Chapter 7

Functional DNA-based systems: conclusions and perspectives

In this chapter the different approaches followed in the construction of functional DNA-based systems are summarized and conclusions are drawn. The advantages of the introduced modular approach are compiled. Furthermore, the research perspectives and future prospects are discussed.
7.1. Introduction

DNA has shown great potential in the construction of complex architectures as well as in the creation of innovative functional systems.\textsuperscript{[1,2]} Thanks to the unique features of the DNA molecule its applications in science are already numerous.\textsuperscript{[3-8]} The research described in this thesis aimed to further explore the application of the DNA molecule for the construction and study of novel DNA-based systems. A new concept has been introduced in this thesis: the modular assembly approach (Figure 1). This concept entails the use of different functionalized and unfunctionalized oligonucleotide modules which can be combined and assembled by hybridization. A key point in the approach lays in the fact that the functional molecules are covalently linked to the oligonucleotides. This allows for a precise positioning in the DNA structures which gives ready access to complex systems in a well-defined manner with the ultimate goal of creating new activity and/or function.

![Figure 1](image)

\textbf{Figure 1.} Schematic representation of the modular assembly approach.

In this chapter, an overview of the science developed in this thesis is presented, followed by a discussion about the research perspectives.

7.2. Research overview

The remarkable features of the DNA molecule have been shown to be perfectly suitable for the construction of complex and versatile functional systems. Based on the specific complementarity between the nucleobases, complex structures could be constructed and functional molecules of diverse nature could be assembled in a predictable fashion. The key features of the DNA-based systems described in this research are the ease of assembly by hybridization and the covalent linkage of functional molecules.
The use of a metal complex covalently linked to a specified position in the DNA resulted in a novel DNA-based catalyst (chapter 2). The modular approach involved the use of three oligonucleotide components, i.e., an oligonucleotide functionalized with a Cu$^{2+}$ complex, an unfunctionalized oligonucleotide and a DNA template complementary to both. This DNA-based catalytic system not only takes advantage of the complementarity between nucleobases to be assembled but furthermore, the chirality of the DNA makes it suitable for asymmetric catalysis. This was demonstrated for the Cu$^{2+}$ catalyzed Diels-Alder reaction in water; up to 71% conversion and 93% ee were obtained using an optimized DNA-based catalyst. The optimization was achieved by exchange of the individual modules. Based on the results, the creation of DNA-based catalysts with increasing complexity can be envisioned. By programming and assembly of diverse functionalized oligonucleotide modules, multicatalytic systems might be constructed. The use of covalently attached catalysts allows for the control over their position and orientation with respect to each other. This is a key point in the design of multicatalytic systems since the optimization of the system can be readily achieved.

Based on the concept of DNA-templated synthesis, different approaches towards the construction of an artificial ribosome have been investigated (chapter 3). The templating action of the DNA is capable of bringing together amino acids linked covalently to oligonucleotides. This increase in the effective molarity can promote the reaction between amino acids. Although a coupling step was not yet observed and further optimization is required, the approach followed holds promise. The optimization should be possible due to the ease of assembly of the system that allows for exchange of the modules. The use of different modules with variable oligonucleotide lengths and distances to the functional molecules might result in an optimized system. The optimization and understanding of such a system would represent a step further in the field of DNA-templated synthesis, mimicking one of the most elegant and versatile synthesis machineries in nature, the ribosome.

Chapter 4 described how, by hybridization of protein fragment-oligonucleotide conjugates to a DNA template, the enzymatic activity of murine dihydrofolate reductase (mDHFR) was modulated. The results showed the dependence of the assembly, and therefore the enzymatic activity of mDHFR, on the DNA hybridization. The presence of mismatches in the template as well as the use of different concentrations resulted in differences in the enzymatic activity. This is of interest for the field of biosensors since it enables the sensing of mutations in the DNA and therefore, could allow a premature and sensitive detection of genetic diseases.

The combination of the enzymatic system described in chapter 4 with a molecular recognition site for the construction of a molecular sensor was described in chapter 5. The approach combined an adenosine triphosphate (ATP) aptamer (a single stranded DNA
capable of specifically recognizing ATP) together with the DNA template necessary for the total hybridization of the oligonucleotides attached to the mDHFR fragments and the subsequent reassembly of split mDHFR. This approach makes use on one hand of the specificity of DNA aptamers in recognizing target molecules and, on the other hand, of the control over enzymatic activity by DNA hybridization. A structural rearrangement is triggered by ATP recognition liberating the DNA sequence necessary for the enzymatic reassembly. The approach shows how two different applications of DNA can be combined for the creation of an innovative biosensor. The design of the DNA strand turned to be crucial in the performance of the sensor and, although noticeable changes were observed, an optimization of the sequence should lead to higher sensitivity.

In the last experimental chapter of the thesis, a DNA G-quadruplex assembly was investigated for the creation of a light harvesting antenna. This particular structure enables the controlled assembly of covalently attached donor molecules and supramolecularly assembled acceptor molecules. Furthermore, the quadruplex provides the right orientation of the donor and acceptor moieties to achieve efficient energy transfer. This approach not only brings new insights in the energy transfer process taking place between those molecules but establishes the foundation for the creation of more complex light harvesting systems based on DNA.

Combined, the results discussed in this thesis show the diversity of systems that can be created based on the DNA-based modular assembly concept. This approach presents many advantages that make it a powerful tool in the construction of novel DNA-based systems:

I) The modular systems can be assembled readily by hybridization.

II) The covalent functionalization of the oligonucleotides is straightforward and allows for the controlled positioning of functional molecules.

III) The complexity of the systems can be increased by designing the suitable DNA strands.

IV) The versatility of the designs allows for the combination of different functionalities for the creation of multifunctional systems.

V) Any desired DNA sequence can be readily obtained by automated synthesis. This makes possible the construction of complex systems based on very diverse predictable structures.

The systems described in this research illustrate some of the possibilities that the DNA molecule offers in the construction of functional devices. Considering the different nature of the fields in scientific research where the described modular approach can be applied, the possibilities seem to be unlimited.
7.3. Research perspectives

The DNA-based modular assembly concept introduced in this research establishes the basis for the creation of complex functional systems with novel and enhanced properties. The ease of the functionalization and assembly makes it an attractive approach to be applied in further research. The immediate steps to follow in the research would involve the increase in the complexity of the DNA-based systems by combination of different functionalities. This can be achieved by precise design of the DNA sequences and subsequent assembly. In this way, 2D and 3D assemblies containing functional molecules can be readily obtained.

The modular nature of the approach enables the combination of different functionalities in a single ensemble. The creation of multifunctional assemblies would result in systems with enhanced properties. Some examples of such multifunctional systems could be:

- artificial photosystems
- systems capable of achieving multiple synthetic steps
- multicatalytic systems
- systems combining biosensors with allosteric enzymes

By combining modules with catalysts of different nature, tailored multicatalytic systems could be created. This would represent a step forward, for instance, in the synthesis of relevant molecules avoiding intermediate purification steps thus, resulting in faster and more economical processes. Furthermore, the combination of a biosensor with an allosteric enzymatic system could result in very interesting and valuable systems. Its application in medicine would be attractive since the excess or lack of a specific analyte could not only be detected but it could also trigger an enzymatic reaction that leads to production or destruction of such a compound by the enzymatic reaction. The work described here may lead to future contributions in the field of medicine by the creation of novel regulatory systems, molecular sensors as well as the synthesis of relevant drugs. Due to the biocompatibility of DNA, its application for medicinal purposes is particularly appealing. However, in vivo applications involve many biological factors that should be taken into account such as the degradation by nucleases, and that can represent a limitation in the creation of such systems.

Furthermore, the functionalization of oligonucleotides with molecules other than the ones described in this work, e.g., molecular switches, organo-catalysts, fluorescent proteins, etc. could lead to the construction of novel functional ensembles. Therefore, the exploration of such functionalizations on DNA would be desired.
After more than 50 years since the discovery of the double helix structure of the DNA\cite{9} this unique molecule has shown to be one of the most versatile building blocks in supramolecular chemistry. DNA nanotechnology\cite{10} is a discipline which still remains in its infant phase therefore the development of numerous novel DNA-based functional systems in the near future can be foreseen.

7.4. References


