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Latest Advances in OBOC Peptide Libraries. Improvements in Screening Strategies and Enlarging the Family From Linear to Cyclic Libraries

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Abstract: Solid phase screenings of one bead one compound (OBOC) libraries have been widely used to find ligands with pharmacological and analytical uses, and to purify or detect proteins in complex mixtures. To improve library screening, in the last years various strategies have been developed to avoid the selection of false positive beads and to obtain selective ligands. Currently, there is great interest in cyclic peptides because of their resistance to enzymatic degradation and higher selectivity compared to their linear counterparts. Lots of cyclic peptide libraries protocols have been recently developed to facilitate hits analysis. The aim of this review is to summarize the latest applications of solid phase screening of OBOC combinatorial peptide libraries, the improvements in the screening methods including mass spectrometry MS/MS techniques and the strategies to synthesize OBOC cyclic peptide libraries.

Keywords: Mass Spectrometry, Tandem MS/MS, peptide, peptoid, cyclic, libraries, affinity.

1. INTRODUCTION

Short peptides are more physically and chemically stable than proteins. They can be synthesized in bulk quantities, at low cost, and under good manufacturing practices. Furthermore, they can be easily modified to improve their resistance against proteolytic cleavage and, unlike proteins, oligopeptides hardly cause poisoning and immune responses. Peptide libraries have been widely used to find ligands for pharmacological and/or analytical purposes, and to purify or detect proteins in complex mixtures [1-16].

To search for new peptide ligands, nowadays there are many strategies to produce synthetic peptide libraries with thousand to millions of compounds. Unlike biological peptide libraries, D-amino acids, unnatural amino acids, and other organic moieties can be used as building blocks together with the L-amino acids and, hence, the diversity is increased. In all approaches, a large family of compounds is generated in a faster way and with less effort that if each member is synthesized individually [17, 18].

The resourceful idea of solid phase peptide synthesis introduced by Merrifield [19] facilitated the development of library synthesis strategies. In 1984 Geysen *et al*. [20] introduced the multipin technology to synthesize a 96-compound peptide library. Since then, different approaches have been developed. First ones consisted on synthesizing simultaneously multiple peptides of known sequences, such as multipin [20], tea-bags [21], spot [22] and the light-directed, spatially addressable, parallel chemical synthesis [23]. The number of members in these libraries is limited to no more than 100-200, and the amino acid sequence of each peptide is pre-determined and known, so there is no need for sequence analysis.

To increase the number of members from hundreds to thousands, a combinatorial synthetic method was developed almost simultaneously by different groups and with different

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names: divide-couple-recombine [24], portion-mixing [25], and split and mix [26]. In this approach, synthesis is performed using solid phase resin beads. First, the resin beads are equally divided. Then, one amino acid is coupled in each portion. Finally, all the portions are mixed in one container. The total of members of the combinatorial library will be k^n, where "k" represents the number of building blocks used, and "n" represents the number of variable residues in the peptide and steps in the process [20, 24-26] (Fig. **1**). The method was developed to overcome the differential coupling rates of each amino acid and ensures an equal representation of all members of the library.

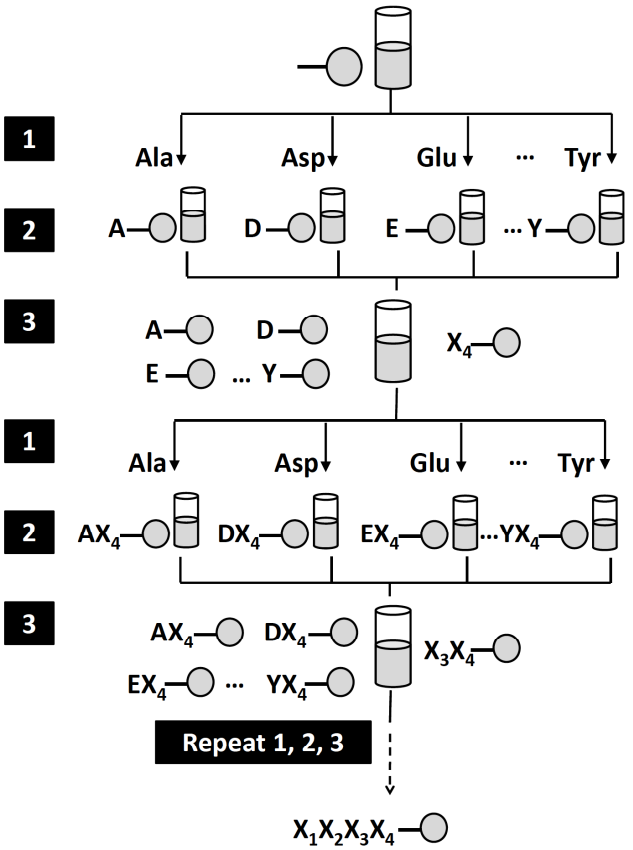

Fig. (1). Divide-couple-recombine, portion-mixing, or split and mix: (1) The resin is divided into equal portions. The number of portions (k) is determined by the number of amino acids varied in each position. (2) In each portion a different amino acid is coupled. (3) All the portions are mixed in a unique container. The process is repeated "n" times, where n represents the number of residues in the peptides synthesized. X=equimolar mixture of the amino acids used.

Library screening can be performed in solution. Peptide mixtures are cleaved from their solid support and assayed as free peptide mixtures using the positional scanning screening methods [24]. The combinatorial mixtures are made up of peptide sequences in which one position is defined while the others consist of equimolar mixtures of the amino acids assayed. For example, a hexapeptide library will consist of six groups of libraries with the format $O_1XXXXX\text{-}NH_2$, $XO_2XXXX\text{-}NH_2$, $XXO_3XXX\text{-}NH_2$, $XXXO_4XX\text{-}NH_2$, $XXXXO_5X\text{-}NH_2$ and $XXXXXO_6\text{-}NH_2$, where X represents the combinatorial mixture of the amino acids and O represents one defined amino acid. The bioactivity of each sub-library is assayed in solution and the remaining mixture positions are determined through an iterative selection process in order to identify active sequences. Although this strategy has been widely used in drug discovery [27, 28] it implies the synthesis of numerous libraries and therefore is very tedious and also expensive.

Lam *et al*. [26] realized that the divide-couple-recombine synthetic method assured a one-bead-one-compound (OBOC) distribution, in which each bead displayed only one peptide entity. Taking advantage of this property, they designed a binding solid phase screening strategy far less expensive than the solution assay. The methods involves: (a) incubating the resin beads with a blocking solution to avoid unspecific interactions; (b) incubating the target with the library; (c) identifying those beads with the target adsorbed using antibodies against the target or by labeling the target with a reporter group; (d) isolating positive beads; (e) washing the beads to desorb the target; (f) deducing their peptide sequences by Edman's degradation; (g) synthesizing peptide hits in higher quantities to assay their bioactivity, specificity, and/or affinity for the target.

Amphiphilic resins suitable for library synthesis and screening, performed in organic solvents and in aqueous buffers respectively, must be used in this approach. Those targets that cannot be detected directly must be labeled. Therefore, there is a risk of selecting hits with affinity for the label. To improve OBOC screening, in the last years various strategies have been developed to avoid the selection of false positive beads and to obtain selective ligands [29-32].

As the peptide contained in each selected bead is unknown, its sequence must be identified. Edman's degradation is a high cost technique and only suitable for peptides with free *N*-terminal. Nowadays, tandem mass spectrometry (MS/MS) using soft ionization techniques, like electrospray ionization (ESI) [33] and matrix-assisted laser desorption/ionization (MALDI) [34, 35], outstrip Edman's degradation. It is less expensive and time-consuming, and allows sequencing of *N*-terminal blocked peptides. OBOC libraries have been designed with linkers suitable to release hit peptides after the screening process for MS/MS analysis [36-39].

Currently, there is a great interest in cyclic peptides because of their resistance to enzymatic degradation and higher selectivity compared to their linear counterparts [40, 41]. However, their sequence elucidation by MS/MS is difficult due to their fragmentation pattern [42]. To facilitate cyclic peptide hit analysis, lots of OBOC cyclic peptide libraries protocols have been recently developed.

The aim of this review is to summarize the latest applications of solid phase screening of OBOC combinatorial peptide libraries, and the improvements in the screening methods and in the strategies to synthesize OBOC cyclic peptide libraries.

2. LATEST APPLICATIONS OF LINEAR OBOC PEPTIDE COMBINATORIAL LIBRARIES

During the last five years, combinatorial linear peptide libraries screening has been used to find peptides with affinity for many biomolecules to apply in affinity chromatography, diagnosis and as bioactive compounds.

Yang *et al*. [43] described the peptide His-Trp-Arg-Gly-Trp-Val that binds specifically to the Fc fragment of human immunoglobulin G. The peptide ligand developed is more chemically stable and less expensive than protein A or B, commonly used for antibody purification. Recently, the peptide was used by Menegatti *et al*. [44] to purify polyclonal antibodies (pAbs) from Cohn fraction II+III of human plasma, bovine skim milk and whey, and afterwards by Liu *et al*. [45, 46] to purify IgA and IgM.

Lund *et al*. [47] identified two novel peptide ligands by OBOC screening for mAb purification: D_2-Ala-Ala-Gly and D-Ala-Ala-Gly. Both contain natural amino acids, Ala and Gly, as well as the synthetic aromatic acid 2,6-di-t-butyl-4-hydroxybenzyl acrylate (D). Wang *et al*. [48], by molecular docking and dynamics simulation, demonstrated that they interact with the Fc fragment of IgGs.

Camperi *et al*., developed peptide ligands for affinity chromatography by screening OBOC peptide libraries using the 4-hydroxymethylbenzoic acid (HMBA) linker immobilized on ChemMatrix resin [49]. The linker HMBA facilitates the peptide cleavage from each bead isolated after the screening and its identification by tandem mass spectrometry [50]. Two peptide ligands were found for rhEPO purification from CHO cell cultures: Phe-His-His-Phe-Ala-His-Ala-Gly-NH_2 and Phe-His-Asn-Phe-Ala-His-Ala-Gly-NH_2 [50]. Recently, we identified peptides to purify *Crotalus durissus terrificus* phospholipase A2 (CDT PLA2) [51] in only one step. Muller *et al*. [52] reported that CDT PLA2 strongly inhibits the yellow fever and dengue viruses growth in VERO E6 cells, and it can be used as a possible antiviral agent against several tropical diseases.

OBOC peptide libraries were also used to find affinity ligands for live cells by Cho *et al*. [53] that could be used in molecular imaging and target drug delivery. They designed a screening of cancer cells using the Complex Object Parametric Analyzer and Sorter (COPAS) BIOBEAD flow sorting equipment (Union Biometrica), which has the capacity to sort large objects (120-300 μm) on the basis of their size, density, and fluorescence [54-57].

Kumaresen *et al*. [58] used an OBOC library to find peptide ligands to block the C-reactive protein (CRP), related to cardiovascular disease. Six peptides were found and their inhibition performances were assayed.

Hepatitis B X-protein (HBx) has been related to the development of hepatocellular carcinoma (HCC). Liao and co-workers [59] used an OBOC peptide library to identify two peptides that bind the promoter region of the HBx gen in order to shut down its expression. They suggested their use as possible therapeutic agents to prevent HCC.

Also, Witucki *et al*. [60], applied the OBOC peptide library approach to find substrates for the focal adhesion kinase, an enzyme that has emerged as an important target for cancer research, and proposed their possible applications in diagnosis.

As was previously reviewed by Gautam *et al*. [61], OBOC libraries have been used, for their applications in cancer therapeutics and diagnosis, to find peptides with potential to detect tumors in vivo and to deliver anticancer agents to the tumor site.

3. RECENT DEVELOPMENTS IN OBOC SCREENING TECHNIQUES

A drawback of solid phase strategies is that low selectivity ligands may be selected. Non-ionic detergents and blocking agents such as gelatin or powdered milk are used during the screening to avoid non-specific interactions. Also, false positive beads may be selected. When the target is labeled with biotin, positive bead identification is performed using streptavidin (SA) coupled with peroxidase (POD) or alkaline phosphatase. It has been reported that beads with His-Pro-Gln and His-Pro-Met motifs have high affinity for SA, giving rise to false positive selection [62]. Furthermore, as we have previously reported [55], when screening the library with the target protein coupled with fluorescent dyes, peptides with high content of hydrophobic amino acids interact with the dye, resulting in false positive beads. We have noticed that those beads showed bright homogeneous fluorescence while true positive beads displayed a heterogeneous fluorescence, exhibiting a halo-like appearance with high fluorescence intensity on the bead surface and low in the core.

To avoid false positive selection when screening a TentaGel OBOC library with an Alexa Fluor 647-labeled protein, Cha *et al*. [29] first sorted all fluorescent beads from above the maximum fluorescence level using the COPAS [54, 55]. In a second stage, all sorted objects were further analyzed to discard less meaningful beads based on the uniformity of fluorescence along the length of a bead by Profiler software (Union Biometrica). Also, they discarded the small broken TentaGel beads because they showed abnormally strong fluorescent intensity due to the exposure of the polystyrene core, resulting in a strong interaction with target proteins.

In our laboratory, we designed a two-stage screening method [30] using bovine serum albumin (BSA) as the model protein. In a first stage, BSA labeled with Texas red was incubated with the OBOC peptide library. All fluorescent beads were selected by using the COPAS. Positive beads were collected in a syringe and washed. Next, they were incubated with the target labeled with biotin. Finally, beads were incubated with SA-POD conjugate and revealed with 3,3'-diaminobenzidine and H_2O_2. Positive beads were isolated and their peptides identified by MS/MS analysis.

Another limitation of OBOC screening process when using proteins labeled with fluorescent dyes, is the autofluorescence of the resin beads. Hintersteiner & Auer [71] equipped the COPAS bead sorting instrument with a high-speed profiling unit and developed a spectral

autofluorescence correction method, making COPAS selection more restrictive.

To avoid the selection of low affinity ligands, Doran *et al*. [32] proposed the use of redundant OBOC libraries in which each compound was represented by many beads. After the screening, only compounds isolated more than once were selected as possible hits.

Magnetic particles have been used to facilitate positive peptide beads isolation. Recently, Liu *et al*. [63] developed a three stage screening protocol using streptavidin magnetic nanoparticles, previouly introduced by Samson *et al*. [64]. In the first stage, they mixed the library beads with the target labeled with biotin and isolated the positive beads using streptavidin-coated magnetic particles (Dynabeads) and a magnet. The selected beads were washed and the bound protein was removed using chaotropic agents. Selected beads were incubated again with the target labeled with biotin, but in this second stage positive bead identification was performed using streptavidin coupled with alkaline phosphatase. After bromo-4-chloro-3-indolyl phosphate (BCIP) addition, those bead that become turquoise colored due to the dye precipitation were selected. Once again, selected beads were washed. Finally, the beads were incubated with the protein labeled with Texas red and red fluorescent halo hits were manually selected under a fluorescent microscope.

Astle and coworkers [65] combined bead-based and microarray methodologies to take advantage of their complementary strengths. They incubated the OBOC library with the target protein and, after washing, they added anti-target protein antibodies linked to magnetic particles. A magnet was used to isolate positive beads. Then they identified the sequence of the hits using tandem mass spectrometry and spoted them onto microarrays for subsequent quantitative analysis without the need for hit re-synthesis. The compound microarrays were probed with different concentrations of the target protein to determine the intrinsic affinity of each hit compound for the target. In that way, no re-synthesis of the hits was necessary until the best binders were identified.

Although magnetic particles seem to be an excelent option to facilitate positive beads isolation, the protocol must be highly optimized in order to select only positive beads and avoid the selection of a large number of false positives, as was recently reported by Mendes *et al*. [66].

4. PROTOCOLS FOR OBOC CYCLIC LIBRARIES SYNTHESIS

In the last years, there has been much interest in the OBOC combinatorial cyclic peptides libraries development. Cyclization lowers conformational flexibility and removes exopeptidases vulnerable sites [40, 41]. Even more, very often cyclic peptides mimic biologically relevant regions of proteins, such as β-turns and loop motifs [67].

Many protocols have been developed for synthesis of OBOC combinatorial cyclic peptide libraries using on-resin homodetic cyclization. This process cyclizes the amino function of the *N*-terminal with the side-chain carboxylic acid function of an Asp or Glu added to the peptide sequence. These protocols require the selective removal of the carboxylic acid protecting group in the presence of all other permanent protection groups. Once the orthogonal protecting group has been removed from Glu or Asp, the activation of the carboxyl group and subsequent intra-molecular aminolysis allows the formation of a lactam bridged cyclic peptide.

When employing solid phase screening, cyclic peptides of hit beads must be identified. Although linear peptides identification can be readily performed by MS/MS, the high complexity of cyclic peptides fragmentation hinders their sequence elucidation [42].

Fluxa & Raymond [68] developed a method for designing combinatorial peptide libraries decodable by amino acid analysis (AAA) using an algorithm called TAGSFREE, which consists in assigning each amino acid building block to a so-called 'unique pair' of variable positions, leading to a library in which each amino acid composition determined by AAA corresponds to only one, sometimes two, or rarely four possible sequences.

On the other hand, many protocols were developed to facilitate cyclic hit identification by MS/MS.

Pei's group [69, 70] developed an homodetic OBOC cyclic library, cyclo-(1,8)-Ala-$X_1X_2X_3X_4X_5$-Val-Glu-β-Ala-β-Ala-Arg-Met-resin using TentaGel S NH_2 resin, where X_1-X_5 represent the random residues. The Met at the *C*-terminus facilitates peptide release with CNBr for peptide analysis by partial Edman degradation/mass spectrometry (PED/MS) (Fig. **2A**). To facilitate peptide cyclization N^{α}-Fmoc-Glu(δ-*N*-hydroxysuccinimidyl)-O-$CH_2CH{=}CH_2$ was used. The allyl ester orthogonal protecting group was selectively removed with a cleavage mixture containing Pd(0) in order to form a cycle by an amide linkage between the α-amino terminus and the carboxylic acid function. To facilitate peptide identification, each bead contained the cyclic peptide together with the corresponding linear counterpart. Fmoc-Glu(tBu)-OH was used to synthesize the linear code. The permanent tBu group was cleaved at the end of the synthesis together with the other permanent protecting groups, avoiding code cyclization. Applying the segregation strategy previously described by Liu *et al*. [71], cyclic peptides were synthesized on the surface of the resin beads, while the corresponding linear peptide codes were synthesized on the inside. As the targets are usually macromolecules, they only interact with the surface of the bead and cannot permeate into the TentaGel pores. The linear peptide inside the bead was used as a code to determine the sequences of the hit beads by PED/MS. With these libraries, cyclic peptides that bind the extracellular domain of human prolactin receptor were found with potential clinical applications in various reproductive disorders caused by high prolactin levels such as prolactinomas. Also, cyclic peptides with antiproliferative activity against the cancer cells and putative immunosuppressive agents [72] and cell-permeable cyclic peptides that induce apoptosis of cancer cells [73] were also found. Afterwards, the strategy was optimized by Bédard *et al*. using commercially available reagents and controlling those parameters that influenced the outer/inner layer ratios in the segregated beads [63, 74].

A similar approach was developed by Kwona & Kodadek [75] for the synthesis of a peptoid cyclic library. In their strategy, they used differential deprotection in order to create two chains that contain the peptoid of interest, but only one containing both, a glutamic acid residue to support cyclization as well as a Cys residue to allow the specific conjugation of only the cyclic peptoid molecule to a maleimide activated microscope slide for the building of a microarray to facilitate screening. The linear molecule was not coupled to the slide, but was present to support tandem MS-based sequencing.

Giudicessi *et al*. [76] designed an OBOC library with homodetic cyclic peptides of the type cyclo-(1,8)-Ala-$X_1X_2X_3X_4X_5$-Val-Asp-Gly-oxymethylbenzamide-ChemMatrix (HMBA-CM) encoded with a minimum proportion of the corresponding linear analogue (Fig. **2B**). HMBA-CM was used as the resin. The ester formed between the *C*-terminal amino acid and the linker (HMBA) can be cleaved with ammonia vapor, which is easily evaporated without leaving contaminants that could interfere with the MS analysis. The cycles were synthesized by an amide linkage between the α-amino terminus and the carboxylic acid function of the Asp added to the peptide sequence. Fmoc-Asp[2-phenylisopropyl

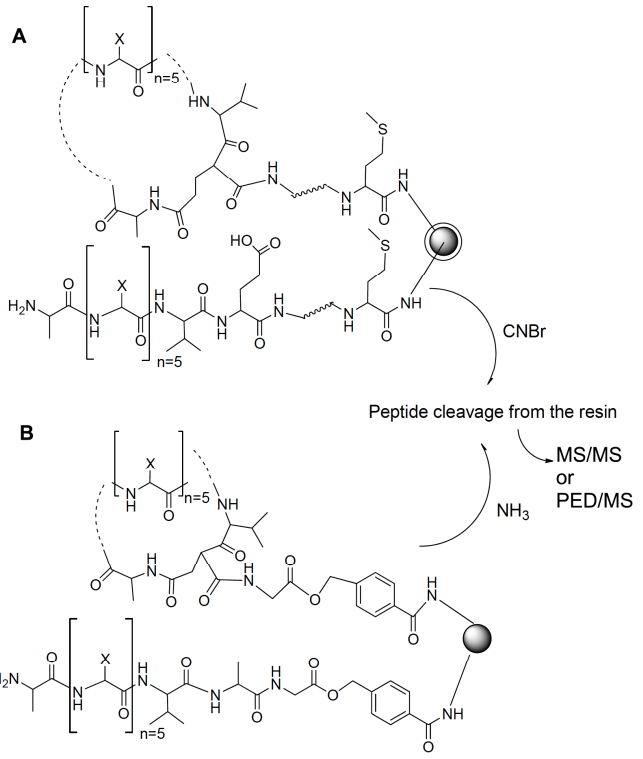

Fig. (2). Cyclic peptide libraries with linear codes. **A**) Cyclo-(1,8)-Ala-$X_1X_2X_3X_4X_5$-Val-Glu-β-Ala-β-Ala-Arg-Met-TentaGel library. The Met at the *C*-terminus facilitates peptide release with CNBr for MS/MS analysis. The segregation strategy provides cyclic peptides on the surface of the resin beads and the corresponding linear peptide codes on the inside. **B**) Cyclo-(1,8)-Ala-$X_1X_2X_3X_4X_5$-Val-Asp-Gly-HMBA-CM library encoded with a minimum proportion of the corresponding linear analogue. The ester formed between the *C*-terminal amino acid and the HMBA can be cleaved with ammonia vapor for MS/MS analysis.

ester (OPp)]-OH was added to the peptide sequence for solid phase ring formation. The semi-permanent group OPp was cleaved with 4% TFA and an amide linkage was formed between the amino terminus and carboxylic acid function to give homodetic cyclic peptides. A minor proportion of Fmoc-Ala-OH was incorporated instead of Fmoc-Asp(OPp)-OH to synthesize the code. To minimize the interference during the screening step due to the interaction of the linear peptides with the target, the minimum proportion of the linear code necessary for its MS analysis was synthesized. Peptide sequencing was acquired by MALDI TOF MS/MS.

Instead of using a linear code to facilitate OBOC cyclic library MS/MS analysis, Lee *et al*. [77] developed a strategy where the cycles were opened after screening. Abruptly, many similar strategies were developed. In all of them OBOC cyclic libraries were designed with a cleavable site in order to open the ring to facilitate MS/MS analysis. The method proposed by Lee *et al*. [77] consisted in incorporating an alkylthioaryl bridge in an OBOC peptoid library to facilitate cyclization as well as a later ring-opening reaction. The cyclic peptoids were used for screening. After selecting hit beads, the thioether linkage on the cyclic peptoids was opened. To separate the peptoids from the resin, the photocleavable linker 3-amino-3-(2-nitrophenyl) propionic acid was incorporated between the resin and the library [78]. Recently they improved their strategy [79] to facilitate sequence determination of cyclic peptide/peptoid libraries. Through a thioether bond formation, and using CNBr for ring-opening, they linearized the molecules to efficiently sequence them by MS/MS analysis. Trityl-protected homocysteine (Fmoc-hCys-(Trt)-OH) was coupled to TentaGel S NH_2 resin. The free amine on the *N*-terminus was chloroacetylated and the trityl group on the hCys was removed with 5% TFA. Macrocyclization of linear peptides/peptoids was accomplished via the nucleophilic attack of the thiol group on the chloride group. The cyclic peptides/peptoids were linearized by CNBr-mediated-ring-opening (Fig. **3A**).

Simpson *et al*. [80] designed an OBOC cyclic library where Met played a role as the linker as well as a building block within the peptide cycle. After the screening process, the hits could be simultaneously opened and released from the beads using CNBr. Then Liang and co-workers improved the synthesis strategy to avoid the presence of two *C*-terminal homoserine lactones that interfered with MS/MS analysis [81] (Fig. **3B**).

Menegatti *et al*. [82] synthesized an OBOC library of cyclic dilactone cyclo-[(N^{α}-Ac)Ser(A)-$X_1X_2X_3X_4X_5X_6$-Lact-E]. First, Fmoc-Glu-OAll was coupled to the Aminomethyl-ChemMatrix resin. Lactic acid was then coupled to glutamic acid to incorporate one ester group. After adding the random sequences, Ac-Ser(Trt)-OH was coupled at the *N*-terminus. The trityl protection on the hydroxyl group of Ser was removed by treatment with 2% TFA. Fmoc-Ala-OH was coupled to the hydroxyl group of Ser and the Fmoc was eliminated. The allyl ester orthogonal protection on Glu was removed with a mixture containing Pd(0), and an amide linkage was formed between the amino of Ala and the carboxylic acid function of Glu. After library screening, the combinatorial region of the peptide, framed between two

ester bonds, was released with NaOH generating the linear peptides Ac-Ser-$X_1X_2X_3X_4X_5X_6$ that could be sequenced by ESI MS/MS (Fig. **3C**). They assayed their protocol with the IgG binding cyclic depsipeptide cyclo-[(N^{α}-Ac)-S(A)-RWHYFK-Lact-E].

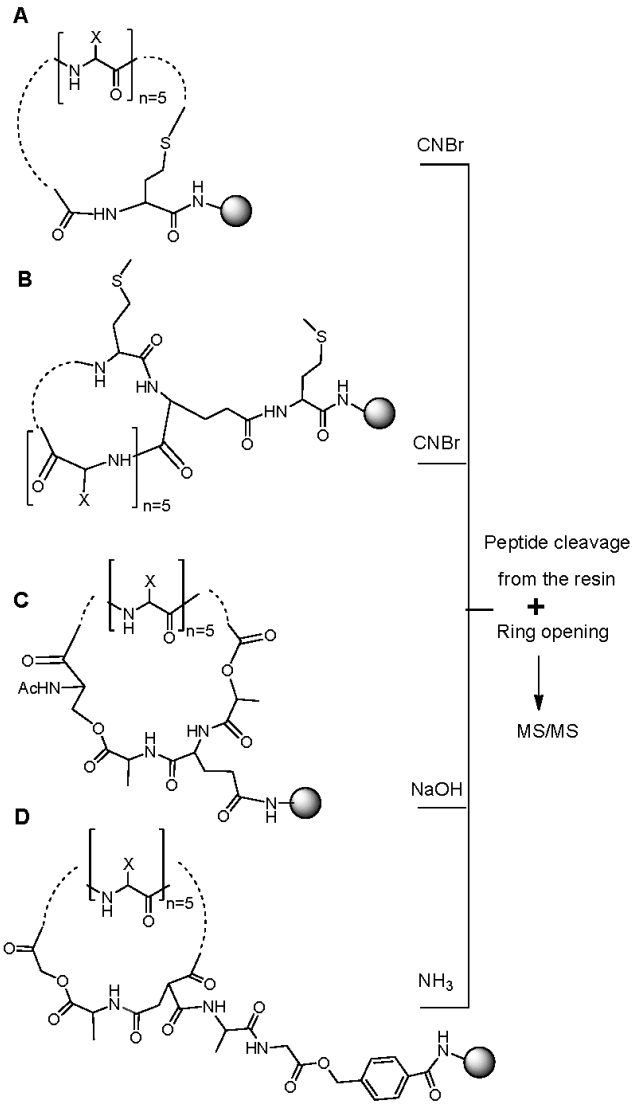

Fig. (3). Cyclic peptide libraries designed with a cleavable site in order to simultaneously open the ring and release the linear peptide from the resin for MS/MS analysis. A) Cyclic peptide library with a thioether bond. The cycle is opened and the peptide released with CNBr. B) Cyclic libraries with Met as a linker and as a building block within the peptide cycle. Peptides can be simultaneously opened and released from the beads using CNBr. C) Cyclic dilactone cyclo-[(N^{α}-Ac)Ser(A)-$X_1X_2X_3X_4X_5X_6$-Lact-Glu] library. The combinatorial region of the peptide, framed between two ester bonds, can be released with NaOH. D) Cyclic depsipeptide library cyclo-(1,8)-Ala-OCH_2CO-$X_1X_2X_3X_4X_5$-Asp-Ala-Gly-HMBA-CM. The ring can be opened with ammonia vapor and the peptide is simultaneously released for MS/MS analysis**.**

Gurevich *et al.* [83] proposed an alternative protocol for the synthesis of OBOC cyclic depsipeptides: cyclo-(1,8)-Ala-OCH_2CO-$X_1X_2X_3X_4X_5$-Asp-Ala-Gly-oxymethylbenzamide-ChemMatrix. Fmoc-Asp[2-phenylisopropyl ester (OPp)]-OH was added to the peptide sequence for solid phase ring formation. The semi-permanent group OPp was cleaved with 4% TFA and an amide linkage was formed between the amino terminus and the carboxylic acid function. HMBA was used as linker and a glycolamidic ester group was incorporated by adding glycolic acid at the end of the combinatorial region. After library screening, hit beads were treated with ammonia vapor to cleave simultaneously the benzyl and glycolamidic esters, opening the ring and releasing the peptide simultaneously for MS/MS analysis. Ammonia was easily evaporated without leaving contaminants that could interfere with the MS analysis [38, 84] (Fig. **3D**). Hazardous reagents such as Pd or CNBr are not used in this strategy and therefore it can be applied in a broad range of laboratories without special expertise in organic synthesis.

CONCLUSION

In the last years much progress has been made in OBOC screening strategies to find peptides useful as therapeutic drugs, in diagnosis, and/or as ligands for affinity chromatography. Cyclic peptides usually show better biological activity compared to their linear counterparts due to the conformational rigidity and their resistance to peptidases. Therefore, great advances have been made in the development of cyclic peptide libraries during the last years. Many strategies were developed to assure cyclic peptide sequencing, specially by mass spectrometry analysis. The new chemical protocols developed together with advances in mass spectrometry technology will ensure, in the near future, the development of many cyclic peptides with special applications in the pharmaceutical industry.

LIST OF ABBREVIATIONS

BSA = Bovine serum albumin

COPAS = Complex Object Parametric Analyzer and Sorter

ESI = Electrospray ionization

HMBA = 4-hydroxymethylbenzoic acid

MALDI = Matrix-assisted laser desorption/ionization

MS/MS = Tandem mass spectrometry

OBOC = One-bead-one-compound

OPp = 2-phenylisopropyl ester

PED/MS = Partial Edman degradation/mass spectrometry

POD = Peroxidase

SA = Streptavidin

TFA = Trifluoroacetic acid.

CONFLICT OF INTEREST

The authors declare that they have no competing financial interests.

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REFERENCES

[1] Yao, N.; Xiao, W.; Wang, X.; Marik, J.; Park, S.H.; Lam, K.S. Discovery of targeting ligands for breast cancer cells using the One-Bead One-Compound combinatorial method. *J. Med. Chem.*, **2009**, *52*, 126-133.

[2] Ding, H.; Prodinger, W.M.; Kopecék, J. Two-step fluorescence screening of CD21-binding peptides with one-bead one-compound library and investigation of binding properties of HPMA copolymer–peptide conjugates. *Biomacromolecules*, **2006**, *7*, 3037-3046.

[3] Dostmann, W.R.; Nickl, C.; Thiel, S.; Tsigelny, I.; Frank, R.; Tegge, W.J. Delineation of selective cyclic GMP-dependent protein kinase Ialpha substrate and inhibitor peptides based on combinatorial peptide libraries on paper. *Pharmacol. Ther.*, **1999**, *82*, 373-387.

[4] Lam, K.S.; Hruby, V.J.; Lebl, M.; Knapp, R.J.; Kazmierski, W.M.; Hersh, E.M.; Salmon, S.E. The chemical synthesis of large random peptide libraries and their use for the discovery of ligands for macromolecular acceptors. *Bioorg. Med. Chem. Lett.*, **1993**, *3*, 419-424.

[5] Liu, R.; Enstrom, A.M.; Lam, K.S. Combinatorial peptide library methods for immunobiology research. *Exp. Hematol.*, **2003**, *31*, 11-30.

[6] Aina, O.H.; Liu, R.; Sutcliffe, J.L.; Marik, J.; Pan, C.X.; Lam, K.S. From combinatorial chemistry to cancer-targeting peptides. *Mol. Pharm.*, **2007**, *4*, 631-651.

[7] Camperi, S.A.; Iannucci, N.B.; Albanesi, G.J.; Oggero Eberhardt, M.; Etcheverrigaray, M.; Messeguer, A.; Albericio, F.; Cascone, O. Monoclonal antibody purification by affinity chromatography with ligands derived from the screening of peptide combinatory libraries. *Biotechnol. Lett.*, **2003**, *25*, 1545-1548.

[8] Wang, G.; De, J.; Schoeniger, J.S.; Roe, D.C.; Carbonell, R.G. A hexamer peptide ligand that binds selectively to staphylococcal enterotoxin B: isolation from a solid phase combinatorial library. *J. Pept. Res.*, **2004**, *64*, 51-64.

[9] Gurgel, P.V.; Carbonell, R.G.; Swaisgood, H.E. Identification of peptide ligands generated by combinatorial chemistry that bind alpha-lactalbumin. *Separ. Sci. Technol.*, **2001**, *36*, 2411-2431.

[10] Gurgel, P.V.; Carbonell, R.G.; Swaisgood, H.E. Fractionation of whey proteins with a hexapeptide ligand affinity resin. *Bioseparation*, **2000**, *9*, 385-392.

[11] Kaufman, D.B.; Hentsch, M.E.; Baumbach, G.A.; Buettner, J.A.; Dadd, C.A.; Huang, P.Y.; Hammond, D.J.; Carbonell, R.G. Affinity purification of fibrinogen using a ligand from a peptide library. *Biotechnol. Bioeng.*, **2002**, *77*, 278-289.

[12] Necina, R.; Amatschek, K.; Schallaun, E.; Schwinn, H.; Josic, D.; Jungbauer, A. Peptide affinity chromatography of human clotting factor VIII. Screening of the vWF-binding domain. *J. Chromatogr. B. Biomed. Sci. Appl.*, **1998**, *715*, 191-201.

[13] Amatschek, K.; Necina, R.; Hahn, R.; Schallaun, E.; Schwinn, H.; Josić, D.; Jungbauer, A. Affinity chromatography of human blood coagulation factor VIII on monoliths with peptides from a combinatorial library. *J. High Resol. Chromatogr.*, **2000**, *23*, 47-58.

[14] D'Agostino, B.; Bellofiore, P.; De Martino, T.; Punzo, C.; Rivieccio, V.; Verdoliva, A. Affinity purification of IgG monoclonal antibodies using the D-PAM synthetic ligand: chromatographic comparison with protein A and thermodynamic investigation of the D-PAM/IgG interaction. *J. Immunol. Methods*, **2008**, *333*, 126-138.

[15] Palombo, G.; Verdoliva, A.; Fassina, G. Affinity purification of immunoglobulin M using a novel synthetic ligand. *J. Chromatogr. B,* **1998**, *715*, 137-145.

[16] Camperi, S.A.; Marani, M.M.; Martinez-Ceron, M.C.; Albericio, F.; Cascone, O. Protein purification by affinity chromatography with peptide ligands selected. *Trends Chromatograp.*, **2010**, *6*, 11-22.

[17] Liu, R.; Lam, K.S.; Begley, T.P. In: Wiley Encyclopedia of Chemical Biology. John Wiley & Sons, Inc.: **2007**.

[18] Kodadek, T. The rise, fall and reinvention of combinatorial chemistry. *Chem. Commun.*, **2011**, *47*, 9757-9763.

[19] Merrifield, R.B. Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide. *J. Am. Chem Soc*, **1963**, *85*, 2149.

[20] Geysen, H.M.; Meloen, R.H.; Barteling, S.J. Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. *Proc. Natl. Acad. Sci. USA*, **1984**, *81*, 3998-4002.

[21] Houghten, R.A. 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. USA*, **1985**, *82*, 5131-5135.

[22] Frank, R. The SPOT-synthesis technique: Synthetic peptide arrays on membrane supports-principles and applications. *J. Immunol. Methods*, **2002**, *267*, 13-26.

[23] Fodor, S.P.; Read, J.L.; Pirrung, M.C.; Stryer, L.; Lu, A.T.; Solas, D. Light-directed, spatially addressable parallel chemical synthesis. *Science*, **1991**, *251*, 767-773.

[24] Houghten, R.A.; Pinilla, C.; Blondelle, S.E.; Appel, J.R.; Dooley, C.T.; Cuervo, J.H. Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery. *Nature*, **1991**, *354*, 84-86.

[25] 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.

[26] Lam, K.S.; Salmon, S.E.; Hersh, E.M.; Hruby, V.J.; Kazmierski, W.M.; Knapp, R.J. A new type of synthetic peptide library for identifying ligand-binding activity. *Nature*, **1991**, *354*, 82-84.

[27] López-Vallejo, F.; Giulianotti, M.A.; Houghten, R.A.; Medina-Franco, J.L. Expanding the medicinally relevant chemical space with compound libraries. *Drug Discov. Today*, **2012**, *17*, 718-726.

[28] Fleeman, R.; LaVoi, T.M.; Santos, R.G.; Morales, A.; Nefzi, A.; Welmaker, G.S.; Medina-Franco, J.L.; Giulianotti, M.A.; Houghten, R.A.; Shaw, L.N. Combinatorial libraries as a tool for the discovery of novel, broad-spectrum antibacterial agents targeting the ESKAPE pathogens. *J. Med. Chem.*, **2015**, *58*, 3340-3355.

[29] Cha, J.; Lim, J.; Zheng, Y.; Tan, S.; Ang, Y.L.; Oon, J.; Ang, M.W.; Ling, J.; Bode, M.; Lee, S.S. Process automation toward ultra-high-throughput screening of combinatorial one-bead-one-compound (OBOC) peptide libraries. *J. Lab Autom.*, **2012**, *17*, 186-200.

[30] Martinez-Ceron, M.C.; Giudicessi, S.L.; Kruszyn, J.N.; Marani, M.M.; Albericio, F.; Cascone, O.; Camperi, S.A. Two-stage screening of combinatorial peptide libraries. Application to bovine serum albumin ligand selection. *Revista CENIC Ciencias Biológicas*, **2015**, *46*, 77-86.

[31] Hintersteiner, M.; Auer, M. A two-channel detection method for autofluorescence correction and efficient on-bead screening of one-bead one-compound combinatorial libraries using the COPAS fluorescence activated bead sorting system. *Methods Appl. Fluoresc.*, **2013**, *1*, 017001.

[32] Doran, T.M.; Gao, Y.; Mendes, K.; Dean, S.; Simanski, S.; Kodadek, T. Utility of redundant combinatorial libraries in distinguishing high and low quality screening hits. *ACS Comb. Sci.*, **2014**, *16*, 259-270.

[33] Fenn, J.B.; Mann, M.; Meng, C.K.; Wong, S.F.; Whitehouse, C.M. Electrospray ionization for mass spectrometry of large biomolecules. *Science*, **1989**, *246*, 64-71.

[34] Tanaka, K.; Waki, H.; Ido, Y.; Akita, S.; Yoshida, Y.; Yoshida, T.; Matsuo, T. Protein and polymer analyses up to m/z 100 000 by laser ionization time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.*, **1988**, *2*, 151-153.

[35] Karas, M.; Hillenkamp, F. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. *Anal. Chem.*, **1988**, *60*, 2299-2301.

[36] Fitzgerald, M.C.; Harris, K.; Shevlin, C.G.; Siuzdak, G. Direct characterization of solid phase resin-bound molecules by mass spectrometry. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 979-982.

[37] Pastor, J.J.; Fernández, I.; Rabanal, F.; Giralt, E. A new method for the preparation of unprotected peptides on biocompatible resins with application in combinatorial chemistry. *Org. Lett.*, **2002**, *4*, 3831-3833.

[38] Camperi, S.A.; Marani, M.M.; Iannucci, N.B.; Côté, S.; Albericio, F.; Cascone, O. An efficient strategy for the preparation of one-bead-one-peptide libraries on a new biocompatible solid support. *Tetrahedron Lett.*, **2005**, *46*, 1561-1564.

[39] Martinez-Ceron, M.C.; Giudicessi, S.L.; Marani, M.M.; Albericio, F.; Cascone, O.; Erra-Balsells, R.; Camperi, S.A. Sample preparation for sequencing hits from one-bead-one-peptide combinatorial libraries by matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry. *Anal. Biochem.*, **2010**, *400*, 295-297.

[40] Mallinson, J.; Collins, I. Macrocycles in new drug discovery. *Future Med. Chem.*, **2012**, *4*, 1409-1438.

[41] Adessi, C.; Soto, C. Converting a peptide into a drug: strategies to improve stability and bioavailability. *Curr. Med. Chem.*, **2002**, *9*, 963-978.

[42] Redman, J.E.; Wilcoxen, K.M.; Ghadiri, M.R. Automated mass spectrometric sequence determination of cyclic peptide library members. *J. Comb. Chem.*, **2003**, *5*, 33-40.

[43] Yang, H.; Gurgel, P.V.; Carbonell, R.G. Hexamer peptide affinity resins that bind the Fc region of human immunoglobulin G. *J. Pept. Res.*, **2005**, *66*, 120-137.

[44] Menegatti, S.; Naik, A.D.; Gurgel, P.V.; Carbonell, R.G. Purification of polyclonal antibodies from Cohn fraction II + III, skim milk, and whey by affinity chromatography using a hexamer peptide ligand. *J. Sep. Sci.*, **2012**, *35*, 3139-3148.

[45] Liu, Z.; Gurgel, P.V.; Carbonell, R.G. Purification of human immunoglobulins A, G and M from Cohn fraction II/III by small peptide affinity chromatography. *J. Chromatogr. A*, **2012**, *1262*, 169-179.

[46] Liu, Z.; Gurgel, P.V.; Carbonell, R.G. Effects of peptide density and elution pH on affinity chromatographic purification of human immunoglobulins A and M. *J. Chromatogr. A*, **2011**, *1218*, 8344-8352.

[47] Lund, L.N.; Gustavsson, P.-E.; Michael, R.; Lindgren, J.; Nørskov-Lauritsen, L.; Lund, M.; Houen, G.; Staby, A.; St. Hilaire, P.M. Novel peptide ligand with high binding capacity for antibody purification. *J. Chromatogr. A*, **2012**, *1225*, 158-167.

[48] Wang, R.-Z.; Lin, D.-Q.; Tong, H.-F.; Yao, S.-J. Molecular insights into the binding selectivity of a synthetic ligand DAAG to Fc fragment of IgG. *J. Mol. Recognit.*, **2014**, *27*, 250-259.

[49] Camperi, S.A.; Martinez-Ceron, M.C.; Giudicessi, S.L.; Marani, M.M.; Albericio, F.; Cascone, O. In: Protein Downstream Processing; N.E. Labrou, ed. Humana Press: New York, **2014**, Volume *1129*, pp. 277-302.

[50] Martinez-Ceron, M.C.; Marani, M.M.; Taules, M.; Etcheverrigaray, M.; Albericio, F.; Cascone, O.; Camperi, S.A. Affinity chromatography based on a combinatorial strategy for rErythropoietin purification. *ACS Comb. Sci.*, **2011**, *13*, 251-258.

[51] Martinez-Ceron, M.C.; Saavedra, S.; Avila, L.; Giudicessi, S.L.; Albericio, F.; Camperi, S.A.; Cascone, O. In: Peptides **2015**. Proceedings of the Twenty Forth American Peptide Symposium, Orlando, U. S. A., June 20-25, **2015**; V. Srivastava, A. Yudin and M. Lebl, eds. American Peptide Society: San Diego, U. S. A., **2015**, pp. 137-138.

[52] Muller, V.D.M.; Russo, R.R.; Oliveira Cintra, A.C.; Sartim, M.A.; De Melo Alves-Paiva, R.; Figueiredo, L.T.M.; Sampaio, S.V.; Aquino, V.H. Crotoxin and phospholipases A2 from Crotalus durissus terrificus showed antiviral activity against dengue and yellow fever viruses. *Toxicon*, **2012**, *59*, 507-515.

[53] Cho, C.F.; Behnam Azad, B.; Luyt, L.G.; Lewis, J.D. High-throughput screening of one-bead–one-compound peptide libraries using intact cells. *ACS Comb. Sci.*, **2013**, *15*, 393-400.

[54] COPAS Biosort. http://www.unionbio.com/copas/product.aspx?id=2. (Accessed September 15, **2015**)

[55] COPAS^{TM}BIO-BEAD. Screen 100,000 drug candidates per hour ~ up to one million compounds a day! *Unión Biométrica*, **2010**

[56] Christensen, C.; Groth, T.; Bruun Schiødt, C.; Tækker Foged, N.; Meldal, M. Automated sorting of beads from a "One-Bead-Two-Compounds" combinatorial library of metalloproteinase inhibitors. *QSAR Comb. Sci.*, **2003**, *22*, 737-744.

[57] Marani, M.M.; Martinez-Ceron, M.C.; Giudicessi, S.L.; de Oliveira, E.; Cote, S.; Erra-Balsells, R.; Albericio, F.; Cascone, O.; Camperi, S.A. Screening of one-bead-one-peptide combinatorial library using red fluorescent dyes. Presence of positive and false positive beads. *J. Comb. Chem.*, **2009**, *11*, 146-150.

[58] Kumaresan, P.R.; Devaraj, S.; Huang, W.; Lau, E.Y.; Liu, R.; Lam, K.S.; Jialal, I. Synthesis and characterization of a novel inhibitor of C-reactive protein–mediated proinflammatory effects. *Metab. Syndr. Relat. Disord.*, **2013**, *11*, 177-184.

[59] Liao, H.-H.; Nawarak, J.; Chang, K.-l.; Hsieh, W.-Y.; Tsai, H.-Y.; Chen, S.-T.; Cheng, S.-L. Screening and identification of peptides that bind specifically to the X gene promoter of hepatitis B virus using a combinatorial peptide library approach. *J. Chin. Chemic. Soc.*, **2011**, *58*, 869-876.

[60] Witucki, L.A.; Borowicz, L.S.; Pedley, A.M.; Curtis-Fisk, J.; Kuszpit, E.G. Identification of FAK substrate peptides via colorimetric screening of a one-bead one-peptide combinatorial library. *J. Pept. Sci.*, **2015**, *21*, 302-311.

[61] Gautam, A.; Kapoor, P.; Chaudhary, K.; Kumar, R. Tumor homing peptides as molecular probes for cancer therapeutics, diagnostics and theranostics. *Curr. Med. Chem.*, **2014**, *21*, 2367-2391.

[62] Lam, K.S.; Lebl, M. Streptavidin and avidin recognize peptide ligands with different motifs. *Immuno. Methods*, **1992**, *1*, 11-15.

[63] Liu, T.; Qian, Z.; Xiao, Q.; Pei, D. High-throughput screening of one-bead-one-compound libraries: identification of cyclic peptidyl inhibitors against calcineurin/NFAT interaction. *ACS Combinatorial Sci.*, **2011**, *13*, 537-546.

[64] Samson, I.; Kerremans, L.; Rozenski, J.; Samyn, B.; Van Beeumen, J.; Van Aerschot, A.; Herdewijn, P. Identification of a peptide inhibitor against glycosomal phosphoglycerate kinase of Trypanosoma brucei by a synthetic peptide library approach. *Bioorg. Medicin. Chem.*, **1995**, *3*, 257-265.

[65] Astle, J.M.; Simpson, L.S.; Huang, Y.; Reddy, M.M.; Wilson, R.; Connell, S.; Wilson, J.; Kodadek, T. Seamless bead to microarray screening: rapid identification of the highest affinity protein ligands from large combinatorial libraries. *Chem. Biol.*, **2010**, *17*, 38-45.

[66] Mendes, K.; Ndungu, J.M.; Clark, L.F.; Kodadek, T. Optimization of the magnetic recovery of hits from one-bead–one-compound library screens. *ACS Combinatorial Sci.*, **2015**, *17*, 506-517.

[67] MacDonald, M.; Aube, J. Approaches to cyclic peptide beeta turn mimics. *Curr. Org. Chem.*, **2001**, *5*, 417-438.

[68] Fluxa, V.S.; Reymond, J.-L. On-bead cyclization in a combinatorial library of 15,625 octapeptides. *Bioorg. Medicin. Chem.*, **2009**, *17*, 1018-1025.

[69] Liu, T.; Joo, S.H.; Voorhees, J.L.; Brooks, C.L.; Pei, D. Synthesis and screening of a cyclic peptide library: discovery of small-molecule ligands against human prolactin receptor. *Bioorg. Med. Chem.*, **2009**, *17*, 1026-1033.

[70] Joo, S.H.; Xiao, Q.; Ling, Y.; Gopishetty, B.; Pei, D. High-throughput sequence determination of cyclic peptide library members by partial Edman degradation/mass spectrometry. *J. Amer. Chem. Soc.*, **2006**, *128*, 13000-13009.

[71] Liu, R.; Marik, J.; Lam, K.S. A novel peptide-based encoding system for "one-bead one-compound" peptidomimetic and small molecule combinatorial libraries. *J. Am. Chem. Soc.*, **2002**, *124*, 7678-7680.

[72] Liu, T.; Liu, Y.; Kao, H.-Y.; Pei, D. Membrane permeable cyclic peptidyl inhibitors against human peptidylprolyl isomerase Pin1. *J. Medicin. Chem.*, **2010**, *53*, 2494-2501.

[73] Upadhyaya, P.; Qian, Z.; Selner, N.G.; Clippinger, S.R.; Wu, Z.; Briesewitz, R.; Pei, D. Inhibition of Ras signaling by blocking Ras–effector interactions with cyclic peptides. *Angewandte Chemie Int. Edit.*, **2015**, *54*, 7602-7606.

[74] Bédard, F.; Girard, A.; Biron, É. A convenient approach to prepare topologically segregated bilayer beads for one-bead two-compound combinatorial peptide libraries. *Int. J. Pept. Res. Ther.*, **2013**, *19*, 13-23.

[75] Kwon, Y.-U.; Kodadek, T. Encoded combinatorial libraries for the construction of cyclic peptoid microarrays. *Chemic. Commun.*, **2008**, 5704-5706.

[76] Giudicessi, S.L.; Gurevich-Messina, J.M.; Martinez-Ceron, M.C.; Erra-Balsells, R.; Albericio, F.; Cascone, O.; Camperi, S.A. Friendly strategy to prepare encoded one bead–one compound cyclic peptide library. *ACS Comb. Sci.*, **2013**, *15*, 525-529.

[77] Lee, J.H.; Meyer, A.M.; Lim, H.S. A simple strategy for the construction of combinatorial cyclic peptoid libraries. *Chem. Commun.*, **2010**, *46*, 8615-8617.

[78] Bryson, D.I.; Zhang, W.; Ray, W.K.; Santos, W.L. Screening of a branched peptide library with HIV-1 TAR RNA. *Mol. BioSyst.*, **2009**, *5*, 1070-1073.

[79] Lee, K.J.; Lim, H.-S. Facile method to sequence cyclic peptides/peptoids via one-pot ring-opening/cleavage reaction. *Org. Lett.*, **2014**, *16*, 5710-5713.

[80] Simpson, L.S.; Kodadek, T. A cleavable scaffold strategy for the synthesis of one-bead one-compound cyclic peptoid libraries that can be sequenced by tandem mass spectrometry. *Tetrahedron Lett.*, **2012**, *53*, 2341-2344.

[81] Liang, X.; Girard, A.; Biron, E. Practical ring-opening strategy for the sequence determination of cyclic peptides from one-bead-one-compound libraries. *ACS Comb. Sci.*, **2013**, *15*, 535-540.

[82] Menegatti, S.; Ward, K.L.; Naik, A.D.; Kish, W.S.; Blackburn, R.K.; Carbonell, R.G. Reversible cyclic peptide libraries for the discovery of affinity ligands. *Anal. Chem.*, **2013**, *85*, 9229-9237.

[83] Gurevich-Messina, J.M.; Giudicessi, S.L.; Martinez-Ceron, M.C.; Acosta, G.; Erra-Balsells, R.; Cascone, O.; Albericio, F.; Camperi, S.A. A simple protocol for combinatorial cyclic depsipeptide libraries sequencing by matrix-assisted laser desorption/ionisation mass spectrometry. *J. Pept. Sci.*, **2015**, *21*, 40-45.

[84] Marani, M.M.; Oliveira, E.; Cote, S.; Camperi, S.A.; Albericio, F.; Cascone, O. Identification of protein-binding peptides by direct matrix-assisted laser desorption ionization time-of-flight mass spectrometry analysis of peptide beads selected from the screening of one bead-one peptide combinatorial libraries. *Anal. Biochem.*, **2007**, *370*, 215-222.

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