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Recent developments in dynamic combinatorial chemistry

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Generating combinatorial libraries under equilibrium conditions has the important advantage that the libraries are adaptive (i.e. they can respond to exterior influences in the form of molecular recognition events). Thus, a ligand will direct and amplify the formation of its ideal receptor and *vice versa*. Proof of principle of this approach has been established using small libraries showing highly efficient amplification of selected receptors. The approach has recently been extended to address folding of macromolecules, including peptides.

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Abbreviations

DCL	dynamic combinatorial library
ESI	electrospray ionisation
FTICR	Fourier transform ion cyclotron resonance
HPLC	high-performance liquid chromatography

Introduction

"A terrible problem." Such was the comment of a synthetic chemist when asked about the reversibility of hydrazone formation. Yet in many cases reversibility can be the solution rather than the problem [1]. Reversibility adds a new dimension to combinatorial chemistry with the potential to solve common problems associated with screening, isolation and even resynthesis of hits.

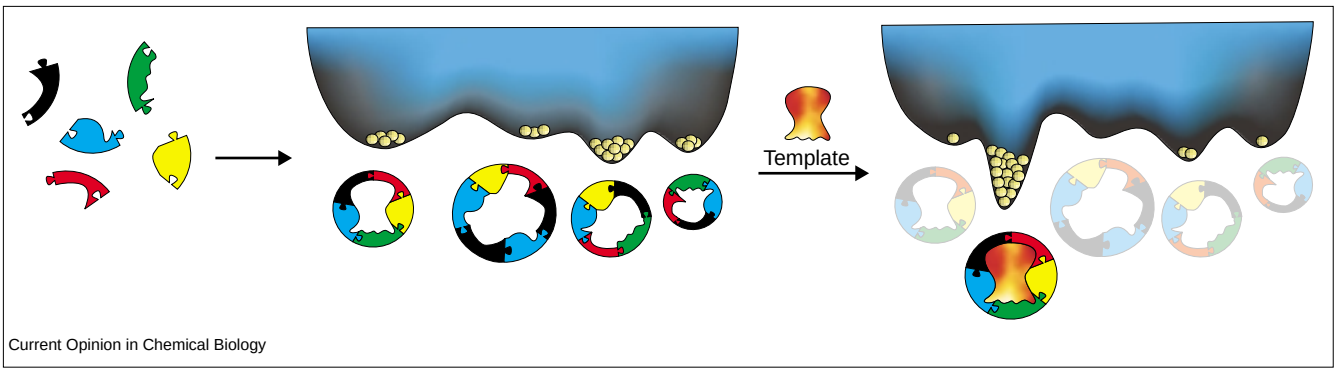
A combinatorial library in which the building blocks are connected through reversible linkages is dynamic and adaptive. The individual members of the library are interconnected by a network of equilibria, and under thermodynamic conditions the concentration of each member is dictated by its stability. Any influences that affect this stability will induce shifts in the equilibria changing the composition of the library. This makes dynamic combinatorial libraries (DCLs) ideally suited for the discovery of species that take part in molecular recognition. For example, when a guest molecule is introduced into a dynamic library of potential hosts it will select and bind the best host. The binding event introduces a new equilibrium in the system adding an additional free-energy well (Figure 1). If this well is sufficiently deep, the equilibria will shift in the direction of the best host at the expense of unfit hosts. The resulting amplification of the ideal host will facilitate identification. Moreover, templated synthesis under the same reversible conditions should provide quick and easy access to large amounts of material.

Since our first publication introducing the concept in 1996 [2], considerable progress has been made. In this short review, we summarise the developments over the past two years. For other reviews discussing dynamic combinatorial chemistry from a drug-discovery standpoint, see [3,4]. The reader is also referred to more general reviews on covalent synthesis under thermodynamic control [5] and template-directed non-covalent synthesis [6].

Reversible chemistries

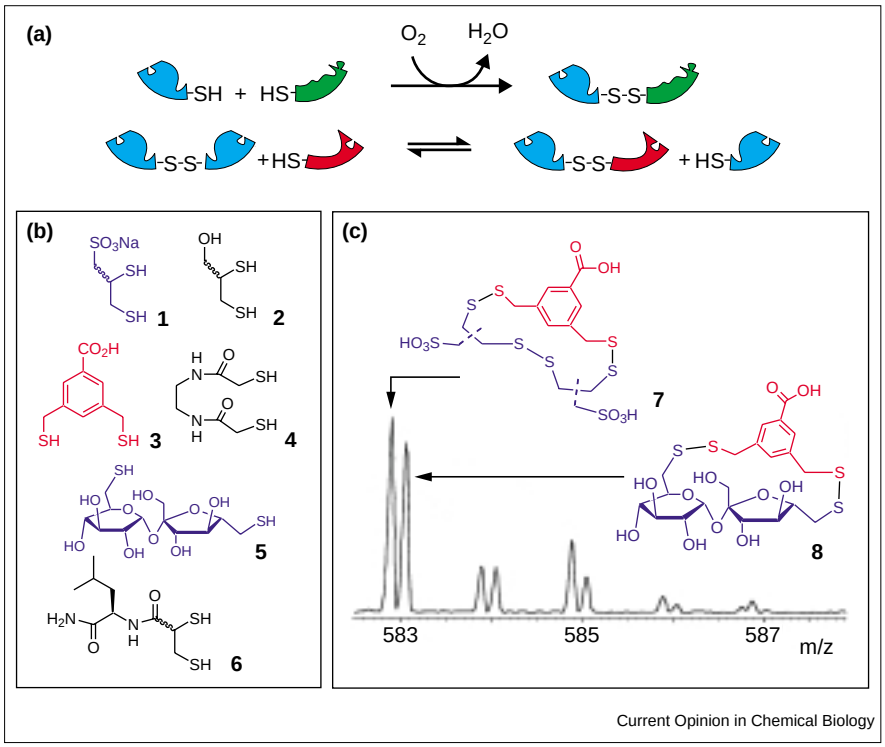
Key to the success of dynamic combinatorial chemistry is the reaction that mediates exchange of the building blocks between different library members. This reaction should be reversible and proceed under conditions that are compatible with the subtle non-covalent interactions with the template and compatible with the template itself.

Figure 1



A small dynamic combinatorial library and its free energy landscape showing the effect of adding a template that strongly and selectively binds to one of the equilibrating species.

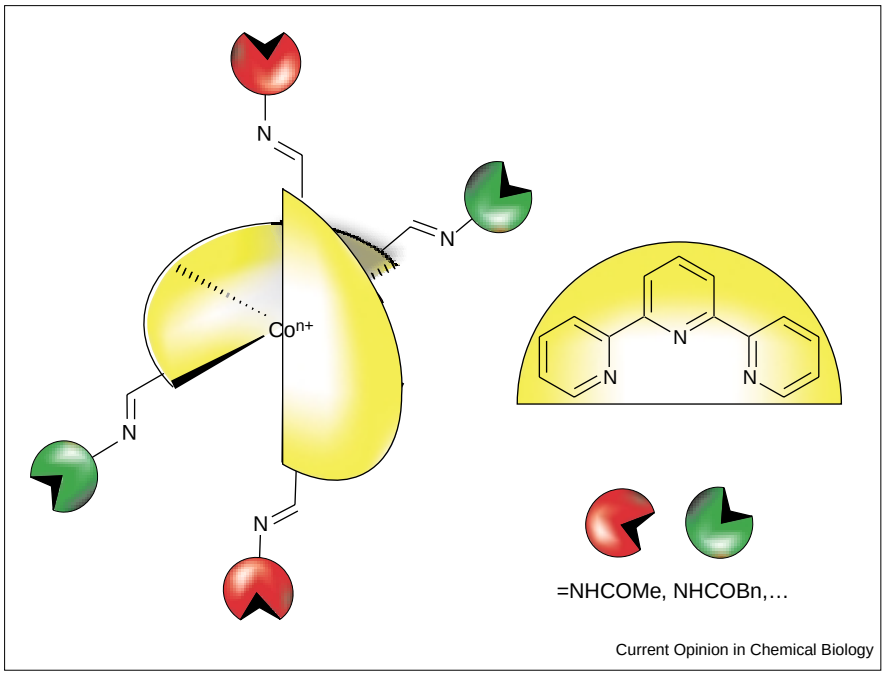
Figure 2



Disulfide exchange as a new reversible reaction for dynamic combinatorial chemistry. (a) Disulfide formation and exchange. (b) Dithiol building blocks. (c) Part of the high-resolution FTICR-ESI mass spectrum of a disulfide library formed after oxidation of a mixture of building blocks **1**, **2**, **3** and **5** showing two macrocyclic disulfides differing by 0.17 atomic mass units. The peaks near m/z = 584, 585, 586 and 587 result from isotopic substitution.

Furthermore, it should be possible to turn the reaction off in order to isolate and handle selected members of the library individually. These are severe restrictions, particularly where nucleic-acid-based and protein-based templates are involved. The reversible reactions that have been studied in this context include metal–ligand coordination [7–9,10•], hydrogen-bond exchange [11,12•,13,14•], ester exchange [2,15,16], transamination [17], transimination [8,18], exchange of oximes [19] and hydrazones [20], and olefin metathesis [21].

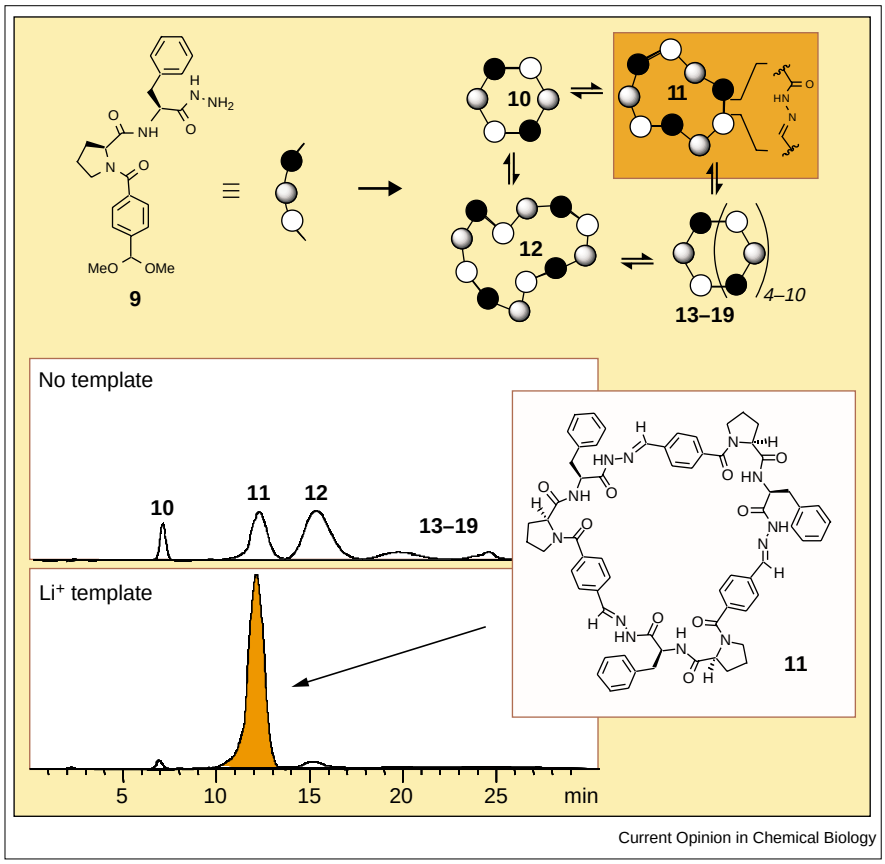
Figure 3



Two orthogonal levels of diversity can be obtained using hydrazone and ligand–cobalt exchange.

Figure 4

A dynamic combinatorial library of hydrazone-based pseudopeptide macrocycles. Shown are the HPLC traces of the library before (upper trace) and after (lower trace) addition of Li^+ template.



During the past two years, only one additional reversible reaction has been explored for its use in DCLs: the disulfide exchange reaction [22,23]. Disulfides form spontaneously at neutral pH upon oxidising a solution of thiol building blocks by exposing it to air. While oxidation progresses, the remaining thiolate anion mediates the exchange (Figure 2a). Exchange stops upon protonation of the thiolate or after removal of the thiol. We have recently prepared DCLs using a wide variety of neutral or charged building blocks including dithiols derived from carbohydrates and α-amino acids (Figure 2b) [22]. At present, disulfide exchange and olefin metathesis are the only reversible reactions that operate under physiological conditions.

Enhanced levels of diversity can be achieved by combining different connective chemistries, provided that these can be controlled independently. Eliseev and co-workers [24•] have demonstrated this by assembling ligands containing imine appendages around a central cobalt ion (Figure 3). The orthogonal levels of diversity include Co–ligand exchange and imine exchange and can be addressed independently.

Library analysis

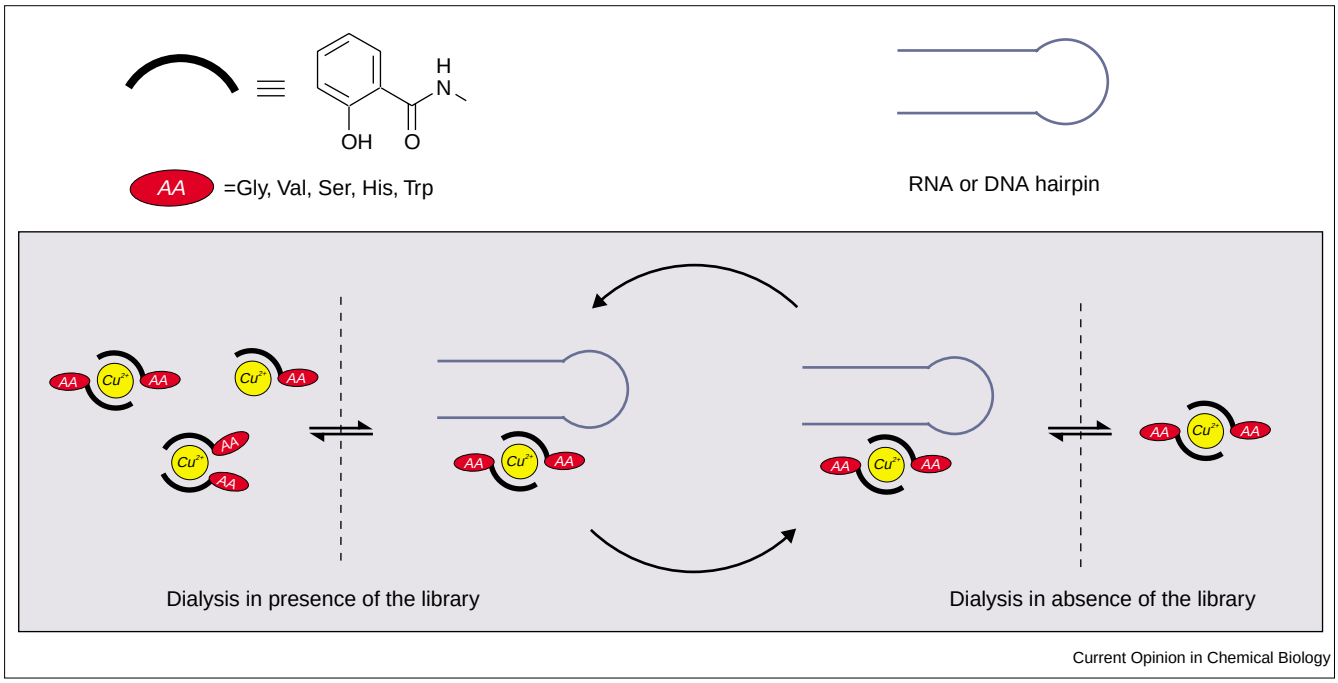
Because most of the DCLs reported to date were aimed at proving the principle, they are limited in size and could be analysed by high-performance liquid chromatography (HPLC). For most practical purposes, however, larger libraries will have to be analysed, requiring more extensive use of high-resolution mass spectrometry. Fourier transform ion cyclotron resonance (FTICR) mass spectrometry is a powerful technique when high mass resolution is required (see, for example, Figure 2c) [22]. Furthermore, MS–MS enables analysis in cases involving sequence isomers [25] and regioisomers [26•].

Template-induced shifts in library composition

Although several papers have been published over the past six years reporting the preparation of DCLs, only very recently examples have appeared that make full use of the unique potential to efficiently select, amplify and isolate re-utilizable molecules.

We have developed the use of hydrazone exchange to prepare DCLs of macrocycles from building blocks containing both a hydrazide and a protected aldehyde functionality [20]. Hydrazone formation and exchange are rapid under acidic conditions, whereas under neutral conditions the reaction is switched off. Using this chemistry, a 10-macrocycle library was prepared from a single dipeptide building block. Addition of Li^+ as a template shifted the equilibrium towards the cyclic trimer while the concentration of all the

Figure 5



Miller's approach for the discovery of a selective RNA-binding Cu^{2+} complex using the receptor to select the best ligand. The template is dialyzed in the presence of the library to select the best binder and in absence of the library to wash out the selected compound.

unselected macrocycles decreased (Figure 4) [27••]. Six hours after the addition of Li^+, the cyclic trimer represented almost all the peptide material in the library. The amplified trimer forms a 1:1 complex with Li^+, with a binding constant of 4×10^4 M^{-1} in chloroform–methanol (98:2).

The dynamic combinatorial approach in this case allows generation and screening of the library as well as detection and preparation of the preferred receptor all in one single process. The outcome of this one-step experiment was the identification and high-yield synthesis in a preparative scale of a new re-usable receptor for lithium. The receptor is flexible and changes conformation upon binding to Li^+. It is unlikely that such a receptor would have been rationally designed.

The same hydrazone chemistry was used also to prepare a simple dynamic system of potential receptors for ammonium ions. When a series of ammonium salts were tested as templates, a cyclic receptor was amplified to varying extents according to the template used [28,29]. Binding studies demonstrated that the relative affinities of the various templates for the isolated receptor correlate well with the effect they produce on the library composition.

Reversible metal–ligand coordination chemistry has also been used for generating mixtures of receptors. Fujita and co-workers [30] have prepared ligands containing multiple pyridine rings and linked them with palladium centres. Different host–guest complexes were isolated depending on the particular guest that was introduced into the dynamic mixtures [31•].

Timmerman, Reinhoudt and co-workers [11,12•] have pioneered hydrogen-bond exchange as a means to prepare DCLs of host molecules containing a varying number of zinc-porphyrins as binding sites. The use of a tripyridine template produced a clear amplification of the receptor that contains three porphyrin moieties per complexed template molecule.

To facilitate analysis of the hydrogen-bond exchange-based libraries, the authors have frozen the exchange process using a separate covalent reaction [13]. This approach leads to stable compounds that can be isolated and characterized by traditional analytical methods.

The complementary approach where a host is used to select its preferred ligand has been explored recently by Karan and Miller [32•] to identify RNA-selective coordination complexes. The authors mixed six salicylamide-amino-acid ligands with Cu^{2+} to generate a 27-membered DCL, based on metal-ligand exchange (Figure 5). The library was exposed to approximately 0.07 equivalents of homologous RNA or DNA hairpin templates in a dialysis tube. After several hours, the template was removed and placed in buffer solution to dialyse away bound compounds. The cycle of dialysis in the presence or absence of the library was repeated three times, and the dialysed ligand solutions combined and analysed to determine which ligands were selected by the templates. The results showed that the histidine-derived ligand is preferred by the

RNA template and to a lesser extent by the DNA hairpin. Binding experiments confirmed that in presence of Cu^{2+} the selected ligand binds RNA with remarkable affinity (152 nM), and with higher than 300-fold selectivity over the homologous DNA sequence. Given the small amount of template used relative to the amount of library members, amplification could not be clearly evaluated in this system.

A related approach has been applied recently by Ramstrom and Lehn [23] to study the interaction of carbohydrates with the lectin concanavalin A. Small DCLs of 10 to 21 carbohydrate dimers were prepared using disulfide exchange and exposed to immobilised lectin as template. The authors concluded that the lectin, to an unquantified extent, acts as a thermodynamic trap. In a similar manner, the same group used acetylcholinesterase as template in a hydrazone-based library [33]. Unfortunately, incompatibility between the enzyme and the acidic conditions required for hydrazone exchange made direct amplification impossible.

Macromolecule folding

Dynamic libraries are powerful tools in the search for the best binder to a given template. They are equally powerful in mapping free-energy surfaces and finding the global minimum in the absence of a template. This is particularly relevant for studies of the folding of polymers and oligomers including peptides and proteins. In pioneering work, Case and McLendon [34••] have analysed a small dynamic library of three-helix bundles. The authors prepared three short α-helices appended with 2,2′-bipyridyl units. The three helices differed in hydrophobicity by replacement of four alanine residues by zero, two or four leucine residues (Figure 6). The peptides (in excess) were allowed to assemble around a central iron ion producing an equilibrium mixture of 11 different three-helix bundles. At equilibrium, the most hydrophobic peptides were preferentially incorporated into the bundles. Unfolding studies corroborated that the thus-formed bundles are indeed the most stable ones. Although predictable, these results demonstrate the ability of dynamic libraries to identify the global minimum of a complex system in a single experiment.

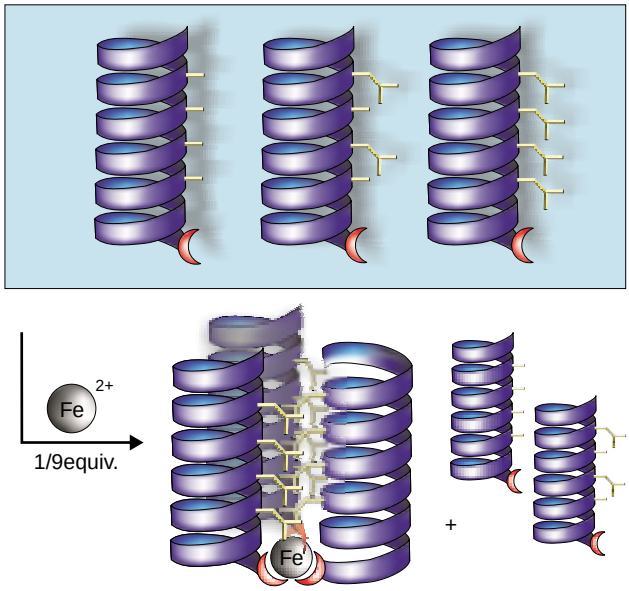
In a similar study, Kumar and co-workers [35] have observed self-recognition of a fluorine-containing α-helix. These authors have prepared a mixed dimer containing a non-fluorinated and a fluorinated α-helix linked by a disulfide bond. Equilibration through disulfide exchange led to the almost complete self-sorting producing a mixture containing only the two homodimers.

Recently, Moore and co-workers [36] have used dimers of fully synthetic oligomers with different lengths linked through an imine bond. Imine exchange produced equilibrium mixtures dominated by the most-stable folded structures.

Conclusions

Over the past two years, ample proof of the viability of the concept of dynamic combinatorial chemistry has accumulated.

Figure 6



Dynamically controlled folding and interactions of macromolecules. Selective incorporation of the most hydrophobic peptides into three-helix bundles.

Research has focussed almost exclusively on small model libraries but has produced encouraging results. We should now start moving towards more diverse libraries that will give rise to new questions: will templating still be efficient enough to produce significant shifts in library composition when library size is increased? And will it still be feasible to isolate selected species directly from larger libraries?

Dynamic combinatorial libraries have considerable potential for drug discovery. Using an enzyme's active site as a template should lead to amplification of potential inhibitors. Although initial results are encouraging, some experimental problems remain to be overcome. In order to achieve significant amplification, introduction of a close to stoichiometric amount of protein template is needed while equilibration is taking place. Given that a reasonable concentration of building blocks is necessary to ensure rapid equilibration, relatively high protein concentrations are required. Moreover, only very few reversible reactions will operate under physiological conditions. Extension of this repertoire is highly desirable.

We should also start thinking about applications in areas such as catalysis where dynamic combinatorial chemistry provides a more controllable and less laborious alternative to the catalytic antibody approach.

Update

In recent work, Storm and Luning [37] demonstrate how small diimine-based dynamic libraries can be biased towards certain macrocycles using alkaline earth ions.

Imine chemistry was also used by Therascope researchers [38] who have identified inhibitors for neuramidase from potentially dynamic libraries.

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