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Universal encoding of next generation DNA-encoded chemical libraries†

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DNA-encoded chemical libraries (DELs) are useful tools for the discovery of small molecule ligands to protein targets of pharmaceutical interest. Compared with single-pharmacophore DELs, dual-pharmacophore DELs simultaneously display two chemical moieties on both DNA strands, and allow for the construction of highly diverse and pure libraries, with a potential for targeting larger protein surfaces. Although methods for the encoding of simple, fragment-like dual-display libraries have been established, more complex libraries require a different encoding strategy. Here, we present a robust and convenient “large encoding design” (LED), which facilitates the PCR-amplification of multiple codes distributed among two partially complementary DNA strands. We experimentally implemented multiple coding regions and we compared the new DNA encoding scheme with previously reported dual-display DEL modalities in terms of amplifiability and performance in test selections against two target proteins. With the LED methodology in place, we foresee the construction and screening of DELs of unprecedented sizes and designs.

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Introduction

DNA-encoded chemical libraries (DELs) are collections of small molecules, each connected to a unique encoding DNA sequence.¹ As all compounds are individually encoded, this system allows for the efficient one-pot screening of an unprecedented number of compounds by affinity-based selections on a protein target of interest. The identity of the selected molecules can be retrieved by PCR amplification of the selected DNA tags and subsequent analysis by high-throughput DNA sequencing.^{2,3} DEL technology is increasingly recognized and implemented as an important tool for the *de novo* discovery of ligands.^{4–8} However, obtaining ligands for “difficult” targets – e.g., protein–protein interactions – may remain challenging when using Lipinski’s rule of five compliant single-pharmacophore DELs, displaying typically compounds consisting of two or three sets of diversity elements.^{9–12} While some approaches are currently explored to create better performing single-pharmacophore DELs, for example by developing new DNA-compatible reactions,^{13–15} adopting diverse scaffolds and building blocks,^{16–18} or exploring different spatial building block arrangements,^{10,19,20} another promising option to tackle difficult targets may be exploring

larger binding surfaces.²¹ This has been attempted, for instance, by generating macrocyclic DELs,^{16,22–25} but large libraries in general will require the introduction of several sets of building blocks, thereby lowering DEL library quality.

The most widely used setup is the single-pharmacophore DEL, built on a “headpiece” linker on double-stranded-DNA, and it is well-suited for defined protein pockets. An attractive strategy to access larger protein surfaces may consist in the simultaneous display of two pharmacophores (“dual display”) as implemented in Encoded Self-Assembling Chemical (ESAC) libraries (Fig. 1A).^{26,27} Dual-display libraries feature the combinatorial assembly of independent sub-libraries, which allows to form large libraries containing multiple diversity elements.²⁸ These large dual-display DELs can be obtained in high quality with rather little synthetic effort. Since building block coupling occurs separately on both strands of the DNA heteroduplex, the self-assembly of mutually complementary DELs may allow the construction of larger and purer combinatorial libraries, compared to conventional split-and-pool single-pharmacophore DELs.^{26,27,29–31} While dual-display technology requires the optimization of the linkage of the displayed pharmacophores,³² the technology may allow for the versatile combination of two single-stranded DELs. To this aim, recent work from our group has explored encoding modalities to assemble pre-existing single-stranded DELs.³³

Initially, self-assembling dual-display DELs described the combination of two sets of building blocks in a “1 + 1” setting, one on each complementary DNA strand, as depicted in Fig. 1A.^{27,31} Then combinatorial display of small molecule

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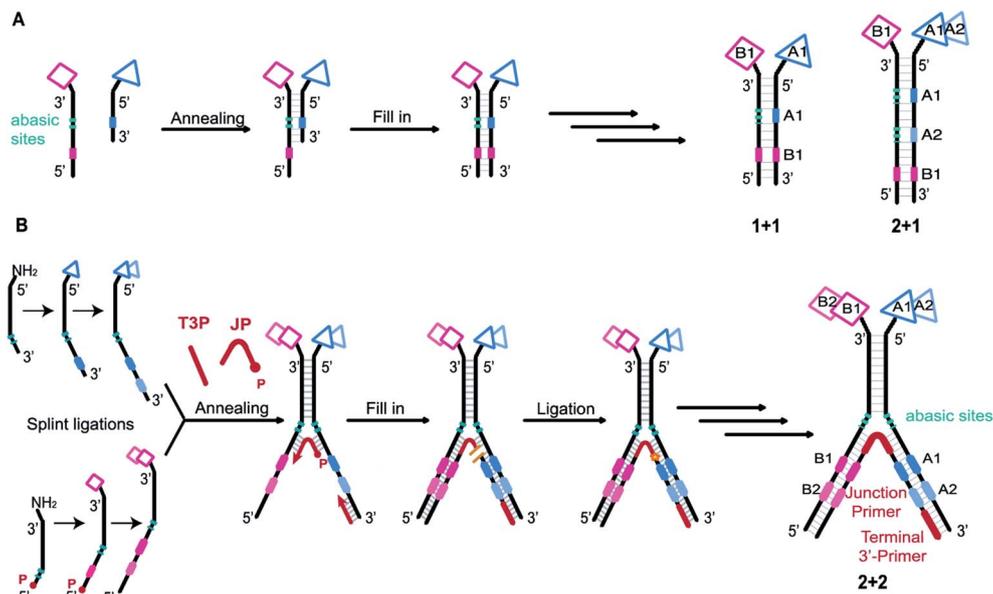


Fig. 1 (A) Previous work. Schematic representation of a conventional ESAC library construction and examples of ESAC formats with “1 + 1” or “2 + 1” chemical building blocks. Two complementary sub-libraries each bearing one building block are annealed. The first coding region (in blue) is opposed to abasic sites while the second coding region (in pink) can be transcribed onto the blue strand by fill-in polymerisation, thus creating a unique DNA strand bearing all the coding information for subsequent PCR amplification and DNA sequencing. This scheme could be applied to “1 + 1” or “2 + 1” setups. (B) This work. Schematic representation of LED library construction and “2 + 2” LED setup used for this study. Two partially complementary sub-libraries are synthesized, hybridized and annealed with a junction primer (JP) and a terminal 3'-primer (T3P). Using T4 DNA polymerase the coding information is transferred onto two DNA strands which are subsequently ligated by T4 DNA ligase. P: phosphate; JP: junction primer; T3P: terminal 3'-primer.

fragments was investigated with DNA-templated synthesis^{34–37} but DNA-recorded synthesis eventually proved to be a more versatile strategy for the construction of large DELs. More recently, the ESAC construct was further developed with the combination of a conventional, single-stranded DEL of two diversity elements, paired with a sub-library of one building block on the complementary strand, in a “2 + 1” setting.³⁸ For the creation of even larger dual-display DELs, this conventional encoding scheme cannot be easily adapted (*e.g.*, for a “2 + 2” or any “*m* + *n*” setting) nor efficiently implemented.

We here propose a new encoding strategy named “Large Encoding Design” (LED), which offers a convenient solution for the construction of dual-display DELs of unprecedented size. We were inspired by the works of Xiaoyu Li's^{39–42} and Yixin Zhang's^{43,44} laboratories on Y-shaped dynamic self-assembling DELs featuring a three-way junction approach. In the dynamic setting, partially complementary sub-libraries bearing one set of building blocks each were allowed to pair in the presence of a target protein and the coding regions of both sub-libraries could be joined after selection into a unique, amplifiable DNA strand.

In contrast, LED features the stable display of two (or more) sets of diversity elements on complementary DNA strands, allowing to produce and store large combinatorial dual-display DELs which can then be submitted to affinity-based selections against the protein targets of interest.

We implemented LED with a unique set of four codes and subsequently tested the system in model selection experiments,

while systematically studying selection conditions, in order to establish robust methods for DEL construction and screening. As high-quality DEL screenings depend on the reliable and sensitive amplification of the DNA after selections,⁴⁵ we identified the minimum amount of DNA construct which could be confidently PCR amplified. We next investigated the minimum amount of DNA which is required for a proper selection experiment and we finally determined the highest possible dilution for a known binder to be retrieved from a dual-display library. To that aim we produced model libraries and compared the LED performance with conventional ESAC model libraries in selections against alpha-glycoprotein 1 (AGP) and carbonic anhydrase II (CAII) thereby demonstrating the potential of LED for future, ambitious dual-display DELs.

Results and discussion

An extended and versatile encoding system

As in the original ESAC approach, LED features the stable display of two combinatorially assembled pharmacophores on complementary DNA strands. Each strand contains a chemical moiety for building block attachment, a hybridization region, an abasic region and one or more coding regions (Fig. 1B). The codes can consecutively be added by splint ligation and encode for the building blocks displayed on their respective DNA strand only. As graphically depicted in Fig. 1B, the process for LED-based DEL library construction starts with the synthesis and encoding of each of the two sub-libraries on its respective DNA



strand. Subsequently, the sub-libraries are annealed together with a terminal 3'-primer (T3P) and a junction primer (JP). DNA polymerase is used to transfer the DNA codes onto the growing primer strands and finally a DNA ligase links the two synthesized DNA strands to form a single amplifiable strand bearing all identification codes. After the affinity-based DEL selection, this strand can be PCR-amplified and sequenced for hit identification.

Implementation

To demonstrate the feasibility of LED, we rehearsed the entire process for two identical sets of codes per DNA strand and we monitored the steps by LC-MS or analytical agarose gel electrophoresis. First, all the required DNA components were formed, starting with the strands II and IV (Fig. 2A). They were built from universal oligonucleotides which possess a hybridization region, four abasic *d*-spacer positions and an overhang of ten nucleotides. With the help of an adaptor, splint ligations were performed between this overhang and a first DNA coding sequence, to obtain strands I and III. Another splint ligation was performed to add the second code, in order to obtain II and IV, respectively. From this point, the sub-libraries were ready to be paired, so the full-length DNA strands II and IV were annealed together through their hybridization regions. The junction primer (JP) and the terminal 3'-primer (T3P) were allowed to anneal as well, to form X which was subsequently submitted to T4 DNA-polymerase mediated polymerization to yield product XI, and finally to T4 DNA ligase-mediated

ligation, yielding XII (Fig. 2B). In XII, the strand depicted in orange bears all the necessary coding information and can be amplified to the PCR product XIII (Fig. 2C). The formation of DNA strands II and IV, as well as the amplification product XIII were easily monitored by analytical agarose gel, while X, XI and XII could be extensively characterized by LC-MS. It is noteworthy to mention that in an actual library setting, selection experiments are performed after the self-assembling step, *i.e.* after the stable formation of XII. With this first experiment we show that the LED encoding could be performed reliably and quantitatively.

Optimization

In contrast to the dynamic setting proposed by Li's group³⁹ we used a stable hybridization region, in line with the ESAC setup previously described by our group.²⁷ Therefore, the LED components are annealed together and submitted to T4 DNA polymerase and T4 DNA ligase before the selection process. This allows for higher concentrations for the enzymatic conversions and for a proper assessment of the library homogeneity. In addition, four abasic nucleotides are added between the coding and the annealing regions in order to avoid the formation of side products when using the T4 DNA polymerase (Fig. 2B, (2)). Finally, a second code was added to each DNA strand, permitting the combinatorial assembly of "2 + 2" (and possibly even more) sets of diversity elements.

The annealing length of the junction primer (JP) to strands II and IV proved to be critical, as the original ten nucleotides

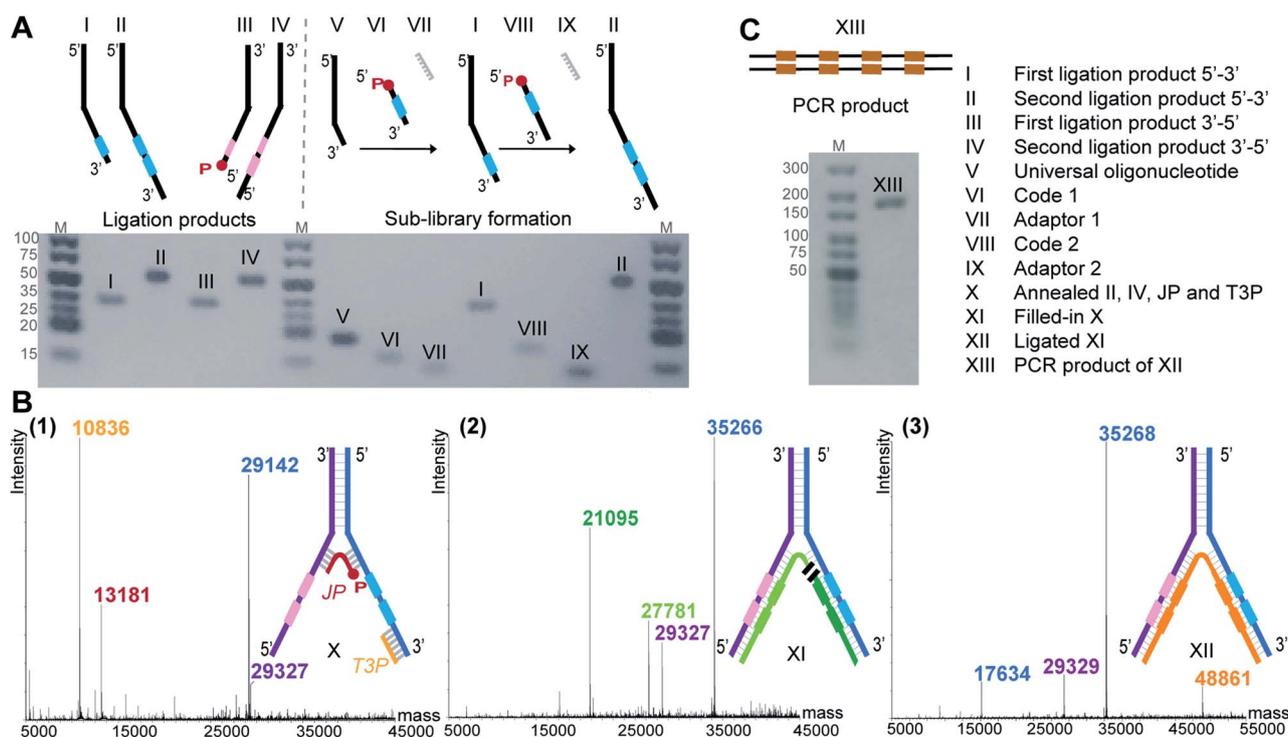


Fig. 2 (A) Agarose gel image (3%) of individual ligation products (I–IV) and of the exemplary preparation of one sub-library (V–VI–VII–I–VIII–IX–II). (B) Formation of the amplifiable DNA strand containing all coding information. (1–3) Deconvoluted LC-MS spectra of (1) assembled strands II, IV, junction primer (JP) and terminal 3'-primer (T3P) forming X, (2) T4 DNA polymerase-mediated fill-in of X yields XI, (3) T4 DNA ligase-mediated ligation of the green XI fragments yields XII. (C) The orange fragment of XII is submitted to PCR yielding product XIII. Agarose gel image (2%).



sequence led to the formation of side products (depicted in orange in Fig. 3A), since the T4 DNA polymerase was able to displace the JP. Such side product leads to a reduction of product **XII**, and hence to a critically reduced amount of DNA that can be sequenced after a DEL selection. In a first attempt to prevent the formation of this side product, two abasic nucleotides were added on strand **II**, just before the annealing region to the JP (Fig. 3B). Even though the formation of the impurity was suppressed, we found that the T4 DNA ligase was now unable to form **XII**, rendering the subsequent PCR amplification impossible. Finally, we decided to increase the length of the JP annealing sequence from ten to twenty bases. The concomitant increase in annealing strength was sufficient to prevent any JP displacement and to ultimately lead to the formation of desired **XII** (Fig. 3C).

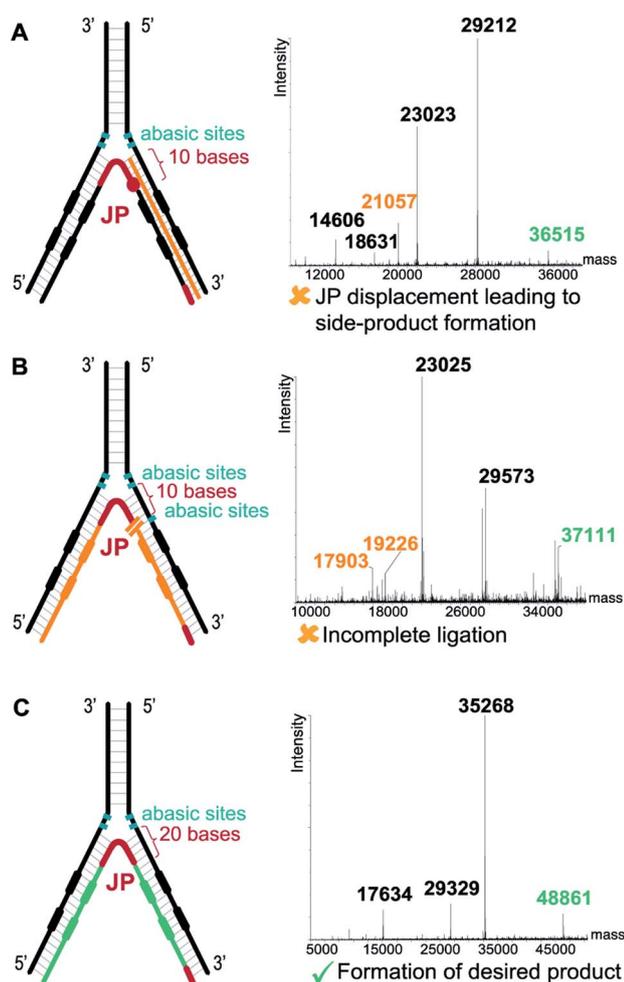


Fig. 3 Critical optimization of the junction primer (JP) system. (A) With a 10-base annealing region the JP is displaced by the T4 DNA polymerase, leading to side product formation (orange strand). (B) Two abasic sites (in green) on the 5' DNA strand prevent JP displacement by the T4 DNA polymerase but hampers the subsequent ligation step. (C) Increasing the JP annealing region to 20 bases prevents displacement. Right panels show the deconvoluted LC-MS spectra with the orange labels depicting the side products/non-ligated fragments, the green labels depicting the desired ligated products.

Quality of the essential ligation step

If more than one set of codes is used, as in the case of an actual DEL, a quantitative ligation step is crucial to assure the correct decoding of simultaneously selected dual-pharmacophores. When, for instance, ligation is incomplete, non-ligated fragments can shuffle in the PCR steps following selections and lead to incorrect combinations of pharmacophores. We assessed this problem for two constructs with distinct sets of codes and hence different molecular masses. Each construct was assembled separately to produce the two distinct constructs represented in dark blue and light blue in Fig. 4. These constructs were then submitted to T4 polymerase and T4 ligase separately as well as a mixture (mimicking a LED library), yielding final constructs which were subsequently PCR-amplified. The PCR products were analyzed by LC-MS and could easily be distinguished for the separate constructs (Fig. 4A and B). The mixture of the constructs showed the expected masses of non-shuffled constructs, as expected from a quantitative ligation (Fig. 4C). However, in a control experiment without ligase, additional masses could be identified as resulting from the shuffling of the two constructs (Fig. 4D). These side-products could not be identified in the positive control (Fig. 4C), indicating that the ligation step proceeded quantitatively, thus reaffirming the integrity of the encoding process.

This essential ligation step was additionally investigated before PCR amplification using fluorescence labeling (Fig. S1†). We repeated the LED construction with a fluoresceinated T3P or with a 5'-fluoresceinated **IV** strand. Polymerization and ligation

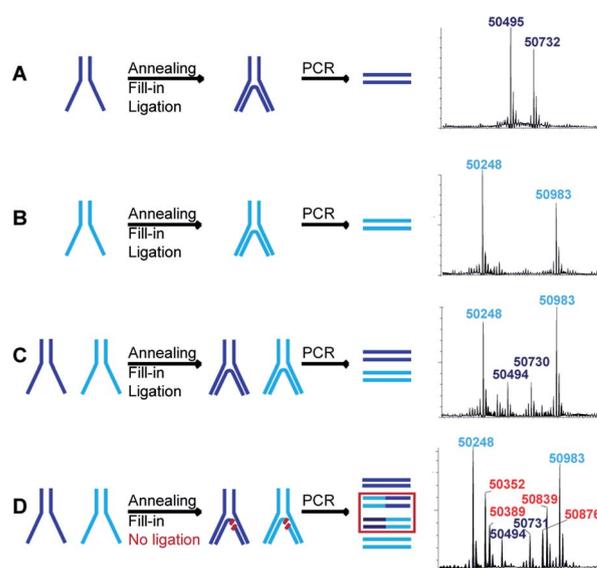


Fig. 4 Assessment of ligation efficiency and concomitant code shuffling potential. Schematic representations of the assembly (annealing, fill-in and ligation) of LED constructs with two distinguishable sets of codes represented in light blue and dark blue. After assembly, the constructs are PCR-amplified and the respective deconvoluted LC-MS spectra of the PCR products are reported. (A) First set of codes assembly and PCR. (B) Second set of codes assembly and PCR. (C) The first and second sets of codes are assembled together and PCR-amplified. (D) Omitting the ligation step results in the production of shuffled side products during PCR amplification.



were analyzed by denaturing SDS-PAGE. As expected, the fluorescent band completely shifted after polymerization and after ligation (Fig. S1, † transition to product 5 and 6), demonstrating quantitative polymerization and ligation.

Lowest level of detection

Since DNA input and recovery are essential parameters during a selection experiment,^{45–47} we wanted to evaluate the sensitivity of our method. To this extent, we performed a dilution series of a LED construct. Each dilution was individually PCR-amplified and analyzed on agarose gel (Fig. 5B), following our established protocol.² The lowest level of detection was found for an input of DNA superior to approximately 3000 copies of LED construct. A negative control without DNA template was included to check for potential contamination. This experiment was additionally performed with single-stranded and double-stranded constructs previously used in our group for benchmarking with single-pharmacophore DEL technology and with a generic ESAC construct for a direct comparison with an already established dual-display system.²⁷

For the other systems, at least 30 000 DNA molecules were necessary to obtain a satisfying PCR amplification. Since all

DNA systems performed quite similarly in this lowest level of detection analysis, we concluded that LED should allow for robust PCR amplification after selection experiments despite the increase of complexity caused by the additional coding regions.

Minimum number of duplexes

For small DEL quantities, determining the precise amount of DNA remaining after a selection experiment can be difficult. Instead, we determined the minimal quantity of library input that, post selection, would yield enough material for reliable PCR amplification. To this end, both for ESAC and LED systems, we prepared simple dual-pharmacophore mixtures containing a positive control against alpha-1-glycoprotein (AGP) and a negative control (Fig. S2 and S3†). AGP is one of the major acute phase proteins of human blood⁴⁸ and was already tested in selection experiments with ESAC libraries by our group.²⁷ A synergistic pair of fragments was identified and optimized, consisting of *N*-([1,1'-biphenyl]-2-yl)-*N*-tosylglycine, and of 3-(5-(2-(trifluoromethyl)phenyl)furan-2-yl)propanoic acid (Table 1).³² Affinity measurements of the optimized ligand displayed on locked nucleic acid yielded a dissociation constant of 108 nM,

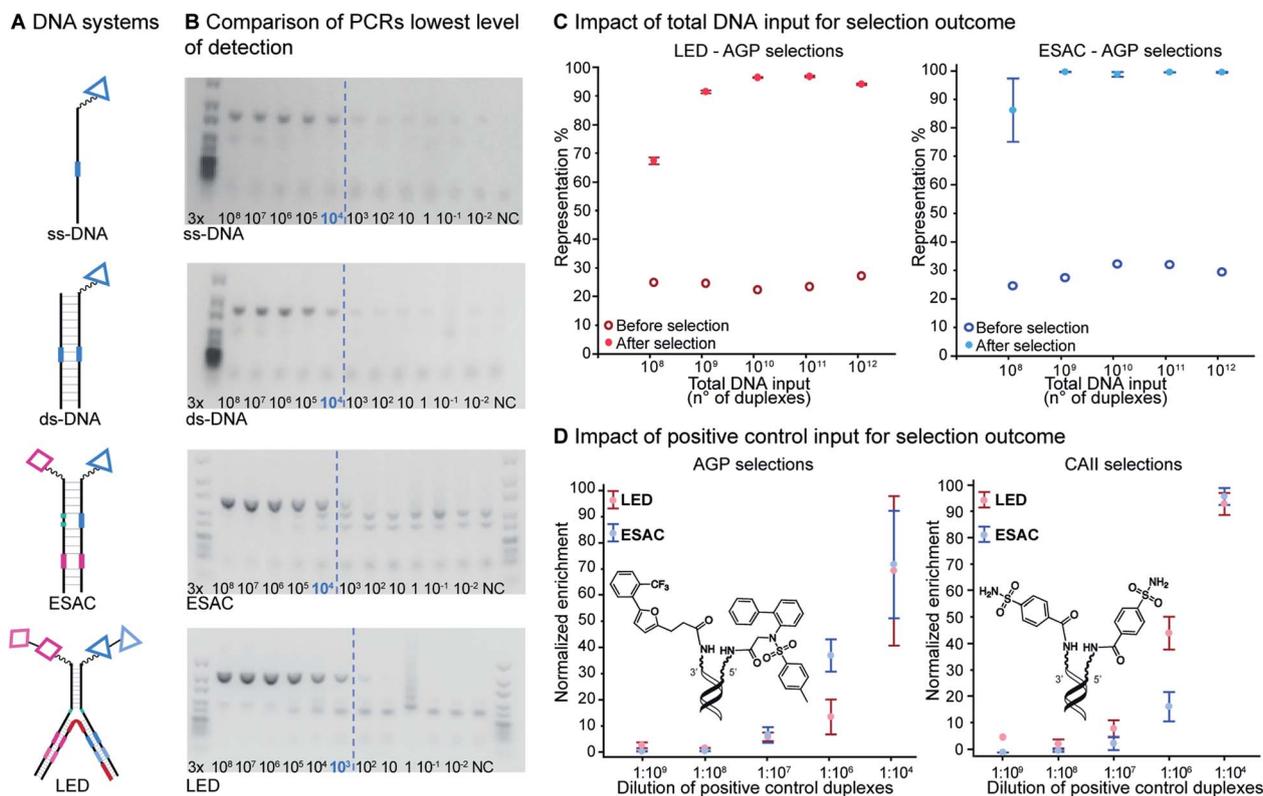
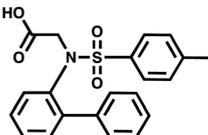
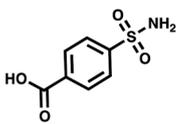
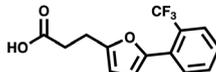
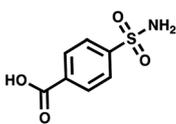


Fig. 5 (A) Representation of different DNA encoding systems, ss: single-stranded; ds: double-stranded. (B) Determination of lowest level of detection for PCR amplification of each DNA encoding system. The input (as number of DNA constructs) for PCR amplification is indicated. The amplification threshold is indicated with a blue dashed line. Negative controls (NC) were performed without DNA construct. Agarose gel image (2%). (C) Model selections results. Representation of positive control duplexes as a function of total number of duplex inputs in affinity-based selections against AGP (filled dots) and, as control, without selection (circles, theoretical value = 25%). Left: LED system, right: ESAC system. (D) Normalized enrichment of positive control duplexes as a function of the dilution of positive control duplexes present in the 10^{13} total DNA construct input, in AGP (left) or CAII (right) selections. Selections were done in triplicates; mean values are represented with corresponding standard error.



Table 1 Building blocks used in the model selections, and the corresponding DNA strands on which they are displayed

DNA strand	Targeted protein	Building block	Chemical structure
5' → 3'	AGP	<i>N</i> -([1,1'-Biphenyl]-2-yl)- <i>N</i> -tosylglycine	
	CA II	4-Sulfamoylbenzoic acid	
	Negative control	Acetic acid	
3' → 5'	AGP	3-(5-(2-(Trifluoromethyl)phenyl)furan-2-yl)propanoic acid	
	CA II	4-Sulfamoylbenzoic acid	
	Negative control	Acetic acid	

rendering this ligand a tool of choice to assess dual-display technologies.

To synthesize the collections of duplexes for ESAC and LED, the corresponding 5'-strands were coupled to the glycine building block and the 3'-strands to the furanpropanoic acid building block. Acetic acid was used as a negative control for both strands (Fig. S2†). The building blocks were encoded on both coding positions of each sub-library, such that the individual readability of each of the four codes could be assessed. Positive and negative control strands were mixed in equimolar ratio to form duplexes. Amplifiable DNA was produced as previously described for LED and ESAC,²⁷ and selections were performed with variable amounts of duplexes, ranging from 10^8 to 10^{12} . Sequencing of the unselected mixtures showed equal repartition of all combinations of building blocks as expected (Fig. S5†). Selections were carried out in triplicate against immobilized AGP following an already described protocol,² and the results are shown in Fig. 5C. The representation of the synergistic binding pair (*i.e.* the fraction of positive control counts against the total number of counts, expressed in percentage (formula (S1)†)), was calculated after selection, PCR amplification and DNA sequencing and is displayed for LED in red and for ESAC in blue. The representation of the three other duplexes was very low, indicating successful selection experiments, and is presented in Fig. S7.† After selection, the representation of the positive control duplex rises with increasing

amounts of DNA in the selection experiment. From the plotted results, we concluded that 10^9 is the minimum number of duplexes needed for reliable selections.

Maximum dilution of positive controls

The last parameter we wanted to investigate in order to establish good selection conditions was the maximum dilution of a positive control in the total of all library members. We performed another set of model selections in which we varied the amount of positive controls while keeping constant the total number of duplexes at 10^{13} , thus introducing different dilutions of the positive controls from $1 : 10^4$ to $1 : 10^9$. Once again, we used AGP and its pair of synergistic fragments as a first positive control. We selected carbonic anhydrase II (CAII) as a second test system, a protein of pharmaceutical relevance,⁴⁹ and as positive control 4-sulfamoylbenzoic acid (SABA), a known CAII ligand. SABA was displayed on both 5' and 3' DNA strands of the duplexes, to assess potential avidity effects (Table 1 and Fig. S4†). Again, acetic acid modification was used as a negative control and each building block was encoded with the two sub-library coding positions. For LED and ESAC, encoded sub-libraries of positive and negative controls were mixed together to produce five model libraries with pre-defined amounts of positive control duplexes, ranging from 10^4 to 10^8 (Table S4†). Sequencing of the unselected model libraries confirmed that the desired dilutions of positive controls



were obtained (Fig. S6†). Selections were carried out in triplicate against AGP and CAII following the same protocol² and PCR amplification was performed. The results of these selections are given in Fig. 5D, where normalized enrichments (formula (S3)†) of the known pairs of binders are plotted against the number of positive duplexes present in each of the model libraries. Results for the other duplexes are reported in Fig. S8 and S9.†

In these selections, we could successfully retrieve the positive controls and, importantly, their enrichments displayed comparable trends for LED (in red) and ESAC (in blue). Significant enrichment was detected when at least 10^7 copies of the positive control duplexes were present, corresponding to a dilution of 1 : 1 000 000 with the negative controls. Interestingly, good enrichment values were observed only for the positive control duplexes consisting of either the full AGP binder duplex or the double-positive SABA duplex. For AGP selections, this means that only the combination of the previously identified simultaneously binding fragments was able to efficiently bind to the protein, as was already reported for the conventional ESAC setup.³² For CAII selections, since monovalent SABA has an $0.6 \mu\text{M}$ affinity for CAII, this means that avidity was greatly helping to retrieve the SABA moiety.⁵⁰

Conclusion

In the present work, we have established a new large encoding design, LED, to widen the potential of DEL technology for more complex library construction and to facilitate approaching more difficult protein targets, such as protein–protein interactions. For this purpose, we designed and implemented a LED construct and we optimized our system until a reliable transfer of four codes partitioned between two sub-libraries onto a unique, amplifiable DNA strand was achieved. PCR sensitivity was investigated and found to be comparable or even superior to already established dual-pharmacophore systems. The system was validated through model selections and it allowed retrieving known pairs of binders against AGP and CAII, by synergistic and avidity effects, respectively. Thorough comparison with conventional ESAC demonstrated the potential of LED in selections and subsequent PCR amplification. Furthermore, optimal affinity-based selection conditions were established in terms of DNA input and dilution of the positive controls within a pool of DNA-encoded compounds. The minimum total DNA amount needed in selections was determined to be 10^9 duplexes both for LED and ESAC. This minimal input permits the retrieval of enough DNA construct after selection to perform reliable PCR amplification and observe enrichment of binders. As we are aware that concentration of potential ligands in the DEL pool is a key factor for determining an appropriate final library size, we determined the maximum dilution of a given small molecule in a model library. A maximal ratio of 1 : 1 000 000 between positive and negative controls allowed us to recover known binders, both in case of LED and of the conventional ESAC system. Knowledge of both minimum input and limiting dilution will help to design and interrogate novel types of very large dual-display libraries and enable DEL technology to become a more reliable source of specific, small- and

medium-sized ligands to difficult protein targets. We described the utility of LED for the construction of “2 + 2” DELs, however, as it is very robust and versatile, the new LED encoding scheme may also well serve the design of a whole range of structurally diverse libraries of several diversity elements, and spanning from linear to branched, or macrocyclic structures. Using LED we foresee the versatile construction of single-pharmacophore DELs and of dual-pharmacophore DELs of unprecedented sizes.

Data availability

The data supporting the findings of this study are given within this article and its ESI.† Further raw DNA sequencing data can be obtained from the corresponding authors upon reasonable request.

Author contributions

LP, DN and JS developed the encoding strategy, designed the validation experiments and wrote the manuscript. LP performed the experiments for implementing the encoding strategy. AM optimized the software for deconvoluting data from NGS sequencing. LP, AM and JS analyzed the data. LP, AL, MK, SO and GB performed the selection experiments and PCR amplifications.

Conflicts of interest

Dario Neri is a co-founder and shareholder of Philochem AG (Otelfingen, Switzerland).

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References

- 1 S. Brenner and R. A. Lerner, *Proc. Natl. Acad. Sci. U. S. A.*, 1992, **89**, 5381–5383.
- 2 W. Decurtins, M. Wichert, R. M. Franzini, F. Buller, M. A. Stravs, Y. Zhang, D. Neri and J. Scheuermann, *Nat. Protoc.*, 2016, **11**, 764–780.
- 3 Y. Song and X. Li, *Acc. Chem. Res.*, 2021, **54**, 3491–3503.
- 4 S. Brenner and R. A. Lerner, *Proc. Natl. Acad. Sci.*, 2006, **89**, 5381–5383.



- 5 R. A. Goodnow and C. P. Davie, *DNA-Encoded Library Technology: A Brief Guide to Its Evolution and Impact on Drug Discovery*, Elsevier Inc., 1st edn, 2017, vol. 50.
- 6 D. Neri and R. A. Lerner, *Annu. Rev. Biochem.*, 2018, **87**, 479–502.
- 7 N. Favalli, G. Bassi, J. Scheuermann and D. Neri, *FEBS Lett.*, 2018, **592**, 2168–2180.
- 8 V. Kunig, M. Potowski, A. Gohla and A. Brunschweiler, *Biol. Chem.*, 2018, **399**, 691–710.
- 9 M. R. Arkin, Y. Tang and J. A. Wells, *Chem. Biol.*, 2014, **21**, 1102–1114.
- 10 R. M. Franzini and C. Randolph, *J. Med. Chem.*, 2016, **59**, 6629–6644.
- 11 T. Kodadek, N. G. Paciaroni, M. Balzarini and P. Dickson, *Chem. Commun.*, 2019, **55**, 13330–13341.
- 12 N. Favalli, G. Bassi, C. Pellegrino, J. Millul, R. De Luca, S. Cazzamalli, S. Yang, A. Trenner, N. L. Mozaffari, R. Myburgh, M. Moroglu, S. J. Conway, A. A. Sartori, M. G. Manz, R. A. Lerner, P. K. Vogt, J. Scheuermann and D. Neri, *Nat. Chem.*, 2021, 41557.
- 13 J. P. Phelan, S. B. Lang, J. Sim, S. Berritt, A. J. Peat, K. Billings, L. Fan and G. A. Molander, *J. Am. Chem. Soc.*, 2019, **141**, 3723–3732.
- 14 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 and P. S. Baran, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, E6404–E6410.
- 15 L. Su, J. Feng, T. Peng, J. Wan, J. Fan, J. Li, J. O'Connell, D. R. Lancia, G. J. Franklin and G. Liu, *Org. Lett.*, 2020, **22**, 1290–1294.
- 16 Y. Li, R. de Luca, S. Cazzamalli, F. Pretto, D. Bajic, J. Scheuermann and D. Neri, *Nat. Chem.*, 2018, **10**, 441–448.
- 17 P. Dickson and T. Kodadek, *Org. Biomol. Chem.*, 2019, **17**, 4676–4688.
- 18 C. Zambaldo, S. N. Geigle and A. L. Satz, *Org. Lett.*, 2019, **21**, 9353–9357.
- 19 N. Favalli, S. Biendl, M. Hartmann, J. Piazzzi, F. Sladojevich, S. Gräslund, P. J. Brown, K. Näreaja, H. Schüler, J. Scheuermann, R. Franzini and D. Neri, *ChemMedChem*, 2018, **13**, 1303–1307.
- 20 C. J. Gerry and S. L. Schreiber, *Curr. Opin. Chem. Biol.*, 2020, **56**, 1–9.
- 21 G. Zhang, J. Zhang, Y. Gao, Y. Li and Y. Li, *Expert Opin. Drug Discovery*, 2021, 1–15.
- 22 D. L. Usanov, A. I. Chan, J. P. Maianti and D. R. Liu, *Nat. Chem.*, 2018, **10**, 704–714.
- 23 Z. Zhu, A. Shaginian, L. C. Grady, T. O'Keeffe, X. E. Shi, C. P. Davie, G. L. Simpson, J. A. Messer, G. Evindar, R. N. Bream, P. P. Thansandote, N. R. Prentice, A. M. Mason and S. Pal, *ACS Chem. Biol.*, 2018, **13**, 53–59.
- 24 L. Plais and J. Scheuermann, *RSC Chem. Biol.*, 2022, **3**, 7–17.
- 25 Q. Nie, S. Zhong, Y. Li, G. Zhang and Y. Li, *Chin. Chem. Lett.*, 2021, DOI: 10.1016/j.ccl.2021.09.041.
- 26 S. Melkko, J. Scheuermann, C. E. Dumelin and D. Neri, *Nat. Biotechnol.*, 2004, **22**, 568–574.
- 27 M. Wichert, N. Krall, W. Decurtins, R. M. Franzini, F. Pretto, P. Schneider, D. Neri and J. Scheuermann, *Nat. Chem.*, 2015, **7**, 241–249.
- 28 Á. Furka, *MedComm*, 2021, **2**, 481–489.
- 29 K. I. Sprinz, D. M. Tagore and A. D. Hamilton, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 3908–3911.
- 30 R. M. Franzini, D. Neri and J. Scheuermann, *Acc. Chem. Res.*, 2014, **47**, 1247–1255.
- 31 J. Scheuermann and D. Neri, *Dual-Pharmacophore DNA-Encoded Chemical Libraries*, 2014.
- 32 M. Bigatti, A. Dal Corso, S. Vanetti, S. Cazzamalli, U. Rieder, J. Scheuermann, D. Neri and F. Sladojevich, *ChemMedChem*, 2017, **12**, 1748–1752.
- 33 S. Oehler, L. Plais, G. Bassi, D. Nario and J. Scheuermann, *Chem. Commun.*, 2021, 12289–12292.
- 34 K. Gorska, K.-T. Huang, O. Chaloin and N. Winssinger, *Angew. Chem.*, 2009, **121**, 7831–7836.
- 35 J. P. Daguer, M. Ciobanu, S. Alvarez, S. Barluenga and N. Winssinger, *Chem. Sci.*, 2011, **2**, 625–632.
- 36 H. Eberhard, F. Diezmann and O. Seitz, *Angew. Chem.*, 2011, **123**, 4232–4236.
- 37 J. P. Daguer, C. Zambaldo, M. Ciobanu, P. Morieux, S. Barluenga and N. Winssinger, *Chem. Sci.*, 2015, **6**, 739–744.
- 38 G. Bassi, N. Favalli, M. Vuk, M. Catalano, A. Martinelli, A. Trenner, A. Porro, S. Yang, C. L. Tham, M. Moroglu, W. W. Yue, S. J. Conway, P. K. Vogt, A. A. Sartori, J. Scheuermann and D. Neri, *Adv. Sci.*, 2020, **7**(22), 2001970.
- 39 Y. Zhou, C. Li, J. Peng, L. Xie, L. Meng, Q. Li, J. Zhang, X. D. Li, X. Li, X. Huang and X. Li, *J. Am. Chem. Soc.*, 2018, **140**, 15859–15867.
- 40 Y. Deng, J. Peng, F. Xiong, Y. Song, Y. Zhou, J. Zhang, F. S. Lam, C. Xie, W. Shen, Y. Huang, L. Meng and X. Li, *Angew. Chem., Int. Ed.*, 2020, **59**, 14965–14972.
- 41 Y. Zhou, J. Peng, W. Shen and X. Li, *Biochem. Biophys. Res. Commun.*, 2020, **533**, 215–222.
- 42 Y. Zhou, W. Shen, J. Peng, Y. Deng and X. Li, *Bioorg. Med. Chem.*, 2021, **45**, 116328.
- 43 F. V. Reddavid, W. Lin, S. Lehnert and Y. Zhang, *Angew. Chem., Int. Ed.*, 2015, **54**, 7924–7928.
- 44 F. V. Reddavid, M. Cui, W. Lin, N. Fu, S. Heiden, H. Andrade, M. Thompson and Y. Zhang, *Chem. Commun.*, 2019, **55**, 3753–3756.
- 45 A. Sannino, E. Gabriele, M. Bigatti, S. Mulatto, J. Piazzzi, J. Scheuermann, D. Neri, E. J. Donckele and F. Samain, *ChemBioChem*, 2019, **20**, 955–962.
- 46 Q. Chen, X. Cheng, L. Zhang, X. Li, P. Chen, J. Liu, L. Zhang, H. Wei, Z. Li and D. Dou, *SLAS Discovery*, 2020, **25**, 523–529.
- 47 A. L. Satz, R. Hochstrasser and A. C. Petersen, *ACS Comb. Sci.*, 2017, **19**, 234–238.
- 48 T. Fournier, N. Medjoubi-N and D. Porquet, *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.*, 2000, **1482**, 157–171.
- 49 C. T. Supuran, *Nat. Rev. Drug Discovery*, 2008, **7**, 168–181.
- 50 L. Prati, M. Bigatti, E. J. Donckele, D. Neri and F. Samain, *Biochem. Biophys. Res. Commun.*, 2020, **533**, 235–240.

