A Perspective for the Peptide Drug Hunting Consortium

Synthetic Combinatorial Libraries: Four Decades of Peptide-Driven Innovation and the Road Ahead

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Abstract

Combinatorial chemistry has transformed the discovery of bioactive molecules by enabling the rapid generation and screening of large, structurally diverse chemical libraries. Since its emergence in the 1980s, the field has evolved from early peptide-based approaches—establishing a versatile chemical space bridging small molecules and biologics—to encompass a wide range of platforms, including one-bead-one-compound (OBOC) libraries, solution-phase mixture libraries, biological display technologies, and DNA-encoded libraries (DEL). These advances have dramatically expanded accessible chemical space and accelerated the identification of ligands for diverse biological targets. Notably, peptide and peptide-like modalities have played a central role in this evolution, serving as a bridge between small molecules and biologics in combinatorial discovery.

Recent progress has shifted the focus from library size alone toward increased molecular complexity, functional relevance, and integration with advanced screening technologies. Innovations in synthetic methodologies—including macrocycle construction, post-translational modification, and DNA-compatible chemistries—have enabled the generation of more diverse and drug-like compounds. In parallel, developments in high-throughput and high-content screening, microfluidics, and encoding strategies have enhanced both the efficiency and depth of library interrogation.

This article highlights the historical perspective and key advances in combinatorial library synthesis, screening, and encoding, with an emphasis on emerging technologies that bridge chemistry and biology. It further examines how the convergence of synthetic innovation, functional screening, and data-driven analysis is reshaping the field. Finally, future directions are outlined in which integrated, function-oriented, and information-rich approaches are expected to drive the next generation of therapeutic and materials discovery. These developments align with emerging community-driven efforts, such as the Peptide Drug Hunting Consortium (PDHC), to integrate chemistry, biology, and data science in next-generation discovery.

Introduction

Combinatorial chemistry involves the generation of large arrays of structurally diverse compounds—referred to as chemical libraries—through the systematic and repetitive covalent assembly of modular building blocks. Once constructed, these libraries can be screened in parallel to identify compounds that interact with biological targets of interest. Active compounds can then be identified either directly in addressable libraries or through decoding strategies that rely on genetic or chemical barcodes. Combinatorial chemistry has played a central role in drug discovery and lead optimization [1-4]. As summarized in **Figure 1**, most combinatorial platforms—including phage display, yeast and bacterial display, mRNA display, one-bead-one-compound (OBOC) libraries, DNA-encoded libraries (DEL), and solution phase mixture peptide libraries (e.g., SPCL, PC-SPCL)—are capable of generating libraries with enormous diversity (often exceeding 10^6 members) while supporting rapid, parallel screening against biological targets. In contrast, parallel synthesis and planar microarray approaches typically produce smaller, more focused libraries (hundreds to thousands of compounds) but offer advantages in structural definition and downstream optimization.

Planar microarrays have been particularly valuable in peptide research; however, other classes of compounds can also be synthesized *in situ* using microtiter arrays or droplet-based reactors in a high-throughput manner [5]. Each combinatorial technology possesses distinct strengths and limitations, and both the chemical nature of the library members and the screening modalities employed vary substantially across platforms (**Figure 1**). Phage-display and mRNA-display libraries are genetically constructed primarily from amino acids, yielding peptides and proteins that inherently reflect a biological bias. In contrast, synthetic libraries accommodate a far broader range of building blocks and are not subject to this biological constraint.

> ***Within this landscape, peptides and peptide-derived modalities occupy a distinct middle ground, combining synthetic accessibility with biological relevance, and are therefore particularly well suited to combinatorial discovery.***

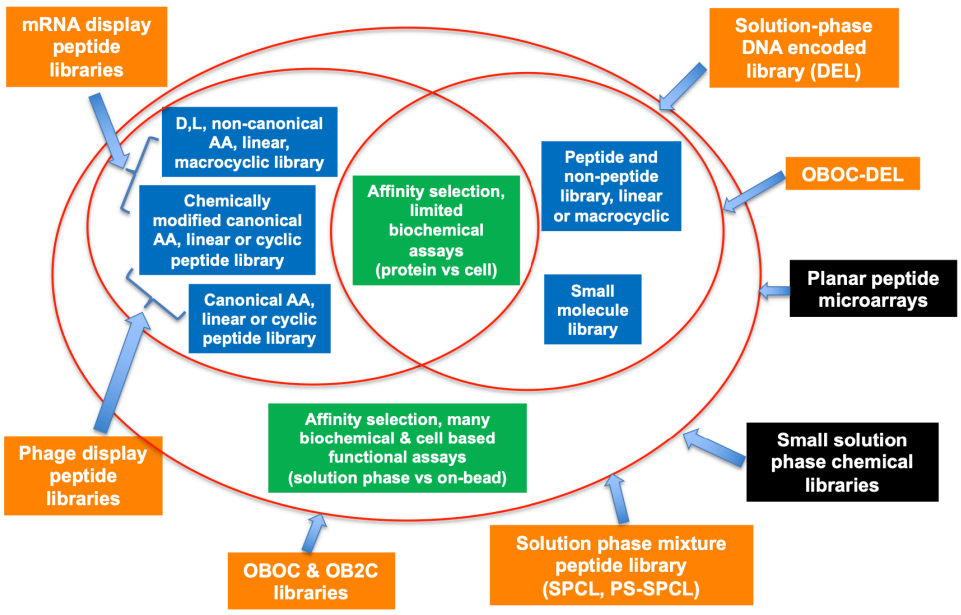

Figure 1. Overview of combinatorial technologies. The various combinatorial platforms are shown in orange (diverse and focused libraries) and black (focused small libraries), the chemical nature of library compounds is shown in blue, and the two broad groups of screening assays are shown in green. Depicted within the red ovals are the screening assays and nature of library compounds pertaining to each combinatorial platform. (Modified from Fig. 1 of Ref. 1).

In general, with some exceptions, affinity-based selection (binding assays) is the primary screening approach used for DEL, mRNA-display, and phage-display libraries. In contrast, OBOC libraries and solution-phase mixture libraries such as SPCL and PS-SPCL are compatible with a wide range of biochemical and cell-based functional assays in addition to binding assays. The diversity afforded by each method also differs substantially: OBOC libraries (90 µm beads) can achieve 10^6–10^7 diversity (and higher with smaller beads), PS-SPCL (10^8-10^{12}), phage-displayed peptide libraries (10^7-10^{11}), mRNA-displayed macrocyclic peptide libraries (10^{12}-10^{14}), bead-based DEL (10^6–10^8), and solution-phase DEL (10^8-10^{11} for dual pharmacophore DEL). While DEL offers exceptional diversity, it is generally limited to small molecules, small macrocycles or short peptides with a restricted number of diversity points. In contrast, peptide and macrocyclic peptide libraries—particularly those derived from OBOC and mRNA display—enable access to higher-order structural complexity and functional diversity that more closely approximates biologics while retaining synthetic control. It may sometimes be beneficial to combine different platforms, for example using DEL, mRNA display or phage display platforms to discover a lead compound and then utilizing OBOC cell-based screening platform to optimize the molecule with desirable cellular effects.

When integrated with computational approaches, focused libraries generated by parallel synthesis are particularly effective for structure-activity relationship (SAR) studies and lead refinement. Emerging computational tools—including AlphaFold-based structure prediction, artificial intelligence-driven design, and ADMET filtering—are increasingly incorporated into combinatorial library design, significantly accelerating the identification of viable drug candidates. Selected combinatorial approaches are highlighted in this mini review, with synthetic combinatorial libraries as the primary focus. Nucleic acid-based libraries (e.g., aptamers [6]) are beyond the scope of this article.

Historical Evolution of Combinatorial Libraries

The historical evolution of combinatorial library platforms is illustrated in **Figure 2**. The foundations of combinatorial chemistry were established in the mid-1980s with Geysen's multi-pin technology [7] and Houghten's tea-bag method [8], both of which enabled parallel synthesis of defined but small peptide sets. These approaches were built upon the seminal solid-phase peptide synthesis method developed by Bruce Merrifield in 1963 [9].

> ***These peptide-based innovations were not only foundational historically, but continue to define the trajectory of combinatorial discovery due to their modularity, synthetic tractability, and compatibility with both chemical and biological screening paradigms.***

In 1991, we [10, 11] introduced the OBOC concept and demonstrated that millions of random peptides could be generated via solid-phase "split-mix synthesis" (also referred to as split-and-pool synthesis), screened, and decoded using automated Edman sequencing. Houghten [12] described solution-phase mixture peptide libraries decoded through iterative screening, which later evolved into positional scanning synthetic combinatorial libraries (PS-SPCL) [13]. Fodor reported the synthesis and screening of 1,024 peptides in ten steps on a glass slide using photolithography and light-directed deprotection to create spatially addressable planar peptide microarrays [14]. Furka independently reported split-and-pool synthesis for solution-phase peptide mixtures, but the OBOC concept was not explicitly articulated [15]. Biological display technologies emerged alongside these chemical approaches. Smith introduced the phage display concept in 1985 [16] and demonstrated its application to peptide libraries in 1990 [17]. Positive binders were enriched through multiple rounds of bio-panning and identified by DNA sequencing.

The 12th American Peptide Symposium (Boston, June 1991) marked a pivotal moment in the combinatorial field. At this meeting, several foundational technologies were presented, including OBOC libraries [18], phage-display peptide libraries [19], photolithographic peptide microarrays [20], and solution-phase mixture libraries [21].

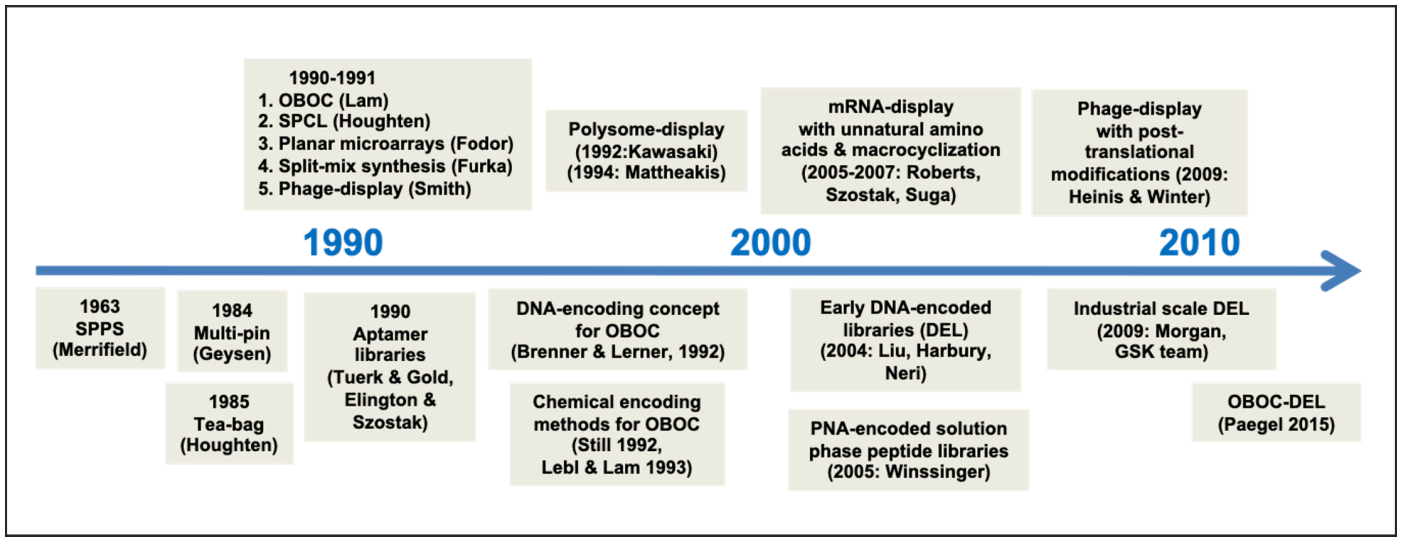

Figure 2. Historical development of combinatorial library platforms

Collectively, these advances established the conceptual and technical framework for modern combinatorial chemistry.

Early biological display systems—including phage, yeast, and polysome display—were initially limited to the 20 canonical amino acids and simple disulfide-mediated cyclization. This limitation was substantially overcome in the mid-2000s through advances in mRNA display and reprogrammed translation systems. Work by Frankel [22], Josephson [23], and Suga [24] demonstrated the incorporation of some non-canonical amino acids into macrocyclic peptide libraries. Subsequently, Heinis and Winter [25] introduced post-translational chemical modification strategies for phage display enabling access to conformationally constrained, proteolytically stable peptide scaffolds with expanded chemical diversity and improved drug-like properties.

Shortly after the introduction of the OBOC platform [10], Brenner and Lerner in 1992 proposed using DNA as an encoding element, leading to the concept of a DNA-encoded library (DEL) that could be prepared with a split-and-pool strategy on a solid support [26]. However, limitations in DNA sequencing technology initially restricted practical implementation. Approximately a decade later, Liu introduced DNA-templated organic synthesis [27], and in 2004 demonstrated a 65-member macrocycle library that could be decoded via PCR amplification [28].

That same year, several groups reported related advances. Harbury described a DNA-display peptide library generated through DNA-routing to identify ligands against anti-enkephalin antibody [29]. Neri introduced DNA-encoded self-assembling chemical libraries comprised of complementary DNA-tagged chemical fragments for small-molecule discovery [30]. Winnsinger reported small PNA-encoded peptidic libraries synthesized via solid-phase methods and decoded with DNA microarrays [31]. With continued advances in next-generation sequencing (NGS), DEL technology has evolved rapidly. In 2009, through the concerted effort by a large team of investigators led by Morgan of GlaxoSmithKline, an $8x10^8$ diversity small molecule solution-phase DEL was created from which inhibitors against Aurora A kinase and p38 MAP kinase were identified [32,33]. In 2015, Paegel reported the development of OBOC-DEL libraries using 10 µm beads, maintaining the original OBOC configuration while incorporating DNA encoding [33,34]. DEL is now widely adopted by many pharmaceutical companies [4]. Several drug candidates currently in clinical trials have been identified through DEL approaches [35-37]. Over the past five years, new encoding and screening strategies, expanded DNA-compatible chemistries, and machine learning–based data analysis have further accelerated the impact of DEL in drug discovery [4].

Preparation of Synthetic Combinatorial Libraries

Small synthetic combinatorial libraries (hundreds to a few thousand compounds) can be generated through parallel synthesis, either manually or using automated platforms, in solution or on solid support, with or without flow chemistry. These libraries offer broad flexibility in reaction chemistry and allow purification and full structural characterization of each member. Because the identity of each compound is predefined, such libraries are inherently addressable and well suited for systematic structure–activity relationship (SAR) studies.

In contrast, OBOC libraries are synthesized on microbeads using split-and-pool strategies, enabling the creation of much larger and more diverse libraries (thousands to tens of millions of compounds) [10]. Each bead displays a single compound species; however, these libraries are non-addressable. Therefore, active compounds identified during screening must be decoded, typically via direct chemical analysis of the library compound or through the introduction of chemical barcodes during synthesis. Unlike DELs, which use DNA barcodes that are unstable under harsh conditions, we encode OBOC libraries with chemical barcodes (e.g., peptide barcode for non-sequenceable polymers or small molecules [38]) that are compatible with a broad range of chemical reactions, including low pH, high pH, oxidizing conditions, and elevated temperatures.

For OBOC encoding, we developed topologically segregated bilayer beads, in which the library compound is displayed on the outer layer and the chemical barcode is embedded within the bead interior [39,40], thus minimizing potential interference of the barcodes with screening. The OBOC platform also allows the option of keeping library compounds tethered to the bead or releasing them into the vicinity of the bead for solution-phase assays. In addition to peptides, peptoids, peptidomimetics, and small molecules, we have successfully used glycosyltransferase enzymes to generate OBOC combinatorial peptide-glycan libraries (unpublished data). We have also developed strategies to convert OBOC peptide libraries into polyamine and small-molecule libraries through global transformation [41]. SPLCs and PS-SPCLs are assembled on solid support via split-and-pool synthesis and subsequently cleaved into separate mixtures for screening in solution. Deconvolution strategies are then used to identify active components [12,13].

In 2004, when various DEL approaches were first developed, library synthesis was cumbersome and often impractical for generating high-diversity libraries. As the technology has evolved, DELs are now primarily prepared in solution using sticky-end enzymatic ligation strategies for DNA encoding, yielding solution-phase single pharmacophore DELs (diversity 10^6-10^9) and dual pharmacophore dynamic DELs (10^8-10^{11}). Typically, three to five cycles of coupling are performed to ensure encoding fidelity. This limits the construction of large macrocyclic structures or library compounds requiring more than six coupling cycles. Even though DEL synthesis is now primarily performed in solution, the original OBOC concept still applies because split-and-pool strategy is necessary to maintain the “one-DNA-one-compound” configuration.

Most pharmaceutical and academic investigators now use solution-phase DELs because of their enormous diversity. Nevertheless, bead-based OBOC-DELs remain very important, particularly due to their broader compatibility with assays beyond affinity selection. OBOC-DELs are prepared like solution-phase DELs, but ~10 µm beads are used as solid support [33,34].

Despite their potential for enormous diversity, DELs are constrained by the requirement for DNA-compatible chemistry. Reactions must typically proceed under mild, aqueous, near-neutral conditions to preserve DNA integrity, which limits the range of accessible chemical transformations and structural complexity. Nonetheless, there has been great progress in this area over the past five years [42]. In principle, PNA-encoded libraries could overcome many of these chemical limitations. However, it remains to be determined whether this approach can be translated to industrial scale, as has been achieved with standard solution-phase DELs.

Screening of Combinatorial Libraries

The choice of screening strategy is closely linked to library format and encoding methodology. Affinity-selection assays are the most widely used; however functional assays—including biochemical, enzymatic, and cell-based systems—provide critical complementary information. In particular, cell-based assays capture higher-order biological responses such as cytotoxicity, apoptosis, autophagy, receptor engagement, cell differentiation, cell motility, metabolism, and signaling pathway modulation, often using optical reporter systems (exogenous or engineered).

With few exceptions, affinity selection assays are the primary screening method for phage-display libraries, mRNA-display libraries, as well as solution-phase and bead-based DELs (**Figure 1**). Purified proteins are commonly used for affinity selection. In the case of cell surface receptors such as GPCRs, which are difficult to express in their native configuration, live cells or cell membranes may be used directly, with appropriate subtraction strategies. Screening methods beyond affinity selection, such as biochemical and cell-based assays, are actively being explored for solution-phase DEL; however, the literature in this area remains relatively sparse [43,44]. OBOC-DEL platform using small (~10 µm) beads has been shown to provide sufficient compound quantities per bead for biochemical assays when integrated with droplet-based microfluidic systems for automated functional screening at the picoliter scale [33,34]. These systems integrate compound release, assay incubation, fluorescence detection, and sorting into a continuous workflow, enabling highly efficient identification of active compounds.

Although the primary screening method for phage-display peptide libraries is biopanning with immobilized target proteins or intact cells, this platform also offers a powerful approach for identifying organ-targeting peptides through *in vivo* "panning," which is very challenging with other combinatorial platforms. Phages recovered from specific organs following tail-vein administration in mice can be amplified in *E. coli* and subjected to repeated rounds of *in vivo* selection [45]. These canonical amino acid-containing organ-targeting peptides can potentially be further optimized with non-canonical amino acid-containing OBOC libraries.

Addressable libraries generated by parallel synthesis or PS-SPCLs are in solution and are therefore well suited for manual or automated high-throughput screening (HTS) in multiwell plate formats (96-, 384-, and 1536-well). These formats are compatible with a wide range of binding, biochemical, and cell-based assays. In recent years, applications of PS-SPCLs have been largely concentrated in immunology, particularly in T-cell epitope mapping and vaccine development [46].

Among all combinatorial platforms, OBOC technology is the most versatile. It enables efficient screening across a wide range of targets, including soluble proteins, living cells, organoids, and even whole organisms such as bacteria, yeasts, and viruses. This versatility is particularly impactful for peptide and peptide-derived libraries, where functional screening can directly capture context-dependent biological activity, including cell penetration, receptor modulation, and tissue targeting. The historical development of methods for OBOC screening and encoding is shown in **Figure 3**. We typically use 90 µm TentaGel beads as solid support for OBOC library synthesis, which are significantly larger than mammalian cells (~10–20 µm in diameter). Each 90 µm bead can generate over 10 pmol of library compound. Compounds may remain tethered to the bead during screening [10] or be released into vicinity of the bead for solution-phase biochemical or cell-based functional assays [47,48]. For protein-binding assays, smaller beads (10–30 µm) may be used to increase library diversity within a given volume of beads, whereas 90 µm beads are preferred for cell-based functional assays.

For protein-binding detection, we have used enzyme-linked colorimetric assays for mapping B-cell epitopes [10], identifying MHC class I anchor residues [49], and discovering high-affinity peptides containing non-canonical amino acids against the SARS-CoV-2 spike protein [50].

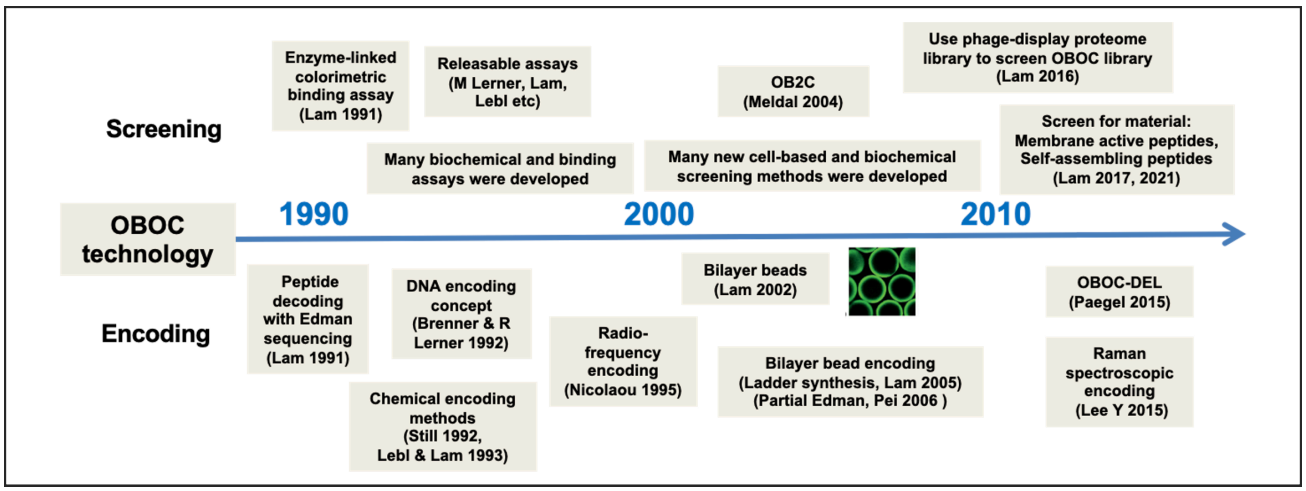

Figure 3. History of OBOC platform: screening and encoding

We have developed radiodetection assays to identify peptide substrate motifs for protein kinases [51,52] and sulfotransferases (unpublished work), as well as fluorescence quenching assays to identify protease substrate motifs [51,52]. We have also used [^{3}H]-strychnine and scintillation autoradiography to screen OBOC libraries for toxin-caging molecules (unpublished work). In addition, we developed a proximity ligation strategy to identify reactive affinity elements capable of site-specific ligation to immunoglobulins [53] and human serum albumin [54] for the development of drug carriers. We have used the OBOC platform to develop an illuminating peptide for instant detection of opioids in blood [55]. We employed a molecular rotor dye to screen OBOC libraries for genetically encoded small illuminants [56]. For cell-based assays (**Figure 4**), we use live cancer cells as probes to screen OBOC libraries for cancer-targeting ligands [57-60]. We have also developed approaches to probe cellular functions, such as multiplexed intracellular staining with antibodies against signaling proteins. Alternatively, engineered cells incorporating optical reporter systems can be employed. Using these strategies, ligands capable of inducing osteogenic progenitor cell differentiation have been identified [61]. We have also utilized human brain endothelial cells to identify cell-penetrating peptides capable of crossing the blood-brain barrier *in vivo* (unpublished work).

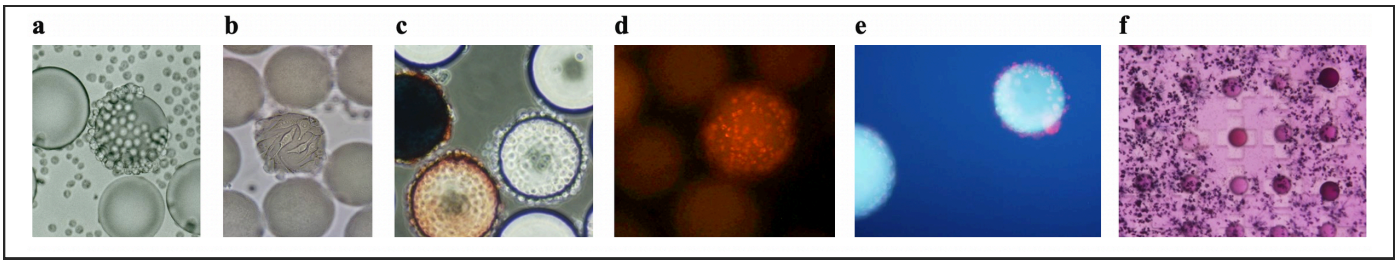

Figure 4. Various cell-based approaches to screen OBOC libraries: (a) cell binding assay, (b) cell binding and morphological changes, (c) cell-signaling assay; in-cell staining (brown) with anti-phospho-Erk antibody, (d) propidium iodide to stain cells on bead undergoing apoptosis, (e) cell-penetrating peptides delivered quantum dots inside brain endothelial cells coated on OB2C library beads, turning the cells pink, (f) library compounds released from immobilized OBOC bead, generating a cell-killing zone after addition of MTT.

We have developed a one-bead-two-compound (OB2C) configuration for cell-based assays, in which each bead displays both a fixed cell-capturing ligand and a random library compound on its surface. Upon incubation with live cells, each bead becomes coated with a monolayer of cells. Cellular responses induced by interactions between tethered ligands and cell surface receptors can then be detected using imaging-based reporter systems [62,63]. This approach has enabled the discovery of pro-apoptotic ligands with anti-tumor activity in xenograft models prior to optimization [62,63].

Library compounds in OB2C or OBOC systems can also be released from the bead via cleavable linkers (e.g. disulfide bonds) to interact with cells bound to the bead surface or embedded within surrounding gel matrices [47,48]. For certain assays, photolithography has been used to fabricate PDMS microwell arrays for bead loading and screening [48] (**Figure 4f**).

In materials science, OBOC methods have been applied to discover membrane-active peptides [64, 65], some of which destabilize lipid bilayers or promote membrane fusion, while others exhibit antifungal activity [66]. OBOC approaches have also been used to identify self-assembling penta-peptides capable of forming nanoparticles, some of which can penetrate intact cells [67]. Methods have been developed to identify stealth peptides [68].

Many other groups have used OBOC approaches for basic research and drug discovery [2]. For example, Miller and others have used OBOC for the discovery of artificial enzymes [69,70]. Pei has successfully utilized OBOC to discover cell-penetrating cyclic peptides [71] and macrocyclic inhibitors against intracellular protein-protein interactions [72]. Meldal developed the OB2C method to display both substrate and library compounds on each bead, facilitating the discovery of protease inhibitors [73]. Heath [74] described an *in situ* click chemistry-based strategy for constructing multivalent protein capture agents directly on OBOC beads. This iterative process enables progressive affinity maturation, in which initial binders serve as templates for subsequent ligand assembly, leading to rapid improvements in affinity and specificity without prior knowledge of high-affinity ligands.

Magnetic and microfluidic devices have been used to increase the throughput of OBOC screening [75, 76]. A standard wide-field fluorescence microscope equipped with LED excitation and a CMOS camera has been shown to be highly efficient for OBOC screening [77]. Weller reported the use of bead arrays on microscope slide for efficient screening and mass spectrometry decoding of OBOC libraries [78].

mRNA-display and phage-display platforms enable ligand maturation through biological evolution via multiple sequential rounds of selection. In contrast, ligand maturation in OBOC and DEL platforms relies on iterative design of focused libraries combined with increasingly stringent screening conditions. Additional cycles of library design and screening can yield very high-affinity ligands. Common strategies to increase stringency include: (1) lowering target protein concentration, (2) increasing washing stringency, and (3) introducing competing ligands. For OBOC libraries, an additional useful parameter is reducing ligand density on the bead surface by down substituting the outer layer prior to library synthesis.

Overall, screening technologies are evolving toward greater integration of throughput, sensitivity, and functional relevance. The convergence of advanced imaging, microfluidics, chemical biology, and data-driven analysis is transforming combinatorial screening from a largely binding-centric process into a multidimensional platform capable of capturing complex biological responses. Continued innovation in this area will be essential for translating the expanding diversity of combinatorial libraries into meaningful therapeutic outcomes.

Encoding and Decoding of Synthetic Combinatorial Libraries

Efficient and high-fidelity encoding and decoding of library members are central to the utility of combinatorial chemistry, particularly for large, non-addressable libraries. For solution-phase mixture libraries, such as PS-SPCL, compound identity is determined through deconvolution. This iterative process involves testing defined sub-mixtures to progressively narrow down active components until the active structure is elucidated [12,13]. While effective, deconvolution can be time-consuming, and the presence of multiple unrelated hits within the library can complicate interpretation.

Single-pharmacophore DEL utilizes covalently attached DNA barcodes to record synthetic history. Following affinity selection, bound library members are identified through PCR amplification and NGS of their associated DNA tags. Enriched sequences

are determined by comparing input library counts with post-selection counts. However, distinguishing true positives from background noise can be challenging. Emerging computational approaches, including artificial intelligence and active learning, are potential solutions to this challenge [79,80]. Dual-pharmacophore DEL evolved from the encoded self-assembling chemical (ESAC) library platform developed by Neri [30]. It affords dynamic screening and fragment based-drug discovery. Although dual-pharmacophore DEL provides substantially greater diversity, its screening and encoding processes are more complex than those of standard single-pharmacophore DEL [1-4].

Traditional OBOC libraries require external encoding strategies, as each bead carries a unique compound without intrinsic positional or genetic identifiers. In addition to DNA encoding, a variety of chemical and physical barcoding methods have been developed to address this challenge. **Figure 3** shows the history on the development of OBOC encoding methods. Peptide or peptoid libraries can be directly decoded on-bead using automated Edman degradation sequencing [81], or compounds can be cleaved from the bead for mass spectrometric (MS) analysis [78, 82,83]. For small-molecule OBOC libraries, we have used peptides as coding tags [38,39], while Still employed halophenol derivatives as tags decoded by gas chromatography [84]. Other decoding strategies include MS analysis of ladder sequences generated by partial Edman degradation [85], or ladder sequences formed during synthesis [86,87], Raman tag encoding [88-90], and colloidal barcoding [91].

Collectively, these encoding and decoding strategies reflect a broader trend toward increasing information density, speed, and compatibility with high-throughput screening platforms. DNA barcoding has enabled unprecedented scaling of library size, while advances in chemical and spectroscopic barcoding continue to enhance the practicality of bead-based systems. Looking forward, integration of encoding technologies with real-time screening, advanced imaging, and machine learning–driven analysis is likely to further streamline hit identification and accelerate the translation of combinatorial libraries into actionable chemical matter.

The Road Ahead: Future Opportunities for Combinatorial Libraries

The vast diversity and spatially segregated architectures enabled by OBOC combinatorial bead technology [10,92,93] and planar peptide microarrays [14], both emerging in the early 1990s, did more than introduce new experimental platforms—they reshaped how discovery is conceptualized. By enabling the simultaneous interrogation of thousands to hundreds of thousands of analytes, these technologies catalyzed a shift toward truly parallel experimentation. This "combinatorial mindset" rapidly extended beyond chemistry, influencing the development of DNA microarrays for high-throughput genomics [94-96] and bead-based multiplexed barcode platforms that underpin modern sequencing and diagnostic technologies [97-99]. In this sense, combinatorial thinking has evolved from a methodological innovation into a foundational framework for modern biology (**Figure 5**).

Proteomics has only begun to exploit this paradigm. OBOC peptide libraries can function as "compressive samplers" of proteomic complexity, selectively enriching subsets of proteins and reducing dynamic range—one of the central challenges in the field. Early demonstrations using gel-based [100] and mass spectrometric analytical approaches [101] highlight this potential, but a true inflection point will likely come from integration with artificial intelligence.

> ***The convergence of combinatorial enrichment and machine learning–driven inference could transform biomarker discovery from a largely empirical process into a predictive, systems-level discipline.***

Peptide and peptide-derived modalities are likely to play an increasingly central role in this evolution. Their ability to access extended binding surfaces, adopt defined secondary structures, and incorporate non-canonical chemistries positions them uniquely between small molecules and biologics. As combinatorial platforms become more integrated with machine learning and high-content screening, peptides provide a tractable and scalable modality for interrogating complex biological systems.

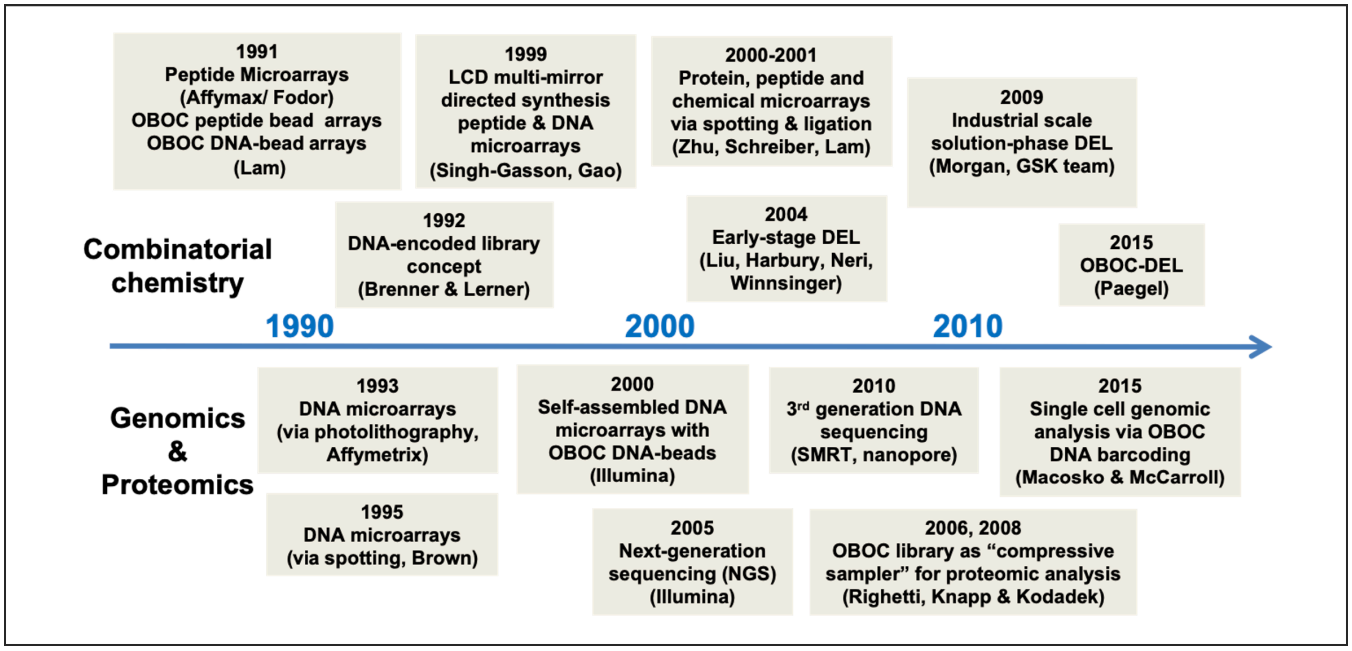

Figure 5. From combinatorial chemistry to genomics and proteomics

A broader frontier lies in the convergence of combinatorial chemistry with omics-scale interrogation. Rather than treating proteomes and cell lysates as passive outputs, they can be repurposed as active probes to map interactions across vast chemical spaces. For example, the use of phage-display proteome libraries to screen OBOC small-molecule libraries [102] begins to generate dense ligand–protein interaction networks. As these approaches scale, they will produce datasets whose complexity necessitates machine learning as an integral, rather than auxiliary, component of discovery.

> ***In this context, OBOC and related platforms may serve as a critical bridge between experimental chemistry and data-driven biology.***

One of the most underappreciated strengths of the OBOC platform is its intrinsic compatibility with functional screening. While many high-throughput platforms remain anchored to binding as a primary readout (i.e. affinity selection), OBOC enables direct selection based on biological activity in live cells (**Figure 4**). This distinction is fundamental: it allows discovery to proceed at the level of phenotype rather than proxy. The identification of chemical compounds that mediate tumor targeting, cell penetration, blood-brain barrier traversal, cell differentiation, metabolic changes, or pathway modulation underscores this capability. Looking ahead, integration with high-content imaging, organoid systems, and immune system mimics using advanced microfluidics [103] will enable functional screening in increasingly physiologically relevant microenvironments, shifting combinatorial discovery from a target-centric to a systems-level paradigm.

Beyond biomedicine, the implications extend to materials science. Current materials discovery efforts remain constrained by relatively low-diversity, planar array screening approaches [104], leaving large regions of chemical space unexplored. OBOC platform offers an alternative paradigm: massively parallel synthesis and screening with built-in diversity and functional readouts.

Early studies on membrane-active peptides [64,65] and self-assembling peptides [67] suggest that such approaches can uncover emergent physicochemical properties of synthetic materials that are difficult to predict *a priori*. Extending this framework could enable the discovery of adaptive, bio-interfacing, and environmentally responsive materials.

OBOC platform also enables unconventional strategies in small-molecule discovery. Beyond identifying ligands, they can be used to discover compounds that sequester or neutralize other molecules. The identification of strychnine-binding "caging" agents, with demonstrated *in vivo* protection (unpublished work), highlights the potential of this concept. Such approaches suggest a complementary paradigm to classical pharmacology—one based on physical capture rather than biochemical antagonism—and could be extended to toxins, metabolites, and signaling molecules.

The need for rapid responses to emerging infectious diseases further underscores the value of combinatorial approaches. OBOC and DEL platforms enable direct screening against microbial targets ranging from isolated proteins to intact pathogens. The rapid identification of SARS-CoV-2 spike protein binding peptides that supported diagnostic development [50] illustrates this capability. More broadly, combinatorial platforms can function as front-line discovery engines, generating ligands for diagnostics, targeting, and therapeutic intervention of global outbreaks under compressed timelines.

A parallel challenge lies in accessing structurally complex chemical space. Many of the most potent natural products are large macrocycles and non-peptidic, yet they remain difficult to translate into combinatorial formats. Overcoming this limitation will require new synthetic strategies that render complex architectures modular and "combinatorially accessible." At the same time, naturally occurring scaffolds such as cyclotides [105,106] provide highly stable frameworks that can be diversified through sequence grafting, genetically [107] or chemically [108], enabling libraries that combine structural precision with functional diversity.

Taken together, these developments point to a broader conclusion: OBOC, DEL, and related combinatorial technologies should not be viewed merely as tools for generating molecular diversity, but as platforms for interrogating—and ultimately engineering—complex biological and chemical systems. Their greatest impact will emerge at the interfaces: between chemistry and biology, experiment and computation, and molecules and systems. In this context, the true promise of combinatorial chemistry lies not only in accelerating discovery, but also in redefining how discovery itself is conducted. Community-driven efforts such as the Peptide Drug Hunting Consortium (PDHC) are helping to formalize this convergence by integrating advances in peptide design, synthesis, screening, and data science into a cohesive discovery framework.

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