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Review

DNA based molecular motors

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Abstract

Most of the essential cellular processes such as polymerisation reactions, gene expression and regulation are governed by mechanical processes. Controlled mechanical investigations of these processes are therefore required in order to take our understanding of molecular biology to the next level. Single-molecule manipulation and force spectroscopy have over the last 15 years been developed into extremely powerful techniques. Applying these techniques to the investigation of proteins and DNA molecules has led to a mechanistic understanding of protein function on the level of single molecules. As examples for DNA based molecular machines we will describe single-molecule experiments on RNA polymerases as well as on the packaging of DNA into a viral capsid—a process that is driven by one of the most powerful molecular motors.

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Contents

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1. Introduction

DNA molecules are the carrier of all genetic information and have evolved both, as extremely stable entities in order to preserve this genetic code, as well as in ways that allow for the controlled access of this information. Thus in addition to understanding the biochemical information of a DNA molecule, it is also important to study its mechanical behaviour and characteristics, since it is these characteristics that control many of the biological processes that take place during gene expression.

To this end, mechanical studies of single biomolecules have in recent years revolutionised our understanding of biological complexes [1–6]. The most prominent methods for single-molecule force spectroscopy are the atomic force microscope (AFM), magnetic tweezers and optical tweezers [7]. In single-molecule force spectroscopy AFMs have mainly been used for experiments which require high forces, i.e. to rupture bonds or unfold proteins [8]. Magnetic tweezers allow for the study of proteins that twist DNA and proteins that are very sensitive to forces [9]. Optical tweezers are probably the most widely used tool for studying the mechanics of molecular motors [10]. It has been shown that forces as low as ∼10 fN [11] and distance changes as low as 0.1 nm [12–14] can be observed using optical tweezers. Moreover, with the use of clever experimental geometries [15] or strong lasers, it is possible to exert forces exceeding 100 pN. These forces and distances are in the range reported for molecular motors [16,17].

One of the most important applications of single-molecule force spectroscopy is to unravel the mechanics of DNA molecules [18,19]. For any DNA the measured force-extension behaviour can be well described (up to forces of about 50 pN) using a simplistic coarse grained polymer model. Moreover, at high forces or in situations when the DNA helix is overwound (i.e. the number of bases per complete turn of the DNA helix is reduced) or underwound, the conformation of the DNA changes completely from the canonical B-DNA structure to a different, stable form in a spontaneous and cooperative manner which resembles a phase transition.

Understanding the mechanical behaviour of single DNA molecules becomes biologically important in the context of DNA–protein interactions. Proteins that bind DNA or translocate along DNA oftentimes exert forces on the DNA substrate due to bending or unwinding [20,21]. Typical forces that are applied are in the range of 1–100 pN and typical displacements that need to be observed can be as small as a single basepair (bp) which is only 0.34 nm. Physically one can understand these force- and distance-scales since the energies important for understanding the underlying phenomena lie between the thermal energy $k_B T$ which in units of force times distance equals to 4.1 pN nm and the energy available through the chemical reaction. In the case of ATP hydrolysis at physiological conditions the free energy liberated by the hydrolysis of a single ATP molecule by a single protein amounts to ∼100 pN nm for the hydrolysis. The time-scales that are important to monitor such processes reach from the millisecond to second regime. Single-molecule manipulation with optical (or magnetic) tweezers allows for investigations at these force-, distance-, and time-scales. Hence, optical tweezers are an ideal tool for the mechanistic investigations of these protein nucleic acid interactions at the single-molecule level. The high sensitivity of the single-molecule experiments allows for a detailed analysis of this enzymatic behaviour and opens up the possibility to unravel the underlying molecular mechanisms. A huge variety of such DNA–protein interactions have been studied using single-molecule manipulation techniques and the results are summarised in recent reviews [9,22]. Of particular interest are proteins that use the free energy liberated by a chemical reaction (most often the hydrolysis of ATP) to either translocate along DNA [22], unwind DNA [23], change the topology of DNA [24] or catalyse a polymerisation reaction [3,25], thus constituting molecular motors.

In the literature one can find many excellent reviews on optical trapping and single-molecule force spectroscopy [10,26–29] and the purpose of this article is not to give another complete overview of the field. Instead, we focus on some key aspects of current research and discuss experimental procedures as well as current challenges (Section 2). In particular we describe in detail the design of an optical tweezers apparatus that allows studying the mechanical properties of DNA molecules, such as the well-known force-extension behaviour and the overstretching transition (Section 3). This is a necessary pre-requisite for investigating the molecular mechanism of molecular motors that