Discovery of highly selective ligands with unique binding modes for challenging oncology targets using DNA-encoded chemical libraries

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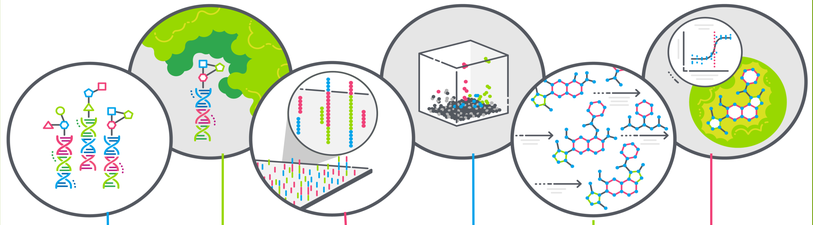
Abstract

DNA-encoded chemical library (DEL) technology enables the efficient screening of large collections of encoded compounds and has emerged as a powerful tool for hit identification in recent years. This technology allows for the multiplexed interrogation of target-ligand binding interactions and results in DEL hits with unusual binding modes and desirable selectivity. The screening methodology is particularly effective for oncology targets. Examples of discovery of highly selective ligands for challenging oncology targets, including kinases, protein-protein interactions, and receptors, will be presented. We will illustrate how DEL campaign strategies directly aim at novel binding modes and provide molecular details around the uniqueness of these interactions. Furthermore, we will show how these novel binding modes lead to unique pharmacology with direct benefit to patients. Lastly, we will demonstrate how X-Chem's high-quality encoded libraries enhance the tractability and novelty of chemical equity discovered with the platform.

X-Chem's Discovery Platform



DNA-Encoded Library Generation- Split and Pool

Small molecule consisting of successively concatenated "building blocks"

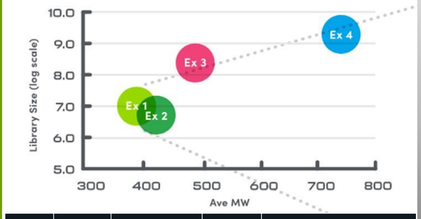
Encoding oligonucleotide consisting of successively concatenated "tags"

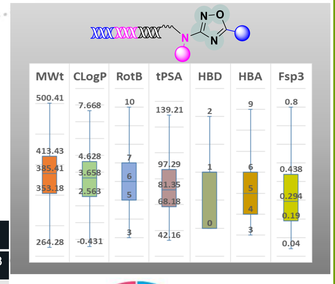
Library Numerical Size = (Green BBs) x (Red BBs) x (Blue BBs)

Oligonucleotide ligation successively records individual library member's synthetic history

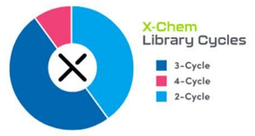
- **Advantages over other encoding methods**
  - Compounds and libraries are screened as a mixture
  - Large split sizes supported: >10,000
  - Numerically large libraries even with only two diversity steps
- **X-Chem's expansive and unique approach to chemistry**
  - 40,000+ Building Blocks representing >10 classes for library synthesis
  - >70% of recent libraries utilize X-Chem proprietary chemistries

X-CHEM Encoded Library Deck Overview

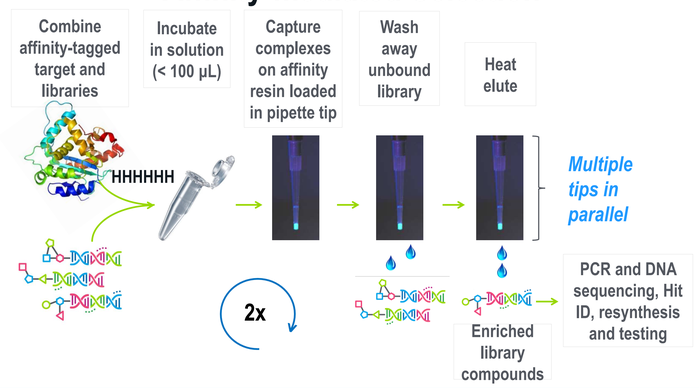




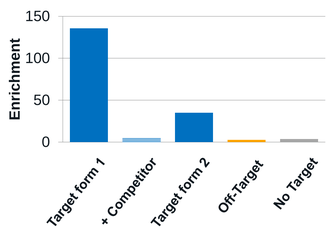
Lib	Cycle #	Chem	Lib Size	Average MW	Average cLogP	Average Fsp³
Ex 1	2	Oxadiazole	7.8 M	385	3.6	0.29
Ex 2	2	Azacycle	4.3 M	417	3.4	0.52
Ex 3	3	Peptide	225 M	490	1.8	0.42
Ex 4	4	Macrocycle	1.5 B	750	3.3	0.37



Affinity-mediated Selection



Insight into Lead Characteristics from Parallel DEL Selections

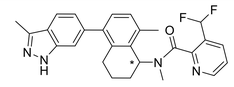


- Entire library is simultaneously selected for affinity to the target protein in a single tip
- **Multiple tips = Multiple distinct selection conditions in one campaign**
  - Read out the characteristic of each enriched compound across multiple conditions
  - Selectivity evaluation directly from selection data
    - –Select against multiple targets from the same target class
  - Binding site indication / Mechanism of Action
    - –+/- Competitor (orthosteric, allosteric binders, binding partner, etc.)
- Multiple forms of the target be used to increase probability of success.

AstraZeneca c-MET Inhibitors

- The dysregulation of c-MET plays a major role in cancers
- Major challenge to the existing therapy: emergence of acquired drug-resistant mutant forms of c-MET
- Inhibitors for both wild-type and drug-resistant mutant forms were identified from DEL screen, achieving the project goal
- Series displays high kinome selectivity

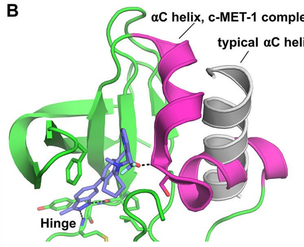
A



1 racemate

2 *R*, IC_{50} (WT | D1228V): 0.9 µM | 0.09 µM

B



Crystal structure (2.01 Å) of D1228V c-MET bound by compound **1** highlighting the highly unusual conformation of the αC helix (magenta) observed in this complex, in comparison to αC helix of c-MET bound by savolitinib (light grey)

Collie, G. W. et al *Bioorg. Med. Chem. Lett.* **2022,** *75*, 128948; Collie, G. W. et al *J. Med. Chem.* **2024**, *67*, 2, 864–884

AstraZeneca MerTK Inhibitors

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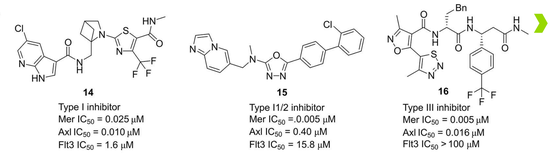
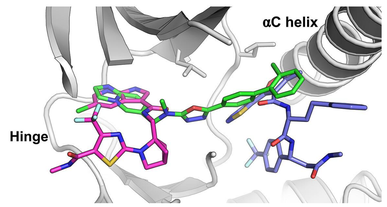


14
Type I inhibitor
Mer IC_{50} = 0.025 µM
Axl IC_{50} = 0.010 µM
Flt3 IC_{50} = 1.6 µM

15
Type I1/2 inhibitor
Mer IC_{50} =.0.005 µM
Axl IC_{50} = 0.40 µM
Flt3 IC_{50} = 15.8 µM

16
Type III inhibitor
Mer IC_{50} = 0.005 µM
Axl IC_{50} = 0.016 µM
Flt3 IC_{50} > 100 µM

B



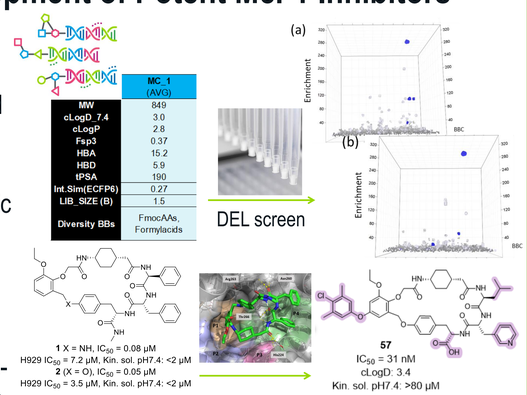
Overlay of crystal structures of all three hits showing the different binding modes in complex with Mer, **14** (magenta), **15** (green) and **16** (blue).

Nissink, J.W. et al *J Med Chem* **2021** *64* (6), 3165; Collie, G. W. et al *J. Med. Chem.* **2024**, *67*, 2, 864

- Inhibiting Mer/Axl will promote tumor immunity by inhibiting M2 macrophage-derived immunosuppressive mechanisms and sustaining Dendritic cell activation
- Multiple series of MerTK inhibitorsof distinct modes of action were identified from X-Chem DEL screen, offered excellent start points for lead development
- Tumor shrinkage in mouse xenograft study validate dual Mer/Axl inhibition as strategy to restore innate immune response

XRx Symeres Development of Potent Mcl-1 Inhibitors

- The pro-survival protein myeloid cell leukemia 1 (Mcl-1) is an antiapoptotic member of the Bcl-2 family, associated with tumor aggressiveness, poor survival, and drug resistance.
- Conformationally privileged macrocyclic Mcl-1 inhibitors were identified from DEL screen.
- Structure-enabled lead optimization of the DEL-derived series demonstrated lead candidates with desirable physico-chemical properties for therapeutic use.



Hekking, et al *J Med Chem* **2024**, *67*, 4, 3039−3065

Acknowledgements

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X-Chem Website

Poster Reprint