Abstract

Over the past decades, the field of DNA biotechnology has attracted paramount interest because of the revolutionary solutions it provides in many applications. Herein this thesis, investigated two fundamental applications of DNA biotechnology.

First, the DNA oligonucleotides (ONs) were introduced as a supramolecular biomaterial which were engineered to aggregate *via* bottom-up technique and form macroscale structures. Basically, a high ionic strength buffer is crucial to stabilize such DNA self-assembles which is a big barrier for utilizing the technique in medicinal applications such as DNA robots. In this part, non-nucleosidic modifications with hydrophobic affinities have been functionalized to enhance the aggregation stability under different ionic strength environments. Different numbers of Twisted Intercalating Nucleic Acid (TINA) monomers were inserted into short complementary sequences. The mechanism of interaction of the TINA-DNA constructs was studied *via the* nano-tracking analysis (NTA) technique. The size of the aggregates was explored in solutions using polyacrylamide gel (PAGE) and the topology of the structures was visualized *via* atomic force microscope (AFM) and fluorescence microscope. Moreover, biophysical and molecular dynamic properties were studied *via* circular dichroism (CD) and molecular modelling, respectively. The presented techniques of modifying the DNA-ONs bearing TINA building blocks afforded a stable macrostructure where its size can be controlled by changing the ionic strength of the solution and the number of incorporated monomers.

Second, engineering the modified ONs for therapeutic purposes presented for gene-silencing. Antisense-modified oligonucleotides (ASOs) were designed to target a single-point mutation which develops a rare genetic disease called progeria. Two different sequences were modified and engineered in order to sterically hinder the splicing of the cryptic splice site, which is activated by the mutation, or stop the translation of a truncated version of lamin protein (progerin). It is known that the lamin proteins are one of the main components of the nuclear envelope, therefore the toxic progerin distorts the morphology of the nucleus which results in increasing the stiffness of the cell nuclei and provides wobbly structures with blebs. Also, the mechanical properties of the nucleus decrease and lead to apoptosis, DNA damage and increasing the nuclear area of the cell nuclei. A novel treatment protocol *in-vitro* was presented, where the modified ASOs were tested. The efficiency of the developed ASOs was evaluated by visualizing the alterations in the nuclear lobulation of the progeroid cells upon repetitive treatment over 10 days using confocal microscopy. In addition, the gene expression of the targeted gene was analyzed using the polymerase chain reaction (PCR) technique.

Finally, the same developed transfection protocol was employed to study the efficiency of six modified ASOs-targeting mRNA with the aim of knocking down the progerin expression. To this end, three nucleotide building blocks were chemically synthesized *via* copper-catalyzed click reaction containing 1-phenyl-1,2,3-triazole moiety together with two further analogues substituted with *p*-sulphonamide and *p*-aminomethyl groups to the 5-position of 2´-O-methyl uridine or thymidine nucleotides The corresponding phosphoramidites were systematically incorporated into ASOs and hybridized with DNA and RNA complements. This chemical addition allows the aromatic stacking of the phenyl group which decreases the ribosomal activity on the targeted strands. A significant decrease in the nuclear area of the treated nuclei was visualized by confocal microscopy, downregulation of the progerin and increase of lamin A/C expression on both RNA and protein levels were observed during qPCR and western blot analyses.