Application of On-bead DEL Technology in Target Protein Degradation (TPD) Based Phenotypic Screen

WuXi Biology

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Introduction

To date, DNA-encoded library is widely applied into various drug discovery fields, including conventional small molecular drug discovery, covalent drug discovery, peptide drug discovery and other modalities, such as RNA-targeting drug discovery and Target Protein Degradation (TPD) drug discovery. However, most of these screenings are still limited to the affinity-based selection methodology, while functional/activity-based assay application on DEL screening is still limited. On-bead DEL utilizes combinatory chemistry to synthesize compounds on solid matrix beads with each bead representing a single type of molecules. Previous studies showed that by combining On-bead DEL with microfluidics technology, biochemical activity based screening could be performed in picoliter-sized droplets and assay-active compounds could thus be identified. To further expand the On-bead DEL technology into cellular assay based screening, we developed in-house microfluidics chips, together with a high-speed droplet sorting system as well as a unique compound loading strategy. To test this cellular assay based On-bead DEL system, we select TPD cellular assay as our proof of concept study. In the study, we showed that the cell could be cultured in droplet for 24hrs with maintained viability and target protein expression. Further reference compound treatment shows that positive compound encapsulated droplets shows significant signal loss compared to negative control compound encapsulated droplets. An additional droplet sorting experiment followed by high-throughput decoding demonstrates that positive compounds could be successfully sorted and identified. Together, there results show that cellular assay could be performed using On-bead DEL and this system could be potentially used for further facilitating target protein degradation drug discovery.

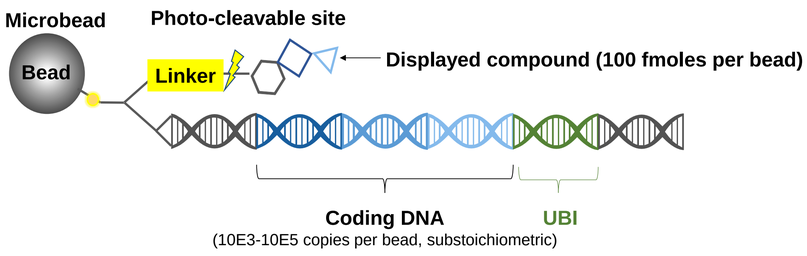
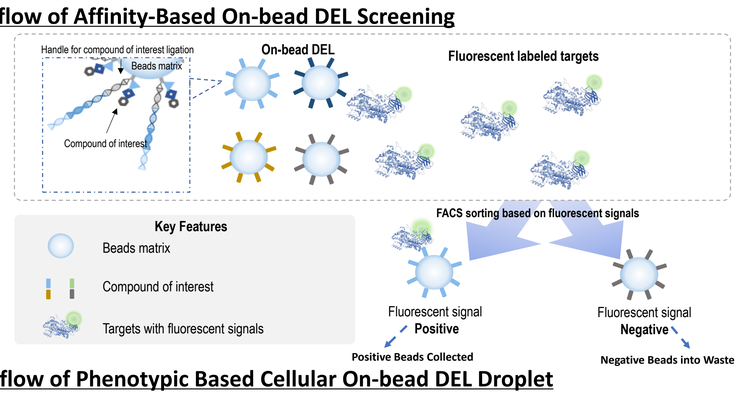


Figure 1.The schematic diagram of on-bead DEL

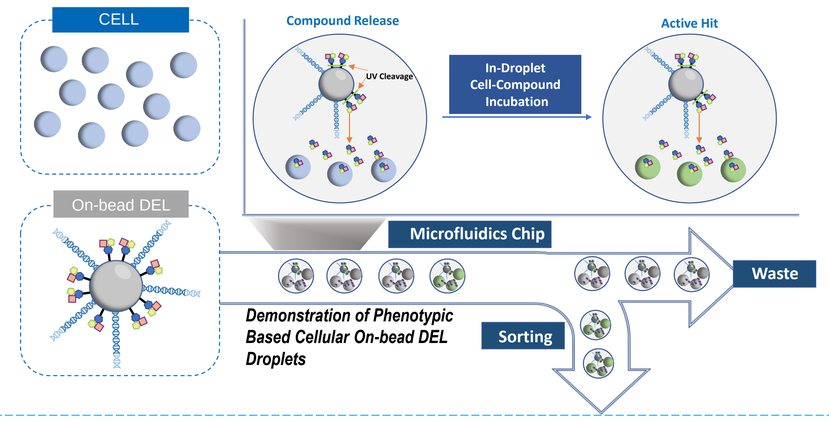
On-bead DEL Screening Options – Affinity and Phenotypic Based Screening

A. Workflow of Affinity-Based On-bead DEL Screening



The affinity-based on-bead DEL screening is capable for molecular glue, binders of target, protein-protein interaction disruptor discovery.

B. Workflow of Phenotypic Based Cellular On-bead DEL Droplet

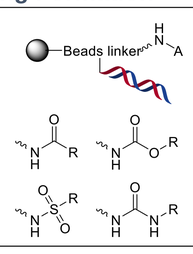


The phenotypic-based On-bead DEL Screening is capable for biochemical assay such as protease inhibitors and cellular assay such as TPD molecules discovery.

Chemistry Reaction & On-bead DEL Library

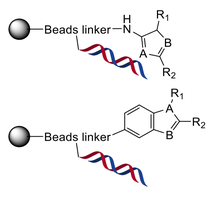
Chemistry Toolbox

Ring Formation



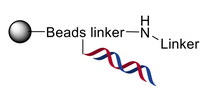
- 1,2,3-Triazole
- 1,2,4-Oxadiazole
- 2-Isoxazoline
- Benzimidazole
- Imidazole
- Isoindole
- Thiodiazole
- 1, 4-Dihydroquinazolinone

Amine Capping



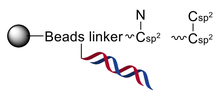
- Amide formation
- Carbamate formation
- Curtius rearrangement
- Guanidination
- Reductive amination
- S_NAr
- Sulfonylation
- Thiourea formation
- Urea formation

Amine + Bifunctional Linker



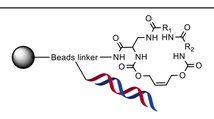
- S_N2
- Michael addition
- Wittig reaction
- Mitsunobu
- Knoevenagel condensation

Coupling

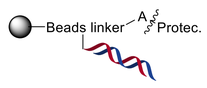


- Suzuki
- Buchwald
- Sonogashira
- Chan-lam

Peptide Cyclization

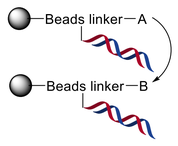


De-protection



- De-Fmoc
- De-Boc
- De-Alloc
- De-Tfa
- De-STMP
- De-Trt

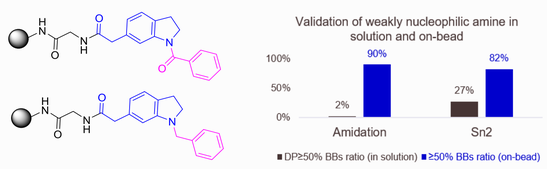
Functional Group Transformation



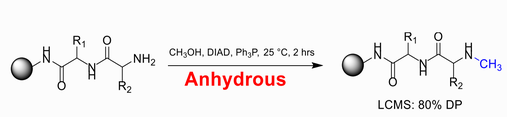
- NH_2 to NCO
- NH_2 to TSC
- N_3 to NH_2
- OH to OTs
- NO_2 to NH_2
- COOR to COOH

Larger Chemistry Toolbox in Organic Solvents

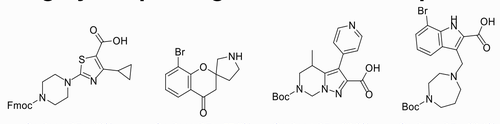
• Weakly nucleophilic amine reacts with electrophiles



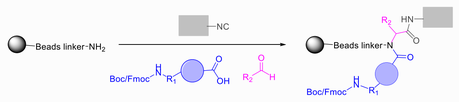
• N-methylation of amine



• High yield privileged scaffold incorporation

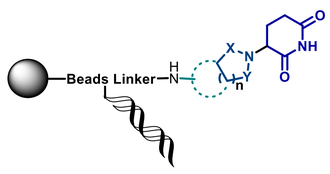


• High compatibility of multi-component reaction



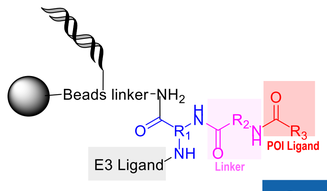
Off-the-Shelf On-bead DEL Libraries

• On-bead CRBN focus library



- Diversity ***~100K-500K***
- ***Diverse warhead*** designed based on reported features, most have at least one aromatic ring
- ***Various attachment strategy*** provides different linker structure

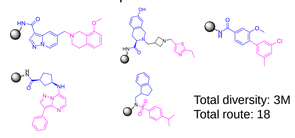
• On-bead PROTAC linker focus library



- Diversity ***~100K***
- ***Intermediate library***: fast assembled various E3 ligand, rational linkers and specific POI ligand

• On-bead diverse library

Presentative Compounds of Libraries



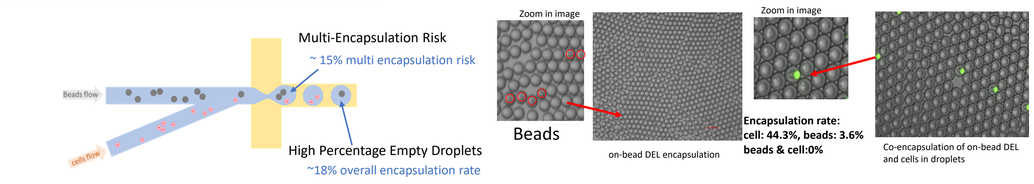
Total diversity: 3M
Total route: 18

On-bead DEL Library in WuXi AppTec

- Expected library size: 50K to 2 M
- MW ≤500 g/mol, logP ≤5, HBD ≤5, HBA ≤10, PSA ≤140 Å
- No PAINS, No cytotoxic structures
- High fidelity, Flexible screening size

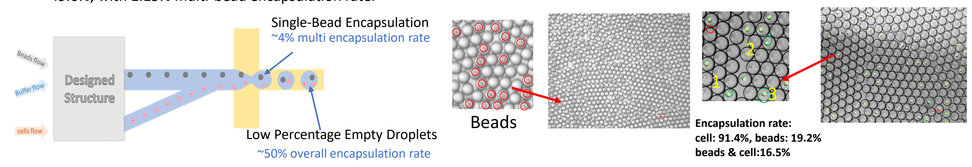
Droplets Encapsulation Capability of On-bead DEL on Cellular Assay

A. Commercial Available Droplet Formation Chip

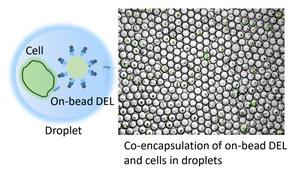


B. In-house High Encapsulation Chip

49.6%, with 1.15% multi-bead encapsulation rate.

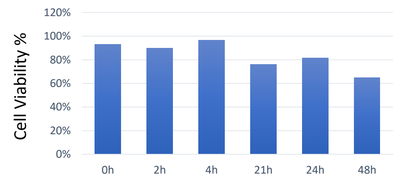


C. Co-encapsulation of Beads and Cells in Droplets



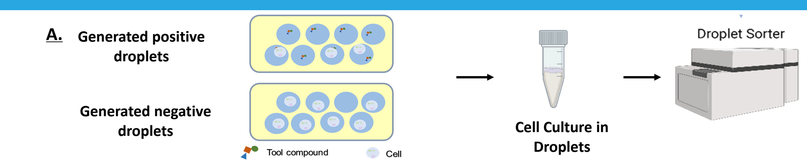
Cell viability in droplets	
Before droplet generation	99%
6h	90.9%

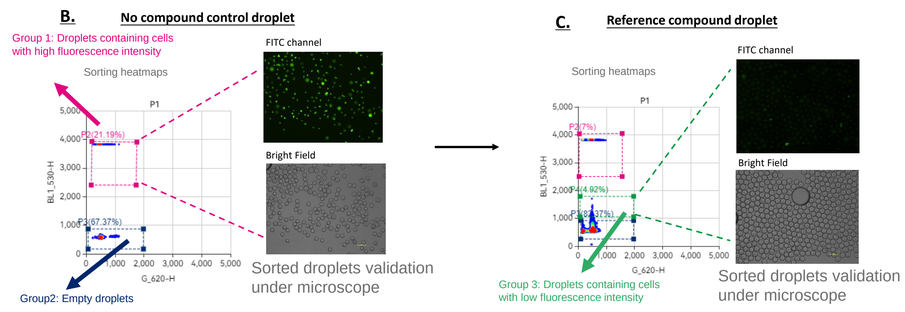
D. Cell Viability Test of Beads & Jurkat Cell Co-encapsulation in Droplet



Feasibility test of beads and cells co-encapsulation 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. (D) Cell viability of Jurkat cells were tested for 48 hours, over 80% at 24 hours.

Droplets Sorting Capability of TPD Cellular Assay





On-bead DEL tool compound sorting test of TPD assay in droplets
(A). Illustration of workflow of tool compound beads sorting. The fused protein with GFP degradation after reference compound treatment (C) with fluorescence signal decrease were observed compared with control (B) group. The cells fluorescence signal in droplets were detected using droplet sorter with high sorting purity.

Summary

- WuXi AppTec has adapted more than 60 protocols for solid phase synthesis and offers more than 30,000 building blocks for On-bead DEL constructions.
- Various linker options are available, including On-bead DEL with photo-cleavable linker for activity screening, and On-bead DEL with PEG linker for affinity screening.
- Both off-the-shelf libraries and customized libraries are available for downstream screening, supported by an extensive chemistry toolbox.
- The focus libraries for molecular glue and TPD are ready to go, with diversities ranging from 100K to 500K.
- Both affinity and activity based (biochemical & cellular) screening are available, with extensive screening experience and a high hit-discovery rate.

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