

DNA Encoded Libraries: A Visitor's Guide

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Abstract: In 1992, Brenner and Lerner hypothesized that individual chemical transformations could be encoded in DNA, allowing the rapid synthesis and screening of large collections of small molecules. Since their report, huge investments into the development of the DNA encoded library (DEL) technology have enabled the acceleration of the drug discovery process especially early phase discovery undertakings such as target validation and hit identification. As DEL lies at the nexus between chemistry and biology, there is an increasing need to expand the toolboxes of both

organic transformations and biological methods. However, the myriad of techniques and reactions already reported can be difficult to digest for practitioners whose expertise resides outside the realm of DEL. This review therefore focuses on a stepwise presentation of DEL from the basic concepts to newest developments. The presentation includes the history, fundamentals, and successes of DEL, different methods for DEL synthesis and affinity selection, the conventional transformations, and finally the latest developments from a synthetic organic perspective.

Keywords: Guide • DNA • Encoded • Libraries • HTS • Chemistry • Biology • Drug discovery • Binding

1. Conceptualization and Early Development

Identification of small molecule chemical ligands that bind to protein targets is often a fundamental early step in the process of drug discovery (Figure 1A).^[1–3] Despite great advances in computational methods, the ability to quantitatively predict ligand binding to a biological target in the absence of structural information is underdeveloped.^[4,5] Thus, a typical workflow involves high-throughput screening (HTS) of large libraries of chemical compounds (typically >1 million) against the target protein. Building and employing such a large chemical library requires that each individual compound is made, stored, and assayed in an automated resource intensive facility. Hence, it is not possible to mix all the compounds with the protein of interest and deconvolute the binders. Instead, each ligand must be tested separately in one-by-one fashion at a very significant cost.

The technique of phage display (Figure 1B) is an important tool for the identification of protein-protein interactions.^[6–15] The process begins with insertion of a DNA sequence that encodes a particular protein into the coat protein gene of a bacteriophage. The protein is expressed on the surface of the bacteriophage. Since a large library of DNA sequences are available, or can be evolved using the phage's biological machinery, a huge library of proteins can be easily constructed. In contrast to HTS, this massive library of protein ligands can be tested for binding to a protein target simultaneously in the same vessel. This process is known as affinity selection and most commonly consists of mixing the library with an immobilized target protein, incubation, then non-binders are washed away. The phage bearing the successful protein ligand is cleaved and the DNA that encodes for the protein is extracted. In contrast to the chemical compounds screened in HTS, the successful ligand can be characterized through amplification and sequencing of the encoding DNA.

The development of phage display in 1985 may have led to Brenner and Lerner's thought experiment in 1992: combine the diversity of chemical compounds with the power of genetic encoding to overcome the limitations of HTS and provide a more powerful and versatile method for drug discovery (Figure 1C).^[16] Their initial proposal consisted of the synthesis of polypeptide ligands on beads in a combinatorial split-and-pool approach. Each building block (amino acid in this case) added to the ligand moiety would be encoded by addition of a DNA oligo to a growing strand of DNA. As in phage display, the DNA encodes the ligand and enables amplification of a small number of binders after affinity selection thereby facilitating the development of massive chemical libraries. At the time of their disclosure, they presciently recognized a significant challenge would be the development of DNA compatible synthetic methods.^[16] The vision of DEL was realized shortly thereafter by the groups of Janda and Gallop who developed peptide libraries on beads. Janda synthesized peptidic DELs by appending a bifunctional linker to a CPG bead and sequentially chain assembling the peptide and chemically ligating the DNA barcoding the encoding molecule.^[17] Shortly after, Gallop reported the synthesis of a ~10⁶ member encoded peptide library which they screened against fluorescent antibodies.^[18] The fluorescent beads were sorted using fluorescence activated cell sorting (FACS), and the binder was PCR amplified. These examples provided the first real world DEL synthesis and applications.

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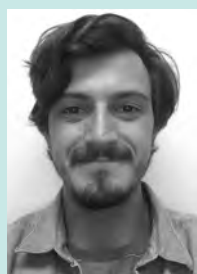
Although the groundwork for DEL was laid in the 1990's, the field sat dormant until the mid 2000's. During this time a flurry of reports emerged from various labs around the world presenting new methods of constructing, screening and evaluating DELs. In 2004 the concept of self-assembling encoded libraries, DNA templated synthesis, and chemical evolution were introduced by the groups of Neri, Liu, and Harbury respectively.^[4,5,19–23] This period of time also saw the first implementation of DELs in their current form. At this point the binders could be selected from complex mixtures, PCR amplified, and decoded to reveal the identity of the binding molecules. In 2009, Morgan and coworkers demonstrated that the DNA barcodes could be enzymatically ligated to the growing encoding chain, bringing DELs into its current form.^[24] This period of intense development coalesced into the current DEL paradigm.

The typical DEL is assembled (Figure 1C) through a series of DNA encodings followed by chemical diversification (although these steps can be inverted).^[11,15,25–27] Usually, the DEL starts as a small DNA (single or double stranded) structure, referred to as a headpiece, linked to a free amine group, or other functional handle, by a spacer.^[27,28] A stock solution of the DNA headpiece is aliquoted into individual wells of a 96-well plate along with

differing strands of DNA that act as barcodes for the identity of the individual building block employed. The barcode is enzymatically ligated to the DNA headpiece, and the tagged headpiece is isolated from the ligation mixture by ethanol precipitation. The dry elongated headpiece is then dissolved in the reaction solution and the free amine is diversified by appending the first building block, usually through an amide coupling. At this point the DNA-small molecule conjugate is again ethanol precipitated, redissolved, and all the wells are pooled. The pooled library members can be split again to repeat the process multiple times and build a collection of ligands (most DELs undergo 3 total cycles of diversification). Upon completion of the final diversification cycle the DEL may be tagged with another barcode containing additional information. One example is the addition of a library ID to identify the library in “super pool” mixtures of multiple libraries.

2. Alternative Methods for DEL Formation

Although the workflow described above is by far the most common method for building a DEL, multiple other techniques



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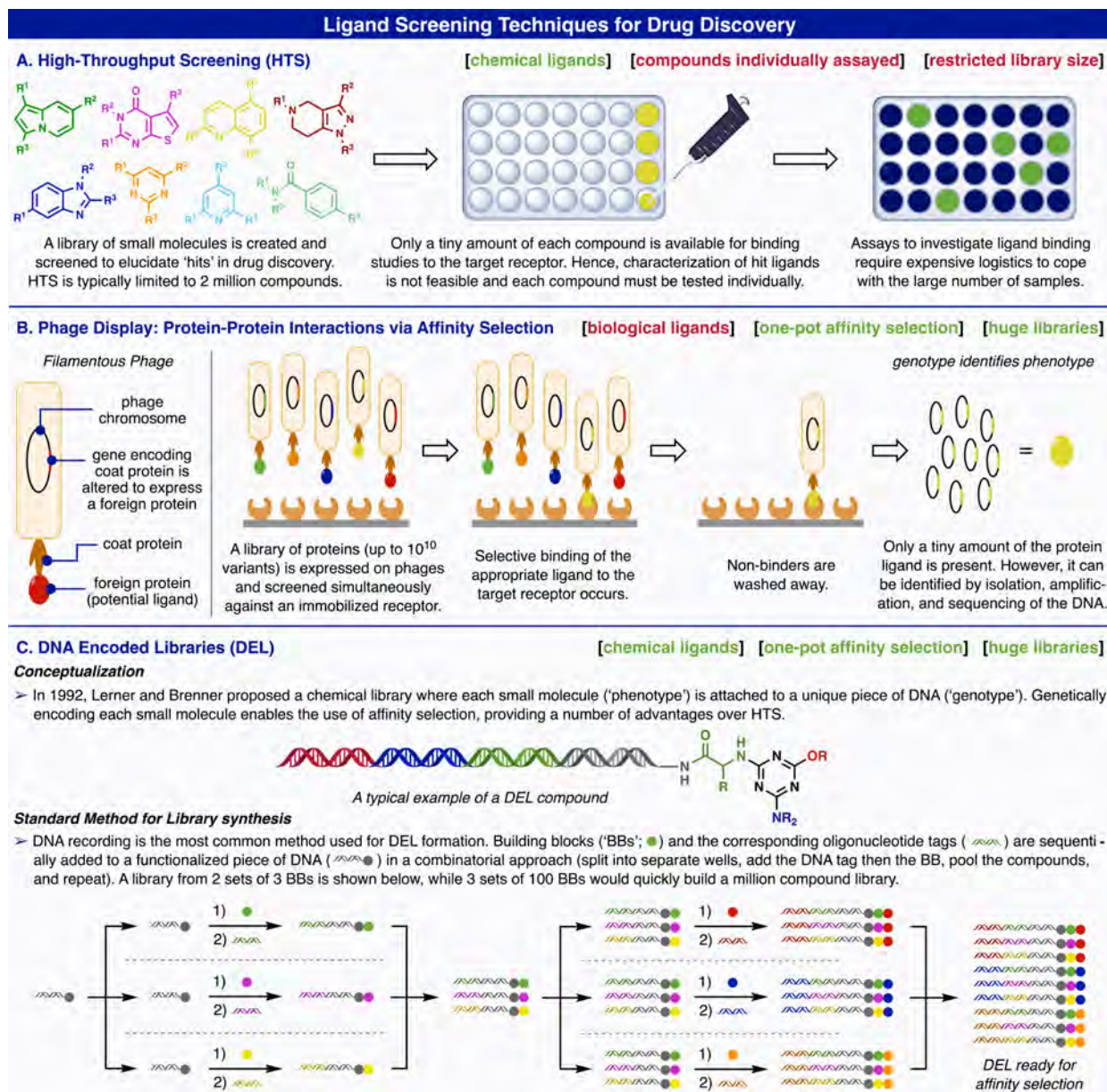


Figure 1. Techniques for ligand screening: (A) High-Throughput Screening, (B) phage display, and (C) DNA encoded libraries.

exist for encoding the synthesis of a small molecule with DNA (Figure 2).^[27] Indeed, the Liu group pioneered an approach subsequently coined DNA templated synthesis, wherein a single stranded DNA template is synthesized bearing all of the information (codons) for the final library member (Figure 2A).^[22,29] In this approach, reactive building blocks were appended to a short DNA oligomer (anti-codons) through a cleavable linker. Upon mixing with the template strands these short oligomers would hybridize to the template strand, and the reactive building blocks would react and couple. The linker is cleaved, and the process could be repeated multiple times to build the elaborated DEL member. Although DNA templated synthesis provides an elegant approach to on-DNA chemistry,

the need to synthesize individual template strands for every intended library member creates a significant hurdle for its widespread adoption while the size of the orthogonal codon set precludes its use in constructing very large libraries.^[11,27]

Another technique that utilizes the self-assembling nature of DNA is the encoding self-assembling chemical library (ESAC, Figure 2B).^[2,23,27] This approach uses small molecule fragments (similar to the fragments sometimes encountered in HTS) linked to a single stranded DNA tag which is comprised of a hybridization region and an encoding region. Upon mixing the library the DNA will hybridize (driven through its hybridization region) in a combinatorial manner, regardless of its coding region. Upon hybridization, the attached small

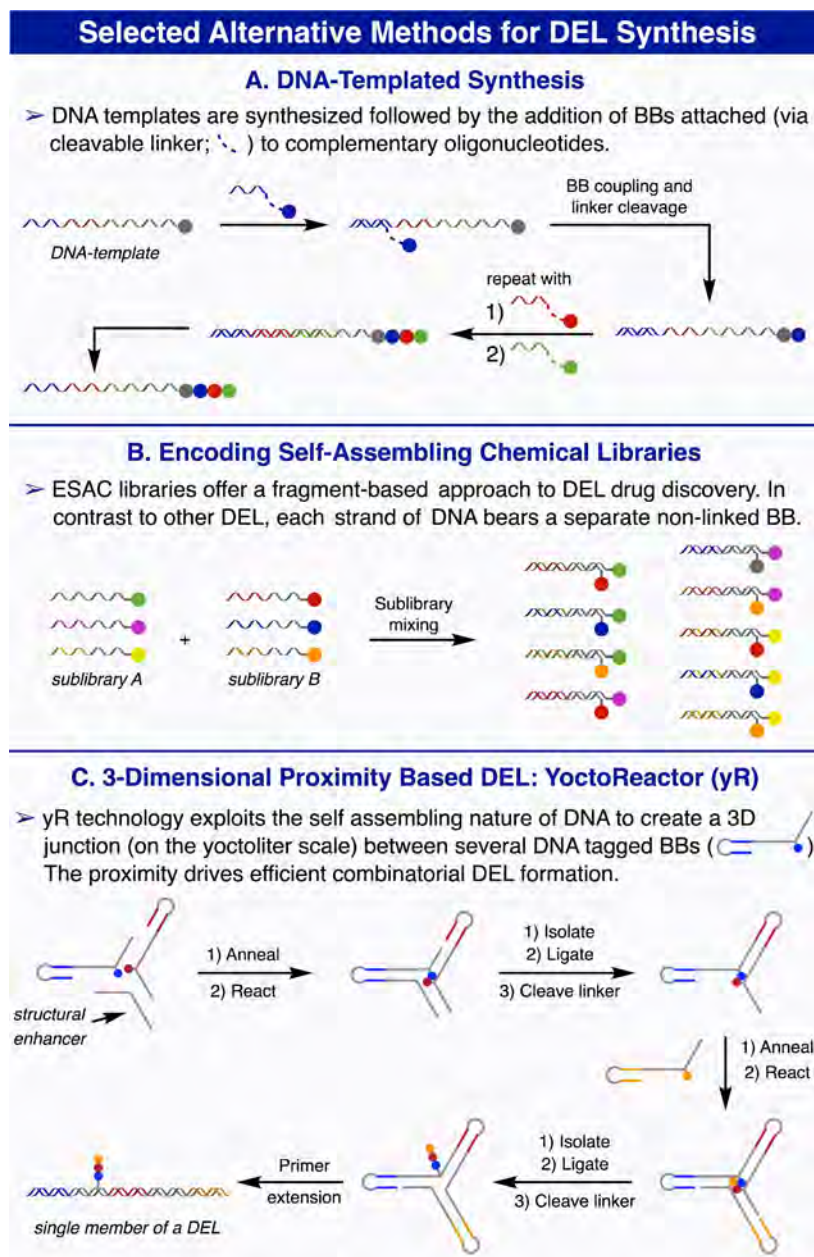


Figure 2. Alternative methods for DEL formation.

molecule fragments do not react (to create a single ligand) and hence this is referred to as dual pharmacophore library. Another interesting advance in the ESAC is the development of dynamic self-assembling libraries.^[30,31] These libraries are created in a similar manner to the typical ESAC described above, but the hybridization region is short and only forms unstable duplexed species that readily convert under dynamic combinatorial control. Upon external influence, such as introduction of the target protein, the library will shift its equilibrium in reaction to the binding events that stabilize the dynamic duplexed interactions. Thus, duplexed fragments that bind to the protein in a favorable interaction are stabilized and

amplified. Both of these strategies can be screened for binding in the same manner as a normal DEL library would be. However, the act of linking the fragment binders to create the proposed ideal ligand can have a major effect on binding and activity.

The YoctoReactor (yR) technology exploits the capacity for self-assembling 3D structures of DNA oligonucleotides to direct DNA-bound building blocks into close proximity and drive highly efficient combinatorial synthesis (Figure 2C).^[32,33] To accomplish this goal, building blocks are covalently attached (by a cleavable or non-cleavable linker) to a DNA oligonucleotide comprised of a self-complementary encoding

region (specific to the building block attached) and a non-variable region that directs the yR self-assembly. The junction formed by the assembly of the annealed oligonucleotides forms a type of ‘reaction vessel’ on the yoctoliter scale, allowing reactions between building blocks to occur at effectively millimolar concentrations. Practically, the yR library is built in a stepwise procedure where two building blocks are reacted, the reaction mixture is gel purified to remove any unreacted substrates, and the linker is cleaved. The next building block pool is subsequently mixed in and the process is repeated. Once diversification is complete any remaining cleavable linkers are cut, and the self-complementary single stranded oligomer is primer extended to create a double-stranded DNA tag. The resulting library can be panned (screened by affinity selection) against an immobilized protein target for selection in the typical manner.

3. Selection Strategies

As discussed above, DNA encoded libraries are typically panned (i.e. screened) through affinity selection against an immobilized protein of interest (Figure 3A).^[18,27,28,31,34–37] Ligands that bind to the target (“binders”) are retained while nonbinders are washed away, followed by elution of the binders from the solid supported protein and identification. The protein of interest can be immobilized on magnetic beads bearing such affinity capture moieties as streptavidin, Ni-NTA, or ANTI-FLAG antibodies.^[25–27] The protein can be immobilized before or after the panning step and multiple wash and elute conditions have been described. Unfortunately, experimental parameters such as panning/incubation conditions, washing procedures, detergent load, and protein immobilization procedures have been shown to profoundly impact the selection outcome.^[27,38] As such, no general method exists, and

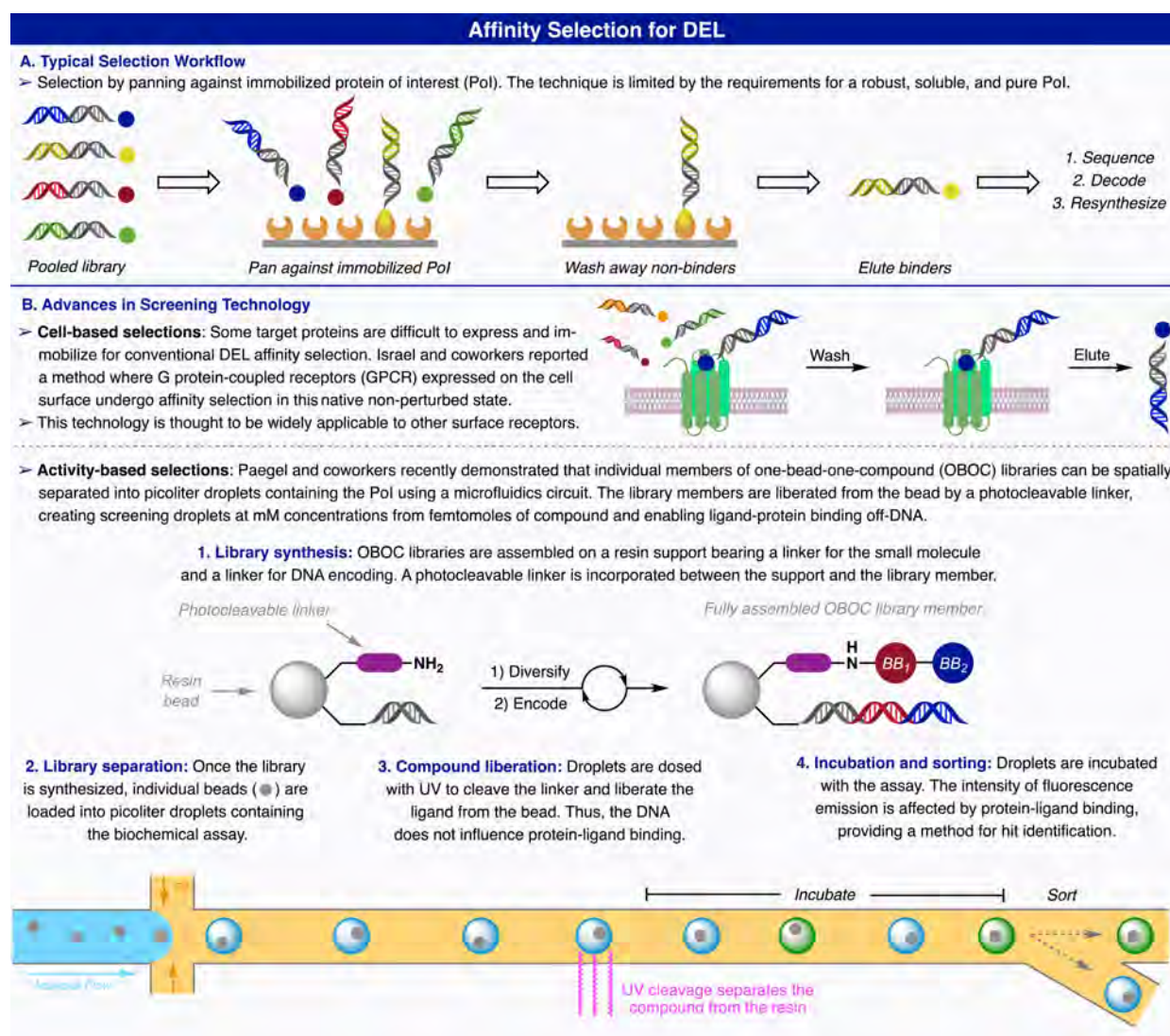


Figure 3. Typical and alternative methods for affinity selection.

experimental systems are usually optimized for each protein of interest.

Although the affinity selection procedure described above has proven useful for identifying hits in numerous DEL campaigns, the empirical nature of the selection is not applicable to many relevant classes of proteins because a soluble, isolated, and purified protein is required.^[31] One advance towards expanding the proteome available for DEL selections is the recently reported cell-based DEL screen (Figure 3B).^[39] In 2015, a team at GSK reported a method in which DNA encoded libraries could be panned/selected against GPCRs overexpressed on a cell surface. In a technique directly analogous to affinity DEL selection, the protein of interest (a tachykinin GPCR called NK3) is overexpressed, and effectively immobilized at the cell surface. A DEL can be panned against a population of cells overexpressing this receptor as well as a population of cells transfected with a negative control. Unbound DEL members are washed away, and the hit ligands are eluted by heat denaturing. The binders that were selected by panning against the cell population overexpressing the protein of interest, were then compared to the binders that were selected when the same library was panned against cells that were not expressing the protein of interest. The molecules that bound to the cells that were not expressing the protein of interest were interpreted to be binders of the endogenous cell surface proteins. This method increases the proteome available to DEL selection by making GPCRs DEL compatible, a class of proteins that would typically be recalcitrant to express, isolate, and purify.

Although expanding the proteome available to DEL selections is a valuable task, these protocols are inherently restricted to small molecule-protein binding. Many relevant pathologies/phenotypes are simply not affected through such interactions, as supported by the large number of DEL hits that ultimately have no activity *in vitro* or *in vivo*.^[25,40] To this end, activity-based DEL selections that rely on more complex biochemical assays would be a powerful tool. Recently, Paegel and coworkers reported a microfluidics-based system that allows a one-bead-one-compound (OBOC) DEL to be selected for activity instead of merely binding (Figure 3C).^[41,42] In this system, a 67,100 member OBOC library was assembled on a bifunctional TentaGel Rink Amide support by standard split and pool methods. The library member (small molecule) is attached to the bead through a photocleavable linker and the DNA barcode is separately attached through an orthogonal linker. The OBOC library was loaded into the microfluidics device such that each bead was contained in its own droplet (at the picoliter scale). The droplet also contains all the components for a phosphodiesterase autotaxin assay. The droplets were flowed through a photoreactor in which the small molecule library members were cleaved from the support by exposure to UV light (365 nm) and femtomoles of now freely soluble library member (at millimolar concentrations) were incubated with the biochemical assay. The droplets were then flowed through a fluorescence detector and separated based on activity (determined if a droplet had a

statistically significant drop in fluorescence compared to the background population). Hits were resynthesized and their activity was confirmed. A standard DEL was then screened against the same protein using the canonical affinity-based approach and, in contrast to the microfluidics approach, the resulting hit population contained a large number of inactive binders (determined after resynthesis). This innovative technology could be expanded to many reporter assays and benefits from the fact that selection is performed on freely soluble small molecules (not attached to the bead or DNA tag) and is performed with activity in mind.

The Krusemark group recently presented a sophisticated solution to the problem of on- and in-cell DEL selections.^[43] A DEL was utilized that included an appended cyclic cell penetrating peptide (cCPP) and a reactive crosslinking agent. cCPP allows the DEL to access the cytosolic space and the crosslinking moiety allows the covalent crosslinking upon formation of a transient interaction between a library member and a HaloTag (a widely used self-labeling protein tag derived from a bacterial haloalkane dehalogenase)^[44] fusion protein of interest. After incubation with the cell penetrating library, the protein of interest was captured on streptavidin beads and the hit pool could be amplified. Although this method is more complex than the classic affinity selection, the ability to select against proteins within native cellular environments should prove extremely advantageous in the world of drug discovery.

4. Recent Successes

Since 1992, several successes for the DEL approach have been registered. In 2016, GSK screened receptor interacting protein 1 (RIP1) kinase – which plays a preponderant role in regulation of tumor necrosis factor (TNF) – against the 7.7 billion compounds contained in the GSK's DNA encoded small-molecule libraries (Figure 4A).^[45] This screening identified a novel highly potent benzoxazepinone inhibitor (GSK 481) with complete monokinase selectivity for RIP1, high potency (RIP1 IC_{50} = 1.3 nM), and good pharmacokinetic profiles in rodents. This compound was therefore chosen as the lead compound for further development into a RIP1 clinical candidate. After optimization, it was found that replacement of the isoxazole of GSK 481 with a triazole (GSK 2982772) was crucial to improve solubility, lipophilicity, and rat oral exposure. GSK 2982772 demonstrated effective binding to RIP1 with exquisite kinase specificity and exceptional activity in suppressing many TNF-dependent cellular responses.^[46,47] This compound was successfully advanced into phase 1 clinical trials in 2015, and phase 2a clinical trials in psoriasis, rheumatoid arthritis, and ulcerative colitis patients in 2017. The highly favorable physicochemical and ADMET properties of GSK 2982772 and its high potency are suggestive of a low oral dose in humans.

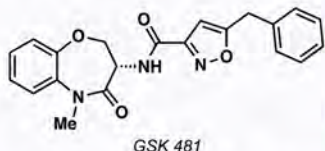
Another industrial success story was reported in 2017 when AztraZeneca, Heptares Therapeutics, and X-Chem disclosed the crystal structure of an allosteric ligand bound to

Successful Applications and Advantages of DEL

- Several drug candidates have been identified using DNA-encoded libraries over the past few years.

A. GSK - RIP1 Kinase Inhibitor

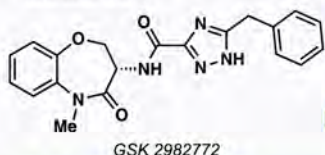
- GSK recently initiated a search for a compound to inhibit receptor interacting protein 1 kinase (RIP1 kinase). Known kinase inhibitors did not offer the desired druglike properties or were not selective for RIP1.
- The extremely potent and highly specific inhibitor GSK 481 was identified by screening a DEL consisting of approximately 7.7 billion compounds.



GSK 481

[From DNA encoded library]
[RIP1 IC₅₀ = 1.3 nM]
[U937 cell IC₅₀ = 10 nM]
[Mono-kinase selectivity]
[Excellent PK profile in rodent]
[Lead compound]

- After subsequent development off DNA, compound GSK 2982772 advanced to Phase IIa clinical trials in patients with psoriasis, rheumatoid arthritis, and ulcerative colitis.

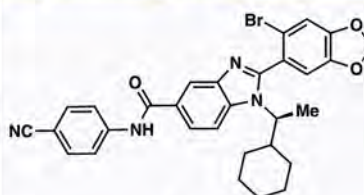


GSK 2982772

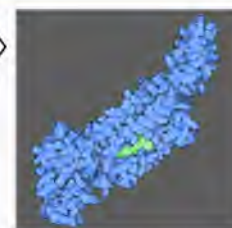
[From GSK 481 after SAR]
[RIP1 IC₅₀ = 1.0 nM]
[U937 cell IC₅₀ = 6.3 nM]
[Excellent ADMET properties]
[Predicted low oral dose in human]

B. AstraZeneca, Heptares Therapeutics, and X-Chem - GPCR ligands

- AstraZeneca, Heptares Therapeutics, and X-Chem reported the crystal structure of two allosteric ligands bound to a GPCR called protease-activated receptor 2 (PAR2). One of those allosteric ligands—AZ3451—was identified using DNA-encoded libraries from X-Chem.



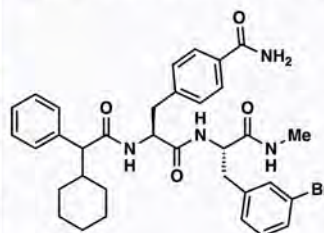
AZ3451



AZ3451/PAR2 cocrystal

C. Lefkowitz Lab - New method for GPCR ligand discovery

- Success stories are not limited to the pharmaceutical industry due to the economic advantages of DEL technologies compared to high-throughput screening collections. For instance, a HTS library of 1 million compounds would cost between \$0.4 and 2 billion (approximately \$400–\$1,000 per compound) to create and screen, whereas a DEL of 800 million compounds would cost approximately \$150,000 (approximately \$0.0002 per compound).
- The Lefkowitz's lab used a 190 million-compound DEL from Nuevolution to discover an allosteric modulator for the β_2 -adrenergic receptor, another GPCR.



[From DNA encoded library]
[Unique chemotype]
[β_2 AR IC₅₀ = 1.9 μ M]
[β_2 AR EC₅₀ = 480 nM]
[Negative allosteric modulator]
[Selectivity for β_2 AR]
[General method for screening DEL against GPCRs]

Figure 4. Successful applications of DEL.

a G protein-coupled receptor (GPCR) called protease-activated receptor 2 (PAR2) (Figure 4B). Compound AZ3451 was identified from several DNA encoded libraries from X-Chem and exhibited a different binding site than the ligand discovered using conventional HTS.^[48] This report was of significant interest as PARs are implicated in diseases such as cancer and inflammation. Indeed, these receptors are irreversibly activated by proteolytic cleavage of the *N*-terminus inducing several cellular events in response to inflammatory signals and other stimuli. PARs have been extensively studied by pharmaceutical companies but the discovery of small-molecule antagonists that effectively bind to them has proven challenging. Therefore, the report of these crystal structures may enable a more rational development of selective PAR2 antagonists for a range of therapeutic uses.

DEL successes are not limited to the pharmaceutical industry, on-the-contrary, several academic labs have harnessed DEL to elucidate new binding molecules for various target proteins.^[49–53] As an example, the group of Prof. Robert Lefkowitz at Duke University screened DNA encoded small-molecule libraries containing 190 million compounds against purified human β_2 -adrenergic receptor (β_2 AR, Figure 4C).^[54,55] This screening campaign afforded the discovery of a small molecule which exhibits a unique chemotype and low micromolar affinity for the target. This particular study is of high

interest as it focuses on a specific GPCR involved in cardiovascular and pulmonary diseases. But more importantly, this report establishes a broadly applicable method for screening DNA encoded small-molecule libraries against purified GPCR targets. Such an approach will certainly facilitate the discovery of GPCR drugs with tailored allosteric effects.

In addition to these success stories, DEL technologies have also exhibited economic advantages compared to HTS collections. Indeed, a hypothetical library of 1 million compounds would cost between \$0.4 and 2 billion (approximately \$400 to \$1,000 per compound) to create and screen, whereas a hypothetical DEL of 800 million compounds would cost approximately \$150,000 (approximately \$0.0002 per compound).^[25]

5. DNA Compatible Chemistry

Achieving efficient chemical transformations on-DNA is not a trivial matter.^[56–60] The idiosyncratic nature of the encoding DNA results in reaction requirements that are incompatible with a significant portion of the conventional medicinal chemistry toolbox. These requirements include (i) an aqueous environment as DNA is insoluble in organic solvents, (ii) highly dilute conditions for solubility considerations, and (iii)

exquisite chemoselectivity to avoid damaging the functional group rich DNA tag. With these requirements in mind, multiple chemical solutions have been devised for the incorporation of structural diversity into DEL (Figure 5). Although new chemistries are disclosed regularly, a small set of reactions is widely used in most DEL builds (and all disclosed DEL hits) including amide bond formations (Fmoc-peptide and peptoid synthesis), amine “capping reactions” (amide, (thio)urea, sulfonylation), cyanuric chloride-based S_NAr , metal-mediated cross couplings (Sonogashira, Suzuki, and more recently Buchwald-Hartwig), cycloadditions, and condensations.^[61] Furthermore, DNA-compatible Fmoc and Boc deprotections along with azide reductions are crucial to the use of bi- and tri-functional DEL building blocks.^[61,62]

Although the reactions presented above are usually accepted as DNA compatible and are widely utilized, difficulties still plague their use by the new practitioner. Reactivities can vary wildly over a class of similar building blocks, and as such, individual building blocks are usually validated and reaction conditions reoptimized before a library build.^[63] Also, it is common for each library member to be purified after the first cycle of diversification.^[27] Another confounding issue is that reactions commonly accepted as “DNA-compatible” are in fact less compatible than previously

thought.^[62,64] Recently, a new method for the evaluation of reaction induced DNA degradation has shed new light on the extent of DNA damage that occurs during commonly utilized DEL reactions (Boc deprotections and Pd-mediated cross couplings).^[64] Such side-reactions can affect both ligation and PCR efficiency.

6. Emerging Techniques

In 2017 our lab (PSB) entered a collaboration with Pfizer to develop chemistry for introducing C(sp³)-rich architectures into a DEL setting via a modular cross-coupling approach (Figure 6A).^[65] The well-documented chemoselectivity of radicals in an aqueous environment suggested that such intermediates might be useful in such a context if side-reactions with DNA could be avoided.^[66,67] Indeed, it is well known that free radicals can damage DNA by reacting with the purine bases. Extensive optimization (including kinetic analysis) off-DNA provided “mock” DEL conditions that were subsequently tuned on-DNA to produce a decarboxylative Giese-type addition that provides access a variety of complex scaffolds even bearing quaternary centers.^[65] The broad scope of this transformation, its use of ubiquitous carboxylic acid

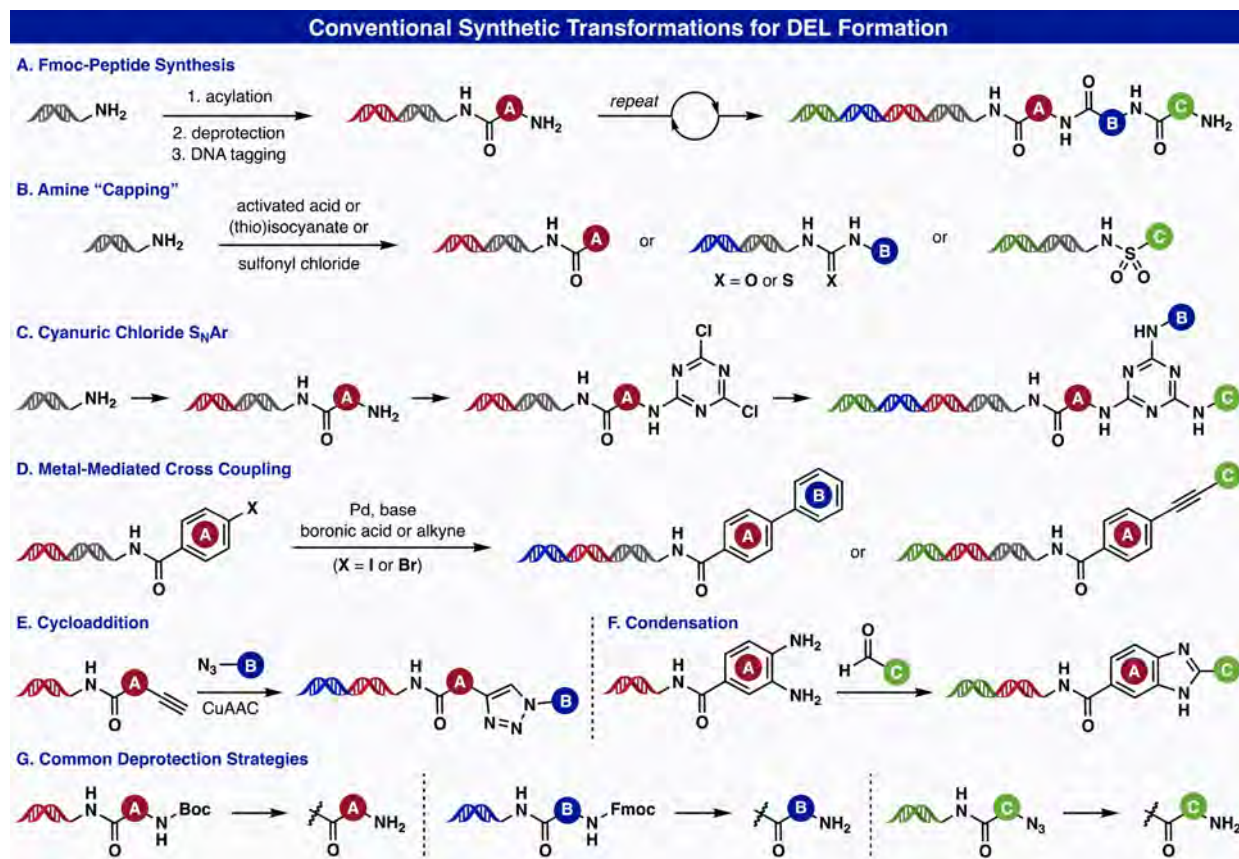


Figure 5. Conventional synthetic transformations used for DEL.

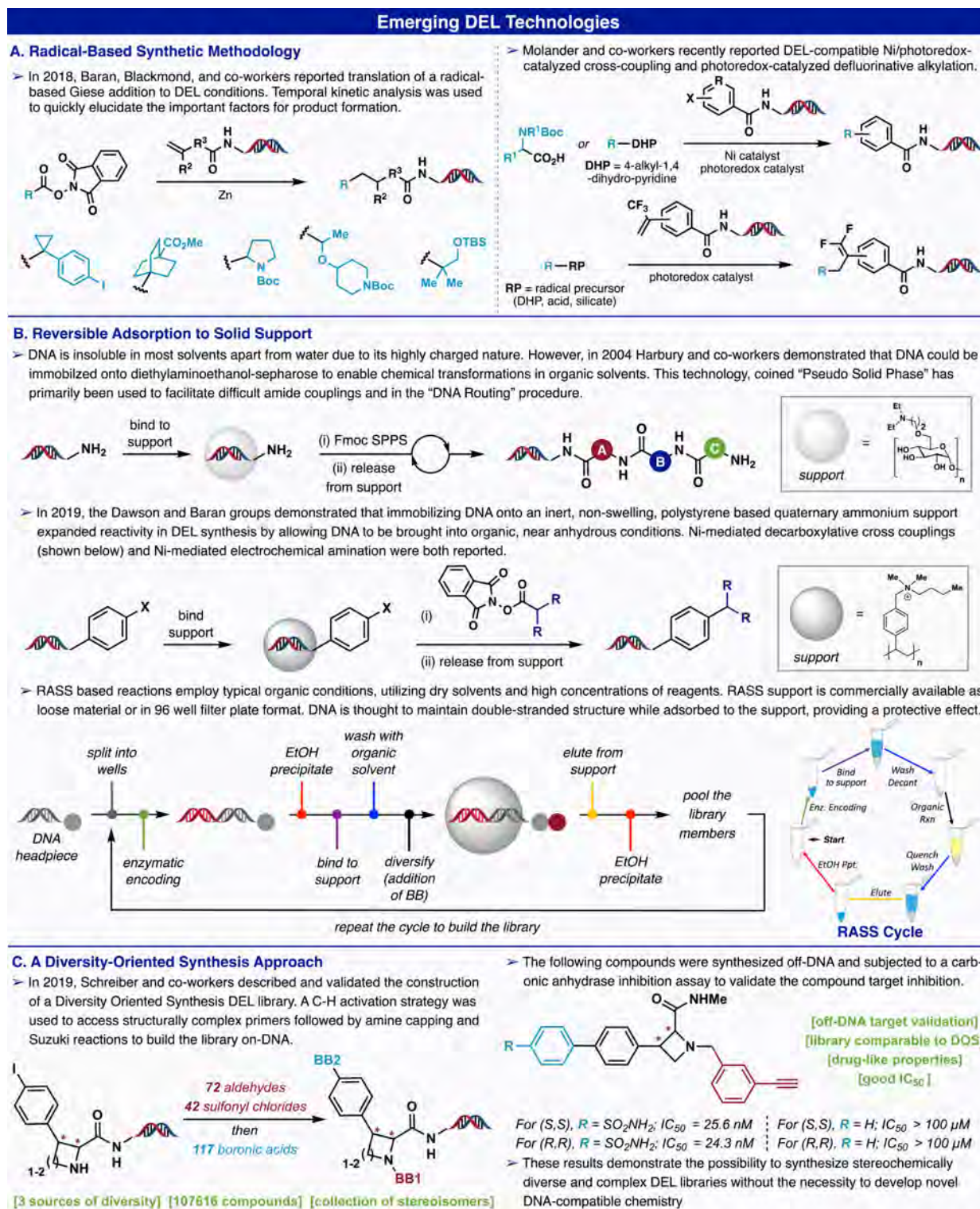


Figure 6. Emerging synthetic transformations and strategies for DEL.

building blocks, and its demonstration on-DNA bode well for its use in DEL and encourage the development of other radical based transformations.^[68] The Molander group, as well as the

Flanagan group, also reported the use of radical chemistry in a DEL-context using photoinduced electron transfer to couple amino acids or other radical precursors (dihydropyridines,

silicates) to aromatic systems.^[69,70] The useful C(sp²)-C(sp³) linkages contained in the structures generated were previously outside the realm of DEL-based chemistry. Taken together, these studies suggest that radical compatibility with DNA is indeed possible despite conventional wisdom.

As mentioned above, DEL are molecular collections of unprecedented size and diversity. However, the hydrophilic properties of the highly charged DNA tag make it insoluble in most solvents apart from water and therefore lessen the scope of applicable organic transformations. In 2004, Harbury and co-workers demonstrated for the first time that DNA could be immobilized onto diethylaminoethanol-sepharose to enable chemical transformations in organic solvents.^[21] This “pseudo solid phase” support has principally been used to ease difficult amide couplings and in the “DNA routing” procedure, which was devised to spatially separate a population of DNA templates based on sequence, obviating the need to a micro-well plate.^[19–21,71–75] This approach has also been applied to biomacromolecules to perform challenging synthetic modifications in an approach described as reversible adsorption to solid support (RASS).^[76,77] As RASS based reactions mostly mimic typical organic conditions utilizing dry solvents and high concentrations of reagents, the Dawson and Baran groups envisioned the adaption of this technology to DEL to enable previously sluggish chemical reactivities.^[78] They demonstrated that immobilizing DNA onto an inert, non-swelling, polystyrene-based quaternary ammonium support facilitates the rapid development of useful transformations including C(sp²)-C(sp³) decarboxylative cross-couplings, the first DNA-compatible electrochemical reactions, and improved reductive amination conditions. Based on these results, RASS should be a valuable tool to access new and diverse DEL reactivities, expand chemical space, and ultimately obtain more drug-like

libraries. In practice, the RASS procedure fits almost effortlessly into the typical DEL workflow. Before the reaction (after the encoding step) the DNA is adsorbed to the resin, which is commercially available in 96 well plates, and the reaction is washed with organic solvents to remove water. Depending on the level of “dryness” that is required by the reaction, the support can subsequently be dried under vacuum. The chemical transformation can be run under conditions similar to a typical small molecule reaction and the unreacted reagents simply washed away. This can be helpful in a multistep reaction in which a reactive intermediate is formed and sequestered on the support while the one set of reagents is removed, and another set is added. The DNA is eluted from the resin with a high salt buffer and ethanol precipitated as normal. From this point the DNA can be utilized in the DEL build as normal.

In 2019, Schreiber and co-workers reported the assembly and validation of a Diversity Oriented Synthesis (DOS)-based platform to incorporate structural complexity and stereochemical diversity into DEL (Figure 6C).^[79] This new library containing 107,616 DNA-barcoded chiral 2,3-disubstituted azetidines and pyrrolidines was built using a stereospecific C–H arylation step to afford complex scaffolds and was diversified with the development of an optimized on-DNA Suzuki reaction. The team investigated both the structural diversity of the library and the physicochemical properties of all compounds using several techniques including cheminformatic and multifusion similarity analyses. These analyses revealed that the DOS-DEL members have similar “drug-like” properties to the compound obtained from diversity synthesis and better than those synthesized from commercially available building blocks. Finally, the authors decided to screen the DOS-DEL against carbonic anhydrase IX and were able to

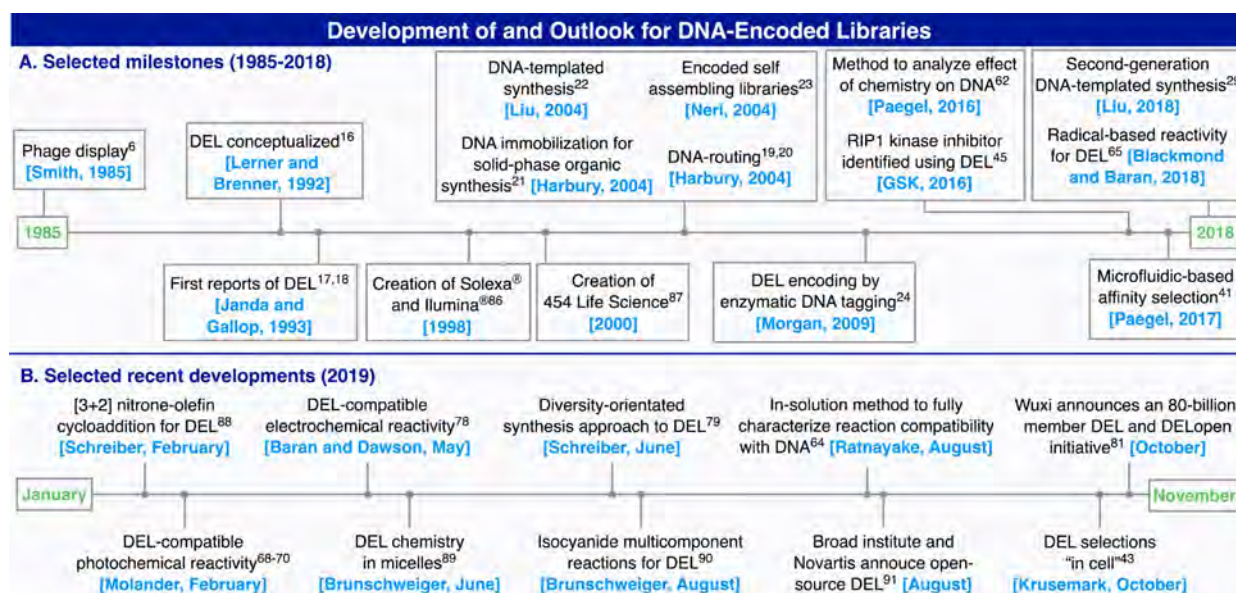


Figure 7. (A) Significant milestones and (B) recent developments for DEL.

find potent carbonic anhydrase inhibitors (IC₅₀ from 20.1 to 68.7 nM). These results demonstrate the possibility of accessing stereochemically diverse and complex DEL libraries without the requirement to develop novel DNA-compatible chemistry.

7. Conclusion and Outlook

In this “visitors guide” we have attempted to provide a rapid overview of the history and current state of affairs in the growing area of DEL. Figure 7 outlines some of the memorable events that laid the foundation for this exciting area from its inspiration and origins to the current era. It is clear that innovation is occurring at a rapid pace both from the standpoint of extending the range of targets amenable to the DEL approach and expanding the toolbox of compatible synthetic transformations leading to increased structural diversity. The debate still continues within this community on whether huge numbers of compounds are desirable over smaller libraries focused on certain chemotypes and scaffolds.^[27,80–82] Despite notable successes, failures still abound in this area stemming from a lack of structural diversity, poorly designed assays, the inability to convert hits to actual leads, and difficulties in rapidly deconvoluting large numbers of false positives from authentic hits.^[82] With regards to larger libraries, improvements in informatic and sequencing technology to rapidly deconvolute hits will have an immediate impact.^[82–84] Finally, a greater understanding of the dynamics of small molecule binding when attached to DNA could facilitate more effective selections. Ultimately the future success of the DEL-based screening paradigm will be reliant on a community-wide collaboration including both industry and academia to advance technology in informatics, sequencing, biochemistry, and synthetic organic chemistry.^[85]

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References

- [1] R. Macarron, M. N. Banks, D. Bojanic, D. J. Burns, D. A. Cirovic, T. Garyantes, D. V. S. Green, R. P. Hertzberg, W. P. Janzen, J. W. Paslay, U. Schopfer, G. S. Sittampalam, *Nat. Rev. Drug Discovery* **2011**, *10*, 188–195.
- [2] D. A. Erlanson, S. W. Fesik, R. E. Hubbard, W. Jahnke, H. Jhoti, *Nat. Rev. Drug Discovery* **2016**, *15*, 605–619.
- [3] P. J. Hajduk, J. Greer, *Nat. Rev. Drug Discovery* **2007**, *6*, 211–219.
- [4] W. L. Jorgensen, *Science* **2004**, *303*, 1813–1818.
- [5] For a recent report on “ultra-large library docking”, see: J. Lyu, S. Wang, T. E. Balius, I. Singh, A. Levit, Y. S. Moroz, M. J. O'Meara, T. Che, E. Algaa, K. Tolmachova, A. A. Tolmachev, B. K. Shoichet, B. L. Roth, J. J. Irwin, *Nature* **2019**, *566*, 224–229.
- [6] G. Smith, *Science* **1985**, *228*, 1315–1317.
- [7] S. F. Parmley, G. P. Smith, *Gene* **1988**, *73*, 305–318.
- [8] G. P. Smith, V. A. Petrenko, *Chem. Rev.* **1997**, *97*, 391–410.
- [9] G. P. Smith, *Angew. Chem. Int. Ed.* **2019**, *58*, 14428–14437.
- [10] L. Ledsgaard, M. Kilstrup, A. Karatt-Vellatt, J. McCafferty, A. H. Laustsen, *Toxins* **2018**, *10*, 236.
- [11] L. Mannocci, M. Leimbacher, M. Wichert, J. Scheuermann, D. Neri, *Chem. Commun.* **2011**, *47*, 12747–12753.
- [12] S. Mimmi, D. Maisano, I. Quinto, E. Iaccino, *Trends Pharmacol. Sci.* **2019**, *40*, 87–91.
- [13] A. Rami, M. Behdani, N. Yardehnavi, M. Habibi-Anbouhi, F. Kazemi-Lomedasht, *Asian Pac. J. Trop. Biomed.* **2017**, *7*, 599–602.
- [14] C.-H. Wu, I. J. Liu, R.-M. Lu, H. -C Wu, *J. Biomed. Sci.* **2016**, *23*, 8.
- [15] G. Zimmermann, D. Neri, *Drug Discovery Today* **2016**, *21*, 1828–1834.
- [16] S. Brenner, R. A. Lerner, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 5381–5383.
- [17] J. Nielsen, S. Brenner, K. D. Janda, *J. Am. Chem. Soc.* **1993**, *115*, 9812–9813.
- [18] M. C. Needels, D. G. Jones, E. H. Tate, G. L. Heinkel, L. M. Kochersperger, W. J. Dower, R. W. Barrett, M. A. Gallop, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10700–10704.
- [19] D. R. Halpin, P. B. Harbury, *PLOS Biology* **2004**, *2*, e173.
- [20] D. R. Halpin, P. B. Harbury, *PLOS Biology* **2004**, *2*, e174.
- [21] D. R. Halpin, J. A. Lee, S. J. Wrenn, P. B. Harbury, *PLOS Biology* **2004**, *2*, e175.
- [22] Z. J. Gartner, B. N. Tse, R. Grubina, J. B. Doyon, T. M. Snyder, D. R. Liu, *Science* **2004**, *305*, 1601–1605.
- [23] S. Melkko, J. Scheuermann, C. E. Dumelin, D. Neri, *Nat. Biotechnol.* **2004**, *22*, 568–574.
- [24] M. A. Clark, R. A. Acharya, C. C. Arico-Muendel, S. L. Belyanskaya, D. R. Benjamin, N. R. Carlson, P. A. Centrella, C. H. Chiu, S. P. Creaser, J. W. Cuzzo, C. P. Davie, Y. Ding, G. J. Franklin, K. D. Franzen, M. L. Geftter, S. P. Hale, N. J. V. Hansen, D. I. Israel, J. Jiang, M. J. Kavarana, M. S. Kelley, C. S. Kollmann, F. Li, K. Lind, S. Mataruse, P. F. Medeiros, J. A. Messer, P. Myers, H. O'Keefe, M. C. Oliff, C. E. Rise, A. L. Satz, S. R. Skinner, J. L. Svendsen, L. Tang, K. van Vloten, R. W. Wagner, G. Yao, B. Zhao, B. A. Morgan, *Nat. Chem. Biol.* **2009**, *5*, 647–654.
- [25] N. Favalli, G. Bassi, J. Scheuermann, D. Neri, *FEBS Lett.* **2018**, *592*, 2168–2180.
- [26] R. M. Franzini, D. Neri, J. Scheuermann, *Acc. Chem. Res.* **2014**, *47*, 1247–1255.
- [27] D. Neri, R. A. Lerner, *Annu. Rev. Biochem.* **2018**, *87*, 479–502.

- [28] A. Kowalczyk, *A Handbook for DNA-Encoded Chemistry: Theory and Applications for Exploring Chemical Space and Drug Discovery* (Ed.: R. E. Goodnow) Wiley-VCH: Weinheim, Germany, **2014**, pp. 1–18.
- [29] D. L. Usanov, A. I. Chan, J. P. Maianti, D. R. Liu, *Nat. Chem.* **2018**, *10*, 704–714.
- [30] F. V. Reddavid, M. Cui, W. Lin, N. Fu, S. Heiden, H. Andrade, M. Thompson, Y. Zhang, *Chem. Commun.* **2019**, *55*, 3753–3756.
- [31] B. Shi, Y. Zhou, Y. Huang, J. Zhang, X. Li, *Biorg. Med. Chem. Lett.* **2017**, *27*, 361–369.
- [32] M. H. Hansen, P. Blakskjær, L. K. Petersen, T. H. Hansen, J. W. Højfeldt, K. V. Gothelf, N. J. V. Hansen, *J. Am. Chem. Soc.* **2009**, *131*, 1322–1327.
- [33] P. Blakskjær, T. Heitner, N. J. V. Hansen, *Curr. Opin. Chem. Biol.* **2015**, *26*, 62–71.
- [34] S. P. Hale, *A Handbook for DNA-Encoded Chemistry: Theory and Applications for Exploring Chemical Space and Drug Discovery* (Ed.: R. E. Goodnow) Wiley-VCH: Weinheim, Germany, **2014**, pp. 281–317.
- [35] A. W. Fraley, *A Handbook for DNA-Encoded Chemistry: Theory and Applications for Exploring Chemical Space and Drug Discovery* (Ed.: R. E. Goodnow) Wiley-VCH: Weinheim, Germany, **2014**, pp. 153–169.
- [36] A. I. Chan, L. M. McGregor, D. R. Liu, *Curr. Opin. Chem. Biol.* **2015**, *26*, 55–61.
- [37] W. Decurtins, M. Wichert, R. M. Franzini, F. Buller, M. A. Stravs, Y. Zhang, D. Neri, J. Scheuermann, *Nat. Protoc.* **2016**, *11*, 764–780.
- [38] J. C. Faver, K. Riehle, D. R. Lancia, J. B. J. Milbank, C. S. Kollmann, N. Simmons, Z. Yu, M. M. Matzuk, *ACS Comb. Sci.* **2019**, *21*, 75–82.
- [39] Z. Wu, T. L. Graybill, X. Zeng, M. Platchek, J. Zhang, V. Q. Bodmer, D. D. Wisnoski, J. Deng, F. T. Coppo, G. Yao, A. Tamburino, G. Scavello, G. J. Franklin, S. Mataruse, K. L. Bedard, Y. Ding, J. Chai, J. Summerfield, P. A. Centrella, J. A. Messer, A. J. Pope, D. I. Israel, *ACS Comb. Sci.* **2015**, *17*, 722–731.
- [40] T. Kodadek, N. G. Paciaroni, M. Balzarini, P. Dickson, *Chem. Commun.* **2019**, *55*, 13330–13341.
- [41] A. B. MacConnell, A. K. Price, B. M. Paegel, *ACS Comb. Sci.* **2017**, *19*, 181–192.
- [42] W. G. Cochrane, M. L. Malone, V. Q. Dang, V. Cavett, A. L. Satz, B. M. Paegel, *ACS Comb. Sci.* **2019**, *21*, 425–435.
- [43] B. Cai, D. Kim, S. Akhand, Y. Sun, R. J. Cassell, A. Alpsoy, E. C. Dykhuizen, R. M. Van Rijn, M. K. Wendt, C. J. Krusemark, *J. Am. Chem. Soc.* **2019**, *141*, 17057–17061.
- [44] G. V. Los, L. P. Encell, M. G. McDougall, D. D. Hartzell, N. Karassina, C. Zimprich, M. G. Wood, R. Learish, R. F. Ohana, M. Urh, D. Simpson, J. Mendez, K. Zimmerman, P. Otto, G. Vidugiris, J. Zhu, A. Darzins, D. H. Klaubert, R. F. Bulleit, K. V. Wood, *ACS Chem. Biol.* **2008**, *3*, 373–382.
- [45] P. A. Harris, B. W. King, D. Bandyopadhyay, S. B. Berger, N. Campobasso, C. A. Capriotti, J. A. Cox, L. Dare, X. Dong, J. N. Finger, L. C. Grady, S. J. Hoffman, J. U. Jeong, J. Kang, V. Kasparcova, A. S. Lakdawala, R. Lehr, D. E. McNulty, R. Nagilla, M. T. Ouellette, C. S. Pao, A. R. Rendina, M. C. Schaeffer, J. D. Summerfield, B. A. Swift, R. D. Totoritis, P. Ward, A. Zhang, D. Zhang, R. W. Marquis, J. Bertin, P. J. Gough, *J. Med. Chem.* **2016**, *59*, 2163–2178.
- [46] P. A. Harris, S. B. Berger, J. U. Jeong, R. Nagilla, D. Bandyopadhyay, N. Campobasso, C. A. Capriotti, J. A. Cox, L. Dare, X. Dong, P. M. Eidam, J. N. Finger, S. J. Hoffman, J. Kang, V. Kasparcova, B. W. King, R. Lehr, Y. Lan, L. K. Leister, J. D. Lich, T. T. MacDonald, N. A. Miller, M. T. Ouellette, C. S. Pao, A. Rahman, M. A. Reilly, A. R. Rendina, E. J. Rivera, M. C. Schaeffer, C. A. Schon, R. R. Singhaus, H. H. Sun, B. A. Swift, R. D. Totoritis, A. Vossenkämper, P. Ward, D. D. Wisnoski, D. Zhang, R. W. Marquis, P. J. Gough, J. Bertin, *J. Med. Chem.* **2017**, *60*, 1247–1261.
- [47] K. Weisel, N. E. Scott, D. J. Tompson, B. J. Votta, S. Madhavan, K. Povey, A. Wolstenholme, M. Simeoni, T. Rudo, L. Richards-Peterson, T. Sahota, J. G. Wang, J. Lich, J. Finger, A. Verticelli, M. Reilly, P. J. Gough, P. A. Harris, J. Bertin, M. -L Wang, *Pharmacol. Res. Perspect.* **2017**, *5*, e00365.
- [48] R. K. Y. Cheng, C. Fiez-Vandal, O. Schlenker, K. Edman, B. Aggeler, D. G. Brown, G. A. Brown, R. M. Cooke, C. E. Dumelin, A. S. Doré, S. Geschwindner, C. Grebner, N.-O. Hermansson, A. Jazayeri, P. Johansson, L. Leong, R. Prihandoko, M. Rappas, H. Soutter, A. Snijder, L. Sundström, B. Tehan, P. Thornton, G. Troast, G. Wiggan, A. Zhukov, F. H. Marshall, N. Dekker, *Nature* **2017**, *545*, 112.
- [49] J. P. Maianti, A. McFedries, Z. H. Foda, R. E. Kleiner, X. Q. Du, M. A. Leissring, W.-J. Tang, M. J. Charron, M. A. Seeliger, A. Saghatelian, D. R. Liu, *Nature* **2014**, *511*, 94–98.
- [50] F. Samain, T. Ekblad, G. Mikutis, N. Zhong, M. Zimmermann, A. Nauer, D. Bajic, W. Decurtins, J. Scheuermann, P. J. Brown, J. Hall, S. Gräslund, H. Schüller, D. Neri and R M Franzini, *J. Med. Chem.* **2015**, *58*, 5143–5149.
- [51] S. Barluenga, C. Zambaldo, H. A. Ioannidou, M. Ciobanu, P. Morieux, J.-P. Daguer, N. Winssinger, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 1080–1085.
- [52] F. Buller, M. Steiner, K. Frey, D. Mircsof, J. Scheuermann, M. Kalisch, P. Buhlmann, C. T. Supuran, D. Neri, *ACS Chem. Biol.* **2011**, *6*, 336–344.
- [53] M. Leimbacher, Y. Zhang, L. Mannocci, M. Stravs, T. Geppert, J. Scheuermann, G. Schneider, D. Neri, *Chem. Eur. J.* **2012**, *18*, 7729–7737.
- [54] S. Ahn, A. W. Kahsai, B. Pani, Q.-T. Wang, S. Zhao, A. L. Wall, R. T. Strachan, D. P. Staus, L. M. Wingler, L. D. Sun, J. Sinnaeve, M. Choi, T. Cho, T. T. Xu, G. M. Hansen, M. B. Burnett, J. E. Lamerdin, D. L. Bassoni, B. J. Gavino, G. Husemoen, E. K. Olsen, T. Franch, S. Costanzi, X. Chen, R. J. Lefkowitz, *Proc. Natl. Acad. Sci. USA* **2017**, DOI: 10.1073/pnas.1620645114, 201620645.
- [55] S. Ahn, B. Pani, A. W. Kahsai, E. K. Olsen, G. Husemoen, M. Vestergaard, L. Jin, S. Zhao, L. M. Wingler, P. K. Rambarat, R. K. Simhal, T. T. Xu, L. D. Sun, P. J. Shim, D. P. Staus, L.-Y. Huang, T. Franch, X. Chen, R. J. Lefkowitz, *Mol. Pharmacol.* **2018**, *94*, 850–861.
- [56] A. D. Keefe, *A Handbook for DNA-Encoded Chemistry: Theory and Applications for Exploring Chemical Space and Drug Discovery* (Ed.: R. E. Goodnow) Wiley-VCH: Weinheim, Germany, **2014**, pp. 45–66.
- [57] K.-C. Luk, A. L. Satz, *A Handbook for DNA-Encoded Chemistry: Theory and Applications for Exploring Chemical Space and Drug Discovery* (Ed.: R. E. Goodnow) Wiley-VCH: Weinheim, Germany, **2014**, pp. 67–98.
- [58] R. M. Franzini, C. Randolph, *J. Med. Chem.* **2016**, *59*, 6629–6644.
- [59] D. Neri, *ChemBioChem* **2017**, *18*, 827–828.
- [60] P. Dickson, T. Kodadek, *Org. Biomol. Chem.* **2019**, *17*, 4676–4688.
- [61] A. L. Satz, J. Cai, Y. Chen, R. Goodnow, F. Gruber, A. Kowalczyk, A. Petersen, G. Naderi-Oboodi, L. Orzechowski, Q. Strebel, *Bioconjugate Chem.* **2015**, *26*, 1623–1632.
- [62] M. L. Malone, B. M. Paegel, *ACS Comb. Sci.* **2016**, *18*, 182–187.
- [63] Y. Li, E. Gabriele, F. Samain, N. Favalli, F. Sladojevich, J. Scheuermann, D. Neri, *ACS Comb. Sci.* **2016**, *18*, 438–443.

- [64] A. S. Ratnayake, M. E. Flanagan, T. L. Foley, J. D. Smith, J. G. Johnson, J. Bellenger, J. I. Montgomery, B. M. Paegel, *ACS Comb. Sci.* **2019**, *21*, 650–655.
- [65] J. Wang, H. Lundberg, S. Asai, P. Martín-Acosta, J. S. Chen, S. Brown, W. Farrell, R. G. Dushin, C. J. O'Donnell, A. S. Ratnayake, P. Richardson, Z. Liu, T. Qin, D. G. Blackmond, P. S. Baran, *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E6404–E6410.
- [66] M. Dizdaroglu, P. Jaruga, *Free Radical Res.* **2012**, *46*, 382–419.
- [67] M. Dizdaroglu, P. Jaruga, M. Birincioglu, H. Rodriguez, *Free Radical Biol. Med.* **2002**, *32*, 1102–1115.
- [68] D. K. Kölmel, R. P. Loach, T. Knauber, M. E. Flanagan, *ChemMedChem* **2018**, *13*, 2159–2165.
- [69] J. P. Phelan, S. B. Lang, J. Sim, S. Berritt, A. J. Peat, K. Billings, L. Fan, G. A. Molander, *J. Am. Chem. Soc.* **2019**, *141*, 3723–3732.
- [70] D. K. Kölmel, J. Meng, M.-H. Tsai, J. Que, R. P. Loach, T. Knauber, J. Wan, M. E. Flanagan, *ACS Comb. Sci.* **2019**, *21*, 588–597.
- [71] S. J. Wrenn, P. B. Harbury, *Annu. Rev. Biochem.* **2007**, *76*, 331–349.
- [72] S. J. Wrenn, R. M. Weisinger, D. R. Halpin, P. B. Harbury, *J. Am. Chem. Soc.* **2007**, *129*, 13137–13143.
- [73] R. M. Weisinger, R. J. Marinelli, S. J. Wrenn, P. B. Harbury, *PLoS One* **2012**, *7*, e32299.
- [74] R. M. Weisinger, S. J. Wrenn, P. B. Harbury, *PLoS One* **2012**, *7*, e28056.
- [75] C. J. Krusemark, N. P. Tilmans, P. O. Brown, P. B. Harbury, *PLoS One* **2016**, *11*, e0154765.
- [76] P. A. Cistrone, P. E. Dawson, *ACS Comb. Sci.* **2016**, *18*, 139–143.
- [77] D. T. Flood, N. L. Yan, P. E. Dawson, *Angew. Chem. Int. Ed.* **2018**, *57*, 8697–8701; *Angew. Chem.* **2018**, *130*, 8833–8837.
- [78] D. T. Flood, S. Asai, X. Zhang, J. Wang, L. Yoon, Z. C. Adams, B. C. Dillingham, B. B. Sanchez, J. C. Vantourout, M. E. Flanagan, D. W. Piotrowski, P. Richardson, S. A. Green, R. A. Shenvi, J. S. Chen, P. S. Baran, P. E. Dawson, *J. Am. Chem. Soc.* **2019**, *141*, 9998–10006.
- [79] C. J. Gerry, M. J. Wawer, P. A. Clemons, S. L. Schreiber, *J. Am. Chem. Soc.* **2019**, *141*, 10225–10235.
- [80] L. H. Yuen, S. Dana, Y. Liu, S. I. Bloom, A.-G. Thorsell, D. Neri, A. J. Donato, D. Kireev, H. Schüller, R. M. Franzini, *J. Am. Chem. Soc.* **2019**, *141*, 5169–5181.
- [81] W. AppTec, WuXi AppTec Provides Easy Access to 80 Billion DNA-Encoded Small Molecules Libraries, (<https://www.prnewswire.com/news-releases/wuxi-apptec-provides-easy-access-to-80-billion-dna-encoded-small-molecules-libraries-300729530.html>).
- [82] A. L. Satz, R. Hochstrasser, A. C. Petersen, *ACS Comb. Sci.* **2017**, *19*, 234–238.
- [83] L. Kuai, T. O'Keeffe, C. Arico-Muendel, *SLAS DISCOVERY: Advancing Life Sciences R&D* **2018**, *23*, 405–416.
- [84] J. Castañón, J. P. Román, T. C. Jessop, J. de Blas, R. Haro, *SLAS DISCOVERY: Advancing Life Sciences R&D* **2018**, *23*, 387–396.
- [85] C. J. Gerry, S. L. Schreiber, *Curr. Opin. Chem. Biol.* **2019**, *56*, 1–9.
- [86] D. R. Bentley, S. Balasubramanian, H. P. Swerdlow, G. P. Smith, J. Milton, C. G. Brown, K. P. Hall, D. J. Evers, C. L. Barnes, H. R. Bignell, J. M. Boutell, J. Bryant, R. J. Carter, R. Keira Cheetham, A. J. Cox, D. J. Ellis, M. R. Flatbush, N. A. Gormley, S. J. Humphray, L. J. Irving, M. S. Karbelashvili, S. M. Kirk, H. Li, X. Liu, K. S. Maisinger, L. J. Murray, B. Obradovic, T. Ost, M. L. Parkinson, M. R. Pratt, I. M. J. Rasolonjatovo, M. T. Reed, R. Rigatti, C. Rodighiero, M. T. Ross, A. Sabot, S. V. Sankar, A. Scally, G. P. Schroth, M. E. Smith, V. P. Smith, A. Spiridou, P. E. Torrance, S. S. Tzonev, E. H. Vermaas, K. Walter, X. Wu, L. Zhang, M. D. Alam, C. Anastasi, I. C. Aniebo, D. M. D. Bailey, I. R. Bancarz, S. Banerjee, S. G. Barbour, P. A. Baybayan, V. A. Benoit, K. F. Benson, C. Bevis, P. J. Black, A. Boodhun, J. S. Brennan, J. A. Bridgham, R. C. Brown, A. A. Brown, D. H. Buermann, A. A. Bundu, J. C. Burrows, N. P. Carter, N. Castillo, M. Chiara E Catenazzi, S. Chang, R. Neil Cooley, N. R. Crake, O. O. Dada, K. D. Diakoumakos, B. Dominguez-Fernandez, D. J. Earnshaw, U. C. Egbujor, D. W. Elmore, S. S. Etchin, M. R. Ewan, M. Fedurco, L. J. Fraser, K. V. Fuentes Fajardo, W. Scott Furey, D. George, K. J. Gietzen, C. P. Goddard, G. S. Golda, P. A. Granieri, D. E. Green, D. L. Gustafson, N. F. Hansen, K. Harnish, C. D. Haudenschild, N. I. Heyer, M. M. Hims, J. T. Ho, A. M. Horgan, K. Hoshler, S. Hurwitz, D. V. Ivanov, M. Q. Johnson, T. James, T. A. Huw Jones, G.-D. Kang, T. H. Kerelska, A. D. Kersey, I. Khrebtukova, A. P. Kindwall, Z. Kingsbury, P. I. Kokko-Gonzales, A. Kumar, M. A. Laurent, C. T. Lawley, S. E. Lee, X. Lee, A. K. Liao, J. A. Loch, M. Lok, S. Luo, R. M. Mammen, J. W. Martin, P. G. McCauley, P. McNitt, P. Mehta, K. W. Moon, J. W. Mullens, T. Newington, Z. Ning, B. Ling Ng, S. M. Novo, M. J. O'Neill, M. A. Osborne, A. Osnowski, O. Ostadan, L. L. Paraschos, L. Pickering, A. C. Pike, A. C. Pike, D. Chris Pinkard, D. P. Pliskin, J. Podhasky, V. J. Quijano, C. Raczy, V. H. Rae, S. R. Rawlings, A. Chiva Rodriguez, P. M. Roe, J. Rogers, M. C. Rogert Bacigalupo, N. Romanov, A. Romieu, R. K. Roth, N. J. Rourke, S. T. Ruediger, E. Rusman, R. M. Sanches-Kuiper, M. R. Schenker, J. M. Seoane, R. J. Shaw, M. K. Shiver, S. W. Short, N. L. Sizto, J. P. Sluis, M. A. Smith, J. Ernest Sohna, E. J. Spence, K. Stevens, N. Sutton, L. Szajkowski, C. L. Tregidgo, G. Turcatti, S. vandeVondele, Y. Verhovsky, S. M. Virk, S. Wakelin, G. C. Walcott, J. Wang, G. J. Worsley, J. Yan, L. Yau, M. Zuerlein, J. Rogers, J. C. Mullikin, M. E. Hurler, N. J. McCooke, J. S. West, F. L. Oaks, P. L. Lundberg, D. Klenerman, R. Durbin, A. J. Smith, *Nature* **2008**, *456*, 53–59.
- [87] D. A. Wheeler, M. Srinivasan, M. Egholm, Y. Shen, L. Chen, A. McGuire, W. He, Y.-J. Chen, V. Makhijani, G. T. Roth, X. Gomes, K. Tartaro, F. Niazi, C. L. Turcotte, G. P. Irzyk, J. R. Lupski, C. Chinault, X.-Z. Song, Y. Liu, Y. Yuan, L. Nazareth, X. Qin, D. M. Muzny, M. Margulies, G. M. Weinstock, R. A. Gibbs, J. M. Rothberg, *Nature* **2008**, *452*, 872–876.
- [88] C. J. Gerry, Z. Yang, M. Stasi, S. L. Schreiber, *Org. Lett.* **2019**, *21*, 1325–1330.
- [89] M. K. Škopić, K. Götte, C. Gramse, M. Dieter, S. Pospich, S. Raunser, R. Weberskirch, A. Brunschweiler, *J. Am. Chem. Soc.* **2019**, *141*, 10546–10555.
- [90] V. B. K. Kunig, C. Ehrh, A. Dömling, A. Brunschweiler *Org. Lett.* **2019**, *21*, 7238–7243.
- [91] Novartis, Broad Institute researchers unveil new DNA-encoded library (<https://cen.acs.org/biological-chemistry/dna/Novartis-Broad-Institute-researchers-unveil/97/i34>).
- [92] D. S. Goodsell, L. Autin, A. J. Olson, *Structure* **2019**, *27*, 1716–1720 e1711.

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