Chapter 1

Dynamic Combinatorial Chemistry as foundation of Systems Chemistry
Chapter 1

1.1 Introduction
The gap between chemistry and biology, though apparently modest, seems to be difficult to bridge in practice. The multiple and not fully understood molecular interactions between molecules is the first difficulty that chemists face when trying to mimic the behavior of biological systems. A second level of complexity arises when multiple species interact with each other, behaving as a single system. In this way, the interactions between individual species affect the state of the complete system. In this thesis we introduce some chemical systems which, by using dynamic combinatorial chemistry (DCC), achieve a certain level of molecular complexity and interactions within the controlled conditions of an organic chemistry bench. These systems will be explored for the discovery of catalysts and the construction of molecular communication networks. Catalysts are key elements of biochemical transformations as well as essential parts in many current industrial chemical processes. Molecular networks are ubiquitous, yet not well understood and the communication between different molecular systems could give important information on how chemistry builds up biological systems.

1.2 Dynamic combinatorial chemistry
1.2.1 Definitions
Combinatorial chemistry deals with the preparation of a large number of different molecules by combination of a relatively limited number of smaller building blocks, often leading to the formation of structurally related compounds (Figure 1.1). The synthesis can involve sequential steps which will exponentially increase the number of different molecules. A time-effectiveness is often related to the expression “combinatorial chemistry” widely known within the pharmaceutical industry, where the rapid screening of large libraries of different but related compounds is needed. Nevertheless, scientists have found that combinatorial chemistry has also some limitations when searching for new molecules. As a tool based on random synthesis, the outputs of combinatorial libraries are likely to lack specific chirality and/or rigidity, characteristics that are frequently found in pharmaceutical drugs. High activation energy can prevent the
formation of potentially interesting compounds as the synthesis of the combinatorial products is usually kinetically driven.

Dynamic combinatorial chemistry\textsuperscript{2,3} involves the synthesis of relatively complex molecules from simpler molecular building blocks that can react among themselves in a reversible way (Figure 1.2). This means that in a dynamic combinatorial library (DCL) all the products of the mixture can interconvert because of the reversible covalent or non-covalent bonds connecting each of the building units. Following the second law of thermodynamics, the composition of the library will change as the building blocks self-assemble towards the thermodynamic minimum of the system where the product distribution reaches an equilibrium. This equilibrium is a dynamic state: there is a constant formation and disassembly of the products of the library, even though the total composition of the system remains stable over time. Because of the reversibility of the system, kinetic products initially formed due to their low activation energy can be displaced by thermodynamically more stable compounds. In particular cases where the building blocks have two functional groups to reversibly interact with each other, the library is usually dominated by small cyclic compounds (provided the building block concentration is not too high). This composition results in a favorable higher entropy of the system in comparison to the situation where a smaller amount of larger macrocycles would be formed.
Figure 1.2 A dynamic combinatorial library made from different combinations of building blocks. The library members are constantly exchanging building blocks. The final composition of the library will depend on the thermodynamic stability of the sum of the library members.

1.2.2 Template effects

The state of a dynamic combinatorial library is influenced by thermodynamic variables such as temperature\(^4\), pressure\(^4\), or entropy\(^5,6\) of the system. The equilibrium of DCLs can be altered by changing any of these variables so that a new composition becomes the one with the lowest energy. This fact implies that, after a stimulus, the library will reorganize itself by amplifying certain species at the expense of others, shifting its composition (Figure 1.3). For example, when a new species is added to a DCL, interactions between the library members and the new molecules can alter the equilibrium, so the strongest binding molecules will become dominant within the library.\(^7,8\) In this case the composition change is driven by the addition of an external template. It is important to realize though, that the composition of the system does not necessarily include the individually most stable species but rather reflects a combination of species which accounts for the lowest Gibbs energy of the whole system for the particular set of conditions of the experiment.\(^7-9\)

Templating effects may also arise from internal changes in the library, leading to new interactions among the library members and resulting in foldamers\(^10\) (covalent oligomers adopting secondary structures stabilized by noncovalent interactions) or fiber-like self-assemblies\(^11\) (noncovalent polymers) that may give rise to fascinating self-replicating species.\(^12,13\) The ability evidenced by DCLs to be templated is the basis of most of the research work presently carried out in the area of DCC.
Figure 1.3 A dynamic combinatorial library will reequilibrate upon addition of a template able to interact with the library members.

1.2.3 Design of dynamic combinatorial libraries

When designing a dynamic combinatorial library, the first step is choosing what type of chemistry will be involved in the formation of the reversible bonds which will build the library members. From this selection, a number of variables may become fixed such as solvent, pH, temperature or chemical species compatible with the library. The rest of the building block structure and chemical functionality should be designed in a way that it addresses the purpose for which the library is prepared. In this regard, hydrogen-bond donors and acceptors are valuable moieties for chemical interactions in libraries in organic solvents while for aqueous libraries, motifs leading to hydrophobic interactions are often preferred. In both cases, charged and aromatic building blocks are useful because of their possibility to generate, respectively, Coulomb interactions and π-π interactions.

Reversible chemistries

As previously mentioned, in order to obtain a dynamic combinatorial library, the bonds formed between molecular building blocks must be reversible. In this way, the connections can be reverted or exchanged allowing for the generation of different combinatorial products. Some examples using noncovalent interactions such as hydrogen bonding\(^{14-18}\) and metal-ligand coordination have been already reported.\(^{19-24}\) However, many applications (see section 1.2.5) require the isolation of the library products, which can be difficult due to the weakness of these types of linkages. To circumvent this problem, an approach using reversible covalent reactions can be used. Acidic or basic conditions, or the presence of metal catalysts are required to achieve most of the reversible reactions used in DCC which are summarized in Scheme 1.1.
Hydrazone exchange and disulfide exchange are the chemistries involved in the work described in this thesis; hence they will be explained in detail in following sections.

**Hydrazone libraries**

Hydrazone exchange is a widely used reversible reaction in DCC. It involves an initial step of formation of a hydrazone by condensation of a hydrazide and an aldehyde, and a second step where the hydrazone undergoes a nucleophilic substitution by attack of a free hydrazide to the carbonylic carbon from the aldehyde moiety (Scheme 1.2). These reactions can efficiently work in pH ranging from 2.5 to 6.0 as they are favored by the protonation of the aldehyde and the Nitrogen atom in the C=N bond of the hydrazone. Lower pH values will inhibit the nucleophilic attack by protonation of the hydrazide, while at neutral conditions the lack of acidic catalysis retards the hydrazone formation and exchange. Alternatively, nucleophilic catalysts like aniline can accelerate the equilibration of a library at neutral pH and building block concentration ranging between 0.1 and 1.0 mM, decreasing the equilibration time from several days to a few hours.\(^{25,26}\)

**Acyl transfer and related**

\[ a) \quad \text{R}_1\text{O}\text{R}_2 + \text{R}_3\text{O}\text{R}_4 \xleftrightarrow{\text{base}} \text{R}_1\text{O}\text{R}_4 + \text{R}_3\text{O}\text{R}_2 \]

\[ b) \quad \text{R}_1\text{O} \text{R}_2 + \text{R}_3\text{O} \text{R}_4 \xleftrightarrow{\text{Pd(0)}} \text{R}_1\text{O} \text{R}_4 + \text{R}_3\text{O} \text{R}_2 \]

\[ c) \quad \text{R}_1\text{N} \text{H} + \text{R}_3\text{N} \text{H} \xleftrightarrow{\text{base}} \text{R}_1\text{N} \text{R}_4 + \text{R}_3\text{N} \text{R}_2 \]

\[ d) \quad \text{HO} \text{R}_1\text{R}_2 \text{OH} + \text{Me} \text{O} \text{Na} \xleftrightarrow{\text{aldolase}} \text{R}_1\text{R}_2 \text{OH} + \text{Me} \text{O} \text{Na} \]

\[ e) \quad \text{R}_1\text{S} \text{R}_2 + \text{HS} \text{R}_3 \xleftrightarrow{\text{base}} \text{R}_1\text{S} \text{R}_3 + \text{HS} \text{R}_2 \]

\[ f) \quad \text{R}_1\text{C} \text{R}_2 + \text{HS} \text{R}_3 \xleftrightarrow{\text{base}} \text{R}_1\text{S} \text{R}_3 + \text{R}_3\text{C} \text{R}_2 \]
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Acetal exchange and related

\[
g) \quad R_1\text{OR}_2 + R_3\text{OR}_4 \xrightleftharpoons{\text{acid}} \xleftarrow{\text{acid}} R_1\text{OR}_3 + R_2\text{OR}_4
\]

\[
h) \quad R_1\text{SR}_2 + R_3\text{SR}_4 \xrightleftharpoons{\text{acid}} \xleftarrow{\text{acid}} R_1\text{SR}_3 + R_2\text{SR}_4
\]

\[
i) \quad R_1\text{N} = \text{N} + R_2\text{O} \xrightleftharpoons{\text{acid}} \xleftarrow{\text{acid}} R_1\text{N} = \text{N}R_2 + R_2\text{H}
\]

C=\text{N} exchange

\[
j) \quad R_1\text{N} = \text{N} + R_2\text{H} \xrightleftharpoons{\text{acid}} \xleftarrow{\text{acid}} R_1\text{N}R_2 + R_2\text{H}
\]

\[
k) \quad R_1\text{O}N\text{R}_2 + R_3\text{N} \xrightleftharpoons{\text{acid}} \xleftarrow{\text{acid}} R_1\text{N}R_4 + R_2\text{N}R_2
\]

\[
l) \quad R_1\text{N}O\text{R}_2 + R_3\text{N}O\text{R}_4 \xrightleftharpoons{\text{acid}} \xleftarrow{\text{acid}} R_1\text{N}O\text{R}_4 + R_3\text{N}O\text{R}_2
\]

Other reversible covalent bonds

\[
m) \quad R_1\text{H} + R_2\text{H} \xrightleftharpoons{\text{Grubbs catalyst}} \xleftarrow{\text{Grubbs catalyst}} R_1\text{H} + R_2\text{H}
\]

\[
n) \quad R_1\text{C} = \text{C} + R_2\text{C} \xrightleftharpoons{\text{Mo catalyst}} \xleftarrow{\text{Mo catalyst}} R_1\text{C} = \text{C} + R_2\text{C}
\]

\[
o) \quad R_1\text{S} \cdot \text{S} \cdot R_1 + R_2\text{S} \cdot \text{S} \cdot R_2 \xrightleftharpoons{\text{RS}^-} \xleftarrow{\text{RS}^-} R_1\text{S} \cdot \text{S} \cdot R_2 + R_2\text{S} \cdot \text{S} \cdot R_1
\]

\[
p) \quad R_1\text{C} = \text{C} + R_2\text{C} \xrightleftharpoons{\text{Mo catalyst}} \xleftarrow{\text{Mo catalyst}} R_1\text{C} = \text{C} + R_2\text{C}
\]

\[
q) \quad R_1\text{OR}_2 + \text{HOR}_2 \xrightleftharpoons{} \xleftarrow{} R_1\text{OR}_2 + \text{HOR}_2
\]
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Non-covalent bonds

\[
M^{(2n)^{\text{II}}} + nL_2 \rightleftharpoons M^{(2n-1)^{\text{II}}} + nL_1
\]

Scheme 1.1 Some reversible reactions used for dynamic combinatorial chemistry: a) transesterification; b) transallylesterification; c) transamidation; d) aldol exchange; e) transthioesterification; f) Michael / retro-Michael reactions; g) acetal exchange; h) thioacetal exchange; i) pirazolotriazine metathesis; j) transimination; k) Hydrazone exchange; l) oxime exchange; m) alkene metathesis; n) alkyne metathesis; o) disulfide exchange; p) Diels-Alder / retro-Diels-Alder reactions; q) boronate ester exchange; r) metal-ligand exchange; s) hydrogen bond exchange.

Hydrazone groups are relatively stable in aqueous media as opposed to other similar functional groups like imines, and that is the reason why they are more frequently used in DCC. This extra stability is due to the lower electronegativity of the N atom of the hydrazide compared to the one of the amine. This difference makes the C=N bond less susceptible to hydrolysis by nucleophilic attack of water. As a result, the typical hydrazone equilibrium provides an approximate 90/10 to 99/1 ratio of hydrazone/hydrazide when both reagents are added in equimolar concentrations. This ratio allows for an excellent product yield which benefits the synthesis and analysis of the library members while still keeping a high enough hydrazide concentration to achieve a high reequilibration rate of the library. The addition of an excess of hydrazide may help to accelerate library equilibration.

\[
\begin{align*}
&\text{R}_1\text{C}=\text{N}\text{NH}_2 + \text{H}_2\text{O} \rightarrow \text{R}_1\text{C}=\text{N}\text{NH}_3 + \text{H}_2\text{O} \\
&\text{R}_1\text{C}=\text{N}\text{NH}_2 + \text{R}_3\text{C}=\text{N}\text{NH}_2 \rightarrow \text{R}_1\text{C}=\text{N}\text{NH}_2 + \text{R}_3\text{C}=\text{N}\text{NH}_2
\end{align*}
\]

Scheme 1.2 Hydrazone formation and exchange.
**Disulfide libraries**

Disulfide exchange is also commonly used in the preparation of DCLs. Thiols are normally employed as starting material. In this case, an initial step of oxidation is needed so that part of the thiol groups in the solution become oxidized to disulfides (Scheme 1.3). The reaction proceeds readily in presence of atmospheric oxygen. The disulfides hence formed, may now exchange by simple nucleophilic attack by the free remaining thiolates in solution. For both steps (oxidation and exchange) to happen, a neutral to mildly basic pH (usually between 7 and 9) is typically used so that thiolates are available in solution.

![Scheme 1.3 Mechanism of disulfide formation and disulfide exchange.](image)

An excess of molecular oxygen available to the library may result in a complete oxidation of the thiols which are required for the exchange, therefore bringing the library into a “frozen” state. At this point, the library is no longer able to shift its disulfide composition to achieve a thermodynamic minimum, since it is not anymore a dynamic system. Consequently, it is important to be able to determine whether the final composition of the library corresponds to the one at equilibrium, or if, on the contrary, the library was fully oxidized before it could reach equilibrium. To be able to tackle these issues, the kinetics of the library reequilibration should be fast compared to the oxidation rate of the building blocks. Alternatively, one can “artificially” keep the library in a dynamic state by periodically adding extra dithiol building blocks or some agent able to reduce part of the disulfides back into thiols. An original approach was established during this research work by keeping the libraries under a nitrogen atmosphere to keep the concentration of the oxidized species at a constant level. Working under these conditions, disulfide systems have proven to be dynamic for periods of time longer than 2 months.
1.2.4 Analysis of libraries

One of the advantages of DCC is the possibility of easily obtaining a large range of compounds which are closely related in terms of molecular reactivity and structure. Unfortunately, this advantage turns into a difficulty at the point of analyzing the library composition. Given that screening DCLs for targeted molecular binders requires the ability to measure any increase in the concentration of any of the library members, an ideal analytical technique would include high sensitivity as well as dynamic range. If these two prerequisites are fulfilled, low and high concentrations of any library member can be quantified. With the exception of small libraries containing simple building blocks, NMR spectra of the complete libraries become difficult to interpret due to the overlap of signals. The combination of two techniques which allow the separation and later identification of the mixture of compounds has proven to be the most general solution to analyze DCLs, in particular liquid chromatography-mass spectrometry (LC-MS) (Figure 1.4). The initial separation of the species by HPLC can give information about how many different molecules are present in the library and the relative amount of each of them. Care should be taken though, not to directly associate signal intensity to compound concentration when working with molecules that exhibit different molar absorptivities ($\varepsilon$). The subsequent analysis of the liquid chromatography output by MS should lead to the identification of each of the detected species. In most of the cases, the value of the mass can unequivocally determine the structure of the species investigated. When different sequence isomers are possible, the analysis of their fragmentation pattern by MS-MS might be required to identify them. Alternatively, nuclear magnetic resonance (NMR) might be a useful technique to fully assign the structure of the library members. The stability of the samples to the LC-MS conditions should of course be tested in advance. For a proper analysis, libraries should be static on the time scale of the analysis, which can normally range from a few minutes to around one hour. In certain cases it is convenient to freeze the library equilibrium prior to its analysis. This can be done by e.g. changing the pH of the solution or by reacting the library members to inhibit their reversible bonds from reacting.
1.2.5 Applications of dynamic combinatorial chemistry

The responsive behavior of DCLs is displayed when the equilibrium of a library is shifted by addition of a templating molecule. This responsiveness is the main advantage of a dynamic combinatorial library over a non-dynamic one, particularly when exploring a recognition process. Examples of uses of DCLs include directed synthesis, discovery of synthetic receptors or sensors.

Directed synthesis

Equilibrium reactions can be tuned towards product formation by following Le Chatelier’s principle through removing the products from the reaction mixture, adding excess of reagents or controlling the temperature and the pressure. The system will counteract the changes applied on it to reach a new minimum energy state by producing more of the desired product. In a dynamic combinatorial library a template may be added to shift the library composition towards the formation of a selected member of the network.$^{30}$
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synthetic approach is specially interesting when the formation of complex supramolecular structures such as catenanes or rotaxanes is required. One of the earliest examples of template-directed synthesis in dynamic systems was achieved by Busch using Ni(II) to synthesize a bis-imino macrocycle (Scheme 1.4).  

![Scheme 1.4 Synthesis of a bis-imino macrocycle directed by Ni(II) templation.](image)

**Receptor discovery / Molecular recognition**

Dynamic combinatorial libraries can be of enormous utility to discover new synthetic receptors for target molecules. The main advantage in using DCLs is that the synthesis and binding analysis of a high number of the potential binders take place within the same medium without the need for isolating any of them. The possible receptors are self-assembled in the library from simple initial building blocks that were selected to contain functional groups complementary to the ones existing in the target molecule. The interactions between the template and the products of the library will select and amplify the preferred library members which are, in principle, the ones binding more strongly. Once the best receptors are detected, they can be either isolated from the library or resynthesized by traditional synthetic methods. Alternatively, a new combinatorial library containing a selection of the building blocks participating in the formation of the amplified binder can be set up. In this way the assembly of the desired ligand will be further amplified. A significant example of this strategy to discover new receptors was accomplished in our group by identifying ephedrine binders from a dynamic combinatorial library of about 10,000 components (Scheme 1.5).  

Aside from biomolecule binders, DCLs prepared for the binding of anions and cations have also been reported.
Scheme 1.5 The presence of ephedrine 9 in a library made from building blocks 1-8 amplifies mainly two tetrameric structures from a set of about 10000 possible compounds.

Sensors

Probably the most recent application of dynamic combinatorial libraries is for analytical purposes. The concentrations of the library members in a DCL can be converted into an output signal which varies depending on different factors such as pH, concentration of target molecules, etc. Although generally HPLC is the most common method to analyze DCLs, for sensor applications it is more convenient to make use of faster methods such as fluorescence,\textsuperscript{47,48} or UV-vis\textsuperscript{49,50} spectroscopy. The spectrum obtained will be a reflection of the analyte interacting with the library. The information about the analyte is distributed along the spectral range of the library members. For this task, the libraries can contain building blocks with dyes covalently attached. Alternatively, the library members can interact through non-covalent bonding with UV-vis active molecules added to the reaction mixture. The presence of a specific molecule able to interact with the same library members can trigger a spectroscopic signal by displacing the UV-vis active molecules out of the library member – dye complex. A clear example by the group of Severin\textsuperscript{51} to illustrate this technique employs a set of three different dyes in combination with CuCl$_2$ and NiCl$_2$ to form an initial mixture of complexes. The addition of a dipeptide results in a
release of the dyes which gives a UV-vis signal. The library gives different signals depending on the amino-acid sequence of the added aminoacids (Figure 1.5).

Figure 1.5 A dynamic combinatorial library of dye-metal-dye complexes gives a specific UV-vis spectrum depending on the amino-acid sequence of dipeptides interacting with the library.

Another remarkable example is represented by the chirality sensing of secondary alcohols achieved by Anslyn by analyzing the Zn(II)-ligand self-assembled complexes by circular dichroism (CD).

1.3 Catalysis

1.3.1 Principles of catalysis

A catalyst is a substance able to increase the rate of a reaction by participating in the chemical transformation of the substrates into products. It works by lowering the activation energy ($E_a$) of a reaction. The presence of a catalyst promotes the formation of a new transition state lower in energy and therefore easier to reach. In consequence, the reaction proceeds faster (Figure 1.6). The catalyst is not consumed in the reaction, although frequently its activity can be reduced by inhibitors which may be produced in the reaction. The opposite case is also possible: a substance can be able to enhance the catalytic activity and in this case it is called catalytic promoter.
Figure 1.6 Graphical representation of a generic exothermic reaction pathway with and without catalyst. The presence of a catalyst opens a new reaction pathway with a lower transition-state energy while the energies of the initial and final state remain the same.

1.3.2 Types of catalysis

Traditionally, a clear division between homogeneous and heterogeneous catalysis is made (Figure 1.7). While in homogeneous catalysis the catalyst is in the same phase as the reagents (typically dissolved in a solvent) in heterogeneous catalysis the catalyst is in a different phase (normally, a solid in a solution of reagents). In the latter case, the properties of the surface of the catalyst become very important as the reagents will interact only with this surface. In many cases, to improve the surface properties (i.e. increase the surface area) the catalyst is supported on a specific solid.

Figure 1.7 Classification of catalysis

Homogeneous catalysis can further be divided in organocatalysis (no metals are employed) or metal catalysis. Transition metals are widely employed as catalysts in small scale and industrial processes. Organocatalysis is less developed than transition-metal
catalysis; however, many of the enzymatic processes lack metal ions and can therefore be considered as organocatalysts, although enzymes are more commonly referred as biocatalysts.

Catalysts can work via covalent interactions with the substrate (DMAP in esterifications) or through non-covalent interactions (hydrogen bonding, hydrophobic interactions, etc.). Mainly the latter are used in supramolecular catalysis.

1.3.3 Supramolecular catalysts

A supramolecular catalyst\textsuperscript{53,54} consists of a molecular structure able to bind a substrate through non-covalent interactions to increase the rate of its transformation into the product(s). Supramolecular catalysts, although usually considered as organocatalysts, can sometimes contain metal ions in their structure.

![Figure 1.8 Breslow’s supramolecular catalyst based on hydrophobic binding interactions with the substrate and nucleophilic attack of a water molecule activated by a metal.](image)

Metals can actively participate in the creation of a new reaction pathway by interacting with the substrate\textsuperscript{55-58} (oxidative addition, transmetallation, reductive elimination, etc.) (Figure 1.8) or they can simply act as scaffolds to give shape to the catalysts. In the latter case, the host is assembled by metal-ligand interactions. In a representative example of supramolecular catalysis by Fujita\textsuperscript{59}, a cage made from \textit{cis}-capped Pd(II) and a triazine-cored tridentate ligand was able to catalyze a Diels-Alder reaction (Figure 1.9). In the presence of 10 mol\% of the host, the conversion was complete after 5 hours, while only 3\% of conversion was achieved in the absence of catalyst.
A common feature shared by supramolecular catalysts is the presence of non-covalent interactions between the catalyst and both the substrate and transition state of the reaction. The supramolecular structure acts as a host for the substrate which is converted into the product within the cavity of the receptor. Raymond and co-workers were able to accelerate the aza-Cope rearrangement of an enammonium cation up to three orders of magnitude by using a self-assembled metal-ligand host in aqueous solvent (Figure 1.10). The hydrophobic cavity created by the supramolecular assembly interacted with the substrate via hydrophobic and cation-π interactions. The restriction of the space in the cavity favors the encapsulation of the tightly packed chairlike conformation of the TS of the aza-Cope rearrangement.
Following Pauling’s concepts, the active site of the catalyst is designed to be complementary to the shape and electronic features of the transition state in analogy to the working principles of enzymes. Nature defines very precisely the environment of a catalyzed chemical transformation by selective fitting of enzyme to substrate and transition state. The goal of designing and synthesizing systems of comparable complementarity seems, however, hard to achieve for the traditional synthetic chemist. For this reason, self-assembling systems such as DCLs, where chemical structures can be influenced by the presence of templating agents, can be regarded as a shortcut to prepare complex structures with potential catalytic properties.

1.3.4 Dynamic combinatorial approach to catalysis

Dynamic combinatorial libraries allow for synthesizing and screening for molecular complementarity in one step. This aspect renders DCLs as potentially valuable systems to discover new catalysts. Based on the trial-and-error approach, DCLs may allow a self-adjusting formation of a catalyst which matches the chemical characteristics of the substrate. A catalyst should of course bind to the substrate in order to perform any chemical transformation. But another crucial step is the lowering of the energy of the transition state of the reaction. A good adjustment between substrate stabilization (which occurs if there is preferential binding by the catalyst) and transition state stabilization must be reached in order to obtain catalytic behavior from a dynamic combinatorial library member. It is impossible to know a priori if a good binder of the substrate will also be a good catalyst for its transformation into the product. A good substrate binder may effectively stabilize the substrate but not the transition state. On the other hand, good catalysts could transform the substrate within the library environment even if their concentration is relatively low compared to other library members. In this case a good catalyst would remain undetected due to lack of (or very small) amplification. To address this problem, transition-state analogs (TSA) are used to detect library members able to stabilize the transition state of the reaction. This idea is based on the catalytic antibody approach for the formation of supramolecular catalysts. It was firstly suggested by Pauling in 1948 and later developed by Jencks, proposing a transition-state analog as antigen to produce catalytic antibodies. If the developed antibodies are able to bind and stabilize
the TSA mimicking the real TS of a reaction, catalysis would be achieved. It has been estimated that there are around $10^{10}$ potential antibodies, which makes this a powerful approach for developing new catalysts. Some promising examples of the use of catalytic antibodies have been already developed. For instance catalytic antibodies were obtained for the hydrolytic degradation of cocaine\textsuperscript{67} (Figure 1.10) or the oxidation of nicotine.\textsuperscript{68}

![Figure 1.10 Molecule of (-)-cocaine and the TSA used by Landry to obtain catalytic antibodies for the hydrolysis of the benzoate moiety of the cocaine.\textsuperscript{67}](image)

In conclusion, when using DCC for discovering catalysts, an important part of the work is done by the system itself. The selection of the preferred functional groups and the spatial configuration that will make a potential catalyst will be determined by the library based on the interactions between catalyst and substrate or transition-state analog. Moreover, the synthesis of the chosen structures will also take place within the dynamic combinatorial library environment.

**State of the art. Catalysis and dynamic combinatorial chemistry**

To date, very few examples of catalytic applications within DCC have been reported. A dynamic combinatorial library of disulfide molecules was used by our group to identify a catalyst for a Diels-Alder reaction.\textsuperscript{64} The product of the reaction itself was used as transition-state analog (TSA) to identify binders of the real transition state (TS) of the Diels-Alder reaction, given the similarity between the TS and the product (Figure 1.11). Two library members were amplified when the TSA was added to the library. Subsequent isolation of these selected compounds allowed to perform catalysis experiments which showed that one of them was active as a catalyst. An improvement of the reaction rate by a factor of 10 was achieved. The resemblance between TS and product had an undesired consequence: the affinity of the catalyst for the product caused inhibition of the catalysis.
In another example involving DCLs as a source for catalysts, the same disulfide macrocycle was found to accelerate an acetal hydrolysis. In this case, a quaternary ammonium salt was chosen as a TSA to mimic the proposed transition state of the reaction which also contained a positive charge (Figure 1.12). Macrocyle 14 was selected by templating a library of disulfide building blocks. The combination of this preferred cyclic disulfide with the substrate of the reaction showed a modest (factor of 2) acceleration of the rate of the hydrolysis reaction.

**Figure 1.11** A macrocycle is amplified by a TSA of a Diels-Alder reaction in a dynamic combinatorial library made from dithiol building blocks. The selected cyclic homotrimer was found to be catalytically active.

**Figure 1.12** Catalyzed acetal hydrolysis reaction and the TSA used to select the catalyst from a disulfide dynamic combinatorial library.
In a different study, Prins and Scrimin compared the catalytic properties of different neighboring functional groups for the hydrolysis of carboxylic esters. Libraries of hydrazones combining a substrate (ester) and different functional groups were tested for differences in rate of hydrolysis of the ester moiety and variations of the rate of reaction up to a factor of 60 were found (see Scheme 1.6). A phosphonate moiety had previously been used as a TSA for the ester hydrolysis. The good correlation between the affinities between the TSA and the selected functional groups and their activity in catalyzing the hydrolysis reaction proved once more the validity of the use of a TSA to identify catalysts.

The group of Nicholas described an approach to develop catalysts from a DCL of imines with the participation of Zn(II) to hydrolyze esters. Adding a TSA, they were able to identify the best combination of amine / aldehyde to achieve catalysis of the hydrolysis reaction (Scheme 1.7). Again, in this example the amplification of a library member within the DCL of imine-Zn(II) complexes of TSAs selected the best imine ligand to stabilize the transition state of the reaction and, consequently, the best catalyst for the reaction.

Scheme 1.6 Scrimin’s DCL of hydrazones. The rate of hydrolysis of the ester moieties attached to the hydrazone molecules is influenced by the R substituent. The rate of hydrolysis for each of the library members corresponded well with the concentration of the matching phosphonated TSAs of a similar DCL, proving that the TSAs were a good model for the TSs.
The rate of the reaction depends on imine coordinated to the Zn(II). The TSA used identified the imine ligand that promotes catalysis best within a DCL of four different imines.

### 1.4 Systems behavior of dynamic combinatorial libraries

#### 1.4.1 Introduction

Similarly to how systems biology deals with biological networks, systems chemistry can be regarded as an attempt to understand the new behaviors arising from the interaction between simple molecules within complex molecular networks. The study of these systems should give a better understanding of how biologically important mechanisms work. This knowledge can ultimately be applied to design new medical therapies or even to engineer new chemical systems able to perform complex tasks that individual chemical entities or simple chemical reactions fail to accomplish. As for now, systems biology is still at the stage of improving the understanding of how the different molecular components interact. Therefore, systems chemistry can be useful at this point by providing some simplified chemical models.

Up to now, most of the applications reported from DCLs were reductionist (section 1.2.5 and 1.3). The different approaches focus on selecting one of the several entities of the system via non-covalent interactions with templating target molecules. The number of
different library members grows exponentially with the different building blocks participating in the DCL. Therefore even a small number of such building blocks can give rise to a relative complex system of interacting species. The study of the behavior of DCLs when considering them as networks of interconnected library members can be regarded as an interesting opportunity to learn about the principles of systems chemistry.

1.4.2 Multiphase dynamic combinatorial libraries

The addition of a new phase (i.e. a second immiscible solvent) to a network of exchanging building blocks can be an advantage from different points of view. On one hand, the possibility to increase the number of library members due to the presence of a new solvent able to solubilize additional compounds can lead to an increase in the number of potential receptors for a target molecule. For synthetic purposes, there is a new possibility of shifting the equilibrium of a library towards the compound of interest depending on the solubility preferences of the library members. By tuning the solubility of the building blocks in the different phases, systems with new properties can be created. A careful design of the molecules existing in the system can achieve a new dimension of complexity by limiting the interactions between specific molecules which prefer different solvent phases. This gives the opportunity to simultaneously use certain building blocks that are otherwise incompatible. For example, the group of Sanders has prepared disulfide combinatorial libraries using systems with two phases to overcome solubility problems. Moreover, three-phase systems have been used for the transport of spermine between two aqueous layers through an organic phase. The building blocks dissolved in the organic phase and one of the aqueous phases self-assembled a carrier that allowed polar spermine molecules to cross the apolar phase (Figure 1.13). This work and the report by Lünинг on the transport of calcium ions through a bulk phase and membranes are the only examples that make use of DCLs for molecular transport.
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Figure 1.13 A disulfide library in an aqueous-organic system made by building blocks 22-24 was templated by spermine (25) to amplify the library member 26 which acted as carrier of 25 from one aqueous phase to the other.

An original work on DCC at the interface of a phospholipid bilayer\textsuperscript{81} reported that this special environment can lead to a change on the outcome of the dynamic combinatorial library, promoting chain-like compounds over small macrocycles.

1.5 Aims and outline of this thesis

An attempt to bridge the gap between chemistry and biology is described in the following chapters. For that purpose, DCC will serve as a tool to create complex dynamic mixtures from which new exclusive properties can be obtained: from the self-sorting of a catalyst to the communication between systems via chemical signals.

In Chapter 2, an approach to catalysis using hydrazone DCLs containing Zn(II) is developed.

In Chapter 3, an alternative type of DCLs based on disulfide exchange is used to explore metal catalysis.

In Chapter 4, a system able to assemble an organic catalyst in response to the presence of substrate in the reaction mixture is presented. After the substrate reacts completely, the DCL shifts back towards its original composition where the catalyst is a minor species.

Chapter 5 gives an example of how a dynamic combinatorial system can be enriched by having a number of different phases. In particular, the communication between two populations of chemical libraries in different phases has been achieved.
Finally, Chapter 6 presents a summary of the results described in the previous chapters.
1.6 References


Chapter 1


