Harnessing dynamic combinatorial chemistry in the search for new ligands for protein targets

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Harnessing dynamic combinatorial chemistry in the search for new ligands for protein targets

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Keywords: drug discovery • dynamic combinatorial chemistry • inhibitors • molecular recognition

Drug discovery is a time-consuming and intricate process. As a result, novel tools to accelerate this process and render it more efficient are urgently needed. Dynamic combinatorial chemistry (DCC) is one such tool and it holds the potential to accelerate both hit identification and optimization. DCC was first introduced in the 1990s [1,2], and since then, numerous examples of applications in medicinal-chemistry and chemical-biology projects have been reported [3–6].

General features of DCC

DCC is a method in which different building blocks react reversibly to generate libraries of chemical compounds under thermodynamic control. A dynamic combinatorial library (DCL) is adaptive if it is formed in the presence of the target, which works as a template, or pre-equilibrated, if the target is added once the DCL has reached equilibrium. The former is preferred, given that it affords a fully responsive system. Here, the system can adapt due to molecular recognition events between the protein target and one or more of the library members, the equilibrium will shift, resulting in amplification of the best binders at the expense of other members of the DCL that contain the same building blocks. Pre-equilibrated DCLs are especially useful when working with targets that are not compatible with the equilibration conditions.

To generate a DCL, it is possible to use a range of chemical reactions; however, some criteria must be fulfilled. The reaction of choice should be biocompatible, meaning that it should take place in aqueous medium and at a temperature and pH at which the protein target is stable (for adaptive DCLs). It is also important that the reactions are chemoselective to avoid the formation of undesired compounds or side reactions with the protein. The building blocks should have similar reactivity and energy to ensure that comparable amounts of each library member are formed in an untemplated DCL. Furthermore, all members of the DCL must be soluble, to avoid any compound being trapped as a solid, biasing the equilibrium. To improve the solubility, a small percentage of a cosolvent, such as DMSO, can be used if tolerated by the protein target. In some instances, when the reaction of choice is slow, the use of a catalyst can speed up the reaction to ensure it proceeds in a time frame during which the protein remains folded. For example, a nucleophilic catalyst such as aniline can be used when the reversible system consists of acylhydrazones, promoting the equilibration of the DCL at a pH compatible with most protein targets rather than the acidic pH required in the absence of a catalyst [7,8].

To analyze the library, the conditions should be modified in order to ‘freeze’ the equilibrium and ensure that the composition...
remains constant. This can be done by changing the pH or irreversible modification of the ligands formed (for example, \textit{in situ} reduction of imines to amines). For relatively small libraries, separation techniques such as HPLC, HPCE (high-performance capillary electrophoresis) or GC are routinely applied. In some cases, MS- or NMR-based techniques have been successfully used. For larger libraries, a combination of chromatographic techniques coupled to MS can simplify the analysis.

In most reports of protein-templated DCC, the potentially bioactive ligands feature reversible covalent bonds connecting the various building blocks, and these ligands interact with the biological target through noncovalent bonds. Once a hit has been identified, bioisosteric modification of the reversible connector is necessary in most cases to obtain stable analogs while preserving the biochemical activity.

The increasing number of publications over the past 20 years is a good reflection of the success of DCC applied to medicinal chemistry and chemical biology. DCC has enabled the discovery of binders of a variety of DNA, RNA and protein targets, including several enzyme classes, such as proteases, anhydrases, kinases and oxygenases [5].

**Advantages & limitations of DCC**

The main advantage of DCC is its potential to accelerate the hit-identification process: library generation and screening of the potential ligands are combined into a single operation, avoiding the individual synthesis, purification, characterization and biochemical evaluation of every single member of the DCL.

The most challenging part of DCC is often the analysis, which becomes more difficult with increasing size and complexity of the DCL. Up to now, mainly small libraries and well-known targets have been studied. Long equilibration times are another limitation, especially for unstable protein targets or library members.

"...identifying more biocompatible, reversible reactions will endow the medicinal chemist with a more diverse ‘toolbox’!"

New approaches have emerged to circumvent the limitations of DCC. For example, the analysis of large and more complex libraries can be simplified by looking only at the bound species by using mild techniques to identify protein–ligand complexes or by ‘fishing’ these complexes from the mixture. For instance, in a proof of concept study reported by Guo and co-workers [9], size-exclusion chromatography (SEC) allows for the separation of the ligand–target complexes from the DCL. After denaturation, the ligands are released from the protein and identified by MS analysis. This pioneering study was carried out using the well-established model enzyme hen egg-white lysozyme, but SEC has not been used for the discovery of inhibitors of a ‘real’ drug target yet. Other options are the immobilization of the target or library members on a solid support. For example, Lehn and co-workers identified binders of the carbohydrate-binding protein concanavalin A by adding the immobilized protein target to the DCL [10]. The protein was immobilized on sepharose beads, allowing for the removal of the nonbinding compounds by filtration and subsequent elution of the bound compounds for characterization. The reverse approach, comprising immobilization of the library members, has been reported by Miller and co-workers. In this so-called resin-bound DCC (RBDCC) [11], the building blocks are attached to a resin and combined with the same building blocks in solution to form a DCL of dimers. After incubation with the fluorescent-tagged DNA target, wash and analysis of the fluorescent beads, they succeeded in the identification of DNA binders. There are a few more proof-of-principle studies regarding the immobilization of the target or library components on a solid support, but these are also carried out using model systems and do not involve protein targets [12-14]. Alternatively, by choosing the right conditions for imine formation [15,16] or a reversible reaction that leads to inherently labile products such as hemithioacetals [17], virtual dynamic combinatorial libraries can be generated. In these systems, little or no products are formed in the absence of the target protein, meaning that only binders have to be detected, thereby facilitating the analytical challenge.

There are a few reports in which challenging targets have been studied. For example, Wanner and co-workers developed MS-binding assays to identify binders of the GABA transporter 1 [18]. This method is highly sensitive, allowing the detection of binders using a low concentration of the target protein. This can be especially useful when studying targets such as GPCRs, ion channels and transporters.

**Future perspective**

Despite the progress, there are still a number of challenges that need to be addressed before DCC could be used by a wider community, including the pharmaceutical industry. For example, identifying more biocompatible, reversible reactions will endow the medicinal chemist with a more diverse ‘toolbox’. Until now, the structural diversity that can be achieved by employing established reversible reactions is arguably somewhat limited and most applications in medicinal-chemis-
try projects are focusing on the formation of imine-type C=N double bonds or disulfides. Having access to more reactions that are compatible with protein-templated DCC will also facilitate the development of doubly dynamic systems: the combination of two different reactions can be useful for example to simultaneously screen/optimize fragments for two different pockets of the active site of an enzyme. This would be easier if both reactions were compatible with the same pH window, which should also be tolerated by the protein target.

The analysis of the DCLs is one of the most important bottlenecks, and in order to simplify it, techniques such as affinity chromatography will be established to successfully identify ligands in larger libraries. Furthermore, it will be possible to study more challenging targets, such as transmembrane proteins or protein–protein interactions.

Apart from improvements of the technical aspects of the method, more applications to a growing set of protein targets are expected, which will demonstrate its general applicability. Even though most reports to date focus on identification of first hits or lead compounds, DCC could equally find application in the optimization of pharmacokinetic (PK) properties. Optimizing PK properties right from the early stages of the drug discovery process is becoming increasingly important and DCC would allow the screening of a relatively large number of derivatives of a hit in a short period of time. Regarding the enhancement of selectivity, DCC can also be exploited to identify isoform-selective inhibitors, which would be extremely useful, for instance in the case of kinases, whose family consists of more than 500 isozymes. Greaney and co-workers used this approach to develop selective inhibitors of two isozymes from the GST class of enzymes [8].

Although most of the publications so far are proof-of-concept studies, establishing either a novel reversible biocompatible reaction or an analytical technique, DCC certainly holds the potential to truly revolutionize the drug-discovery process by combining the in situ synthesis and screening of structurally diverse potential ligands for a specific target into a single operation.

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References

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