Summary
The title of this thesis is “Single-protein motion on microtubules and in cell membranes”. As this title implies, the central theme of this thesis was motion, a change of position in time. We can distinguish two main types of motion: random motion and directed motion. An example to illustrate these types of motion is the walk of a man on the street. Let us consider first that, after work, the man walks straight home. He will thus make all his steps in the same direction and the distance he will cover will increase linearly in time. This is a typical illustration of directed motion. Let us now imagine that the man stops on his way in a bar and gets drunk. After leaving the bar, he will have equal probability to step either home or away from home, resulting in a random walk, which will take longer to bring him home. The types of motion described above are also present in biology, from the level of the cell to the level of the molecule. The first scientific observation of random motion was made in 1828 by the British botanist Robert Brown while studying pollen particles in water. Later on, this phenomenon was explained by the random collision of pollen particles with water molecules and was named Brownian motion.

The cell can be viewed as a small compartment delimited by a membrane and crowded with proteins and biomolecules. All these molecules undergo some kind of motion that can be directly related to their function. The shape and the structure of the cell are supplied by the cytoskeleton. A major component of the cytoskeleton are the microtubules (MT), which, besides providing mechanical structure, are also crucial for protein transport and cell division. MTs are long hollow tubes that display a polarity (− end and + end). Molecular motors, such as Kinesin-1, take advantage of this polarity to transport cargo from one side of the cell to another. Kinesin-1 “walks” over the MT towards the + end using ATP as an energy source and it can make more than hundred steps before it detaches from the MT. The velocity of this “walk” is about 1 µm/s, making this mode of cargo transport more effective and faster over µm distances than diffusion (2s to directional transport of cargo across a cell of 2 µm long vs. 8s for diffusion with a diffusion constant of 10 nm² s⁻¹). Chapter 3 deals with Kinesin-1 walking over MTs without transporting cargo. We describe novel methods to successfully determine 3 important parameters that define the directional motion of such a molecular motor: the velocity, the run length (the average distance travelled) and the randomness (a measure of the stochasticity of stepping).

Besides its role as a “track” for molecular motors, MTs are also involved in the separation of the genetic material between the two daughter cells during the cell division. This segregation is coordinated by a combination of molecular motors and a specific MT structure, namely the mitotic spindle. The mechanism of action of the mitotic spindle depends on the polarity patterns of its constituent MTs. In Chapter 4, we studied one of the MT-based molecular motor, KLP61F. We show that KLP61F is capable of crosslinking and sliding adjacent spindle MTs and that it displays a preference for crosslinking MTs in the
antiparallel orientation. These results explain its crucial role in the formation of the mitotic spindle.

The architecture of the cytoskeleton depends on the interplay of the aforementioned molecular motors, which can crosslink and move directionally along MTs, and of non-motor proteins which can also crosslink MTs but do not drive directional motion. In Chapter 5, we investigated such a non-motor protein, PRC1, and show that it binds to individual MTs and diffuses along them. This diffusive motion slows down upon binding of PRC1 to a second MT, leading to an accumulation of PRC1 on overlapping MTs and thus to the formation of stable MT bundles.

Proteins can also diffuse within the cell membrane. This is the case of the Tat system (twin arginine translocation), a protein complex present in the cell membrane of the bacterium *E.coli*. The Tat complex consists of 3 different proteins, TatA, TatB and TatC, and translocates proteins over the inner membrane. In Chapter 6 we measured the number of TatA present in single Tat complexes and its diffusive motion. We showed that the motion of the complex depends on the concentration of the protein that is transported and on the presence of the energy source needed for the translocation. In Chapter 7 we perform computer simulations to explain the motions of the Tat complexes obtained in Chapter 6. The simulations reveal the existence of two distinct types of complexes: complexes formed by only TatA and much larger ones, likely formed by a combination of TatA, TatB and TatC.

Almost everything in biology is in motion; either in passive motion (e.g. the diffusion of a protein in the cell membrane due to the constant collisions with surrounding molecules), or active motion (e.g. the directional motion of Kinesin-1, which requires the energy released by ATP hydrolysis) or a combination of both. To be able to quantitatively and qualitatively describe the motion of a particle, one has to be able to visualize the particle and accurately determine its position at different times to reconstitute its trajectory. The tool of choice for this challenge is single-molecule fluorescence microscopy, as introduced in Chapter 2. Indeed, it allows the specific visualization of biomolecules labelled with fluorescent dyes – numerous procedures are available for fluorescent labelling- against a dark background. In combination with the use of specific optics and high speed camera’s this yields to a high position accuracy as well as high time resolution. Single-molecule fluorescence microscopy is thus an excellent tool with spatial and temporal resolution required to unravel all kinds of motion in biology.