Dynamic combinatorial chemistry

Sijbren Otto, Ricardo L.E. Furlan and Jeremy K.M. Sanders

A combinatorial library that responds to its target by increasing the concentration of strong binders at the expense of weak binders sounds ideal. Dynamic combinatorial chemistry has the potential to achieve exactly this. In this review, we will highlight the unique features that distinguish dynamic combinatorial chemistry from traditional combinatorial chemistry, and that could make a useful addition to the set of combinatorial techniques used in drug discovery.

Sijbren Otto and
Jeremy K.M. Sanders*
University of Cambridge
Dept of Chemistry
Lensfield Road
Cambridge
UK CB2 1EW
tel: +44 1223 336411
fax: +44 1223 336017
*e-mail: jkms@cam.ac.uk
Ricardo L.E. Furlan
Universidad Nacional de Rosario
Facultad de Ciencias
Bioquimicas y Farmaceuticas
Suipacha 531
2000 Rosario
Argentina

▼ Dynamic combinatorial chemistry is a young but rapidly growing field; the first publication clearly introducing the concept dates back to 1996 (Ref. [1]). As the field has been reviewed extensively and comprehensively [2–7], we focus here on the tools presently available, addressing not only the merits but also the problems associated with the technique.

Dynamic versus traditional combinatorial chemistry

The main difference between dynamic combinatorial chemistry and traditional combinatorial chemistry is that the reaction that links the building blocks together in dynamic combinatorial chemistry is reversible [8]. There is a continuous interchange of building blocks between the different members of the dynamic combinatorial library (DCL) and so the composition of the library is governed by thermodynamics rather than kinetics. A DCL is therefore able to respond to external influences. More specifically, molecular recognition events selective for one member of the library will stabilize this member, thereby inducing a shift of the equilibrium towards the formation of this species and away from the species that are not recognized (Fig. 1). Thus, the desired compounds are amplified at the expense of undesired ones that contain the same building block. This is the major advantage of dynamic combinatorial chemistry over traditional combinatorial chemistry. In addition, amplification of the desired compound can be efficient enough to enable isolation of the molecule straight from the library on a preparative scale and in high yield, which is a second important advantage. Ideally, preparation and screening of the library and isolation of the selected compound will be achieved in one step. However, it might sometimes be necessary to make a second biased library comprising only the selected building blocks in the ratio in which they occur in the selected compound. To prevent the isolated species from re-equilibrating, it should be possible to switch the exchange process off.

For building blocks with relatively simple architectures, the structures that can be accessed using dynamic combinatorial chemistry are generally similar to those used in traditional libraries. For example, in the absence of any specific recognition events, a library of dimers of different building blocks will have a similar composition independent of whether it is under kinetic or thermodynamic control (Fig. 2a). More complex topologies become accessible by increasing the valency of the building blocks (that is, the number of functional groups per building block that can take part in reversible bond formation). For these more complex structures (see Fig. 2b–d), kinetic chemistry will probably produce undesired polymeric material, whereas entropy dictates that a thermodynamically controlled library operating at low concentration will consist largely of low molecular weight closed structures. Within combinatorial chemistry, these more complex structures have, to date, been largely ignored as they are difficult to access. We believe that the development of dynamic combinatorial chemistry will change this situation in the very near future.

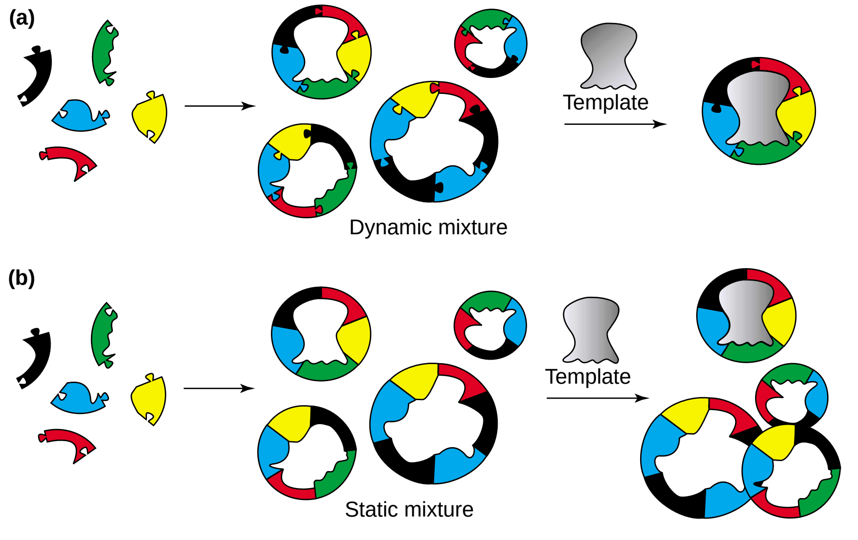
Aside from the merits of dynamic combinatorial chemistry (summarized in Table 1), there are also some limitations. Perhaps the most important constraint (and thus one that is hardly ever mentioned in the research papers) is that every single member of a given DCL must be sufficiently soluble. Frequently, the

1359-6446/02/$ – see front matter PII: S1359-6446(01)02086-4

re-dissolving rate of the precipitated material is so slow that the material becomes effectively trapped in the solid form, resulting in a shift of the equilibria towards this kinetic trap, and affecting all members of the library that contain any of the involved building blocks. Furthermore, the reversibility of the linkage between building blocks puts stringent requirements on the types of reactions that can be used for the construction of DCLs. This significantly limits the number of chemical reactions that can be used (discussed later in this review).

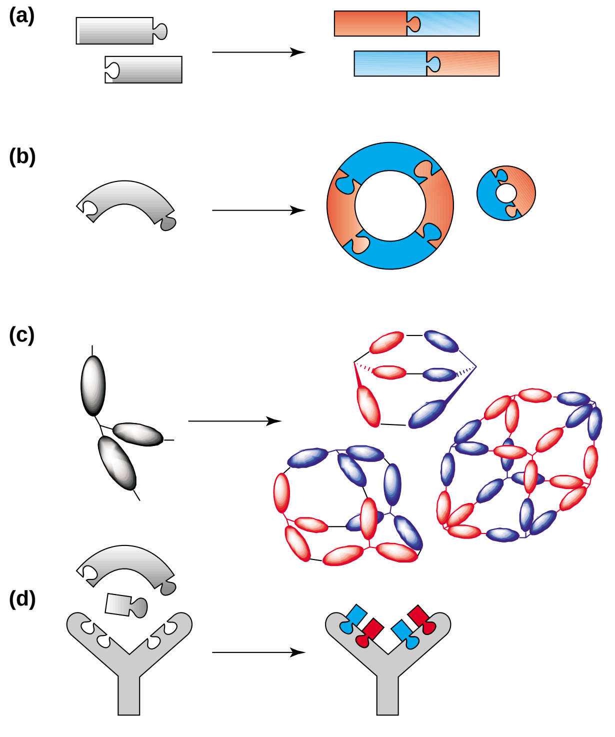
Finally, the level of control that can be asserted in traditional combinatorial chemistry generally exceeds that of dynamic combinatorial chemistry, where no bond is fixed until exchange is turned off. In traditional combinatorial chemistry, every bond that is formed normally remains fixed and unaffected by subsequent reactions. Consequently, many of the techniques developed in traditional combinatorial chemistry (for example, split-and-mix methods) are not compatible with dynamic combinatorial chemistry.

In summary, dynamic combinatorial chemistry is a promising technique in cases where recognition events can be recruited to select, stabilize and thus amplify the desired library members. No previous understanding of the interactions involved in the recognition process is required. A receptor needs no guidance to select its best ligand and vice versa.



Drug Discovery Today

Figure 1. (a) Dynamic combinatorial chemistry versus **(b)** traditional combinatorial chemistry.



Drug Discovery Today

Figure 2. Libraries made from building blocks with different numbers of functional groups (valency) available for reversible bond formation. **(a)** Mono-functionalized building blocks generate libraries of dimers, **(b)** di-functionalized building blocks give access to macrocycles, and **(c)** tri-functionalized building blocks generate libraries of capsule-like molecules. **(d)** Mixing building blocks with different valencies further extends the range of possible architectures.

Reversible reactions for dynamic combinatorial libraries

The processes involved in a dynamic combinatorial approach include: (1) preparation of a mixture of inter-converting molecules; (2) amplification of the best binder(s) through non-covalent interactions with a template; and (3) isolation (or re-synthesis) of the best binder(s). The success of each step depends largely on the type of reversible reaction used to connect the building blocks. Ideally, a rapid reversible reaction is required that is tolerant towards a wide-range of functional groups, that proceeds under mild conditions, and that does not interfere with the recognition events. Moreover, isolation and handling of a library member in its pure form requires another set of conditions where the compound does not undergo any exchange reactions. Hence, it should also be possible to turn the exchange process on or off as required. The switching-off process should not affect the characteristics

Table 1. A comparison of traditional combinatorial libraries with dynamic combinatorial libraries[a]

Traditional combinatorial libraries	Dynamic combinatorial libraries
Concentrations unaffected by recognition events	Recognition can induce amplification
Selected compounds need to be synthesized independently	Selected compounds can be isolated from the library
Complex topologies are difficult to access	Complex topologies are easier to access
Many irreversible reactions available	Number of suitable irreversible reactions is limited
Insolubility of some library members of no consequence	All library members need to be soluble
Stepwise control over every individual reaction step	Limited control because of reversibility

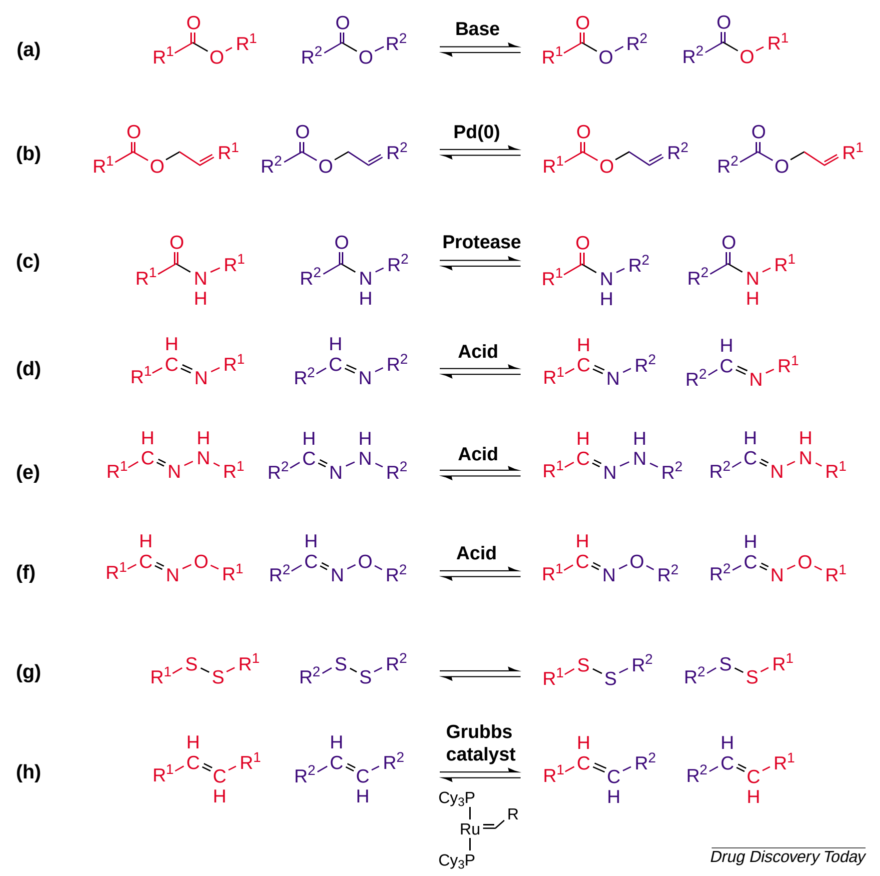
[a] Green and red text represents advantages and disadvantages of the libraries, respectively.

of the members of the library, thereby avoiding changes in the binding properties of the best binder. Finally, the isolated compound has to be stable under the conditions in which it is going to be used.

Non-covalent exchange processes involving hydrogen bonds [9–11] and metal-ligand coordination [12–15] have been used in the preparation of DCLs. These processes are usually fast and proceed under mild conditions. Several examples of receptor amplification have been documented [9–15]. However, removal of the template as well as isolation and re-use of the amplified molecules is problematic because of the labile connections between building blocks.

One way of overcoming this problem could be to covalently link the building blocks after the templating step using a separate irreversible reaction. This approach, which has been implemented by Timmerman, Reinhoudt and colleagues, produces libraries of stable compounds that can be characterized by traditional analytical methods [16]. Although promising, this strategy should be used carefully because there is always the danger that covalent capture will affect the binding properties of library members.

Reversible covalent reactions do not have this problem. Figure 3 provides an overview of the most versatile reversible covalent reactions that have been investigated in the context of dynamic combinatorial chemistry.



Drug Discovery Today

Figure 3. Reversible chemistries that have been used to date for construction of dynamic combinatorial libraries. Red and blue colouring is used to distinguish the different building blocks.

Trans-esterification

Base-catalyzed trans-esterification was the first covalent reversible reaction investigated by our group for the generation of dynamic combinatorial libraries (Fig. 3a) [1,17]. Although base-catalysed ester exchange requires harsh conditions (potassium methoxide in refluxing toluene), template effects by alkaline cations have been observed [18].

A somewhat milder alternative for the preparation of ester-based DCLs is Pd(0)-catalyzed exchange of allyl esters (Fig. 3b) [19]). These characteristics were used for the templated synthesis of cyclic porphyrin dimers under reversible conditions [20].

Peptide-bond exchange

Dynamic combinatorial libraries have also been prepared by exchange of peptide bonds (Fig. 3c). Venton and colleagues have demonstrated that pools of diverse peptides can be prepared by incubating peptide material with low specificity proteases under conditions in which the enzymes catalyze both synthesis and hydrolysis of peptide bonds [21]. Some templating has been observed using this approach (discussed later in this review).

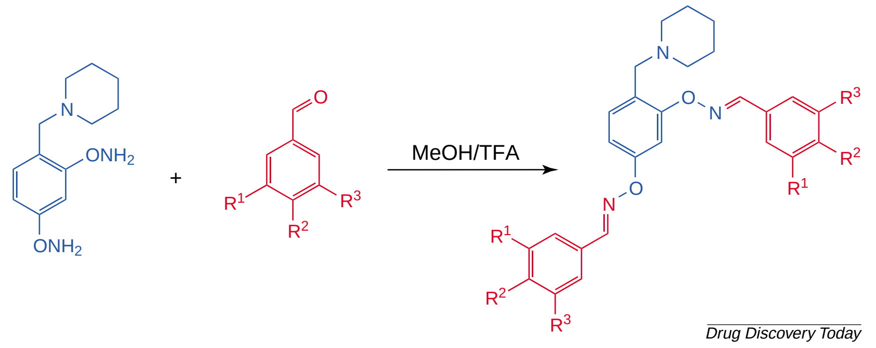


Figure 4. A scaffold-based approach for a dynamic combinatorial library based on oxime exchange. The reaction was carried out at room temperature. R^1, R^2 and R^3 are different combinations of H, OH, Me, OEt, OMe, OAc, and NHAc. Red and blue colouring is used to distinguish the different building blocks.

Exchange over the C=N double bond

Several groups have explored exchange reactions that benefit from the labile C=N double bond. Schiff base exchange (Fig. 3d) has been used for the preparation of a dynamic library of potential enzyme inhibitors in H_2O at pH 6 [22]. However, in practical terms, a disadvantage of the imine functionality is its sensitivity to hydrolysis, which means that for most purposes, reduction of the products to amines is required. Such covalent modification changes both geometry and electronics, and might affect the binding properties of the target compound.

Hydrazones and oximes are structurally related to imines but have the advantage of being hydrolytically stable so that no reduction step is required (Fig. 3e,f).

Eliseev *et al.* have reported on the mechanism of transimination of oximes in H_2O [23]. Equilibration was rapid at higher temperatures and/or under acidic conditions, whereas ambient temperature and neutral conditions effectively switched-off exchange. The same group constructed a DCL of O-aryl and O-alkyl oximes based on a common aromatic scaffold in methanol solution (Fig. 4) [24].

We have developed a hydrazone exchange method for the preparation of DCLs of receptor-like molecules. Macrocycles can be generated by incorporating a hydrazide and a protected aldehyde into the same building block. Under acidic conditions (trifluoroacetic acid in chlorinated solvents), hydrazone formation occurs, leading to the formation of macrocycles. The acid also catalyzes the hydrazone exchange reaction that is responsible for interconversion of the macrocycles. Exchange can be switched-off by neutralization. Cyclization of two dipeptide building blocks can generate libraries of more than 50 macrocycles with unique masses (Fig. 5) (Ref. [25]). The large number of compounds that can be generated from a small number of di-functionalized building blocks requires the use of powerful techniques for the analysis of library composition. Electro-spray ionization Fourier-transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) has been shown to be suitable for this purpose, also enabling the analysis of sequence isomers [26].

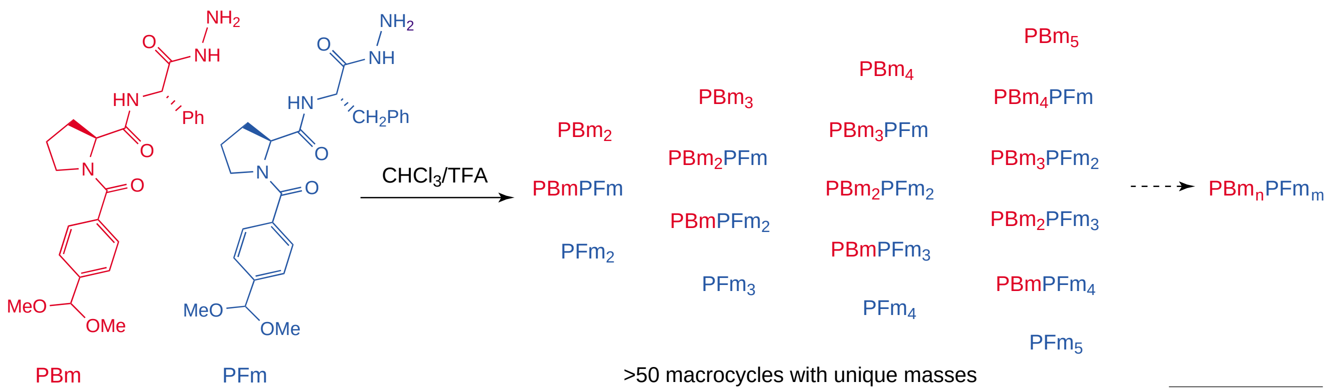
Disulfide exchange

Disulfide exchange is perhaps one of the most promising reactions for dynamic combinatorial chemistry (Fig. 3g). Mechanistic studies [27–29] indicate that: (1) in solution, thiols readily oxidize to disulfides upon exposure to air; (2) disulfide exchange takes place under mild conditions in the presence of a catalytic amount of thiol; and (3) the exchange is switched-off under slightly acidic conditions.

We used these characteristics for the preparation of a DCL of structurally diverse macrocycles in H_2O by mixing neutral or charged building blocks (Fig. 6) derived from amino acids, carbohydrates, or fully synthetic thiols [30]. FTICR-MS analysis of a library made from four of these building blocks indicated the presence of over 100 library members. Lehn *et al.* have also explored disulfide exchange for the preparation of DCLs of sugar dimers in H_2O [31]. Disulfide exchange is also effective in organic solvents, as demonstrated by Hioki and Still [32].

Olefin metathesis

With the development of efficient catalysts [33], olefin metathesis has become a promising reaction for the preparation of DCLs (Fig. 3h). Catalysts such as the carbene-ruthenium complexes developed by Grubbs *et al.*, are commercially available, only moderately air-sensitive, suitable for reactions in H_2O [34], and claimed to be tolerant towards many functional groups [35]. Brändli and Ward have demonstrated that the cross-metathesis of two di-substituted



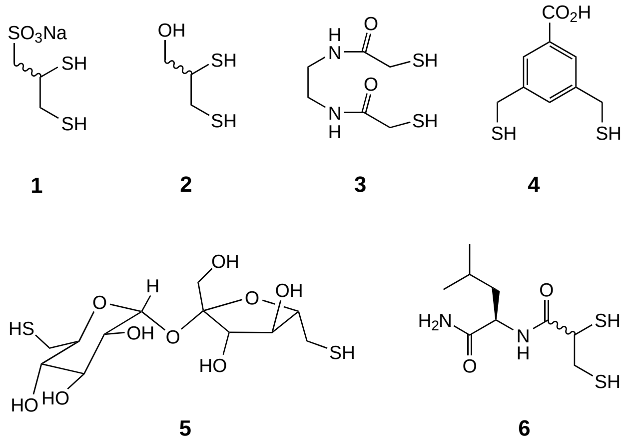
Drug Discovery Today

Figure 5. A hydrazone-based dynamic combinatorial library. Red and blue colouring is used to distinguish the different building blocks.

internal olefins can generate up to 20 different compounds [including (E)/(Z) isomers] [36]. Olefin metathesis ligation was used by Nicolaou *et al.* to prepare a library of vancomycin dimers in H_2O. These authors observed a kinetic template effect in the presence of the peptide Ac_2-L-Lys-D-Ala-D-Ala [37]. Template effects have also been observed for the ring-closing metathesis macrocyclization in the presence of $LiClO_4$ [38].

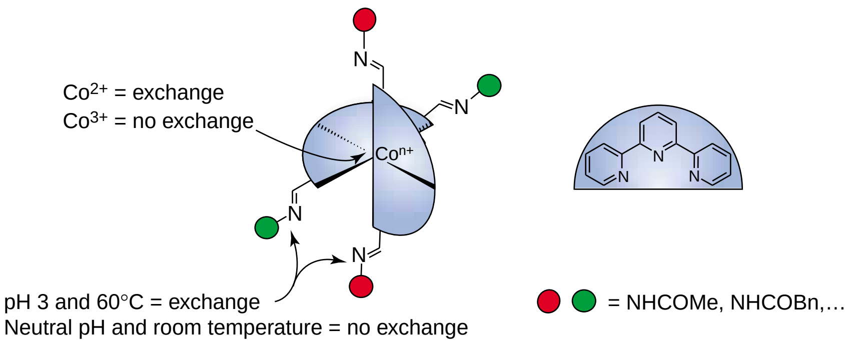
Orthogonal libraries

As in conventional combinatorial chemistry, additional degrees of library diversification can be achieved in a DCL by using two or more reactions to connect the building blocks. This approach has recently been used in the preparation of a DCL, where hydrazones are incorporated into the tridentate ligands that bind to a central cobalt ion (Fig. 7) [39]. The library has two orthogonal levels of diversity because of: (1) exchange of the ligand around the cobalt centre [which is normally rapid but freezes on oxidation to form the kinetically inert Co(III) complexes]; and (2) hydrazone exchange (which is efficient under acidic conditions but inactive under neutral conditions).



Drug Discovery Today

Figure 6. Compounds **1**–**6** are dithiol building blocks used to generate highly diverse dynamic combinatorial libraries. These compounds illustrate the broad functional group tolerance of the disulfide exchange reaction.



Drug Discovery Today

Figure 7. Two orthogonal levels of diversity can be obtained using hydrazone and ligand-cobalt exchange.

Effect of molecular recognition on dynamic combinatorial library composition

Initial studies aimed merely at generating diverse libraries using reversible chemistry; the emphasis now is shifting towards templating these DCLs by exposing them to targets. These targets can either be receptors or ligand molecules (Fig. 8). Here, we review a selection of the most successful examples of

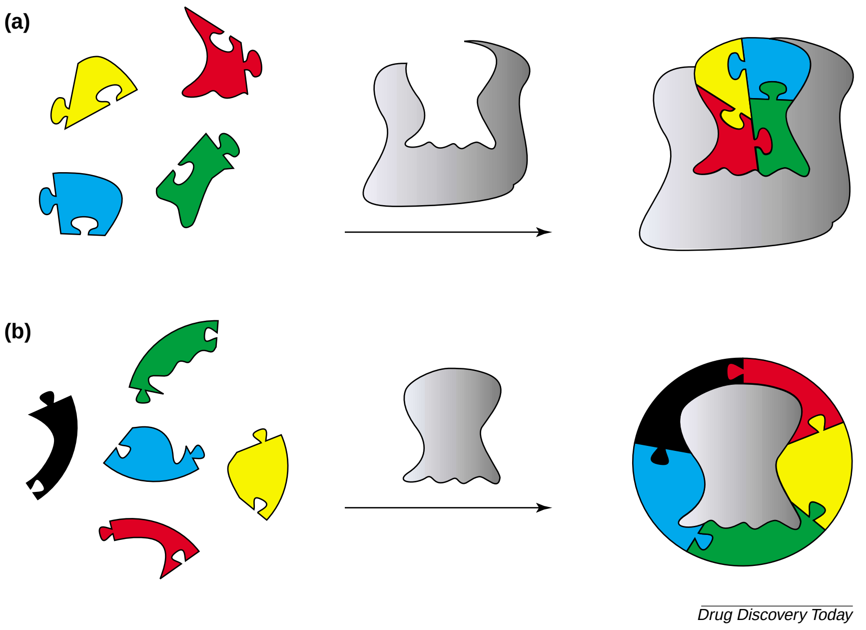


Figure 8. Dynamic combinatorial libraries can be templated by **(a)** receptor molecules or **(b)** ligand molecules.

templating. For a more complete overview, see Refs [2–7].

The most significant examples of templating have been observed when a molecule selects its best receptor from small dynamic libraries of macrocycles of different ring sizes (Fig. 1a and 8b) [40–42]. For instance, a hydrazone-based dynamic system was prepared from a building block derived from L-proline (Fig. 9). Acid-catalyzed cyclization of this building block in chloroform initially yields a mixture of 15 macrocycles, which changes over time to give, mainly, cyclic dimers. At equilibrium, this represents 88% of the library accompanied by 11% of cyclic trimers (as a mixture of conformers). Addition of acetylcholine to the reaction mixture significantly changes the equilibrium to produce a 50-fold amplification of the cyclic trimer (as a

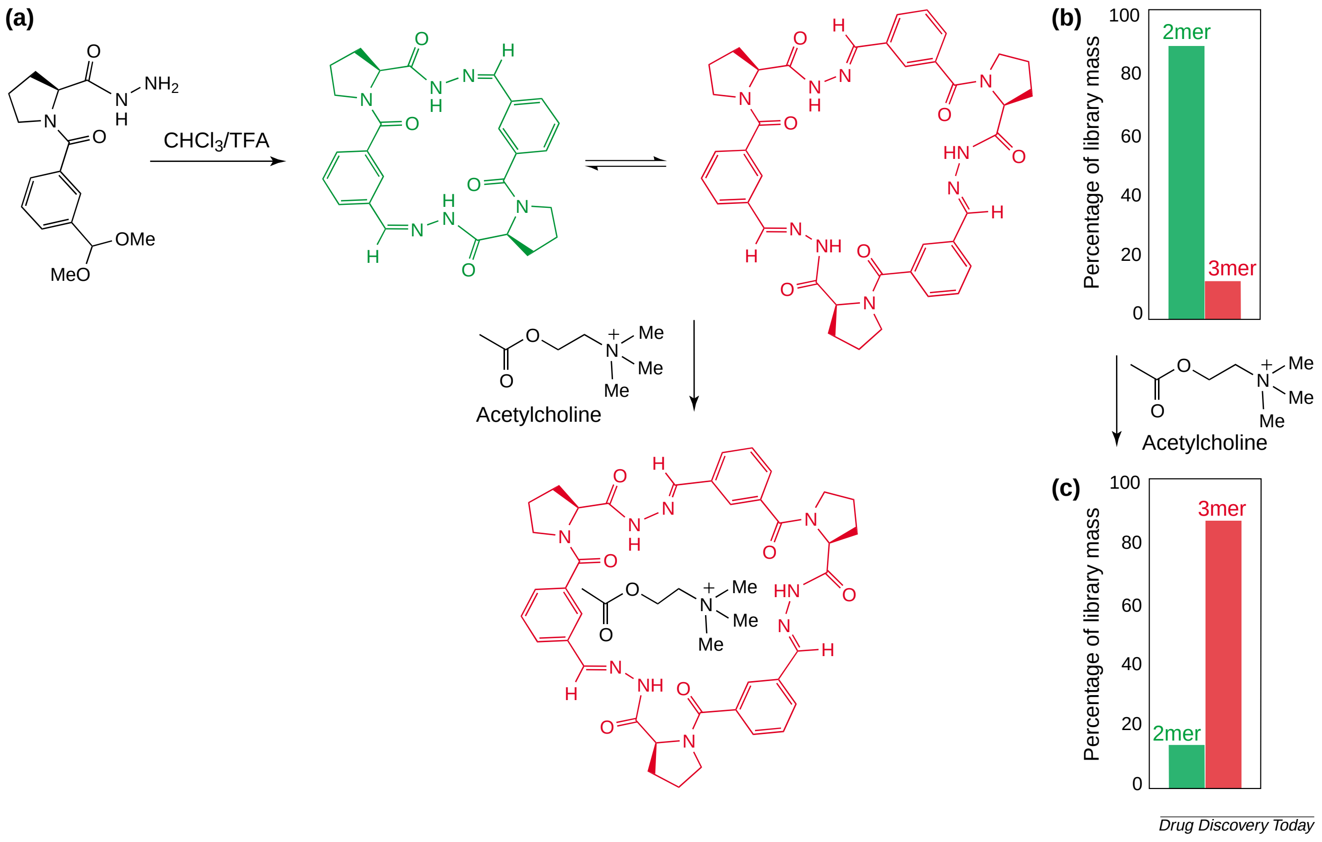


Figure 9. (a) Templating of a hydrazone-based library by acetylcholine. The graphs show the mass percentages of the dimer (green) and trimer (red) in the library in **(b)** the absence and **(c)** the presence of acetylcholine.

single conformer) relative to cyclic dimer. A series of ammonium cations were also tested as templates, and produced different responses from the dynamic library. Binding constants for the complexes between the selected receptor and the different templates were estimated and correlate well with the influence these templates produce in the product distribution [43].

The complementary approach, in which a receptor templates the formation of the strongest binder (Fig. 8a), is probably more relevant in the context of medicinal chemistry. The first detailed report in this area originated in 1996 [21]. Venton and colleagues generated a dynamic library of peptides (discussed previously) and used an antibody as the molecular trap. Despite significant analytical problems (the templating experiments were performed at nanomolar concentrations), the authors concluded that the antibody weakly amplified the formation of library members that had an affinity for the antibody.

In 1997, Huc and Lehn reported on the use of imine chemistry to target carbonic anhydrase [22] and observed modest amplification of a compound structurally similar to a known inhibitor. The most convincing example of the shifts in product distribution observed in these systems was reported in the same year by Sakai *et al.* [44]. These authors covalently linked *N*-acetyl galactose to a bipyridine ligand (Fig. 10). Three of these ligands were coordinated to a central Fe(II) ion, thereby generating a small library of four stereoisomeric carbohydrate species. Rapid ligand exchange ensured that the mixture was at thermodynamic equilibrium. Exposure of the library to one equivalent of lectin (*Vicia villosa* B_4) yielded a substantial shift in product distribution. One of the stereoisomers that had constituted only 15% of the total carbohydrate material in the absence of lectin became the major product (85%) in the presence of the lectin template. Binding experiments confirmed that out of all the stereoisomers present, this compound was indeed the one with the highest affinity for the protein. Interestingly, when different lectins were used as the template, different stereoisomers were selected.

Since these early examples, two additional papers have appeared in which enzymes were used as templates. The first involved dynamic libraries, again investigating carbohydrate–lectin interactions, but using covalent disulfide chemistry [31]. The quantity of isolated, selected carbohydrate dimer depended on the amount of time the library was exposed to the lectin, and the paper concluded that although amplification occurs, the effect is small. The second paper reported on the use of acetylcholinesterase as the target in a hydrazone-based library [45]. However, unfortunately, the enzyme is not stable under the acidic conditions required for hydrazone exchange, and equilibration and selection had to be separated: the method therefore lost most of the advantages of DCLs.

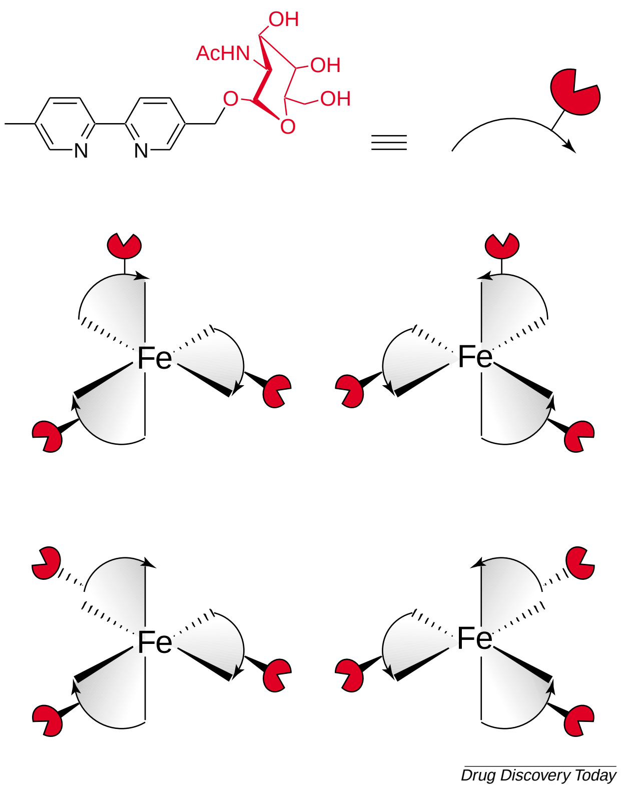


Figure 10. A small dynamic combinatorial library of four diastereomeric complexes, which transforms into a mixture containing one major compound upon exposure to *Vicia villosa* B_4 lectin. The parts of the structures involved in recognition are shown in red.

In summary, there are still experimental difficulties involved with the use of proteins under conditions required for the library to reach equilibrium. When restricted to physiological conditions, disulfide exchange and olefin metathesis are the only reversible reactions presently available that involve covalent bond formation. Another problem, in practical terms, is the concentration of protein required. At least a stoichiometric quantity of (protein) template is required to take full advantage of templating a DCL. There are several orders of magnitude between the concentrations at which researchers tend to dissolve proteins and the typical concentrations at which DCLs equilibrate on a reasonable timescale (usually millimolar).

Similar problems will probably be encountered when using nucleic acids as templates. A degree of success has been achieved in the pioneering work by Miller and colleagues, who used a library assembled from salicylaldimines linked to Zn(II) [46,47]. Some members of the dynamic library are selectively retained by an immobilized DNA template.

Recently, the Miller group has used a DCL based on salicylamides bound to Cu(II) to identify compounds that bind to an RNA hairpin sequence with more than 300-fold selectivity over the homologous DNA sequence [48].

Conclusions and outlook

Dynamic combinatorial libraries have the potential to become a versatile tool in the drug discovery process. Target-induced amplification of the desired products in a DCL will not only facilitate analysis but might also provide quick access to the compound through direct isolation from the library. Furthermore, subsequent larger-scale production of hit compounds might be feasible using the same dynamic conditions, because these enable conversion of side products into desired products through re-equilibration.

To date, although research in dynamic combinatorial chemistry has provided proof of principle, it has also unveiled problems, but these are inevitable when developing a new technique. The next few years will reveal whether the approach is viable. In terms of medicinal applications, this will depend significantly on whether we can bridge the gap between template concentration and library concentration. Furthermore, it would be advantageous to increase the number of reversible covalent reactions that can be used for formation of covalent DCLs under physiological conditions.

Acknowledgements

We are grateful for support from the Marie Curie Individual Fellowships Program to SO (HPMF-CT-1999-00069); the Fundación Antorchas and the Consejo Nacional de Invertigaciones Científicas y Tecnicas (Argentina) to RLEF; and from EPSRC to JKMS.

References

1 Brady, P.A. *et al.* (1996) Living macrolactonisation: thermodynamically-controlled cyclisation and interconversion of oligocholates. *Chem. Commun.* 319–320

2 Ganesan, A. (1998) Strategies for the dynamic integration of combinatorial synthesis and screening. *Angew. Chem., Int. Ed. Engl.* 37, 2828–2831

3 Lehn, J.M. (1999) Dynamic combinatorial chemistry and virtual combinatorial libraries. *Chem. Eur. J.* 5, 2455–2463

4 Klekota, B. and Miller, B.L. (1999) Dynamic diversity and small-molecule evolution: a new paradigm for ligand identification. *Trends Biotechnol.* 17, 205–209

5 Karan, C. and Miller, B.L. (2000) Dynamic diversity in drug discovery: putting small-molecule evolution to work. *Drug Discov. Today* 5, 67–75

6 Cousins, G.R.L. *et al.* (2000) Molecular evolution: dynamic combinatorial libraries, autocatalytic networks and the quest for molecular function. *Curr. Opin. Chem. Biol.* 4, 270–279

7 Lehn, J-M. and Eliseev, A.V. (2001) Dynamic combinatorial chemistry. *Science* 291, 2331–2332

8 Rowan, S.J. *et al.* Dynamic covalent chemistry. *Angew. Chem., Int. Ed. Engl.* (in press)

9 Crego Calama, M. *et al.* (2000) Guest-templated selection and amplification of a receptor by non-covalent combinatorial synthesis. *Angew. Chem., Int. Ed. Engl.* 39, 755–758

10 Hof, F. *et al.* (2000) Diversity and selection in self-assembled tetrameric capsules. *J. Am. Chem. Soc.* 122, 4251–4252

11 Huc, I. *et al.* (1999) Dynamic combinatorial chemistry: substrate H-bonding directed assembly of receptors based on bipyridine-metal complexes. *Eur. J. Inorg. Chem.* 1415–1420

12 Baxter, P.N.W. *et al.* (2000) Adaptative self-assembly: environment-induced formation and reversible switching of polynuclear metallocyclophanes. *Chem. Eur. J.* 6, 4140–4148

13 Hiraoka, S. *et al.* (2000) Self-and hetero-recognition in the guest-controlled assembly of Pd(II)linked cages from two different ligands. *Chem. Commun.* 1509–1510

14 Albrecht, M. *et al.* (1999) An expansible metalla-cryptand as a component of a supramolecular combinatorial library formed from di(8-hydroxyquinoline) ligands and Gallium(III) or Zinc(II) ions. *Chem. Eur. J.* 5, 48–56

15 Hasenknopf, B. *et al.* (1997) Self-assembly of tetra-and hexanuclear circular helicates. *J. Am. Chem. Soc.* 119, 10956–10962

16 Cardullo, F. *et al.* (2000) Covalent capture of dynamic hydrogen-bonded assemblies. *Chem. Commun.* 367–368

17 Rowan, S.J. and Sanders, J.K.M. (1998) Macrocycles derived from Cinchona alkaloids: a thermodynamic vs kinetic study. *J. Org. Chem.* 63, 1536–1546

18 Brady, P.A. and Sanders, J.K.M. (1997) Thermodynamically-controlled cyclisation and interconversion of oligocholates: metal ion templated living macrolactonisation. *J. Chem. Soc. Perkin Trans.* 1, 3237–3253

19 Amatore, C. *et al.* (1999) Evidence of the reversible formation of cationic π–allylpalladium(II) complexes in the oxidative addition of allylic acetates to palladium(0) complexes. *Chem. Eur. J.* 5, 466–473

20 Kaiser, G. and Sanders, J.K.M. (2000) Synthesis under reversible conditions of cyclic porphyrin dimers using palladium-catalysed allyl transesterification. *Chem. Commun.* 1763–1764

21 Swann, P.G. *et al.* (1996) Nonspecific protease-catalyzed hydrolysis/synthesis of a mixture of peptides: product diversity and ligand amplification by a molecular trap. *Biopolymers* 40, 617–625

22 Huc, I. and Lehn, J.M. (1997) Virtual combinatorial libraries: dynamic generation of molecular and supramolecular diversity by self-assembly. *Proc. Natl. Acad. Sci. U. S. A.* 94, 2106–2110

23 Polyakov, V.A. *et al.* (1999) Imine exchange in O-aryl and O-alkyl oximes as base reaction for aqueous 'dynamic' combinatorial libraries. A kinetic and thermodynamic study. *J. Phys. Org. Chem.* 12, 357–363

24 Nazarpack-Kandlousy, N. *et al.* (1999) Synthesis and characterization of a mixture-based library of oxime ethers based on a common aromatic scaffold. *J. Comb. Chem.* 1, 199–206

25 Cousins, G.R.L. *et al.* (1999) Dynamic combinatorial libraries of pseudo-peptide hydrazone macrocycles. *Chem. Commun.* 1575–1576

26 Poulsen, S.A. *et al.* (2000) Electrospray ionization Fourier-transform ion cyclotron resonance mass spectrometry of dynamic combinatorial libraries. *Rapid Commun. Mass Spectrom.* 14, 44–48

27 Wallace, T.J. *et al.* (1963) The base-catalyzed oxidation of mercaptans. III. Role of the solvent and effect of mercaptan structure on the rate determining step. *J. Org. Chem.* 28, 1311–1314

28 Lees, W.J. and Whitesides, G.M. (1993) Equilibrium constants for thiol-disulfide interchange reactions: a coherent, corrected set. *J. Org. Chem.* 58, 642–647

29 Gilbert, H.F. (1995) Thiol/disulfide exchange equilibria and disulfide bond stability. *Methods Enzymol.* 251, 8–28

30 Otto, S. *et al.* (2000) Dynamic combinatorial libraries of macrocyclic disulfides in water. *J. Am. Chem. Soc.* 122, 12063–12064

31 Ramström, O. and Lehn, J.M. (2000) *In situ* generation and screening of a dynamic combinatorial carbohydrate library against Concanavalin A. *Chem. BioChem.* 1, 41–48

32 Hioki, H. and Still, W.C. (1998) Chemical evolution: a model system

that selects and amplifies a receptor for the tripeptide D-Pro-L-Val-D-Val. *J. Org. Chem.* 63, 904–905

33 Grubbs, R.H. and Chang, S. (1998) Recent advances in olefin metathesis and its application in organic synthesis. *Tetrahedron* 54, 4413–4450

34 Lynn, D.M. *et al.* (1996) Living ring-opening metathesis polymerization in aqueous media catalyzed by well-defined ruthenium carbene complexes. *J. Am. Chem. Soc.* 118, 784–790

35 Grubbs, R.H. *et al.* (1995) Ring-closing metathesis and related processes in organic synthesis. *Acc. Chem. Res.* 28, 446–452

36 Brändli, C. and Ward, T.R. (1998) Libraries via metathesis of internal olefins. *Helv. Chim. Acta* 81, 1616–1621

37 Nicolaou, K.C. *et al.* (2000) Target-accelerated combinatorial synthesis and discovery of highly potent antibiotics effective against vancomycin-resistant bacteria. *Angew. Chem., Int. Ed. Engl.* 39, 3823–3828

38 Marsella, M. *et al.* (1997) Template-directed ring-closing metathesis: synthesis and polymerisation of unsaturated crown ether analogs. *Angew. Chem., Int. Ed. Engl.* 36, 1101–1103

39 Goral, V. *et al.* (2001) Double-level ‘orthogonal’ dynamic combinatorial libraries on transition metal template. *Proc. Natl. Acad. Sci. U. S. A.* 98, 1347–1352

40 Furlan, R.L.E. *et al.* (2000) Molecular amplification in a dynamic combinatorial library using non-covalent interactions. *Chem. Commun.* 1761–1762

41 Cousins, G.R.L. *et al.* (2001) Identification and isolation of a receptor for *N*-methyl alkylammonium salts: molecular amplification in a pseudo-peptide dynamic combinatorial library. *Angew. Chem., Int. Ed. Engl.* 40, 423–428

42 Furlan, R.L.E. *et al.* (2001) A new cyclic pseudo-peptide receptor for Li^+ from a dynamic combinatorial library. *J. Am. Chem. Soc.* 123, 8876–8877

43 Furlan, R.L.E. *et al.* Molecular amplification in a dynamic system by ammonium cations. *Tetrahedron* (in press)

44 Sakai, S. *et al.* (1997) A self-adjusting carbohydrate ligand for Gal*N*Ac specific lectins. *Tetrahedron Lett.* 38, 8145–8148

45 Bunyapaiboonsri, T. (2001) Dynamic deconvolution of a pre-equilibrated dynamic combinatorial library of acetylcholinesterase inhibitors. *Chem. BioChem.* 2, 438–444

46 Klekota, B. *et al.* (1997) Generation of novel DNA binding compounds by selection and amplification from self-assembled combinatorial libraries. *Tetrahedron Lett.* 38, 8639–8642

47 Klekota, B. and Miller, B.L. (1999) Selection of DNA-binding compounds via multistage molecular evolution. *Tetrahedron* 55, 11687–11697

48 Karan, C. and Miller, B.L. (2001) RNA-selective coordination complexes identified via dynamic combinatorial chemistry. *J. Am. Chem. Soc.* 123, 7455–7456