Chapter 6
DNA-based micellar catalytic system

In this chapter efforts towards the assembly of a DNA-based micellar catalytic system are presented; in the proposed design DNA has a dual role: it acts as a scaffold for catalyst assembly and as a tool to control the position of the catalyst and to fine-tune the reactivity in a chemical reaction conducted in a micellar medium. In order to do that, we used hybridization of specific DNA sequences, complementary to each other, specifically functionalized to control the catalysis and, at the same time, the interaction with the micellar aggregate.
6.1 Introduction

In the living cell a wide variety of different chemical reactions take place with high efficiency and specificity. Sometimes, these reactions are coupled in time and space (e.g. cascade reactions) in such a way that the products of one reaction act as substrate/catalyst of the subsequent one. However, many of the transformations occurring are chemically incompatible; yet, nature manages to ensure the integrity of the individual synthetic pathways and to realize the coupling of the different processes and the tuning of products (Figure 1, a). Among the several approaches adopted by nature, one of the most important ones is compartmentalization: by spatially separating incompatible processes and by regulating the flux of molecules in and out of the microenvironment, they can take place simultaneously without interfering with each other. The resulting well-defined reaction environments vary from relatively simple systems (nanometer scale), like enzymes, to extremely complex ones (micrometer scale), like cells (Figure 1, b).

Figure 1. a) Metabolic maze and b) schematic representation of a cell.

Each chemical reaction is represented by a filled circle. The reactions of the glycolytic pathway and the citric acid cycle are shown in the middle of the picture.

As often, Nature represents a source of inspiration for chemists: mimicking the complex medium that is the cell, many efforts have been done to couple reactions in time and space by designing confined reaction environments where the conversion(s) could take place. To create such reactors, early attempts were directed to synthesize low molecular weight receptors, which often
implied the covalent attachment of a transition metal complex to one or more binding cavities such as cyclodextrins (CDs),[2-6] cyclocholates,[7] calixarenes[8] and other concave binding sites.[9] These cavities, which are capable of recognizing/binding substrates, are located in proximity of a catalytic center/active site. However, as soon as the complexity of the target-product of the reactions increases, the construction of highly specific catalysts and reactors in a covalent fashion starts to require time-consuming and complicated multistep syntheses.

The development of more accessible systems demanded a supramolecular approach, that is the multicomponent self-assembly of small molecular complementary building blocks. A key example is represented by the ‘molecular softball’ reported by Rebek as nanoreactor for bimolecular reactions, which allowed for rate acceleration and selective formation of the products.[10] In most cases, the enclosed cavities of this type of reactors were formed reversibly by non-covalent interactions and the encapsulation process of the substrates was driven by the size and shape complementarity of the guest in the host. Not only hydrogen-bonding motifs, but also the interaction between ligand and metal centers can be used in the building blocks to construct reactors: several groups have built cage-like architectures by multicomponent transition metal mediated self-assembly.[11] Virus, and other protein capsids or polymersomes, that can be prepared, analogous to amphiphilic phospholipids of the cell membrane, from macromolecular amphiphiles molecule (block copolymers) building blocks, can be envisioned as well to achieve spatial separation of chemical moieties.[1,12,13] Even relatively simple systems, like micelles and vesicles, can be useful for this purpose.[1] Although micelles are not well-shaped/defined systems compared to self-assembled capsules or vesicles, they can be used as nanoreactors. An example, discussed in the previous chapter, is represented by the so-called Lewis acid-surfactant combined catalysts (LASCs) that act both as Lewis acid to activate the reactants and as surfactant capable of forming stable colloidal dispersions consisting of micrometer-sized spherical particles.[14] The concept was successfully applied to several C-C bond forming reactions such as allylation, Mannich-type, aldol and Diels-Alder reactions.[15] The difference in polarity between the hydrophobic core and the hydrophilic surface of the micellar aggregate allowed for solubilization of polar/non-polar substrates from the aqueous phase leading to reduction or increase of the rate of the reaction.[16] In the latter case, the enhancement of the rate of the
reaction has been ascribed to the stabilization of the transition state of the reaction as a consequence of a favourable interaction with the surfactant, to a combined effect charge-polarity-microviscosity inside the micellar aggregate\cite{17} or to the increased local concentration of the reactants at the micellar surface.\cite{1} An example where the rate enhancement is due to the ability of the micelles to bring reaction partners together causing them to react in a small reaction volume, was reported by Engberts and Otto.\cite{18} Cu(DS)$_2$ micelles provided a confined reaction environment which caused a 1.8 million fold acceleration compared to the uncatalyzed reaction for the Diels-Alder reaction between aza-chalcone and cyclopentadiene in water.

However, in order to create an efficient bio-inspired catalytic system, simply creating compartments is not sufficient: the reactants not only need to be separated, but also to be positioned at a specific time in specific sites within the cell. Hence, the predictable and programmable positioning of the catalyst and reactants in or with respect to these compartments is an important goal. DNA-based amphiphilic molecules (surfactants) that can be anchored to micelles or vesicles, are ideal for this purpose.

DNA-based surfactants consist of a long hydrophobic moiety (tail) and a short DNA strand as the polar headgroup.\cite{19-30} Mainly, these have been investigated as anti-sense oligonucleotides, where the hydrophobic tail acts as molecular recognition agent in the adhesion event to the living cell or micellar-like systems \cite{21,22,26} or they have been used to stabilize either duplex or triplex strand formation\cite{23,24,28} or to functionalize fluid surfaces.\cite{20}

Recently, several examples of anchoring single and double stranded DNAs to vesicular surface, via cholesterol or multiple lipid chains, were reported.\cite{30-32} A single lipid modified DNA was used to create DNA-functionalized reverse micelles for sequence-selective extraction of DNA.\cite{33} Finally, DNA-block copolymer micelles have been employed in DNA-templated synthesis\cite{34} and drug delivery (Figure 2).\cite{35,36}

Using the simple rules of Watson-Crick base pairing, the DNA head groups of the DNA-amphiphilic molecules can be used to bind other DNA strands with complementary sequence and properly functionalized with a catalyst. This would allow for precise positioning of the catalyst in a specific location on the surface of a micellar/vesicular system which in turn may determine its reactivity
and selectivity.

**Figure 2.** Members of the family of amphiphilic oligonucleotides: a) ON$_1$-TEG-Chol (cholesteryl-tetraethylenglycol ss-18mer-oligonucleotide) for insertion into lipidic surfaces; b) sequence selective extraction of DNA oligonucleotides using DNA-functionalized reverse micelles; c) DNA block copolymer micelles for drug delivery.

In this chapter a DNA-based micellar catalytic system will be presented. In the
first design DNA is proposed to act as a scaffold for catalyst assembly; later in the chapter we will discuss a different approach where, by using specific functionalized DNA sequences, different components can potentially be placed at predetermined positions in the system by hybridization with complementary DNA strands.

6.2 A DNA-based micellar catalytic system

6.2.1 Enantioselective catalysis

The increasing importance of catalysis in water has fuelled interest in the application of micelles, since they can potentially be used to overcome solubility problems associated with performing organic reactions in water. However, micelles can affect other features of a chemical reaction, for example the reaction rate, leading to inhibition or acceleration.\[^{16}\] The rate acceleration provided by micellar aggregates has been referred to as micellar catalysis. For the Diels-Alder reaction between aza-chalcone and cyclopentadiene an enhancement up to $10^6$ fold was observed in presence of micelles of Cu$^{2+}$ dodecylsulfate (Cu(DS)$_2$) compared to the uncatalyzed reaction.\[^{18}\] Recently, even stronger effects of the micelles of SDS on the reaction rate were found by our group in the Cu$^{II}$-catalyzed Friedel-Crafts alkylation.\[^{37}\]

On the other hand the introduction of the concept of DNA-based asymmetric catalysis has been proven really versatile, leading to high enantioselectivity and selectivity in a wide range of Cu$^{II}$-catalyzed reactions.\[^{38}\] The initial supra-molecular approach was based on the non-covalent anchoring of the copper bound catalyst to the double helix of DNA. In an alternative approach, the covalent anchoring of the catalyst to a modified DNA oligomer\[^{39}\] and the subsequent assembly by hybridization with a second and a third oligonucleotide strand (one of them acting as a template to bring the system together) allowed for precise positioning of the metal complex and, hence, control over the structure and geometry of the catalytic site (Scheme 2). Also, the second coordination sphere around the metal centre could be optimized readily by exchange of the unfunctionalized modules.

Inspired by these phenomena, the research initially aimed at using DNA-based amphiphiles to combine the high enantioselectivities obtained with the DNA-
based covalent approach\textsuperscript{[39]} and the high rate acceleration provided by SDS aggregate in the Cu\textsuperscript{II}-catalyzed Diels-Alder reaction in water.\textsuperscript{[18]}

\textbf{Scheme 1.} Cu\textsuperscript{II}-catalyzed Friedel-Crafts reaction in the presence of SDS micelles.

Conceptually, most straightforward would have been using a DNA-duplex that had an hydrophobic tail at one of its termini and the non-covalent binding of a copper complex, as in our original DNA-based asymmetric catalysis concept.

\textbf{Scheme 2.} Modular assembly of a DNA-based catalyst for asymmetric Cu\textsuperscript{II}-catalyzed Diels-Alder reaction in water.
However, in this approach the catalyst is not expected to be close enough to the surface to be able to interact with the substrates. Therefore the design depicted in Figure 3, which involves the modular assembly concept, was proposed.

The system was created by modular assembly of three oligonucleotides building blocks: one with 5’-terminally attached Cu-bpy complex (ON-A), one with a 3’ attached twelve carbon lipid tail (ON-B) and a template strand that had a sequence complementary to both (ON-C). After hybridization, both the Cu\textsuperscript{II} complex and the hydrophobic tail were located at the interface of the oligonucleotides. The tail ensured the interaction with the SDS micelle and the covalent linkage ensured that the copper complex was located at a predetermined site that is the micellar surface.

**Figure 3.** Schematic representation of the proposed DNA-based micellar catalytic system.

The driving force for insertion into the micelle would be the hydrophobic effect. Finally the chiral environment provided by the DNA would cause the reaction to proceed in an enantioselective fashion.

The sequences of the three oligonucleotides were selected based on previous observations:\textsuperscript{[39]} in the Cu\textsuperscript{II}-catalyzed Diels-Alder reaction, using System I (X = 3) (Scheme 4), an ee up to 93% was obtained after 3 days (Table 1, entry 2). The sequence of the oligonucleotides had been demonstrated to affect the ee significantly and the three bases closest to the copper complex were demonstrated to be critical for the ee observed (GTA and TAC in the template). Therefore, initially System I (X = 3) was selected. The reaction was analyzed
after one day: in the presence of SDS an increase in conversion was observed, accompanied by a significant drop in ee (entry 3). By functionalizing one of the oligonucleotide strands with a chain of twelve carbons and in the presence of SDS, a further decrease of enantioselectivity was observed but a similar conversion value was detected after 1 day (entry 4). Using a construct (System III) which differs from System I and II in the DNA sequence of the oligonucleotide strands (entry 5) both the ee and conversion were affected negatively by the presence of SDS.

Put together, these results showed the incompatibility of the three components (SDS, lipid chain functionalized oligo and oligo-bound catalyst).

**Scheme 4.** Catalytic systems used in this study.

<table>
<thead>
<tr>
<th>System</th>
<th>ON-A</th>
<th>ON-B</th>
<th>ON-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3'-GTTCCAGTCTGTACAG-(CH$_2$)$_X$</td>
<td>GTAATGCTTAAGCGAG-5'</td>
<td>5'-CAAGGTCAGACATGTCCATTACGAATTCGCTC -3'</td>
</tr>
<tr>
<td>II</td>
<td>3'-GTTCCAGTCTGTACAG-(CH$_2$)$_X$</td>
<td>CH$_3$-(CH$<em>2$)$</em>{11}$-O-3'-GTAATGCTTAAGCGAG-5'</td>
<td>5'-CAAGGTCAGACATGTCCATTACGAATTCGCTC -3'</td>
</tr>
<tr>
<td>III</td>
<td>3'-GACATGTGTCTGACCTTG-(CH$_2$)$_X$</td>
<td>CH$_3$-(CH$<em>2$)$</em>{11}$-O-3'-GAGCGAATTCGTAATG</td>
<td>5'-CTGTACACAGACTGGACCTGCCTAAGCATTAC -3'</td>
</tr>
</tbody>
</table>

The presence of SDS did provide a significant acceleration of the reaction but a drop in enantioselectivity and the presence of the lipid chain-modified oligonucleotide did not seem to provide further acceleration. Moreover, a further decrease in ee was observed. Probably the electrostatic repulsion between the sulfate groups of SDS and the negatively charged DNA, affected the catalysis negatively. Most likely, Cu$^{2+}$ ions bind preferentially to the head groups of the SDS instead of the ligand attached to the DNA strand, catalyzing the reaction in a racemic fashion.
Table 1. Cu\textsuperscript{II}-catalyzed Diels-Alder reaction in the presence of DNA-based micellar catalytic system I-III.\textsuperscript{[a]}

<table>
<thead>
<tr>
<th>entry</th>
<th>System</th>
<th>linker</th>
<th>conversion (%)\textsuperscript{[b]}</th>
<th>ee (%)\textsuperscript{[b]}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I \textsuperscript{[k]}</td>
<td>X = 6</td>
<td>65</td>
<td>89 (+)</td>
</tr>
<tr>
<td>2</td>
<td>I \textsuperscript{[k]}</td>
<td>X = 3</td>
<td>71</td>
<td>93 (+)</td>
</tr>
<tr>
<td>3</td>
<td>I</td>
<td>X = 3</td>
<td>90</td>
<td>69 (+)</td>
</tr>
<tr>
<td>4</td>
<td>II</td>
<td>X = 3</td>
<td>80</td>
<td>32 (+)</td>
</tr>
<tr>
<td>5</td>
<td>III</td>
<td>X = 3</td>
<td>42</td>
<td>21 (+)</td>
</tr>
</tbody>
</table>

\textsuperscript{[a]} All experiments were carried out with 0.13 mM oligonucleotides, MOPS buffer, 20 mM, pH = 6; [Cu(NO\textsubscript{3})\textsubscript{2}] = 0.1 mM; [1a] = 1 mM; [Cp] = 33 mM; [SDS] = 8 Mm, for 1 day at 5 °C, unless noted otherwise. \textsuperscript{[b]} ee and conversion values were determined by HPLC and are reproducible within ±5%. \textsuperscript{[c]} 3 days, no SDS.

6.2.2 New design: control over the activity of the DNA-based system by positioning of the catalyst at specific sites.

An interesting aspect of DNA-based surfactants is that they can interact with micellar aggregates formed by other surfactant molecules, via their hydrophobic moiety. Upon hybridization of the lipid chain-functionalized oligonucleotide with a complementary oligonucleotide strand functionalized with small molecule ligand (capable of binding metal ions) at the 3’ or 5’ terminus, it is possible to control the position of the catalyst with respect to the surface of the micellar aggregate and, consequently, its activity.

The aim of the present study was the creation of a DNA-based micellar system in which the catalysis of a metal catalyzed reaction could be tuned or, ideally, switched on/off, depending on the location of the catalyst with respect to the micellar surface (Figure 4). Ultimately, it was envisioned that different reactions could be catalyzed depending on the position of the catalyst within the micellar system.

The strategy towards the modular assembly of our catalytic system is outlined in Scheme 5.
It involved two oligonucleotide components: one that is functionalized with a 2,2’-bipyridine ligand (bpy) at the 5’ or 3’ terminal phosphate moieties (ON-1), and another that is functionalized with an alkyl chain of twelve carbons at its 5’ terminus (ON-2), which had a sequence complementary to ON-1. The hybridization in the presence of a metal salt (in our case copper (II) nitrate) gave rise to a DNA duplex where the catalytically active metal complex was positioned in proximity of the alkyl chain or at the other terminus, far away from it. By combining the oligonucleotide ON-2 with another surfactant molecule - in the present case, SDS - at the concentration where the latter starts aggregating, the catalytically active Cu\textsuperscript{II} complex can be positioned either at/near the micellar surface or further away from it. Based on its location, the active copper metal center is expected to catalyze more or less efficiently the reaction.

In case of the Cu\textsuperscript{II}-catalyzed Diels-Alder reaction between aza-chalcone (1\textit{a}) and cyclopentadiene (2), a higher reactivity was observed in the presence of micellar aggregates, which was explained by the high local concentration of the reaction partners at the Stern region (region at the interface between the non-polar interior of the surfactant and the aqueous exterior, which contains the head group and a variable fraction of counterions) where also the metal ion would be located.\textsuperscript{[18]}

Hence, one would expect that, by controlling of the distance of the Cu\textsuperscript{II}-catalyst respect with the surface of the aggregate, it would be possible to tune the catalytic activity. When the catalyst is pointing towards the micellar surface, it
is likely that the reaction would proceed faster compared to the opposite situation where the Cu-bpy complex is placed away from the micellar surface, pointing towards the aqueous phase. In principle, in the latter case, the limited access to the substrates would lead to a reduced reactivity.

**Scheme 5.** Assembly and design of the catalytic micellar system(s).

ON-1: 5'-GGCATTCTAAGCCCAT-O-(CH$_2$)$_x$-NH$_2$

ON-2: CH$_3$-(CH$_2$)$_{11}$-O-5'-ATGGGCTAGAATGCC-3'

ON-3 = ON-2 (no lipid chain attached)

ON-4 = ON1 (no ligand attached)

In the proposed design, the DNA would act as tool to position the catalyst: the average distance of the copper ion with respect to the surface of the SDS aggregate will be dependent on the length of the DNA strands. Ideally, this distance would be ~3.3 Å per base pair in case of a B-DNA structure; obviously, micelles are not ideal spheres but disordered dynamic aggregates, constantly disintegrating and reforming, which means that the average distance for DNA-catalyst moiety will deviate from this ideal value. Since a 16-mer was used for the assembly of the DNA-based micellar system, the distance of the
catalyst from the micellar surface in both cases (either the catalyst would be placed at 3’ or 5’ terminus of the oligonucleotide strand) would be sufficient to observe differences in reactivity.

6.3 Effect of the design of the DNA-based catalytic system on the rate of the CuII-catalyzed Diels-Alder and Friedel-Crafts reactions

A synthetic oligonucleotide (16-mer) with a defined sequence was functionalized, according to known procedures,[39] with a ligand (2,2’-bipyridine (bipy)) at the 5’ or 3’ terminal phosphate moieties (ON-1). The functionalized oligonucleotide ON-1 was obtained in 80% yield after purification by size exclusion chromatography (see Experimental Part) and it was characterized by Maldi-TOF mass spectrometry and reversed phase-HPLC (rp-HPLC). A second oligonucleotide, functionalized at the 5’ terminus with a hydrophobic chain of twelve carbons (ON-2), was commercialy available. Two catalytic systems were assembled which implied a different positioning of the catalyst with respect to the lipid tail of the oligonucleotide ON-2 and to the micellar surface. In one case the ligand (bpy) was attached at the 3’ terminus of the oligonucleotide ON-1, so that it would point towards the hydrophobic chain of the complementary oligonucleotide (that is towards the micellar surface). In the other design, the ligand was attached at the 5’ terminus of the oligonucleotide ON-1. In this case, in the presence of SDS and after hybridization with the oligonucleotide ON-2, the catalytically active metal complex would be protruding towards the aqueous phase.

As proof of principle, the CuII-catalyzed Diels-Alder reaction of aza-chalcone (1a) with cyclopentadiene (2) was selected as benchmark reaction (Scheme 6, a); the constructs were used on a scale of 0.04 micromol in the presence of a concentration of SDS above its cmc; the products were analyzed, after extraction, by normal phase-HPLC (np-HPLC; ODH 98:2); the retention times corresponded to those of the Diels-Alder adducts.[39] No enantioselectivity was observed, which was expected for a construct where the catalyst is placed at the terminus of the DNA helix. However, a small but encouraging difference in conversion between the two constructs was observed: for the system where the catalyst was located closer to the micellar surface, 76% conversion was
found; for the second design, in which the copper complex was placed at the other terminus of the oligonucleotide strand, further away from the micellar surface, 60% conversion was observed after the same time.

Scheme 6. Cu$^{II}$-catalyzed reactions used in this study.

a) 

\[
\text{ON-1/ON-2 Cu(NO}_3)_2 \text{ SDS H}_2\text{O} \rightarrow \begin{array}{c}
\text{1a} \\
\text{2} \\
\text{endo} \\
\text{exo}
\end{array}
\]

b) 

\[
\text{ON-5/ON-6 Cu(NO}_3)_2 \text{ SDS H}_2\text{O} \rightarrow \begin{array}{c}
\text{4f} \\
\text{7} \\
\text{8f}
\end{array}
\]

As next step, a kinetic study was performed to determine to which extent the different positioning of the catalyst with respect to the micellar surface was reflected in differences in reactivity.

The reactions were performed at 25 °C and monitored by UV/Vis spectroscopy, following the decrease of the enone substrate in time, as described previously (Chapter 2). Firstly, the reaction in the presence of the oligo ON-4 (without ligand covalently attached), the lipid chain-functionalized oligonucleotide ON-2, SDS and Cu(NO$_3$)$_2$ was analyzed: it was found that the reaction was two orders of magnitude faster (Table 2, entry 1) than the reaction in which SDS was not present (entry 2). Hence, the presence of the DNA-amphiphilic surfactant does not affect the acceleration expected for the Diels-Alder reaction in the presence of SDS. In the presence of ON-1 with ligand covalently attached, a significant difference of up to an order of magnitude between the two designs was found for the apparent second order rate constant ($k_{app}$) (Figure 5; Table 2, entries 3, 4-5, 6). This can be rationalized as hypothesized (vide supra): when the catalyst is placed further away with respect to the micellar surface (entries 4, 6), it is
likely that the reaction is not significantly affected by the presence of the SDS aggregate. In contrast, when the catalyst is pointing towards the surface of the micellar aggregate, the hydrophobicity of the environment would force eventually the substrates to stay in close contact, in a limited reaction volume (at the Stern region) so that they could be reached easily by the catalyst. As consequence, the reaction occurs faster (entries 3, 5). With a shorter linker between the bases and the ligand-bound catalyst (X = 3) (entries 5, 6) and with a slightly lower concentration of SDS (5 mM; entries 9, 10) the same trend was observed.

**Figure 5.** UV-Vis traces for the Cu$^{II}$-catalyzed Diels-Alder reaction in the presence of ON-1/ON-2/SDS.

The traces refer to the conversion to the product after the first 2 h of reaction. Completion is reached after longer reaction times (7 h for reaction a). **Line a** = 3’-coupled ON-1/ON-2/ SDS 8 mM; **line b** = 5’-coupled ON-1/ON-2/ SDS 8 mM.

The lack of the lipid chain in the oligonucleotide ON-3 (entry 7) led to a $k_{\text{app}}$ value that was similar to that measured for the construct in which the ligand-bound catalyst was placed further from the micellar surface (entries 4, 6).

The absence of SDS (entry 8) resulted in a value of the apparent rate constant similar to the situation where both the ON-2 and SDS were present (entries 3, 5). These data together suggest that in this system (coupled-ON1/ON2), the presence of SDS does not lead necessarily to acceleration of the reaction in itself (see entries 3, 5, 8) but it acts mainly as a handle for the positioning of the catalyst; the chain of the ON-2 is needed to allow for the interaction between
the DNA-components and the micellar aggregate formed by the SDS molecule; the catalysis of the reaction in the presence of all components is mediated by the ligand bound copper attached to the oligonucleotide and not by the Cu\(^{2+}\) ions exchanging with the Na\(^{+}\) ions at the surfactant head group shell (in this case usually a significant acceleration is detected (entry 1)).

The observed differences in reactivity are promising. By modifying the design, varying the length of the spacer ‘X’, the number of base pairs of the oligonucleotides, it may be possible to make these differences even larger.

**Table 2. Effect of the design of the DNA-based catalyst on the rate of the reaction of 1a with 2.**\(^{[a]}\)

<table>
<thead>
<tr>
<th>entry</th>
<th>oligonucleotide</th>
<th>[SDS] (mM)</th>
<th>linker</th>
<th>(k_{app} \times 10^5 \text{[M}^{-1} \cdot \text{s}^{-1}])</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ON-4/ON-2/Cu(^{2+})</td>
<td>8</td>
<td>C6</td>
<td>129(^{[c]})</td>
</tr>
<tr>
<td>2</td>
<td>ON-4/ON-2/Cu(^{2+})</td>
<td>-</td>
<td>C6</td>
<td>7.93(^{[c]})</td>
</tr>
<tr>
<td>3</td>
<td>3’-coupled ON-1/ON-2</td>
<td>8</td>
<td>C6</td>
<td>3.0 ± 0.45</td>
</tr>
<tr>
<td>4</td>
<td>5’-coupled ON-1/ON-2</td>
<td>8</td>
<td>C6</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>5</td>
<td>3’-coupled ON-1/ON-2</td>
<td>8</td>
<td>C3</td>
<td>6.8 ± 0.50</td>
</tr>
<tr>
<td>6</td>
<td>5’-coupled ON-1/ON-2</td>
<td>8</td>
<td>C3</td>
<td>0.77 ± 0.02</td>
</tr>
<tr>
<td>7</td>
<td>3’-coupled ON-1/ON-3</td>
<td>8</td>
<td>C6</td>
<td>0.59(^{[c]})</td>
</tr>
<tr>
<td>8</td>
<td>3’-coupled ON-1/ON-2</td>
<td>-</td>
<td>C6</td>
<td>5.55(^{[c]})</td>
</tr>
<tr>
<td>9</td>
<td>3’-coupled ON-1/ON-2</td>
<td>5</td>
<td>C6</td>
<td>4.20(^{[c]})</td>
</tr>
<tr>
<td>10</td>
<td>5’-coupled ON-1/ON-2</td>
<td>5</td>
<td>C6</td>
<td>0.38(^{[c]})</td>
</tr>
</tbody>
</table>

\(^{[a]}\) Conditions: MOPS buffer, 20 mM, pH = 6.5; [Cu\(^{2+}\)] = 0.01 mM; [1a] = 0.1 mM; [2] = 2 mM. \(^{[b]}\) The data refer to the first 15% of the reaction. \(^{[c]}\) Result from a single experiment.

Since a dramatic effect on the rate of the Cu\(^{II}\)-catalyzed Friedel-Crafts reaction in the presence of SDS micelles (Chapter 6) was observed,\(^{[37]}\) our DNA-based micellar
system was tested also in this transformation (Scheme 6, b). The procedure for assembling the catalytic system was similar to what previously described (*vide supra*). The 2-acyl-imidazole (4f) was chosen as a benchmark substrate and the 5-methoxy indole (7) as nucleophile since the most dramatic effect of rate acceleration was found for this combination substrate/nucleophile. Fixed concentrations in copper (0.015 mM), SDS (8 mM), substrate ((4f), 0.01 mM) and indole (1.2 mM) were used and the decrease in the substrate absorption was followed in time at 340 nm. The results are reported in Table 3.

**Table 3.** Effect of the design of the DNA-based catalyst on the rate of the reaction of 4f with 7.^[a]

![Reaction Scheme](image)

Sequence oligonucleotides:

ON-5: 5'‐TACCGAATCTTACGG

ON-6: CH₃(CH₂)₁₁-O-5'-CCGTAAGATCCGGTA

ON-7 = ON-6 (no lipid chain attached)

ON-8 = ON-5, linker = (CH₂)₆-NH-COO-CH₂CH₂

<table>
<thead>
<tr>
<th>entry</th>
<th>oligonucleotide</th>
<th>[SDS]/mM</th>
<th>linker</th>
<th>( k_{app}/[M^{-1} \cdot s^{-1}] \times 10^{-2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3'-coupled ON-5 / ON-6</td>
<td>8</td>
<td>C6</td>
<td>2.38 ± 0.26</td>
</tr>
<tr>
<td>2</td>
<td>5'-coupled ON-5 / ON-6</td>
<td>8</td>
<td>C6</td>
<td>1.65 ± 0.25</td>
</tr>
<tr>
<td>3</td>
<td>5'-coupled ON-5 / ON-8</td>
<td>8</td>
<td>C6</td>
<td>2.45 ± 0.02</td>
</tr>
<tr>
<td>4^[c]</td>
<td>3'-coupled ON-5 / ON-7</td>
<td>8</td>
<td>C6</td>
<td>0.066^[b]</td>
</tr>
<tr>
<td>5^[d]</td>
<td>5'-coupled ON-5 / ON-6</td>
<td>-</td>
<td>C6</td>
<td>24 ± 4.6</td>
</tr>
</tbody>
</table>

^[a] Conditions: MOPS buffer, 20 mM, pH = 6; [Cu²⁺] = 0.015 mM; [4f] = 0.01 mM, [7] = 1.2 mM; results correspond to the average of at least two experiments unless noted otherwise. [b] Result from a single experiment. [c] [7] = 7.2 mM. [d] [7] = 0.053 mM

Unfortunately, we did not observe significant differences in the \( k_{app} \) value depending on the location of the catalyst with respect to the micellar surface and the reasons for this different behaviour compared to the Diels-Alder are not understood at the moment.
To confirm that the insertion of the functionalized oligonucleotide ON-2 into the micellar aggregate formed by SDS could occur, we determined the critical micellar concentration (cmc) of the DNA-amphiphilic molecule using fluorescence spectroscopy and, as fluorescent probe, Nile Red (NR, Figure 6).[^41]

**Figure 6.** Nile red fluorescent probe.

![Nile Red Fluorescent Probe](image)

Nile Red has been found to exhibit a really sensitive response to changes in the microenvironment in the surfactant aggregates.[^43] Therefore, it can be used to detect the micelle formation of surfactants in aqueous solution.[^41,42] This hydrophobic probe contains both electron donor (dialkylamino) and acceptor (carbonyl oxygen) groups within its molecule. While in its ground state it has low polarity, in the excited state the molecule undergoes an intramolecular transfer of an electron from the donor to the acceptor group, which is accompanied by a twist between the donor and the acceptor moieties in two perpendicular parts. This twisted intramolecular charge transfer (TICT) process gives to the excited state a highly polar character with a large dipole stabilized in more polar solvents. With increasing solvent polarity, the probe shows a large bathochromic absorbance shift; this leads to a sensitive response to the change of the microenvironment, making it possible to excite the probe molecules selectively in different environments (excitation-dependent emission maximum $\lambda_{\text{max}}$) by using different excitation wavelengths.

In micelles, Nile Red is usually situated at the interface; some molecules are facing the water, whereas others are located more towards the hydrophobic tails of the amphiphiles. If there is no aggregation of the surfactant molecules, for example when the concentrations of surfactant molecule are below the critical micelle concentration (CMC) or in a solvent in which surfactant molecules are randomly mixed,[^43] no excitation-dependent emission is found.

The value of cmc measured for the oligonucleotide ON-2 (1.65 mM) was close to that obtained by measuring the surface tension of the surfactant solution (4.66
mM). Considering that the value of cmc measured for SDS in an aqueous solution is in the mM range (8.3 mM),\textsuperscript{44} an interaction between the aggregate formed by SDS at a concentration higher than its cmc and the oligo-ON-2, present as a monomer in solution, that is, at a concentration lower than its cmc, would be likely. Moreover, to confirm the interaction between the two components (SDS and ON-2), the excitation-dependent fluorescence of Nile Red probe was measured in the presence of both SDS (at a fixed concentration of 8 mM, under reaction conditions) and increasing concentration of oligonucleotide ON-2 (0.1 - 3.5 mM) (Figure 7). In the graph it is shown that an excitation dependent behavior exists.

Increasing the concentrations (0.1 - 3.5 mM) of the ON-2 to the SDS solution causes a progressive change in the overall polarity of the environment: an increase in polarity (increase in NR $\lambda_{\text{max}}$) was observed at 520 nm; at these high excitation wavelengths NR molecules located in proximity of the aqueous shell are selectively excited. Upon addition of the oligo ON-2, at 490 nm (which refers mainly to the molecule located in the hydrophobic core), changes in NR $\lambda_{\text{max}}$ were observed which are not significant.

**Figure 7.** Excitation dependent behaviour of the fluorescent probe Nile Red in the presence of SDS-ON-2.

The slight increase in the $\lambda_{\text{max}}$ is probably an indication that penetration of water into the hydrophobic core occurs, thus increasing the polarity of the environment.
The changes in the polarity of the environment are more pronounced at the surface of the aggregate (NR molecules excited at 550 nm) due to the larger hydrophilic moiety represented by the DNA strands compared to the hydrophobic alkyl chain. Since an excitation-dependence behavior of the NR molecules within the aggregate was found, it could be concluded that the two amphiphilic moieties interact.

6.4 Conclusions

In this chapter efforts to develop a new DNA-based catalytic system are shown. Early attempts were directed to combine the high enantioselectivity achieved with DNA-based asymmetric catalysis with the rate acceleration due to the presence of micellar aggregates. Secondly, the self-assembly of a micellar catalytic system where DNA acted as tool to position the catalyst differently with respect to the micellar surface and thus to tune the reactivity, was presented. The differences in rate detected by positioning of the catalyst in proximity of the micellar surface or further away from it, are small for the Cu$^{II}$-catalyzed Friedel-Crafts reaction but significant for the Diels-Alder reaction. However, further optimization of the system is required in order to achieve the ultimate goal which is the construction of a system where two different catalysts can be anchored to the micelle (one on the surface and the other in the aqueous phase), to allow for different reactions, chemically incompatible, proceeding simultaneously.
6.5 Experimental Section

General remarks:

All the substrates were synthesized following published procedures;\textsuperscript{[45]} 5-methoxyindole (7), SDS and Cu(NO$_3$)$_2$ were purchased from Sigma-Aldrich and used without further purification. Cyclopentadiene (2) was freshly distilled from its dimer prior to use. Nile Red was obtained from ACROS. Synthetic oligonucleotides were obtained from BioTez GmbH (Berlin, Germany). Enantiomeric excess determination was performed by HPLC analysis using UV detection (Daicel-Chiralcel ODH 98:2 n-heptane/i-PrOH, flow: 0.5 mL/min). Reversed phase-HPLC (rp-HPLC) analysis were performed on a Shimadzu LC-10AD VP, Waters Xterra MS C18 column (3.0 x 150 mm, particle size 3.5 μm) using a gradient of CH$_3$CN/TEAA buffer 50 mM, pH = 7; gradient: 05/95 0 to 10 min, to 35/65 at 60 min, to 70/30 at 65 min, flow: 0.5 mL/min. MALDI TOF mass spectrometry: the measurements were done on a Voyager-DE Pro apparatus. (Matrix: 20 μL of a solution of 2,4,6-Trihydroxyacetophenone 0.5 M in ethanol + 10 μL of a solution of ammonium citrate dibasic 0.1 M in Milli Q water + 2 μL sample solution in Milli Q water).

Physical methods, general remarks:

The UV/Vis absorption spectra were measured on JASCO V-560 and JASCO V-570 spectrophotometers. Nile Red fluorescence was measured on a JASCO FP-6200 spectrofluorimeter at 20 °C using an excitation wavelength between 490 and 550 nm. Emission spectra were measured from 550 to 700 nm at 5 nm band width and 1 nm data interval. A 1 nm data interval for the emission was found to be sufficient to obtain a resolution of several tenths of a nanometre after a log-normal fit. The values of λ$_{\text{max}}$ emission were calculated with SigmaPlot using a 4-parameters log-normal fit and those were plotted against the excitation wavelength.\textsuperscript{[46]} The cmc was determined using a drop-volume tensiometer device (LAUDA-TV51) or fluorescent probe technique (Nile Red). In this case a 2.5 mM 9-diethylamino-5H-benzo[a]phenoxazine-5-one (C$_{20}$H$_{18}$N$_2$O$_2$) (Nile Red) stock solution was made in ethanol and diluted 2000-fold in the surfactant systems. The concentration of surfactant ON-2 was plotted against the corresponding fluorescent intensity obtained at a excitation wavelength (λ$_{\text{exc}}$) of 550 nm.
Kinetic measurements:

All kinetic measurements were performed using UV/Vis absorption spectroscopy (JASCO V-560 or JASCO V-570 spectrophotometers) monitoring the decrease of the absorption of the dienophile at 25 °C (at 326 and 340 nm). The kinetic analysis for the Diels-Alder reaction was conducted as described in Chapter 2; the decrease of the absorption was followed for the first 15% of substrate conversion. For the Friedel-Crafts reaction, the \( k_{\text{app}} \) was determined by initial-rate kinetics with the following expression:

\[
k_{\text{app}} = \frac{d[A4f]}{dt} \cdot \frac{1}{d(A\epsilon) \cdot [4f]_0}
\]

in which \( d(A4)/dt \) is the slope of the decrease of absorption in time, \( d \) the path length of the cuvette (1 cm) and \( A\epsilon \) the difference in molar absorptivities of the substrate and the product (determined separately) and \([4f]_0\) is the initial substrate concentration. Apparent second-order rate constants were then deduced by dividing by the concentration of nucleophile.

Synthesis of bipyridine-DNA conjugates, representative procedure:

250 μL of a stock solution of amino modified-oligonucleotide (200 μM in H₂O) was mixed with 200 μl of phosphate buffer (200 mM, pH = 7.2) and 50 μL of dimethylformamide. To this solution, 100 μL of a stock solution of \( N \)-hydroxy-succinimide 2,2'-bipyridine-5-carboxylate (20 mg/mL in dimethylformamide) was added in fractions of 25 μL over a period of 4 h with continuous shaking. The mixture was shaken overnight and the coupled product was purified by size exclusion chromatography (SephadexTM G-25 DNA Grade, Triethylammonium acetate 50 mM, pH = 7). Yields were detected by UV-Vis and they were typically around 80%. The products were analyzed by reversed phase-HPLC (rp-HPLC) and MALDI-TOF mass spectrometry. After freeze drying, the sample was redissolved in a minimum amount of buffer (MOPS 20 mM, pH = 6.5) and warmed up to 60 °C while shaking. The concentration was calculated using the Nanodrop ND-1000 from Thermo Scientific or by UV using the following formula to calculate the extinction coefficient:

\[
\epsilon = A \cdot (15.2) + C \cdot (7.05) + G \cdot (12.01) + T \cdot (8.4)
\]
Diels-Alder reaction on 0.04 μmol scale:

0.04 μmol of the bpy-conjugate (ON-1) and of the lipid-modified oligonucleotide (ON-2), dissolved in MOPS buffer, were mixed. After freeze drying, the sample was redissolved in 242 µL of a 0.12 mM solution of Cu(NO₃)₂ × 3H₂O in MOPS buffer (20mM, pH = 6.5); the solution was warmed to 94 °C for 15 min and then cooled down slowly to 0 °C. Prior to use the solution was kept at 5 °C for 1.5 h. Next, 48 µL of a 50 mM SDS solution in MOPS buffer (final SDS concentration 8 mM) were added and the solution was warmed up to 25 °C for 15 min. To the catalyst solution was added 3 µL of a stock solution 100 mM of aza-chalcone (1a) in acetonitrile to a final concentration of 1 mM. The reaction was started by the addition of 0.8 µL (9.8 μmol) of cyclopentadiene (2). After one day, the sample was diluted with ethyl acetate (3 × 1 mL), filtered over Na₂SO₄ and after removal of the solvent the product was analyzed by HPLC (Daicel chiralcel-ODH column, n- heptane/i-PrOH 98:2, flow: 0.5 mL/min). Conversion were calculated using the formula:

\[ \text{conv.} \%(\%) = \frac{\text{area } P}{\text{area } S + c \times \text{area } P} \times 100 \]

where area P is the total peak area of the product of the reaction, area S is the peak area of the starting material and c is a correction factor of 1.21, which was determined using a calibration curve.

Preparation samples for kinetic experiments:

**Diels-Alder reaction:** 0.04 μmol of the bpy-conjugate (ON-1) and of the lipid chain-modified oligonucleotide (ON-2), dissolved in MOPS buffer (ratio 1:1), and 60 µl of a stock solution of 50 mM SDS in MOPS buffer (20 mM, pH = 6.5) were mixed. After freeze drying, the sample was redissolved in 300 µL of a 0.1 mM solution of Cu(NO₃)₂ × 3H₂O in MOPS buffer (20 mM, pH = 6.5); the solution was warmed to 94 °C for 15 min and then cooled down slowly to 0 °C. Prior to use the solution was kept at 5 °C for 1.5 h. The solution was equilibrated at 25 °C for 15 min 2 μL of a solution of aza-chalcone (1a) in acetonitrile (4.78 mM, 0.03 mM final concentration) and 1 μL of a stock solution of freshly distilled cyclopentadiene (2) in acetonitrile (2 mM final concentration) were added. The decrease in absorption of aza-chalcone (1a) was followed in time at 326 nm.
Friedel-Crafts reaction: 0.066 μmol of the bpy-conjugate and of the lipid chain-modified oligonucleotide, dissolved in MOPS buffer, were mixed. After freeze drying, the sample was redissolved in 100 μL of a 0.075 mM solution of Cu(NO₃)₂ × 3H₂O in MOPS buffer (20 mM, pH = 6.5) to a final concentration of 0.015 mM; the solution was warmed to 94 °C for 15 min and then cooled down slowly to 0 °C. 400 μL of a SDS solution in MOPS buffer (3 - 8 mM final concentration) were added and the solution was stabilized at 25 °C for 15 min. To the catalyst solution was added 2 μL of a stock solution (3.76 mM) of acyl-imidazole (4f) in DMSO (0.01 mM final conc). The reaction was started by the addition of 3 μL of a stock solution of 5-methoxy indole (7) (final concentration 1.2 mM). The decrease in absorption of substrate was followed in time at 340 nm.

6.6 References


DNA-based micellar catalytic system


