A Review on: Combinatorial Chemistry in Drug Discovery

Author Name: Ms. Priyanka Gajanan Wagh
Co-author: Mr. Vishal Shivaji Gaikwad
Pratibhatai Pawar College of Pharmacy, Wadala Mahadev.

Abstract:

Several combination methods were developed to create it Focused or diverse chemical libraries with broad linear ranges or macrocyclic chemical molecules: peptides, non-peptides Oligomers, peptidomimetics, small molecules and natural Product-like organic molecules. any combination method Has its own unique high-throughput screening and encoding strategy. In this article we provide a brief overview of it Combinatorial Chemistry in Drug Discovery, with emphasis on In recent years, design, synthesis, Combinatorial library screening and decoding. Example Successful application of combinatorial chemistry in thermal shock Discovery and lead compound optimization are presented. restrictions and The advantages of combinatorial chemistry are also briefly discussed. We are now better able to actually take advantage of Combination technologies for discovery and development Next generation medicines.

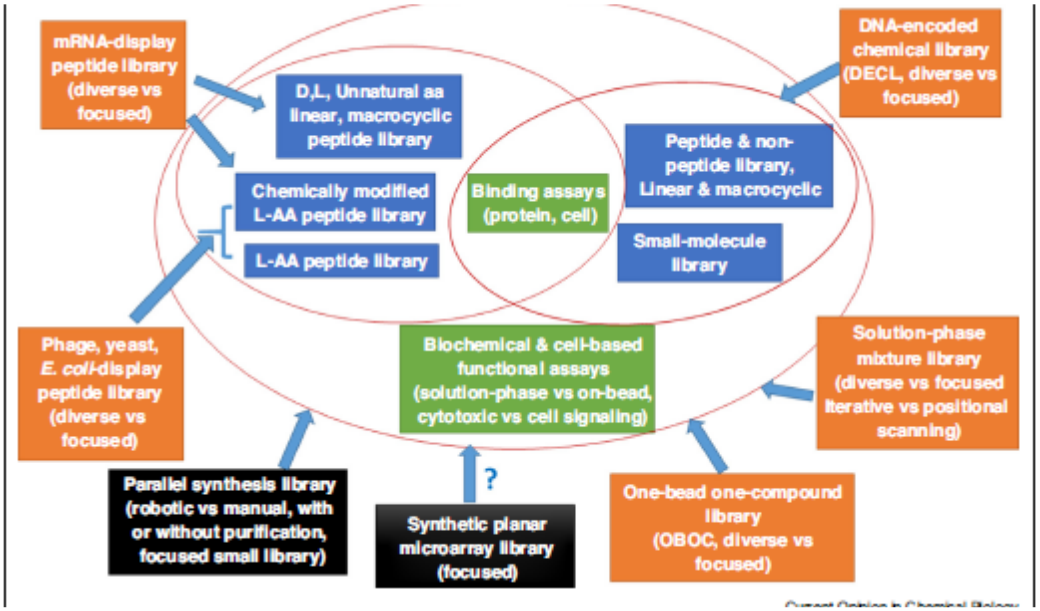
Keywords – Non-peptides, Oligomers, macrocyclic, peptidomimetics

Introduction:

Chemical library building involves systematic, repetitive and covalent linkagevarious of' building blocks'. Once prepared, these compounds can be screened for individual interactions with biological targets of interest. Positive compounds can then be identified either directly (in position-addressable libraries) or via decoding (using genetic or chemical means) combinatorial chemistry is a new method developed by academic and researchers to reduce the time and cost of producing effective marketable and competitive new drugs.Scientist use combinatorial chemistry to create large number of molecule that can be defected efficiently.This technique has captured the attention of many areas such as pharmaceutical,biotechnology and agrochemistry.

The concept of combinatorial chemistry is based on Geysen's multi-needle technology was adopted in the mid-1980s Synthesized with Houghten's tea bag technique Hundreds of thousands of peptides on a solid support parallel lines. In 1991, Lam et al.]Introduction to single beads Single compound combinatorial peptide library (OBOC). and Horton et al. describes the resolution phase Mixtures of combinatorial peptide libraries. 1992 The first example was reported by Bunin and Ehrman Small molecule combinatorial library also Microspheres, peptides and other synthetic compounds can be displayed on flat or Solid support, such as glass, to form a planar microarray. In 1985, Smith described phage display peptides library method . Similar to the OBOC library, one for each M13 Phage displays unique peptide unit (five copies); That is, phages and peptides. Positive phages can then Isolated for reinforcement, re-rotation, etc. decodingand DNA sequencing. Compared to synthetic libraries Methods, early biological libraries (phage display, yeast display, polyribosome display peptide libraries) are limited 20 Natural L-Amino Acids Used and Easy Cyclization with a disulfide bridge. In the mid-2000s, Frankel et al. Josephson et al. and Murakami et al. Macrocyclic peptide library with mRNA display reported Use unnatural amino acids and D-amino acids as building blocks. exist In 2009, Heinis et al. Methods for post-translational chemical modification of phage display peptides are introduced library. The latter approach enables the creation of conformationally constrained peptide libraries Have greater chemical diversity and resistance to proteolysis and therefore may be more useful as pharmaceuticals.

Parallel synthesis library and synthetic planar microarray library methods black boxes,The throughput of the box is much lower, and The resulting library is more targeted than the approaches mentioned above. Planar microarray methods have Mainly used as a tool for peptide research; although, In theory, other types of compounds could also be chemical On-site preparation through automation. The highly concentrated one Parallel synthesis of small molecule libraries (hundreds to Particularly useful when developed in conjunction with computational chemistry Optimize drug candidates. The topic is Combinatorial chemistry has been extensively documented and reviewed , hence this brief review Covers only the latest advances in combinatorial libraries Design, synthesis, and high-throughput screening methods. (e.g., aptamer libraries)



Role Of Combinatorial Synthesis In Drug Divcovery :

1.find a target

2.find a lead

3.Isolate active structure

4.identify structure

5.optimize lead

Technique Used In Combinatirial Chemistry:

1.Solid phase synthesis

2.parallel synthesis

3.Mixed combinatorial synthesis

1.Solid phase synthesis:

In this method ,the reaction is carried out on solid support such an resin beads ,the beads is treated with different starting materials which bound together. Then it is mixed with another reagent to get the product which bound to solid support. The excess reagent or by product can be easily removed by washing with appropriate solvent.

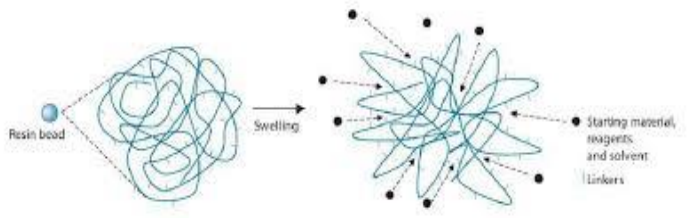


Fig Swelling of Resin Bead

2. Parallel Synthesis:

- In parallel synthesis approach each starting material is reacted with each building block separately.After each reaction step,the product is split in to number of portions before it is reacted with next building block.
- Compounds are synthesized in parallel using spatially separated compartments solid supported as well as solution chemistry is possible

3.Mixed Combinatorial Synthesis:

- To use a standard synthetic route to produce large variety of different analogues where each reaction vessel or tube contains a mixture of products.Capable of synthesizing large number of compounds quickly.
- Inactive mixtures are stored in combinatorial libraries.
- Active compounds are studied further to identify active compound.

Computational chemistry for combinatorial library design:

The integration of combinatorial chemistry and computational chemistry has proven to be advantageous in achieving higher hit rates. This approach offers a more cost-effective so prior to the actual synthesis and screening of libraries, it is now common practice in drug discovery programs to utilize computer-assisted drug design techniques. This includes the generation of virtual libraries, analogue docking, and in silico screening. In fragment-based drug design (FBDD), libraries of small chemical fragments are experimentally screened using techniques such as nuclear magnetic resonance (NMR) spectroscopy or surface plasmon resonance (SPR). For low affinity hits, in silico screening of virtual fragments can also be employed if the structural information of the target is available. The identified fragment hits are then connected using proper linkers while maintaining their relative positions in the sub-pockets. These approaches have successfully led to the discovery of high-affinity ligands, with Vemurafenib being the first drug discovered via FBDD to gain FDA approval. To increase the likelihood of obtaining drug-like hits, ADMET filters, which consider factors such as absorption, distribution, metabolism, excretion, and toxicity, have been incorporated into the library design algorithm. Other methods for library design include multi-objective optimization methods, the 'adaptive' library chemicals.approach with a simulated evolutionary process, and the multiple copy simultaneous sealution for designing and screening virtual

A de novo structure-based design tool and active site mapping are employed in a method. Li et al. recently introduced a Python-based approach for target-focused combinatorial library design . This method utilizes flexible SMILES strings, concatenated using Python language, to encode molecule structures and generate libraries at a remarkable rate of approximately 70,000 molecules per second. To enhance hit rates and optimize library size, the authors utilized the hybrid 3D similarity calculation software SHAFTS. While these computational methods are applicable to both diverse and focused

library design, they hold particular significance in the development of focused libraries with limited diversity, thereby increasing the likelihood of successful hits.

Generation of combinatorial libraries:

Combinatorial libraries can be generated through parallel synthesis, either manually or robotically, in solution or on solid support. These libraries typically have a small diversity, ranging from hundreds to a few thousands, but the choice of coupling chemistry is not limited. Each compound in the library can be purified using automatic chromatography if necessary, and the intended structures of each compound are known. On the other hand, OBOC libraries are synthesized on microbeads using the split-pool synthesis strategy, resulting in a greater diversity of bead-bound compounds, ranging from thousands to millions. However, these compounds are non-addressable, and the identification of positive beads during screening requires decoding through a chemical or physical barcode. Solution-phase positional scanning libraries can also be prepared on solid support through split-pool synthesis, and later the compounds can be cleaved off the beads into a solution mixture. Different methods for generating biological peptide libraries, such as phage-display, yeast-display, mRNA-display, and chemically modified phage-display libraries, have been extensively described in the literature and will not be discussed here. DECL libraries, on the other hand, can be assembled by ligating DNA-tagged building blocks to form peptides, small molecules, or macrocycles. The available coupling chemistries for DECL libraries are more limited as they need to be mild and compatible with the oligonucleotide tags. For comprehensive reviews on the synthesis of chemical libraries, please refer to references and the series of 'Comprehensive Survey of Combinatorial Library Synthesis' in the Journal of Combinatorial Chemistry (currently ACS Combinatorial Science). In this context, we would like to highlight several recently developed chemical approaches and technologies in the preparation of combinatorial libraries.

Huang and Bode have recently presented a novel approach called the 'synthetic fermentation' technique, which eliminates the need for organisms, enzymes, or reagents in the production of a diverse collection of intricate organic compounds. This method enables the synthesis of complex molecules by assembling small building blocks in a water-based environment.

authors adapted ketoacid ligation, which produces b-amino acid linkages. By adjusting the reaction conditions and the building blocks, products with different sequences, structures and compositions can be modulated. The authors prepared a 6000-membered library from 23 simple building blocks and discovered a 1.0-mM inhibitor against hepatitis C virus NS3/4A protease.

Litovchik et al. A chemical ligation method was developed Construction of DECL [32 . This method is based on Capacity of the Klenow fragment of DNA polymerase I Translocation via triazole bonds into

the DNA backbone via click cycloaddition. The author develops a strategy that allows for repetition and specific things Install multiple oligonucleotide tags. Comparison of This chemical connection can be achieved by the previous DECL method Methods represent progress and can be extended The range and diversity of chemistries available for DECL.Many bioactive peptide natural products contain macrocyclic structures. Suga and Bashiruddin recently published an article on architecture and Screening a large library of natural product macrocyclic peptides using a reconstituted translation system certain codons are released and then Switch to unnatural amino acids. ribosome synthesis The synthesis of macrocyclic peptides can be achieved through a custom in vitro translation system containing Flexizyme, Amino acids (natural and unnatural) and unnatural Amino acids that can be cross-linked with other amino acids. Fasan et al. A novel and versatile method was recently reported Generation of side-chain-to-tail cyclic peptide macrocycles from ribosome-derived peptides in vitro pH control or directly in live bacterial cells.

Screening of combinatorial chemistry:

Screening combinatorial libraries can be divided into Divided into two categories: virtual screening and experimental Real demonstration. Virtual screening using computer A method of predicting or modeling how a specific compound will interact with a specific target protein. three of them Virtual screening methods used in modern drug research Including molecular docking, pharmacopoeia maps, and quantitative structure-activity relationships. thisdisadvantages of virtual screening are that it cannot replace real screening, and generated hits may be very difficult to chemically synthesize. Real screening approaches, such as high-throughput screening (HTS), can test the activity of hundreds of thousands of compounds experimentally, providing real results; however, these methods are far more expensive and slower than virtual screening methods. The most common assay to screen a combinatorial library is to determine the binding of the library compounds to the target protein. Other common assays are functional assays, such as biochemical and enzymatic assays, or cellbased assays. Cell-based assays can be direct cytotoxic assays, receptor-binding assays, or cell-signaling assays using cell lines with specific genetic reporter systems. Selection of screening methods greatly depends on theCombinational libraries to be screened are position-addressable soluble library prepared from parallel synthesis that can be screened using automated high tensile test (HTS) methods in 96, 384, and 1536 well plates. Solid supports libraries (OBOC library) can easily be screened against a wide range of biological targets (e.g. proteins, cells, viruses,etc. for binding or functional actions or released in situ for solutions phase functional assays (PPF assays). Phage- display peptide library can

be screened using biopanning or limited cell based functional assays such as cell-binding and cell uptake assays . Structure based virtual libraries can be screened in silico Several new screening approaches have recently been developed for combinatorial library.

A standard wide field fluorescence microscope with LED based excitation and an advanced CMOS camera is used to detect signals related to target proteins attached to beads in the OBOC library (see Heusermann, et al., 2015). Optical image subtraction (OCT) is used to overcome the autofluorescing issue. The screening system is very high throughput and can screen >200,000 beads-bound compounds in 1.5 hours. Perez-Pineira et al. report a direct, label-free, ultra-fast method of identifying and spectroscopically classifying hits from OBOX peptide libraries (see MacConnell, 2015). They synthesize peptides on Tenta Gel beads decorated with Bimetallic Ag Clusters on the surface and then use surface enhanced Raman Scattering Analysis to detect the peptide signals on each bead. As the Raman Scattering intensity is closely related to the distance to surface, the peptide is limited to small peptide lengths (7–10 amino acids). MacConnell, et al., describe an automated and quantifiably functional screening of DNA encoded compound beads.

In situ click chemistry is a webbing approach to assembling multiple-ligand proteins. This system has several advantages, including

1) the product of the prisoner agent doesn't bear knowledge of affinity agents against the target protein;%

2) the in situ click webbing covers a large chemical space;

3) the process can be repeated until ligands with the asked affinity and particularity are linked. For illustration, once a bi-ligand has been linked, it can serve as the anchor ligand to click back to the same OBOC library for discovery of atri-ligand, and so forth. Upon the addition of each ligand to the prisoner agent, the affinity and selectivity of the prisoner agent for its tar.

Death ligands can be identified through the screening of one-bead two-compound (OB2C) libraries. In an OB2C library, a fixed cell-capturing ligand and a random library compound are co-displayed on each bead surface, and a coding tag resides inside the bead to exclude interference . When live cells bind to the capturing ligand on the bead surface, the cells are forced to expose their cell membrane proteins to the OB2C library compounds . After incubation, dead cells or apoptotic cells can be readily detected using propidium iodide (PI) staining or anticleaved caspase 3 antibody staining .

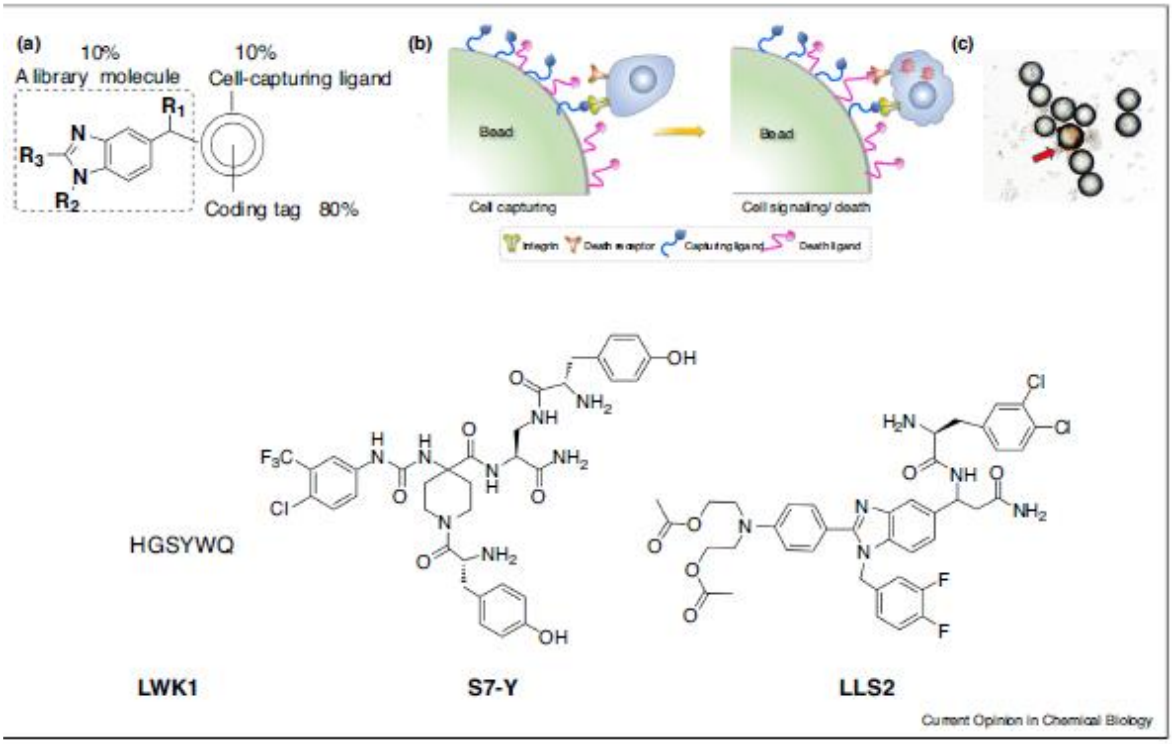
Encoding and decoding of combinatorial libraries:

Since the chemical structure of individual compounds in conventional addressable combinatorial libraries or planar microarray libraries are known, there is no need to encode and decode the chemical hits. For mixture libraries in solution, such as positional-scanning libraries, deconvolution is needed to determine the identity of the hits. Biological-displayed peptide libraries (e.g., phage, yeast or mRNA-display) can be decoded with PCR and DNA sequencing. DECL decoding can easily be achieved through PCR-amplification of the DNA barcode, followed by high-throughput DNA sequencing. Buller et al. reported another approach called 'Illumina sequencing of DECLs' which can yield over 10 million DNA sequence tags per flow-lane . This technology can reduce decoding cost and be used in a multiplex format, allowing the encoding and subsequent sequencing of multiple selections in the same experiment.

. There have been many encoding and decoding strategies before was developed for the OBOC library with chemical Barcodes are often decoded using automatic Edmanmicrosequencing of bead-bound peptide tags or mass spectroscopy of released coding tags. Marcon et al. recently reported a fluorescence-based encoding method called 'on-the-fly' encoding using colloidal barcoding . In this method, 10–20 mm beads were encoded contain specific and identifiable combinations of fluorescent dye. After screening, the colloidal barcode can be decoded with confocal microscopy. Recently, Lee et al. reported a simple and efficient surface-enhanced Raman spectroscopic (SERS) barcoding method using highly sensitive SERS nanoparticles (SERS ID). More than one million codes can be generated by using combinations of 44 different SERS IDs, which are highly stable and reliable under bioassay conditions.

Applications of combinatorial chemistry for drug discovery:

The past decade has seen the development of combinatorial library approaches Has been successfully used in various applications Including drug discovery. Table 1 summarizes some of thesePublished applications of various combination library methods. The following are two reports Latest report on the use of DECL for drug developmentBlackskjaer et al. reported on a 'Binding Trap Enrichment' enables libraries Robust and uniform screening . at this With this approach, the building blocks are spatially restricted DNA connects centers (called yoctorreactors) and facilitates chemical reactions between buildings Block and library encoding. Filtering by DECL Can be combined in a single tube. this This approach is increasingly used as a viable technique to identify small



molecule modulators of protein targets. Weichert et al. recent reports Efficient identification using dual pharmacophore DECL method, small molecules are first conjugated 30s and 50s ends of complementary DNA strands Contains a unique identification number followed by DNA Hybridization and subsequent code transfer between strands. Authors identify binders in the low micromolar rangeThe authors also applied dual-display technology to affinity maturation of an inhibitor of carbonic anhydrase IX (CAIX). They successfully developed a high affinity bidentate ligand of CAIX (KD 0.2 nM) which showed %efficient tumor targeting in a SK-RC-52 kidney cancer xenograft mouse model%.

Conclusion and perspectives:

Combinatorial chemistry has sped up the development of a whole host of combinatorial tools, comprising combinatorial library design, efficient synthetic methods, reagents for library synthesis (including solid supported reagents), linkers, bilayer beads, library encoding and decoding strategies, HTS methods and equipment, and so on. The large diversity within this field allows for many unique applicationmicroarrays in the early 1990's had inspired investigators in fields beyond chemistry to think 'combinatorially'; this change in thinking led to the development of oligonuleotide bead and planar microarrays, genomics and many other '-omics' technologies that involve the concurrent interrogation of thousands to hundreds of thousands of analytes or biomolecules. A recent report on

single-cell RNAseq analysis with nanodroplet, indeed usesthe ‘splitpool’ synthesis approach to prepare sets of DNA barcodes on microbeads, for subsequent tracking of sequences derived from the same cell . Many investigators, particularly in the pharmaceutical industry, are now working on smaller target-focused solution-phase libraries of compounds with drug-like properties, and incorporating ADMET filters and structure-based drug design approaches into library development . However, for novel lead discovery against a large number of therapeutic targets, particularly for those targets with little structural information, the various high diversity library methods outlined in this mini-review will undoubtedly be invaluable.

Numerous macrocyclic natural products exist that are not peptides. Some of these natural products are based on polyketides. There is a significant demand for the development of innovative and efficient chemistry techniques to create macrocycles that imitate these structures. By incorporating chemical characteristics of these molecules into the design of easily combinable building blocks, it will be possible to create extensive and diverse libraries of macrocycles that resemble natural products. These libraries can then be utilized to discover new drug leads. Another promising approach in combinatorial chemistry involves using nature's highly stable peptides, such as cyclotides, as scaffolds for library design. Random peptide loops can be chemically or recombinantly grafted into cysteine knots to form cyclotide libraries. Although the ambitious goals of combinatorial chemistry in drug discovery have not been fully achieved, substantial advancements have been made in the last thirty years. Various innovative tools in the fields of chemistry, biology, computation, and screening have been developed, enhancing our understanding of the strengths and limitations of combinatorial chemistry. Currently, we are better positioned to exploit the potential of combinatorial technologies in the search for future pharmaceuticals. The outlook for utilizing combinatorial chemistry in drug discovery seems encouraging.

Reference:

1. Geysen HM, Meloen RH, Barteling SJ: Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. Proc. Natl. Acad. Sci. U. S. A. 1984, 81:3998-4002.

2. Houghten RA: General-method for the rapid solid-phase synthesis of large numbers of peptides—specificity of antigen–antibody interaction at the level of individual amino-acids. Proc. Natl. Acad. Sci. U. S. A. 1985, 82:5131-5135.

3. Lam KS, Salmon SE, Hersh EM, Hruby VJ, Kazmierski WM, Knapp RJ: A new type of synthetic peptide library for identifying ligand-binding activity. Nature 1991, 354:82-84.

4. Houghten RA, Pinilla C, Blondelle SE, Appel JR, Dooley CT, Cuervo JH: Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery. Nature 1991, 354:84-86.

5. Bunin BA, Ellman JA: A general and expedient method for the solid-phase synthesis of 1,4-benzodiazepine derivatives. J. Am. Chem. Soc. 1992, 114:10997-10998.

6. Fodor SPA, Read JL, Pirrung MC, Stryer L, Lu AT, Solas D: Light-directed, spatially addressable parallel chemical synthesis. Science 1991, 251:767-773.

7. Smith GP: Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science 1985, 228:1315-1317.

8. Frankel A, Millward SW, Roberts RW: Encodamers: unnatural peptide oligomers encoded in RNA. Chem. Biol. 2003, 10:1043-1050.

9. Josephson K, Hartman MCT, Szostak JW: Ribosomal synthesis of unnatural peptides. J. Am. Chem. Soc. 2005, 127:11727-11735.

10. Bollag G, Tsai J, Zhang J, Zhang C, Ibrahim P, Nolop K, Hirth P: Vemurafenib: the first drug approved for BRAF-mutant cancer. Nat. Rev. Drug Discov. 2012, 11:873-886.

11. Rose S, Stevens A: Computational design strategies for combinatorial libraries. Curr. Opin. Chem. Biol. 2003, 7:331-339.

12. Nicolaou CA, Kannas CC: Molecular library design using multi-objective optimization methods. Methods Mol. Biol. 2011, 685:53-69.

13. Schneider G, Schuller A: Adaptive combinatorial design of focused compound libraries. Methods Mol. Biol. 2010, 572:135-147.

14. Evensen E, Joseph-McCarthy D, Weiss GA, Schreiber SL, Karplus M: Ligand design by a combinatorial approach based on modeling and experiment: application to HLA-DR4. J. Comput. Aid Mol. Des. 2007, 21:395-418.

15. Li SL, Song YW, Liu XF, Li HL: A rapid Python-based methodology for target-focused combinatorial library design. Comb. Chem. High Throughput Screen. 2016, 19:25-35.

16. Furka A, Sebestyen F, Asgedom M, Dibo G: General method for rapid synthesis of multicomponent peptide mixtures. Int. J. Pept. Protein Res. 1991, 37:487-493.

17. Gray BP, Brown KC: Combinatorial peptide libraries: mining for cell-binding peptides. Chem. Rev. 2014, 114:1020-1081.

18. Barot KP, Nikolova S, Ivanov I, Ghate MD: Liquid-phase combinatorial library synthesis: recent advances and future perspectives. Comb. Chem. High Throughput Screen. 2014, 17:417-438.

19. Shin DS, Kim DH, Chung WJ, Lee YS: Combinatorial solid phase peptide synthesis and bioassays. J. Biochem. Mol. Biol. 2005, 38:517-525.

20. Armstrong RW, Combs AP, Tempest PA, Brown SD, Keating TA: Multiple-component condensation strategies for combinatorial library synthesis. Acc. Chem. Res. 1996, 29:123-131.

21. Huang YL, Bode JW: Synthetic fermentation of bioactive nonribosomal peptides without organisms, enzymes or reagents. Nat. Chem. 2014, 6:877-884. An excellent paper describing a novel approach to synthesize b-amino acid peptides under mild aqueous conditions without workup. The reaction can be initiated or terminated at any point by choosing an appropriate building block.

22. Litovchick A, Dumelin CE, Habeshian S, Gikunju D, Guie MA, Centrella P, Zhang Y, Sigel EA, Cuozzo JW, Keefe AD et al.: Encoded library synthesis using chemical ligation and the discovery of sEH inhibitors from a 334-million member library. Sci. Rep. 2015, 5:10916. This paper describes the synthesis of a huge DNA-encoded smallmolecule library using Click chemistry ligation and identification of a 2 nM sEH inhibitor from the library.

23. Bashiruddin NK, Suga H: Construction and screening of vast libraries of natural product-like macrocyclic peptides using in vitro display technologies. Curr. Opin. Chem. Biol. 2015, 24:131-138.

24. Frost JR, Jacob NT, Papa LJ, Owens AE, Fasan R: Ribosomal synthesis of macrocyclic peptides in vitro and in vivo mediated by genetically encoded aminothiol unnatural amino acids. ACS Chem. Biol. 2015, 10:1805-1816. An elegant method for ribosomal synthesis of side chain-to-tail macrocyclic peptides.

25. Jongkees SAK, Umemoto S, Suga H: Linker-free incorporation of carbohydrates into in vitro displayed macrocyclic peptides. Chem. Sci. 2017, 8:1474-1481.

26. Townsend JB, Shaheen F, Liu R, Lam KS: Jeffamine derivatized TentaGel beads and poly(dimethylsiloxane) microbead cassettes for ultrahigh-throughput in situ releasable solutionphase cell-based screening of one-bead-one-compound combinatorial small molecule libraries. J. Comb. Chem. 2010, 12:700-712.

27. Wu CH, Liu IJ, Lu RM, Wu HC: Advancement and applications of peptide phage display technology in biomedical science. J. Biomed. Sci. 2016, 23:8.

28. Heusermann W, Ludin B, Pham NT, Auer M, Weidemann T, Hintersteiner M: A wide-field fluorescence microscope extension for ultrafast screening of one-bead one-compound libraries using a spectral image subtraction approach. ACS Comb. Sci. 2016, 18:209-219.

29. Perez-Pineiro R, Correa-Duarte MA, Salgueirino V, Alvarez-Puebla RA: SERS assisted ultra-fast peptidic screening: a new tool for drug discovery. Nanoscale 2012, 4:113-116.

30. MacConnell AB, Price AK, Paegel BM: An integrated microfluidic processor for DNA-encoded combinatorial library functional screening. ACS Comb. Sci. 2017, 19:181-192.

31. Agnew HD, Rohde RD, Millward SW, Nag A, Yeo WS, Hein JE, Pitram SM, Tariq AA, Burns VM, Krom RJ et al.: Iterative in situ click chemistry creates antibody-like protein-capture agents. Angew. Chem. Int. Ed. Engl. 2009, 48:4944-4948.

32. Kumaresan PR, Wang Y, Saunders M, Maeda Y, Liu R, Wang X, Lam KS: Rapid discovery of death ligands with one-bead-two-compound combinatorial library methods. ACS Comb. Sci. 2011, 13:259-264.

33. Liu R, Shih TC, Deng X, Anwar L, Ahadi S, Kumaresan P, Lam KS: Design, synthesis, and application of OB2C combinatorial peptide and peptidomimetic libraries. Methods Mol. Biol. 2015, 1248:3-22.

34. Shih T-C, Liu R, Fung G, Bhardwaj G, Ghosh PM, Lam KS: A novel galectin-1 inhibitor discovered through one-bead twocompound library potentiates the anti-tumor effects of paclitaxel in vivo. Mol. Cancer Ther. 2017. in press.