# Molecular Machines under Tension: Bionanomechanics with Optical Tweezers

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Molecular machines—assemblies of macromolecules, often fueled by nucleotide hydrolysis—are fascinating devices that drive self-organization in cells. While the protein components of many biological machines have been identified, and in many cases their structures have been solved, the mechanical principles that govern the operation of biological machines are poorly understood. For example, how much force can they generate; and what limits their speed and efficiency? Eventually, how do cells use energy to create pattern and order? And how can these principles that nature has optimized be helpful for the design and construction of new biological and nanotechnological devices? These questions have been difficult to answer because the tools needed to study nanometer-sized machines and motors that generate minute forces on the order of piconewtons have not been available until recently. We use and develop high-resolution, (i) single-molecule fluorescence, (ii) light-force microscopy—so-called optical tweezers, and (iii) novel trapping probes to measure intermolecular forces that are central to biological questions such as how kinesin motor proteins translocate and diffuse along microtubules or how damaged DNA is repaired via homologous recombination. In the talk, I will introduce our optical tweezers, recent improvements of the latter, and their use in measuring the nanomechanics of individual kinesins and a DNA repair protein involved in homologous recombination. I will give an outlook on the technology development and how one can fabricate assemblies of molecular machines, which may be utilized in the future for biological, medical, or nanotechnological applications.

Optical tweezers are very high-resolution position and force transducers, which are extensively used in physics, biology, and materials science. Small dielectric particles are trapped in a highly focused laser beam and often used as probes and handles for sensitive force measurements. For many experiments, the maximum force of optical tweezers is limiting. To produce larger forces, only the instrument apparatus and the employed laser have been optimized so far. Through theory-optimized photonic structuring of the probes, we managed to use new high refractive-index materials for anti-reflection coated microspheres. Using these core-shell particles, we have demonstrated to date the highest optical forces for the investigation of biological and other nanoscopic systems. Higher forces enable new applications that were previously not possible or reserved to other instruments with lower resolution. In addition to these high-force probes, we develop small nanospheres to measure fast translational and rotational dynamics of biological mechanisms. To this end, we employ high-refractive index materials such as titania and nanodiamonds, in addition to birefringent substances. Apart from the probe size, its inherent drag and Brownian motion, measurements are ultimately limited by the drift of the setup. To this end, we isolate the apparatus with respect to mechanical and acoustic vibrations, electromagnetic radiation, and thermal drift. In addition, we stabilize the trapping objectives to within one thousandth of a degree. By these measures, we can achieve molecular resolution in force and position.

With our equipment, we investigate the function of individual cellular machines, molecular motors and DNA repair proteins that have essential mechanical functions during cell division. Thus, the mechanical analysis of the nanomachines and the development of new probes also have medical relevance because information gained can be used to specifically interfere with the cell division function, for example, during uncontrolled cell proliferation. With respect to the motor proteins, we measure the maximum force they generate, how fast they translocate, either in a directed manner or via diffusion, and how efficient they are. As an example, I will focus on the latter point: we measured how friction arises between proteins when they interact by making and breaking weak intermolecular bonds. When a bond breaks, the energy stored in its deformation is dissipated. Protein friction is a useful concept because it provides mechanical insight and allows for a quantitative theoretical understanding of the dynamics and energy balance of mechanical cellular processes. In cells, many motor proteins often cooperate to drive motility. Open questions remain, how friction and force-generation arise and scale with the number of motors elucidating how collective behavior and self-organization emerge. In part, these are questions also relevant for nanotribology. In the sense of a synthetic biology and biomimetic approach, principles

of how nature has optimized protein friction may be helpful for the design and construction of new biological and nanotechnological devices. In the long term, we hope that our versatile, avant-garde nanotechnological tools and instruments will be applicable to a wide range of problems to discover and understand phenomena of the biological nano-cosmos and to develop novel applications employing molecular machines for designed purposes.