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Structural and mechanical insights into DNA and chromosomes under strain using optical tweezers and fluorescence microscopy

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ABSTRACT

In our lab, we have developed the combination of optical tweezers and single-molecule fluorescence microscopy as a powerful tool to study biomolecules and biomolecular complexes. Combining these two technologies allows holding a sample molecule or complex, extending or deforming it, and measuring forces acting on it, while, at the same time, visualizing it with single-molecule sensitivity. We have applied this approach to different biological systems, including DNA, whole chromosomes, cytoskeletal components and membranes. Here, I will explain the concept of the technology, its potential and limitations. I will explain the technology by highlighting its application to our research on the mechanical properties of DNA and force-induced conformational transitions. I will also discuss our latest breakthroughs in applying this technology to intact mitotic human chromosomes, which provide novel insights in the mechanical properties of chromosomes and the way they are condensed in mitosis.

Keywords: Optical tweezers, fluorescence microscopy, single-molecule techniques, DNA, DNA-binding proteins, chromosomes

1. INTRODUCTION

Since the invention of optical tweezers by Arthur Ashkin and Steve Chu in 1986 [1], optical tweezers have become one of the techniques to study the mechanical properties of biomolecules at this single-molecule level. To this end, single or multiple-trap optical tweezers are used to apply piconewton forces to glass or plastic microspheres attached to the biomolecule of interest [2]. At the same time, nanometer-scale changes in position of the microspheres can be measured, providing insight in how the biomolecule reacts to force or changes position in time. Optical-tweezers studies have, for example been instrumental in increasing our understanding how motor proteins like kinesin or RNA polymerase convert chemical free energy into mechanical work [2].

In our lab, we have mostly focused on optical-tweezers studies of DNA and DNA-binding proteins using and experimental assay where both ends of a double-stranded DNA molecule are each attached to a microsphere. Each microsphere is then held in an independent trap and the distance between the traps can be changed to apply forces in a controlled way and changes in DNA length (caused e.g. by structural transitions, the binding of proteins, or enzymatic activity) can be measured very accurately. Although very powerful, this approach has as key limitation that the measured forces and displacements are integrated over the whole length of trapped DNA construct, hiding heterogeneous response along the DNA. To address this, we have implemented fluorescence microscopy in our optical-tweezers instruments. In a first iteration, we used epi-illuminated wide-field fluorescence microscopy allowing continuous visualization of the trapped DNA molecule while applying forces with the traps [3]. In particular, we used fluorescent markers for different states of DNA –fluorescent intercalators specific for double-stranded DNA and fluorescent single-stranded DNA binding proteins (SSB or RPA) specific for single-stranded DNA– and studied structural transition of double-stranded DNA at forces of ~65 pN, the so-called overstretching transition. We found that different transitions occur depending on the exact condition, but remarkably all take place at forces of ~65pN and have very similar mechanical signatures. At low salt concentration and when the double-stranded DNA has free ends, overstretching will zipper open the double-stranded DNA from these ends, forming single stranded DNA. When there are no free ends, the DNA will melt (base pairing between strands get lost) in small regions, so-called melting bubbles. At high salt concentrations, when the negative charges of the DNA backbone are effectively screened, a new base-paired structure is formed that has lost most of its helicity, called S-DNA [4]. The additional information provided by fluorescence was instrumental in unraveling this complex behavior.

Although epi-illuminated wide-field fluorescence microscopy is generally regarded to suffer from issues with out-of-focus background signals, we figured that, given the right conditions, laser illumination and EMCCD detection, it is sensitive

enough for single-molecule detection. We have used single-molecule fluorescence measurements in our tweezers instruments to study how the detachment of DNA-repair protein RAD51 is governed by force on the DNA template and ATP-hydrolysis [5]. We also implemented this fluorescence scheme in a quadruple-trap optical tweezers instrument, which allows the independent manipulation of two DNA molecules. We used this instrument to show that complexes of other DNA-repair proteins, XLF/XRCC4, can very stably crosslink two DNA molecules, while forming highly mobile 'sleeves' around these DNA molecules [6].

It is also possible to implement confocal fluorescence microscopy in an optical tweezers instrument, with as key benefit better out-of-focus background suppression [7]. The implementation is, however, more complex since the confocal excitation beam needs to be scanned over the sample. We have also included STED (STimulated Emisson Depletion) super-resolution fluorescence microscopy allowing an optical resolution of <20 nm [7]. Since in a typical (single) DNA experiment only the spatial resolution along (and not perpendicular to) the trapped DNA molecule is relevant, we used 1-dimensional STED. This substantially simplifies the implementation since super-resolution scanning is than only required along the DNA (in 1 dimension) and since it alignment of excitation and STED beams and DNA molecule, in the direction perpendicular to the DNA long axis, is more straightforward. The combined single-molecule fluorescence and optical tweezers instruments developed in our lab and described above, have formed the basis of our spin-off company LUMICKS b.v. (I declare financial interest), which has further developed the instruments to make them more reliable and easier to use, also for non-experts. LUMICKS has sold more than 100 of these combined fluorescence optical tweezers instruments worldwide.

2. THE NEXT STEP: OPTICAL TRAPPING OF INTACT CHROMOSOMES

So far, most optical tweezers experiments on DNA have been on 'naked' DNA. In the cell, however, DNA is hardly ever naked, but in an intricate complex covered by many proteins (including nucleosomes) called chromosomes. Chromosomes not only have different mechanical properties, but also serve as a completely different substrate to DNA-processing proteins than naked DNA. During the cell cycle, chromosomes undergo major structural transformations, prior to mitosis they condense into the archetypical X-shaped structures, metaphase chromosomes, that are ready to withstand the forces employed by the mitotic spindle to segregate them over the two daughter cells, later in mitosis.

One approach employed by us [8] and others [9] is to *in vitro* build up towards the complexity of chromosomes, by reconstituting DNA with histones and use this DNA-histone complexes, chromatin, as a substrate in optical or magnetic tweezers experiments. We recently took another approach, by isolating metaphase chromosomes from human cells and manipulating them with optical tweezers [10]. To be able to attach microspheres firmly and specifically to the chromosomes, we employed an *in cellulo* biotinylation protocol, specific to the telomer ends of the chromosomes. In this way, metaphase chromosomes could be caught, extended, and visualized (Figure 1), which provides a new way of studying the properties of chromosomes and the roles of many players involved.

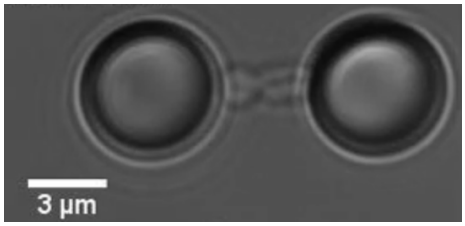

Figure 1. Brightfield microscopy image of a human metaphase chromosome held and extended between two optically trapped microspheres. Figure adapted from [10].

So far, we have specifically focused on assessing the mechanical properties of metaphase chromosomes. To this end, we measure many force-extension curves of individual chromosomes. Since we do not have control over the particular chromosome that is captured, interpretation of these measurements is complicated by the large variation in length between the different chromosomes. By plotting chromosome stiffness as a function of force applied, this problem was overcome and all the curves collapsed to a single master curve showing nonlinear stiffening of the chromosomes upon increasing load, different from the prediction of classical polymer models like the worm-like chain. This stiffening behavior of the chromosomes could be understood in terms of a novel polymer model, the hierarchical worm-like chain model, in which the chromosome is portrayed as an assembly of multiple worm-like chains with different contour and persistence lengths, highlighting the mechanical heterogeneity of chromosomes on different structural and spatial scales.

3. CONCLUSIONS

Over the years, optical tweezers, often in combination with fluorescence microscopy has become and essential tool to unravel the behavior of DNA and DNA-bind proteins on the single-molecule scale. Since recently, we have been applying optical tweezers to much more complex DNA-based assemblies, metaphase chromosomes, providing exciting new insight in their surprisingly complex mechanical properties. Currently, we are unraveling what the molecular basis is of chromosome mechanics, focusing on the specific roles of proteins like topoisomerase IIα [10], condensins, histones, and polyamines [11].

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