Modular assembly of functional DNA-based systems
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Summary

DNA is the molecule containing the genetic information in living organisms and is usually found as a dimer of two complementary polymers of nucleotides that are linked via phosphodiester bonds. Each nucleotide consists of a phosphate group, a sugar moiety and a base (Figure 1). There are four types of bases, i.e., adenine (A), guanine (G), cytosine (C) and thymine (T).

![Figure 1. General structure of a nucleotide and structures of the different bases.](image)

The specific interaction between the bases (adenine with thymine and guanine with cytosine) by hydrogen bonding results in the assembly of both strands in a double helix structure (Figure 2).

![Figure 2. Hydrogen bond interaction between DNA bases and schematic representation of the DNA double helix.](image)

The genetic information is coded in the sequence of the bases. Making use of the specificity of the assembly between them the structural possibilities in the DNA assemblies go beyond the double helix. This unique feature together with the possibility of synthesizing virtually any desired DNA sequence allows for the assembly of predictable structures of variable complexity, ranging from 2D to 3D structures.

The DNA molecule has been used in diverse fields of scientific research such as catalysis, synthesis and nanotechnology due to its special properties. This versatility of the DNA shows the potential of such a molecule to be used in an increasing number of research fields and to create innovative functional systems. The application of DNA in nanotechnology remains still in its initial stage therefore, the possibilities appear endless.
The work described in this thesis aimed to develop novel functional systems based on DNA structures. A modular assembly approach has been followed for this purpose, involving functionalized and unfunctionalized oligonucleotides. The resulting DNA-based systems showed application in the fields of catalysis, synthesis, molecular sensing and light harvesting and all were based on the use of oligonucleotides with covalently linked functional molecules. This allows for the precise location of the functional molecules in the assembly, resulting in a better understanding of the system and therefore, in an easier optimization.

In the first chapter of the thesis an overview of the applications of the DNA molecule in science is given. This chapter shows the diversity of fields in which the DNA has been successfully applied. Although the applications are already numerous, multiple possibilities are still to be explored.

Chapter 2 describes the construction of two DNA-based catalysts with a covalently linked metal complex. The catalysts were studied in the asymmetric copper (II)-catalyzed Diels Alder reaction between azachalcone and cyclopentadiene (Figure 3). Different results in conversion and enantioselectivity were obtained when using two monodentate ligands, i.e., pyridine, or a bidentate ligand, i.e., bipyridine. The modular nature of the system allowed for a rapid optimization by exchange of any of the modules. This approach makes use not only of the ease of assembly by DNA hybridization but it also utilizes the inherent chirality of the DNA to obtain one of the possible enantiomers in higher ratio with respect to the other. The enantiomeric excesses obtained using the bipyridine-based catalyst were up to 93%.

![Figure 3. DNA-based catalysts using covalently linked pyridine (left) and bipyridine (right) moieties.](image)

In chapter 3 two different approaches were studied for the creation of an artificial ribosome. In this chapter, the templating action of the DNA was used to promote the coupling between amino acids attached to independent oligonucleotides and complementary to the DNA template (Figure 4). The ribosomal design followed implies the use of protected amino acids thus, a deprotection step is required prior the peptide coupling can take place. The two approaches studied make use of different deprotection methods namely, palladium catalyzed deprotection of alloc protected amino acids and
photoinduced deprotection of o-nitrobenzylcarbamate derivatives. Attempts to perform the deprotection via the first approach proved unsuccessful. However, the deprotection by photolysis was possible not only using the protected amino acid-oligonucleotide conjugate but also when the conjugate was integrated in the artificial ribosome. Despite the fact that the coupling between amino acids was not yet observed after the deprotection, the results are encouraging. Coupling reactions between similar reactive groups are observed in nature thus, an optimization in the design of the system might lead to the desired peptide bond formation.

Figure 4. Schematic representation of an artificial ribosome.

The templating action of the DNA is also a key point in the research described in chapter 4. In this study a DNA template complementary to independent oligonucleotides covalently linked to two halves of an enzyme, i.e., murine dihydrofolate reductase (mDHFR) was used. By hybridization of the oligonucleotide components with a template strand a reassembly of the enzyme was possible, thus restoring its catalytic activity (Figure 5). The catalytic activity of the enzyme was found to be modulated by the incorporation of different number of mismatches in the DNA template. Furthermore, its activity was shown to be dependent on the concentration of the DNA template used.

Figure 5. Schematic representation of split enzyme reassembly by DNA hybridization.

A direct application of this enzymatic system is discussed in chapter 5 for the creation of a molecular sensor. The design involves the combination of the system described in chapter 4 together with a molecular recognition site, i.e., an adenosine triphosphate (ATP) aptamer (Figure 6). The approach studied relies on the liberation of a fragment of the
DNA template necessary for the DNA hybridization and subsequent enzyme reassembly as a result of the recognition of ATP. Differences in the enzymatic activity were observed in the presence and absence of the target molecule indicating that the structural change required for the enzymatic reassembly takes place when ATP is recognized. A balance between the stability of the folded aptamer and the hybridized enzymatic system is required for the optimal performance of the system. Therefore, the design of the aptamer-template DNA strand is crucial and the sensitivity of the sensor might be increased by optimization of the DNA sequence.

Figure 6. Schematic representation of ATP-triggered split enzyme reassembly.

The last experimental chapter of the thesis describes the construction of a light harvesting system based on DNA G-quadruplexes (Figure 7).

Figure 7. Schematic representation of the G-quadruplex-based artificial light harvesting antenna. E. T. = energy transfer.
This particular structure that DNA can adopt allows for the placement of donor (coumarin) and acceptor molecules (porphyrin) in the proper orientation for energy transfer processes to take place. When DNA sequences not able to form quadruplex structures were used, energy transfer was not observed.