Chapter 1
An introduction to DNA-based asymmetric catalysis

An emerging field in catalysis is the use of hybrid biomolecules for asymmetric catalysis. A variety of transition-metal catalyzed reactions can proceed in an enantioselective manner due to the presence of proteins. But also polynucleotides such as RNA and DNA can be harnessed for chiral recognition and asymmetric catalysis. This chapter aims to provide a literature survey on these concepts which form the foundation of DNA-based asymmetric catalysis.
1.1 Asymmetric catalysis and water

1.1.1 Asymmetry in catalysis
Molecules can have different three-dimensional structures even though they have the same combinations of bonds between the atoms. If these molecules are non-superimposable mirror images, they are considered to be chiral, and are called enantiomers. In 2007, 70% of the new small-molecule approved drugs contained at least one stereogenic center. The isolation of a single enantiomer is highly desirable, because the function of the drug in biological systems is related to its chiral configuration. This has resulted in the development of several methodologies for the preparation of enantiopure drugs. The three main strategies to obtain enantiopure compounds are i) resolution techniques, i.e. separating enantiomers by changing their physical properties upon interaction with other chiral molecules, ii) synthesis using enantiopure starting materials available from nature’s chiral pool, and iii) asymmetric synthesis and catalysis. Resolution techniques are widely used, but are costly and often a bottleneck in multistep synthesis due to the limiting yield of 50%, which in turn can be overcome by recycling the unwanted enantiomer. In asymmetric synthesis, a prochiral substrate is transformed into a single enantiomer by inducing a relative change in Gibbs energy between the two activated complexes by the formation of diastereomeric transition-state structures. In order to obtain an enantiomerically enriched compound, either a chiral auxiliary can be attached to the substrate, or a chiral catalyst can be employed. Since in a catalytic event the catalyst by definition lowers the Gibbs energy of the activated complex, the latter is an ideal tool to create diastereomeric transition states.

1.1.2 Asymmetric catalysis in water
The asymmetric catalysts can roughly be divided in two groups; enzymes and small molecule catalysts, both having advantages and disadvantages. Enzymes generally function as highly enantioselective catalysts for their natural substrates, and are generally applied in water. Most enzymes however, with some notable exceptions such as lipases, display a high substrate selectivity, which limits the scope of the substrates. This can be overcome by engineering an enzyme, by means of mutagenesis techniques, or post-synthetic modification. Most enzymes require mild conditions, although engineered and wild-type enzymes have been used at higher reaction temperatures (70-110 °C), in organic solvents, and at non-physiological pH. Wild-type enzymes are used for the transformation of their natural substrate on industrial scale. For example, fumarase catalyzes the asymmetric hydration of fumarate to provide maleate. This process is performed with 400 g/L concentrations for the synthesis of maleate.

The small molecule catalysts have the advantage over enzymes that they are generally easier to modify, have larger substrate scopes, and perform chemistry that is not available for enzymes.
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with enzymes. Although small molecule catalysts can have high catalytic activity, they are generally still outperformed by enzymes. However, the possibility to catalyze a wide variety of reactions, renders small molecule catalysts very important for applications of asymmetric catalysis in industry, and on smaller scale in total synthesis. The solvents of choice for small molecule catalysis have been organic solvents, due to the complications associated with water as solvent, i.e. the insolubility of many small molecule catalysts in water, the deactivation of several catalysts by water, loss of non-bonding interactions in water, and the often low substrate solubility in water. On the other hand, the use of water as a solvent has been subject to numerous research efforts, since water is arguably the most non-toxic and cheapest solvent. Indeed, several examples of small molecule catalysts to accomplish asymmetric catalysis in water as a solvent have been described. The often low solubility of small molecule catalysts in water is overcome by using micellar media, attaching solubilizing ionic groups to the catalyst, or attaching the catalyst to a solid support. Examples of these strategies are the rhodium catalyzed hydrogenations, the rhodium catalyzed hydrogen transfer reductions, Sharpless dihydroxylations, and the proline-catalyzed C–C bond forming reactions. In these examples, the reaction occurs “in water”, but the role of the water in the catalytic event is not always clear: does the catalysis occur “in water”, “on water”, or “with water”? This is often not known because concentrations of reactants are used that exceed the solubility limits in water.

In some cases, water has a beneficial effect on the catalytic event. For example in the case of concerted reactions such as the Diels-Alder reaction and the Claisen rearrangement. The rate increase has been ascribed to both hydrophobic effects and hydrogen bonding of water with the activated complex. The first report of a chiral Lewis-acid catalyst that was able to catalyze an asymmetric transformation in water was a copper(II)-amino acid catalyst (Scheme 1).

![Scheme 1: Asymmetric catalysis in water using a small molecule catalyst.](image-url)
The copper(II) ion was shown to act as a Lewis acid, and catalysed the Diels-Alder reaction between aza-chalcone 1 and cyclopentadiene 2. Here, the water did not only increase the rate of Diels-Alder reactions, but also increased the enantioselectivity. It was postulated that π-stacking interactions of the aromatic side group of the α-amino acid were enhanced due to hydrophobic effects. The π-stacking of the arene on the dienophile or copper(II) is important for the enantioselectivity since it provides shielding of one face of the dienophile.

1.2 Asymmetric catalysis with biohybrid catalysts

A catalyst that can combine the attractive properties of biocatalysis and chemocatalysis would be highly desirable. An emerging approach is to modify a protein post-translationally with a catalytically active metal complex, resulting in a hybrid or artificial metalloenzyme. In this way, the scope and promiscuity are adopted from the transition metal catalyst, and the high enantioselectivity from the protein. These features can be optimized separately by chemical and molecular biology techniques. Variations in the primary coordination sphere provided by the metal complex have the most significant effect on the enantioselectivity, while the fine tuning of the enantioselectivity is governed by the modification of the secondary coordination sphere provided by the protein. These two parameters combined should in principle give an additional advantage over the two conventional approaches. The enantioselectivity does not need to originate from the metal complex, since the protein acts as a chiral scaffold also. This excludes the need of an enantiopure metal complex, and prevents the formation of different diastereomers upon combination of the metal complex with the protein. The application of the biohybrid catalysts in asymmetric catalysis was pioneered by Wilson & Whitesides, who showed that a protein can act as a source of enantioselectivity when a biotin-containing rhodium-phosphine catalyst was anchored into avidin. An asymmetric hydrogenation was performed with a modest selectivity of 41% ee (Scheme 2).

![Scheme 2: Asymmetric hydrogenation using a biohybrid catalyst by Wilson & Whitesides.](image)
Later, Ward and co-workers improved this design and obtained selectivities up to >95% ee, using a combination of chemical and genetic engineering. A critical choice in the design of a biohybrid catalyst is the mode of attachment of the catalyst to the protein, which can be in a covalent, dative, or non-covalent fashion.

### 1.2.1 Covalent anchoring strategies
The catalyst can be bound to the protein via a covalent bond, in which the metal-binding ligand contains a reactive moiety that binds selectively to an α-amino acid such as cysteine. The advantages of a covalent mode of attachment are i) precise knowledge of the location of the metal complex, ii) access to different sites on the protein, and iii) no dependence on the binding affinity of the complex to the protein. An important drawback of this design is that the optimization is time-consuming due to the necessity of extra synthesis and purification steps. This could be a reason why examples of successful asymmetric catalysis with a covalently attached catalyst are less prevalent in the literature compared to the dative and the non-covalent approaches. The most notable example using this strategy is an asymmetric sulfoxidation reaction catalyzed by a manganese salen complex anchored to myoglobin with moderate ee’s up to 51%. The covalent approach has among others also been employed for rhodium-catalyzed hydrogenations using papain as host, which induced rate accelerations or enantioselectivities up to 65%.

### 1.2.2 Dative anchoring strategies
In the dative anchoring strategy, the catalytically-active metal is bound to the protein by coordination of one of the α-amino acid side chains, e.g. a cysteine or a histidine. Several examples exist in which high ee is generated. A prominent example is the combination of bovine serum albumin (BSA) and copper(II) phthalocyanine, which was inspired by the earlier work by Otto & Engberts (vide supra) and the first generation DNA-based catalysts (vide infra). The copper(II)phthalocyanine is most likely anchored to the BSA via a dative bond, originating from a tyrosine (Tyr161) that binds to the axial coordination site on the copper(II). Ee’s up to 98% were obtained in the Diels-Alder reaction between aza-chalcone and cyclopentadiene (Scheme 3). Surprisingly, the use of a tetradentate chelating ligand such as phthalocyanine, which leaves only the axial coordination site on the copper available, still renders the copper ion active in catalysis. The authors propose that the aza-chalcone was activated via the coordination of the ketone to the free axial coordination site on copper. The issue that a low pH of 4 is beneficial for the ee and conversion, is very important because the Diels-Alder reaction can also be Brønsted-acid catalyzed, however, the authors do not comment on this issue. Another important observation is that the rate of the catalysis by copper(II) phthalocyanine decreased due to the presence of the BSA. This means that the copper(II) complex has to be bound tightly since any unbound copper(II) complex will have a strong negative effect on the ee.
Scheme 3: A copper(II)phthalocyanine/BSA conjugate for catalytic asymmetric Diels-Alder reactions.

1.2.3 Non-covalent anchoring strategies

The non-covalent, or supramolecular, approach is based on the binding of a transition metal complex to a protein via hydrophobic interactions, hydrogen bonding, or electrostatic interactions. Most successful examples of asymmetric catalysis using a hybrid enzyme were reported following this approach. The main advantage of the non-covalent approach is the modular assembly of the catalyst, which makes the optimization process less time-consuming. Different transition-metal complexes can be tested, and the protein can be modified using mutagenesis. The non-covalent approach based on the biotin-avidin technology was first reported by Wilson and Widesides, and further developed by Ward. The biotin-streptavidin approach induced high ee’s in hydrogenations, ketone reductions, alcohol oxidations, and allylic alkylations. The ruthenium catalyzed ketone reduction has been optimized successfully using both chemical and genetic optimization (Scheme 4). The chemical optimization of the ruthenium complex, i.e. the primary coordination sphere, affected the ee and the conversion most. This was exemplified by the sensitivity of the catalysis to the substitution pattern of the aryl group in the linker, since only the para substitution resulted in a catalytically active complex. Also, the ee and the enantiopreference, i.e. which enantiomer is obtained in excess, were influenced to a large extend by the nature of the aryl group in the piano-stool complexes. When, for example, benzene was applied as capping arene instead of cymene, the other enantiomer of the
product was obtained. The ee in the reduction of acetophenone 6 ranged between 56% of the S-enantiomer and 57% of the R-enantiomer, when employing wild-type streptavidin.

Scheme 4: A ruthenium-piano stool complex non-covalently attached to streptavidin via biotin for enantioselective ketone reductions. S112Y Sav is a mutant streptavidin with serine S112 substituted for tyrosine.

Optimization of the secondary coordination sphere, i.e. the protein, is less straightforward, since the α-amino acids which comprise the active site need to be identified. From docking studies it was found that serine S112 was placed close to ruthenium catalyst. Hence, position 112 was subjected to saturation mutagenesis giving 20 mutants of streptavidin. The mutants gave rise to changes in the enantiomeric excess as well as the enantioselectivity, and a relation was found between the charge or the hydrophobicity of the α-amino acid side group and the enantioselectivity. Using saturation mutagenesis at position 112, several mutants were identified that gave rise to an increased ee for a variety of aromatic ketones. In the case of acetophenone 6, the mutant containing a tyrosine at position 112 increased the ee to 90%. For more challenging substrates, for example dialkyl ketones, a more elaborate modification of the protein was needed. With the availability of the X-ray structure of the mutant streptavidin in combination with a ruthenium complex, two new locations that were in close contact with the catalyst were identified, i.e. K121 and L124. Saturation mutagenesis of these two positions gave 114 mutants, which were tested in the reduction of dialkyl ketones. Also in this case, the primary coordination sphere had the largest effect on the ee, but by optimization of the second coordination sphere the ee could be increased, as is illustrated by an increase from 30% to 90% ee for a dialkyl ketone.
1.3 RNA and DNA enzymes

1.3.1 RNazymes
The use of biopolymers in catalysis is not restricted to proteins. Cech and Altman discovered the catalytic properties of RNA, for which they were awarded the Nobel Prize in chemistry in 1989. In Nature, RNA catalyzes reactions as part of the ribosome in the synthesis of proteins, and RNA cleaves phosphodiester bonds of other RNAs. Artificial RNazymes have been isolated from random pools of RNAs, via rational and iterative methods such as in vitro selection procedures, e.g. SELEX-type methodologies. These RNazymes have been subject to continuous simplification and modification. Artificial and natural RNazymes often form a tertiary structure called a hammerhead, which consists of three double stranded regions, and a pocket in the middle containing unpaired bases. Many reactions have been catalyzed by artificial RNazymes, including C-C bond forming reactions such as the Diels-Alder reaction and the aldol reaction. Using an RNAzyme, a Diels-Alder reaction between anthracene and maleimide was performed in an asymmetric fashion, with an ee of 90% in the corresponding Diels-Alder product (Scheme 5).

![Scheme 5: Ribozyme catalyzed enantioselective Diels-Alder reaction. Crystal structure reproduced with permission from the Nature Publishing Group.](image)

Crystal structure reproduced with permission from the Nature Publishing Group.
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In this reaction, the mechanism for both the induction of the ee and the catalytic activity relies on the presence of a preformed catalytic pocket in the central part of the RNAzyme. Upon reaction with maleimide, the anthracene undergoes a large structural change from a planar to a bent structure. The preformed catalytic pocket complements the bent shape of the activated complex of the Diels-Alder reaction, resulting in an increase in the rate of the reaction and an enantioselective cycloaddition.

1.3.2 DNAzymes

In contrast to RNAzymes, natural occurring DNAzymes have not been found to date. The absence of DNAzymes in Nature is maybe not surprising since DNA is the most important carrier of genetic information. Artificial DNAzymes, on the other hand, have become more common recent years. The examples of catalytic DNAs are highly related to the RNAzymes, as the design is based on similar tertiary structures. DNA can also form a variety of different topologies such as loops, junctions, etc., and these tertiary structures can be programmed by means of the DNA sequence. Other similarities between RNAzymes and DNAzymes is that i) the stability of these tertiary structures is often increased by the presence of divalent metal ions such as magnesium(II), and ii) the possibility to use in vitro selection procedures of random pools of oligonucleotides. The first example of a DNAzyme was capable of cleaving RNA. Several other types of reactions have been catalyzed using catalytic DNA, of which the majority is nucleotide chemistry. Recently, a Diels-Alder reaction was catalyzed by a DNAzyme. The RNAzyme capable of catalyzing the Diels-Alder reaction between anthracene and maleimide was taken as a starting point for the optimization of the DNAzyme, resulting in the catalysis of the same Diels-Alder reaction as efficiently as the RNAzyme. Previously, DNAzymes were considered to be an inferior catalyst compared to RNAzymes due to absence of the 2'-OH. The Diels-Alder case proves that DNA can be as good as a catalyst as RNA. To date, the ee of the Diels-Alder product was not reported.

1.4 Applications of DNA in chiral recognition and catalysis

DNA has found many applications in vitro, in addition to its role as the principle carrier of genetic information in Nature, and its recent use as a DNAzyme (vide supra). Due to the programmability of the hybridization event and its structural robustness, many of its applications are found in nanotechnology. The applications that are relevant to the research described in this thesis are the enantiomeric selection of chiral molecules, and the attempts to modify DNA with small molecule catalysts. It must be noted that the majority of the latter applications were published after our initial reports of DNA-based asymmetric catalysis as described in this thesis.
1.4.1 Chiral recognition of molecules containing stereogenic carbons

The chirality of DNA’s right handed helix can be exploited to distinguish between enantiomers of DNA-binding molecules. In fact, a common observation is an increased binding affinity for one of both enantiomers, or diastereomers, to the DNA.\textsuperscript{31} For example, daunarubicin intercalates preferentially in B-DNA, but when the opposite enantiomer of daunarubicin was employed, a higher affinity for Z-DNA, i.e. DNA with a left handed helical conformation, was observed.\textsuperscript{58} Also, oxidized polycyclic aromatic hydrocarbons (PAH) interact with DNA via a reaction of the DNA with the epoxide ring.\textsuperscript{29} The epoxide ring reacts with an exocyclic amino group of a nucleobase leading to a covalent adduct of which the chirality of its ligand is important for its toxicity.\textsuperscript{60}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{scheme6.png}
\caption{Structures of daunarubicin, an oxidized polycyclic aromatic hydrocarbon, and oxaliplatin.}
\end{figure}

1.4.2 Chiral recognition of octahedral metal complexes

The majority of research on the recognition of enantiomers by DNA has been performed on the chiral octahedral metal complexes. When combined with bi- or tridentate ligands, these complexes can form right (\(\Delta\)) or left handed (\(\Lambda\)) propellor-like shapes (Scheme 7). Nordén and Tjerneld showed that the addition of the conformationally labile iron(II) tris(bipyridyl) to DNA resulted in a preferred binding of the \(\Delta\)-enantiomer, while the complex remaining in the bulk solution racemized.\textsuperscript{61} Stable chiral octahedral complexes of ruthenium, rhodium, or cobalt have been studied extensively.\textsuperscript{52} The group of Barton has developed several applications for these complexes, such as DNA-light switch,\textsuperscript{63} and DNA cleavage agents.\textsuperscript{64} All these applications rely on which enantiomer of the complex is applied, because the symmetry of the complex matches that of the double helix. Moreover, small changes in the ligands changed the symmetry of the complex, resulting in binding to different sites in the DNA. Hence, the dependence on the chirality of the binding affinity of a molecule to DNA is a common observation, and is in some cases critical.
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1.5 Applications of DNA in catalysis

DNA is a very suitable chiral scaffold in synthesis, since it is chemically stable, commercially available in large quantities, and the costs are in the range of small molecule catalysts. The chiral features of DNA are predictable to a certain extent: the relatively stable right handed helix of B-DNA is the predominant conformation, while variations in the conformation of the helix can be expected when a variety of parameters are changed, such as the DNA sequence, the extent of hydration of the grooves, the ionic strength of the solvent, and the presence of DNA-binding molecules. Moreover, many examples exist in which DNA is bound by molecules in an enantioselective fashion (vide supra). DNA has several other advantages, such as the possibility to design other topologies, the programmability of the hybridization by its sequence, and the possibility to modify it covalently or non-covalently with a catalytically active molecule.

It is important to distinguish between the different forms of DNA catalysis, which accelerate reactions based on different principles. The different classes are DNAzymes (vide supra), DNA-templated synthesis, DNA-based catalysis, DNA-directed catalysis, and finally DNA-based asymmetric catalysis. These approaches will be discussed in more detail in the remainder of this chapter.

1.5.1 DNA-templated synthesis

Many examples exist in which the rate of the reaction is increased by increasing the effective molarity of the reactants, making use of the hybridization of the DNA to which they are attached, i.e. DNA-templated synthesis. But the reactants do not necessarily be attached covalently to DNA: Dervan showed that in the case of the dimerization of two DNA binding molecules the rate was enhanced by specific sequences of DNA, sequences that brought the two reagents into close contact. The difference between DNA catalyzed reactions and DNA-templated syntheses can be rather subtle, because in both cases the rate...

Scheme 7: Structures of the octahedral complexes iron(II) (bpy)_2, and the Δ-enantiomer of ruthenium(II) (phi)(bpy)_2.
of the reaction is increased. Hence, the claim of catalytic DNA deserves a thorough investigation. For example, the group of Wang and Li showed that under heterogeneous conditions (20 mg/ml DNA) the presence of unmodified natural DNA had a favourable effect on the conversions observed in an Aldol and a Henry reaction.\textsuperscript{70,71} These reactions occur most likely due to an increase in the effective concentration of the reactants.

The first examples in which template-directed synthesis displayed stereoselectivity dealt with the polymerization of nucleotides that hybridized on a nucleotide strand, such as DNA.\textsuperscript{72} The growth of the polymer was faster when the same enantiomer of the nucleotides was used as the template strand, whereas the growth was inhibited when the opposite enantiomer was employed. In another example, DNAzymes were found to cleave RNA upon hybridization of the RNA strand with enantiopreference for D-ribonucleotides.\textsuperscript{73}

Liu et al. showed that in DNA-templated synthesis one enantiomer was preferred over the other (Scheme 8).\textsuperscript{74} The $S\textsubscript{N}2$ displacement of an $S$-bromide by a thiol nucleophile proceeded 4 times faster compared to the reaction with an $R$-bromide. Moreover, when the reaction was performed with Z-DNA, the selectivity reversed, and the rate towards the $R$-bromide was 2 times higher compared to the $S$-bromide. Hence the conformation of the DNA can be used to control the diastereoselectivity.

\begin{center}
\includegraphics{scheme8.png}
\end{center}

\textbf{Scheme 8:} Enantioselective displacement of an $S$-bromide with DNA-templated synthesis.

1.5.2 DNA-directed catalysis

In DNA-directed catalysis the DNA is used to increase the effective concentration of the catalyst and the reactant, and is thus necessary for the catalysis. This strategy has mainly been employed for site specific cuts in a complementary DNA strand using a localized catalytically active metal complex.\textsuperscript{75-77} An example is the approach followed by Sheppard et al., in which a nickel(II) salen complex is incorporated into a single strand of DNA.\textsuperscript{76} Upon combination with a complementary strand, the complementary strand is cleaved by the nickel(II) salen complex.

Also C-C bond forming reactions have been performed using DNA-directed catalysis, using proline as a catalyst attached covalently to the 5'-end of DNA (Scheme 9).\textsuperscript{78} An aldehyde was attached to the 3'-end of the complementary strand, bringing it in close contact with
the proline via hybridization, which effectively increases of the concentration of the aldehyde. Combining proline with a ketone such as acetone gave the enamine ion, which reacted with the aldehyde furnishing the corresponding aldol product is obtained. By heating the duplex above its melting temperature, and subsequently cooling the mixture, the proline modified strand recombined with a strand containing an unreacted aldehyde, and catalytic turnover was achieved. The same catalytic aldol reaction was also feasible using another design, in which the aldehyde was attached to a porphyrin.\textsuperscript{79} Porphyrins stabilize the G-quadruplex conformation of a DNA strand via intercalation, and by attaching a proline to the G-quadruplex, the reactants were brought again into close contact. No enantioselectivity has been reported in these two examples.

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\textbf{Scheme 9}: DNA-directed catalysis of an aldol reaction.
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\subsection*{1.5.3 DNA-based catalysis}

As in the case of the hybrid enzymes, hybrid constructs of catalytic small molecules and DNA can also be applied in catalysis. The strategies for anchoring the small molecule catalyst to the DNA are the same as in the case of the proteins, i.e. covalent, dative, or non-covalent anchoring. An example of the dative anchoring strategy is the combination of an iron porphyrin with a G-quadruplex forming oligomer.\textsuperscript{80} The porphyrin intercalates in the G-quadruplex, and the iron is chelated by a guanine.\textsuperscript{81} The peroxidase activity of the iron porphyrin was increased for a variety of substrates due to the presence of the DNA, albeit that in the case of chiral substrates no enantiomeric preference was displayed.\textsuperscript{82} Mechanistic studies indicated that the increase in catalytic activity was caused by a favorable conformational change of the porphyrin during catalysis, which was stabilized by the DNA.\textsuperscript{80}

The covalent approach has been explored as well. Four groups have developed strategies to modify the DNA post-synthetically with chelating moieties, of which three groups have used modified nucleobases, providing a handle for selective functionalization (Scheme 10). The group of Kamer modified a uridine nucleobase chemically with a diphenylphosphine via a palladium(II) catalyzed cross coupling (Scheme 10).\textsuperscript{83} A single uridine nucleotide was modified, as well as a uridine containing a flanking adenine and thymine. The diphenylphosphine modified uridine was applied in the Pd(II)-catalyzed allylic amination, inducing ee's up to 82\% in THF as solvent. The application of trimers containing the modified base, and the presence of water, lead to a lower reactivity and a loss of ee. No explanation has been put forward for this observation. The group of Jäschke showed that...
DNA could also be modified post-synthetically by reaction of a phosphane with an oligonucleotide containing a reactive group (Scheme 10). The phosphanes contained an activated ester, that coupled efficiently with the amine of the modified nucleobase. To date, no catalysis using these modified oligomers has been reported, possibly due to the competition by the nucleobases for the free coordination sites on the transition metal. The group of Vogel connected a polyaza crown ether to an oligonucleotide, and incorporated a copper(II) ion. Applying the hybrid catalyst in the Diels-Alder between aza-chalcone 1 and cyclopentadiene 2 did not lead to a significant ee (<10%).

To date, incorporation of modified nucleobases into oligonucleotides did not lead to enantioselectivity. A recently reported approach followed by Sancho Oltra and Roelfes involved the attachment of a copper(II) complex to a single stranded DNA, via the 3’ or 5’ end of a commercially available modified oligomer (Scheme 11). This design is highly modular, and the combination with different complementary single stranded DNAs gave a variety of DNA based catalysts. Given the success of the Diels-Alder reaction of aza-chalcone 1 and cyclopentadiene 2 catalyzed by copper(II) in DNA-based asymmetric catalysis (this thesis), the same reaction was performed in the presence of these catalysts, resulting in ee’s up to 93%.

Scheme 10: Synthesis of ligand modified oligonucleotides by the groups of a) Kamer and b) Jäschke for potential asymmetric catalysis.
Critical features in the design were the length of the spacer between the bipyridine and the DNA, and the sequence of the DNA close to the copper(II) bipyridine. The optimal spacer length was found to be an n-propyl spacer. The sequence affected the ee to a large extent, and the straightforward modular assembly with other strands made rapid optimization feasible. The three bases closest to the copper complex were critical for the ee observed, and it was found that the triplet GTA, TAC in the template strand, gave the highest ee of 93%.

1.6 DNA-based asymmetric catalysis

1.6.1 Concept of DNA-based asymmetric catalysis

The key issue in DNA-based asymmetric catalysis is to perform a reaction in the vicinity of the helix, which then provides the chiral information for the activated complex of the reaction, resulting in the preferential formation of one of the enantiomers of the reaction product. In analogy with the hybrid enzymes, the approach to perform DNA-based asymmetric catalysis is to make use of a small-molecule catalyst anchored to the DNA. The noncovalent approach leads to a modular self-assembly of the catalyst, leading to a rapid optimization of the catalyst. A catalyst can be bound non-covalently to DNA for instance via intercalation. Moreover, to ensure the chiral information originates from the DNA, the catalyst should be non-chiral or racemic.
1.6.2 Design of the first DNA-based catalyst

A catalytically active copper(II) ion was bound to DNA via its ligand (Scheme 12). The ligand comprised a bidentate copper(II) ion binding site, a DNA-intercalating 9-amino acridine, connected via a spacer. The advantage of this design is that the spacer length (n) and the amine substituent (R) can be used to optimize the design. Notably, a stereogenic center is created at the nitrogen upon binding of copper(II). In the absence of DNA the complex will be formed as a racemate, but in the presence of DNA an enantiomeric excess in the complex could in principle be induced, similar to the case of iron(II)(bipy)$_2$ (vide supra). In this way, the chirality transfer might proceed in two steps; first from the DNA to the copper(II) complex, and then from the copper(II) complex to the product of the reaction. The model reaction that was chosen for proof-of-principle was the copper(II) ion catalyzed Diels-Alder reaction between aza-chalcone 1 and cyclopentadiene 2 in water, furnishing the chiral Diels-Alder product 3. This reaction was chosen because it undergoes efficient Lewis-acid catalysis in water,$^{23}$ and enantioselective examples of this transformation have been reported in water as a solvent, using α-amino acids as ligands.$^{22}$

Scheme 12: The first generation of DNA-based asymmetric catalysts.
1.6.3 Asymmetric catalysis with a DNA-based catalyst

Using the described DNA-based catalysis concept it is possible to obtain ee’s in the Diels-Alder reaction. The results obtained in the presence of salmon testes DNA (st-DNA) and ligands L1 – L6, indicated that the enantiomeric excess obtained is highly dependent on the ligand employed. A crucial feature was the spacer length: the n-propyl and ethyl spacers led to the highest ee’s, whereas longer spacers such as n-pentyl (L5) showed a diminishing ee. Hence, close contact of the copper(II) ion to the DNA appears to be a necessity to induce significant ee’s. Also the R substituent on the ligand influenced the stereochemical outcome of the reaction to a large extend. The highest ee’s were obtained with R = 1-methylnaphthyl, i.e. ee’s up to 49%, whereas a change in the positioning of the naphthyl group with R = 2-methylnaphthyl L6 resulted in a racemic product. Moreover, in the case of R = 1-methylnaphthyl, using an n-propyl spacer instead of an ethyl spacer led to the formation of the opposite enantiomer of 3. Hence, although DNA consisted of only the right handed helix, both enantiomers could be obtained by a judicious choice of the achiral ligand.

1.7 Aims and Outline

The goal of the research described in this thesis is to develop the general concept and methodology of DNA-based asymmetric catalysis, with the aim of using this concept to discover novel reactivities, and to develop mechanistic understanding of successful catalysts. This knowledge can be used to further improve the class of hybrid enzymes, and might increase our insight into the mechanistic features of enzyme catalysis.

In Chapter 2, a new design of DNA-based asymmetric catalysts is introduced. The 2nd generation DNA-based catalysts are presented, which are capable of catalyzing the Diels-Alder reaction between aza-chalcone and cyclopentadiene with >99% ee. The key issue of removal of the coordinating auxiliary is solved by the application of a new substrate class.

Chapter 3 provides mechanistic insight, describing a thorough kinetic and sequence dependence study of the Diels-Alder reaction catalyzed by the 2nd generation catalysts. The results provide the basis to explain why salmon testes DNA can give such high enantioselectivities.

Chapter 4 describes a structural study of the DNA-based catalysts, in which the DNA-binding mode of the copper(II) complexes is investigated.

Chapter 5 deals with the second part of the structural studies, in which the binding of aza-chalcone to the copper(II) complexes, and its effect on the enantioselectivity of the reaction, is investigated.
Chapter 6 describes the first asymmetric Friedel-Crafts reaction with an olefin in water. This reaction was catalyzed by the 2nd generation DNA-based catalysts and provided ee’s up to 93%.

Chapter 7 displays another example of novel reactivity, in which the first non-enzyme catalyzed asymmetric syn-hydration of an α,β-unsaturated carbonyl compound is demonstrated.

Finally, in chapter 8 the results described in this thesis are summarized in a comprehensive fashion, and overall conclusions are drawn.

1.8 References

4. Not all enzymes are highly enantioselective: (-)-Menthol is isolated from rose oil with an ee of 80%. It is made synthetically on multiton scale via homogeneous asymmetric catalysis with an ee of 94-96%. See also: Noyori, R., Nobel Lecture 2001.
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70. Sun, G; Fan, J.; Wang, Z.; Li, Y. *Synlett*, **2008**, 2491.
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