucose oxidase, 0.008 mg/mL catalase, 1% β-mercaptoethanol)) all in BRB80 buffer. Microtubules were polymerized from 5 µL of bovine brain tubulin (4 mg/mL, 1 rhodamine labeled unit, 3 unlabeled units, Cytoskeleton, Denver, CO) in the presence of 4 mM MgCl₂, 1 mM GTP and 5% DMSO in BRB80 buffer (37 °C for 45 min). Then the microtubules were stabilized and 400× diluted in BRB80 containing 10 µM Taxol.

References

[9] Duke *et al.* [8] provide a relation between the average distance ⟨d⟩ of kinesin molecules bound to a microtubule and the surface concentration σ of active kinesins. It is assumed that kinesin motors can reach isotropically over a distance w ≈ 20 nm to attach to a microtubule. If the motor density is very high, such that the microtubule tip can bend much less than its diameter, σ ∼ 1/⟨d⟩w. If the surface density is lower, the microtubule end can explore a larger area and σ ∼ √p/⟨d⟩5, where p is the persistence length of the tip. The boundary between the two regimes is at σ* ∼ w−5/3p−5/3. If we estimate ⟨d⟩ ≤ 0.5 µm and p ∼ 0.5 mm, then we find in both regimes that σ ∼ 10^2 µm^−2, which is also approximately the value of σ*, justifying the use of these relations. Finally, the critical kinesin surface density, below which buckling of the microtubule tip is expected to occur, is about 4 orders of magnitude lower than σ*.

We simulated 1137 microtubule trajectories with a velocity that is Gaussian distributed with $0.78 \pm 0.07 \mu m$ (mean $\pm$ STD). The distribution of simulated trajectory lengths was made similar to the experimental data by randomly picking a predetermined length for each simulated trajectory from an exponential distribution that resembled the experimental data. For the microtubule tip length, we assumed that it was exponentially distributed with mean $0.26 \mu m$, although the length distribution of the tip is of no relevance to the evolution of the trajectory angle Eq. 8.1). For the persistence length we took $p = 195 \mu m$. The coordinates of the microtubule after each step of random length were calculated based on its initial position, its momentary tip length and tip orientation that were randomly picked from an exponential and Gaussian distribution, respectively. Afterwards, the positions of the leading tip were collected with 1 s intervals. We confirmed that adding a random variance onto the tip coordinates, representing the effects of pixel noise and errors in the tracing routine, did not affect the results.


Chapter 8: Tracking stochastic microtubule trajectories
Using fluorescence microscopy, we have directly observed an unexpected reversible bending deformation of homogeneously charged microtubules in electrophoresis. The bending accompanies a reorientation of the microtubules perpendicular to the electric field. Similar phenomena have been inferred from anomalous electrical birefringence experiments that measure an ensemble-averaged orientation. We attribute the bending and orientation to hydrodynamic interactions between different parts of the molecule, leading naturally to a translation-rotation coupling. We provide measurements of this effect for electric fields in the range $2 - 100 \text{ kV/m}$ and find that parallel orientations are favored for low and high fields, while anomalous perpendicular orientations are measured in the intermediate regime. Interestingly, the distributions of individual microtubule orientations reveal an unexpected two-state system, with microtubules being in either perpendicular or parallel orientations. As such, our results provide new insights in the orientation of cylinders in an electric field and are strong evidence for the hypothesis that hydrodynamic coupling is responsible for the anomalous signals often reported in electrical birefringence experiments.
Chapter 9: Hydrodynamic coupling observed for microtubules

9.1 Introduction

Electro-optical techniques such as electric dichroism or electrical birefringence measurements are powerful tools for the study of rotational dynamics and structural properties of anisotropic rod-like colloids and macromolecules in solution. In electrical birefringence experiments, an applied electric field causes an alignment of the molecules which induces a measurable optical birefringence. Switching off the electric field leads to a time-dependent decay of the birefringence, from which the rotational diffusion constant and dimension of the particles can be calculated. Electric birefringence has been used to study a variety of systems such as biomolecules [1, 2], synthetic polyelectrolytes [3], and micelles [4].

The ionic polarizability of a rod-like molecule is largest along its major axis. Consequently, the birefringence signal that is usually measured in these experiments corresponds to an alignment of the particles with their long axes parallel to the applied electric field. However, under circumstances such as high particle concentrations, long particles, or low salt concentrations, a reversal of the birefringence signal has been reported. This so-called anomalous birefringence indicates that the particles are, instead, aligned perpendicular to the electric field. This has been observed for polyelectrolytes [3], micelles [4], short DNA fragments [5], and fd viruses [6].

Different mechanisms have been proposed to explain anomalous birefringence. Explanations for high particle concentrations are based on clustering of particles [7], overlapping ion clouds [4], and steric interactions [6], but these mechanisms do not account for anomalous birefringence at lower particle concentrations. A more universal mechanism is based on hydrodynamic interactions between different parts of the molecule that lead to an elastic bending deformation of the rods [8–10]. The reduction in symmetry due to bending then leads to a coupling between translational and rotational motion of the molecule [11]. This proposal of hydrodynamic coupling has been explored numerically [8–10] but has remained untested experimentally.

In this work, we present direct microscopic evidence of two of the central predictions of the hydrodynamic coupling hypothesis, namely (i) the bending deformation of rods, and (ii) their subsequent perpendicular orientation. Using microtubules as a model system for rod-like particles, we measure their orientation and shape under application of an electric field inside microfluidic channels by fluorescence microscopy. Microtubules are perfectly suited for this because they have a large persistence length of $\sim 5$ mm [12], which makes them stiff rods on the $\mu$m length scales that are optically accessible. Microtubules have a highly negative charge and move toward the positive electrode in our channels [13].
The use of 1 µm high fluidic channels confines the motion of microtubules to the focal plane of the objective, facilitating prolonged optical observation. We observe that microtubules become strongly bent upon application of an electric field and that the bending increases with contour length and field strength. We also measure that the orientation is predominantly perpendicular for strongly bent microtubules. Both observations are strong evidence for the hydrodynamic-coupling hypothesis.

9.2 Results and discussion

The principle of hydrodynamic coupling is depicted in Fig. 9.1a-b. In Fig. 9.1a we show a rod that moves through a quiescent liquid under an external force field \( F_e \) that is homogeneous along the length of the rod. The hydrodynamic drag forces \( F_{\text{drag}} \) that act on the rod are not homogeneous due to hydrodynamic interactions between different parts of the rod. The fluid disturbance induced by the motion of the extremities of the rods act to reduce the drag force in the middle, whereas the hydrodynamic disturbance of the middle of the rod increases the drag on its extremities [9]. This distribution of the net force causes the ends of the molecule to bend backward with respect to the direction of motion (Fig. 9.1a).

The bending of the rod reduces its symmetry. In general [11], this causes the translational motion to become coupled to the rotational motion of the object (Fig. 9.1b). For an object of arbitrary shape, a unique point can be defined on which the resultant external driving force acts, which is usually denoted as the center of mass (CM). This point is generally distinct from the center of hydrodynamic reaction (CR), which is the point at which the net friction force acts [11, 14]. Consequently, a torque will develop that rotates the bent molecule in such a way that the end-to-end vector becomes oriented perpendicular to the driving force, as indicated in Fig. 9.1b.

To test this hydrodynamic-coupling hypothesis, we use fluorescence microscopy to directly image the conformation and orientation of a large number of microtubules in a microfluidic channel under the application of an electric field \( E \). Microtubules and channels were treated as described previously [13]. In short, fluorescent microtubules were polymerized at 37 °C for 45 min from 5 mg/mL tubulin (1:3 stoichiometry of rhodamine labeled:unlabeled tubulin) and stabilized in 80 mM Pipes buffer containing 100 µM paclitaxel (Taxol). Unpolymerized tubulin was removed by centrifugation of the microtubules and resuspending the pelletized microtubules again in buffer containing taxol. Microfluidic channels of 500 µm width and 1 µm depth were fabricated in fused-silica substrates be-
tween entrance holes separated by 5 mm and precoated with casein to prevent interactions of the microtubules with the channel walls. A microtubule solution (supplemented with 0.13 M D-glucose, 0.13 mg/mL glucose oxidase, 0.06 mg/mL catalase, and 5 % beta-mercapthoethanol to prevent photobleaching) was added to the channel and microtubules could be observed inside the channel through the glass (Fig. 9.1c). Images were recorded using a CCD camera. The concentration of microtubules was well below the overlap concentration and steric effects due to mutual microtubule interactions can be ignored. Electric fields were applied via platinum electrodes inside the reservoirs at either end of the channel. As discussed previously, Joule heating is negligible due to the large surface-to-volume ratio of our channels [15]. We previously showed that microtubules move in the direction opposite to an electric field inside our channels. This motion reflects a superposition of the positive electro-osmotic flow mobility inside the channels and the negative electrophoretic mobility of the microtubules, the latter being the dominant effect [13].

In Fig. 9.1d we show a kaleidoscopic overview of fluorescence images of microtubules inside a microfluidic channel without any applied field. The lengths of the microtubules are approximately exponentially distributed with an average length of 7 µm and, as is evident from Fig. 9.1d, at these length scales microtubules are straight and stiff rods. However, under the application of an electric field we observe that microtubules start to deform. Fig. 9.1e shows a kaleidoscopic overview of microtubule shapes that were recorded while they moved downward in the image at $E = 4$ kV/m. We observe that some microtubules get highly bent, in particular the longer microtubules, and that they are oriented with their ends directed away from the driving force, in accordance with the predictions of hydrodynamic coupling (Fig. 9.1a-b). Note that there is a large dispersion in orientation and shape. Long microtubules tend to get deformed more than medium-length microtubules, and their shapes are in many cases asymmetric (Fig. 9.1e). The left part of Fig. 9.1e shows a selection of exceptionally long and deformed microtubules, that are oriented perpendicular to the direction of motion. Microtubules of a shorter lengths, which occur much more frequently, are shown in the middle of Fig. 9.1e, and are deformed less. The right-hand side of Fig. 9.1e shows examples of microtubules of different length that are oriented in parallel orientations to the field.

To quantify these observations, we measure the bending angle $\alpha$ of microtubules as a function of the microtubule contour length $L$ [16]. We define the bending angle $\alpha$ positive for microtubules that are bent with their ends directed away from the driving force, and negative if the ends are directed toward the force (inset Fig. 9.2a). Figure 9.2a shows binned values of $\alpha$ as a function of
Figure 9.1: (a) Deformation of a semi-flexible rod due to hydrodynamic interactions. Essential is that the drag force on the rod is modified due to hydrodynamic interactions between different parts of the rod, resulting in a bending moment. (b) An arbitrarily oriented bent rod will have a center of reaction (CR) that is different from the center of mass (CM), resulting in an orienting moment. (c) Schematic of the experimental setup. (d) Collage of different fluorescence images of microtubules inside a channel. (e) Collage of different fluorescence images of microtubules inside a channel under application of the same electric field $E = 4 \text{kV/m}$. Note the large variety of microtubule shapes. Strongly deformed and perpendicularly oriented microtubules (left part) occur in the same experiment as straight and approximately parallel oriented microtubules (right part), together with intermediate deformations (middle part). During the 20 ms integration time of the camera, the microtubule movement is estimated to be less than 1 µm at this field strength.

length for two different electric fields. In accordance with our qualitative observations in Fig. 9.1e, we observe that the microtubule deformation increases with increasing contour length. Moreover, for the higher $E = 8 \text{kV/m}$ the bending is consistently larger than for the lower $E = 2 \text{kV/m}$ (red points versus black points in Fig. 9.2a).

The average bending of microtubules shows a clear increase with $E$ and $L$, but there is a considerable variance between individual microtubules. Figure 9.2b shows the population distribution of microtubule deformations $\alpha$ that were measured for microtubules with an average contour length $\langle L \rangle = 10 \mu\text{m}$ at $E = 8 \text{kV/m}$. The shape of the distribution resembles a gaussian, with the
Figure 9.2: (a) Binned values of the bending deformation $\alpha$ (mean $\pm$ standard error of the mean (SEM) as a function of contour length $L$ for two different values of the electric field. Bending with the ends away from the driving force corresponds to positive $\alpha$. Lines are guides to the eye. (b) Distribution of $\alpha$ for the data points contained in the bin of $E = 8$ kV/m and $\langle L \rangle = 10$ µm. (c) Snapshots of three different microtubules with 0.1 s intervals directly after switching off the external field. Time is on the vertical axis. The vertical distance between microtubules is arbitrary. (d) The relaxation of the bending angles of the three microtubules in panel c as a function of time, normalized with respect to the deformation $\alpha$ at $t = 0$. Lines are guides to the eye.

width of the distribution approximately 4 times larger than its mean. Note the difference in scale of $\alpha$ between Figs. 9.2a and b.

We now show that the bending deformation of microtubules is a dynamic effect which disappears after switching off the electric field. Figure 9.2c shows time-lapse snapshots of three different microtubules that were taken at 0.1 s intervals immediately after switching off a driving field $E = 40$ kV/m. At $t = 0$ (top snapshots) all three microtubules are highly bent. During the first second after switching off the field (the total time period that is displayed in Fig. 9.2c) the microtubules rapidly relax and straighten again. The shortest microtubule, with a contour length of 12 µm relaxes most rapidly and has returned to its straight shape again within 0.5 s, although some residual bending seems to persist. In contrast, the longest microtubule of the three still has a considerable curvature 1 s after switching off the field.
Figure 9.2d quantifies the relaxation by plotting the bending angle as a function of time, normalized with respect to the bending angle at $t = 0$. The relaxation time of the microtubules is governed by their stiffness and hydrodynamic drag. Longer microtubules, with a larger hydrodynamic drag, are expected to relax more slowly, which is in qualitative agreement with the data in Fig. 9.2d. An exact analytical expression for the time-dependent relaxation is difficult to obtain because the microtubules are so strongly bent that the usual small-bending approximation does not hold in this case.

We have demonstrated that a reversible bending deformation of microtubules does occur through an electric driving force, and that the bending is similar to what is expected from hydrodynamic interactions between different parts of the molecule. The second essential ingredient of the hydrodynamic-coupling hypothesis is that translation becomes coupled to rotation of the molecules (Fig. 9.1b). The anomalous orientation process that is measured in birefringence experiments is fast ($\sim$ms) and cannot directly be imaged due to the relatively long integration times that are needed for our CCD camera. However, we can measure the resulting orientation of microtubules and correlate this to the bending deformation to probe if there is a coupling between translation of bent objects and their orientation.

In Fig. 9.3 we plot the bending deformation $\alpha$ as a function of the orientation of the microtubule end-to-end vector $\theta$. We define $\theta$ between 0 and $\pi/2$ (inset Fig. 9.3), with $\theta = 0$ denoting the orientation perpendicular to the electric field. The data in Fig. 9.3 show that microtubules in perpendicular orientations to the electric field are much more bent than microtubules that are oriented parallel to $E$. This observation holds for all field strengths between 2 and 10 kV/m and is expected if bending deformations lead to a coupling between translation and rotation. The data presented in Figs. 9.2 and 9.3 are thus in agreement with the pictorial mechanism shown in Fig. 9.1a-b.

In electric birefringence experiments the orientation is measured as an ensemble property of many molecules, as opposed to the single-molecule picture that we present here. To facilitate comparison, we quantify the orientation in an ensemble average over all the molecules as a function of electric field. We express the overall orientation of the end-to-end vector using the orientation parameter $\psi$, adopting the definition of Ref. [9]:

$$\psi = \frac{3 \sin^2 \theta}{2} - \frac{1}{2}.$$  \hspace{1cm} (9.1)
Figure 9.3: (a) Binned values of the bending deformation $\alpha$ (mean ± SEM) as a function of microtubule orientation $\theta$ for two different values of the electric field. The orientation $\theta$ is defined between 0 and $\pi/2$. A clear correlation between bending and perpendicular orientation is observed. Microtubules that are oriented perpendicular to the field ($\theta \rightarrow 0$) are more deformed than microtubules oriented parallel to the field, which is expected as a result from hydrodynamic coupling. Lines are guides to the eye.

In this way, the orientation parameter can vary between $-\frac{1}{2}$ and $+1$, with anomalous orientation (microtubules move in perpendicular orientation to the field) corresponding to values of $-\frac{1}{2} \leq \psi < +\frac{1}{4}$ and normal orientation (microtubules move parallel to the field) corresponding to $+\frac{1}{4} < \psi \leq 1$. An isotropic distribution of rods yields $\psi = +\frac{1}{4}$.

The data presented in Figs. 9.1–9.3 was obtained for electric fields $E = 2 - 10$ kV/m. At higher field strengths, the velocity of the microtubules becomes so large that we cannot obtain fluorescence images without significant blurring due to the finite displacement during the integration time of the camera. Therefore, at higher electric fields, measurements were obtained by applying an electric field during 1 s and taking an image of the resulting microtubule distribution within 30 ms after switching off the field. The latter time is sufficiently short to limit dispersion of the orientation distribution due to rotational diffusion [17]. For an electric field of $E = 10$ kV/m we confirmed that the two measurement methods, viz. the pulsed-field method and the continuous-field method, yielded the same results for the orientation distribution.

In Fig. 9.4a we present measurements of the ensemble orientation parameter as a function of electric field ($E = 2 - 100$ kV/m) for different microtubule contour lengths. The solid lines are drawn as guides to the eye. For the shortest microtubules (red curve in Fig. 9.4a), with an average contour length $\langle L \rangle = 3.5 \, \mu\text{m}$ (the width of each $L$-bin is 3 $\mu$m), we observe that the orientation parameter...
for low field strengths is indicative of orientations parallel to the field, namely $\psi > \frac{1}{4}$. Upon increasing $E$, the orientation becomes initially more parallel reaching a maximum $\psi = 0.53$ for $E = 8 \text{ kV/m}$. For higher fields, $\psi$ starts to decrease rapidly to a minimum $\psi = -0.14$ that is measured for the highest field strength used of $E = 100 \text{ kV/m}$, indicative of anomalous orientation perpendicular to the field. For slightly longer microtubules with $\langle L \rangle = 6.5 \ \mu\text{m}$ (black curve) we observe a similar increase and subsequent decrease in $\psi$ as a function of the electric field, with the notable difference that the maximum value of $\psi$ only reaches 0.45 and occurs at lower $E = 4 \text{ kV/m}$.

We speculate that these measurements can be interpreted as a balance between the mechanisms of counter ion polarizability, which tends to orient rods parallel to the electric field, and hydrodynamic-coupling that induces bending and perpendicular orientation. To explain the nonlinear $\psi$ vs $E$ dependence, both mechanisms need to scale differently with the magnitude of the electric field, with the hydrodynamic coupling growing faster with increasing $E$. Around the field strength corresponding to the maximum in $\psi$ the hydrodynamic-orientation mechanism begins to become significant. From the relative location of the maxima (red line vs black line in Fig. 9.4a) we conclude that hydrodynamic coupling becomes important at lower $E$ if the rod length increases, which can be explained by the increased deformation angle for longer rods (Fig. 9.2a).

For even longer rod lengths ($\langle L \rangle = 9.5 \ \mu\text{m}$, $\langle L \rangle = 13 \ \mu\text{m}$, and $\langle L \rangle = 16 \ \mu\text{m}$, green, blue, and orange curves, respectively) we only measure a monotonic decrease in $\psi$ in the range $E = 2 - 20 \text{ kV/m}$, with the cross-over from parallel to perpendicular orientations happening at increasingly lower fields for longer lengths ($E \approx 6 \text{ kV/m}$ for $\langle L \rangle = 9.5 \ \mu\text{m}$, $E \approx 2.5 \text{ kV/m}$ for $\langle L \rangle = 9.5 \ \mu\text{m}$ and $E < 2 \text{ kV/m}$ for $\langle L \rangle = 16 \ \mu\text{m}$). According to the mechanism proposed above it implies that for these longer rods the maximum in $\psi$, that is observed for the shorter lengths (black and red curves in Fig. 9.4a), is occurring at fields strengths below 2 kV/m.

At higher field strengths $E = 20 - 200 \text{ kV/m}$ we observe an unexpected minimum in the orientation parameter for longer microtubules, above which $\psi$ increases again. The position of this minimum is again dependent on the rod length. In this case however, the electric field at which the minimum occurs seems to decrease with increasing rod length. Based on the guides to the eye we estimate that the longest rods have a minimum $\psi$ around $E = 20 \text{ kV/m}$, whereas the minimum for rods of $\langle L \rangle = 9.5 \ \mu\text{m}$ is around $E = 70 \text{ kV/m}$. Again, we propose that the absence of a minimum in $\psi$ for the shortest microtubules is because this minimum occurs for electric fields that are above the measurement range. At fields higher than 70 kV/m, the orientation parameter is consistently
larger for longer rods, which is opposite to the low field region. Although we do not measure a value of $\psi$ that is significantly larger than $\frac{1}{4}$, extrapolation of our data suggests a return to parallel orientation for $E > 200 \text{ kV/m}$.

Disappearance of the anomalous electric birefringence signal at high fields has been observed experimentally [3, 6, 18, 19] and in simulations [10]. Oppermann observed anomalous birefringence of synthetic polyelectrolytes at low fields ($\sim 10 \text{ kV/m}$), which disappeared when the field strength was increased [18]. Kramer et al. reported similar observations on synthetic polypeptides and also reported that the anomaly disappeared for field strengths above $10 - 100 \text{ kV/m}$ [3]. Kramer et al. also performed low field strength ($E \leq 10 \text{ kV/m}$) birefringence measurements on fd virus, and reported a disappearance of the anomaly at higher fields [6]. Finally, for micelles the anomalous birefringence was reported to dis-
appear for fields much larger than $\sim 10^2 \text{kV/m}$ [19]. Although all these measurements are performed on different particles and at much higher concentrations (leading to particle interactions) than in our experiments, the results do illustrate that anomalous birefringence can disappear above fields strengths that are similar to the values that we have probed.

A possible explanation for the disappearance of the anomalous electric birefringence signal at high fields comes from Brownian dynamics simulations by Porschke et al. [10]. These authors simulated the interplay between hydrodynamic coupling and polarization alignment for a rigid bent rod (mimicking a short stretch of DNA with fixed curvature) as a function of the electric field strength. They observed that for low fields the rods (with fixed curvature) had a tendency for perpendicular orientation due to hydrodynamic coupling, which reverses for higher electric fields in a parallel orientation due to polarization. The explanation they provide is that the polarization-induced parallel aligning torque increases with $E^2$, whereas the torque resulting from hydrodynamic-coupling increases more slowly for a rod with a fixed bending. Note that the bending of the rod was set in the simulations and was not allowed to increase with the electric field.

A qualitatively similar explanation, combined with the field-dependent bending of microtubules, could be invoked to explain our data as follows. Initially, at very low fields, microtubules become oriented parallel with the field through the polarizability of the counter ions, and $\psi > \frac{1}{4}$. Then, with increasing field strength, hydrodynamic coupling starts to bend microtubules and a torque develops that tends to perpendicular orientation. Above a certain field strength, the bending becomes so pronounced that hydrodynamic coupling is the strongest effect and the microtubules start to orient perpendicular to the field, leading to a decrease in $\psi$ with $E$ and values of $\psi < \frac{1}{4}$. This transition comes first for the longest microtubules because they are most strongly deformed. Then, at even higher $E$, the bending deformation of microtubules reaches a maximum, with microtubules deformed into U-shapes. From this point onward (invoking the simulation results of Porschke [10]), the hydrodynamic-coupling torque increases more slowly with $E$ because the bending no longer increases with $E$, whereas the polarizability retains the same $E^2$-dependence as before. The simulations in Ref. [10] indicate that in this situation the perpendicular orientation will eventually be reduced and turn into parallel orientation, leading again to an increase in $\psi$ with $E$. This situation will occur first for the longer rods, because their bending saturates at lower fields.

This mechanism is further clarified by presenting the same data for the orientation parameter as a function of length. Figure 9.4b plots $\psi$ as a function of
contour length for different values of $E$. For low and constant $E$ (black curve), the orientation is initially governed by polarization effects and $\psi > \frac{1}{4}$. Bending becomes more pronounced with increasing length, leading to perpendicular orientation for longer rods. If the rod length increases even further, the bending saturates, whereas the dipole moment will still increase with length, leading eventually to a reversal to parallel orientation for the longest rods. For higher constant electric fields, the maximum in $\psi$ occurs for lower rod lengths because bending saturates at lower rod length. For the same reason, the point of maximum bending, and thus the minimum in $\psi$ is also found at lower $L$.

A surprising insight is obtained from considering the microscopic distribution of orientation parameters obtained from individual microtubules. In Fig. 9.4c, we show histograms of the orientational distribution for microtubules of $\langle L \rangle = 3.5 \, \mu m$ (top row) for different field strengths, corresponding to the red curve in Fig. 9.4a. The bottom row shows the distributions of $\psi$ for microtubules of $\langle L \rangle = 13 \, \mu m$, corresponding to the blue data points in Fig. 9.4a. Strikingly, the orientation obtained from the ensemble-average value of $\psi$ as displayed in Fig. 9.4a, and possibly also from electric birefringence experiments, does not correspond to the actual microscopic orientation of most of the individual microtubules. Instead, microtubules are mostly in either a perpendicular or a parallel orientation, and hardly ever in intermediate orientations. By changing the electric field strength, it seems that it is the relative occupation of the two extreme orientations that is changing, and that there is not a smooth variation of $\psi$ on a microscopic level. This is a surprising new finding which has not been addressed before. Understanding of the mechanism for this apparent two-state behavior requires more theoretical modeling of the interplay between elasticity and electrohydrodynamic coupling and is beyond the scope of the present work. It is clear that a microscopic single-molecule observation of the orientational distribution can provide valuable additional insights in the anomalous orientation that is measured in the ensemble-average electric birefringence experiments.

## 9.3 Conclusions

We have directly observed a bending deformation of microtubules under application of a homogeneous electric field. The bending is such that the ends of the microtubule are directed away from the direction of the applied force. The deformation increases with electric field strength and contour length and can be explained by hydrodynamic interactions between different parts of the molecules. We demonstrate that the bending is reversible and disappears if the driving field
is removed. Furthermore, we confirmed that bending deformation leads to orientation of the molecules perpendicular to the driving field, which is expected from a coupling between translation and rotation for asymmetric molecules. We thus demonstrated two essential ingredients, namely hydrodynamic bending deformations and the effects of translation-rotation coupling, of recent hydrodynamic coupling theories that aim to explain the anomalous perpendicular orientation measured in electric birefringence experiments.

We have provided a systematic investigation of the ensemble-averaged orientation of microtubules as a function of electric field and contour length. We found that for low electric fields, microtubules become initially oriented parallel to the electric field. For intermediate electric fields the anomalous perpendicular orientations were favored and for the highest electric fields a reversal toward parallel orientation was observed. The boundaries between the three electric-field ranges were found to be consistently dependent on rod length. We proposed a mechanism to explain these observation based on the interplay between polarization effects, which favors parallel orientations, and hydrodynamic coupling effects, which induce perpendicular orientations.

Finally, interesting insights are obtained from considering the distribution of individual microtubule orientations instead of their ensemble average. Interestingly, we find that most microtubules are in either perpendicular or parallel orientations to the electric field, and much less in orientations in between. Changing the electric field strength modifies the relative occupation of the two states, rather than causing a continuous change in orientation of individual microtubules. As such, our observations reveal details about the anomalous orientation of rod-like molecules that are obscured in bulk measurements such as electric birefringence experiments.

References


Microtubule positions and shapes were digitized using Matlab routines in an automated way and manually checked. Coordinates of the microtubule skeleton were obtained for all microtubules in a large number of images. The angle α for each microtubule was calculated as the angle between its end segments that were taken of 1 μm length.

An upper bound to the orientational dispersion is set by the rotational diffusion for the shortest microtubules of $L = 2 \mu m$ that we included in our analysis. The variance of the orientation due to rotational diffusion can be calculated from the rotational drag coefficient for a cylinder. Using the drag coefficient for a cylinder in an unbounded solution [20], the variance in $\theta$ can be estimated as maximum 0.1 rad$^2$ during the 30 ms time interval between switching off the field and recording the camera image. The presence of the channel surfaces will likely increase the drag, making the variance even smaller.


Summary

This thesis describes experimental work at the interface of nanotechnology and biology. We combine fabrication techniques from nanotechnology with motor proteins from the biological cell. Motor proteins are fascinating protein complexes with nanometer dimensions that are involved in force generation and motion inside cells. One intriguing opportunity to consider is whether these motor proteins can be used as active force-generating components in nanofabricated devices. The exploitation of motor proteins for nanotechnological tasks, in particular the motor protein kinesin that translocates along microtubules, is the focus of the first part of this thesis. Furthermore, we show that nanofabricated devices form an excellent system for fundamental studies of the physics of individual biomolecules. The exploration of their properties is described in the second part of this thesis.

We start with a general introduction that motivates the use of motor proteins as active components in nanofabricated devices. The challenges of controlling and directing motion at a nanoscale are considerable and is currently beyond our capabilities. Motor proteins, optimized for their specific tasks after billions of years of evolution, can provide a solution to this challenge and research into their use in nanotechnology has only just begun. We give an overview of the current state of this field, and review the progress that has been made in recent years. We conclude that clever ideas for applications have evolved, but that most of the demonstrations are still proof-of-principles.

We then describe how nanostructured surfaces can be used to rectify the motility of microtubules gliding over kinesin-coated gold surfaces, which is of use in e.g. sorting applications. We have developed a novel fabrication technique for the creation of gold tracks for microtubule motility recessed in silicon oxide (SiO$_2$) substrates. We block kinesin absorption on the SiO$_2$ using the well-understood poly(ethyleneglycol)-silane chemistry, resulting in excellent confinement of the
motility to the gold tracks. Using this fabrication route, we presented three designs of advanced rectifier geometries. By analyzing the traversal of microtubules through these structures we find that up to 92% rectification can be achieved.

An advantage of metal tracks for microtubule motility is that it allows for electrical control. We demonstrate that the docking of microtubule shuttles from solution onto the gold tracks can be controlled through application of a voltage. We find that after applying a positive voltage to the gold the number of moving microtubules increases exponentially with time, while switching off the voltage resulted in an exponential decrease. We proposed a kinetic model incorporating the electrophoretic transport of microtubules and an equilibrium between free and surface-bound microtubules to account for these data.

One major challenge in the use of biomotors for nanotechnological tasks, such as transport or sorting, is control of directionality on the level of an individual microtubule. Another issue with the ‘open trench’ structures that were previously used is that the shuttles can easily detach from their tracks. Therefore, we integrated kinesin motors in enclosed submicrometer fluidic channels, fully confining the microtubules to their tracks and, more importantly, enabling a localized application of strong electric fields. In this way we realized electrical control of the direction of even an individual microtubule approaching a Y-junction. To unambiguously demonstrate this control, we used our device to sort a population of green- and red-labeled microtubules depending on their color.

To investigate the nature of the steering mechanism of microtubules, we performed experiments aimed at determining the magnitude of the electric-field-induced force by measurement of the electrophoretic mobility of microtubules. To this end we used fluorescence microscopy to directly image the electrophoretic motion of microtubules in microfluidic channels. We found that the velocity of microtubules was dependent on orientation and not necessarily collinear with the electric field. From these observations we inferred the anisotropic electrophoretic mobility of microtubules, which, for the first time, confirmed the predicted mobility anisotropy of cylindrical particles. Moreover, we inferred an effective charge of $-23 \pm 0.2$ e per tubulin dimer, and we compare this to measurements performed on subtilisin-digested microtubules that have their negatively charged C-termini removed. Finally, we discuss the importance of counter ions for interpreting mobility experiments, that is often underestimated, and the large implication that counterions have for the mobility anisotropy and the value of the effective charge as measured from electrophoresis experiments.
We then study the trajectories of kinesin-propelled microtubules under perpendicular electric forces. From this we conclude that the steering of a microtubule is caused by a field-induced bending of its leading end. We related the microscopic bending of the microtubule tip to the macroscopic curvature of its trajectory. In experiments we quantified the trajectory curvature of a large number of microtubules as a function of the electric field. This provides an elegant technique to probe the stiffness of submicrometer microtubule ends. Using the results of the mobility measurements to determine the magnitude of the electric-field induced force, we infer a value for the stiffness of the microtubule tip. We find a persistence length of $0.55 \pm 0.22$ mm, which is an order of magnitude shorter than the value that is usually measured on long microtubules. These experiments also illustrate that electric fields are an excellent tool to apply forces of well-defined magnitude and direction on biomolecules for biophysics studies.

An independent measurement for the persistence length of the microtubule tips is obtained from tracing their stochastic trajectories over kinesin-coated surfaces. The evolution of the tangent-angle of a microtubule trajectory is similar to a random-walk and solely dependent on the microtubule velocity and the stiffness of its leading tip. The time-dependent variance of the trajectory angle thus provides an estimate of the tip persistence length. From the average over a large number of microtubule trajectories we measure a persistence length of $0.24 \pm 0.03$ mm, which does not rely on a calibration of the electric force, nor on a knowledge of the length of the leading tip. The validity of this approach is confirmed by simulations of microtubule trajectories that are subjected to the same analysis.

Finally, we describe experiments that yield surprising new insights in hydrodynamic effects on semiflexible rods, for which microtubules serve as an excellent model system. Using fluorescence microscopy we observe an unexpected and reversible bending deformation of microtubules in electrophoresis. We also observe that microtubules orient perpendicular to the electric field. We attribute the bending to hydrodynamic interactions between different parts of the molecule. The bending leads naturally to a translation-rotation coupling, inducing perpendicular orientation. Interestingly, the distributions of individual microtubule orientations reveal an unexpected two-state system, with microtubules being in either perpendicular or parallel orientations. Our results are strong evidence for the hypothesis that hydrodynamic-coupling is responsible for the anomalous signals often seen in electrical birefringence experiments.
In conclusion, we have used the techniques of nanotechnology to fabricate devices for the exploitation and exploration of individual biomolecules. The experimental work in this thesis contributes to the development of the young field of bionanotechnology, in which motor proteins are used as active components. Furthermore, we use nanofabricated devices for the study of mechanical and electrical properties of individual biomolecules and we contributed to the understanding of hydrodynamic interactions in electrophoresis experiments.

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July 2007
Samenvatting

Dit proefschrift beschrijft experimenteel werk op het grensvlak van de nanotechnologie en de biologie. Hierbij combineren we fabricatietechnieken uit de nanotechnologie met motoreiwitten uit de biologische cel. Motoreiwitten zijn fascinerende eiwitcomplexen met een grootte van enkele nanometers, die betrokken zijn bij bijna alle bewegingen en bij het krachtenspel in een cel. Het is een intrigerende optie om te onderzoeken of deze eiwitmotoren gebruikt zouden kunnen worden als actieve, krachtgenererende componenten in structuren die gemaakt zijn met behulp van nanofabricagetechnieken. Het gebruik van motoreiwitten, in het bijzonder het motoreiwit kinesine dat zich voortbeweegt langs microtubuli, voor toepassingen in de nanotechnologie is het onderwerp van het eerste gedeelte van dit proefschrift. Daarnaast laten we zien dat nanostructuren ook een uitstekende mogelijkheid bieden om fundamentele studies te verrichten op het gebied van de fysica van biomoleculen. De exploratie van deze fundamentele eigenschappen is beschreven in het tweede gedeelte van dit proefschrift.

We beginnen met een algemene introductie waarin we het gebruik van motoreiwitten voor toepassingen in de nanotechnologie verder motiveren. De uitdagingen in het controleren en sturen van beweging op nanoschaal zijn aanzienlijk en op dit moment nog grotendeels buiten onze mogelijkheden. Eiwitmotoren, die na miljarden jaren van evolutie geoptimaliseerd zijn voor hun specifieke taken, zouden mogelijk een antwoord kunnen vormen op deze uitdaging en het onderzoek hieraan staat nog in de kinderschoenen. We geven een overzicht van de huidige stand van zaken in dit veld, en we beschouwen de ontwikkelingen van de laatste jaren. Hieruit concluderen we dat er al slimme toepassingsmogelijkheden bedacht zijn, maar dat de meeste gerealiseerde voorbeelden toch nog voornamelijk principedemonstraties zijn.

Vervolgens beschrijven we hoe nanostructuren gebruikt kunnen worden om de beweging van microtubuli, die over met kinesine bedekte oppervlakten glijden, te kunnen gelijkrichten. Dit is van belang voor bijvoorbeeld sorteertoepassingen.
We hebben een nieuwe techniek ontwikkeld voor de fabricage van kleine goudbanen, verzonken in siliciumoxide (SiO$_2$) substraten, voor de beweging van microtubuli. We verhinderden de absorptie van kinesine op het SiO$_2$ door het gebruik van de goed begrepen poly(ethyleenglycol)-silaan chemie, hetgeen geresulteerd heeft in een bijna volledige beperking van de bewegingen van de microtubuli tot de goudbanen. Met deze fabricatiemethode hebben we drie geavanceerde gelijkrichtersstructuren gemaakt. Door de beweging van microtubuli door deze structuren te analyseren hebben we gevonden dat we tot 92% efficiënt zijn geweest in het gelijkrichten van de beweging van microtubuli.

Een voordeel van het gebruik van metaal voor de microtubule-banen is dat het gemakkelijk toestaat via elektrische velden een vorm van controle uit te oefenen. We laten zien dat het landen van microtubuli uit de vrije oplossing op de goudbanen gecontroleerd kan worden door middel van het aanleggen van een elektrische spanning. We merkten dat na het aanleggen van een positieve spanning op het goud het aantal bewegende microtubuli exponentieel toenam in de tijd, terwijl het uitschakelen van de spanning leidde tot een exponentiële afname. We hebben een kinetisch model uitgewerkt om deze data te verklaren, waarin het elektroforetische transport van microtubuli en het evenwicht tussen aan kinesine gebonden microtubuli en vrije microtubuli verwerkt is.

Een grote uitdaging in het gebruik van biomotoren voor toepassingen in de nanotechnologie, zoals het transport of sorteren van materialen, is de controle over de richting waarin een individuele microtubule zich beweegt. Een ander punt is dat de microtubule ‘treintjes’ gemakkelijk kunnen loslaten en ontsporen uit de baan van de open greppelachtige structuren die tot nu toe werden gebruikt. Daarom hebben we kinesine motoren geïntegreerd in geheel omsloten, submicrometer vloeistofkanalen, waarmee we automatisch de microtubuli volledig opsluiten in hun baan. Belangrijker nog, het gebruik van afgesloten vloeistofkanalen maakt het mogelijk om lokaal een sterk elektrisch veld aan te leggen. Op deze manier hebben we elektrische controle kunnen uitoefenen over het sturen van zelfs individuele microtubuli die op een Y splitsing afkwamen. Om deze controle onomstotelijk te demonstreren hebben we onze structuren gebruikt om een mix van rood en groen gekleurde microtubuli op kleur te scheiden.

Om het stuurmechanisme van microtubuli verder te onderzoeken hebben we experimenten uitgevoerd die gericht waren op het bepalen van de grootte van de kracht die door het elektrische veld wordt uitgeoefend. Hiervoor wilden we de elektroforetische mobiliteit van microtubuli meten. Om dit te doen hebben we fluorescentiemicroscopie gebruikt om de elektroforetische beweging van microtubuli in microvloeistofkanalen direct te kunnen volgen. We merkten dat de snelheid van microtubuli afhankelijk was van hun orientatie, en niet noodzakelijk
samenviel met de richting van het aangelegde elektrische veld. Uit deze waar-
meningen konden we de anisotrope elektroforetische mobiliteit van microtubuli
afleiden, waarmee we voor het eerst de voorspelde mobiliteits-anisotropie van ci-
llandervormige deeltjes konden bevestigen. Bovendien konden we een effectieve
landing voor het tubuline dimer afleiden ter grootte van $-23 \pm 2 \text{ e}$. We hebben
dezelfde waarden vergeleken met metingen aan met subtilisine behandelde microtubuli,
waardoor ze hun zeer sterk negatief geladen C-termini kwijtgeraakt waren.
Tenslotte bespreken we het belang van tegenionen voor de interpretatie van elek-
troforese experimenten, hetgeen vaak onderschat wordt, en de grote implicaties
die de tegenionen kunnen hebben voor de mobiliteitsanisotropie en de effectieve
landing zoals die gemeten worden uit elektroforese experimenten.

Vervolgens bestuderen we de trajecten die microtubuli volgen terwijl zij on-
derworpen zijn aan een elektrische kracht loodrecht op hun bewegingsrichting.
Hieruit concluderen we dat het elektrisch sturen van een microtubule veroorzaakt
wordt door het buigen van zijn voorste uiteinde door toedoen van het elektrische
veld. Dan relateren we de microscopische buiging van de uiteinde van de micro-
tubule aan de macroscopische kromming van zijn afgelegde baan. Door middel
van experimenten kwantificeerden we de baankromming van een groot aantal mi-
crotubuli als functie van het elektrische veld. Dit biedt een elegante manier om
de stijfheid van de uiteinden van microtubuli te meten, die lengtes hebben van
minder dan een micrometer. Met de resultaten van de mobiliteitsexperimenten
kunnen we de grootte van de door het elektrische veld aangelegde kracht bepalen,
en kunnen we uit de huidige metingen de stijfheid van de microtubule-uiteinden
bepalen. We vinden een persistentielengte van $0.55 \pm 0.22 \text{ mm}$, dat een orde van
grootte kleiner is dan de waarde die normaliter gemeten wordt aan lange micro-
tubuli. Deze experimenten laten ook zien dat elektrische velden een uitstekende
mogelijkheid bieden om krachten van goed gedefinieerde grootte en richting uit
te oefenen op individuele biomoleculen voor fundamenteel biofysisch onderzoek.

Een onafhankelijk meting van de persistentielengte van de uiteinden van mi-
crotubuli haalden we uit het volgen van hun stochastische banen over met kinesine
bedekte oppervlakten. Het verloop van de hoek van de raaklijn aan het pad van
een microtubule is vergelijkbaar met een stochastische wandeling en is slechts
afhankelijk van de snelheid van de microtubule en de stijfheid van zijn voorste
uiteinde. De tijdafhankelijke variantie van de hoek van de raaklijn aan het pad
van een microtubule geeft dus een indicatie van de persistentielengte van zijn
uiteinde. Uit het gemiddelde over een groot aantal paden van microtubuli meten
we een persistentielengte van $0.24 \pm 0.03 \text{ mm}$. Deze meting is niet afhankelijk van
een calibratie van de grootte van een elektrische kracht, en ook niet van de kennis
van de lengte van het uiteinde van de microtubule. We bevestigen de geldigheid
van de methode door middel van simulaties van stochastische microtubule paden die aan dezelfde analyse werden onderworpen.

Tenslotte beschrijven we experimenten die verrassende nieuwe inzichten geven in hydrodynamische effecten op semi-flexibele stokken, waarvoor microtubuli als een uistekend model systeem kunnen fungeren. Door middel van fluorescentiemi-
croscopie zien we een onverwachte en reversibele buigervorming van microtubuli optreden gedurende elektroforese. Ook zien we dat microtubuli zich oriënteren in richtingen dwars op het elektrische veld. We schrijven het buigen van de microtu-
bule toe aan het optreden van hydrodynamische interacties tussen de verschillen-
de delen van dezelfde microtubule. Dit buigen leidt dan op natuurlijke wijze tot een koppeling tussen translatie en rotatie van het molecuul, hetgeen een dwarse oriëntatie op het veld induceert. Interessant is dat de verdelingen van oriëntaties van individuele microtubuli een onverwachte splitsing in een systeem met twee toestanden te zien geven, waarbij microtubuli ofwel in een oriëntatie parallel aan het veld zijn, ofwel in een oriëntatie loodrecht op het veld. Deze resultaten die-
nen als sterk bewijs voor de hypothese dat hydrodynamische koppelingseffecten verantwoordelijk zijn voor de anomale signalen die gezien worden in elektrische dubbele-brekingsexperimenten.

Concluderend kunnen we stellen dat we fabricagetechnieken uit de nanotech-
nologie gebruikt hebben om structuren te maken voor de exploitatie en de ex-
ploratie van individuele biomoleculen. Het experimentele werk in dit proefschrift draagt bij aan de ontwikkeling van het jonge veld van de bionanotechnologie, waarin motoreiwitten gebruikt worden als actieve componenten. Daarnaast heb-
ben we nanostructuren gebruikt voor het bestuderen van elektrische en mecha-
nische eigenschappen van individuele biomoleculen en hebben we een bijdrage geleverd aan een begrip van hydrodynamische interacties in elektroforese experi-
menten.

Martin van den Heuvel
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Acknowledgement

During the final weeks of the project for my master’s thesis I started orienting myself on the possibilities for a continuation of my physics education. I had become quite enthusiastic about research and moreover, I felt that I was not really ‘finished’ without a PhD. Thus, I went to visit Delft, and a tour around a number of groups was arranged. At that moment I first got to know the enthusiastic impatience of Cees Dekker; while I was still talking to people of the Nanophysics group because, as usual, my tour schedule was too tight, you came and stuck your head around the door and asked why I wasn’t yet at MB... After that things went fast: because I came from Twente, you convinced me that fundamental research was much more fun than applied research, and your enthusiasm about molecular motors was very catching. I must admit that not much persuasion was needed, because with you I had the opportunity to start a new line of research on a subject which still had some technical touch at that time, which appealed a lot to me. But perhaps it was the atmosphere and communicative attitude of the people in MB that really made the difference. I negotiated some free time between my graduation and start of my PhD, and in February 2002 I arrived in Delft.

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Martin van den Heuvel
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Curriculum Vitae

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1990-1996  Secondary school (V.W.O. Gymnasium)
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List of Publications

1. Persistence length measurements from stochastic single-microtubule trajectories
   M.G.L. van den Heuvel, S. Bolhuis, M.P de Graaff, C. Dekker.
   Under review with Nano Letters

2. Microtubule curvatures under perpendicular electric forces reveal a low persistence length
   M.G.L. van den Heuvel, M.P de Graaff, C. Dekker.

3. Motor proteins at work for nanotechnology
   M.G.L. van den Heuvel, C. Dekker.

4. Electrophoresis of individual microtubules in microchannels

5. Op afstand bestuurbare eiwitbuisjes
   M.G.L. van den Heuvel, M.P de Graaff, C. Dekker.

6. Molecular sorting by electrical steering of microtubules in kinesin-coated channels
   M.G.L. van den Heuvel, M.P de Graaff, C. Dekker.

7. High rectifying efficiencies of microtubule motility on kinesin-coated gold nanostructures

8. Electrical docking of microtubules for kinesin-driven motility in nanostructures
9. *Improved method for determining inversion layer mobility of electrons in trench MOSFETs*

10. *Luminescence of Bi$^{3+}$ and Eu$^{3+}$ doped Gd$_{(1-x)}$La$_x$VO$_4$ system*
    M.G.L. van den Heuvel, B.K. Chandrasekhar.
    Inorganic Materials: Recent Advances, Eds. D. Bahadur, S. Vitta, Om Prakash,

11. *Electric force-velocity relation of kinesin-driven microtubule motility*
    In preparation

12. *Single-molecule observation of hydrodynamic deformation and anomalous orientation of microtubules in electrophoresis*
    M.G.L. van den Heuvel, S.G. Lemay, C. Dekker.
    In preparation