TECHVIEW: DRUG DISCOVERY

Integrating Combinatorial Synthesis and Bioassays

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In the early 1990s, combinatorial chemistry started off with nothing less than the ambition of revolutionizing drug discovery (*1*). The pharmaceutical industry hoped that large collections of new compounds would accelerate the identification of suitable drug candidates. Today, the standard industrial approach to the synthesis of small drug-like molecules either in solution or on a solid support is highly parallel (*2*). In the ideal case, each compound is purified and characterized for identity and purity with techniques such as high performance liquid chromatography (HPLC) and mass spectrometry (MS) before testing. Screening is carried out mostly in 96- and 384-well formats.

But the route from design and synthesis of compound libraries to identification of a biologically valid lead structure is still long and tedious. Highly parallel synthesis requires automation not just in synthesis, but also in analysis and screening. In addition, enormous efforts are necessary to handle the amount of data generated. A number of challenges must be met before the current processes can become routine tools for drug discovery. As more and more potential protein targets become available, ever more diverse compound libraries will have to be made and ways to efficiently extract information from such libraries will have to be developed. At the same time, the bottleneck in drug development has shifted from the generation of lead structures to their transformation into orally active drugs with the desired physiological properties and performance results in clinical trials. It will therefore no longer be enough to synthesize large and diverse libraries; rather, it will become more and more important to find ways to integrate demands such as bioavailability at an early stage of the drug search.

These challenges can only be met by creating efficient interfaces between combinatorial syntheses and bioassays. This article will focus on a few recent developments in this area, namely the screening of compound mixtures, screening on beads, the use of surface microarrays, and new label-free methods for detecting binding interactions.

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Screening Highly Complex Mixtures in Solution

Synthesis of and screening for ligands of immunological receptors can be linked directly by using equimolar mixtures of compounds in solution. These mixtures can, for example, be obtained in the form of peptide subcollections synthesized by solid-phase synthesis with mixtures of activated amino acids in randomized coupling. In each subcollection, one amino acid position is left unchanged and the others are randomized. For example, 220

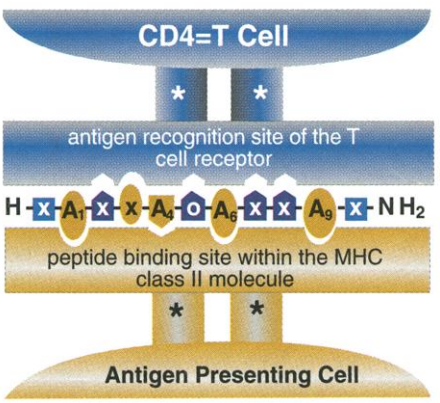

Fig. 1. Screening of highly complex mixtures. 2×10^{13} peptides were tested simultaneously in solution. By synthesizing subcollections of 11-mer peptides with one unchanged position (O) and all other positions randomized (X), superagonistic peptides for the activation of a multiple sclerosis related T cell clone could be identified (*5*).

11-mer subcollections, each containing 2×10^{13} peptides, can be tested separately in a solution assay to extract the relevant biological information from such complex mixtures (Fig. 1) (*3*). By collecting information on all of the positions, single active peptides from protein databases can be directly defined with the program Actipat (*4*) and the experimental data obtained for each major histocompatibility complex (MHC) allele and T cell clone. Activity patterns for a representative number of human leucocyte antigen (HLA) types and T cell clones associated with autoimmune disease have been determined (*3*, *5*, *6*). In a recent application of this approach, superagonistic peptides have been identified for a multiple sclerosis–related human T cell clone (*5*). These single peptides exhibited 10,000 to 100,000 times as much activity as the well-known natural autoantigen. The approach has also been applied to a T cell clone related to chronic Lyme disease (*6*). First examples of the identification of peptidomimetic T cell antagonists from oligomer collections have been reported (*7*). A revival of the use of complex combinatorial oligomer collections is expected, reinforced by advances in analysis. For example, high-resolution Fourier transform–ion cyclotron resonance MS is a new powerful technique that has been demonstrated recently in the high-throughput analysis of organic compound collections, yielding the elemental constitution of each analyte in a single MS experiment and from single beads (*8*).

Screening in the Interior of Resin Beads

The closest link between synthesis and screening can arguably be achieved by conducting both on the same support, that is, on pins and resin beads. A broader application of this very early concept (*9*) was long prohibited by the lack of resin materials compatible with the in-bead use of enzymes. The super permeable resin for organic combinatorial chemistry (SPOCC) was recently introduced to further extend the use of biocompatible resins such as polyethylene glycol dimethyl acrylamide copolymer (PEGA) (*10*). SPOCC contains only primary ether bonds and is fully compatible with organic chemistry and enzyme assays (*11*). These polar resins have been used to synthesize peptide libraries by the split-and-combine technique. Peptide substrates carrying a fluorescent dye and a quencher can be used for protease substrate assays; upon cleavage of a substrate, the resin bead becomes strongly fluorescent. This fluorescence resonance energy transfer (FRET) assay has been used with large libraries of peptides to select for good substrates by collecting the most fluorescent beads (*12*, *13*). Furthermore, Meldal *et al.* have demonstrated the selection of inhibitors from a one-bead–two-compound library prepared by ladder synthesis (Fig. 2) (*14*). The FRET concept saves isolation and cleaning steps because only the beads carrying the most active compounds have to be analyzed and only the identified molecules have to be resynthesized and purified. The structures of active compounds can be elucidated by cleavage of a photolabile linker followed by matrix-assisted laser desorption ionization

(MALDI)–MS analysis. This combined FRET approach has now been demonstrated for the elucidation of substrate specificity of several proteases and for the selection of inhibitors for an osteoporosis-related metalloprotease (*15*).

Screening of Microarrays

A viable alternative to on-bead screening is the screening on functionalized surfaces. On surfaces, compounds synthesized on solid-phase or in solution can be used. Furthermore, surfaces are accessible for larger proteins and protein complexes as well as for whole cells. The classical example for an arrayed microassay is the spot technique developed by Frank (*16*). In this method, synthesis and screening is conducted on cellulose by adding droplets of reagents or screening solutions with a robot. Such arrays have been used in assays for epitope mapping, substrate analysis, and protease inhibition. Today, most bioassays use 96- or 384-well plates, which immobilize one of the binding partners while the other is applied in solution. One crucial drawback of this classical assay format is the relatively high amount of protein and test solution needed (sample volume in the microliter to milliliter range). Miniaturization of this assay concept to nanoliter volumes is being approached to create a high-throughput system when only small amounts of target protein are available.

DNA microarrays are used routinely in expression analysis and genomics, and their use for expression profiling of small drug-like molecules is being investigated intensely. Schreiber and his group recently presented a method of small molecule printing that could be useful for arraying collections of synthetic compounds (*17*). They used glass surfaces to immobilize compounds from nanoliter droplets. A square centimeter of glass can fit 1000 samples and can be inspected under a fluorescence microscope. The Schreiber group also described a similar microarray system for screening whole cells (*18*). These microarray techniques allow the handling of very small volumes of solution by using a microfluidic system with a pipetting robot as its cornerstone. So far, the techniques have only been used in assays of known binding interactions, and their success in detecting protein ligands from a compound library remains to be demonstrated. One potentially interesting feature of these microarrays is the preparation of screening chips, which could be reused in several assays with different protein targets and could become a valuable tool for target evaluation.

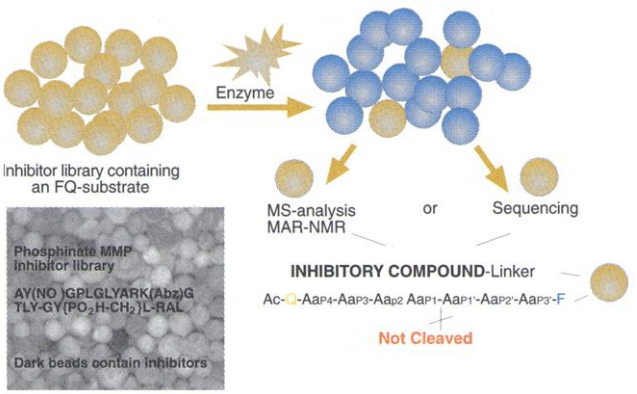

Fig. 2. Screening of libraries with on-bead assays. By the split-and-combine method, a phosphinate matrix metallo-proteinase (MMP)–inhibitor library was synthesized on novel biocompatible polyethylene glycol–based resins. Each bead carried a potential inhibitor compound and a fluorescent-quenched substrate (one-bead–two-compound library). Following enzyme digest of the library, beads carrying the most active inhibitors remain dark and can be selected and analyzed, preferably by MS techniques (*15*).

Label-Free Detection of Affinity-Binding

The mode of detection is a critical element in surface-based techniques for the screening of binding interactions. Classically, fluorescent or radioactive labels have been used, but labeling requires extra synthesis and isolation steps. Considering the many new proteins that are or will be delivered from the isolation or expression of human genes, label-free detection of interactions with small amounts of protein samples is desirable. Plasmon resonance is one commercially available method that allows label-free detection for single samples, but this method is not useful for high-throughput processing. Other label-free detection methods, such as ellipsometry and polarization methods, suffer from the same limitation. A detection technique based on white light interference, reflective interference spectroscopy (RIfS), which overcomes this limitation, was recently developed by Brecht and Gauglitz (*19*). The method exploits the interference of light reflected from a reference layer of known thickness with the partial reflection from the layer formed in the binding event under investigation. RIfS has been demonstrated in studies of the binding kinetics of antibodies, competitive binding of thrombin substrates, and low–molecular weight inhibitors (*20*). Recently, the Gauglitz group presented a microarray setup for the method, which can be used to screen for small-molecule binding to proteins. Furthermore, RIfS sensor arrays with cyclopeptide collections have been successfully applied to detect low–molecular weight analytes in the gaseous (*21*) and aqueous phase (*22*).

Outlook

Bridging the gap between combinatorial synthesis and bioassays is important if combinatorial chemistry is to achieve its ambitious goal of supplying efficient methods for the selection of biologically active molecules. The examples discussed here demonstrate how crucial the combination of knowledge from different areas of expertise is for success. Other developments in analytical techniques and computational library design are also strong contributors to the advancement of combinatorial chemistry. It remains difficult to judge how much combinatorial chemistry has changed the drug-finding processes in the pharmaceutical industry so far, but techniques for finding active compounds for proteins quickly and reliably will be of great value for companies and for the biological sciences.

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