Dynamic combinatorial chemistry: on the road to fulfilling the promise

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Dynamic combinatorial chemistry makes use of reversible reactions between functionalised monomeric building blocks to generate a mixture of products (dimers or oligomers) under thermodynamic equilibrium. This system reorganises upon addition of a target so that species that bind to, and are therefore stabilised by the target, are favourably formed and are thus amplified. Since the mid-1990's, dynamic combinatorial chemistry has been successfully applied to the identification/selection of ion receptors, enzyme inhibitors, catalysts, materials and nucleic acid ligands. Although it is now established as a powerful tool with broad applications some intrinsic limitations appeared when working on systems of increasing complexity. We present here the most recent advances in the field of dynamic combinatorial chemistry that have been developed to overcome these limitations and explore new areas of application.

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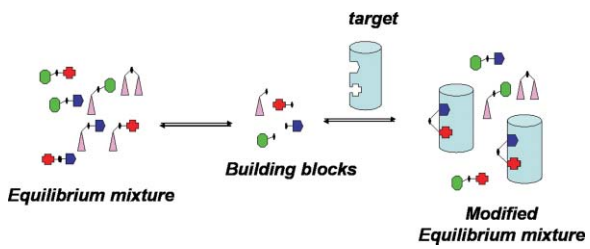
Introduction

Dynamic combinatorial chemistry (DCC) is a powerful concept that takes a number of molecular elements and allows them to reversibly combine *via* covalent or non-covalent linkages to generate a dynamic combinatorial library (DCL) of interchanging products under thermodynamic equilibrium. These DCLs represent chemical networks, the composition of which can be modified in response to changes in the surrounding medium or through specific molecular recognition events. According to Le Châtelier's rules, upon addition of a target, the system re-equilibrates as the mole fractions of individual library members are perturbed as a function of their affinity for that target (Scheme 1). This reorganisation can then be used to identify within a library of molecules the members with a high affinity for the target.[1]



Scheme 1 Re-equilibration of a DCL upon addition of a target, leading to the amplification of the tightest binders at the expense of the non-binders.

In the mid-1990's, DCC has emerged as a new approach to the self-organisation of molecular libraries, thermodynamically driven by the target.[2] It is a more sophisticated approach than "traditional" combinatorial chemistry due to the fact that the library synthesis and the affinity screening step have been combined in a unique process. Therefore, there is no need for the individual synthesis, purification and characterisation of every single library product.

In recent years, dynamic combinatorial chemistry has received considerable attention because of its successful use in identifying new receptors, ligands and catalysts. The method has been validated for a number of relatively simple systems based on small

molecule targets but a broader goal in this field is to employ this concept for ligand evolution as part of a drug discovery programme. Towards this aim, there have been some early proof-of-concept examples of using target assisted dynamic chemistry to evolve ligands against complex targets that include proteins[3] and nucleic acid secondary structures (DNA[4] and RNA[5]).

Assuming it is possible to use analytical techniques that are sensitive enough to detect amplification effects upon addition of a target, DCC could potentially be employed to generate large DCLs to be screened for the identification of molecules that bind to the target. However, almost all examples published in the literature so far have been limited to relatively small libraries of building blocks as part of proof-of-concept studies. Does this reflect the limits of the technique or does it correspond to a deliberate choice of the authors to solely demonstrate possible new applications based on this elegant concept? Some comprehensive reviews[1] have been published recently that summarize all the theoretical and historical aspects of DCC as well as some traditional applications of DCLs for the discovery of new catalysts, receptors or protein ligands. In the present article, we will present and discuss the intrinsic limitations to a broad applicability of DCC and the recent advances to overcome these constraints. The focus is on the very recent applications of DCC for the design of biosensors or "smart" materials for example.

DCC: overcoming the intrinsic limitations

Regardless of the type of chemistry or the nature of the target, the large majority of the reported examples of DCC selection experiments have been limited either to proof-of-concept or to highly simplified systems with a small number of building blocks. This deliberate simplification was initially justified by the need for the authors to demonstrate the validity of the method by (i) proving thermodynamic control over the equilibrating mixture, (ii) analysing, characterising and quantifying each library component and (iii) measuring the affinity of amplified and deamplified species to validate the selection process. Now that the general concept of DCC has been clearly established, its broad applicability to the screening of significantly larger libraries against a relevant target has yet to be demonstrated.

Large DCLs and weak binders

As already highlighted by the groups of Severin,[6] Sanders[7] and Huc,[8] a major drawback when expanding the size of a library is the entropic limitation that arises when a large number of products with weak affinity compete with the strongest hit(s) for binding to the target. Indeed, the amplitude of the equilibrium shift is not only linked to the absolute binding energy of the hit(s) to the target but also to the binding energy of the hit(s) relative to that of all the other products. An "ideal" DCL would therefore possess not only a limited number of hits exhibiting a very strong affinity for the target but also a very high selectivity when compared to the other DCL members. Although it is possible to carefully design a library so that only very few building blocks can combine to form strong binders, what about using DCC for screening a random library of structurally and functionally diverse building blocks against a given target?

Theoretical models have demonstrated that in some extreme cases, the addition of a target molecule to a DCL leads to a decreased concentration of the best binders at the steady-state.[6] Although such competition between a unique moderately tight binder and a large number of weak binders is very unlikely when using small sized DCLs (10s of entities), there is a risk that it becomes a major issue when increasing significantly the size of the library and especially when using bifunctionalised building blocks that can combine to form an infinite number of linear and cyclic DCL species. Among all the parameters which influence the outcome of a selection experiment, the target concentration has been shown to be one of particular importance. There is now theoretical and experimental evidence that the amplification factor does not necessarily correlate with the binding affinity at high target concentration. It has been suggested that it is generally preferable to work with relatively small amounts of target, or under conditions where the building blocks (and not the assemblies) are the dominant species in solution.[6]

Does this mean that DCC is by definition due to be limited to elegant proof-of-concept experiments on miniaturised working systems with little or no chance to see it one day replacing the more traditional combinatorial chemistry in drug discovery or materials science? Fortunately, recent progresses in the field of DCC have proven that these limitations can be overcome. To maximize the chances of success, each selection experiment requires an appropriate and optimized design (*e.g.* building block and target concentrations).

Small amplification of pre-stabilised DCL species

In a perfect DCL, each of the species would be represented at equilibrium in almost identical proportions. However, one of the major complications of the DCC concept is the possibility for library components to strongly self-associate or to favourably associate with another building block in the absence of any template. Under such conditions, the detection of template-induced amplification of species already present in large proportion even in the absence of template remains small and therefore difficult to detect or quantify.

We have recently developed a novel approach using DCC and reversible thiol–disulfide chemistry for selecting DNA binding ligands.[4] In such studies involving complex and expensive biomacromolecules as targets, selection experiments were designed that required only small quantities (micromolar concentrations, tens of nanomoles of target only) of both target and building blocks. To ensure a quick and thermodynamically controlled thiol–disulfide exchange between the thiol building blocks during the DCC experiment, we decided to use a glutathione containing buffer which was previously reported by Ghadiri and others.[9] A 4 : 1 ratio of reduced and oxidized disulfide-bridged glutathione (GSH and GSSG respectively) at low millimolar concentrations was shown to represent favourable conditions for reversible thiol–disulfide exchange of thiol building blocks (each present at a micromolar concentration) under thermodynamic equilibrium. Since we were targeting DNA, negatively charged natural tripeptide glutathione was unlikely to interact in any way with our target and was therefore acting exclusively as a mediator/accelerator of the thiol–disulfide exchange reaction.

More recently, we demonstrated that glutathione could be used not only as an exchange mediator but could also be exploited

as a useful library competitor. This strategy was successfully exemplified for selecting pyrrole-based oligomers binding to different nucleic acid secondary structures (Fig. 1).[4a] When using low millimolar concentrations of glutathione buffer, pyrrole building blocks with the largest aromatic surface were shown to preferentially dimerize even in the absence of target (probably *via* π-stacking interactions). Assuming those dimers were strong DNA binders, only very limited amplification of these species upon addition of the DNA template would have been possible. It was therefore necessary to make sure that the possible hit candidates were not preferentially formed and stabilized before interaction with the target. In this study, we demonstrated that increasing the amount of glutathione within the exchange buffer (10–20 times) was an efficient way to drive the equilibrium mainly towards the formation of glutathione based heterodisulfides in the absence of target.[4a] Therefore, the presence of thermodynamically stable disulfide adducts of library components is limited in the absence of template. This approach was shown to sensitize the system toward the detection of amplified species that are otherwise significantly pre-formed in the absence of template. This may also be particularly useful for complex and large libraries in which there is an increased likelihood of mutual recognition between library members.

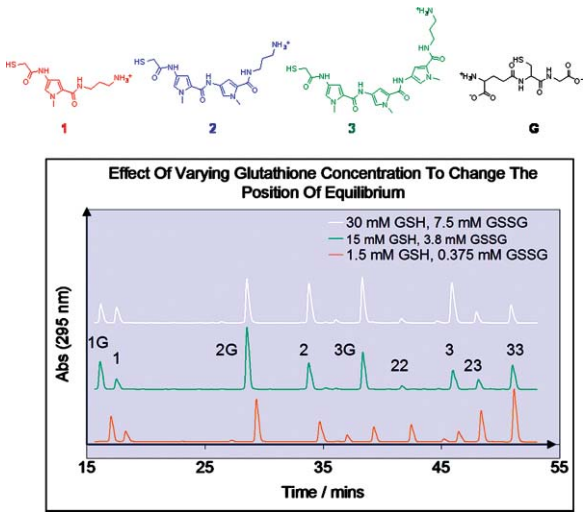
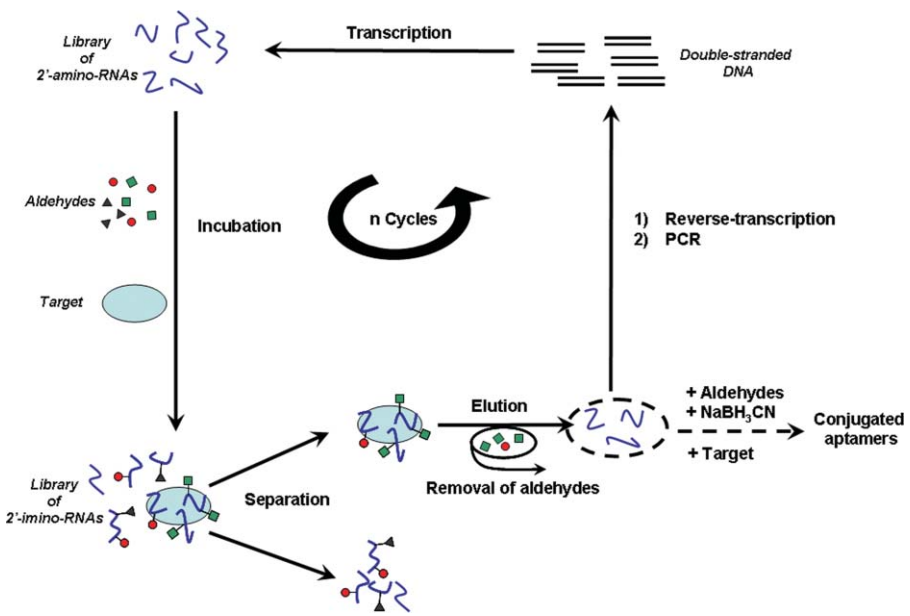


Fig. 1 (Top) Structures of the three thiol building blocks (1, 2 and 3) and of exchange mediator reduced glutathione (G); (bottom) HPLC traces of the reaction mixtures at thermodynamic equilibrium in the presence of increasing glutathione concentrations (bottom to top). At high concentration, glutathione also acts as a competitor and drives the equilibrium towards the formation of glutathione adducts.

DCC and evolutionary systems

An interesting approach consists in refining the selection using an iterative process of selection and amplification. Until recently, only one such evolutionary system had been reported, by Eliseev and Nelen, which makes use of a photochemical isomerization reaction.[10] Using a small DCL of three different compounds that can be completely interconverted, they demonstrated that it is possible to amplify the DCL member with the highest affinity for the target. However, this approach involved freezing the equilibrium within a pool of compounds that can interconvert prior to "fishing out" the best binders with an immobilised target. The best ligands are then removed from the equilibrium mixture and the rest of the unbound compounds allowed to re-equilibrate. More recently, Bugaut *et al.* reported a more advanced evolutionary selection that represents an extension to single DCL selection experiments by combining the techniques of DCC and SELEX (for systematic evolution of ligands by exponential enrichment).[11] This process was applied to the selection of conjugated RNA aptamers that bind to the transactivation-responsive (TAR) element of HIV-1 (Scheme 2).

Briefly, in Bugaut *et al.*'s approach an equilibrium mixture of products is formed from a large library of aptamers containing 2′-amino-pyrimidines and a limited set of aldehydes. Upon addition



Scheme 2 *In vitro* selection of conjugated RNA aptamers using an original evolutionary process that combines DCC and SELEX.

of the target molecule (TAR) the amine–imine equilibrium is shifted due to the stabilising interactions of some of the conjugated imine aptamers with TAR. This process is known as dynamic combinatorial selection. The products that have bound to the target are then isolated, and the aldehydes released, giving an improved set of aptamers. The originality of this approach resides in the fact that this limited number of selected amine aptamers can be used for a refined selection process after being copied during an amplification process (reverse-transcription–PCR–transcription). The whole selection–amplification process can be repeated by increasing progressively the selection pressure until only the best aptamers are left. Using a library of random amino RNAs and a set of three aldehydes, new TAR conjugated ligands were selected from a virtual DCL of 7^{14} possible candidates. This example proves that in certain cases (like conjugated aptamers) dynamic selection can be applied to large DCLs, although using an extra amplification step.

From all the examples reported in the literature, it appears that one main limitation to a broader use of DCC for ligand screening and drug discovery is essentially analytical. Detection of small amplification factors and deconvolution of all the DCL members to enable unambiguous and easy identification of the selected hits (especially when targeting biomacromolecules that need to be removed before library analysis) remain very challenging. Most commonly used techniques for analysing DCL composition and quantifying hit amplification involve HPLC, NMR and mass spectrometry. While HPLC enables accurate monitoring of library re-equilibration as well as separation/isolation of each library component, it is not always suitable when working with large libraries because of the likely overlapping of DCL fragments with similar structures/properties. In order to overcome these intrinsic limitations, new approaches have been recently developed that involve the use of alternative analytical detection methods and spectroscopic methods in particular.

Fluorescence, UV–vis and polarimetry for sensing DCLs: alternative analytical methods for new applications

As an alternative to the frequently used HPLC or HPLC-MS that require the ability to separate individual library members, each having a unique molecular weight inside the DCL, fast and cheap spectroscopic (UV or fluorescence) methods have also the advantage of a very high sensitivity. In a typical DCC experiment, the system re-equilibrates upon addition of the target, thus leading to a change of the physical properties of the medium. These physical properties can therefore be monitored in real time and used as an indicator of the DCL composition. This approach implies that either the target or *all* the DCL building blocks have characteristic physical properties that one can monitor accurately in solution.

Laser polarimetry for the discovery of enantioselective receptors

Gagné and co-workers have recently reported an elegant approach for the selection of enantioselective receptors for (−)-adenosine from a racemic DCL of cyclic hydrazone oligomers (from a racemic proline-containing building block rac-1) using laser polarimetry (LP) associated to HPLC.[12] Rac-1 was shown to form a mixture of cyclic oligomers under reversible conditions which can be easily resolved by HPLC-MS. Upon addition of (−)-adenosine, the dimer was moderately amplified at the expense of the higher oligomers suggesting a modest binding affinity of the dimer for the target. Interestingly, this dimer amplification was accompanied by the appearance of a signal in the LP trace at a retention time corresponding to the dimer. Since achiral compounds and racemates are polarimetrically silent, only receptors enriched in one enantiomer can give a detectable signal whilst the background signal is otherwise null. In the particular case of their proof-of-concept study using rac-1, the appearance of an LP signal associated with the cyclic dimer clearly indicated that this dimer had had one of its homochiral diastereoisomers (*S*,*S*) or (*R*,*R*) selectively enhanced over the other (given that heterochiral dimer (*R*,*S*) is achiral). In order to confirm which homochiral dimer has been preferentially amplified, selection experiments were repeated starting from either (*S*)-rac-1 or (*R*)-rac-1. A significantly larger dimer amplification was observed when starting with the (*S*)-DCL, thus proving that the enantioselective receptor of (−)-adenosine from the racemic DCL of rac-1 is the (*S*,*S*) homochiral diastereoisomer of the cyclic dimer. This example demonstrates the possible selection/characterisation of enantioselective receptors for biomolecules using a sensitive and highly specific laser polarimetric detection method. It should be applicable to significantly larger libraries and enable the discovery of new enantioselective receptors for a number of biological metabolites.

DCC and UV–vis chemosensors

Severin and Buryak have created a DCL that can act as a colorimetric sensor, with any target induced re-equilibration of the library resulting in a measurable perturbation of the global UV–vis spectrum of the equilibrating mixture. The first example reported was using three commercially available dyes in combination with two copper and nickel salts so that metal–dye complexes can form under reversible conditions allowing ligand exchange reactions (Fig. 2).[13]

After showing that dyes, metals and the corresponding complexes are in a dynamic equilibrium which corresponds to a unique UV–vis spectrum, various dipeptides (sensor analytes) were introduced and the resulting changes in UV–vis spectra of the mixture recorded. Dipeptides are known to form stable complexes with Cu^{2+} and Ni^{2+} and were expected to displace some of the dyes from the metal ions, thus leading to an increase in the amount of free dye in solution. Interestingly, specific changes in the UV spectrum were obtained for each of the six dipeptides tested, thus demonstrating the potential of DCLs as colorimetric sensors. This sensor was also notably successfully used to discriminate between the two stereoisomers L-Phe-Ala and D-Phe-Ala which would have inevitably been much more challenging if using HPLC coupled to MS. In this first utilization of a DCL as a colorimetric sensor, each analyte is attributed a "finger-print" distributed over the entire UV–vis spectrum. However, and like for a traditional DCC experiment, the sensor needs to be optimized depending on the sensing problem to be addressed. In a follow-up study, the same group reported on an optimized sensor in which they showed that the concentrations and ratios of the sensor constituent building blocks can also be critical for obtaining a sensitive detection.[14] Despite this inherent limitation, this DCL sensor compares very

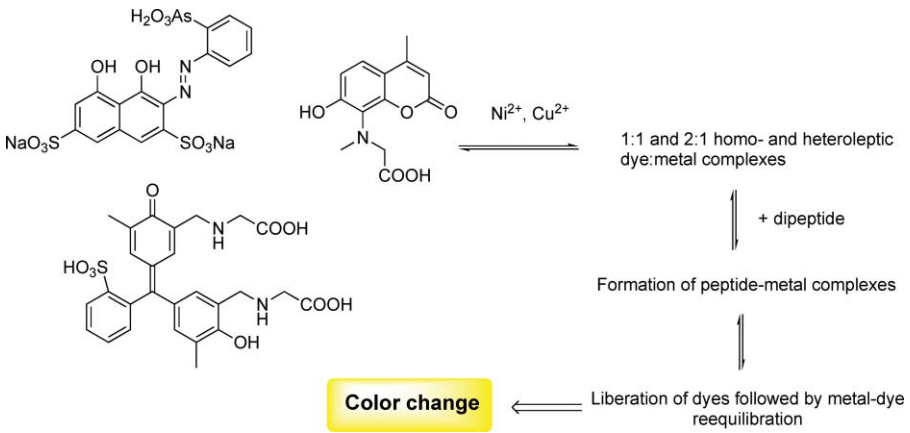


Fig. 2 Structures of the three dyes used for generating a DCL of metal–dye complexes, and mechanism of metal displacement leading to a color change.

well with other small peptide chemosensors and opens the way to a new possible application for DCC in analytical chemistry.

Fluorescence and constitutional dynamic chemistry (CDC)

DCC is an implementation of the broader concept of constitutional dynamic chemistry (CDC) that has been developed in the past few years, mainly in the field of drug discovery.[15] CDC is also of special interest for materials science as it offers the possibility of designing dynamic smart materials. One particular aspect has been developed by Giuseppone and Lehn and deals with iminofluorene-based dynamers (Fig. 3).[16] Using fluorescent fluorene building blocks functionalised with either amino or carboxaldehyde groups they first demonstrated the formation of oligo- and polyimine species and second showed that the system can undergo constitutional reorganization in response to external stimuli.

Interestingly, this reorganization results in the generation of optical signals varying in both wavelength and intensity depending on the nature of the stimulus. The authors investigated the double-effect of Zn^{II} ions on the composition of constitutional dynamic libraries (CDLs). Zinc ions were already shown to promote imine exchange within a set of polyimine dynamers and therefore can act as an effector inducing the selective formation of certain polymeric structures. In addition, zinc can also interact with the fluorene-based polymers formed and by doing so lead to significant changes in their spectroscopic properties. This "self-signalling" process, whereby the effector induces the formation of that CDL constituent that enables its own detection, offers the advantage of an easy detectability of the CDL composition using standard spectroscopic methods. The introduction of the first two equivalents of zinc ions was shown to induce a re-equilibration of the system only, whilst for higher amounts of zinc ions marked fluorescence spectra changes were observed. Taken all together, those examples demonstrate a synergistic adaptative behaviour of a dynamic system in which an external effector (or template) induces the amplification of selected species resulting in the generation of an optical (fluorescence) signal. This signal can therefore be used as an easily detectable indicator of the presence of this effector in solution. Such systems are of particular interest in materials science for the design of novel "smart" materials. The use of optical detection associated with the self-sensing could allow the resolution of new dynamic systems of increasing complexity.

Fluorescent targets and resin-bound DCC (RB-DCC)

When using DCC approaches for targeting biomacromolecules for example, a challenge arises as to how to analyse the DCL in

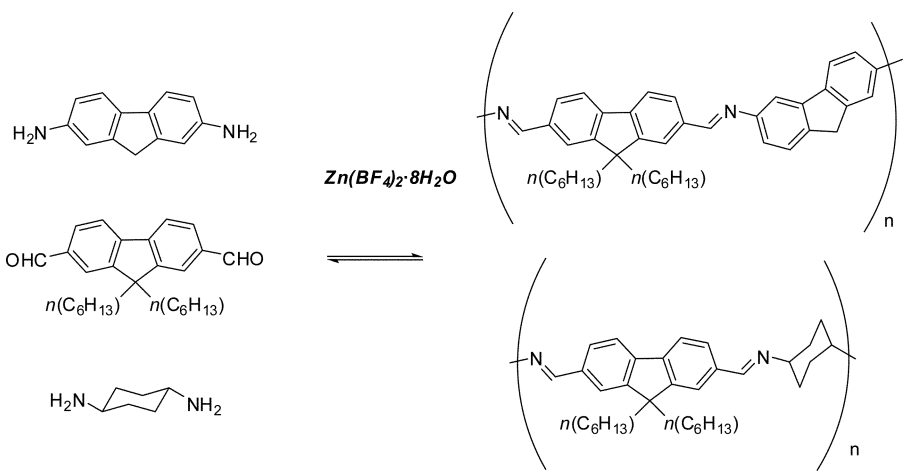


Fig. 3 Structures of the fluorene building blocks and metal-templated formation of iminofluorene dynamers.

the presence of this target or alternatively how to remove it from the DCL without disturbing the thermodynamic equilibrium that has been reached. Phase separation has proven a useful tool in many DCC experiments using complex templates. For instance, resin-immobilized targets have often been used but there are also examples that involve extraction of the DCC products into a different solution or gel phase from that of the target. These additional steps to the DCC selection process offer the advantage of a simplified and more accurate analysis of the target-free DCLs.

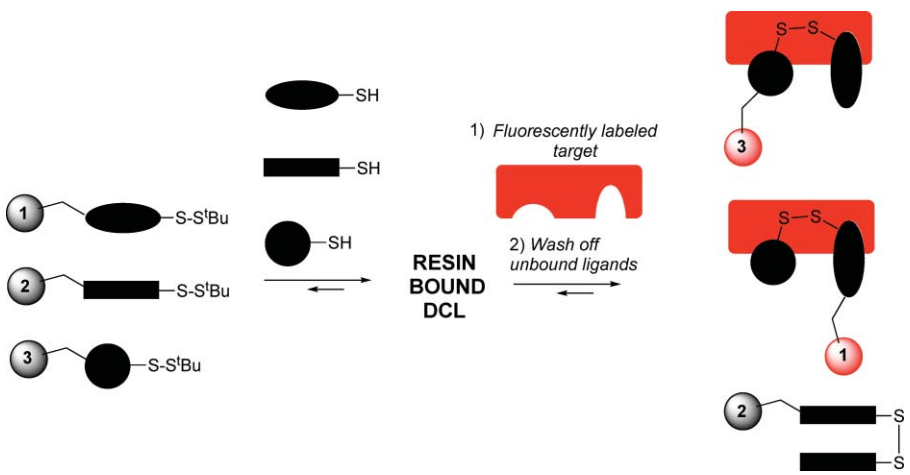
DCC studies in the literature have been reported where the target is immobilised during the equilibration and selection processes, as exemplified by Miller and Karan for targeting RNA,[17] Eliseev and Nelen for targeting guanidinium derivatives,[10] Lehn and Ramström for selecting concanavalin A ligands[18] or by Sanders and co-workers for identifying ammonium receptors.[19] Alternatively we and others have developed a different strategy where the entire DCL equilibration and selection process is carried out with the template in solution, target immobilisation on magnetic beads being carried out in a second step only once dynamic exchange has been frozen and for analytical purposes solely.[4]

Recently, the group of Miller and McNaughton developed a new technique termed resin-bound DCC (RB-DCC) which involves (1) phase-tagging of the library components by immobilisation on a solid support and (2) detection of selected hits using fluorescently labelled targets. In an elegant proof-of-concept experiment, the authors have combined a library of spacially segregated resin-bound thiol building blocks (masked as disulfides) with an identical library of monomers in solution and allowed them to equilibrate (reversible formation of disulfides between thiol building blocks in solution and those immobilised on resin) in the presence of the fluorescent target. A simple two step analysis process involves washing the beads and detecting protein containing "bead-hits" by fluorescence microscopy (Scheme 3).[20]

Considering any dimeric library of *n* monomers, if only beads containing monomer 1 are found to bind the target, then one can conclude that dimer 1–1 is the sole strong binder; if beads containing monomers 1 and 3 are found to bind the target, then dimers 1–1, 1–3 and 3–3 are three potential binders.

This new strategy offers significant advantages over more conventional DCL analysis techniques like HPLC or NMR. The use of fluorescence spectroscopy for example allows a quick and easy detection of the hits. Moreover this approach does not require the isolation and/or characterisation of every library component, which becomes a real advantage when working with libraries of increasing complexity. One last advantage relies on the ease of detection of the selected hits without having to compare the obtained DCL with a target-free experiment run in parallel, as is the case with most DCC experiments monitored by HPLC. However, RB-DCC is still at a very early stage and it also presents some intrinsic limitations. While its restriction to fluorescent targets can be easily overcome by the possibility of tagging most non-fluorescent biomacromolecules with readily available fluorescent flags, this approach is limited to monofunctionalised building blocks (*e.g.* bearing one thiol only) and cannot be easily extended to a selection of macrocycles, oligomers or polymers. Indeed, the principle of RB-DCC as described by Miller and McNaughton[20] requires the detection and identification of the building blocks involved in the constitution of the selected ligand by fluorescence spectroscopy. Although this approach could enable the identification of the different building blocks involved in the selected oligomers or polymers, it would not allow the determination of the number of every building block involved nor their position within the oligomer.

This year, the same group demonstrated the successful application of RB-DCC to significantly more complex systems.[21] They reported the identification, *via* RB-DCC, of selective small molecule ligands of the HIV-1 frameshift regulatory mRNA stem-loop from the largest DCL prepared so far (>11 000 virtual members). The library design was based on the octadepsipeptide family of bis-intercalating nucleic acid binding agents, and 150 cysteine containing tripeptides capped with either a 2-ethylquinoline or a piperazine group were synthesized on Tentagel resin. Beads of three different sizes were used depending on the position of the cysteine amino acid in the tripeptide (1st, 2nd or 3rd) and 50 tripeptide–heterocycle conjugates per resin size were synthesized, each of which was characterised by a unique mass. Using a protocol similar to that established previously in their proof-of-concept article, Miller and co-workers have identified a selective and high-affinity ligand for the biologically relevant HIV-1 frameshift-inducing mRNA stem-loop. The affinity of the



Scheme 3 Principles of resin-bound dynamic combinatorial chemistry as developed by Miller and McNaughton.[20]

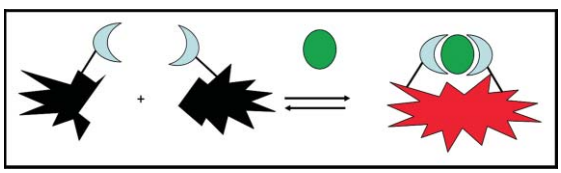
selected hit for the RNA target was confirmed by SPR, thus proving the validity of RB-DCC technology even when using large libraries. Despite the intrinsic limitations described above, this represents a real break-through in the DCC world and paves the way for new applications in the area of drug discovery in particular.

Towards new applications for DCC

As a consequence of all the recent efforts investigating the development of novel DCC methodologies (*e.g.* new types of chemistries, new analytical techniques…), a number of new applications have appeared in the literature that could give some indications on what the future of DCC may look like. Three selected examples are detailed below.

Fluorescent dynamic biosensors

There is currently a growing interest in the development of new chemosensors based on supramolecular systems that have the ability to recognise and bind to a specific metabolite with high affinity. As a result of this interaction, the system re-equilibrates thus leading to a change of the physical properties of the medium. Of particular interest are fluorescent chemosensors for which specific molecular recognition events are associated with changes in the excitation/emission fluorescent properties of the sensor dye.[22] We are currently developing a completely novel fluorescent chemosensor in which two non-fluorescent entities can combine by the formation of a covalent imine bond under reversible conditions, thus leading to a highly fluorescent molecule (Scheme 4).



Scheme 4 Fluorescent biosensor: two dark entities can combine under reversible conditions upon addition of a target to generate an easily detectable fluorescent molecule.

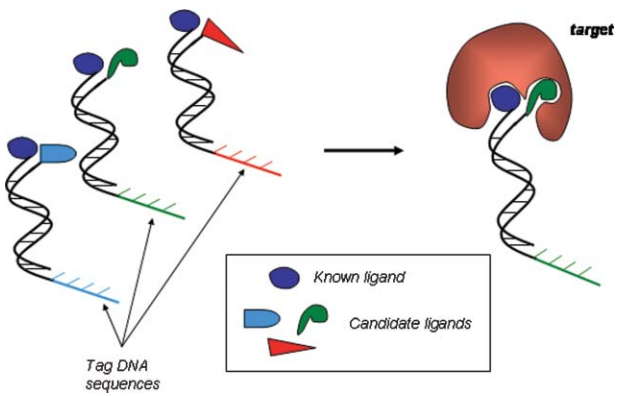
The system is designed so that formation of the fluorescent dye is highly disfavoured under standard conditions (due to the low reactivity of both components) but can be templated upon addition of a metabolite that can bring both components in close proximity. Within our system, the sensor part is covalently attached but distinct from the fluorescent detector which offers the advantage of a high versatility/modularity. The design of such fluorescent sensors as well as more complex ones based on the same principle but capable of sensing different metabolites simultaneously, each sensed metabolite being assigned a unique fluorescence signal, is in progress in our laboratory and will be reported elsewhere.

Encapsulation and redox-triggered drug delivery

Thiol–disulfide chemistry has been used extensively in DCC for the selection of dimeric or polymeric species depending on the number of thiol groups each building block bears. Mixtures of mono- and bis-thiols generate libraries of linear or macrocyclic disulfide-linked structures. The group of Otto and co-workers, pioneers in the field of DCC based on thiol chemistry, has recently reported the generation of DCLs containing water-soluble cages held together *via* disulfide bonds.[23] Using thermodynamically controlled synthesis, they described the first example of a DCL containing covalent cages and obtained from a mixture of trithiol and dithiol building blocks. Although it is still at its early stage the use of DCC for producing covalent cages of diverse structures under thermodynamic control offers new perspectives and applications in the field of drug delivery/targeting both *in vitro* and *in vivo*. Reversible disulfide chemistry seems ideal for linking such subunits since disulfides tend to be stable in the bloodstream whereas they are readily degraded in intracellular fluids, which may allow for the controlled release of the encapsulated guest upon reduction of the disulfide linkages.

Encoding chemical libraries using DNA

Because of their ability to be replicated and their chemical stability, nucleic acids are ideally suited for being used as a tag to encode small molecules. It is particularly interesting to use such an approach to facilitate the identification of active compounds within libraries formed from large numbers of building blocks. A recent approach that uses DNA tags to encode small molecules has been developed by Neri and co-workers and is named Encoded Self-Assembling Chemical Library (ESAC).[24] The ESAC method uses libraries of small molecule pharmacophores linked to DNA oligonucleotides that both identify each pharmacophore and bring together pairs of pharmacophores non-covalently upon base-pairing (Scheme 5).



Scheme 5 Principles of Encoded Self-Assembling Chemical Library (ESAC) using libraries of pharmacophores attached to DNA oligonucleotides.

This approach is particularly attractive for improving a known ligand by identifying new fragments that bind the target in the same binding pocket (or active site). In a proof-of-concept experiment using carbonic anhydrase as a target, a known protein ligand attached at the 3′ end of a DNA oligonucleotide was incubated with a library of small molecule building blocks attached at the 5′ end of a complementary oligonucleotide also comprising the small molecule tag-sequence. Pairs of pharmacophores are then formed upon hybridisation of the two complementary DNA sequences and can be incubated with the target protein. Subsequently, those pairs that survive the selection process can be easily identified by

sequencing of the DNA tag. ESAC is a very elegant approach for elaborating new protein ligands using a fragment-based approach. Although Neri and co-workers discovered bidentate ligands that exhibited a 40-fold increase in affinity over that of the known ligand alone, this approach requires an optimization of the linker connecting the two selected pharmacophores which can possibly be very laborious, thus losing the benefits of the ESAC approach.[25]

Conclusions

Discovered a little bit more than ten years ago, the concept of DCC has quickly emerged as an elegant supramolecular approach relying on the dynamic generation of molecular and supramolecular diversity through the reversible combination of covalently and non-covalently linked building blocks. In recent years, DCC has actively developed and is now recognised as a powerful tool for exploring systems based on molecular recognition, either in materials science, catalysis or drug discovery.

In most of the successful DCC applications, the response of a DCL to an added template (*i.e.* stabilisation of selected species upon interaction with the template resulting in an increase in their concentration) has enabled the identification of specific library members that can act as ligand, host or receptor for the molecule/protein chosen as a template. Giuseppone and Lehn have also investigated the influence of different environmental stimuli—as an alternative to molecular recognition—on the composition of a DCL. They demonstrated the adaptative behaviour of amine–aldehyde based DCL towards various external physical or chemical stimuli (*e.g.* electric field, temperature, pH. . .) which is of particular interest for the development of novel responsive dynamic materials.[26] Although it offers a very broad range of potential and proven applications, DCC does not compete yet with combinatorial chemistry. This may certainly be due to some intrinsic limitations of DCC that one needs to tackle when working on DCLs of high complexity. When increasing the number of building blocks/pharmacophores, experimental conditions (*e.g.* template concentration) need to be carefully adjusted, and alternative techniques to HPLC for the analysis of DCLs and easy detection of the selected hits need to be found. Reported here are a number of recent examples that demonstrate that efforts have been made to not only overcome those limitations but also expand considerably the DCC field of application to new areas of chemistry. Recent examples of DCC on solid support and DCC coupled to SELEX are two promising proofs that DCC can indeed be applied to large and diverse libraries. The development of UV–vis and fluorescent biosensors based on this concept is also very appealing due to their high modularity and their ease of use.

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