

Impact of DNA-Encoded Chemical Library Technology on Drug Discovery

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Abstract: DNA-Encoded Chemical Libraries (DELs) have gained momentum over the recent years for the discovery of small molecule ligands and the technology has been integrated in most of the larger pharmaceutical companies. With this perspective we would like to summarize the development of DEL technology and present some representative DEL-derived hits which may soon enter the pharmaceutical market.

Keywords: DNA-Encoded Chemical Library · small molecule drug discovery · affinity-based screening



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Jörg Scheuermann studied Chemistry at the University of Heidelberg, Germany and at the ETH Zürich, Switzerland. Following his PhD. studies at the ETH under the supervision of Prof. Dario Neri, he co-pioneered the development of DNA-Encoded Library (DEL) technology in different implementations together with Dario Neri. He published over 65 peer-reviewed manuscripts on DEL, wrote his habilitation

thesis on “DNA-Encoded Chemical Library Technology for Drug Discovery” and is co-founder and organizer of the “International Symposium on DNA-Encoded Chemical Libraries”. J S is Principal Investigator at ETH Zürich, heading a group of currently 5 PhD students and 1 Postdoc. His main research interests are the development of novel DEL architectures, selection methodologies and the tailored construction of DELs for difficult targets.

1. Introduction

1.1 Traditional Small Molecule Drug Discovery

Small molecule drug discovery on individual targets of interest has traditionally been pursued by screening conventional, arrayed libraries of small organic molecules, which are screened one-by-one by high-throughput screening (HTS).^[1] The respective arrayed libraries typically do not exceed a few million compounds, and are produced as individual molecules or as small mixtures of compounds by combinatorial split-and-pool methodology. Typically, due to the necessary large infrastructure, the production of larger libraries and HTS is mainly performed by pharmaceutical companies rather than in an academic context. Interesting strategies for the diversification of chemical structures have been devised, e.g., through diversity-oriented synthesis (DOS)^[2] but also these methods suffer from the same restrictions concerning manageable library size and screening infrastructure. On the side of larger compounds, natural display technologies, such as phage display,^[3] yeast display,^[4] mRNA-display^[5] or ribosome display^[6] have proven useful for the creation of encoded libraries using proteinogenic building blocks. With these natural display technologies, valuable high-molecular weight binders could be identified and developed.^[7]

1.2 DNA-Encoded Chemical Libraries

In analogy to the natural display technologies, DNA-Encoded Chemical Libraries (DELs) feature small molecules individually tagged with a DNA barcode, which allows for the identification of target-binding ligands after PCR amplification and DNA-sequencing.^[8] A DEL typically comprises 10⁵–10⁸ library members which may all be tested for binding to a target protein in a single experiment. Thanks to the DNA tag a DEL can be stored in a single tube which greatly reduces the need for complicated and expensive infrastructure for storage and screening.

Over the last couple of years, DEL technology has been implemented as a tool for small molecule ligand discovery by essentially all big pharmaceutical companies and also by academic groups.^[9] While the latter generally focus on the development of novel DNA-compatible chemistries, just a few academic groups practice DEL technology from A-Z, i.e. from DEL generation to in-house screening of targets of interest, hit validation and development. Small molecule ligands derived from DELs are increas-

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ingly fueling the drug pipelines of pharmaceutical companies in various fields and are likely to enter the market within the next couple of years (*vide infra*).

2. DEL Selections

DNA-Encoded Chemical Libraries (DELs) are collections of small molecules which are individually tagged with DNA fragments encoding the respective small molecule's identity.^[8b,10] Compared to HTS, where compounds are screened one-by-one, DEL selections can be performed at once for all library members together. This allows for the performance of affinity-based selections against targets of interest in a rapid fashion compared to other small molecule drug discovery methods.

To perform DEL selections, the target protein typically is immobilized on a solid support (Fig. 1).^[11] Proteins used in such DEL selections require a tag such as biotin or poly-histidine for immobilization onto the solid support coated with streptavidin, or nickel/cobalt, respectively. Magnetic beads or coated tips are often used as the solid support, which also allows for automated DEL selections.^[11-12] After immobilization of the target protein on the solid support it is incubated with the DEL. During this process, some library members may bind to the protein preferentially compared to others. After the incubation period, the solid support is usually washed repeatedly to remove non-bound library members. The DEL members which remain bound to the target protein can then be released from the solid support, for example by heat denaturation of the target protein.^[11] The eluted DEL members should then reflect the strength of binding to the target protein by their relative abundance. To uncover the identity and relative abundance of library members obtained after selections, the DNA barcodes of the eluted library members are first amplified by PCR. Through this process, a sufficient amount of DNA for high-throughput DNA-sequencing (HTDS) is produced.^[8d] The sequencing data can be processed and visualized with 2-D or 3-D plots, known as selection "fingerprints", reflecting how often each library member was counted in the HTDS.^[11] As a result, if a library member binds preferentially to the target protein, its normalized sequence counts are higher compared to those of the other library members.

In some instances, single points show higher enrichments compared to the background, or the counts of other library mem-

bers. In other cases, multiple library members with an identical building block x, for example, may be enriched. This gives additional information concerning structure-activity relationships which may help medicinal chemists to prioritize certain hits.^[13]

Several advancements in DEL selection methodologies have recently been made and are about to be implemented, such as photocrosslinking,^[14] reversible-covalent anchoring,^[15] and selections performed in solution.^[16] While still not well established, also selections on cells and in even in cells have been reported.^[16]

3. Architectures of DELs

DELs exist in many different formats, and can be constructed using different approaches. Both the small molecule and the DNA moiety of a DEL may be assembled to give rise to different geometries, and different methods may be used for library synthesis. Most commonly, the small molecules displayed in a DEL are made up of different subunits, or building blocks. The small molecule part in a DEL can be synthesized by consecutively attaching sets of building blocks. The DNA moiety of the DEL comprises a coding sequence for each of the building blocks and sequencing of the DNA tags after selections will identify the structure of the small molecule.

Two main approaches for the synthesis of DELs are DNA-templated synthesis^[17] and DNA-recorded synthesis (Fig. 2).^[8d, 18] DNA-templated synthesis commences by the synthesis of a library of DNA templates comprising coding portions for the building blocks in the library, and containing a linker which can be chemically modified and on which the final chemical portion can be assembled.^[17] For library synthesis, the DNA templates are incubated with the mixture of building blocks linked to oligonucleotides which are complementary to the coding regions on the template. The template hybridizes with the complementary oligonucleotide-linked reagents, so that the correct corresponding building block reacts with the template-linked chemical structure. By hybridization of the reagents with the template, the effective molarity of reagents is increased. Liu and co-workers, which have pioneered DNA-templated synthesis, have shown that this approach is suitable for the synthesis of macrocyclic DELs (Fig. 3).^[17] While DNA-templated synthesis conceptually is fascinating and interesting, macrocyclic libraries have been constructed

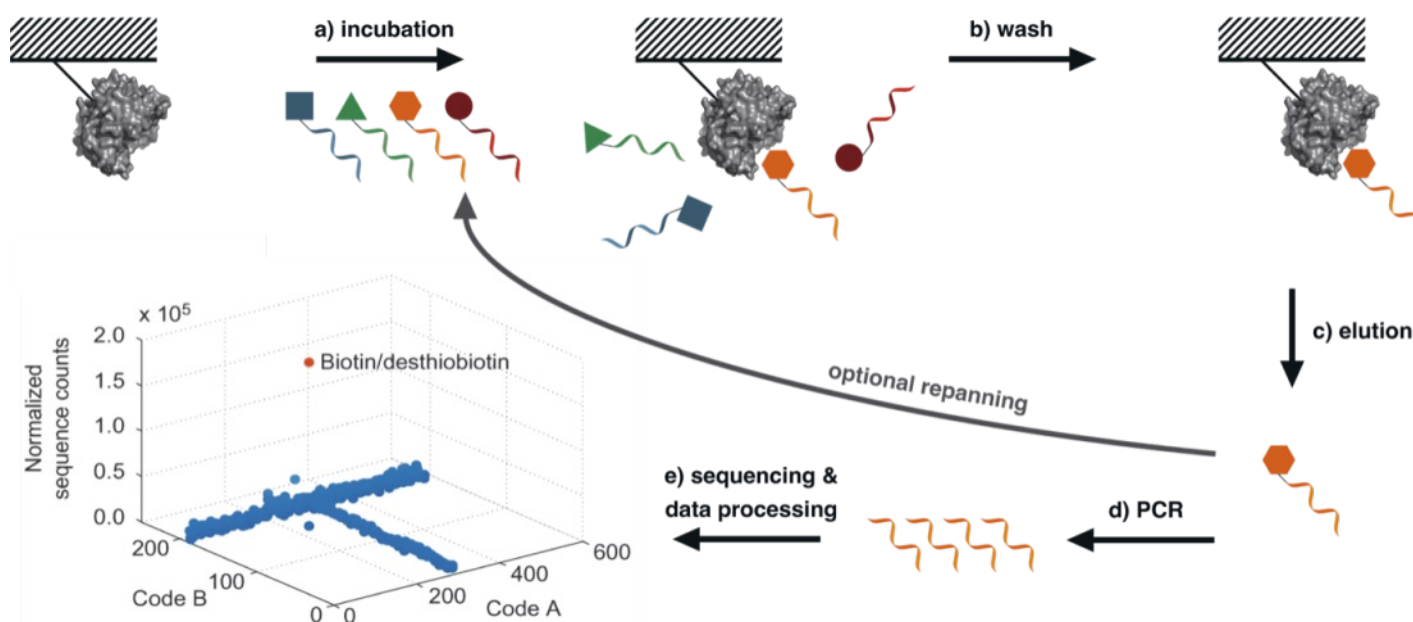


Fig. 1. DNA-Encoded Chemical Library (DEL) selection procedure. a) The protein target immobilized on a solid support is incubated with the DEL. b) Non-bound DEL members are removed by washing. c) The library members bound to the target are eluted. d) The DNA tags of the eluted library members are amplified by PCR. e) Sequencing of the DNA tags provides the enrichment of individual binders and can be visualized through data processing in a "fingerprint" plot.

using this approach, it has not often been used in practice for the synthesis of very large DELs. This is likely due to the fact that the reagent oligonucleotide conjugates need to be prepared, which becomes more tedious with increasing library size. Furthermore, the correct hybridization of templates with reagent oligonucleotides may become less precise with an increasing number of codes. In a similar templated approach, the company Viperger employs three-way junction DNA-templated synthesis.^[19]

The second and in fact most applied approach for the synthesis of DELs is DNA-recorded synthesis.^[8d,18] In this method the DNA tag of the DEL generally is extended by ligation steps, in order to add distinct encoding sequences. Library synthesis starts by attaching a set of first building blocks to the DNA tag. The resulting DNA conjugates are subsequently encoded by ligation and potentially purified. All individual conjugates are then mixed and split into different wells for the attachment of a further building block and ligation of a corresponding DNA code. Depending on the number of split-and-pool steps performed, the theoretical library size grows exponentially and DELs of very large sizes can theoretically be obtained. In practice, due to decreasing display yields, the quality and performance of a DEL typically suffers with an increasing number of split-and-pool steps.

DELs which comprise the small molecules attached to the DNA via one attachment point are termed single-pharmacophore DELs. Single-pharmacophore DELs with 2–4 building blocks are most commonly synthesized,^[8d,16,18b,18c] and also macrocyclic libraries have been reported (Fig. 3).^[20] Using the universal head-piece or the Klenow fill-in approach a wide range of DELs with a double-stranded DNA have been constructed.^[8b,21] More recently, single-pharmacophore DELs have become increasingly popular, as they offer distinct advantages in terms of pairing with complementary DNA strands carrying either a photocrosslinker,^[14] a covalent or reversible-covalent moiety^[15] or an already existing hit compound.^[22]

In 2004, our group at ETH first reported recorded DEL technology, in the implementation of a dual-pharmacophore DEL.^[18a]

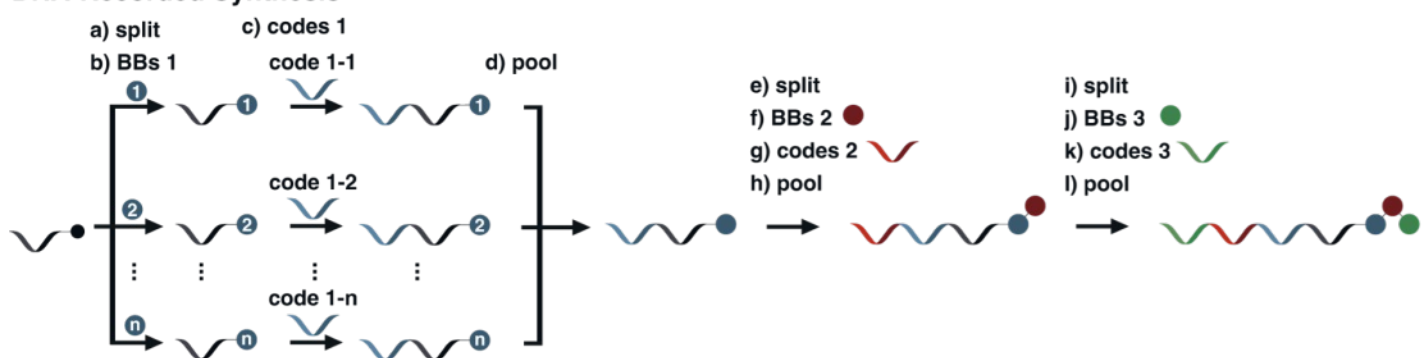
In this approach, two single-stranded sublibraries, each comprising a building block attached to an encoding DNA fragment, are hybridized to give an encoded self-assembling chemical library (ESAC).^[18a] This approach benefits from the avidity effect for combination of fragments. Several ESAC libraries have been constructed in a fragment-like ‘1+1’ fashion, where one building block is presented on each DNA strand,^[8b,18a] and more drug-like ‘2+1’ and ‘2+2’ libraries are currently being constructed. A distinct benefit of dual-display libraries may be the potential to affinity-mature an already existing hit by displaying it on one DNA strand and pairing it with a library on the complementary strand.^[22] Similarly, dual-display PNA-Encoded Chemical Libraries have been pioneered by Winssinger and co-workers, where peptide nucleic acid (PNA) is used instead of DNA.^[23] Recently, an elegant strategy for evolving and diversifying PNA-Encoded libraries has also been reported by Winssinger and co-workers.^[24]

DNA-recorded synthesis has also been used for the synthesis of DNA-tagged one-bead one-compound (OBOC) libraries.^[25] OBOC DELs comprise displayed small molecules attached to a solid support, as well as a DNA barcode attached to the solid support. Typically, the DNA tag is used in a substoichiometric amount compared to the displayed small molecule. This approach can be useful in activity-based assays which have been shown to be automatable using microfluidic systems for not too large DEL sizes, as each compound is screened individually.^[26]

The DELs described above display a fixed population of library members. In contrast, DNA-Encoded Dynamic Libraries (DEDLs) are dual-display libraries featuring a tunable hybridization of complementary DNA strands, so that the target-binding dual-display library members are dynamically formed on the target, which may allow for detecting weaker binders.^[15a,27]

Nowadays, most pharmaceutical companies increasingly use DEL technology for in-house small molecule drug discovery. In 2009, following ETH's initial publications on split-and-pool DEL technology, Praecis, now GlaxoSmithKline (GSK), published a seminal work on the split-and-pool DNA-recorded synthesis of

DNA-Recorded Synthesis



DNA-Templated Synthesis

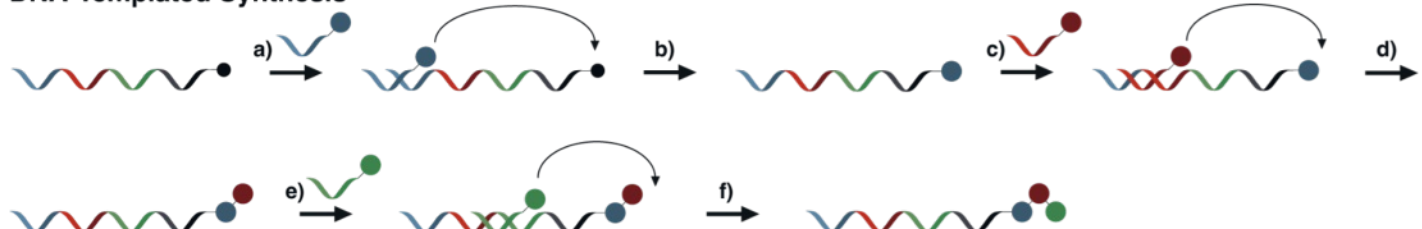


Fig. 2. Methods for the synthesis of DNA-Encoded Chemical Libraries (DELs). DNA-recorded synthesis (top): a) Splitting of the DNA conjugate into separate wells. b) Addition of a structurally distinct building block (BB) 1 for reaction. c) Ligation of barcode 1. d) Pooling into one pot. e) Splitting into separate wells. f) Reaction with BBs 2. g) Ligation to codes 2. h) Pooling into one pot. i) Splitting into separate wells. j) Reaction with BBs 3. k) Ligation to codes 3. l) Pooling into one well.

DNA-templated synthesis (bottom): a) Hybridization of BB 1 reagent oligonucleotides with the templates. b) Transfer of BBs 1 onto the templates. c) Hybridization of BB 2 reagent oligonucleotides with the templates. d) Transfer of BBs 2 onto the templates. e) Hybridization of BB 3 reagent oligonucleotides with the templates. f) Transfer of BBs 3 onto the templates. Potentially, a ring-closing reaction may follow.

1) One-Bead One-Compound DELs

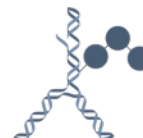


Scripps 1993
Scripps / UC Irvine 2015, 2017, 2019
POSTECH 2019
Scripps 2020

2) DNA-Templated Synthesis



Harvard University
2004, 2008, 2018
Peking University 2013
Ensemble Therapeutics 2015



YoctoReactor
ViperGen 2009, 2016

3) DNA-Recorded Synthesis
a) Single Pharmacophore DELs

2–4 BB double-stranded
ETH Zürich 2008, 2008, 2008,
2010, 2011, 2012, 2015, 2018
GSK 2009, 2015, 2016, 2017
Philochem 2015
X-Chem 2015, 2017, 2020
Roche 2019
University of Utah 2019
Baylor 2020, 2020
TU Dortmund 2020



2–4 BB single-stranded
ETH Zürich 2020, 2021



Directed BB display
ETH Zürich 2018



Macrocytic
GSK 2018
University of Basel 2019
ETH Zürich 2021
Nankai University 2021

b) Dual Pharmacophore DELs



ESAC '1+1'
ETH Zürich 2004, 2007, 2015, 2017
Northwest University, Xi'an 2022



Large Encoding Design
ETH Zürich 2022



PNA-encoded libraries
University of Geneva
2009, 2015, 2022



ESAC '2+1'
ETH Zürich 2020



DNA-Encoded Dynamic Libraries (DEDLs)
University of Peking 2015, 2018, 2020, 2020
TU Dresden 2015, 2019

Fig. 3. Overview of published DNA-Encoded Chemical Library architectures

large 3- and 4-building block DELs based on a double-stranded DNA headpiece.^[18c] Since then, DEL synthesis in industry has mostly been based on this headpiece approach. Pharmaceutical companies typically synthesize large DELs by employing a maximal number of building blocks per synthetic step,^[18c] typically consisting of 2–4 sets of building blocks. This approach has proven very successful for certain protein classes, such as proteins with defined binding pockets. However, it is conceivable that other protein classes, such as proteins with extended binding surfaces, may require screening with DELs of more diverse or extended architectures. Some groups, including ours, are focused on expanding DEL technology beyond the current constraints by developing methods to include more diverse chemistries, more building block positions, or achieving more diverse DEL architectures, as well as novel encoding strategies and selection methods.

4. Considerations for Drug Discovery by DELs

DEL technology has proven to be a useful pillar for small molecule drug discovery. For reliable results from DEL selections, two crucial aspects must be considered, the quality of the constructed DELs and the quality of DEL selections. DEL quality is determined by several factors. Ideally, a constructed DEL comprises an equal amount of each library member, and every small molecule as well as DNA barcode has been properly assembled with complete conversion. Firstly, the small molecule synthesized by the assembly of building blocks with the scaffold should be obtained in high yield. Additionally, the reactions involved in DEL synthesis should be DNA compatible, i.e., the reagents used should not damage the DNA tag. In split-and-pool DEL synthesis, the individual emergent library members can only be individu-

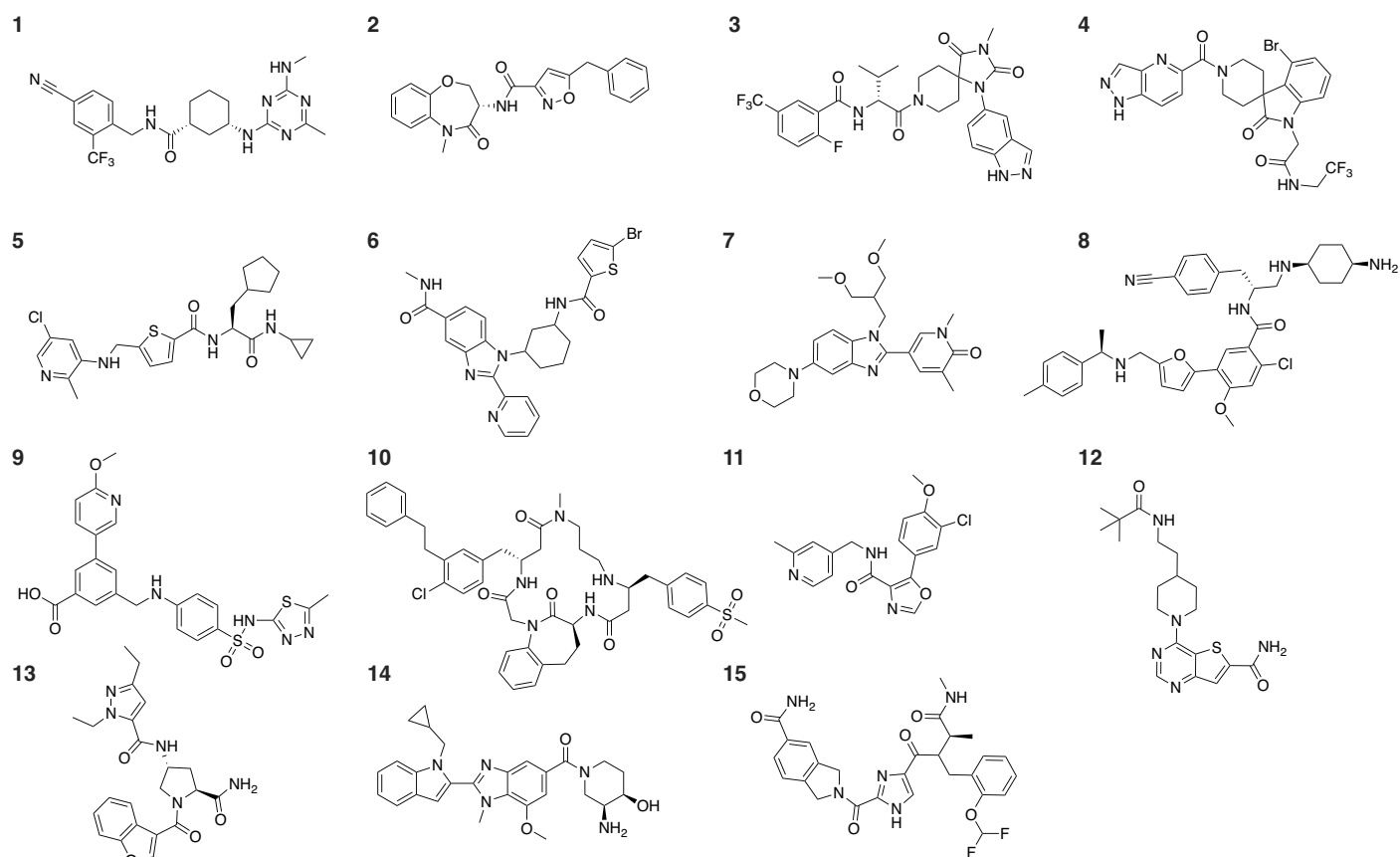
ally purified by HPLC after the attachment of the first building block, since at a later stage in DEL construction, compounds are always present in mixtures. It is obvious that with each additional transformation or building block attachment step, the overall library yield and purity will decrease. Further, the quality of a DEL is determined by its encoding quality. Our group is focusing on developing new DEL synthesis methods to enable the construction of DELs with greater structural diversity and has recently reported a novel large encoding design (LED) which may incorporate several codes into one amplifiable DNA strand.^[28] Obtaining a high purity of individual library members will eventually lead to a uniform distribution of DEL members and hence minimize the background and variability in DEL selections.

A further major impact on the overall quality of DEL selections is given by the quality of the target, particularly the recombinant protein itself. The first obstacle arises during the purification process. Affinity- and solution based selections are ideally performed with pure recombinant protein, however, the production and a limited stability of some proteins can be challenging. For affinity-based selections the capture of proteins is crucial. Suitable affinity tags for non-covalent attachment of proteins, as His-tag and Avi-tag, feature different binding strengths, requiring optimized washing and buffer conditions for the respective DEL selection. However, there is still great potential in improving DEL selection performance. The field will benefit from alternative selection strategies, such as *in vivo* selections on living cells^[15c,29] and in living cells,^[29b,30] however these approaches are still in the fledgling stages.

5. DEL Technology in Industry and Academia

The concept of DELs was originally proposed by Richard Lerner and Sydney Brenner at Scripps Research in 1992^[10a] who aimed at facilitating the identification of combinatorially synthesized library members by genetically encoding each chemical moiety. While first tests of this strategy were performed^[31] the approach was stalled in practice due to the difficulties presented by the need to alternately perform efficient peptide and oligonucleotide synthesis. The DEL concept revived only about a decade later, with the advancement of DNA-compatible reactions and encoding methods, and the advent of high-throughput DNA se-

quencing methodologies. The first DELs were presented by only a few academic groups. David Liu at Harvard University constructed a 65-member macrocyclic library by *DNA-templated synthesis* and performed first test selections on *Carbonic Anhydrase*^[17a]. At the same time our group at ETH Zürich synthesized and screened a first DNA-recorded 137-member ESAC library^[18a]. Concomitantly, industry started investigating DEL technology as drug discovery tool. In 2009, GSK performed affinity selections on a 800 million member DEL against *Aurora A kinase* and *p38 MAP kinase* and selections of a DEL against enzyme targets resulted in the *de novo* discovery of potent inhibitors.^[18c] From



DEL Lead	Target Protein	IC50	Indication	References
1	sEH*	27 pM	Aneurysmal Subarachnoid Hemorrhage	Martini 2021 ^[35] , Podolin 2013 ^[34]
2	RIPK1*	1.3 nM	Psoriasis, Rheumatoid Arthritis, Ulcerative Colitis	Harris 2016 ^[36] , Weisel 2021 ^[37]
3	ATX*	55 nM	Pulmonary Fibrosis	Cuozzo 2020 ^[21e]
4	DDR1	29 nM	Renal Fibrosis	Richter 2019 ^[21b]
5	WIP1	6 nM	Cancer	Gilmartin 2014 ^[38d]
6	BCATm	50 nM	Metabolic Disorders	Deng 2016 ^[38a]
7	BRD4	13 nM	Cancer, Inflammatory Diseases, Viral Infections	Wellaway 2020 ^[38j]
8	ATAD2	166 nM	Cancer	Fernandez-Montalvan 2017 ^[38c]
9	PI3Ka	10 nM	Cancer	Yang 2015 ^[38k]
10	Mcl-1	3 nM	Cancer	Johannes 2017 ^[38e]
11	GSK-3b	32 nM	Cancer, Neurological Disorders, Autoimmunity	Panda 2019 ^[38g]
12	SIRT1	15 nM	Metabolic, Oncologic and Neurodegenerative Disorders	Disch 2013 ^[38b]
13	InhA	4 nM	Tuberculosis	Soutter 2016 ^[38h]
14	PAD4	50 nM	Autoimmune, Cardiovascular and Oncologic Diseases	Lewis 2015 ^[38f]
15	TAK1	2 nM	Cancer, Inflammatory Diseases	Veerman 2021 ^[38i]

Fig. 4. Representative lead compounds after optimization of *de novo* discovered hits from DEL selections. *in clinical trials phase 1/2

today's perspective, the total number of pharmaceutical companies and academic groups working on DEL technology significantly grew within the last 10 years. Currently, by far most academic groups working in the DEL field are chemistry-related. On the other hand, only a few academic groups in Switzerland, Germany, the USA and China are specializing on the whole DEL drug discovery process, featuring library design, synthesis, selections, hit validation and affinity maturation.

6. Representative Clinical Candidates and Leads Discovered Using DEL Technology

Selection experiments against disease-related antigens have resulted in the identification of several lead compounds, some of which later entered clinical development (Fig. 4). In 2013, GSK published the discovery of 1-(1,3,5-triazin-2-yl)piperidine-4-carboxamide inhibitors against soluble epoxide hydrolase (sEH).^[32] The respective compound, GSK2256294, was derived from a triazine scaffold-based DEL which was synthesized in 2009.^[18c,33] After lead optimization, GSK2256294 resulted in a 27 pM (IC₅₀) binder,^[34] that by now has entered clinical phase 1 and 2 for the treatment of neuroinflammatory diseases.^[35] In 2016, GSK published the discovery of benzoxazepinone inhibitors against the receptor-interacting protein kinase 1 (RIPK1) by screening in-house DELs. After lead optimization, the kinase inhibitor GSK2982772 reached an IC₅₀ of 1.3 nM.^[36] At present, the compound is in phase 1 and phase 2 clinical trials for the treatment of inflammatory diseases, such as ulcerative colitis and rheumatoid arthritis.^[37] Furthermore, the FDA recently accepted the application for the autotaxin inhibitor X-165, developed by the biotech company X-Chem. The compound was discovered from a 225 million-member DEL, showing inhibitory effects in a mouse model of human lung fibrosis.^[21e] Additionally, dozens of low nanomolar leads which have not yet reached clinical trial, were discovered using DEL technology.^[21b,38] Many of them include binders against tumor-associated antigens and inflammatory diseases (Fig. 4). Recently, also inhibitors against SARS CoV-2 main protease M^{pro} were discovered using the DEL platform, displaying low micromolar IC₅₀ values.^[39] In the past decade, a collaboration between our group at ETH and the company Philochem resulted in the development of small molecule drug conjugates (SMDCs) based on the discovery of ligands from *de novo* and affinity maturation experiments. A ^{99m}Tc-labeled derivative of a binder that was previously discovered by affinity maturation experiments from a dual-display DEL^[40] is currently examined in a clinical phase 1 trial for nuclear imaging in renal cell carcinoma patients.^[41]

7. Outlook

Over the last two decades, DEL technology has become a trusted resource for small molecule ligand discovery and has made its way into the pharmaceutical industry. Small molecule ligands can be linked to cytotoxic effector functions, serving as therapeutic small molecule drug conjugates, in full analogy to antibody-drug conjugates (ADCs). In fact, SMDCs may display certain pharmacokinetic and targeting advantages over ADCs, as extravasation of smaller molecules occurs more easily.

On the other hand, also basic biological science is likely to profit from DEL technology, as cell-permeable ligands to intracellular targets may be obtained, which can serve as important biochemical tool to study cellular function. Indeed, an initiative termed "Target 2035" has been started which seeks to develop specific small molecule ligands for all proteins of the human proteome. While this is obviously an extremely challenging task, DEL technology may be the technology of choice to approach this aim. DEL technology is not a "finalized" technology improvement, but can certainly be further developed in terms of chemical diversity, scaffolding, quality and uniformity of synthesis, as well

as tailored selection development. DEL technology does not redundantly medicinally chemistry at all but in fact might open the door to efficient lead development by intrinsic structure activity relationship (SAR) information. DEL technology-derived drugs will likely enter the pharmaceutical market in the coming years and fuel the small molecule drug pipelines, and hence further grow in importance.

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