Here, I will briefly discuss the highlights of the work presented in this thesis by guiding you through the five chapters one by one.

The introduction (Chapter 1) takes you from the beginning of the modern theory of evolution to the current status of evolutionary thinking at the molecular level. Charles Darwin is widely recognized as the founding father of this theory. The variety of several types of flora and fauna he observed during his voyage to South America led him to formulate a new theory of coming into existence of species. Instead of species being stable, he hypothesized that they could evolve into another. Furthermore, he insisted that the driving force of evolution was natural selection. Small adaptations within one species could lead to an advantage, leading to more offspring and higher survival rates. The missing link to the modern evolutionary synthesis theory was provided by Gregor Mendel’s experiments on pea plants. He showed that certain traits were inheritable through clear patterns. Eventually, this led to the founding of a new field: molecular biology. A milestone in this process is the discovery of the structure of DNA by Watson and Crick in 1953. The next decades more and more details on life at a microscopic level were revealed. The flow of information, from DNA to RNA to proteins, was confirmed and showed DNA as being at the origin of life, containing the blueprint for every cell.

In the past two decades, single-molecule techniques have sparked the field of biophysics. By studying single proteins at a time, the exact mechanisms of molecular machines can be exposed. At the same time, we are able to learn more about the intricate mechanisms Nature has deployed to sustain the complexity of life. Optical tweezers is one of the most used techniques for single-molecule experiments. A coherent laser beam can be strongly focused in a fluid. In the focus, a small micrometer-sized bead can be held. A single laser beam can be used to generate two foci and therefore two beads can be trapped. By treating the bead with a specific protein, it is possible to attach the ends of a DNA molecule between the beads. A focus can be moved in three dimensions by steering the laser beam using mirrors. In this way, the beads can be moved with respect to each other and the DNA molecule can be stretched. Because proteins can alter the elasticity of DNA, the properties of proteins can be studied.

An extensive review of the use of optical tweezers to study DNA-protein interactions can be found in Chapter 2. Assays that allow for binding characterization are discussed, as well as several applications to study the mechanism of proteins that alter DNA. First, the different assays are discussed with their respective advantages and limitations. Next, a comprehensive section provides the details of a DNA molecule when stretched in optical tweezers. The characteristics of DNA as it is stretched are used in most assays. Finally, the contribution of optical tweezers studies to the understanding
Summary of four essential DNA processes is discussed: (i) DNA organization, on storing DNA in cells; (ii) DNA replication, or how a copy of DNA is made for a daughter cell; (iii) DNA transcription, reading of the DNA to synthesize mRNA for the production of proteins; and (iv) DNA repair, which is needed whenever the DNA is broken or damaged.

The use of DNA molecules with known properties is essential to study single proteins. The length of the DNA molecule and the method of attachment are important to reveal specific properties of proteins. In Chapter 3, protocols are presented for DNA construct synthesis. Moreover, the DNA molecules are extensively tested and the experimental conditions are optimized. This study discusses many practical aspects of generating DNA molecules for different type of experiments.

DNA replication is the process that makes sure that DNA is copied before a cell is able to duplicate itself. Faithful copying is vital to generate offspring with the same traits. For DNA to be replicated, the double helix is first unwound into two single strands of DNA by DNA helicase. Because of the complementary nature of DNA, a single strand can be used as template to synthesize the complementary strand. DNA polymerase (DNAp) is the enzyme that catalyzes this process. It generates two copies of the DNA by reading the single-stranded templates and the one-by-one addition of complementary nucleotides to the newly synthesizing strands.

In Chapter 4, a close look is taken to the extreme high fidelity DNAP achieves. It is known that the DNAP is extremely good at selecting the correct nucleotide out of a pool of the four different nucleotides. DNAP can also proofread in the case it has added a wrong nucleotide; it will excise the last added nucleotides. It is revealed that DNAP is actually fairly ’paranoid’: it removes way more nucleotides than errors it has made. This suggests that the process of DNA replication is by no means efficient. The removal of correct nucleotides means that DNAP has to redo a job it had done well in the first place. This could only make sense in the light of the required fidelity. The persistence of an error in the DNA of the progeny would be more catastrophic than wasting energy and time over what minimally would be required for DNA replication. Hence, the cost of copying DNA correctly is paid by the removal of nucleotides and re-addition of the same.

The last chapter (Chapter 5) consists of a study of the replisome, a complex of proteins necessary for DNA replication. Here, the cooperative mechanism of DNA helicase and DNAP are studied with optical tweezers. Because the complementary strands of DNA are antiparallel orientated and DNAP can only add nucleotides in one direction, the two strands of DNA cannot be synthesized continuously at the same time. Only one strand, the leading strand is synthesized continuously, while the other, the lagging strand, is synthesized in fragments. Intuitively, copying of the lagging strand would be slower, but this is very undesirable. Therefore, synchronization of the two processes is required. In this chapter, results are presented that suggest that this synchronization is achieved by stalling the leading-strand synthesis when a new fragment needs to be initiated on the lagging strand. Additionally, a new mechanism for complete
synchronization is provided based on these results, while in agreement with currently available studies.