On-bead DEL's Application and Case Study on Drug Discovery

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Introduction

DNA-encoded library (DEL) technology has been recognized as one of the major screening methods with its unique advantages in vast chemical diversity and multiplexed affinity-based screening. Recently, solid-phase On-bead DEL, also known as one bead one compound (OBOC), has been developed to further expand DEL screening from affinity-based screening into biochemical activity screening. Here, we present multiple application case study of OBOC, including biochemical activity screening and traditional affinity screening. In biochemical screening, with high-encapsulation rate microfluidics chip design, we successfully screened a 200K-member OBOC library. Further validation shows that potent inhibitor can be directly identified. In affinity screening, we show that molecular glue can be screened using special focus library along with affinity-based screening methods. Our study demonstrates that OBOC technology could be used for wide-range of drug discovery scenarios.

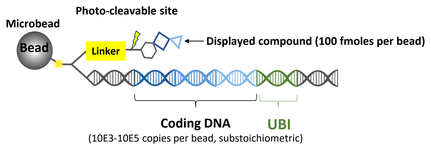


Figure 1.The schematic diagram of On-bead DEL

Chemistry Reaction & On-Bead DEL Library

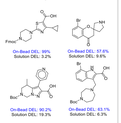
On-Bead DEL Reaction Tool Box

Type	Reaction	Type	Reaction
Amine	Amide formation	Aldehyde	Reductive amination
	Urea		Knoevenagel
	Thiourea	Alcohol	Mitsunobu
	Carbamate	Reduction	Azide reduction
	Sulfonylation	Macrocycle	Cyclic Peptoid
	Tosylation	Hydrolysis	Methyl/Ethyl ester
	S_NAr	Cyclization	Van Leusen for imidazole
	SN2		Click for triazole
	Chan-lam		Oxadiazole formation
	Michael addition		Thiadiazole formation
	Curtius rearrangement	De-protection	De-Fmoc, De-Boc, De-STMP, De-Alloc, De-Tfa, De-Trt
	Guanidination		
Coupling	Suzuki	Under development	Heck
	Sonogashira		Isoxazole/Isoxazoline
	Buchwald		Isoindole
	Wittig		Hayashi Miyaura

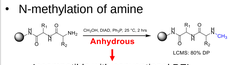
Larger Chemistry Toolbox in Organic Solvents

- Weakly nucleophilic amine reacts with electrophiles

- High yield privileged scaffold incorporation

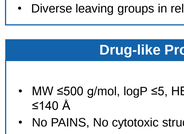


- N-methylation of amine



Incompatible with conventional DEL

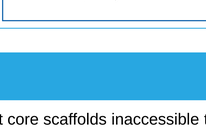
Off-the-Shelf On-bead DEL Libraries



Mpro Focus Library
Diversity: 86K

CRBN Molecular Glue Focus Library
Diversity: 300K

Presentative Compounds of Diversity Libraries



Total diversity: 3M
Total route: 16

Primarily 2-cycle DEL

- Privileged and target-specific scaffolds
- Expected library size: 50-300K
- Photocleavable linker: o-nitrobenzene or coumarine-based core, reacted group (-X, -OH,-NH2,-CO
- Diverse leaving groups in released compounds

Drug-like Properties

- MW ≤500 g/mol, logP ≤5, HBD ≤5, HBA ≤10, PSA ≤140 Å
- No PAINS, No cytotoxic structures

On-bead DELs Enable Molecular Glue (MG) Discovery

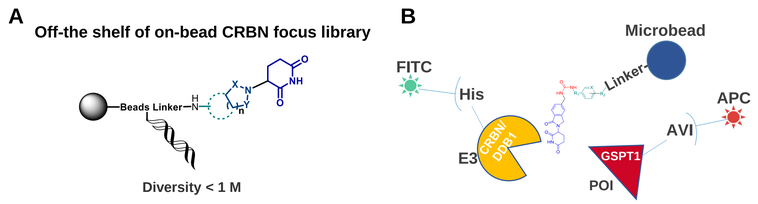


Figure 2.The schematic diagram of CRBN focused DEL and FACS-based glue screening
（A） The schematic design of on-bead DEL. The on-bead CRBN focus library is off-the shelf.
（B） The overall principle of on-bead DEL MG screening. Following complex formation, protein staining is performed using corresponding fluorescence-conjugated antibodies. Beads exhibiting positive fluorescence signals are subsequently sorted out by FACS.

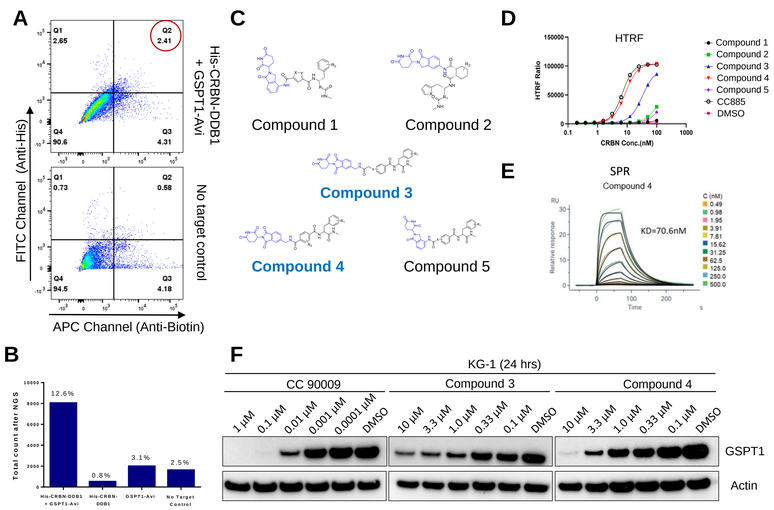
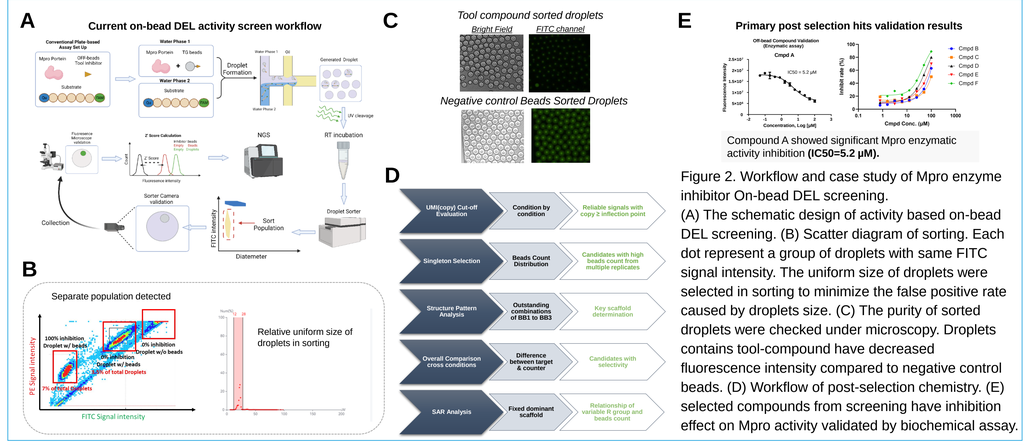


Figure 3.On-bead CRBN focused library screening identified novel molecular glue
(A) The representative FACS sorting figures in the indicated condition during on-bead CRBN focus library screening. (B) The total bead count and recovery rate of spike-in reference compound across different post-selection samples after NGS sequencing. (C) Structures of the five selected compounds. Compound 3 and 4 highlighted in blue were successfully validated. (D) Validation results of hit compounds by HTRF assay. IC50 of compound 3 and 4 are at nM level. (E) Representative validation results of off-DNA compound 4 by SPR. KD value is 70.6 nM. (F) Cellular degradation assay of GSPT1 in KG-1 after 24-hour treatment with the indicated concentrations of CC 90009 or compound 3 or 4.

Activity-Based On-bead DEL Screen for Enzyme Inhibitor

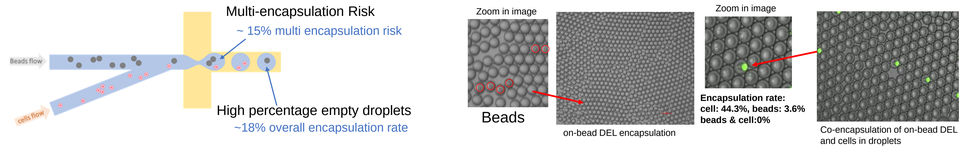


Compound A showed significant Mpro enzymatic activity inhibition **(IC50=5.2 μM).**

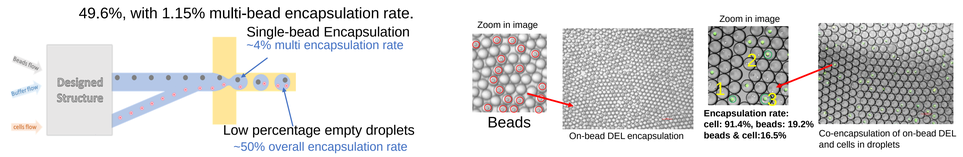
Figure 2. Workflow and case study of Mpro enzyme inhibitor On-bead DEL screening.
(A) The schematic design of activity based on-bead DEL screening. (B) Scatter diagram of sorting. Each dot represent a group of droplets with same FITC signal intensity. The uniform size of droplets were selected in sorting to minimize the false positive rate caused by droplets size. (C) The purity of sorted droplets were checked under microscopy. Droplets contains tool-compound have decreased fluorescence intensity compared to negative control beads. (D) Workflow of post-selection chemistry. (E) selected compounds from screening have inhibition effect on Mpro activity validated by biochemical assay.

Capability of On-bead DEL on cellular assay

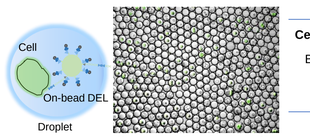
A. Commercial Available Droplet Formation Chip



B. In-House High Encapsulation Chip



C. Co-encapsulation of Beads and Cells in Droplets



Cell viability in droplets	
Before droplet generation	99%
6h	90.9%

Figure 5. Feasibility test of on-bead DEL on cellular assays.
(A). Commercial available droplet formation chip have single bead capsulation rate of around 3.6% and cellular encapsulation rate 44.3% and co-encapsulation rate was under detect.
(B).In-house high encapsulation chip have single bead capsulation rate of around 19.2 % and cellular encapsulation rate 91.4% and co-encapsulation rate was 16.5%.
(C). Bead and cell co-encapsulation rate was over 17%.Bead encapsulation rate reached 22.68%, with over 21% single bead encapsulation rate. Cell encapsulation rate was over 75%. Cell viability was over 90% after 6 hours incubation

Summary

1. Solid-phase synthesis enables reactions and hot core scaffolds inaccessible to traditional DEL. Build 2-cycle 100K library, expect timeline: 4-6 weeks
2. Affinity based screening capability. Combined with FACS-based sorting, On-bead DEL facilitate molecular glue drug discovery by enabling the direct detection of the ternary complex. Screening Library size <500K, 30x library, expect timeline: 8 weeks for final report (pre-selection, selection, data analysis)
3. High encapsulation chip enables higher throughput of on-bead DEL screening and cellular on-bead DEL assays. Fast & accurate sorting system with sorting purity higher than 95%. On-Bead DEL specialized NGS & post selection data analysis pipeline. Mpro Assay based on-bead DEL selection shows > 28% validation success rate. High-throughput screening enables 2 day screening and sorting of library size 100 K, 20X library.

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