doi:10.2533/chimia.2025.158 Chimia 79 (2025) 158–161 © B. Sauter, P. Cai, K. Dagher, C. Disraeli, A. K. Prakash, L. Schneider, A. Cores Esperon, D. Gillingham

Challenges and Opportunities in DNA Encoded Library Screens

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Abstract: In our lab we have been developing techniques that attempt to capture or amplify signals in pooled compound mixtures for several years. DNA encoded libraries (DELs) are the most widely used pooled mixtures in early drug discovery. DELs are massive collections of small molecules, where each individual molecule is covalently linked to a unique DNA strand that can serve as an identification tag by sequencing. The industry standard for selecting DELs is affinity enrichment, which inherently can only search for direct binding. We outline here two of the ways that we are attempting to extend the potential of DEL screens into new areas.

Keywords: Biotechnology · Chemical biology · DNA encoded libraries · Medicinal chemistry

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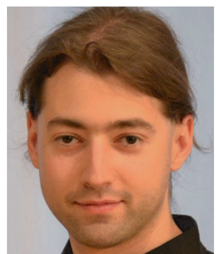

Basilius Sauter obtained his PhD in chemistry from the University of Basel in 2020. As a senior postdoc in the Gillingham group, his research focuses on DNA encoded libraries and medicinal chemistry. He is an important part of the group as a data scientist and a consultant to younger group members.

Lukas Schneider obtained his PhD in chemistry in the Gillingham research group in 2023 where he developed DELSTAR and received the Best PhD thesis award from the DMCCB in 2024. After graduation, he remained as a postdoc to continue his work on DELSTAR.

Koder Dagher joined the group in 2020 and worked on developing a novel selection system for DNA encoded libraries. He graduated from the group in 2024.

Pinwen Cai joined the group in 2020 and worked on developing a novel, functional selection system for DNA encoded libraries based on *in vitro* ubiquitination. He graduated from the group in 2024.

Athira Kakkolliyil Prakash joined the group as a PhD candidate in 2023. She is focused on expanding DELSTAR for new targets and is working on a technology to sequence the tail length of elongated members.

Chiara Disraeli joined the group as a PhD candidate in 2023. She continues the work of Pinwen Cai and works on expanding DELSTAR for new targets.

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1. Introduction

The identification and validation of hits that bind to novel therapeutic targets is the first step in the development of new drugs. Traditionally, the synthesis of new molecules individually and the screening of collections of these well-defined and characterized unique chemical entities through high-throughput screening (HTS) methods were the rate-limiting steps in drug discovery. Although HTS is a tried and trusted method for screening, it comes with significant drawbacks: the highly specialized equipment needed, the difficulty in scaling up the assays to screen a large number of unique compounds, the logistical difficulty of handling these compound collections individually. These and other factors place practical and cost limitations on the number of targets that can be reasonably screened.

However, our growing understanding of disease biology, the emergence of omics methods, and innovations in the way small molecules can address disease has only increased the demand for primary screens. As such, we think it is critical that the small molecule community puts significant thought and effort into streamlined methods for discovering new chemical matter at a fraction of the cost of current approaches. It is against this backdrop that the development of DNA-encoded library (DEL) screening methods, in which each of the chemical entities, whether small molecules, peptides, or proteins, are encoded by a unique DNA sequence, continues to grow in popularity. DNA encoding allows each of the entities to be present as a small number of individual molecules within libraries of billions of compounds in a single vessel, and yet despite the low numbers, hit molecules can be identified due to the ease of PCR amplification and high-throughput sequencing of the DNA tags. The DNA tag also enables the rapid and efficient synthesis of libraries of billions of members using a split-and-pool approach, since DNA can be easily purified by ethanol precipitation.

DEL technology has seen rapid growth since the first practical demonstrations of its potential,[1–7] and yet we think there is still a lot that remains to be discovered for this extraordinary technology. Here are some of the challenges that will guide our research over the coming decade:

Traditional DEL selections primarily identify binders to target proteins through affinity-based enrichment, which inherently limits the technology to finding simple binders.

- ***Functional screens*** that measure activities such as ubiquitin transfer, transcriptional activation, or protein-protein interactions are far more complex to implement, often requiring bespoke methods tailored to individual targets. DNA-encoded One-Bead-One-Compound (OBOC) libraries have an advantage here, but are inherently more limited in terms of library size.[8] Such screens are critical for discovering small molecules that influence protein behavior beyond simple binding.
- ***Sensitivity***. While DELs excel at identifying strong binders, weak or moderate binders as well as binders with a fast k_{off} rate can be missed due to low signal-to-background ratios. Overlooking weak or moderate binding is especially problematic for efforts to apply DEL data toward machine learning.
- ***Direct information on binding affinity***. Despite the correlation between read counts and affinity, interpreting binding affinity from read counts alone can be challenging, particularly in large libraries with low sequencing coverage.
- ***False positive discovery rate***. Let's face it, unless the read count of hits is significantly above baseline, distinguishing false from true positives is impossible without resynthesis due to the large noise from matrix binders.

Despite these challenges, we believe the power of encoded science is just beginning to be appreciated. As a community it is incumbent on us to face the open challenges head-on. Several years ago, we began to brainstorm how we could move beyond affinity selection in pooled screens, ideally conquering the challenges outlined above. The studies outlined here represent our first publications emerging from these ideas. Both projects attempt to tackle the specific challenges in important areas of DEL science: offering novel functional screens, improved sensitivity, or the ability to record binding information.

2. Functional Ubiquitin Transfer Selections

In Cai *et al.*[9] we introduce a novel method that expands DELs beyond traditional binding screens by enabling ***functional selections*** for ubiquitin (Ub) transfer. This work addresses the challenge of identifying small molecules that not only enable protein-protein interactions, but also facilitate or modulate protein degradation pathways. Better tools to find such molecules are highly relevant for the discovery of molecular glue degraders (MGDs) and proteolysis-targeting chimeras (PROTACs).

The key innovation in this work was to create a DEL-based system that identifies small molecule-protein pairs capable of catalyzing Ub transfer. By combining DNA-linked small molecules with DNA-linked proteins of interest (POIs), the method leverages DNA hybridization to induce proximity between library members and POIs (Fig. 1, panel A). Specifically, we set up the assay such that the ternary complex involving a small molecule, the POI, and a ubiquitin ligase complex catalyzes the transfer of Ub from the ligase complex onto the POI.

This functional event is captured through affinity enrichment of the ubiquitinated POI and then read out *via* DNA sequencing. This approach transforms DEL selections into functional screens that measure the catalytic activity, specifically ubiquitin transfer (Fig. 1, panel B). The encoding enables that we can screen for multiple POIs at the same time (Fig. 1, panel C).

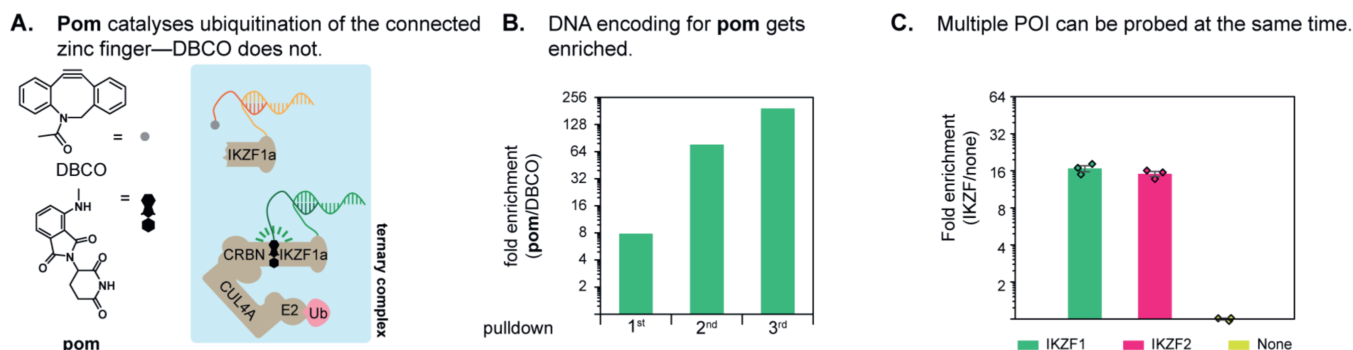

Fig. 1. (A) Pomalidomide (pom) forms a ternary complex with the CRBN-E3-E2 complex and IKZF1, the protein of interest. The constructed proximity catalyzes the transfer of ubiquitin to the zinc finger. (B) Affinity enrichment of the ubiquitinated POIs leads to enrichment of the DNA binding pomalidomide compared to the DNA binding DBCO. (C) Multiple zinc fingers can be tested at the same time. In this example, we use NVP-DKY709 bound to the DNA instead of pomalidomide.

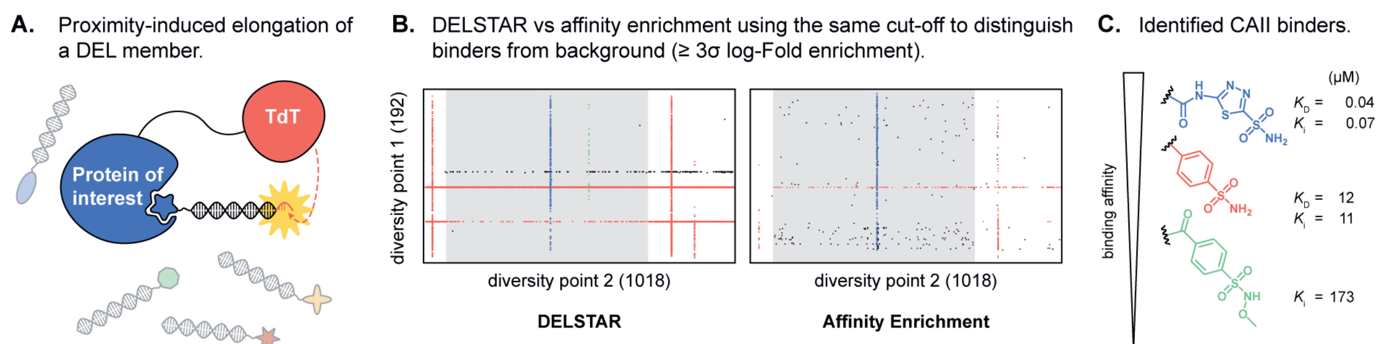

Fig. 2. (A) If a DEL member binds the POI, TdT is in proximity and extends the 3' end of the encoding DNA. (B) Sequencing results of DELSTAR and a classical affinity enrichment. In both cases, only DEL members with an log-Fold enrichment ≥ 3σ were plotted. Identified CAII binding motifs are hinted in the respective colors (blue > red > green). (C) K_D and K_i of the three binding motifs. K_D are from acetazolamide and sulfanilamide. K_i are inhibition of the CAII CO_2 hydration reaction for acetazolamide, 4-methylbenzenesulfonamide and *N*-methoxy-4-methylbenzenesulfon-amide from Briganti *et al*.[10]

Once we fully scale the method, it will enable the identification of molecular glues that reprogram ubiquitination pathways, paving the way for targeted protein degradation strategies in drug discovery. By simultaneously screening small molecules and protein substrates, the method has the potential to identify optimal Ub-transfer pairs, offering a scalable solution for functional DEL selections.

3. Recording Binding Events Using TdT

In Schneider *et al.* we introduce DELSTAR (DEL selections using TdT polyadenylation recording), a solution-phase method that uses terminal deoxynucleotidyl transferase (TdT) to record binding events into the DNA of DELs.[11] This work addresses the limitations of classical affinity-based DEL selections, including poor sensitivity to weak binders and the technical burden of protein immobilization.

TdT is a DNA polymerase which adds untemplated nucleotides to DNA ends consuming deoxynucleoside triphosphates (dNTPs).[12-14] By only providing deoxyadenosine triphosphate (dATP), it is possible to create a homopolymeric polyA tail. In DELSTAR, we use this to record binding events on the DEL members. To implement this technology, target proteins are expressed as fusions with TdT. When a small molecule binds the target protein, its connected DNA comes into proximity to TdT, thus inducing TdT-mediated polyA tailing (Fig. 2, panel A). The length of the polyA tail correlates with the binding affinity, effectively encoding binding information into the DNA. PolyA-tailed DNA can be enriched using magnetic poly(dT)$_{25}$ beads and analyzed *via* next-generation sequencing (NGS). The DELSTAR method identifies strong, moderate, and even weak binders, whereas classical affinity enrichment often prefers strong binders (Fig. 2, panels B and C).

Moreover, DELSTAR requires significantly lower protein quantities (1 pmol of fusion protein for one selection can be enough) and operates in solution, avoiding immobilization artifacts. The polyA tail provides a stable molecular record of binding events, enabling high-fidelity selections. DELSTAR represents a robust and scalable solution for DEL selections, particularly for large libraries where low-affinity binders are often missed. Additionally, the technology is backwards compatible with all existing DELs bearing a free 3'-end. By finding all kind of binders, DELSTAR improves sensitivity and selectivity, offering a powerful tool for early-stage drug discovery.

4. Conclusions

Taken together, the work described above addresses key challenges in DEL technology:

1. **Functional Screens**: Cai's ubiquitin transfer method enables catalytic and proximity-driven functional selections.
2. **Sensitivity**: Schneider's DELSTAR is equally good at identifying strong and weak binders and manages to uncover even a very weak binder.
3. **Direct information on binding affinity**: Schneider's DELSTAR records binding events directly into DEL DNA. The longer the tail, the higher the affinity.
4. **False positive discovery rate**: Although a preprint on finding molecules that enable ternary complex formation with affinity enrichment exists, having the ternary complex does not necessarily lead to a productive ubiquitination. Cai's ubiquitination transfer exclusively selects for productive complexes. Furthermore, Schneider's DELSTAR returns clean data, distinguishing binders from non-binders more clearly.

These advancements collectively expand the capabilities of DELs, enabling functional, high-sensitivity screens for small molecule discovery. By overcoming longstanding limitations, they open new opportunities for identifying small molecules that bind, activate, or modulate protein targets in diverse biological contexts.

Acknowledgment

We would like to thank all former and current group members who contributed on encoded library projects, both in the lab and at the chalkboard. We would also like to thank Syngenta and the SCS for the opportunity to present these results at their annual symposium in Stein.

Received: January 23, 2025

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