14.1 INTRODUCTION TO DYNAMIC COMBINATORIAL CHEMISTRY

Molecular recognition is difficult! The design of artificial receptors, especially biomimetic receptors that work in water, has proved challenging. Dynamic combinatorial chemistry seeks to meet this challenge by combining aspects of design (molecular engineering) with the selection approach inherent in self-assembly. In dynamic combinatorial chemistry, building blocks are allowed to react with one another using reversible chemical reactions to give mixtures of oligomers—dynamic combinatorial libraries (DCLs) [1]. Because the connections between the building blocks are reversible, the mixture is dynamic, and library members are constantly interconverting. Once the DCL reaches equilibrium, its composition is determined by the relative stabilities of the different library members. Dynamic combinatorial chemistry, therefore, is combinatorial chemistry that works under thermodynamic control (Scheme 14.1) [1–3].

DCLs respond to external stimuli. If a target molecule is added to a DCL, it will act as a template for the self-assembly of library members that recognize it. Library members that interact favorably with the template will be stabilized, and their abundance in the library will therefore increase, at the expense of other library members [4]. By analyzing these template-induced changes, the binding properties of all library members are screened simultaneously without having to isolate each species. Through this amplification process, the effective receptors are not just identified but preferentially synthesized (Scheme 14.1), which makes dynamic combinatorial chemistry an efficient discovery strategy for new receptors.

The compositions of DCLs are often analyzed using high-performance liquid chromatography (HPLC). By comparing the chromatograms for the templated and untemplated libraries, amplified species can be identified. The ratio between the concentration of the amplified species in the templated and untemplated libraries is termed the amplification factor. In Figure 14.1 a DCL of peptide-based acylhydrazone macrocycles...
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is shown to exemplify this concept [5]. The DCL was generated from the aldehyde/hydrazide dipeptide building block, 1, and the recognition of cations by the macrocycles formed was investigated by adding NaI and LiI to the library as templates. The cyclic trimer was identified as a receptor for Na$^+$ and Li$^+$, as it was amplified at the expense of the cyclic dimer, tetramer, and higher macrocycles in both cases.

In dynamic combinatorial chemistry, the template selects its host from among all the possible combinations of building blocks in the DCL, and the library amplifies this host at the expense of other library members. This *survival-of-the-fittest* approach has invoked some authors to make comparisons between the dynamic combinatorial idea and Darwinian evolution [6, 7]. A strong motivation for the development of dynamic combinatorial chemistry stems from the difficulties associated with the design and preparation of strong and selective receptors, especially ones that function in aqueous solution. It has been realized that most designed receptors bind their guests much less efficiently than naturally occurring hosts [8]. Receptor design is challenging because it is hard to predict (i) the spatial structure of the designed molecule, (ii) the influence of the solvent, (iii) the strengths of the supramolecular interactions that hold the complexes together, and (iv) the superposition of multiple mutually reinforcing or

Scheme 14.1. Schematic representation of the template effect in a dynamic combinatorial library (DCL) of macrocycles. See color insert.

Figure 14.1. Left: A building block for acylhydrazone dynamic combinatorial chemistry. Middle: Schematic illustration of a DCL of acylhydrazone macrocycles. Right: HPLC analyses of the untemplated (a) and templated (b and c) libraries showing the amplification of macrocycle 1, in the presence of alkali-metal ions [5]. Source of HPLC chromatograms: [5]. Reproduced with permission of the American Chemical Society.
INTRODUCTION TO DYNAMIC COMBINATORIAL CHEMISTRY

Competing interactions. Dynamic combinatorial chemistry can be seen as a strategy where the focus is on designing an experiment to find receptors, rather than designing the receptor. In the last decade, this approach to molecular engineering has led to the identification of high-affinity receptors with complex topologies and unexpected recognition motifs [9].

The fundamental ideas of dynamic combinatorial chemistry were conceived independently by Lehn and coworkers [10, 11] and Sanders and coworkers [12–14], and implemented in proof-of-principle experiments published in 1996. Meanwhile, Benner had suggested some of the concepts in a patent in 1995, but did not report any experimental results [15]. Lehn described a dynamic mixture of circular double helicates formed from the coordination of Fe(II) with tris-2,2′-bipyridine ligands (2) (Scheme 14.2). It was observed that the mixture could be biased toward a cyclic hexamer or pentamer by varying the counterion.

Sanders and coworkers explored reversible transesterification to generate libraries of cyclic oligoesters starting from a cholate-based building block (3) equipped with an alcohol and a methylester moiety [12, 13]. Some of the macrocycles were found to interact with alkali-metal salts (Scheme 14.3) [14, 16]. In order to facilitate exchange and ensure thermodynamic control over the library, harsh reaction conditions were required; KOMe and di-cyclo-hexyl-[18]-crown-6 were combined with the building blocks in boiling toluene. Where harsh conditions are required for reversibility, the stability of the building blocks, the template, and the oligomers formed in the DCL can

Scheme 14.2. Lehn’s template adjustable DCL containing different-sized circular helicates. On the right, a sulphate-induced hexamer (Fe(II)₂₆(SO₄)₆) and on the left a chloride-binding pentamer (Fe(II)₂₅Cl₁₀). The location of the chloride anion in the cavity of the pentamer was verified using single crystal X-ray crystallography [10, 11].

- **Scheme 14.2.** Lehn’s template adjustable DCL containing different-sized circular helicates. On the right, a sulphate-induced hexamer (Fe(II)₂₆(SO₄)₆) and on the left a chloride-binding pentamer (Fe(II)₂₅Cl₁₀). The location of the chloride anion in the cavity of the pentamer was verified using single crystal X-ray crystallography [10, 11].
become an issue. Such harsh conditions may limit the types of functionalities that can be incorporated into the building blocks, as well as the types of supramolecular interactions that may be involved in recognition of a template.

The search for suitable reversible covalent reactions that operate under mild conditions has led to the exploration of many different reactions for dynamic combinatorial chemistry [1, 17]. These include various C=N exchange reactions (e.g., imines, hydrazones, and oximes) [18], C–C bond forming reactions (e.g., the Diels–Alder reaction [19], the Henry reaction [20], and olefin metathesis [21]), various acyl-transfer reactions (e.g., transesterification and transthioesterification) [16, 22], and disulfide exchange [23]. Noncovalent interactions (e.g., metal–ligand coordination and hydrogen-bonding motifs) have also been explored but will not be discussed herein [1]. Due to their mild exchange conditions and fast reaction times, imine, hydrazone, and disulfide exchanges have become the most widely used reactions in dynamic combinatorial chemistry [24, 25].

Scheme 14.3. Sanders’ transesterification library used to identify cyclic oligocholates receptors for sodium ions [14, 16].
When a new receptor has been identified in a DCL, it is often desirable to isolate the receptor so that its recognition properties may be studied. At this point, the lability of the reversible bond is no longer beneficial, as the isolated receptor might start equilibrating again, reforming the DCL. Therefore, an additional requirement for a suitable exchange reaction is that the reversibility of the process must be tamable. What is required is that the reaction is reversible under one set of condition and static (non-equilibrating) under another set of conditions. This is nicely illustrated with the disulfide exchange process. Studies on thiol oxidation and thiol–disulfide exchange have shown that (i) thiols oxidize spontaneously at 25°C in the presence of O$_2$ under weakly alkaline conditions [26]; (ii) thiol–disulfide interchange takes place under mild conditions at weakly basic pH [27, 28]; (iii) thiol–disulfide interchange is negligible under acidic conditions [29]; (iv) disulfide exchange does not happen when the all thiols are oxidized to disulfides; and (v) disulfide and thiol functionalities are compatible with a wide variety of other functionalities [27]. This means that disulfide DCLs may be initiated by dissolving building blocks containing thiol functionalities in water at slightly alkaline pH, and after equilibration, the reversible reaction can either be stopped by complete oxidation to disulfides or by making the solution acidic.

It is important always to check that a DCL has reached thermodynamic equilibrium so that the relative abundances of library members may be correctly interpreted as a reflection of their relative stabilities, and so that addition of a template will successfully alter the library distribution to lower the free energy of the system by amplifying effective receptors. The approach to equilibrium can sometimes, however, be hampered by precipitation or by the occurrence of kinetic traps. Kinetic traps may occur when certain library members are overly stabilized by favorable inter- or intramolecular interactions (e.g., aggregation). Once formed, these stabilized library members can be very slow to exchange their building blocks with other library members and so the library becomes trapped in a kinetically controlled, rather than thermodynamically controlled, regime.

Due to the reversibility of the reactions that link the building blocks in DCLs, thermodynamic equilibrium in a DCL can be approached from different starting points (Scheme 14.4). It is therefore possible to test if equilibrium has, in fact, been reached by combining building blocks or library members in different orders at different time points and observing whether they ultimately achieve the same distribution. If a DCL is set up using only one building block, it is most convenient to simply isolate a library member and allow this to reequilibrate. If two or more building blocks are being used, there are more possible ways to test for thermodynamic control. For example, two DCLs could be set up: one in which all the building blocks are combined together and one in which the building blocks are first equilibrated alone and then mixed together. Once equilibrium has been reached, the two libraries will be identical. If one is studying a DCL that is affected by the addition of a template, then it is also possible to test for thermodynamic control by adding the template at different times and checking that the same library composition results.

This introduction has focused on how the equilibrium composition in a DCL can be affected by the addition of templates. There are other external stimuli, such as pH, pressure, temperature, light, and electric field that can alter library compositions, and the interpretation of these changes can lead to other types of information about the studied system [24, 30]. In the following sections, we will focus on how dynamic combinatorial chemistry is used to study molecular recognition and, in particular, to discover new receptors.
In this chapter, we will describe selected examples from the recent literature on dynamic combinatorial chemistry. We will illustrate how the use of a small number of dithiol building blocks can create large diversity in disulfide-DCLs (Section 14.2); this approach has given some of the strongest known receptors for ammonium ions. We will show how DCLs can be used to optimize the binding properties of already known receptors for anions (Section 14.3) and how the exploration of DCLs has led to new knowledge about mechanically interlocked molecules (Section 14.4). We will describe pseudopeptide DCLs that have been used to identify receptors for both anions and cations using hydrazine exchange chemistry (Section 14.5), and how boronate trans-esterification can be applied in dynamic combinatorial chemistry (Section 14.6). Section 14.7 describes how DCLs can target biologically interesting molecules such as proteins, DNA, and carbohydrates. Section 14.8 describes how modeling studies using computational methods have been used to aid in library design and how the determination of binding constants between DCL members and templates can be achieved by means of modeling studies without actually isolating the receptors.

### 14.2 CREATING DIVERSITY WITH FEW DISULFIDE BUILDING BLOCKS

One of the most attractive features of combinatorial chemistry is the ability to quickly and easily generate very large libraries of molecules with diverse structures.
and properties by mixing together only a few building blocks. This section describes how a small number of dithiol building blocks have been used to generate receptors [31] that bind ammonium ions in water with some of the highest binding affinities observed for synthetic receptors.

Sanders and coworkers have explored DCLs [32] formed from dithiol building blocks (5, 6, and 7) that resemble the aromatic components of an ammonium ion receptor (4) reported by Dougherty and coworkers (Scheme 14.5) [33–35]. Air oxidation of the three building blocks in water at pH 9 gave rise to a DCL, and analysis using mass

**Scheme 14.5.** Structure of ammonium receptor 4 [35] together with dithiol building blocks 5, 6, and 7, and the two macrocycles, 8 and 9, that are amplified in the dynamic combinatorial library upon addition of two different guests (2-methylisoquinolinium iodide and N-methylated morphine) [32].
spectrometry revealed that the DCL contained more than 45 different macrocyclic oligodisulfides [32]. The library was exposed to 2-methylisoquinolinium iodide and a change in library composition was observed; macrocycle 8 was significantly amplified at the expense of most of the other library members. The amplified macrocycle was not the tetradisulfide analog of receptor 4 but rather contained one phenyl linker fewer. A high binding constant of $2.5 \times 10^5$ M$^{-1}$ was measured for the interaction between receptor 8 and the 2-methylisoquinolinium iodide guest, which was similar to the affinity reported for the known receptor [32, 35].

When the same library was templated instead with N-methylated morphine, macrocyclic trimer 9 was amplified. Trimer 9 was isolated, and binding studies with the guest confirmed a strong binding interaction ($K_a = 7.1 \times 10^5$ M$^{-1}$) [32]. Biased libraries that contained, in the appropriate ratios, only those building blocks that made up macrocycles 8 and 9, respectively, were prepared. When templated with their respective guests, macrocycles 8 and 9 were efficiently amplified in the libraries to constitute 60% and 95% of the total library material [32].

The possibility of uncovering unexpected receptors in DCLs is illustrated by the identification of tetrameric receptor 10, which was amplified in a DCL formed from building block 5 upon templating with Me$_4$NI (Scheme 14.6) [36]. Building block 5 was used as a racemic mixture and was therefore capable of generating four different diastereomeric cyclic tetramers. However, it was stereoisomer 10, with alternating RR and SS building blocks, that was amplified with an amplification factor of 400 while the other three possible diastereomers were only slightly amplified. This result shows how it is possible to obtain diastereoselective amplification in DCLs. The amplification of tetramer 10 was rather surprising, given that Me$_4$NI is much smaller than the morphine derivative that amplifies trimer 9. It was speculated that 10 folds into a four-stave barrel shape so as to create a suitably small cavity to bind Me$_4$NI. The binding constant between tetramer 10 and Me$_4$NI was found to be $4.0 \times 10^6$ M$^{-1}$, while trimer 9 binds Me$_4$NI with a binding constant of only $8.0 \times 10^2$ M$^{-1}$ [36].

![Scheme 14.6](image-url)

**Scheme 14.6.** Building block 5 formed a DCL of oligomers from which tetrameric receptor 10 was amplified upon addition of Me$_4$NI [36].
Dynamic combinatorial chemistry can also reveal interesting structures even when no guest is present. An example of this behavior was the discovery of octameric [2]catenane 12, which forms quantitatively (as a mixture of isomers) from naphthalenedithiol building block 11 (Scheme 14.7) [37]. It was proposed that 12 assembles as a result of the hydrophobic effect; unfavorable exposure of the hydrophobic interior of the macrocycles to water is minimized by catenane formation. It was shown to be possible to separate the rings of the catenane and transform 12 into tetrameric macrocycle 13 by introducing an adamantane-derived ammonium ion guest that binds tightly inside the cavity of 13.

Dynamic combinatorial chemistry has produced some of the strongest synthetic receptors known that are selective in a highly competitive aqueous environment. The high binding affinities shown by many of the disulfide-based macrocycles discovered are close to those seen for recognition events in biological systems. Tetrameric receptor 15, for example, formed from the teraphthalic acid-derived building block 14, was identified from a DCL templated with the polyamine spermine, which it was found to bind with an association constant of \((4.5 \pm 0.3) \times 10^7\) M\(^{-1}\) (Scheme 14.8) [38]. It has been shown that receptor 15 can compete with the interaction between spermine and DNA [38]. The addition of spermine to solutions of certain DNA sequences can cause their helicity to change from right handed to left handed. By addition of 15 to the spermine–DNA complex, the helicity of the DNA was reverted to its original right-handed helicity, as monitored by circular dichroism (CD) spectroscopy.

Scheme 14.7. Building block 11 forms [2]catenane 12, which is transformed into tetrameric receptor 13 upon binding to the adamantane ammonium ion guest [37].

Scheme 14.8. A DCL from which the cyclic tetramer (15) was amplified by templating with spermine [38].
If dynamic combinatorial chemistry is used to seek receptors for guest molecules that closely resemble the transition states of given reactions—also known as transition-state analogs—it may be possible to thereby identify supramolecular catalysts from DCLs [39]. This approach has been explored for the Diels–Alders reaction [40] and for acetal hydrolysis [41]. It is most appropriate for reactions where the transition states for the rate-determining steps are markedly different from both the starting material and the product, as this minimizes the risk of product or substrate inhibition. This is the case for the acetal hydrolysis reaction shown in Scheme 14.9; a neutral reactant is converted to neutral products via a positively charged transition state, which resembles an ammonium salt that can be used as the transition-state analog. To find a receptor for the transition-state analog, a DCL was established from dithiol building blocks \( \text{5, 6, and 11} \). Upon addition of the transition-state analog, cyclic trimer \( \text{9} \) was amplified. It was subsequently isolated, and kinetic studies showed that the rate of hydrolysis of the acetal was accelerated by a factor of 2 in the presence of \( \text{9} \).

In the above-mentioned examples, the obtained catenanes and macrocyclic receptors are based on dithiol building blocks. However, in a study by West et al., it was shown possible to expand this array of structures to include organic cages by combining di- and trithiol building blocks [42]. Stefankiewicz et al. later described the template-induced amplification of large cages in a DCL [43]. Dithiol \( \text{6} \) and trithiol \( \text{16} \) were combined to generate a self-sorted mixture containing macrocycles of \( \text{6} \) (trimer and tetramer) and a dimeric cage of \( \text{16} \). When templated with protonated polyamine guests (e.g., spermine), disulfide-linked cages containing up to 11 components were amplified (Scheme 14.10).

From the examples presented, it can be seen that by using only a few different building blocks (\( \text{5, 6, 7, 11, 14, and 16} \)), it has been possible to prepare synthetic receptors with diverse structures for a wide range of guests, which in many cases display very high affinities. Using optimized conditions, DCLs can produce receptors in high yields while avoiding long and complicated syntheses.
Dynamic combinatorial chemistry can be used not only to discover new hosts but also to optimize the structures of known receptors. This is exemplified in the development of high-affinity cyclo-peptide-based receptors for anions in aqueous environment.

Kubik and coworkers have studied cyclo-peptide receptors for molecular recognition of anions [44], cations [45], and ion pairs [46]. They described a cyclic hexapeptide 17 (Figure 14.2) with alternating L-proline and 6-aminopicolinic acid subunits which was shown to form sandwich-type 2:1 complexes with anions such as halides and sulfates [47].

In subsequent work, it was found that these complexes could be stabilized by covalently linking the two cyclo-peptide units together via 1,6-hexanedioic acid to give receptor 18a. The binding affinity for SO\text{4}^{2−} was found to be $K_a = 3.5 \times 10^5$ M\textsuperscript{−1} in 50% CD\textsubscript{3}OD/D\textsubscript{2}O [48]. Minor improvements in binding affinities were achieved by replacing the flexible linker with, for example, a more rigid aromatic linker to give receptor 18b [49]. It was reasoned that increased rigidity would decrease the entropically unfavorable loss of conformational flexibility upon complexation of the anion.

While variation of the linker by a designed approach led to small improvements, it was found that dynamic combinatorial chemistry was an alternative and effective method to optimize the linker. The cyclo-peptide scaffold was functionalized with a thiol to enable the use of disulfide exchange to explore linker possibilities. Cyclo-Peptide dimer 19 was equilibrated with six different dithiol linkers (a–f) in 67% MeCN/H\textsubscript{2}O to generate a DCL containing cyclo-peptide rings separated by one or more different dithiol linkers (Figure 14.3). The library was exposed to different anions and a series of amplifications were observed. Binding studies showed that receptors 20b and 20c bound SO\text{4}^{2−} an order of magnitude more efficiently than receptor 18a [50].
Rodriguez-Docampo et al. extended these studies to receptors in which the two cyclo-peptide rings were connected via two linkers [51]. A DCL was prepared by mixing bis-cyclo-peptide 21a with two dithiol spacers (1,2-ethanedithiol and 1,3-benzenedithiol) in 67% MeCN/H$_2$O at pH 9 with various anions as templates (Figure 14.4). After equilibration, significant amplifications of the doubly linked dimer of macrocycles 21b and 21c were observed with all added anions but most notably with the SO$_4^{2-}$ anion. The new anion receptors possessed extraordinary binding affinities for the SO$_4^{2-}$ anion of $4.7 \times 10^8$ and $3.9 \times 10^7$ M$^{-1}$, respectively [51].

These examples illustrate how elements of design and selection may work together to discover highly effective receptors. The building blocks are carefully designed, but in the DCL, self-selection of the most suitable linker enables efficient synthesis of improved receptors.

### 14.4 DONOR–ACCEPTOR MECHANICALLY INTERLOCKED MOLECULES FROM DYNAMIC COMBINATORIAL LIBRARIES

Mechanically interlocked molecules may be synthesized either by means of a kinetically controlled or a thermodynamically controlled approach. When self-assembled under thermodynamic control, as in a DCL, the components of the interlocked molecule are allowed to react reversibly with one another until an equilibrium mixture is reached. Ideally, the desired interlocked structure will be stabilized by intramolecular interactions (e.g., donor–acceptor interactions and hydrophobic interactions) between the interlocked components such that minimization of the total free energy of the DCL
Figure 14.3. Dynamic combinatorial optimization of bis-cyclo-peptide anion receptors using disulfide DCLs [50].
Figure 14.4. Optimization of doubly linked bis-cyclo-peptide (21) using dynamic combinatorial chemistry [51].

will lead to the amplification of this structure. If well designed, such self-assembled interlocked molecules may be obtained in high yields that are not easily achieved using kinetically controlled synthesis.

One example of the use of dynamic combinatorial chemistry in the synthesis of [2] catenanes was provided by Miljanić and Stoddart [52]. They described the thermodynamically controlled synthesis of a donor–acceptor [2]catenane (22) in MeCN from two isolated macrocycles: \( \pi \)-acceptor \textit{cyclo}-bis(\textit{paraquat}-\textit{p}-phenylene) (23) and \( \pi \)-donor bis-\textit{p}-phenylene[34]crown-10 (24) (Scheme 14.11). The assembly was achieved using a Bu\(_4\)NI-catalyzed reversible benzylic nucleophilic substitution and was initiated by the nucleophilic opening of the \textit{cyclo}-bis(\textit{paraquat}-\textit{p}-phenylene) ring (23) by the iodide ion. Thermodynamic control of the system was demonstrated by the exchange of the donor ring, bis-\textit{p}-phenylene[34]crown-10 (24) for 1,5-dinaphthol[38]crown-10.

Using dynamic nucleophilic substitution, Stoddart and coworkers have also synthesized a [3]catenane [53], bis[2]catenanes [54], and a side-chain donor–acceptor polycatentane [54]. The high yields obtained for these complex structures highlight the efficiency of a thermodynamic rather than kinetic approach to catenane synthesis.

Au-Yeung et al. have described a series of donor–acceptor catenanes assembled via disulfide exchange from \( \pi \)-electron-rich dialkoxy-naphthalene donor units (D) and \( \pi \)-electron-deficient naphthalenediimide acceptor units (A) (Figure 14.5a). The building blocks consist of aromatic scaffolds that are functionalized with cysteine units at
Scheme 14.11. Reaction scheme for the formation of [2]catenane 22 from 23 and 24 via reversible benzylic nucleophilic substitution with Bu₄NI acting as a catalyst [52].

Figure 14.5. (a) Structures of the two building blocks: π-donor dialkoxynaphthalene (D) and π-acceptor naphthalenediimide (A), together with their cartoon representations. (b) Arrangements of the π-units in the donor–acceptor [2]catenanes discovered using dynamic combinatorial chemistry [61].
the end of a linker to give both water solubility in weakly alkaline solution and access to reversible covalent chemistry through disulfide exchange [55–61].

DCLs were prepared from these building blocks in water and in aqueous NaNO$_3$ solutions. By increasing the salt concentration, and therefore the ionic strength of the aqueous solution, it was found possible to amplify the formation of catenanes at the expense of macrocycles. It was reasoned that increasing the polarity of the solution would increase the role of the hydrophobic effect in dictating which library members were formed. Catenane formation would be favored as this leads to a decrease in the amount of solvent-exposed hydrophobic surfaces. In the DCLs, catenanes with previously unobserved stacking arrangements were formed. Not only was the conventional alternating DADA catenane identified but also catenanes with AADA, DAAD, and DADD arrangements of the $\pi$-donor and $\pi$-acceptors were discovered (Figure 14.5b) [61].

It was also found possible to increase the yield of the DAAD [2]catenane by the presence of a template [56, 60]. Considering that the DAAD [2]catenane is anionic (due to the carboxylic functionalities) and contains a $\pi$-electron-deficient interior cavity, the cationic $\pi$-electron-rich compound 25 was chosen as template for the system. The addition of dialkoxynaphthalene-based template 25 led to amplification of the DAAD [2]catenane at the expense of all other macrocycles and catenanes, and by means of nuclear magnetic resonance (NMR) studies, it was determined that the template was located inside the cavity of the [2]catenane, as shown in Scheme 14.12 [56].

Lessons learned from the discovery and exploration of this family of [2]catenanes have been exploited to synthesize higher order interlocked structures. Cougnon et al. reported that by combining a $\pi$-donor (26) with an extended version of the $\pi$-acceptor (27), it was possible to obtain a donor–acceptor [3]catenane (28) in a highly polar solvent (1 M aqueous NaNO$_3$) or in the presence of spermine acting as a template (Scheme 14.13) [62].

In DCLs formed via disulfide exchange, the exchange reaction will stop once all the thiol building blocks have been completely oxidized to disulfides. It is therefore possible that exchange will be halted and the library composition will be fixed before the libraries have reached thermodynamic equilibrium. This was the case for many of

the disulfide-linked donor–acceptor catenanes just described. While it is important to be aware of this phenomenon, such kinetically trapped molecules discovered in DCL may nevertheless exhibit fascinating structures. Furthermore, dithiothreitol (a dithiol) may be added to the library as a reducing agent to reinitiate exchange in a fully oxidized library and promote equilibration toward the thermodynamic minimum of the system [61].

The examples highlighted in this section demonstrate how dynamic combinatorial chemistry can be used to generate complex and delicate interlocked structures. Knowledge about donor–acceptor interactions, gleaned from years of molecular engineering work, has been exploited to design sophisticated DCLs from which unexpected architectures and molecular recognition motifs have been revealed.

14.5 HYDRAZONE-BASED DYNAMIC COMBINATORIAL LIBRARIES

Dynamic combinatorial chemistry using acylhydrazone exchange as the reversible reaction has gained significant popularity since it was introduced in 1999 [63]. Acylhydrazones are formed from a hydrazide and an aldehyde, and they equilibrate under acidic conditions in aqueous solution and in a variety of organic solvents (Scheme 14.14). Libraries of oligomers may be formed from building blocks containing both a hydrazide and an aldehyde or from dialdehydes and dihydrazides. When using building blocks that contain both aldehyde and hydrazide functionalities, it has proven convenient to protect the aldehyde with an acid-labile acetal that allows stable
building blocks to be synthesized. By adding acid to the isolated building block, the acetal deprotects to the aldehyde and acylhydrazone formation and exchange begin immediately.

The lability of the hydrazone linkage coupled with the high dependency of the rates of formation, hydrolysis, and exchange upon pH—therefore the ability to switch on the reaction in acid and off in base—makes hydrazone exchange a good reaction for use in DCLs. The acylhydrazones are less prone to hydrolysis than imines, which make it possible to isolate library members from DCLs.

In the first publication describing acylhydrazone-based DCLs, the building block used was a dipeptide with the C-terminus equipped with a hydrazide and the N-terminus elongated via an amide linkage to an aromatic aldehyde (1) (Scheme 14.15) [63]. The use of an aromatic aldehyde ensured that the equilibration proceeded smoothly at room temperature and side reactions such as aldol condensations were avoided. The acylhydrazone DCL was formed by dissolving 1 in CHCl₃ and adding a small amount of trifluoroacetic acid (TFA). Analysis by HPLC and electrospray ionization–mass spectrometry (ESI-MS) showed a mixture of acylhydrazone macrocycles. The ability of the library to adapt in the presence of a template was demonstrated by addition of [18]-crown-6. By binding to the protonated hydrazides on the monomer building block (1) and other linear oligomers, addition of [18]-crown-6 caused a shift in the constitution of the DCL to amplify these species. The constitution of the DCL could then be shifted back to the original mixture of macrocycles by addition of K⁺-ions to complex the crown ether and release the hydrazides.

A series of pseudopeptide building blocks for acylhydrazone DCLs has since been explored. The most noteworthy example of receptor amplification in these libraries was the discovery of a hexameric catenated high-affinity receptor (30) for acetylcholine by Lam et al. (Scheme 14.16) [64]. The [2]catenane (30) was amplified and isolated in 67% yield from a DCL formed from building block 1, which contained mainly cyclic dimer, trimer, and tetramer in the absence of a template. The [2]catenane had an exceptionally high binding affinity for acetylcholine chloride of $K_a = 1.4 \times 10^7$ M⁻¹ in a 95:5 CHCl₃:DMSO mixture, while the cyclic trimer and the cyclic tetramer had binding affinities of $1.5 \times 10^7$ and $5.7 \times 10^7$ M⁻¹, respectively. The $^1$H-NMR spectrum of the [2]catenane alone exhibited very broad features indicative of a mixture of

Scheme 14.15. The first reported DCL using acylhydrazone exchange [63]. The composition of a hydrazone DCL can be shifted toward the linear hydrazides by addition of [18]-crown-6 [130].
conformations in solution. Upon addition of one equivalent of acetylcholine, however, the spectrum simplified to show sharp signals, which suggested that the acetylcholine was bound tightly by a single conformation of the catenane. The 2]catenane (30) has the same molecular weight as the cyclic hexamer, but the two different species were convincingly differentiated using MS-MS. The cyclic hexamer gives the pentamer, tetramer, and smaller oligomers as daughter ions, while the 2]catenane fragments directly to the trimer. The identification of this completely unexpected receptor illustrates the power of dynamic combinatorial chemistry to reveal instances of molecular recognition that would perhaps not have been uncovered using a purely designed approach to receptor synthesis.

From DCLs of acylhydrazones generated in CHCl₃ or CH₂Cl₂ (often containing small amounts of MeOH or dimethyl sulfoxide [DMSO]) in the presence of TFA [65–67], macrocyclic receptors for alkali-metal ions [5], tetraalkylammonium ions [68], and cationic alkaloids have been identified. The chirality of the amino acids has been varied and diastereoselective and enantioselective recognition have been achieved [69]. Additional catenanes have been isolated and characterized by the group headed by Gagné and Waters using dipeptide-based building blocks. They described [2]catenanes of tetramer-macrocycles that form in the absence of a template [69–71] & in similar libraries, they found strong receptors for nucleic acids and derivatives such as adenosine [72].

Most of the studies described earlier were performed in an organic solvent using a strong acid (typically 1–5% TFA) to facilitate the hydrazone exchange reaction. These rather harsh reaction conditions preclude the use of building blocks containing delicate functional groups. The use of a large excess of TFA in libraries may also interfere with molecular recognition events guided by hydrogen bonding. If milder acids are used, there is a risk that the exchange reaction will be slowed down such that the time frame of the DCL experiment becomes too long and the risk of problematic kinetic traps.
increases. In the case of hydrazone-based DCLs, some practical solutions to prevent kinetic traps and to speed up the equilibrium process have been introduced. It has been shown that addition of a large excess of aniline to hydrazone DCLs accelerates the rate of exchange significantly in aqueous solutions [73]. By addition of aniline it has also been possible to reach equilibrium at the relatively high pH 6 [74]. Addition of a smaller excess of monohydrazide has been shown to facilitate exchange and avoid the occurrence of kinetic traps in acylhydrazone DCLs [75]. Furthermore, when 10 equiv. of monohydrazide is added to the library, a milder acid (e.g., 1-naphthoic acid or acetic acid) may be used to promote the exchange reaction.

Using this last approach, it has been shown that peptide-based building blocks may also be utilized to form anion receptors [76, 77]. DCLs were prepared from a ferrocene containing dihydrazide building block (31, unstable to prolonged exposure to TFA), an aromatic dialdehyde, excess of 4-toluic-hydrazide (10 equiv.), and 1-naphthoic acid. This gave a DCL containing a mixture of linear and cyclic library oligomers. Upon addition of dihydrogenphosphate anions to the library, a series of linear oligomers were amplified. The five-component linear receptor (32) adopts a helical conformation and binds two anions in a cooperative manner (Figure 14.6).

Hydrazone exchange has been used in many interesting conceptual studies in dynamic combinatorial chemistry; acylhydrazones have been used in the recognition of proteins [74], in the dynamic self-assembly of hydrogels based on G-quadruplex DNA structures [78], in the dynamic synthesis of cages, and in self-replicating systems [78]. In a series of papers, Lehn and coworkers have used hydrazone exchange to produce dynamic polymers, the so-called dynamers [79, 80]. This section is by no means a comprehensive review. Hydrazone exchange has, together with disulfide exchange, become the reversible reaction of choice in dynamic combinatorial chemistry.

14.6 BORONATE ESTER EXCHANGE IN DYNAMIC COMBINATORIAL CHEMISTRY

Boronic acids react with 1,2-diols and 1,3-diols to give boronate esters, and this property has been exploited extensively in sensors for carbohydrates [81, 83]. The lability of B–O bonds causes a number of processes involving boronic acids to be reversible, including boroxoaromatic transesterification [91] and boroxine exchange [92] as well as boronate transesterification (Scheme 14.17) [93, 94]. Herein we discuss the use of boronate transesterification to generate DCLs of adaptive macrocycles and capsules [95, 96] and to seek enzyme inhibitors [97].

Boronate transesterification has been used to prepare libraries of oligoboronate esters by reacting bis-boronic acids with tetraols [98–101]. When, for example, 1,4-benzene-bis-boronic acid and tetraol 33 were reacted together in a methanol solution containing either toluene or benzene, the macrocyclic 2:2-tetramer (34) or 3:3-hexamer (35), respectively, were precipitated and subsequently isolated in high yield (Scheme 14.18) [100]. Analysis by NMR spectroscopy and single crystal X-ray crystallography showed that the macrocycles were isolated as inclusion complexes with one toluene molecule and two benzene molecules, respectively, bound in the center.
Figure 14.6. A hydrazone DCL that amplifies a linear tetrahydrazone (32) at the expense of cyclic structures in the presence of dihydrogenphosphate. The receptor binds two $\text{H}_2\text{PO}_4^-$ ions in a cooperative manner and adopts a helical conformation upon binding. Source of HPLC chromatograms: [77]. Reproduced with permission of the American Chemical Society.
In this example, the products precipitated from the mixture, which means that the library was no longer operating under thermodynamic control. In such situations, the library composition will not necessarily reflect the receptors’ respective binding abilities [102]. It will be dependent on the relative solubility of the macrocycles in the particular solvents, as well as on the binding interactions and crystal packing forces. While not strictly a DCL, the authors showed here how the reversibility of boronate transesterification may be exploited for the template-assisted self-assembly of oligoboronate esters. They also showed how to halt the equilibration of boronate esters by changing the solvent composition. It was found that if a suspension of tetrameric macrocycle 34 was stirred in a mixture of methanol and benzene, the nature of the suspended material changed, and hexameric macrocycle 35 was isolated (Scheme 14.19) [102]. Without methanol, no boronate ester interconversion was observed despite changing the nature of the template.

Kubo and coworkers described the reversible formation of boronate-ester-based capsule 36 from cyclo-tricatechylene and a boronic-acid-appended hexahomotrioxacalix[3]arene in the presence of Et₄NOAc (Scheme 14.20) [103]. When the two building blocks were mixed in a combination of methanol and acetonitrile, no boronate ester formation was observed, whereas when Et₄NOAc was added as a template, the 1:1 dimer was generated quantitatively. NMR analysis of the templated mixture showed several pieces of evidence that the Et₄N⁺ ion was bound in the cavity of the capsule: (i) new signals for the bound guest’s ethyl groups appeared that were shifted upfield.
Scheme 14.19. Transesterification takes place in the presence of methanol (top) but is switched off in its absence (bottom). In the absence of MeOH, the bound guests exchange upon changing the solvent, but only with MeOH as a cosolvent does the interconversion of tetramer 34 and hexamer 35 take place [102].

Scheme 14.20. Dynamic formation of a boronate-ester-based capsule (36) [103].

by −1.5 ppm, indicative of an aromatic shielding effect; (ii) nuclear Overhauser effect cross peaks were observed between these new ethyl group signals and selected protons on the capsule; and (iii) almost identical diffusion coefficients for the capsule and the ethyl group signals were obtained by diffusion-ordered spectroscopy (DOSY) [103].

When different Et₄N⁺ salts were tested, more modest capsule formation was observed compared with Et₄NOAC, which indicated that capsule formation was not just cation,
but ion-pair driven. Titrations using UV/Vis spectroscopy of the capsule mixture with 
different templates showed that the equilibrium values increased in the order 
$\text{Et}_4\text{N}^+ > \text{Me}_4\text{N}^+ > \text{K}^+$ with $\text{AcO}^-$ salts and in the order $\text{AcO}^- > \text{F}^- > \Gamma^-$ with $\text{Et}_4\text{N}^-$ salts 
[103]. From these results, the authors concluded that capsule formation was dictated 
mainly by size of the cation and basicity of the anion. Kubo also demonstrated that the 
capsule assembly could be turned on and off simply by switching the pH of the solution. 
An NMR study showed that when HCl was added to a solution of the $\text{Et}_4\text{NOAc}$-bound 
capsule, it collapsed but was reformed when the pH was readjusted by addition of 
$\text{NaHCO}_3$ to the solution [103].

In a subsequent study, Kubo and coworkers showed that formation of the same 
capsule (36) could also be triggered by addition of $\text{Et}_3\text{N}$, which was encapsulated in 
the host as its conjugate acid, $\text{Et}_3\text{NH}^+$ [104]. Furthermore, addition of $\text{Bu}_3\text{N}$ also led to 
capsule formation; but in this case, no guest was bound in the capsule. The molecular 
recognition behavior of the now empty capsule was then investigated. Using competi-
tive binding assays, it was found that the relative affinity of the capsule for certain 
organic cations was $\text{Et}_4\text{N}^- > \text{Me}_4\text{N}^- > \text{Me}_4\text{P}^+$, and additionally, that $\text{Cs}^+$ could be encap-
sulated [104].

Schofield and coworkers recently described the use of boronate transesterification 
in DCLs aimed at discovering new enzyme inhibitors [97, 105]. In their latest study 
[97], an aromatic boronic acid was appended to a known binder of an oxygenase 
enzyme (propyl hydroxylase) and then combined in aqueous buffer at pH 7.5 with a 
range of diols. The DCL was then incubated with the enzyme, and the best binder was 
identified using mass spectrometry (Scheme 14.21). Binding studies with the selected 
boronate ester revealed high-affinity binding to the enzyme in a micromolar binding 
regime. A covalent analog of the identified boronate ester was then prepared, and it 
too displayed strong binding to the enzyme.

The use of boronate transesterification as the exchange reaction in dynamic com-
binatorial chemistry has the advantage that it is reversible both in organic solvents and 
in water at physiological pH. It can also be used in combination with other exchange 
processes such as metal–ligand exchange [106] and imine exchange [107]. Boronate 
esters, however, are often unstable, and it is not trivial to identify condition under which 
they do not exchange or hydrolyze. The analysis of DCLs of boronate esters and the 
isolation of library members can therefore be challenging. As a relatively new reaction 
in dynamic combinatorial chemistry, the development of exchange conditions for boro-
nate transesterification and the exploration of template effects and selection approaches 
are ongoing.

14.7 TARGETING BIOLOGICAL MOLECULES USING DYNAMIC 
COMBINATORIAL CHEMISTRY

The recognition of biomacromolecules is an area of particular importance as it has 
close ties to medicinal chemistry, molecular biology, and other biological chemistry 
disciplines. The use of dynamic combinatorial chemistry is ideal to address the goal of 
recognizing complex biomacromolecules and other natural products in a strong and 
selective manner in their natural surroundings. It is perhaps not surprising that some 
of the first studies using dynamic combinatorial chemistry had the aim of recognizing 
biomacromolecules. These early studies have been followed by a number of reports 
targeting proteins and DNA, and the progress of these studies has been extensively
reviewed recently [1, 108]. In this section, we will highlight the power of DCLs by describing a few examples where the targets are proteins, duplex DNA, G-quadruplex DNA, and carbohydrates.

When searching for molecules that recognize proteins, or more specifically, for enzyme inhibitors, it is perhaps appropriate to use Fisher’s classical metaphor, the lock-and-key principle. In a DCL designed to discover enzyme inhibitors, the library generates a series of possible keys for the lock that is the enzyme. The DCL may not necessarily contain library members corresponding to all possible combinations of the building blocks. There may be virtual keys in the DCL—keys (or library members) that are not present in amounts that can be detected in the DCL in the absence of the enzyme, but which may still be amplified after the addition of the enzyme. The role of the DCL is then to pick the correct key, amplify it, and synthesize it at the expense of the wrong keys.

The first efforts to recognize a protein using a DCL were described by Huc and Lehn in 1997 [109]. They used libraries of imines to target carbonic anhydrase, which is a Zn(II)-containing enzyme that is involved in the interconversion of CO\textsubscript{2} and HCO\textsubscript{3}\textsuperscript{−}. They used a series of amines and aldehydes to generate small libraries of imines (Scheme 14.22). To halt the exchange, and to enable the analysis of the DCLs, the
imines were reduced to the corresponding amines using NaBH₃CN. The most amplified imine in Huc and Lehn’s DCL was the structure shown in Scheme 14.22 (37 and 38) that had a similar structure to a known carbonic anhydrase inhibitor.

When using dynamic combinatorial chemistry for the recognition of biologically interesting molecules, it is desirable to use reversible reactions that equilibrate at or close to physiological pH. The use of imine exchange chemistry enables the reversible chemistry and templating to be performed at pH 6 [109]. Ramström et al. have described the use of acylhydrazones for the identification of enzyme inhibitors [110]. However, in their study, the DCLs were equilibrated at pH 4, and the inhibition studies were performed on the static library mixtures at physiological pH.

Greaney and coworkers recently established biocompatible conditions for hydrazone exchange by adding a large excess of aniline to a DCL of a mixture of an electron-deficient aromatic aldehyde and a series of hydrazides at pH 6.2 (Scheme 14.23) [74]. The aniline equilibrator allowed thermodynamic equilibrium to be reached within a few hours, which is fast compared to the 2 weeks used in Huc and Lehn’s imine DCLs [109]. Greaney and coworkers used two different isozymes of the glutathione S-transferase (GST) class of enzymes as their templates and found that two different acylhydrazones (39) were amplified by the enzymes.

In a conceptually different approach for the recognition of proteins, Waters and coworkers used disulfide exchange chemistry to prepare DCLs to target the trimethyllysine moiety in a histone peptide [111]. It has been shown that significant protein–protein interactions that can result in gene silencing may be induced by methylation of the side-chain amines of lysine residues. Building blocks with similar structures to those described earlier in the chapter (Section 14.3) were used to generate DCLs of oligodisulfide macrocycles (Scheme 14.24). These were template with a series of dipeptides: Ac–K–G–NH₂, Ac–KMe–G–NH₂, Ac–KMe₂–G–NH₂, and Ac–KMe₃–G–NH₂.

Scheme 14.22. Identification of an amine-based inhibitor (38) for carbonic anhydrase using an imine-based DCL [109].
Scheme 14.23. Structure of the electron-deficient aldehyde and the 10 hydrazides used for acylhydrazone-based DCLs to target glutathione S-transferase [74].

Scheme 14.24. Left: The eight dithiol monomers used to prepare the DCL. Right: The amplified receptor (40) that is selective for the trimethyllysine moiety [111].

(K = lysine, G = glycine), and it was observed that the use of Ac–KMe₃−G−NH₂ gave rise to a large amplification of a cyclic trimer (40). The cyclic trimer (40) was isolated as a mixture of stereoisomers and found to exhibit almost native protein-like affinity. The trimethyl derivative Ac–KMe₃−G−NH₂ was bound more than twice as strongly as the dimethyl derivative Ac–KMe₂−G−NH₂ (Kₐ = 25 µM vs. 58 µM), and the monomethyl derivative and the nonmethylated lysine were bound only weakly.

It is important to emphasize that in this example, a small motif of a protein rather than the actual protein was targeted. This approach could, in principle, be important in the study of biological systems using supramolecular chemistry.
The biomimetic recognition of carbohydrates is a topic of great current interest, and the complexity of carbohydrate structures makes a dynamic combinatorial approach attractive to explore. Ravoo and coworkers studied a DCL of disulfide macrocycles assembled from different tripeptide building blocks (41) of the general form Cys–(amino acid)–Cys [112]. It was found possible to amplify the cyclic homodimers of the tripeptides Cys–Thr–Cys and Cys–His–Cys in the presence of the monosaccharide α-D-methylfucopyranoside (MFP) and the neurotransmitter N-acetyl neuraminic acid (NANA), respectively. Furthermore, a cyclic homo-dimer of the tripeptide Cys–Tyr–Cys (42) was formed preferentially in the library in the presence of the disaccharide trehalose (Scheme 14.25). A binding constant of $2.2 \times 10^3$ M$^{-1}$ was determined for this complex, which makes the receptor among the strongest biomimetic carbohydrate receptors to have been reported at this time. The selection of synthetic lectins (carbohydrate-binding proteins) from DCLs could prove valuable in further studies and manipulations of the biological functions of carbohydrates.

The recognition of specific DNA and RNA strands in their various folded and unfolded forms has attracted attention in medicinal and bioorganic chemistry. Molecular recognition of double-stranded DNA has been especially thoroughly studied, and reliable guidelines for the design of major- and minor-groove binders have been established. The past decade has also experienced an increased interest in the study of DNA-based three-dimensional structures that fold in different ways other than the well-known double helix structure. Among these, the so-called G-quadruplex DNA (Figure 14.7) has played a prominent role. G-quadruplexes are present in G-rich regions of DNA, and they form in a templated fashion around a metal ion as a consequence of a combination of π-stacking between several G-tetrads, Watson–Crick base pairing, and Hoogsteen base pairing. The G-quadruplex DNA structures are overrepresented in gene promoter regions, which has made G-quadruplexes attractive targets to recognize and stabilize using supramolecular chemistry approaches.

The first example of the use of a dynamic combinatorial mind-set for the recognition of DNA structures was carried out by Miller and coworkers [113]. They prepared a DCL of coordination complexes based on salicylaldimines and Zn$^{2+}$ and used a resin-immobilized DNA target (Scheme 14.26). Binding of a specific component from the
DCL resulted in its removal from the solution since the DNA was immobilized on cellulose beads, which is different from most other DCL experiments where the amplification of a binding species is what is observed. Using this elegant approach, the micromolar DNA binder 43f was identified.

Balasubramanian and coworkers have targeted DNA in a DCL approach using disulfide exchange chemistry [114]. In most of the work using disulfide exchange chemistry in DCLs, the pH of the aqueous mixtures has been slightly alkaline (pH 8–9).

**Figure 14.7.** Left: Schematic illustration of a bimolecular G-quadruplex DNA structure. Right: Chemical structure of a DNA G-tetrad structure.

**Scheme 14.26.** A small DCL for the identification of a duplex DNA binder (43) [113].
Here a glutathione buffer (a thiol–disulfide buffer) was used to promote and enable equilibration within 24 h while using very dilute solutions of the disulfide building blocks at physiological pH. Inspired by a known duplex DNA minor-groove binder containing N-methylpyrroles and N-methylimidazoles, monothiol building blocks were designed that contained amide-linked N-methylpyrroles and closely resembled the minor-groove binder distamycin (44, Figure 14.8). It was found that upon equilibrating DCLs containing building blocks 45, 46, and 47 in the presence of either a duplex or a G-quadruplex DNA target, the conjugates 46·47 and 47·47 were amplified. The amplification factors were five to six times larger for the duplex DNA compared with the G-quadruplex DNA.

When disulfide DCLs are generated using a large excess of glutathione redox buffer, most of the thiol building blocks will be present in the DCLs as disulfide conjugates with glutathione. In this example, however, this did not pose a problem as both the DNA and the glutathione are negatively charged, so a bias toward the amplification of disulfides containing only the desired positively charged building blocks was expected in the presence of the DNA template. The relative affinities of the isolated disulfides toward the DNA templates correlated well to the relative sizes of the observed amplifications of the disulfide conjugates in the DCL. This was shown using a number of techniques, including measuring the DNA melting temperatures in the presence of the disulfides [114].

In another study, Bugaut et al. targeted G-quadruplex DNA in a similar fashion to that described earlier [115]. In this case, they explored an oxazole-peptide macrocycle—a type of quadruplex-binding ligand they had previously described [116]. A monothiol version of the quadruplex-binding ligand (48) was prepared and used as a scaffold upon which different monothiols could be appended (Figure 14.9). In one part of the study, they chose to explore different side chains (amines and guanidiniums) that would be positively charged at physiological pH (Figure 14.9, A–E). The largest amplifications were observed for the guanidinium-appended macrocycle in the presence of the c-kit G-quadruplex DNA sequence (a naturally occurring G-quadruplex DNA sequence). They isolated the various disulfides and studied their binding properties toward duplex DNA and G-quadruplex DNA. They found that there was a correlation between the amplification factors observed in the DCLs and the relative binding affinities of the isolated ligands. Furthermore, the disulfides that were amplified in the
The examples given earlier using proteins and DNA as templates in dynamic combinatorial chemistry serve to illustrate that DCLs may be used in complex systems. Several other studies using dynamic combinatorial chemistry to target biomacromolecules have been described using a variety of exchange reactions [1]. Most of these studies focus on relatively simple libraries so there is a lot of opportunity for exploration of more complex DCLs in the context of biomacromolecule recognition. While most of the studies using DCL to recognize biomacromolecules are at the proof-of-principle stage, the concept has the potential to be a valuable tool in drug discovery.

14.8 MODELLING OF DYNAMIC COMBINATORIAL LIBRARIES

14.8.1 DCL Simulations to Optimize Library Design

The distribution of library members in a DCL is governed by a complex network of equilibria that describe the exchange of building blocks, the intermolecular interactions between library members, and the intermolecular interactions between library members and added templates. As a consequence, the behavior of libraries in response to certain stimuli is complex and can sometimes appear counterintuitive; addition of a template, for example, will lead to the amplification of receptors, but not necessarily the best
possible receptor [4]. The library, as a whole, responds to the template to minimize the free energy of the entire DCL, and the response will be dependent on the relative stability of all library members and complexes formed, the template concentration, the building block concentrations, and the building block composition of the various library members. The development of computational methods to study DCLs has facilitated an investigation of the parameters that affect library distributions in order to gain a better understanding of how to optimize the design of DCLs.

In an ideal situation, when a template is added to a DCL, the best host that can be formed from the building blocks would be amplified above all other possible hosts. Unfortunately, this is not always the case. Severin and coworkers first highlighted two important situations where the correlation between amplification factor and the binding affinity of the host can break down [117]. In the first study, computational models of simple DCLs containing trimeric macrocycles formed from up to three different building blocks were generated using the computer program, Gepasi [118]. The effects on the DCLs’ distributions of addition of a template were investigated and revealed that there is an intrinsic tendency to amplify mixed macrocycles in preference to those containing only one type of building block. In a second study, DCLs containing only one building block, but that were capable of generating macrocycles with different sizes, were investigated [119]. Here it was observed that there is a tendency to amplify smaller macrocycles in preference to larger macrocycles.

These tendencies were first predicted on the basis of computer-simulated DCLs but have subsequently been confirmed experimentally [4, 120]. Saur and Severin demonstrated the effect of template concentration on the amplification of homo- and hetero-oligomers in a simple DCL of self-assembled trinuclear metallamacrocycles (Figure 14.10) [120]. Homotrimers AAA and BBB were mixed together to generate,

![Figure 14.10](image_url)

*Figure 14.10.* Left: A DCL of trinuclear metallamacrocycles AAA, AAB, ABB, and BBB formed via the self-assembly of [(p-cymene)RuCl$_2$]$_2$ and [Cp*IrCl$_2$]$_2$; together with the ligand 3-hydroxy-4-dimethylaminomethyl-2-(1H)-pyridone in phosphate buffer at pH 8. Right: $^7$Li NMR spectra in D$_2$O of the DCL after equilibration with (a) 40, (b) 15, (c) 5, (d) 2.5, and (e) 0.2 equiv. of Li$^+$ [120]. Source of NMR spectra: [120]. Reproduced with permission of The Royal Society of Chemistry.
at equilibrium, an approximately 1:3:3:1 statistical mixture of AAA, AAB, ABB, and BBB. AAA had been found to exhibit a strong affinity for Li$^+$ ($K_{\text{AAA}} \sim 10^3 \text{ M}^{-1}$), while BBB was a very poor receptor ($K_{\text{BBB}} \sim 0.1 \text{ M}^{-1}$). Library distributions upon addition of different amounts of Li$^+$ were analyzed by means of $^7$Li-NMR spectroscopy. Addition of 0.2 equiv. of Li$^+$ resulted in an amplification of AAA (the best binder), but as the Li$^+$ concentration was increased up to 40 equiv., the concentration of AAA decreased, and a mixed dimer AAB (a moderate binder) was amplified to the greatest extent.

These counterintuitive observations may be explained by the fact that the effect of adding a template to a DCL is determined by the additive effects of the interactions of each library member with the template. When there is a choice between producing a large number of moderately good hosts rather than a small number of stronger binders, the first of these options will sometimes be preferred. For example, the formation of two moderately binding heteromacrocycles may be preferable to the formation of only one strongly binding homomacrocycle. In the same way, a DCL may choose to amplify moderately binding dimers instead of stronger-binding larger oligomers because it can thereby increase the total number of available hosts. Ultimately, these effects are minimized when the different hosts have to compete for a limited number of templates, and therefore, the use of low template concentrations is advised [119].

In order to explore these trends in more complex systems and learn how to best design DCLs so that there is a good correlation between amplification factor and binding affinity, Corbett et al. developed a program called DCLsim that is capable of rapidly simulating thousands of DCLs containing more than 200 components with a wide variety of building block and template concentrations [4, 121, 122]. DCLs were generated computationally from combinations of up to seven different building blocks that were capable of forming dimers, trimers, and tetramers. The building block and template concentrations were systematically varied, and for each of 289 different sets of experimental conditions, 50 libraries were generated, with different randomly assigned binding affinities for each library member. The simulated amplification factors were then compared with the assigned binding affinities, and the probability that the best binder was among the most amplified species in the library was mapped against building block concentration and template concentration. It was concluded that the template concentration should be restricted to one-tenth of the total building block concentration to obtain an approximately 90% chance that the best host is one of the three most amplified [122]. It should be noted, however, that as the template concentration is decreased, there is an increase in the probability that good hosts are not detected because their concentrations are too low. It is therefore advisable to do preliminary screens of DCLs using a higher template concentration in order to increase the chance of seeing some kind of templating effect and then to repeat the setup of the DCLs with low template concentration to better ascertain the identity of the best hosts.

Additional computational studies have resulted in further suggestions for library design. Ludlow and Otto noted that increasing the size of the library (i.e., the number of building blocks) not only increases the probability of generating a strong binder, but it also increases the probability that a strong binder will be identified as a result of amplification [123, 124]. Orrillo and Furlan showed that the use of low template concentrations is also useful to promote the amplification of the best binder in DCLs in which library members have a tendency to interact with each other and form aggregates [125]. Severin also recommended that where possible, DCLs should be designed under reaction conditions such that the equilibria lie toward the building blocks rather than
the oligomers because when the library is mainly composed of free building blocks, there is an increased probability that the best host will be the most amplified [119].

14.8.2 Estimating Association Constants Directly from the DCL

The analysis of DCL composition is most frequently achieved by means of LC-MS and occasionally NMR spectroscopy. A qualitative assessment of the influence of a template on the library distribution may be obtained by visually comparing the HPLC chromatograms of templated and untemplated libraries. Quantification of binding affinity has then traditionally required the isolation of selected receptors followed by complexation studies using techniques such as NMR, UV/Vis, fluorescence, or CD spectroscopy, isothermal titration calorimetry (ITC), or a combination of these. The development of computational methods to model DCLs has recently enabled the direct estimation of association constants from the DCLs, circumventing the need for individual binding studies on isolated receptors.

In 2007, Ludlow et al. developed a program called DCLfit, which was built upon the aforementioned simulation program, DCLsim, and may be used to calculate binding constants between the hosts and templates in a DCL directly from the changes in library distributions observed in the presence of varying amounts of template [126]. The use of DCLfit requires first setting up a series of libraries in which the concentration of the guest is varied while keeping the building block concentrations and all other experimental condition constant. After equilibration of the libraries is complete, the concentrations of all the library members in each library are measured, typically using HPLC. A guess of the various association constants for each library member with the template, and also for the equilibrium constants between the library members, is entered into the program. The program then simulates a set of DCLs and calculates the expected concentrations of the library members in the presence of varying concentrations of template. It compares these to the experimentally determined concentrations and, thus informed, makes a new guess of the equilibrium and association constants. Through an iterative approach, association and equilibrium constants that fit the concentration data are determined.

DCLfit was first applied to a DCL of peptide-based hydrazone macrocycles formed from building block 49 (Scheme 14.27) [126]. It had been observed that addition of acetylcholine chloride to the library resulted in a significant amplification of the cyclic trimer. A series of DCLs with different concentrations of acetylcholine were set up, and the concentration of cyclic dimer, trimer, and tetramer were measured by means of HPLC. Using DCLfit, the data were fitted to the model equilibrium network shown in Scheme 14.27, and binding energies of 14.1, 22.1, and 20.3 kJ/mol were determined for complexation of the guest with the dimer, trimer, and tetramer, respectively. In order to reconcile the calculated binding energies with experimentally determined values, the cyclic trimer was isolated, and its complexation with acetylcholine chloride was studied using ITC. A binding energy of 22.2 kJ/mol was determined, confirming the viability of the computational approach [126].

The possibility to assess binding constants directly from DCLs has been particularly advantageous in situations where either the amplified library member is not isolable; it is unstable or fails to interact with the template under conditions where it is stable [69], it is not soluble at concentrations required for the binding studies [126], or it gives complex spectra and multiple isomers that complicate or frustrate binding studies [55]. It may also be used to study aggregation phenomena in DCLs [127].
Au-Yeung et al. described the amplification of a naphthalenediimide-based tetramer from a DCL of dithiol building block 50 in the presence of an electronically complementary dialkoxynaphthalene guest 51 (Figure 14.11) [55]. The tetrameric receptor was isolated with the intention of conducting binding studies. However, the signals in the $^1$H-NMR spectrum of the receptor were broad and complex over a wide temperature range presumably due to the presence of multiple conformations in solution. The $^1$H-NMR spectrum of the host–guest complex was also broad, and binding studies by
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UV/Vis spectroscopy, while suggesting a 2:1 binding mode, were inconclusive, possibly due to the complexity of the electronic transitions involving the host and guest. The authors turned to DCLfit to estimate association constants. By fitting the equilibrium concentrations of the library members to a model of the DCL, $K_1 = 10^8$ M$^{-1}$ and $K_2 = 10^4$ M$^{-1}$ were determined for the 2:1 interaction between 51 and the cyclic tetramer. From these values, it was concluded that each electron-rich guest 51 interacts with two electron-deficient naphthalenediimide moieties in the tetramer.

The wider application of DCLfit to systems of equilibria not usually classified as DCLs was recently demonstrated by Beeren and Sanders [77]. Having isolated a ferrocene-hydrazone-based ditopic receptor (32) for H$_2$PO$_4^-$, which was identified from a DCL (Section 14.5), an association constant for the 2:1 binding interaction was sought (Scheme 14.28). The $^1$H-NMR spectrum of the receptor was complicated by the existence of two conformational isomers (one with $C_2$ symmetry, 32A, and the other with $C_1$ symmetry, 32B) in slow exchange with each other on an NMR chemical shift timescale. Titration with H$_2$PO$_4^-$ resulted not only in the downfield shifting of electron-positive protons involved in binding the anion, but in the conversion of 32B into 32A, thus indicating the superior binding of the symmetrical isomer 32A. The mixture was considered as a dynamic conformational library. The $^1$H-NMR titration was thus viewed as a series of DCLs with different template concentrations and the composition of each library was determined by analyzing the relevant peaks in the NMR spectrum. DCLfit was used to fit the data to the network of equilibria shown in Scheme 14.28. Association constants for the interaction were thus determined ($K_{A1}K_{A2} = 800,000$ M$^{-1}$, $K_{A1} << K_{A2}$, and $K_{B1}K_{B2} = 100,000$ M$^{-1}$), and the cooperative nature of the binding—first noted in UV/Vis studies—was confirmed.

DCLs are complex, and their behavior under the influence of a template is difficult to predict. Through theoretical modeling and the computational generation of simulated libraries, a clearer understanding of DCL behavior has been achieved. Experimental guidelines have been established to help design successful DCLs, and elegant methods have been developed to obtain a quantitative assessment of association constants directly from the equilibrium library composition. DCLs do not always behave in the simple way that was hoped for when the concept was conceived, but by acknowledging their complexity and the interconnectivity of their networks of equilibria, they can be powerful tools for the study of molecular recognition and systems behavior [128, 129].

![Scheme 14.28](image-url)

Scheme 14.28. Left: Ferrocene hydrazone-based ditopic receptor for H$_2$PO$_4^-$ (32). Right: Network of equilibria that describe the template-induced isomerization of 32 [77].
14.9 CONCLUDING REMARKS

In this chapter we have described the use of dynamic combinatorial chemistry in the search for new receptors through a selection of state-of-the-art examples. In the coming years, we foresee the discovery of new, unexpected, strong, selective, and exciting receptors with unforeseen geometries and binding motifs. We expect the concept will influence medicinal chemistry with more biologically significant molecules being targeted and larger DCLs being explored. As new, reversible chemical reactions are introduced, the possibilities in dynamic combinatorial chemistry will expand. Dynamic combinatorial chemistry has inspired the exploration of adaptive chemical networks, with the field of systems chemistry emerging from this [129]. In dynamic combinatorial chemistry, the chemist can design the experiments, but ultimately, the molecules engineer themselves.

REFERENCES


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