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Review

DNA-Encoded Chemical Libraries: A Comprehensive Review with Succesful Stories and Future Challenges

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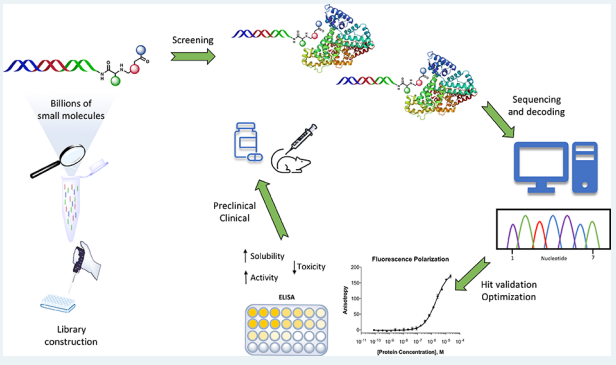
ABSTRACT: DNA-encoded chemical libraries (DELs) represent a versatile and powerful technology platform for the discovery of small-molecule ligands to protein targets of biological and pharmaceutical interest. DELs are collections of molecules, individually coupled to distinctive DNA tags serving as amplifiable identification barcodes. Thanks to advances in DNA-compatible reactions, selection methodologies, next-generation sequencing, and data analysis, DEL technology allows the construction and screening of libraries of unprecedented size, which has led to the discovery of highly potent ligands, some of which have progressed to clinical trials. In this Review, we present an overview of diverse approaches for the generation and screening of DEL molecular repertoires. Recent success stories are described, detailing how novel ligands were isolated from DEL screening campaigns and were further optimized by medicinal chemistry. The goal of the Review is to capture some of the most recent developments in the field, while also elaborating on future challenges to further improve DEL technology as a therapeutic discovery platform.



KEYWORDS: *DNA-encoded libraries, affinity selections, next-generation sequencing, small molecules, machine learning*

INTRODUCTION

The isolation and validation of specific ligands against targets of pharmaceutical relevance represents one of the most important steps in the discovery of new drugs.[1−3] A variety of screening paradigms has been developed to identify protein ligands. Among these, high-throughput screening (HTS) and fragment-based discovery procedures have often been used to evaluate large collections of small molecules (ranging between few thousands to 1 million) against protein targets of interest.[4−6] Effective exploitation of HTS requires the individual synthesis and testing of hundreds of thousands of chemical compounds, which demands substantial resources and complex logistics.[7,8] In spite of progress in the HTS field, it is still difficult to identify high-quality ligands, even from libraries comprising more than 1 million molecules. Larger repertoires are difficult and expensive to produce, store, and screen. In principle, virtual libraries could be analyzed using computational approaches, but reliable ligand binding predictions remain challenging.[9] For this reason, substantial efforts have been committed to the development of alternative methodologies for protein ligand discovery. Improved methodologies are particularly needed for the identification of specific binders toward more difficult targets, such as those involved in protein−protein interactions.[10,11]

Advances in genomics and genetics have led to the identification and validation of protein targets, for which it would be desirable to discover small organic ligands and, potentially, develop drugs. Yet, the power of molecular biology techniques needs to be matched by more efficient chemical methodologies, which may facilitate the isolation of specific binding molecules. In this context, it is interesting to note that large encoded combinatorial libraries of biologics (e.g., antibody libraries) routinely deliver binders against virtually any protein target of interest.[12−14] Those libraries typically contain billions of different members, suggesting that construction and screening of much larger chemical libraries compared to those used in HTS may be needed, in order to drug a larger proportion of protein targets.

Progress in the field of encoded protein libraries inspired Brenner and Lerner to propose the construction of encoded chemical libraries, in which synthetic chemical entities on beads would be linked to individual DNA fragments, acting as

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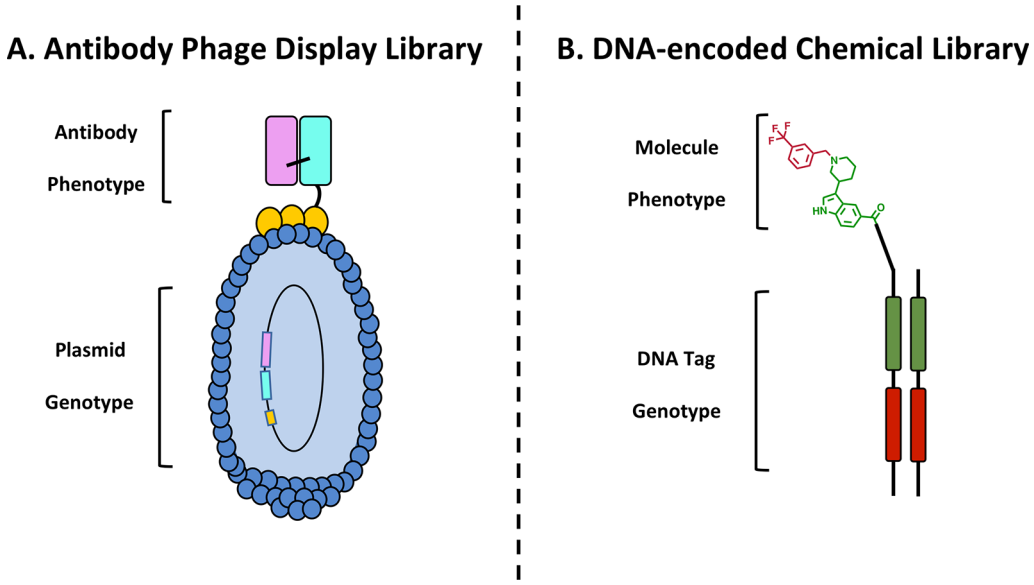

Figure 1. Schematic representations of (A) antibody phage display and (B) DNA-encoded chemical libraries.

identification barcodes (Figure 1).[15,16] Further elaboration of this original proposal has led to a variety of different experimental schemes for the synthesis of DNA-encoded chemical libraries (DELs), even in the absence of beads.[17−25]

HTS activities are typically carried out on multi-well plates (384 or 1536), interrogating single compounds against the protein target of interest or directly against cells expressing this target. As mentioned before, this approach requires expensive infrastructures for compound handling, dispensing, and data analysis.[4−8]

In contrast to conventional HTS methodologies, combinatorial protein libraries (e.g., phage display libraries, ribosome display libraries) and DELs comprise a huge variety of protein variants and small molecules, respectively, which are individually connected to the DNA sequences that code for them. The encoding of each library member allows these libraries to be tested simultaneously in the same vessel, using the concept of affinity selection. Usually, the protein target of interest, modified with an appropriate tag, is captured using solid supports (e.g., streptavidin beads) and then incubated with the library, containing billions of molecules. Enrichment of the most active binders is typically achieved by different washing steps, and the ligands are then recovered by suitable elution procedures (e.g., change of pH or of temperature).[20−24] The identification of selectively enriched compounds is performed by decoding their genetic information, using a PCR amplification step followed by high-throughput DNA sequencing.[25]

Improvements in the field have made DEL technology a very attractive platform for early-stage drug discovery campaigns, both in academia and in pharmaceutical companies.[16−24] Several DEL-derived lead compounds have already reached clinical stages. While the synthesis of conventional chemical libraries for HTS may cost as much as USD 1 billion, the synthesis and screening of DELs comprising billions of compounds only requires standard laboratory infrastructure and moderate investments.[22] The implementation of new DNA-compatible reactions has stimulated the exploration of broader chemical spaces, expanding the structural diversity of DELs.[26−37] In addition, the incorporation of chemical complexity for library synthesis has been enhanced by the development of new synthetic approaches.[36]

HISTORICAL ACCOUNT ON DIFFERENT STRATEGIES FOR DNA-ENCODED LIBRARY CONSTRUCTION

Shortly after the conceptual scheme proposed by Brenner and Lerner, the synthesis and screening of encoded peptide libraries comprising positive control peptides of known binding properties was experimentally implemented.[38,39] However, for more general applications, the bead-based approach was limited by the lack of efficient orthogonal strategies for the simultaneous synthesis of both peptides and oligonucleotides on a solid phase. Two years later, Kinoshita and colleagues demonstrated the possibility to enzymatically ligate oligonucleotides for DEL applications, a feature which has since been frequently used for library encoding purposes.[40]

In 2004, three methodologies for DEL construction were proposed and experimentally implemented, which featured the direct coupling of chemical matter to double-stranded DNA fragments, without the use of beads. Avoiding beads may be important, both in terms of library size (there is a physical limit for the number of solid particles that can be suspended in a given volume) and in terms of versatile procedures for synthesis and screening.

Liu and co-workers, from Harvard University, introduced an approach called DNA-templated synthesis.[41] Neri and colleagues, from ETH Zürich, developed an encoding strategy based on self-assembling of partially complementary DNA strands (ESAC).[42] A few years later, they also reported the first library based on DNA-recorded synthesis strategies.[43] Finally, the group of Harbury reported a strategy, named DNA routing, in which DNA conjugates are sequentially captured on sepharose resin coated with complementary oligonucleotides, allowing an iterative procedure of sequential chemical transformations in organic solvents.[44−46] These methods will be described in more detail in subsequent sections of this Review.

While the basic ideas behind DEL technology had been postulated in 1992, it took approximately 15 years before an

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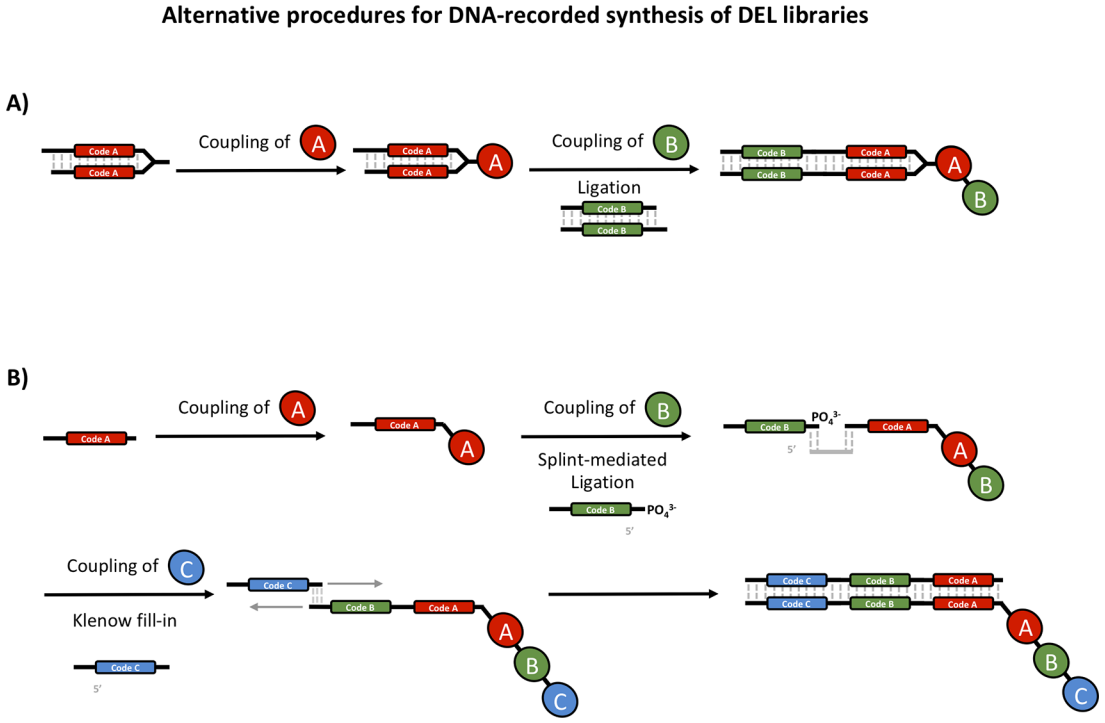

Figure 2. Schematic representation describing encoding procedures for DNA-recorded chemical libraries. (A) The small molecules are appended to the dsDNA by a linker (head-piece) and encoded by subsequent DNA ligations using overhang DNA heteroduplexes. (B) Building blocks A and B are encoded by splint ligation, in which the additional DNA fragment is ligated to the nascent DNA structure. Building block C is encoded by the annealing of a partially complementary DNA fragment, followed by a Klenow polymerization procedure.

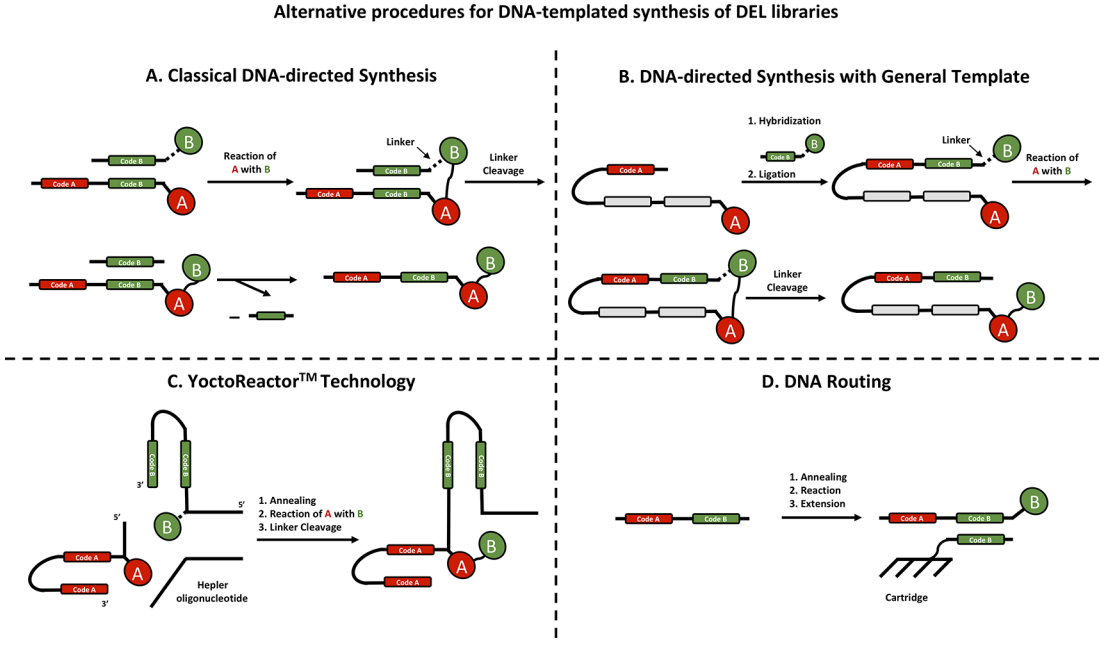

Figure 3. Schematic representation detailing the encoding strategies for DNA-templated synthesis of chemical libraries. (A) During classical DNA-templated synthesis, short oligonucleotides bearing small-molecule reactants are annealed with the DNA to promote the reaction between the two chemical entities. After the reaction is completed, the linker between the oligonucleotide and the reactant is cleaved and the oligonucleotide is removed by affinity capture. (B) Using a polyinosine segment, universal DNA-templated synthesis avoids the use of high-fidelity annealing between the oligonucleotide promoter and the DNA. (C) The YoctoReactor technology promotes highly efficient intermolecular reactions by close proximity, using 3D DNA junctions. (D) On-resin immobilized DNA complementary codons are used to promote annealing with the different DNA-conjugated small molecules and subsequent reaction and elution.

increasing number of groups started reporting alternative methods for library construction and screening. Those methodologies have crucially contributed to the popularity that DEL enjoys today, both in industry and in academia.[17−24] In the following sections, we present some of the most

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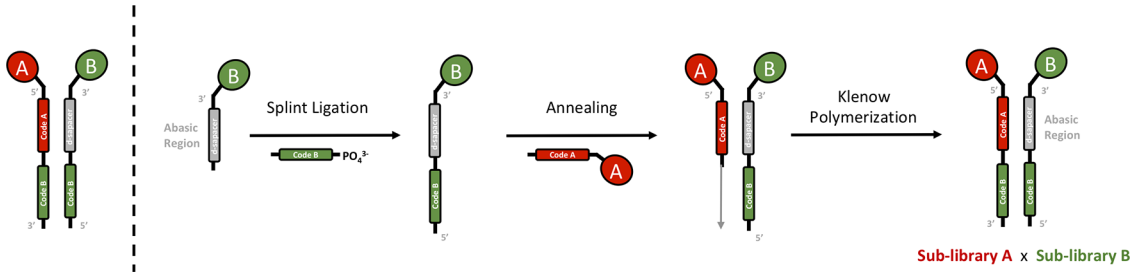

Figure 4. Encoding strategies for dual-pharmacophore encoded chemical libraries. During encoded self-assembling chemical libraries (ESAC) synthesis, both strands A and B bearing the two distinct chemical moieties are prepared and purified individually, offering an extremely high degree of purity to the final library. Upon annealing between the code A and the d-spacer oligonucleotide, the code B is transferred onto the complementary strand by Klenow polymerization.

frequently used strategies for library encoding, synthesis, and screening.

■ ENCODING STRATEGIES

When considering strategies for DEL encoding, it may be convenient to make a distinction between "single-pharmacophore libraries" and "dual-pharmacophore libraries". In single-pharmacophore libraries, individual chemical moieties (no matter how complex) are coupled to distinctive DNA fragments (either in single-stranded or double-stranded format). In dual-pharmacophore libraries, two different chemical moieties are attached to the extremities of complementary DNA strands, acting synergistically for specific protein recognition. Moreover, it is convenient to distinguish between pre-defined DNA sequences that drive library construction ("DNA-templated chemistry") and synthetic procedures in which the identity of individual building blocks that make up the final molecule is encoded by the iterative ligation of small DNA fragments ("DNA-recorded chemistry"). This latter procedure was first introduced in a 2008 publication, describing the synthesis and screening of DEL comprising 4000 members.[43] Shortly afterward, scientists at GSK reported the construction and screening of a library comprising 800 million compounds.[47]

Single-Pharmacophore Libraries. Single-pharmacophore libraries are most commonly constructed using DNA-recorded synthesis, which relies on the use of split-and-pool procedures.[48]

Libraries are built up through a series of chemical transformations in multiple steps, each of which is encoded by the addition of either double-stranded or single-stranded DNA fragments (dsDNA or ssDNA) that uniquely identify them (Figure 2).[17−20,24] In a typical construction procedure, *n* different chemical building blocks are encoded using the same number of DNA fragments. In general, DNA fragments only differ by a short sequence, typically 6−7 base pairs, which serve as a barcode. After the first step, all the individual DNA-conjugated small molecules are pooled together, which can be subsequently split into *m* number of different wells, allowing a second cycle of chemical transformation and DNA tag elongation to yield *m*×*n* library compounds. The split-and-pool procedure can be iterated multiple times (most of the DELs typically involve 2−3 cycles). In the case of dsDNA, DNA fragments are ligated using overhang tags, while ssDNA fragments can be assembled using splint-mediated ligations.[17,49] If desired, ssDNA DEL encoding can be converted into the corresponding dsDNA format by the use of Klenow polymerization with a complementary oligonucleotide primer.[50,51] Alternative strategies such as chemical ligation have also been developed.[52]

Chemical modifications during this approach are performed using a large excess of reagents and reactants to ensure high conversions, therefore minimizing the amount of remaining starting materials and the so-called truncated libraries.

Liu and co-workers designed and implemented library construction methodologies using pools of pre-encoded DNA templates (Figure 3A).[41] This methodology relies on DNA-directed chemical reactions to promote the coupling of diverse sets of building blocks. The interaction of two nucleobases through hydrogen bonds is known to accelerate bimolecular reactions and to increase the local concentration of the reactants in solution. The authors described the use of a 48-mer DNA-linked lysine derivative used as "DNA template" to mediate three steps of DNA-directed amine acylation reactions with building blocks linked onto 10-mer or 12-mer biotinylated complementary oligonucleotides. After each coupling, suitable cleavage steps are required, and the reagent oligonucleotides are biotinylated for purification by affinity capture with streptavidin-linked magnetic beads.

The group of Xiaoyu Li optimized the encoding method by using a single "universal template code", which is capable of directing chemical reactions with multiple small molecule DNA conjugates displaying various encoding sequences (Figure 3B).[53] The universal DNA oligonucleotide contains a polyinosine segment that serves as a promiscuous hybridization stretch for short oligonucleotides including transferable chemical moieties.

A tridimensional extension of the classical linear DNA-templated encoding strategy was developed and implemented by scientists at Vipergen (Figure 3C).[54,55] This methodology, named YoctoReactor, relies on the annealing and subsequent enzymatic ligation of three-way DNA-hairpin-looped junctions carrying chemical moieties. In the first step, two single-stranded DELs are mixed together with a third oligonucleotide, which assists the self-assembling of the libraries. Upon formation of a three-way junction construct, two sets of building blocks are then coupled by DNA-templated reaction. The resulting library is then purified by polyacrylamide gel electrophoresis, which confers high fidelity to the process. All incomplete intermediates are removed, and truncations are eliminated from the combinatorial library. After cleavage of the linker and DNA ligation, a PCR primer extension is carried out to generate the complementary strand. After hybridization, the building blocks are transferred onto a core acceptor site, and the final library is obtained in a double-stranded format.

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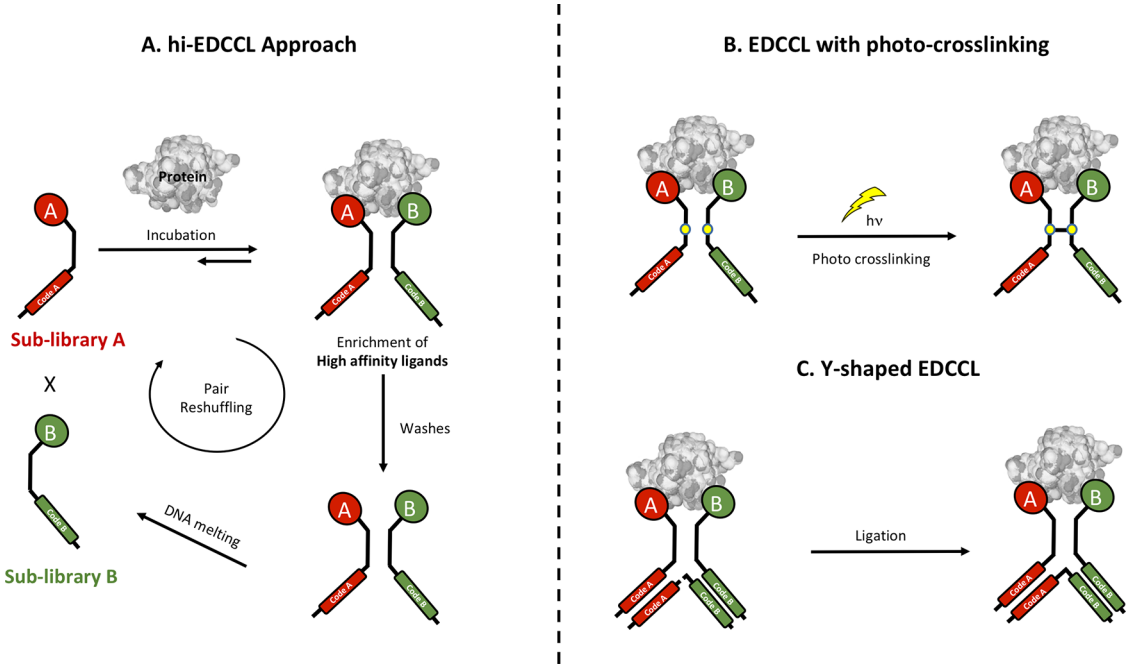

Figure 5. Schematic representation of different hi-EDCCL approaches. (A) hi-EDCCLs are based on partial annealing of two complementary sub-libraries. The DNA duplex is unstable until the addition of the target displaces the equilibrium toward the high-affinity combinations. The non-binding moieties can be re-shuffled to identify the best combinations. (B) An evolution of hi-EDCCL is based on the generation of a physical linkage ([2+2] UV-promoted cycloaddition) between both DNA strands once the equilibrium gets shifted upon addition of the target. (C) The latest advancement on EDCCL relies on the formation of Y-shaped DNA constructs to facilitate the dynamic enrichment of synergistic binding pairs.

Harbury and co-workers described an alternative synthetic approach known as "DNA-routing" (Figure 3D). This technology requires, at the start of the library construction process, the availability of as many pre-synthesized DNA oligonucleotides as the final complexity of the library. In a sequential fashion, the mixture of oligonucleotides is captured on resin coated with partially complementary DNA fragments, allowing the selective modification of captured oligonucleotides on the solid phase.[44,45] The technology may become difficult to implement if large libraries are desired, in view of the numerous capture steps that would be required and the risk of sub-optimal fidelity in the hybridization process. Nonetheless, in principle, DNA-routing procedures may facilitate the Darwinian evolution of library complexity, as the oligonucleotides recovered at the end of a selection experiment could be used to drive the synthesis of chemical variants of preferentially enriched compounds. Similar considerations have also been made for DNA-templated synthesis strategies.

Dual-Pharmacophore Libraries. In 2004, the Neri group developed an encoding strategy based on the self-assembly of partially complementary DNA strands, leading to the so-called encoded self-assembling chemical (ESAC) libraries (Figure 4). In essence, two mutually complementary sub-libraries are annealed in order to create large combinatorial diversity.[42,56] In this dual-pharmacophore library setting, the first sub-library displays chemicals at the 5′ extremity of a tagged oligonucleotide, whereas the second sub-library bears chemical moieties attached at the 3′ extremity and features abasic portions to facilitate the hybridization of complementary strands.[42−58] The strategy for construction of ESAC libraries is described in Figure 4 and makes use of Klenow polymerization in order to transfer code information from one sub-library to the other one.

ESAC libraries feature the simultaneous display of pairs of building blocks that could synergistically interact with the target protein of interest. The flexible linkers between chemical moieties and DNA may facilitate the protein recognition procedure, but also create an element of complexity when ESAC binders are converted into small organic molecules devoid of DNA. Individual members of ESAC sub-libraries are HPLC purified, which contributes to an extremely high quality of the resulting assembled library. By comparison, library construction using split-and-pool procedures features a purification step only after the first building block coupling, which may lead to insufficient library purity due to low reaction yields.

The practical use of ESAC allows the identification and recognition of adjacent binding fragments (chelate-binding effect), which can be exploited in fragment-based drug discovery (FBDD) programs, where each single moiety can be engaged in two distinct non-overlapping binding events. In analogy with FBDD, the two chemical moieties have to be linked to yield a single organic molecule. Bigatti et al. reported an optimized process to expedite the fragment-linking strategy on ssDNA using a set of pre-defined bifunctional scaffolds.[59] The DNA conjugate is hybridized with a complementary locked nucleic acid (LNA) bearing a fluorescent molecule (fluorescein or Bodipy) and used in fluorescence polarization (FP) assays to determine their dissociation constant with the target protein. From this screen, the best candidates are re-synthesized by conventional off-DNA organic synthetic methods and the binding affinity confirmed by orthogonal methodologies.[60,61]

Reddavide et al. reported the application of "dynamic recombination" for the synthesis of "ESAC-type" libraries (Figure 5A).[62] The principle, based on "dynamic combinatorial

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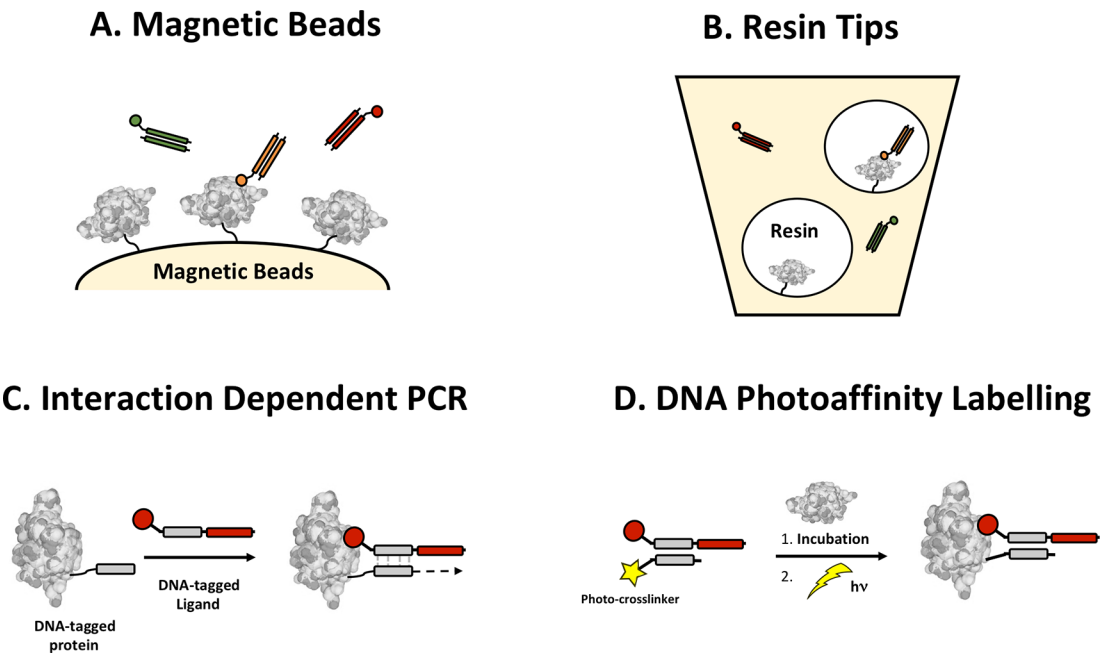

Figure 6. Schematic representation of selection methods for DNA-encoded chemical libraries. (A) Affinity capture of the desired target using magnetic beads is the most common approach for DEL screenings. Upon immobilization, the library is incubated with the target, and the non-binding molecules are washed away by stringent washing procedures. (B) The use of resin tips avoids the necessity of magnetic capturing of the protein−ligand complexes. Once the target is immobilized in the core of the tip, the library passes through it, and the binding molecules get retained in the matrix. After subsequent heat elution, the binding moieties are recovered. (C) The target of interest is modified to include a DNA tag, which hybridizes with a short complementary region in the library once the ligands approach the protein. Only binder−protein complexes are PCR amplified. (D) Photo-cross-linking methodologies allow the formation of a covalent bond between the binding molecules and the target protein. The photo-cross-linking library is incubated with the target protein, and upon irradiation the ligand−protein complex is fixed. Subsequent stringent washings discard the non-binders.

chemistry" (DCC), employs a reversible covalent bond (e.g., disulfide bond, Schiff-base formation) to construct dynamic systems of transient small-molecule adducts in a thermodynamic equilibrium. In analogy to ESAC, the two sub-libraries have a short complementary DNA strand, which allows the formation of a heat-induced DNA-encoded dynamic combinatorial chemical library (hi-EDCCL). Due to thermodynamic instability, the two sub-libraries can hybridize to form unstable dsDNA interactions. Upon addition of the target, the equilibrium is shifted toward the high-affinity duplexes. Thus, the protein can be considered as a template for the *in situ* generation of potent binders via chemical stabilization of unstable adducts. After a round of selection, the non-binding pairs can be "shuffled" by heating and used for a second round of selection to identify the best combinations. Zhou et al. described a conceptually similar method, generating EDCCL libraries from two sub-libraries that could be covalently connected after a binding event to the target protein of interest, thanks to a *p*-stilbazole moiety (Figure 5B).[63] Upon UV-irradiation, an intermolecular [2+2] cycloaddition locks the dynamic exchange of each binding pair. The resulting duplexes, which synergistically interact with the target protein, are then isolated, PCR amplified, and decoded by high-throughput DNA sequencing.

In 2019, a second generation of EDCCL libraries was reported, in which a Y-shaped DNA construct was designed for the dynamic enrichment of synergistic binding pairs (Figure 5C).[64] The approach features the use of a third DNA strand that anneals to the complementary ESAC components, contains the code information for the pair of chemical moieties, and can be PCR amplified and sequenced at the end of the selection procedure.

In principle, peptide nucleic acids (PNAs) could be considered as an alternative to DNA for the construction of DELs. PNAs are more stable than DNA and may enable a broader set of chemical reactions. However, PNAs are not compatible with PCR amplification and may require the use of complementary DNA fragments for hybrid formation and subsequent library decoding. Winssinger and co-workers have pioneered the design, synthesis, and screening of PNA-encoded libraries.[65,66] For many applications, DNA microarrays have been used for the hybridization of PNA-based ligands recovered at the end of affinity capture procedures, followed by a fluorescent readout detection.

SCREENING METHODOLOGIES

Compared to traditional HTS, DEL technology offers the possibility to screen billions of molecules in a single experiment.[17,67] Various screening methodologies have been implemented to select and identify binders to a specific protein of interest on solid support or in solution (Figure 6).

Solid-phase affinity-based selections rely on the immobilization of the proteins of interest on solid supports, which are successively incubated with a DNA-encoded library.[68−75] Preferentially enriched binders are separated from other library members after affinity capture by stringent washing steps. Suitable solid supports for affinity capture include magnetic beads (Figure 6A) and resin-filled tips (Figure 6B). Proteins may be immobilized either by covalent modification of lysine residues or by non-covalent capture of suitable tags (e.g., biotin, His-tag, or other peptide tags). Washing conditions and

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the use of detergents may significantly influence the selection output. In rare cases, immobilization of the protein of interest on a solid support may impair its folding, thus contributing to the isolation of false positives. Non-specific binding with the matrix may also lead to an increase in the noise level of the selections, thus hindering the discrimination between real binders and artifacts.[76] Non-specific binding events can be avoided using herring sperm DNA, bovine serum albumin, biotin, or imidazole as blocking agents. Despite the above-mentioned disadvantages, the simplicity of affinity capture methodologies has made them attractive for many screening campaigns and they have successfully been used for the identification of DEL-derived hits.

In principle, it would be desirable to perform selections in solution, without the need to immobilize the target on a solid support. In 2010, Liu and co-workers described the use of interaction-dependent PCR (IDPCR) for the identification of small-molecule binders of oligonucleotide-tagged proteins (Figure 6C).[77] In this approach, single-stranded DNA DELs are used, and the target protein is covalently tagged with an oligonucleotide which is complementary to the DEL tags and may stabilize the ligand−protein interaction. Such an oligonucleotide can also act as a primer for the selective PCR amplification of library members which preferentially interact with the protein target.[78] Pseudo-hairpin structures may be particularly suited for IDPCR. Later, the same group developed an extension of their first in-solution selection methodology, named as interaction determination using unpurified proteins (IDUP).[79] Using cell lysates, they were able to identify binders for targets that are otherwise very difficult to purify, enhancing the potential uses of in-solution methodologies. Instead of covalently attaching oligonucleotides to the protein of interest, DNA can be coupled to an antibody or to a SNAP-tagged protein.[79]

Vipergen developed binder trap enrichment (BTE) as a screening methodology which occurs in water-in-oil emulsions. In a first step, a DNA-tagged protein target is incubated with a DEL library. By subsequently forming water-in-oil emulsions at high dilution of target protein, the simultaneous incorporation of protein target and bound DEL member within the same water droplet can be promoted. A ligation step can then be performed within the droplet, thus leading to a hybrid DNA molecule that contains both the DEL barcode and the DNA fragment originally coupled to the protein. The resulting ligation product can then be PCR amplified and sequenced.[80]

Li et al. described an innovative methodology called DNA-programmed photoaffinity labeling (DPAL) to select binders from a DEL without the need of tagging or immobilizing the protein prior to the screening.[81−83] DNA conjugates are capable of forming a stable heteroduplex with a short complementary oligonucleotide containing a photo-reactive group that reacts with proteins of interest. When DNA conjugates bind to the target protein in solution, an irradiation step ensures that preferential binders are "captured" on the target protein by photo-cross-linking. The same approach has been followed by Philochem AG, in which a critical evaluation of different parameters of photo-cross-linking methodologies using model selections against Carbonic Anhydrase IX (CAIX) was done.[84]

Although DEL screenings on immobilized targets and in solution have been demonstrated to be successful approaches for the identification of new chemical entities, certain targets may be difficult to stabilize in those conditions, or their isolation, out of the context of the living cells, can lead to a loss of their biological activity (e.g., misfolding). It may therefore be desirable to develop new methodologies for the execution of DEL selections against targets in their natural environment (e.g., membrane proteins on cells). In 2015, scientists at GSK described the first cell-based DNA-encoded library screening. In this report, transduced HEK293 cells overexpressed the tachykinin receptor neurokinin-3 (NK3). A DEL repertoire containing approximately 15 billion compounds was incubated with the living cells, using reagents to avoid target internalization and unspecific binding (sodium azide and sheared salmon sperm). After a series of washing steps, binding molecules were eluted by heat denaturation of the cells, and the resulting supernatant was collected after centrifugation. The authors were able to identify a series of different NK3 antagonists, from four different DEL libraries, which display different structural motives one to another. Moreover, some of these compounds have potencies and specificities comparable to those of other known NK3 antagonists optimized by medicinal chemistry campaigns (Talnetant and Osanetant).[85]

In 2019, the group of Krusemark reported DEL screening experiments against protein targets located either in the cytosol or on the surface of live cells. The authors used a cyclic cell-penetrating peptide (cCPP) conjugated to DNA-linked molecules to facilitate the penetration of the DEL members into the cytosol. A photo-reactive group was used to enrich DEL members capable of selective interaction with the protein target of interest.[86] In spite of these encouraging results, the absence of more reports on successful DEL selections on cells makes it difficult to evaluate how generally applicable such procedures may be. In principle, the binding interaction of two binding partners present at very low concentration (i.e., the target protein on the cell surface and a DEL member) could be challenging.

In an effort to improve the performance of cell-based selections, Xiaoyu Li and co-workers have recently described the possibility to specifically label cell membrane proteins with a DNA tag, in a manner similar to that previously described by Liu et al.[77] The presence of such DNA tags on the target protein of interest may be able to promote a specific hybridization of DEL libraries based on ssDNA, thus increasing the local concentration of DEL compounds in the cell proximity. The affinity of the DNA guide for DEL library members should not be too high, in order to allow for a further affinity gain as a result of ligand−protein interaction. After suitable washing steps, preferential binders could be recovered, PCR amplified, and sequenced.[87]

Early this year, scientists at Vipergen described a new methodology for DEL screening on live cells. The authors reported the use of oocytes, which are more than 100 000 times bigger than typical somatic cells, to specifically express the target protein of interest (POI) in the cytosol. The target POI is expressed in the oocytes fused to a "prey" protein domain. A "bait" DNA conjugate (a specific binder for the prey protein) is co-injected with the DEL library into the oocytes. The purpose of the bait−prey complex is to ensure the subsequent PCR amplification of the DEL library binders to the POI. Using Vipergen's proprietary BTE methodology, a water-in-oil emulsion is formed by high dilution, thus isolating the complex between POI−prey fusion protein, bait, and a library member. A ligation step can then be performed to form a hybrid that contains both the "bait" DNA barcode and the

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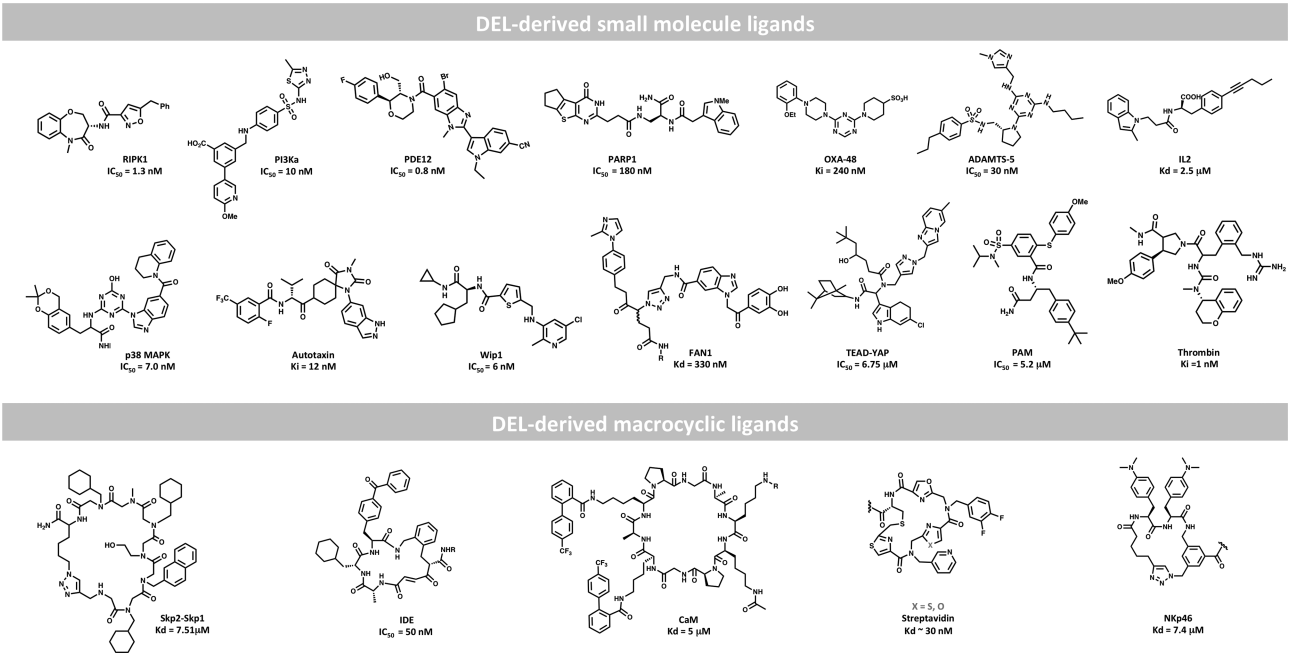

Figure 7. Non-exhaustive list of validated initial hits from DEL screenings against targets of pharmaceutical relevance.

DEL member barcode. The resulting DNA hybrid can be PCR amplified and sequenced.[88]

APPLICATIONS OF DEL TECHNOLOGY TO DRUG DISCOVERY

DEL technology offers the ability to screen large collections of molecules of unprecedented size and functionality, which opens new opportunities for drug discovery.[17−24] Over the years the design and construction of novel DELs has led to the discovery of high-affinity binders against a wide range of proteins of pharmaceutical interest (Figure 7).[50,51,85,89−107] DEL-derived molecules may possess physical properties analogous to those obtained from traditional screening platforms. Moreover, the increasing number of DNA-compatible reactions combined with the availability of a large panel of building blocks provides higher chemical diversity and complexity for more drug-like structures.[17]

Figure 7 describes the structures, targets, and potency of a set of DEL-derived compounds, isolated either in academic laboratories or in companies. When considering DEL synthesis and screening approaches over the past two decades, two main strategies can emerge. On one side, certain DELs were constructed by split-and-pool procedures, aiming at "drug-like molecules" which would be compliant with the Lipinski's rule of five (RO5). From such libraries, binders were isolated against various types of targets, including proteins with defined pockets such as kinases, phosphatases, or proteases. In some cases, libraries with three sets of building blocks were screened, but only one or two chemical moieties contributed to specific protein recognition. For this reason, even if the parental compounds were larger than 500 Da, DEL results served as a basis for the discovery of drug-like hits. When looking at the structures depicted in Figure 7, one often finds molecules comprising two large building blocks (average MW > 200 Da) or three smaller ones (average MW < 160).[89,108,109] To the other extreme, certain DELs have been synthesized with the specific goal of stepping outside of the RO5 chemical space. The synthesis of larger molecular entities (e.g., macrocyclic compounds) may facilitate the recognition of large and flat protein surfaces, which are otherwise difficult to drug with small ligands.[17,58] Several reports of macrocyclic DELs, based on peptidic structures or on natural products derivatives, have demonstrated promising properties against targets which would typically be targeted by larger macromolecular binders such as antibodies.[105−107,110] Alternatively, macrocyclic peptides have been used as scaffolds for the combinatorial modification of side chains.[50] These repertoires have been screened against dozens of different proteins and have yielded binders with affinities in the low micromolar to low nanomolar range.

Despite an extensive effort in the field, chemical optimizations of initial hits from DEL screening campaigns are typically required.[108,109,111] Table 1 summarizes the different chemical transformations of DEL hit structures for the generation of lead molecules, which have progressed to late industrial developments. Six out of seven hits display dissociation constants in a single- or double-digit nanomolar range, and optimization studies were performed with few structural alterations, mainly with unvaried or decreased molecular weight and lower cLogP (Table 1, entries 1, 2, 4−6). Three DEL-derived molecules are currently in clinical trials: inhibitors of receptor interacting protein 1 (RIP1) kinase (entry 1), soluble epoxide hydrolase (sEH; entry 2), and autotaxin (ENPP2; entry 3). Interestingly, removal of unnecessary chemical moieties from the initial hits regularly led to improvement of physicochemical properties. Some examples reported in Table 1 (entries 2, 3, and 7) indicate that DEL-derived hits undergo a different chemical approach during their structure optimization but give a broad space of maneuver during subsequent chemical transformations.

One common feature that can be observed, when considering hit-to-lead conversion of DEL-derived binders, is the relatively small number of chemical transformations which were needed to substantially improve binding affinity and potency. For example, scientists at GSK described a RIP1 kinase inhibitor from a DEL library comprising three sets of

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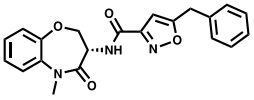
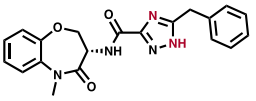
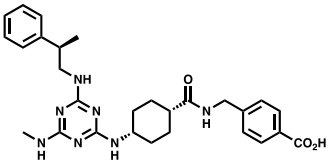
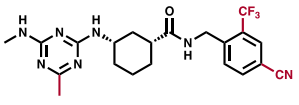
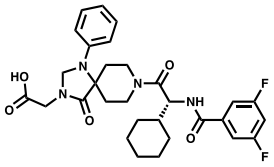
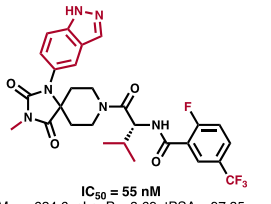
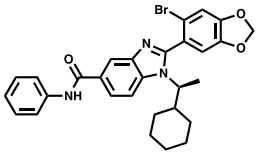
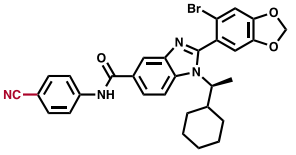
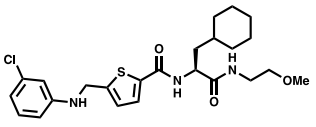
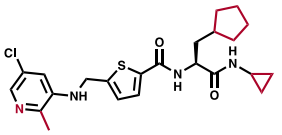
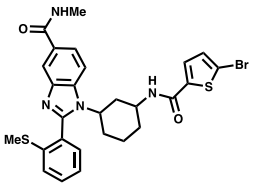
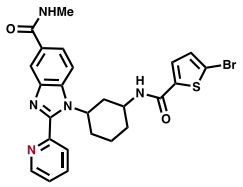
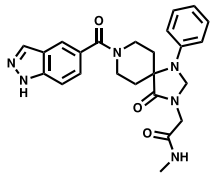
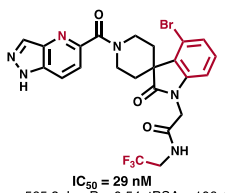
Table 1. Optimized Compounds Derived from DEL Screens

Entry	Target	DEL Hits	Optimized Structures	Status
1	RIPK1 GSK	 IC_{50} = 1.6 nM Mw = 377.4; cLogP = 1.83; tPSA = 80.23	 IC_{50} = 1.0 nM Mw = 377.4; cLogP = 0.81; tPSA = 95.39	Phase 2a
2	sEH GSK	 IC_{50} = 8 nM Mw = 517.6; cLogP = 0.04; tPSA = 139.57	 IC_{50} = 0.027 nM Mw = 447.2; cLogP = −0.22; tPSA = 114.03	Phase 2a
3	ENPP2 X-Chem	 IC_{50} = 86 nM Mw = 568.6; cLogP = 4.13; tPSA = 110.26	 IC_{50} = 55 nM Mw = 624.6; cLogP = 3.69; tPSA = 97.35	Phase 1
4	PAR2 AstraZeneca / X-Chem	 IC_{50} = 90 nM Mw = 546.5; cLogP = 7.23; tPSA = 63.16	 IC_{50} = 23 nM Mw = 571.5; cLogP = 6.67; tPSA = 86.95	Lead
5	Wip1 GSK	 IC_{50} = 13 nM Mw = 478.1; cLogP = 4.70; tPSA = 79.46	 IC_{50} = 6 nM Mw = 461; cLogP = 3.30; tPSA = 82.59	Lead
6	BCATm GSK	 IC_{50} = 251 nM Mw = 583.6; cLogP = 4.76; tPSA = 73.8	 IC_{50} = 13 nM Mw = 538.0; LogP = 2.92; tPSA = 86.16	Lead
7	DDR1 Roche	 IC_{50} = 1.5 μM Mw = 466.5; LogP = 0.79; tPSA = 97.35	 IC_{50} = 29 nM Mw = 565.3; LogP = 0.54; tPSA = 106.47	Lead

building blocks, in which only two of the chemical moieties were necessary for binding. The confirmed DEL hit displayed sufficient activity (IC_{50} = 1.4 nM), and only a few chemical modifications were performed in order to improve pharmacokinetic properties.[109] Analysis of medicinal chemistry activities from entry 2 in Table 1 revealed that, by eliminating certain chemical features, tuning the regiochemistry of the central core, and judicious substitutions in other parts of the molecule, the authors were able to strikingly increase the activity of the hit compound (IC_{50} = 0.027 nM) and reduce its molecular weight by almost 100 Da.[111] Recently, AstraZeneca has described the discovery of a potent protease-activated receptor-2 (PAR2) ligand from DEL campaigns. Rapid optimization of the hit compound (entry 4), by introduction

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of a nitrile group in the para-position, led to the isolation of a lead compound (IC_{50} = 23 nM) which acts as a negative allosteric modulator of PAR2.[112] Roche reported a novel and selective inhibitor (entry 7) against discoidin domain receptor 1 (DDR1) from two different library pools of 83 and 85 billion compounds, respectively. Structure-guided optimization of DEL-derived hits resulted in few modifications and the generation of an optimized lead compound with 50-fold IC_{50} improvement (IC_{50} = 29 nM).[113]

Direct applications of DEL technology also led to the development of small-molecule−drug conjugates (SMDCs) and small-molecule−radio conjugates (SMRCs) for oncology applications (treatment and imaging).[114−116] Philochem AG and ETH Zürich reported a high-affinity acetazolamide-derived ligand for carbonic anhydrase IX (CAIX), a well-known tumor-associated antigen overexpressed in renal cell carcinoma and hypoxic tumors.[58] The discovery of such a ligand led to the development of a 99mTc-radiolabeled ligand, which is currently in Phase I clinical trials for the imaging of clear renal cell carcinoma and hypoxia.[117]

CONCLUSION AND FUTURE CHALLENGES

The field of DELs is rapidly growing, and it has become a powerful technology platform for the discovery of ligands against targets of pharmaceutical interest. DELs are now being used as complements to traditional HTS in industry due to their cost-effective and time-efficient approaches, which facilitate ligand discovery and contribute to a better understanding of biological processes. Despite numerous success stories reported in the literature and four DEL-derived ligands in clinical trials, DEL occasionally fails to deliver hits to certain classes of protein targets, thus underlining the need for additional technical progress.[17,47,118−120]

Coverage of chemical space is one essential factor for the successful hit identification of drug-like molecules. Initial DELs reported in the literature were dominated by chemical structures of poor physicochemical properties and low chemical diversity, with a restricted toolbox of possible reactions.[35]

Over the past 5 years, significant efforts have gone into the development of robust DNA-compatible transformations, to circumvent the restrictions in the presence of the oligonucleotide tag.[35,36] These synthetic transformations, along with better structural complexity of available building blocks, significantly enhance the potential of identifying new hits against a broader selection of target proteins. Therefore, compounds with pharmaceutically interesting structural features are increasingly being represented in DELs.[108] New trends in the development of DNA-compatible reactions are focused on transition-metal-promoted reactions, radical-based reactions, and *in situ* heterocycle formations.[121]

Conventional screening strategies have some limitations due to possible conformational changes of the immobilized protein on solid support (e.g., streptavidin-coated beads or resin). Advances in the field include new strategies to perform selections in solution (e.g., IDPCR) and/or in their native environment. Interaction-dependent PCR techniques enabled screening in cell lysates for the selection of hits out of complex biological samples.[78] Nevertheless, covalent modifications of the protein target of interest, with DNA fragments, are required in order to stabilize and specifically amplify the real binders.[77,78] Proteins in live cells can interact with other cofactors or undergo post-translational modifications. Thus, the possibility to screen a protein target in its native environment has recently gained the attention of the DEL community. New approaches in screenings on live cells have already been demonstrated and have successfully identified ligands against targets of interest. These new methodologies represent future avenues of the DEL screening paradigm.[86−88]

Despite efforts in optimizing library synthesis and selections, efficient identification of ligands (e.g., in the micromolar range) from the background noise remains a crucial challenge in the field. In order to study how to efficiently decipher the sequencing output, researchers in academia have focused their efforts on studying the DNA integrity, which may influence the PCR outcome during the whole process.[118,119,122−124] One could assume that the number of counts for a particular compound identified after affinity selections can be correlated with the binding affinity, but it is important to understand all the possible variables that can determine the selection outcome, such as selection parameters, chemical yields, library size, library PCR amplifiability, sequencing depth, and sequencing errors. It is then fundamental to better shape statistical tools, which take into consideration all these parameters toward a higher success rate of DEL screening campaigns. In 2017, Satz et al. showed that libraries above 10^8 members lead to a higher rate of false negatives due mainly to the higher amount of truncates and byproducts during library synthesis.[118] More recently, Sannino et al. and Chen et al. concluded that a minimum of 10^4 copies per library member may be required in order to efficiently identify potential binders with dissociation constants below 10^{-6} M, which may be lost in the selection process.[125,126] Those aspects may be essential to consider if one is attempting to build up very large libraries (e.g., $>10^{11}$ library members).

The development of statistical methods for the evaluation and comparison of DEL experiments is likely to gain importance in the future. Neri and co-workers first described the use of the negative binomial distribution as a model for the analysis of the sequencing readouts after affinity selection experiments.[127] Further work on the statistical evaluation of selection suggests that the determination of the relative enrichment of sequence counts over the noise may rely on alternative data-processing solutions.[119,122−124] While most DEL selections are performed in solution, library construction and screening on beads brings certain attractive features. For instance, Paegel and co-workers first reported the use of the Poisson distribution for the calculation of the false negative rate.[122] In 2018, Kuai et al. from GSK reported similar results using the same model for the classical DEL screening platform.[119] In 2019, Faver et al. implemented an attractive *z*-score metric approach to determine the enrichment of compounds during DEL screening, which heeds the selection sampling bias.[123] More recently, Kómár and Kalinić have reported the use of machine learning (open source *Deldenoiser*) to empower the determination and discrimination of real potential binders from the background noise.[124] The fidelity of DNA sequencing has been inspected with algorithms to assess and integrate sequencing errors or PCR duplicates in analytical models, thus enhancing the detection, quantification, and informativeness.[128] Other methods aim to efficiently determine the chemical yields and estimate the binding affinity of ligands coming from DEL selection primary data.[129,130]

The emergence of "big data" and machine learning sets of tools presents a great opportunity to rationalize DEL approaches for efficient drug discovery programs toward

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innovative therapeutic applications.[131,132] Developments of chemical informatic tools have helped medicinal chemists to widen the chemical space coverage and improved the physical properties of the DELs. Martin et al. from Eli Lilly recently disclosed a new application called eDESIGNER.[133] This algorithm, which solves efficiently the properties of molecules upon pre-restrictions of heavy-atom distributions and overall molecular weight, allows for the generation of all the potential library designs, based on a rational selection of building blocks and subsequent on-DNA compatible transformations. The evaluation of subsets of compounds stemming from all the possible library designs enables researchers to classify libraries according to their diversity properties.

Nowadays, DEL selections datasets offer large and highly structured information, which constitutes a requisite for the implementation of machine learning. Thus, artificial intelligence (AI) using neural networks has demonstrated robust performance in molecular property prediction.[134−140] X-Chem and Google have recently applied the concept of machine learning to DEL selection data by identifying high-affinity compounds from large libraries of commercially and easily synthesizable molecules.[141]

Despite the efforts for developing algorithms for molecular generation, a remaining challenge is to assess the soundness of the generated molecules, due to the small amount of published data regarding their applications and evaluation. The selection of the appropriate algorithms will be a key parameter in the success of AI for the discovery of new medicines.[142]

In summary, DEL technology is becoming a mature platform and is now fully integrated in most pharmaceutical companies (by either internal programs or strategic partnerships) and in academia. Fast evolutions in machine learning and DEL technology constitute a promising momentum to address wider ranges of pharmaceutical challenges.

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Notes

The authors declare the following competing financial interest(s): D.N. is a cofounder and shareholder of Philogen (www.philogen.com), a Swiss-Italian biotech company that operates in the field of ligand-based pharmacodelivery. A.G.-M., E.J.D., and F.S. are employees of Philochem AG, the daughter company of Philogen, acting as discovery unit of the group.

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REFERENCES

(1) Hajduk, P. J., and Greer, J. (2007) A decade of fragment-based drug design: strategic advances and lessons learned. *Nat. Rev. Drug Discovery 6*, 211−219.

(2) Macarron, R., Banks, M. N., Bojanic, D., Burns, D. J., Cirovic, D. A., Garyantes, T., Green, D. V. S., Hertzberg, R. P., Janzen, W. P., Paslay, J. W., Schopfer, U., and Sittampalam, G. S. (2011) Impact of high-throughput screening in biomedical research. *Nat. Rev. Drug Discovery 10*, 188−195.

(3) Erlanson, D. A., Fesik, S. W., Hubbard, R. E., Jahnke, W., and Jhoti, H. (2016) Twenty years on: the impact of fragments on drug discovery. *Nat. Rev. Drug Discovery 15*, 605−619.

(4) Jorgensen, W. L. (2004) The Many Roles of Computation in Drug Discovery. *Science 303*, 1813−1818.

(5) Folmer, R. H. (2016) Integrating biophysics with HTS-driven drug discovery projects. *Drug Discovery Today 21*, 491−498.

(6) Lyu, J., Wang, S., Balius, T. E., Singh, I., Levit, A., Moroz, Y. S., O'Meara, M. J., Che, T., Algaa, E., Tolmachova, K., Tolmachev, A. A., Shoichet, B. K., Roth, B. L., and Irwin, J. J. (2019) Ultra-large library docking for discovering new chemotypes. *Nature 566*, 224−229.

(7) Lloyd, M. D. (2020) High-Throughput Screening for the Discovery of Enzyme Inhibitors. *J. Med. Chem. 63*, 10742−10772.

(8) Blay, V., Tolani, B., Ho, S. P., and Arkin, M. R. (2020) High-Throughput Screening: today's biochemical and cell-based approaches. *Drug Discovery Today 25*, 1807−1821.

(9) Wang, L., Wu, Y., Deng, Y., Kim, B., Pierce, L., Krilov, G., Lupyan, D., Robinson, S., Dahlgren, M. K., Greenwood, J., Romero, D. L., Masse, C., Knight, J. L., Steinbrecher, T., Beuming, T., Damm, W., Harder, E., Sherman, W., Brewer, M., Wester, R., Murcko, M., Frye, L., Farid, R., Lin, T., Mobley, D. L., Jorgensen, W. L., Berne, B. J., Friesner, R. A., and Abel, R. (2015) Accurate and Reliable Prediction of Relative Ligand Binding Potency in Prospective Drug Discovery by Way of a Modern Free-Energy Calculation Protocol and Force Field. *J. Am. Chem. Soc. 137*, 2695−2703.

(10) Lazo, J. S., and Sharlow, E. R. (2016) Drugging Undruggable Molecular Cancer Targets. *Annu. Rev. Pharmacol. Toxicol. 56*, 23−40.

(11) Dang, C. V., Reddy, E. P., Shokat, K. M., and Soucek, L. (2017) Drugging the 'undruggable' cancer targets. *Nat. Rev. Cancer 17*, 502−508.

(12) Smith, G. P. (1985) Filamentous Fusion Phage: Novel Expression Vectors That Display Cloned Antigens on the Virion Surface. *Science 228*, 1315−1317.

(13) Parmley, S. F., and Smith, G. P. (1988) Antibody-Selectable Filamentous Fd Phage Vectors: Affinity Purification of Target Genes. *Gene 73*, 305−318.

(14) Sioud, M. (2019) Phage Display Libraries: From Binders to Targeted Drug Delivery and Human Therapeutics. *Mol. Biotechnol. 61*, 286−303.

(15) Brenner, S., and Lerner, R. A. (1992) Encoded Combinatorial Chemistry. *Proc. Natl. Acad. Sci. U. S. A. 89*, 5381−5383.

(16) Lerner, R. A., and Neri, D. (2020) Reflections on DNA-encoded chemical libraries. *Biochem. Biophys. Res. Commun. 527*, 757−759.

(17) Neri, D., and Lerner, R. A. (2018) DNA-Encoded Chemical Libraries: A Selection System Based on Endowing Organic Compounds with Amplifiable Information. *Annu. Rev. Biochem. 87*, 479−502.

(18) Favalli, N., Bassi, G., Scheuermann, J., and Neri, D. (2018) DNA-encoded chemical libraries − achievements and remaining challenges. *FEBS Lett. 592*, 2168−2180.
(19) Dickson, P., and Kodadek, T. (2019) Chemical composition of DNA-encoded libraries, past present and future. *Org. Biomol. Chem. 17*, 4676−4688.
(20) Flood, D. T., Kingston, C., Vantourout, J. C., Dawson, P. E., and Baran, P. S. (2020) DNA Encoded Libraries: A Visitor's Guide. *Isr. J. Chem. 60*, 268−280.
(21) Halford, B. (2017) How DNA-Encoded Libraries Are Revolutionizing Drug Discovery. *Chem. Eng. News 95* (25), 28−33.
(22) Goodnow, R. A. J., Dumelin, C. E., and Keefe, A. D. (2017) DNA-Encoded Chemistry: Enabling the Deeper Sampling of Chemical Space. *Nat. Rev. Drug Discovery 16*, 131−147.
(23) Castañon, J., Román, J. P., Jessop, T. C., de Blas, J., and Haro, R. (2018) Design and Development of a Technology Platform for DNA-Encoded Library Production and Affinity Selection. *SLAS Discovery 23*, 387−396.
(24) Conole, D., Hunter, J. H., and Waring, M. J. (2020) The maturation of DNA encoded libraries: opportunities for new users. *Future Med. Chem. 13*, 173−191.
(25) Decurtins, W., Wichert, M., Franzini, R. M, Buller, F., Stravs, M. A, Zhang, Y., Neri, D., and Scheuermann, J. (2016) Automated Screening for Small Organic Ligands Using DNA-Encoded Chemical Libraries. *Nat. Protoc. 11*, 764−780.
(26) Satz, A. L., Cai, J., Chen, Y., Goodnow, R., Gruber, F., Kowalczyk, A., Petersen, A., Naderi-Oboodi, G., Orzechowski, L., and Strebel, Q. (2015) DNA Compatible Multistep Synthesis and Applications to DNA Encoded Libraries. *Bioconjugate Chem. 26*, 1623−1632.
(27) Malone, M. L., and Paegel, B. M. (2016) What is a "DNA-Compatible" Reaction? *ACS Comb. Sci. 18*, 182−187.
(28) Gironda-Martínez, A., Neri, D., Samain, F., and Donckele, E. J. (2019) DNA-Compatible Diazo-Transfer Reaction in Aqueous Media Suitable for DNA-Encoded Chemical Library Synthesis. *Org. Lett. 21*, 9555−9558.
(29) Dawadi, S., Simmons, N., Miklossy, G., Bohren, K. M., Faver, J. C., Ucisik, M. N., Nyshadham, P., Yu, Z., and Matzuk, M. M. (2020) Discovery of potent thrombin inhibitors from a protease-focused DNA-encoded chemical library. *Proc. Natl. Acad. Sci. U. S. A. 117*, 16782−16789.
(30) Wen, X., Duan, Z., Liu, J., Lu, W., and Lu, X. (2020) On-DNA Cross-Dehydrogenative Coupling Reaction toward the Synthesis of Focused DNA-Encoded Tetrahydroisoquinoline Libraries. *Org. Lett. 22*, 5721−5725.
(31) Chen, Y.-C., Faver, J. C., Ku, A. F., Miklossy, G., Riehle, K., Bohren, K. M., Ucisik, M. N., Matzuk, M. M., Yu, Z., and Simmons, N. (2020) C−N Coupling of DNA-Conjugated (Hetero)aryl Bromides and Chlorides for DNA-Encoded Chemical Library Synthesis. *Bioconjugate Chem. 31*, 770−780.
(32) Fan, Z., Zhao, S., Liu, T., Shen, P.-X., Cui, Z.-N., Zhuang, Z., Shao, Q., Chen, J. S., Ratnayake, A. S., Flanagan, M. E., Kolmel, D. K., Piotrowski, D. W., Richardson, P., and Yu, J.-Q. (2020) Merging C(sp3)−H activation with DNA-encoding. *Chem. Sci. 11*, 12282−12288.
(33) Ruff, Y., Martinez, R., Pellé, X., Nimsgern, P., Fille, P., Ratnikov, M., and Berst, F. (2020) An Amphiphilic Polymer-Supported Strategy Enables Chemical Transformations under Anhydrous Conditions for DNA-Encoded Library Synthesis. *ACS Comb. Sci. 22*, 120−128.
(34) Li, K., Liu, X., Liu, S., An, Y., Shen, Y., Sun, Q., Shi, X., Su, W., Cui, W., Duan, Z., Kuai, L., Yang, H., Satz, A. L., Chen, K., Jiang, H., Zheng, M., Peng, X., and Lu, X. (2020) Solution-Phase DNA-Compatible Pictet-Spengler Reaction Aided by Machine Learning Building Block Filtering. *iScience 23*, 101−142.
(35) Gotte, K., Chines, S., and Brunschweiger, A. (2020) Reaction development for DNA-encoded library technology: From evolution to revolution? *Tetrahedron Lett. 61*, 151889.
(36) Fitzgerald, P. R., and Paegel, B. M. (2020) DNA-Encoded Chemistry: Drug Discovery from a Few Good Reactions. *Chem. Rev.*, DOI: 10.1021/acs.chemrev.0c00789.
(37) Priego, J., de Pedro Beato, E., Benavides, J., Gironda-Martínez, A., González, F., Blas, J., Martín-Ortega, M. D., Rama-Garda, R., Ezquerra, J., Toledo, M. A., and Torrado, A. (2021) On-DNA Palladium-Catalyzed Hydrogenation-like Reaction Suitable for DNA-Encoded Library Synthesis. *Bioconjugate Chem. 32*, 88−93.
(38) Needels, M. C., Jones, D. G., Tate, E. H., Heinkel, G. L., Kochersperger, L. M., Dower, W. J., Barrett, R. W., and Gallop, M. A. (1993) Generation and Screening of an Oligonucleotide-Encoded Synthetic Peptide Library. *Proc. Natl. Acad. Sci. U. S. A. 90*, 10700−10704.
(39) Nielsen, J., Brenner, S., and Janda, K. D. (1993) Synthetic methods for the implementation of encoded combinatorial chemistry. *J. Am. Chem. Soc. 115*, 9812−9813.
(40) Kinoshita, Y., and Nishigaki, K. (1995) Enzymatic Synthesis of Code Regions for Encoded Combinatorial Chemistry (ECC). *Nucleic Acids Symp. Ser. 34*, 201−202.
(41) Gartner, Z. J., Tse, B. N., Grubina, R., Doyon, J. B., Snyder, T. M., and Liu, D. R. (2004) DNA-Templated Organic Synthesis and Selection of a Library of Macrocycles. *Science 305*, 1601−1605.
(42) Melkko, S., Scheuermann, J., Dumelin, C. E., and Neri, D. (2004) Encoded Self Assembling Chemical Libraries. *Nat. Biotechnol. 22*, 568−574.
(43) Mannocci, L., Zhang, Y., Scheuermann, J., Leimbacher, M., De Bellis, G., Rizzi, E., Dumelin, C. E., Melkko, S., and Neri, D. (2008) High-throughput sequencing allows the identification of binding molecules isolated from DNA-encoded chemical libraries. *Proc. Natl. Acad. Sci. U. S. A. 105*, 17670−17675.
(44) Halpin, D. R., and Harbury, P. B. (2004) DNA Display I. Sequence-Encoded Routing of DNA Populations. *PLoS Biol. 2*, e173.
(45) Halpin, D. R., and Harbury, P. B. (2004) DNA Display II. Genetic Manipulation of Combinatorial Chemistry Libraries for Small-Molecule Evolution. *PLoS Biol. 2*, e174.
(46) Halpin, D. R., Lee, J. A., Wrenn, S. J., and Harbury, P. B. (2004) DNA Display III. Solid-Phase Organic Synthesis on Unprotected DNA. *PLoS Biol. 2*, e175.
(47) Clark, M. A., Acharya, R. A., Arico-Muendel, C. C., Belyanskaya, S. L., Benjamin, D. R., Carlson, N. R., Centrella, P. A., Chiu, C. H., Creaser, S. P., Cuozzo, J. W., Davie, C. P., Ding, Y., Franklin, G. J., Franzen, K. D., Gefter, M. L., Hale, S. P., Hansen, N. J., Israel, D. I., Jiang, J., Kavarana, M. J., Kelley, M. S., Kollmann, C. S., Li, F., Lind, K., Mataruse, S., Medeiros, P. F., Messer, J. A., Myers, P., O'Keefe, H., Oliff, M. C., Rise, C. E., Satz, A. L., Skinner, S. R., Svendsen, J. L., Tang, L., van Vloten, K., Wagner, R. W., Yao, G., Zhao, B., and Morgan, B. A. (2009) Design, synthesis and selection of DNA-encoded small-molecule libraries. *Nat. Chem. Biol. 5*, 647−654.
(48) Furka, Á., Sebestyén, F., Asgedom, M., and Dibó, G. (1991) General method for rapid synthesis of multicomponent peptide mixtures. *Int. J. Pept. Protein Res. 37*, 487−493.
(49) Kowalczyk, A. (2014) *A Handbook for DNA-Encoded Chemistry: Theory and Applications for Exploring Chemical Space and Drug Discovery* (Goodnow, R. E., Ed.), Wiley-VCH, Weinheim, Germany, pp 1−18.
(50) Li, Y., De Luca, R., Cazzamalli, S., Pretto, F., Bajic, D., Scheuermann, J., and Neri, D. (2018) Versatile protein recognition by the encoded display of multiple chemical elements on a constant macrocyclic scaffold. *Nat. Chem. 10*, 441−448.
(51) Leimbacher, M., Zhang, Y., Mannocci, L., Stravs, M., Geppert, T., Scheuermann, J., Schneider, G., and Neri, D. (2012) Discovery of small molecule interleukin-2 inhibitors from a DNA-encoded chemical library. *Chem. - Eur. J. 18*, 7729−7737.
(52) Litovchick, A., Clark, M. A., and Keefe, A. D. (2014) Universal Strategies for the Dna-Encoding of Libraries of Small Molecules Using the Chemical Ligation of Oligonucleotide Tags. *Artif. DNA PNA XNA 5*, e27896.

(53) Li, Y., Zhao, P., Zhang, M., Zhao, X., and Li, X. (2013) Multistep DNA-Templated Synthesis Using a Universal Template. *J. Am. Chem. Soc. 135*, 17727−17730.
(54) Hansen, M. H., Blakskjaer, P., Petersen, L. K., Hansen, T. H., Højfeldt, J. W., Gothelf, K. V., and Vest Hansen, N. J. (2009) A yoctoliter-scale DNA reactor for small-molecule evolution. *J. Am. Chem. Soc. 131*, 1322−1327.
(55) Blakskjaer, P., Heitner, T., and Hansen, N. J. (2015) Fidelity by design: Yoctoreactor and binder trap enrichment for small-molecule DNA encoded libraries and drug discovery. *Curr. Opin. Chem. Biol. 26*, 62−71.
(56) Neri, D., and Melkko, S. (2002) *Encoded self-assembling chemical libraries*, Patent No. WO03/076943.
(57) Scheuermann, J., Dumelin, C. E., Melkko, S., Zhang, Y., Mannocci, L., Jaggi, M., Sobek, J., and Neri, D. (2008) DNA-Encoded Chemical Libraries for the Discovery of MMP-3 Inhibitors. *Bioconjugate Chem. 19*, 778−785.
(58) Wichert, M., Krall, N., Decurtins, W., Franzini, R. M., Pretto, F., Schneider, P., Neri, D., and Scheuermann, J. (2015) Dual-display of small molecules enables the discovery of ligand pairs and facilitates affinity maturation. *Nat. Chem. 7*, 241−249.
(59) Bigatti, M., Dal Corso, A., Vanetti, S., Cazzamalli, S., Rieder, U., Scheuermann, J., Neri, D., and Sladojevich, F. (2017) Impact of a Central Scaffold on the Binding Affinity of Fragment Pairs Isolated from DNA-Encoded Self-Assembling Chemical Libraries. *ChemMedChem 12*, 1748−1752.
(60) Zimmermann, G., Li, Y., Rieder, U., Mattarella, M., Neri, D., and Scheuermann, J. (2017) Hit-Validation Methodologies for Ligands Isolated from DNA-Encoded Chemical Libraries. *ChemBioChem 18*, 853−857.
(61) Prati, L., Bigatti, M., Donckele, E. J., Neri, D., and Samain, F. (2020) On-DNA hit validation methodologies for ligands identified from DNA-encoded chemical libraries. *Biochem. Biophys. Res. Commun. 533*, 235−240.
(62) Reddavide, F. V., Lin, W., Lehnert, S., and Zhang, Y. (2015) DNA-Encoded Dynamic Combinatorial Chemical Libraries. *Angew. Chem., Int. Ed. 54*, 7924−7928.
(63) Zhou, Y., Li, C., Peng, J., Xie, L., Meng, L., Li, Q., Zhang, J., Li, X. D., Li, X., Huang, X., and Li, X. (2018) DNA-Encoded Dynamic Chemical Library and Its Applications in Ligand Discovery. *J. Am. Chem. Soc. 140*, 15859−15867.
(64) Reddavide, F. V., Cui, M., Lin, W., Fu, N., Heiden, S., Andrade, H., Thompson, M., and Zhang, Y. (2019) Second generation DNA-encoded dynamic combinatorial chemical libraries. *Chem. Commun. 55*, 3753−3756.
(65) Zambaldo, C., Barluenga, S., and Winssinger, N. (2015) PNA-encoded chemical libraries. *Curr. Opin. Chem. Biol. 26*, 8−15.
(66) Gorska, K., Huang, K. T., Chaloin, O., and Winssinger, N. (2009) DNA-templated homo- and heterodimerization of peptide nucleic acid encoded oligosaccharides that mimic the carbohydrate epitope of HIV. *Angew. Chem., Int. Ed. 48*, 7695−7700.
(67) Chan, A. I., McGregor, L. M., and Liu, D. R. (2015) Novel selection methods for DNA-encoded chemical libraries. *Curr. Opin. Chem. Biol. 26*, 55−61.
(68) Arnau, J., Lauritzen, C., Petersen, G. E., and Pedersen, J. (2006) Current strategies for the use of affinity tags and tag removal for the purification of recombinant proteins. *Protein Expression Purif. 48*, 1−13.
(69) Kimple, M. E., Brill, A. L., and Pasker, R. L. (2013) Overview of affinity tags for protein purification. *Curr. Protoc. Protein. Sci. 73*, 9.9.1−9.9.23.
(70) Chapman-Smith, A., and Cronan, J. E., Jr. (1999) The enzymatic biotinylation of proteins: a post-translational modification of exceptional specificity. *Trends Biochem. Sci. 24*, 359−363.
(71) Cull, M. G., and Schatz, P. J. (2000) Biotinylation of proteins in vivo and in vitro using small peptide tags. *Methods Enzymol. 326*, 430−440.
(72) Spriestersbach, A., Kubicek, J., Schäfer, F., Block, H., and Maertens, B. (2015) Purification of His-Tagged Proteins. *Methods Enzymol. 559*, 1−15.
(73) Block, H., Maertens, B., Spriestersbach, A., Brinker, N., Kubicek, J., Fabis, R., Labahn, J., and Schäfer, F. (2009) Immobilized-metal affinity chromatography (IMAC): a review. *Methods Enzymol. 463*, 439−473.
(74) Wong, L. S., Khan, F., and Micklefield, J. (2009) Selective covalent protein immobilization: strategies and applications. *Chem. Rev. 109*, 4025−4053.
(75) Rusmini, F., Zhong, Z., and Feijen, J. (2007) Protein immobilization strategies for protein biochips. *Biomacromolecules 8*, 1775−1789.
(76) Dumelin, C. E., Scheuermann, J., Melkko, S., and Neri, D. (2006) Selection of Streptavidin Binders from a DNA-Encoded Chemical Library. *Bioconjugate Chem. 17*, 366−370.
(77) McGregor, L. M., Gorin, D. J., Dumelin, C. E., and Liu, D. R. (2010) Interaction-dependent PCR: identification of ligand-target pairs from libraries of ligands and libraries of targets in a single solution-phase experiment. *J. Am. Chem. Soc. 132*, 15522−15524.
(78) McGregor, L. M., Jain, T., and Liu, D. R. (2014) Identification of ligand-target pairs from combined libraries of small molecules and unpurified protein targets in cell lysates. *J. Am. Chem. Soc. 136*, 3264−3270.
(79) Chan, A. I., McGregor, L. M., Jain, T., and Liu, D. R. (2017) Discovery of a Covalent Kinase Inhibitor from a DNA-Encoded Small-Molecule Library × Protein Library Selection. *J. Am. Chem. Soc. 139*, 10192−10195.
(80) Blakskjaer, P., Heitner, T., and Hansen, N. J. (2015) Fidelity by design: Yoctoreactor and binder trap enrichment for small-molecule DNA encoded libraries and drug discovery. *Curr. Opin. Chem. Biol. 26*, 62−71.
(81) Zhao, P., Chen, Z., Li, Y., Sun, D., Gao, Y., Huang, Y., and Li, X. (2014) Selection of DNA-Encoded Small Molecule Libraries Against Unmodified and Non-Immobilized Protein Targets. *Angew. Chem., Int. Ed. 53*, 10056−10059.
(82) Li, G., Liu, Y., Yu, X., and Li, X. (2014) Multivalent Photoaffinity Probe for Labeling Small Molecule Binding Protein. *Bioconjugate Chem. 25*, 1172−1180.
(83) Li, G., Liu, Y., Liu, Y., Chen, L., Wu, S., Liu, Y., and Li, X. (2013) Photoaffinity Labeling of Small-Molecule-Binding Proteins by DNA-Templated Chemistry. *Angew. Chem., Int. Ed. 52*, 9544−9549.
(84) Sannino, A., Gironda-Martínez, A., Gorre, É. D., Prati, L., Piazzi, J., Scheuermann, J., Neri, D., Donckele, E. J., and Samain, F. (2020) Critical Evaluation of Photo-cross-linking Parameters for Efficient DNA-Encoded Chemical Library Selections. *ACS Comb. Sci. 22*, 204−212.
(85) Wu, Z., Graybill, T. L., Zeng, X., Platchek, M., Zhang, J., Bodmer, V. Q., Wisnoski, D. D., Deng, J., Coppo, F. T., Yao, G., Tamburino, A., Scavello, G., Franklin, G. J., Mataruse, S., Bedard, K. L., Ding, Y., Chai, J., Summerfield, J., Centrella, P. A., Messer, J. A., Pope, A. J., and Israel, D. I. (2015) Cell-based selection expands the utility of DNA-encoded small-molecule library technology to cell surface drug targets: identification of novel antagonists of the NK3 tachykinin receptor. *ACS Comb. Sci. 17*, 722−731.
(86) Cai, B., Kim, D., Akhand, S., Sun, Y., Cassell, R. J., Alpsoy, A., Dykhuizen, E. C., Van Rijn, R. M., Wendt, M. K., and Krusemark, C. J. (2019) Selection of DNA-Encoded Libraries to Protein Targets within and on Living Cells. *J. Am. Chem. Soc. 141*, 17057−17061.
(87) Huang, Y., Meng, L., Nie, Q., Zhou, Y., Chen, L., Yang, S., Fung, Y. M. E., Li, X., Huang, C., Cao, Y., Li, Y., and Li, X. (2021) Selection of DNA-encoded chemical libraries against endogenous membrane proteins on live cells. *Nat. Chem. 13*, 77−88.
(88) Petersen, L. K., Christensen, A. B., Andersen, J., Folkesson, C. G., Kristensen, O., Andersen, C., Alzu, A., Sløk, F. A., Blakskjær, P., Madsen, D., Azevedo, C., Micco, I., and Hansen, N. J. V. (2021) Screening of DNA-Encoded Small Molecule Libraries inside a Living Cell. *J. Am. Chem. Soc. 143*, 2751−2756.

https://doi.org/10.1021/acsptsci.1c00118
ACS Pharmacol. Transl. Sci. 2021, 4, 1265−1279

(89) Franzini, R. M., and Randolph, C. (2016) Chemical space of DNA-encoded libraries. *J. Med. Chem. 59*, 6629−6644.
(90) Samain, F., Ekblad, T., Mikutis, G., Zhong, N., Zimmermann, M., Nauer, A., Bajic, D., Decurtins, W., Scheuermann, J., Brown, P. J., Hall, J., Gräslund, S., Schuler, H., Neri, D., and Franzini, R. M. (2015) Tankyrase I inhibitors with drug-like properties identified by screening a DNA-encoded chemical library. *J. Med. Chem. 58*, 5143−5149.
(91) Ding, Y., O'Keefe, H., DeLorey, J. L., Israel, D. I., Messer, J. A., Chiu, C. H., Skinner, S. R., Matico, R. E., Murray-Thompson, M. F., Li, F., Clark, M. A., Cuozzo, J. W., Arico-Muendel, C., and Morgan, B. A. (2015) Discovery of potent and selective inhibitors for ADAMTS-4 through DNA-encoded library technology (ELT). *ACS Med. Chem. Lett. 6*, 888−893.
(92) Petersen, L. K., Blakskjær, P., Chaikuad, A., Christensen, A. B., Dietvorst, J., Holmkvist, J., Knapp, S., Korinek, M., Larsen, L. K., Pedersen, A. E., Rohm, S., Sløk, F. A., and Hansen, N. J. V. (2016) Novel p38α MAP kinase inhibitors identified from yoctoReactor DNA-encoded small molecule library. *MedChemComm 7*, 1332−1339.
(93) Dougherty, P. G., Qian, Z., and Pei, D. (2017) Macrocycles as Protein-Protein Interaction Inhibitors. *Biochem. J. 474*, 1109−1125.
(94) Usanov, D. L., Chan, A. L., Maianti, J. P., and Liu, D. R. (2018) Second-Generation DNA-Templated Macrocycle Libraries for the Discovery of Bioactive Small Molecules. *Nat. Chem. 10*, 704−714.
(95) Wood, E. R., Bledsoe, R., Chai, J., Daka, P., Deng, H., Ding, Y., Harris-Gurley, S., Kryn, L. H., Nartey, E., Nichols, J., Nolte, R. T., Prabhu, N., Rise, C., Sheahan, T., Shotwell, J. B., Smith, D., Tai, V., Taylor, J. D., Tomberlin, G., Wang, L., Wisely, B., You, S., Xia, B., and Dickson, H. (2015) The role of phosphodiesterase 12 (PDE12) as a negative regulator of the innate immune response and the discovery of antiviral inhibitors. *J. Biol. Chem. 290*, 19681−19696.
(96) Gilmartin, A. G., Faitg, T. H., Richter, M., Groy, A., Seefeld, M. A., Darcy, M. G., Peng, X., Federowicz, K., Yang, J., Zhang, S. Y., Minthorn, E., Jaworski, J. P., Schaber, M., Martens, S., McNulty, D. E., Sinnamon, R. H., Zhang, H., Kirkpatrick, R. B., Nevins, N., Cui, G., Pietrak, B., Diaz, E., Jones, A., Brandt, M., Schwartz, B., Heerding, D. A., and Kumar, R. (2014) Allosteric Wip1 phosphatase inhibition through flap- subdomain interaction. *Nat. Chem. Biol. 10*, 181−187.
(97) Taylor, D. M., Anglin, J., Park, S., Ucisik, M. N., Faver, J. C., Simmons, N., Jin, Z., Palaniappan, M., Nyshadham, P., Li, F., Campbell, J., Hu, L., Sankaran, B., Prasad, B. V. V., Huang, H., Matzuk, M. M., and Palzkill, T. (2020) Identifying Oxacillinase-48 Carbapenemase Inhibitors Using DNA-Encoded Chemical Libraries. *ACS Infect. Dis. 6*, 1214−1227.
(98) Ahn, S., Pani, B., Kahsai, A. W., Olsen, E. K., Husemoen, G., Vestergaard, M., Jin, L., Zhao, S., Wingler, L. M., Rambarat, P. K., Simhal, R. K., Xu, T. T., Sun, L. D., Shim, P. J., Staus, D. P., Huang, L.-Y., Franch, T., Chen, X., and Lefkowitz, R. J. (2018) Small-Molecule Positive Allosteric Modulators of the β2-Adrenoceptor Isolated from DNA-Encoded Libraries. *Mol. Pharmacol. 94*, 850−861.
(99) Ye, N., Chen, C. H., Chen, T., Song, Z., He, J. X., Huan, X. J., Song, S. S., Liu, Q., Chen, Y., Ding, J., Xu, Y., Miao, Z. H., and Zhang, A. (2013) Design, synthesis, and biological evaluation of a series of benzo[de]- [1,7]naphthyridin-7(8H)-ones bearing a functionalized longer chain appendage as novel PARP1 inhibitors. *J. Med. Chem. 56*, 2885−2903.
(100) Dawadi, S., Simmons, N., Miklossy, G., Bohren, K. M., Faver, J. C., Ucisik, M. N., Nyshadham, P., Yu, Z., and Matzuk, M. M. (2020) Discovery of potent thrombin inhibitors from a protease-focused DNA-encoded chemical library. *Proc. Natl. Acad. Sci. U. S. A. 117*, 16782−16789.
(101) Cuozzo, J. W., Clark, M. A., Keefe, A. D., Kohlmann, A., Mulvihill, M., Ni, H., Renzetti, L. M., Resnicow, D. I., Ruebsam, F., Sigel, E. A., Thomson, H. A., Wang, C., Xie, Z., and Zhang, Y. (2020) Novel Autotaxin Inhibitor for the Treatment of Idiopathic Pulmonary Fibrosis: A Clinical Candidate Discovered Using DNA-Encoded Chemistry. *J. Med. Chem. 63*, 7840−7856.
(102) Kunig, V. B. K., Potowski, M., Akbarzadeh, M., Škopić, M. K., Smith, D. D. S., Arendt, L., Dormuth, I., Adihou, H., Andlovic, B., Karatas, H., Shaabani, S., Zarganes-Tzitzikas, T., Neochoritis, C. G., Zhang, R., Groves, M., Guéret, S. M., Ottmann, C., Rahnenführer, J., Fried, R., Dömling, A., and Brunschweiger, A. (2020) TEAD−YAP Interaction Inhibitors and MDM2 Binders from DNA-Encoded Indole-Focused Ugi Peptidomimetics. *Angew. Chem., Int. Ed. 59*, 20338−20342.
(103) Shin, M. H., Lee, K. J., and Lim, H.-S. (2019) DNA-Encoded Combinatorial Library of Macrocyclic Peptoids. *Bioconjugate Chem. 30*, 2931−2938.
(104) Bassi, G., Favalli, N., Vuk, M., Catalano, M., Martinelli, A., Trenner, A., Porro, A., Yang, S., Tham, C. L., Moroglu, M., Yue, W. W., Conway, S. J., Vogt, P. K., Sartori, A. A., Scheuermann, J., and Neri, D. (2020) A Single-Stranded DNA-Encoded Chemical Library Based on a Stereoisomeric Scaffold Enables Ligand Discovery by Modular Assembly of Building Blocks. *Adv. Science 7*, 2001970.
(105) Stress, C. J., Sauter, B., Schneider, L., Sharpe, T., and Gillingham, D. G. (2019) A DNA-encoded library incorporating elements of natural macrocycles. *Angew. Chem., Int. Ed. 58*, 9570−9574.
(106) Onda, Y., Bassi, G., Elsayed, A., Ulrich, F., Oehler, S., Plais, L., Scheuermann, J., and Neri, D. (2021) A DNA-encoded chemical library based on peptide macrocycles. *Chem. - Eur. J. 27*, 7160.
(107) Koesema, E., Roy, A., Paciaroni, N. G., and Kodadek, T. (2020) Synthesis and Screening of A DNA-Encoded Library of Non-Peptidic Macrocycles. *ChemRxiv Preprint*, DOI: 10.26434/chemrxiv.13490151.v1.
(108) Madsen, D., Azevedo, C., Micco, I., Petersen, L. K., and Hansen, N. J. V. (2020) An overview of DNA-encoded libraries: A versatile tool for drug discovery. *Prog. Med. Chem. 59*, 181−249.
(109) Harris, P. A., Berger, S. B., Jeong, J. U., Nagilla, R., Bandyopadhyay, D., Campobasso, N., Capriotti, C. A., Cox, J. A., Dare, L., Dong, X., Eidam, P. M., Finger, J. N., Hoffman, S. J., Kang, J., Kasparcova, V., King, B. W., Lehr, R., Lan, Y., Leister, L. K., Lich, J. D., MacDonald, T. T., Miller, N. A., Ouellette, M. T., Pao, C. S., Rahman, A., Reilly, M. A., Rendina, A. R., Rivera, E. J., Schaeffer, M. C., Sehon, C. A., Singhaus, R. R., Sun, H. H., Swift, B. A., Totoritis, R. D., Vossenkamper, A., Ward, P., Wisnoski, D. D., Zhang, D., Marquis, R. W., Gough, P. J., and Bertin, J. (2017) Discovery of a First-in-Class Receptor Interacting Protein 1 (RIP1) Kinase Specific Clinical Candidate (GSK2982772) for the Treatment of Inflammatory Diseases. *J. Med. Chem. 60*, 1247−1261.
(110) Zhu, Z., Shaginian, A., Grady, L. C., O'Keeffe, T., Shi, X. E., Davie, C. P., Simpson, G. L., Messer, J. A., Evindar, G., Bream, R. N., Thansandote, P. P., Prentice, N. R., Mason, A. M., and Pal, S. (2018) Design and Application of a DNA-Encoded Macrocyclic Peptide Library. *ACS Chem. Biol. 13*, 53−59.
(111) Belyanskaya, S. L., Ding, Y., Callahan, J. F., Lazaar, A. L., and Israel, D. I. (2017) Discovering Drugs with DNA-Encoded Library Technology: From Concept to Clinic with an Inhibitor of Soluble Epoxide Hydrolase. *ChemBioChem 18*, 837−842.
(112) Kennedy, A. J., Sundstrom, L., Geschwindner, S., Poon, E. K. Y., Jiang, Y., Chen, R., Cooke, R., Johnstone, S., Madin, A., Lim, J., Liu, Q., Lohman, R.-J., Nordqvist, A., Friden-Saxin, M., Yang, W., Brown, D. G., Fairlie, D. P., and Dekker, N. (2020) Protease-activated receptor-2 ligand reveal orthosteric and allosteric mechanisms of receptor inhibition. *Commun. Biol. 3*, 782.
(113) Richter, H., Satz, A. L., Bedoucha, M., Buettelmann, B., Petersen, A. C., Harmeier, A., Hermosilla, R., Hochstrasser, R., Burger, D., Gsell, B., Gasser, R., Huber, S., Hug, M. N., Kocer, B., Kuhn, B., Ritter, M., Rudolph, M. G., Weibel, F., Molina-David, J., Kim, J.-J., Santos, J. V., Stihle, M., Georges, G. J., Bonfil, R. D., Fridman, R., Uhles, S., Moll, S., Faul, C., Fornoni, A., and Prunotto, M. (2019) DNA-Encoded Library-Derived DDR1 Inhibitor Prevents Fibrosis and Renal Function Loss in a Genetic Mouse Model of Alport Syndrome. *ACS Chem. Biol. 14*, 37−49.
(114) Cazzamalli, S., Dal Corso, A., and Neri, D. (2016) Acetazolamide Serves as Selective Delivery Vehicle for Dipeptide-Linked Drugs to Renal Cell Carcinoma. *Mol. Cancer Ther. 15*, 2926−2935.

(115) Cazzamalli, C., Ziffels, B., Widmayer, F., Murer, P., Pellegrini, G., Pretto, F., Wulhfard, S., and Neri, D. (2018) Enhanced Therapeutic Activity of Non-Internalizing Small-Molecule-Drug Conjugates Targeting Carbonic Anhydrase IX in Combination with Targeted Interleukin-2. *Clin. Cancer Res. 24*, 3656−3667.
(116) Cazzamalli, S., Dal Corso, A., Widmayer, F., and Neri, D. (2018) Chemically Defined Antibody− and Small Molecule−Drug Conjugates for in Vivo Tumor Targeting Applications: A Comparative Analysis. *J. Am. Chem. Soc. 140*, 1617−1621.
(117) Kulterer, O. C., Pfaff, S., Wadsak, W., Garstka, N., Remzi, M., Vraka, C., Nics, L., Mitterhauser, M., Bootz, F., Cazzamalli, S., Krall, N., Neri, D., and Haug, A. R. (2021) A microdosing study with 99m Tc-PHC-102 for the SPECT/CT imaging of primary and metastatic lesions in renal cell carcinoma patients. *J. Nucl. Med. 62*, 360−365.
(118) Satz, A. L., Hochstrasser, R., and Petersen, A. C. (2017) Analysis of Current DNA Encoded Library Screening Data Indicates Higher False Negative Rates for Numerically Larger Libraries. *ACS Comb. Sci. 19*, 234−238.
(119) Kuai, L., O'Keeffe, T., and Arico-Muendel, C. (2018) Randomness in DNA Encoded Library Selection Data Can Be Modeled for More Reliable Enrichment Calculation. *SLAS Discovery 23*, 405−416.
(120) Satz, A. L. (2018) What Do You Get from DNA-Encoded Libraries? *ACS Med. Chem. Lett. 9*, 408−410.
(121) Song, M., and Hwang, G. T. (2020) DNA-Encoded Library Screening as Core Platform Technology in Drug Discovery: Its Synthetic Method Development and Applications in DEL Synthesis. *J. Med. Chem. 63*, 6578−6599.
(122) MacConnell, A., and Paegel, B. M. (2017) Poisson Statistics of Combinatorial Library Sampling Predict False Discovery Rates of Screening. *ACS Comb. Sci. 19*, 524−532.
(123) Faver, J. C., Riehle, K., Lancia, D. R., Jr., Milbank, J. B. J., Kollmann, C. S., Simmons, N., Yu, Z., and Matzuk, M. M. (2019) Quantitative Comparison of Enrichment from DNA-Encoded Chemical Library Selection. *ACS Comb. Sci. 21*, 75−82.
(124) Kómár, P., and Kalinić, M. (2020) Denoising DNA Encoded Library Screens with Sparse Learning. *ACS Comb. Sci. 22*, 410−421.
(125) Sannino, A., Gabriele, E., Bigatti, M., Mulatto, S., Piazzi, J., Scheuermann, J., Neri, D., Donckele, E. J., and Samain, F. (2019) Quantitative Assessment of Affinity Selection Performance by Using DNA-Encoded Chemical Libraries. *ChemBioChem 20*, 955−962.
(126) Chen, Q., Cheng, X., Zhang, L., Li, X., Chen, P., Liu, J., Zhang, L., Wei, H., Li, Z., and Dou, D. (2020) Exploring the Lower Limit of Individual DNA-Encoded Library Molecules in Selection. *SLAS Discovery 25*, 523−529.
(127) Buller, F., Zhang, Y., Scheuermann, J., Schäfer, J., Bühlmann, P., and Neri, D. (2009) Discovery of TNF Inhibitors from a DNA-Encoded Chemical Library based on Diels-Alder Cycloaddition. *Chem. Biol. 16*, 1075−1086.
(128) Amigo, J., Rama-Garda, R., Bello, X., Sobrino, B., de Blas, J., Martín-Ortega, M., Jessop, T. C., Carracedo, A., García Losa, M. I., and Domínguez, E. (2018) tagFinder: A Novel Tag Analysis Methodology That Enables Detection of Molecules from DNA-Encoded Chemical Libraries. *SLAS Discovery 23*, 397−404.
(129) Hall, J., Foley, T. L., Chen, Q., Israel, D. I., Xu, Y., Ford, K. K., Xie, P., Fan, J., and Wan, J. (2020) A simple method for determining compound affinity and chemical yield from DNA-Encoded library selection. *Biochem. Biophys. Res. Commun. 527*, 250−256.
(130) Chen, Q., Hall, J., Foley, T. L., Wan, J., Li, Y., and Israel, D. I. (2020) A method for estimating affinity from primary DEL selection data. *Biochem. Biophys. Res. Commun. 533*, 249−255.
(131) Brown, N., Cambruzzi, J., Cox, P. J., Davies, M., Dunbar, J., Plumbley, D., Sellwood, M. A., Sim, A., Williams-Jones, B. I., Zwierzyna, M., and Sheppard, D. W. (2018) Big Data in Drug Discovery. *Prog. Med. Chem. 57*, 277−356.
(132) Lo, Y.-C., Rensi, S. E., Torng, W., and Altman, R. B. (2018) Machine learning in chemoinformatics and drug discovery. *Drug Discovery Today 23*, 1538−1546.
(133) Martin, A., Nicolaou, C. A., and Toledo, M. A. (2020) Navigating the DNA encoded libraries chemical space. *Commun. Chem. 3*, 127.
(134) Smith, J. S., Isayev, O., and Roitberg, A. E. (2017) ANI-1: an Extensible Neural Network Potential with DFT Accuracy at Force Field Computational Cost. *Chem. Sci. 8*, 3192−3203.
(135) Schütt, K. T., Sauceda, H. E., Kindermans, P.-J., Tkatchenko, A., and Müller, K.-R. (2018) SchNet - A Deep Learning Architecture for Molecules and Materials. *J. Chem. Phys. 148*, 241722.
(136) Li, X., Yan, X., Gu, Q., Zhou, H., Wu, D., and Xu, J. (2019) DeepChemStable: Chemical Stability Prediction with an Attention-Based Graph Convolution Network. *J. Chem. Inf. Model. 59*, 1044−1049.
(137) Wu, Z., Ramsundar, B., Feinberg, E. N., Gomes, J., Geniesse, C., Pappu, A. S., Leswing, K., and Pande, V. (2018) MoleculeNet: a Benchmark for Molecular Machine Learning. *Chem. Sci. 9*, 513−530.
(138) Lenselink, E. B., Ten Dijke, N., Bongers, B., Papadatos, G., van Vlijmen, H. W. T., Kowalczyk, W., IJzerman, A. P., and van Westen, G. J. P. (2017) Beyond the Hype: Deep Neural Networks Outperform Established Methods Using a ChEMBL Bioactivity Benchmark Set. *J. Cheminf. 9*, 45.
(139) Kearnes, S., McCloskey, K., Berndl, M., Pande, V., and Riley, P. (2016) Molecular Graph Convolutions: Moving Beyond Fingerprints. *J. Comput.-Aided Mol. Des. 30*, 595−608.
(140) Ma, J., Sheridan, R. P., Liaw, A., Dahl, G. E., and Svetnik, V. (2015) Deep Neural Nets as a Method for Quantitative Structure−Activity Relationships. *J. Chem. Inf. Model. 55*, 263−274.
(141) McCloskey, K., Sigel, E. A., Kearnes, S., Xue, L., Tian, X., Moccia, D., Gikunju, D., Bazzaz, S., Chan, B., Clark, M. A., Cuozzo, J. W., Guié, M.-A., Guilinger, J. P., Huguet, C., Hupp, C. D., Keefe, A. D., Mulhern, C. J., Zhang, Y., and Riley, P. (2020) Machine Learning on DNA-Encoded Libraries: A New Paradigm for Hit Finding. *J. Med. Chem. 63*, 8857−8866.
(142) Bush, J. T., Pogany, P., Pickett, S. D., Barker, M., Baxter, A., Campos, S., Cooper, A. W. J., Hirst, D., Inglis, G., Nadin, A., Patel, V. K., Poole, D., Pritchard, J., Washio, Y., White, G., and Green, D. V. S. (2020) A Turing Test for Molecular Generators. *J. Med. Chem. 63*, 11964−1197.

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