Accelerating CIP Discovery with Affinity Selection Mass Spectrometry and DNA-Encoded Libraries

WuXi Biology

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Abstract

Chemical inducers of proximity (CIPs) are small molecules that induce the proximity of two biological targets, usually proteins. CIPs are widely used to control and study biological target interactions. Molecular glues and PROteolysis TArgeting Chimeras (PROTACs) are among the most well-known CIPs. These molecules hijack the endogenous E3-based proteolysis pathway to induce proximity between the target protein and E3 ligase, thereby triggering targeted protein degradation.

Here we showed that the small molecule diversity library can be used for CIP discovery by affinity selection mass spectrometry (ASMS). We successfully identified several molecular glue molecules from a collection of 2,000 compounds, which showed ternary complex-inducing capabilities.

Additionally, DNA-encoded libraries (DELs) which have enormous diversity (Bn+) and are constructed through modular combinatory chemistry, make them indispensable tools for the direct discovery of CIPs.

Here, we revealed that by using affinity-based ternary screening methods, the focused bivalent DELs and diversity DELs can be used for the direct discovery of PROTAC and molecular glue, respectively. The most potent PROTAC hits derived from primary DEL screening have ~nM EC_{50} in the ternary complex formation assay. Moreover, the DEL screening process only takes 1 month, which is much more efficient than the conventional CIP discovery process.

Hit Identification in CIP Discovery

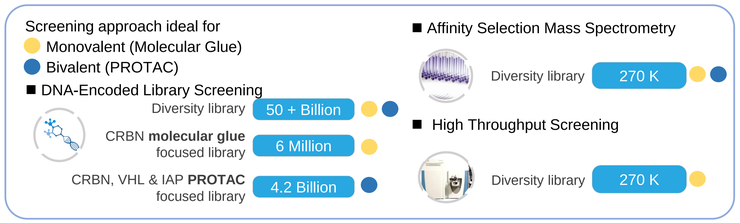


Figure 1. WuXi AppTec's library collection that can be used for CIP discovery

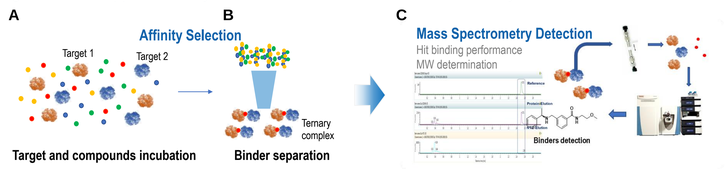


Figure 2. The workflow of ASMS screening method for CIP discovery

A. The small molecules were incubated with target 1 and target 2.
B. The molecules that can induce the ternary complex formation are portioned from the pool.
C. The positive molecules are detected by mass spectrometry.

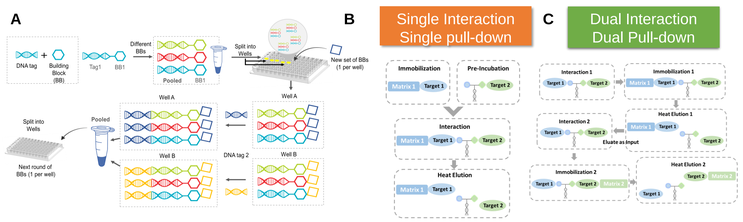


Figure 3. The general principle of DEL and the ternary screening methods for direct CIP discovery

A. The DNA encoded libraries are constructed by combinatory chemistry via a split-and-pool approach.
B. Single interaction and single pull-down method. In the positive condition, target 1 was immobilized while the target 2 was first incubated with DELs, which is followed by mixture of the two. After that the potential CIPs were portioned by a pull-down approach.
C. Dual interactions and dual pull-downs method. In the positive condition, differentially tagged target 1 and target 2 were incubated together. Later the corresponding matrix will be used to capture target 1. The heat eluted molecules are subjected to a new round of incubation and capture by the second affinity matrix.

Diversity Libraries Facilitate Molecular Glue Discovery

ASMS

A Small molecule diversity library screened by ASMS for molecular glue discovery

2000 molecules diversity library → Primary ASMS screen → Dose-response ASMS measurement → Binary Validation → Ternary complex detection → Validated molecular glues

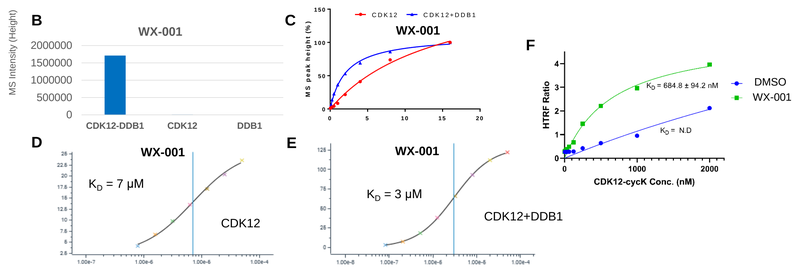
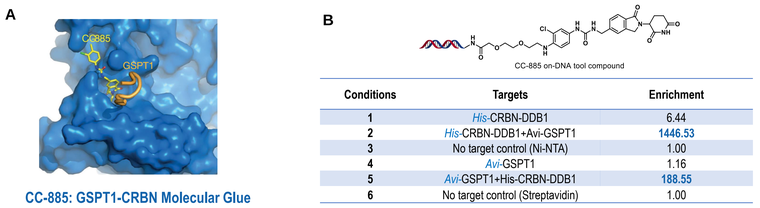


Figure 4. ASMS discovered functional molecular glue from small molecule diversity library

A. The workflow of ASMS based molecular glue discovery.
B. The identified molecular glue hit (WX-001) showed high recovery in dual protein condition (CDK12-DDB1) and undetectable recovery in single protein conditions.
C. WX-001 showed dose response for CDK12 and CDK12+DDB1 in ASMS.
D. WX-001 showed 7 μM affinity for immobilized CDK12.
E. WX-001 showed 3 μM affinity for immobilized CDK12 at the presence of DDB1.
F. WX-001 showed sub-μM EC50 in the HTRF based ternary complex formation assay.

DEL



Conditions	Targets	Enrichment
1	*His*-CRBN-DDB1	6.44
2	*His*-CRBN-DDB1+Avi-GSPT1	1446.53
3	No target control (Ni-NTA)	1.00
4	*Avi*-GSPT1	1.16
5	*Avi*-GSPT1+His-CRBN-DDB1	188.55
6	No target control (Streptavidin)	1.00

Figure 5. Molecular glue can be captured using the single interaction and single pull-down method

A. Binding mode of CC-885: GSPT1-CRBN molecular glue.
B. The on-DNA tool compound derived from CC-885 was found enriched in C2 and C5 using the single interaction and single pull-down method. His-CRBN-DDB1 was immobilized by Ni-NTA matrix through C1 to C3, while Avi-GSPT1 was immobilized by streptavidin matrix through C4 to C6.

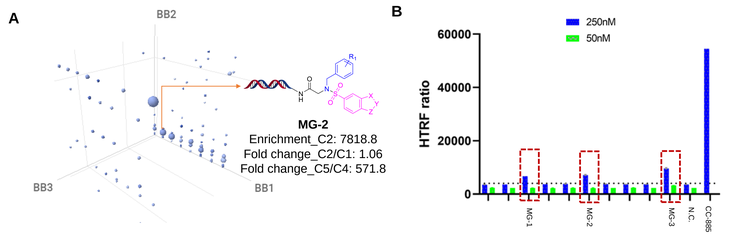
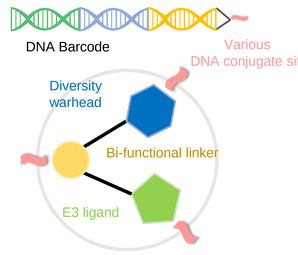


Figure 6. Diversity DEL screening results and validation data.

A. The representative profile of identified candidate MG-2.
B. Preliminary 2-dose HTRF validation data identified 3 potential positive MG molecules.

Focused Bivalent DEL Accelerate Direct PROTAC Discovery



- **Diverse warhead:** linear & branched scaffolds cover wide chemical space
- **Diverse linker:** length and type (flexible and rigid) are selected based on statistics of reported PROTAC
- Various validated **DNA conjugate site**
- Widely used reported **CRBN, VHL & IAP ligands**
- Intermediate library with handle for **fast customized design**

Figure 7. The modular structure of DELs allow for focused PROTAC DEL design and its key features

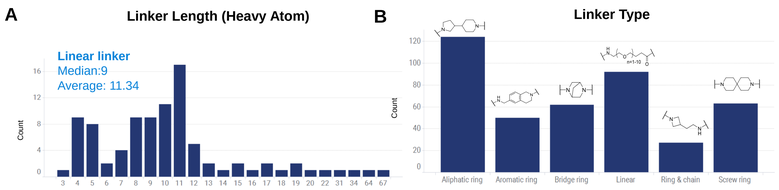


Figure 8. The design of the linkers in the PROTAC DEL

The linkers selection is based on the statistics of the published PROTAC molecules in the public database (http://cadd.zju.edu.cn/protacdb/).

A. The length distribution of WuXi AppTec's linker collection in PROTAC DEL.
B. The linker type distribution of WuXi AppTec's linker collection in PROTAC DEL.

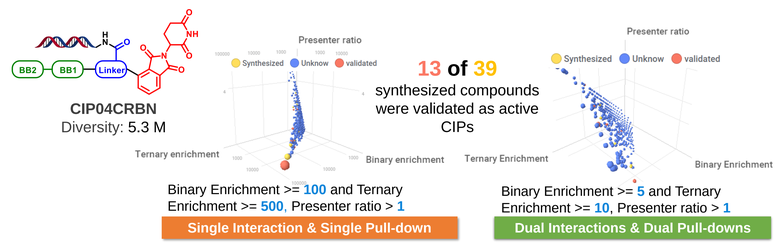
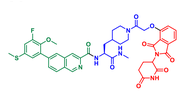
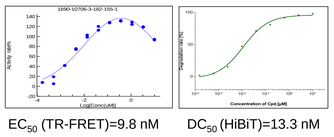


Figure 9. Analysis of screening data

The filtered screening data were presented in cube. The binary enrichment represents the enrichment value in apo POI condition, while the ternary enrichment represents the enrichment in dual proteins (POI + E3) conditions. The presenter ratio is calculated as ternary enrichment/binary enrichment.



Methods / Conditions	Enrichment: Single interaction & single pull-down	Enrichment: Dual interactions dual pull-downs
Binary	91252.8	130.4
Ternary	108995.7	137.2



EC_{50} (TR-FRET)=9.8 nM

DC_{50} (HiBiT)=13.3 nM

Figure 10. Representative PROTAC structure and validation

A. The structure and screening profile of the representative validated PROTAC molecule 10706-3-182-155-1.
B. The potency to induce ternary complex formation in vitro was determined by TR-FRET assay.
C. The potency to induce cellular targeted protein degradation was determined using HiBit BRD4 A549 cells.

Summary

- Utilizing ASMS and pull-down-based ternary screening, both the conventional small molecule diversity library and the DNA encoded library can be utilized for the discovery of molecular glues, respectively.
- Through the pull-down-based ternary screening method, rationally designed, customized PROTAC DELs can accelerate the discovery of PROTACs.

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