Using DEL for Targeted Protein Modulation

Marie Malone, PhD
DEL & Protein Sciences

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Nurix Drugs Engage Ligases for the Treatment of Cancer

Targeted Protein Modulation: TPM = TPD + TPE

A Powerful Cellular System

Ubiquitin is ligated to target proteins to tag them for degradation by the proteasome

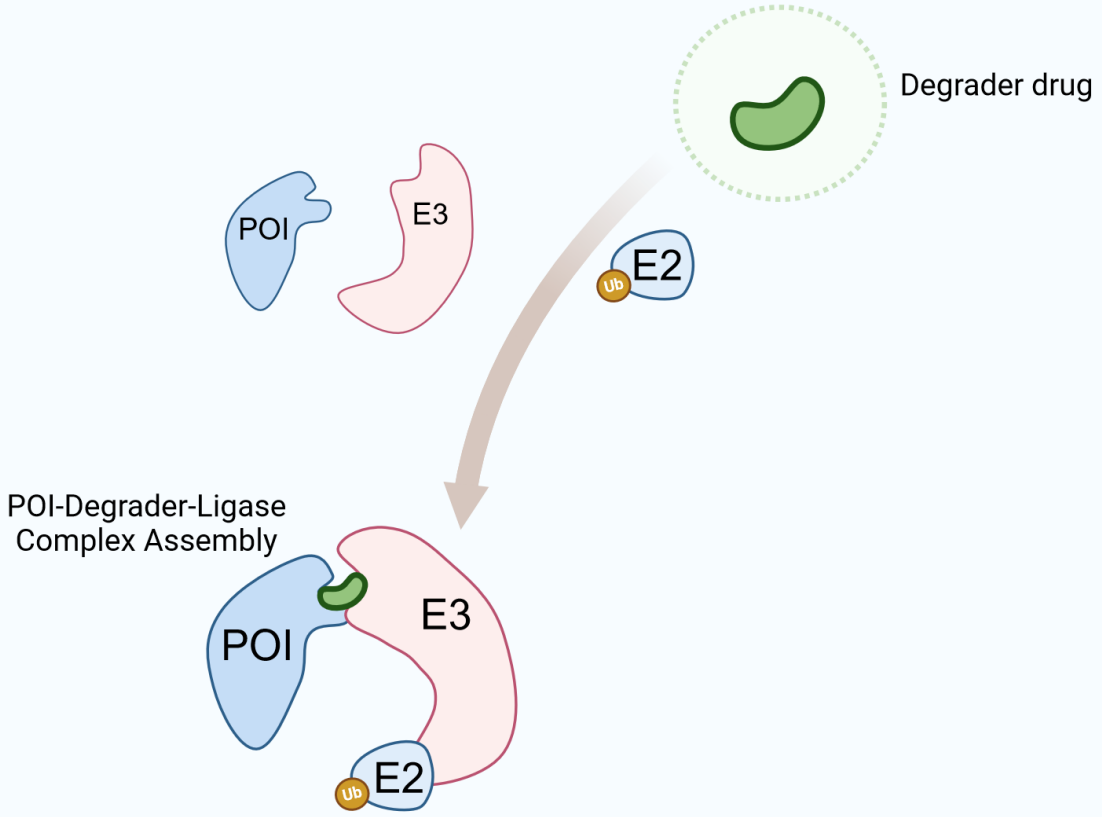
Harness ligases to decrease specific protein levels

Targeted Protein Degradation (TPD)

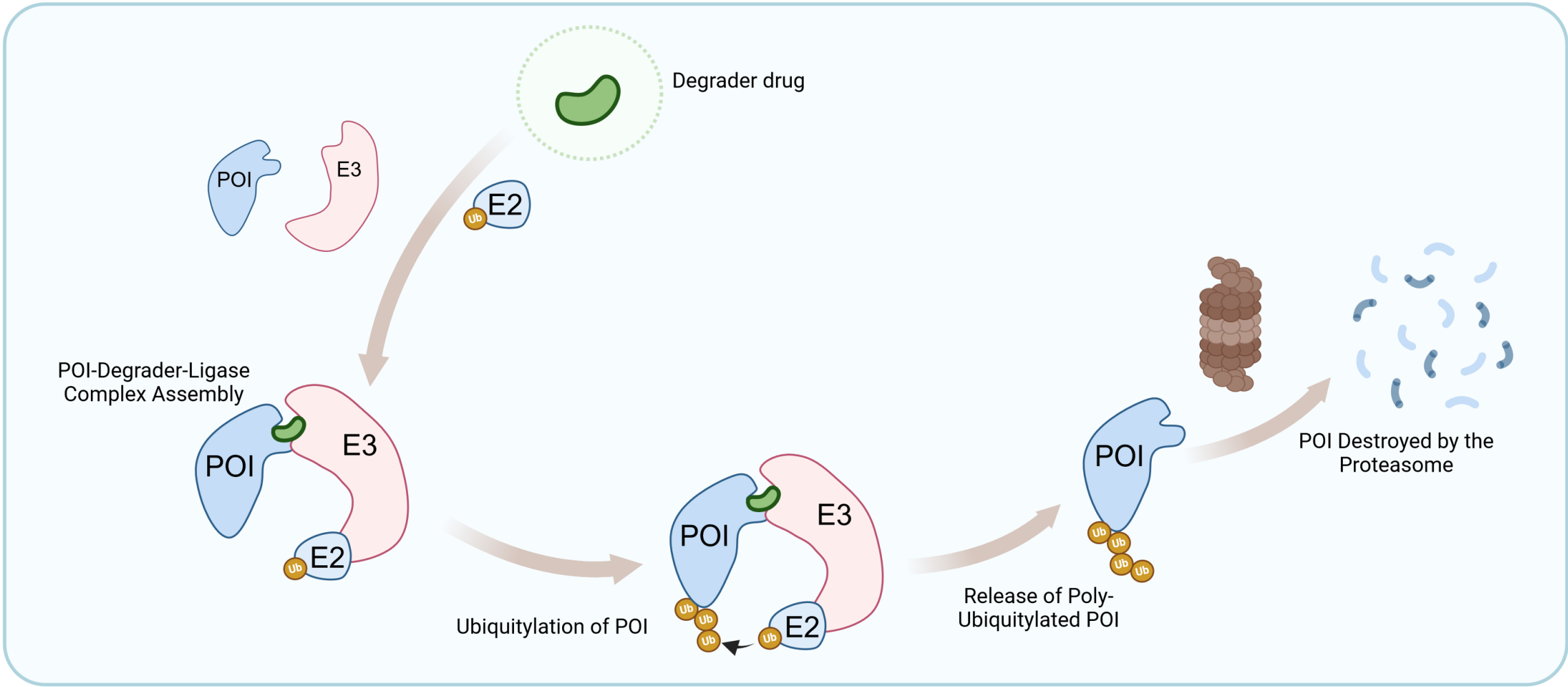
Targeted Protein Elevation (TPE)

Inhibit ligases to increase specific protein levels

Harnessing the ubiquitin proteasome system for therapeutic benefit



Harnessing the ubiquitin proteasome system for therapeutic benefit



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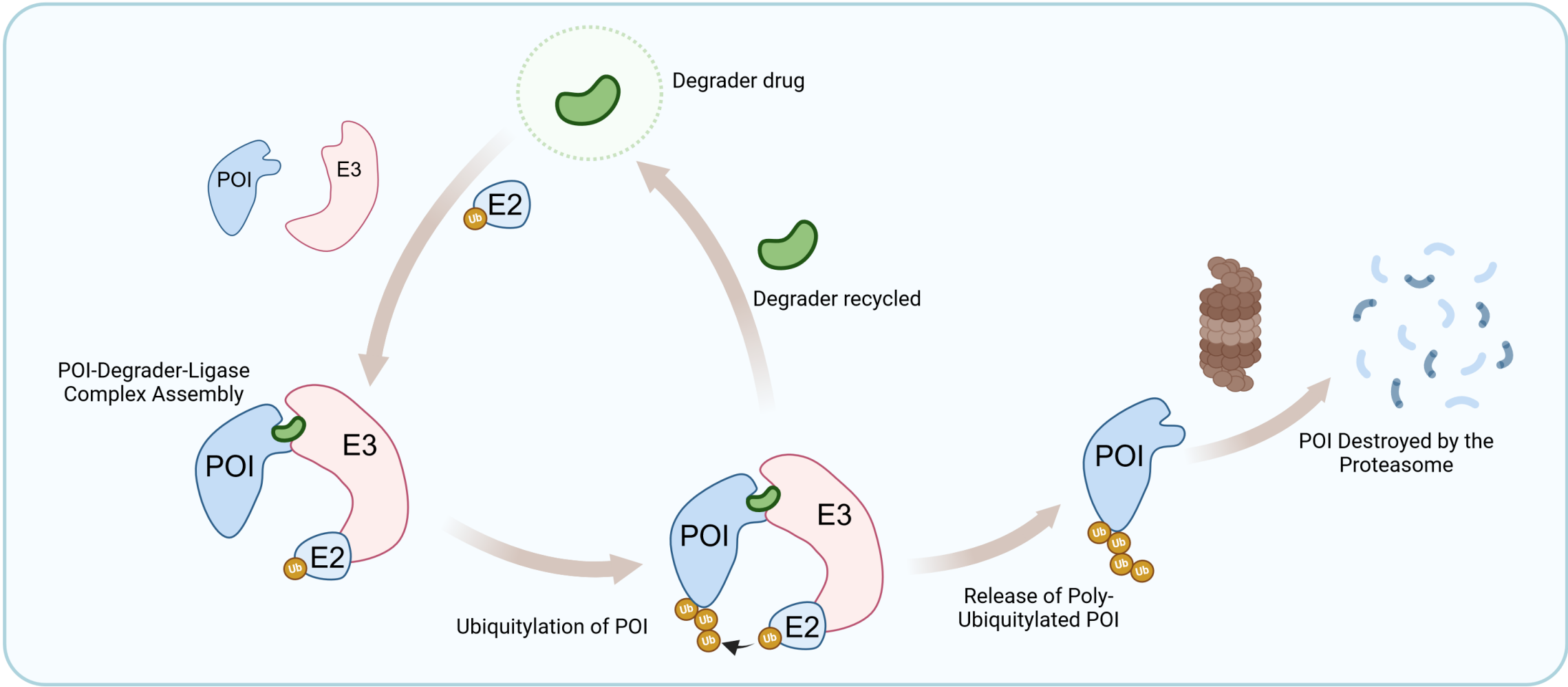
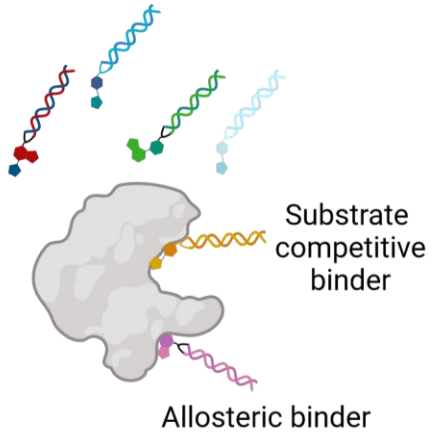
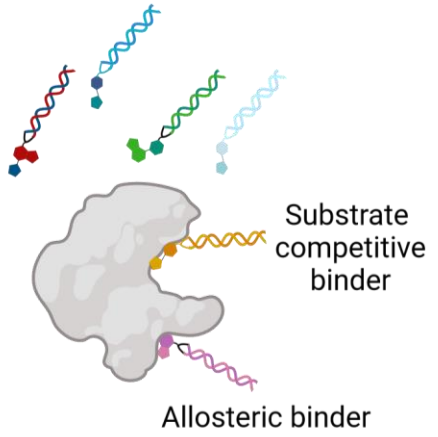


Affinity-based DEL screening is an ideal approach to enable new binder discovery for induced proximity

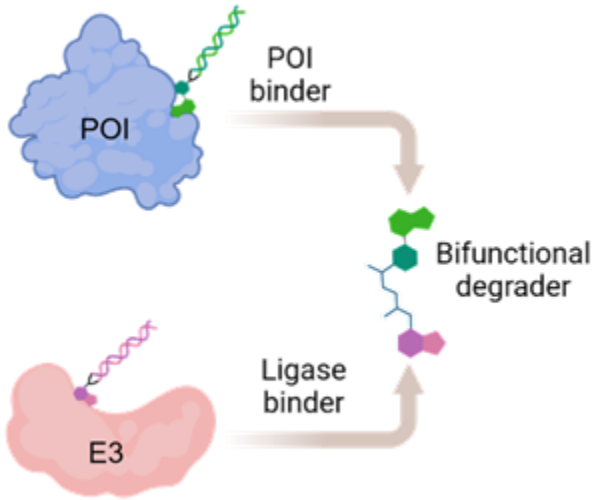


- **Affinity-based ligand discovery is the ideal approach to enable induced proximity**
  - **Affinity-based screening of effectors is MoA agnostic**
- Low per screen cost allows for a broad exploration of target and ligase chemical space

Bifunctional degrader synthesis can simultaneously leverage DEL binders to many ligase or POI binding sites



Protein of interest (POI)



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- **DNA attachment provides initial handle for bifunctional molecule synthesis**

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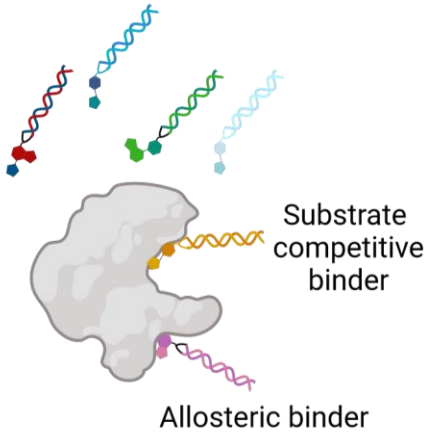

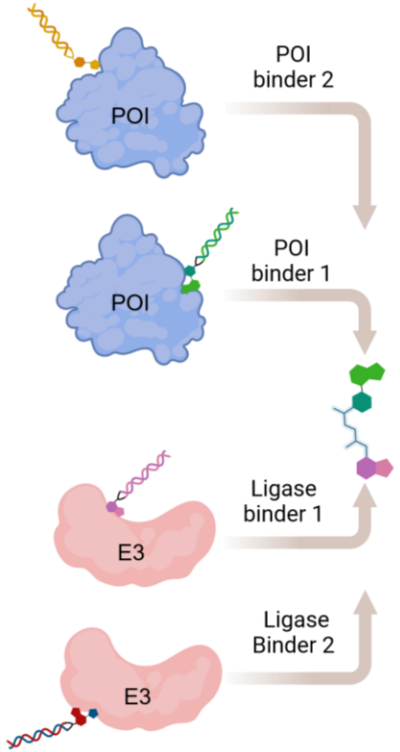

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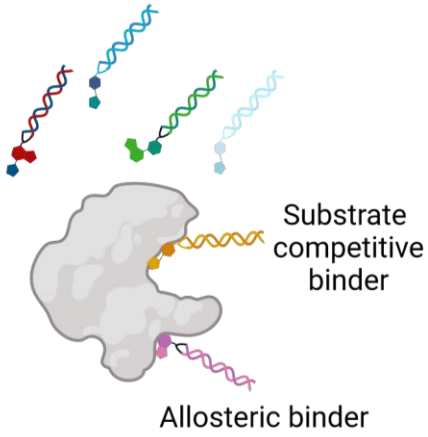

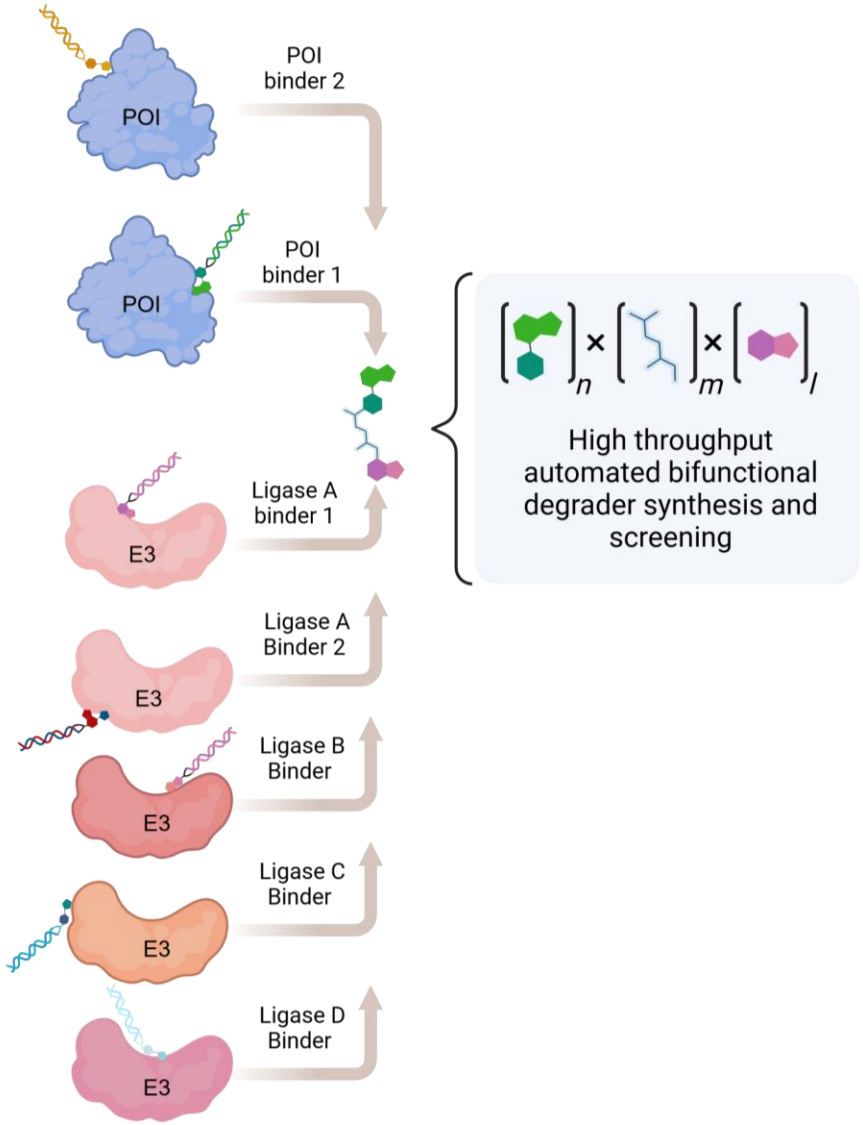

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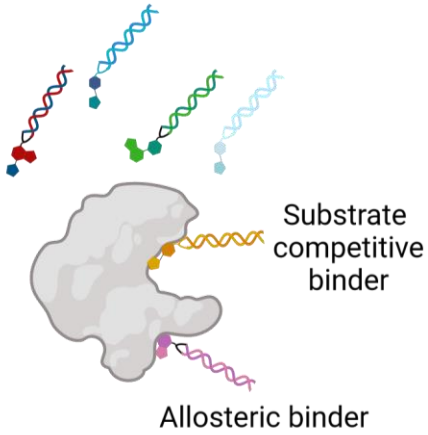
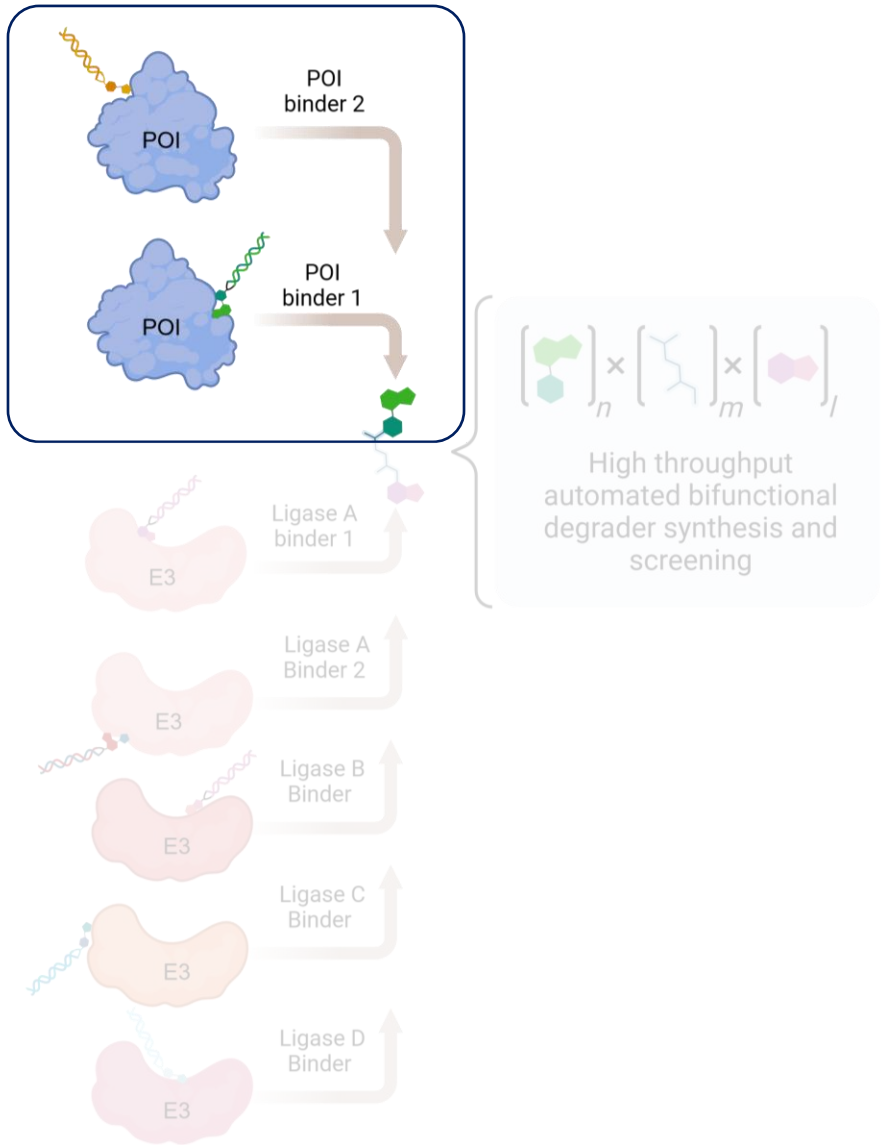
- Low per screen cost allows for a broad exploration of target and ligase chemical space
- DNA attachment provides initial handle for bifunctional molecule synthesis
- **Combinatorial degrader design and synthesis enable rapid hit follow up and optimization**

Leveraging DEL to identify binders for challenging targets





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  - Affinity-based screening of effectors is MoA agnostic
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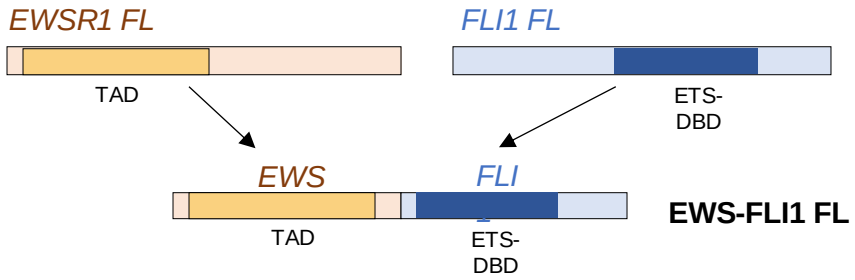
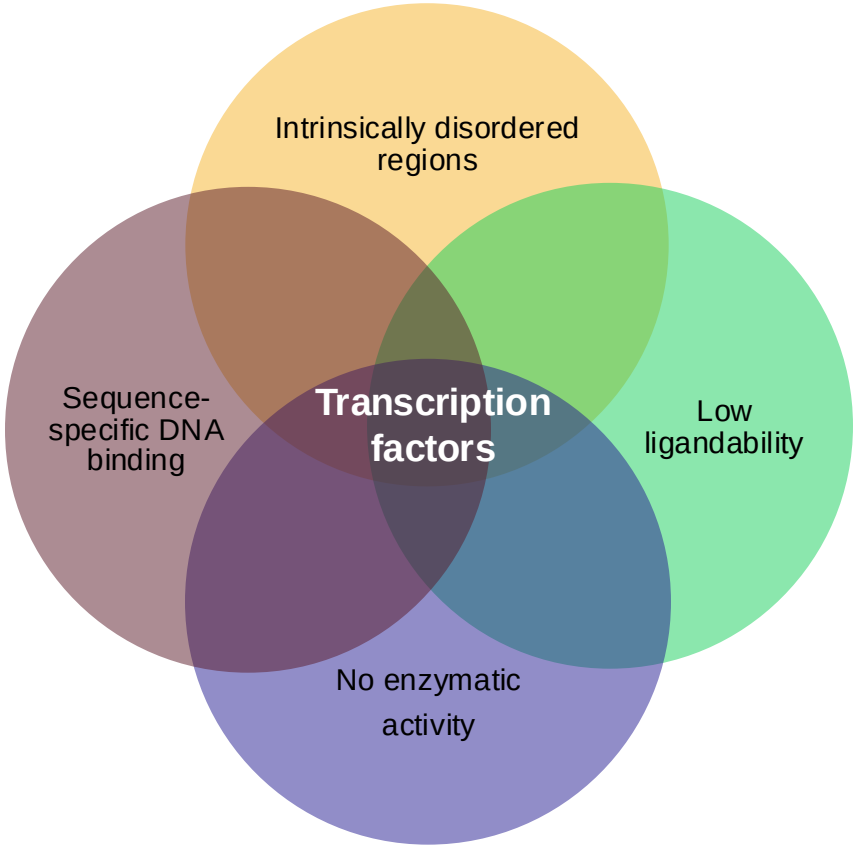
- **Combinatorial degrader design and synthesis enable rapid hit follow up and optimization**

The Nurix DEL screening and analysis platform is designed to unlock challenging targets, including transcription factors, for TPD

- **EWS-FLI1** is a fusion protein caused by chromosomal translocation
  - **EWSR1 -** strong transactivation domain (TAD)
  - **FLI1 –** ETS-DBD transcription factor
    - Binds to 5’ GGAA 3’ dsDNA sequences
  - This leads to **aberrant transcription of oncogenes in Ewing sarcoma**
- EWS-FL1 fusion present in >85% of patients with Ewing sarcoma
- **Ewing sarcoma (ES)** is a pediatric bone and soft tissue cancer with **no therapies available**
- Ewing sarcoma impacts children and young adults, constituting 10-15% of all bone sarcomas
- ~200 patients are diagnosed with Ewing sarcoma each year in the United States

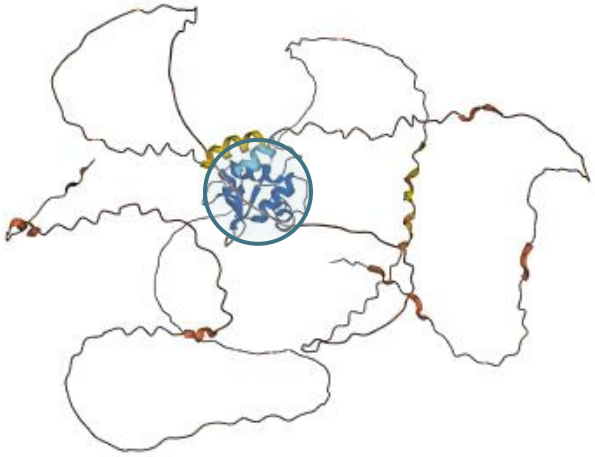


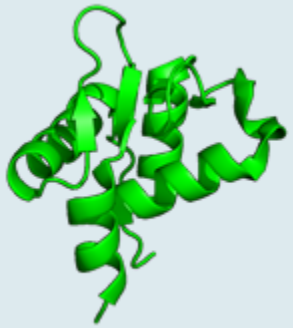
Challenges in Transcription Factor Ligand ID



EWS-FLI1 DEL screen focused on DNA-binding domain (DBD)

EWS-FLI1 (Alphafold)





EWS-FLI1 FL

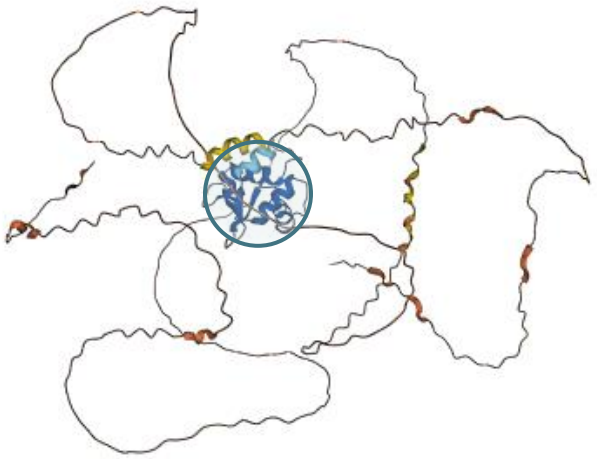
WT FLI1 DBD

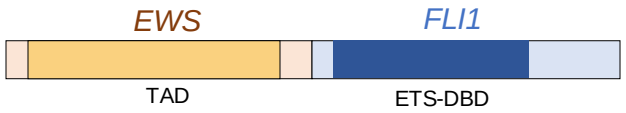
304-445

Hou and Tsodikov, *Biochemistry* (2015)

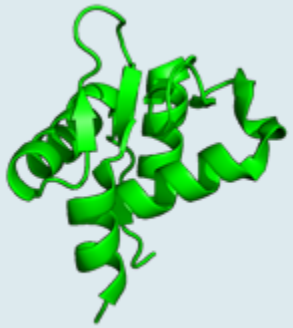
EWS-FLI1 DEL screen focused on DNA-binding domain

EWS-FLI1 (Alphafold)





EWS-FLI1 FL



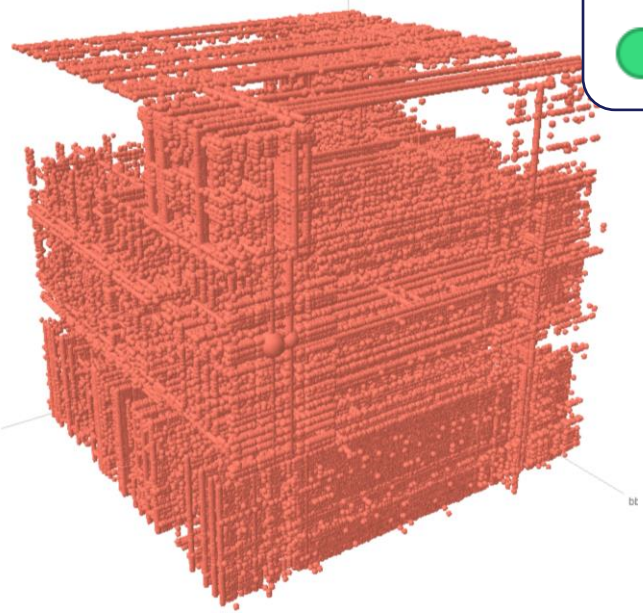
WT FLI1 DBD

304-445

bb2

bb1

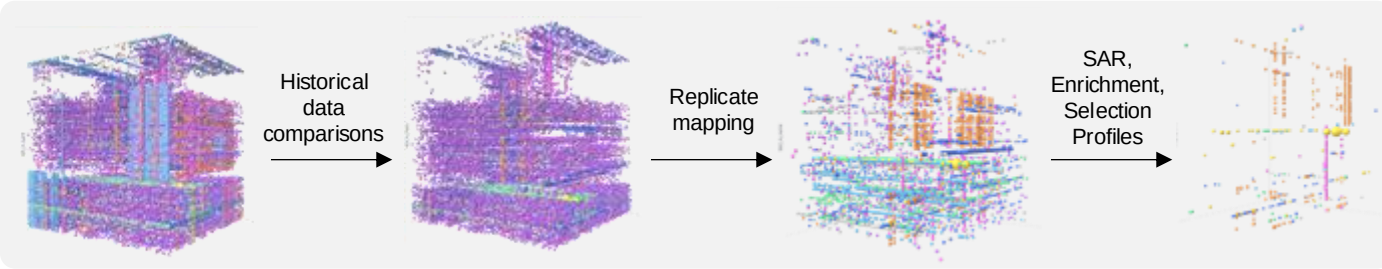
bb3



DNA barcode contains consensus sequence

Consensus sequence free DNA barcode

72% FASTA reads contain known DNA consensus sequence

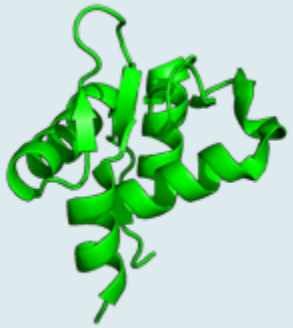


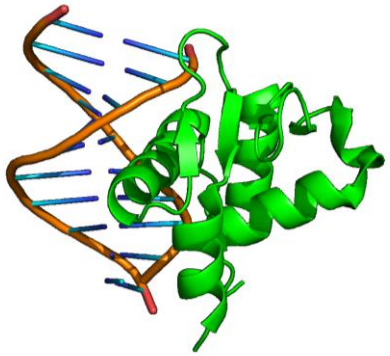
Two strategies used in parallel to reduce sequence-driven enrichment: blocking with a consensus sequence and introducing mutations that prevent DNA binding

Strategies to mitigate DNA tag-driven enrichment of consensus sequence

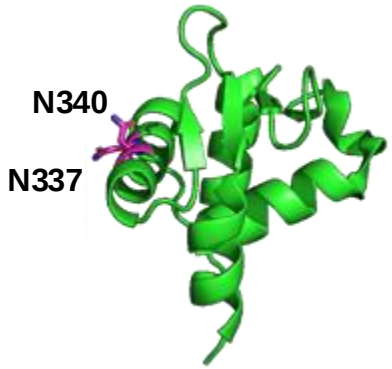
- **DNA blockers**
  - Literature-reported DNA consensus sequence
  - Computationally identified DNA consensus sequence from DEL sequencing output

- **DEL selections performed against a mutated DBD that fails to bind DNA**

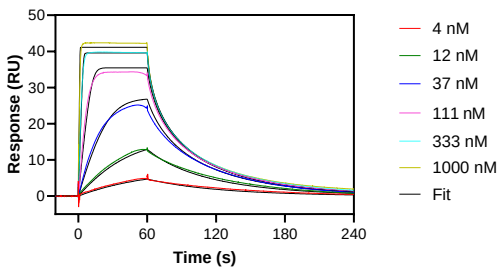




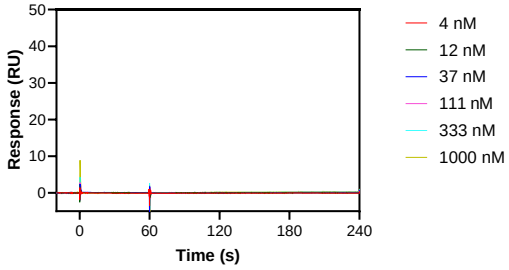
+DNA consensus sequence blocker



DBD$^{R337N/R340N}$ mutant



Consensus sequence DNA K_D = 20 nM



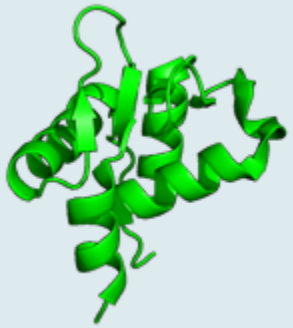
***No binding* of consensus sequence DNA**

Hou and Tsodikov, Biochemistry (2015)

Consensus sequence blocking reduces DNA-driven DEL enrichment

Strategies to mitigate DNA tag-driven enrichment of consensus sequence

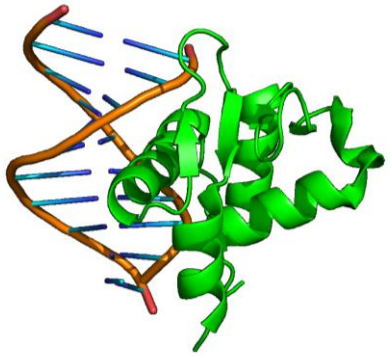
- **DNA blockers**
  - Reduce % FASTA reads containing consensus sequence to levels similar to bead only & Naïve library samples
  - Encoding tags of hits enriched in the presence of consensus sequence blocker contain consensus sequence -> ***how to distinguish between DNA driven vs. true small molecule driven binding?***



WT FLI1 DBD

304-445

ETS-DBD



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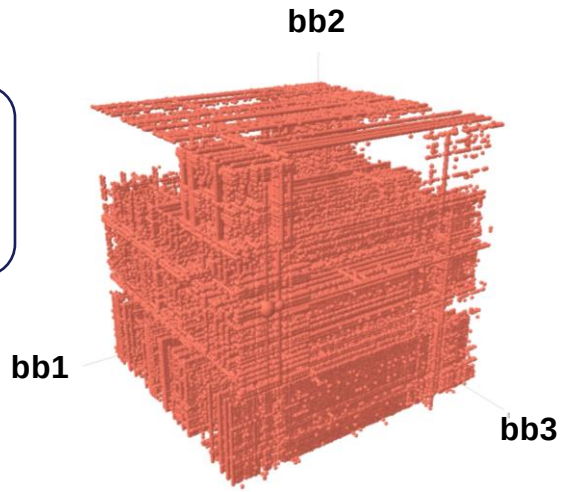
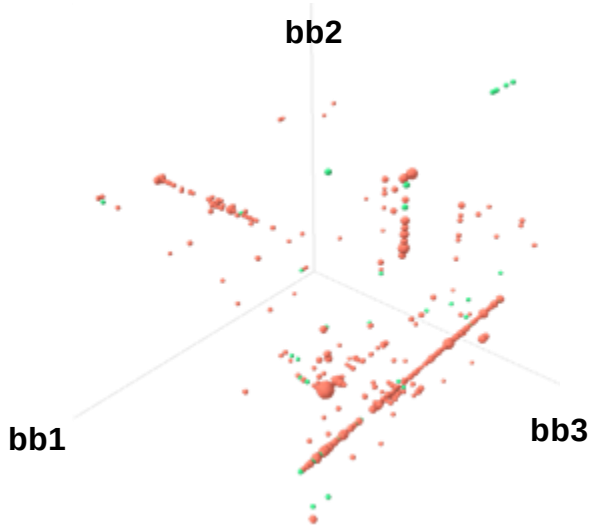


72% Consensus Sequence



25% Consensus Sequence

Hou and Tsodikov, Biochemistry (2015)

Orthogonal screening methods show highly consistent DEL output

Strategies to mitigate DNA tag-driven enrichment of consensus sequence

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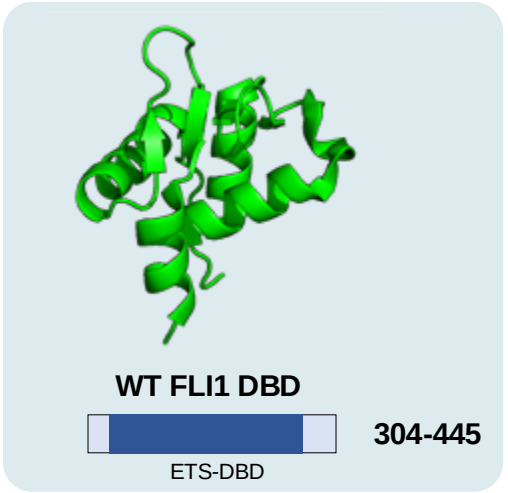
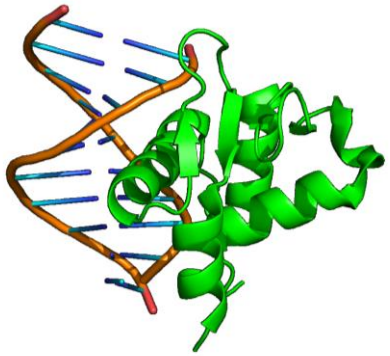
- **DEL selections performed against mutant proteins**
  - *Hits that enrich in both +blocker and DNA-binding mutant conditions are likely true binders, even if the DNA encoding tags contain the consensus sequence*





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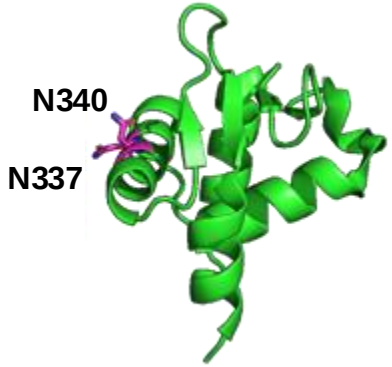

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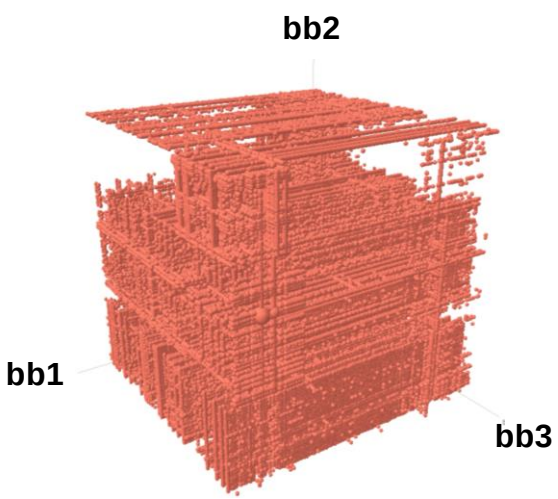

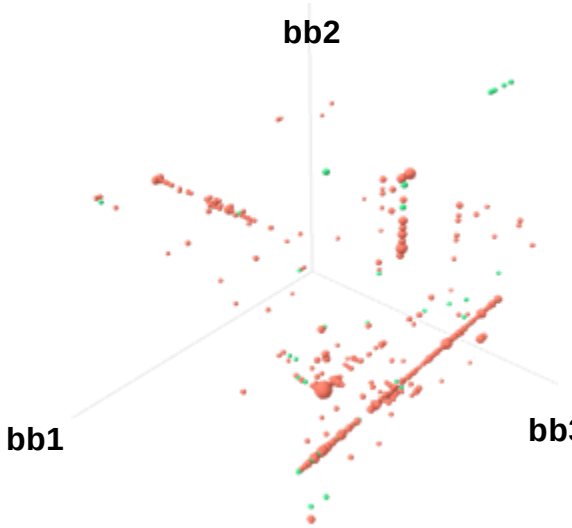

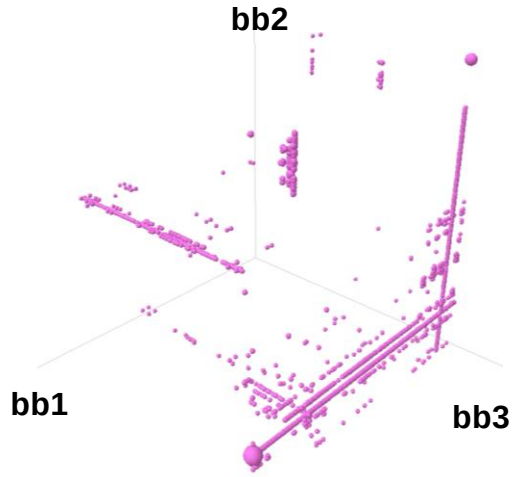

nurix

Hou and Tsodikov, Biochemistry (2015)

Robust enrichment of 4 distinct chemical series in orthogonal screening formats

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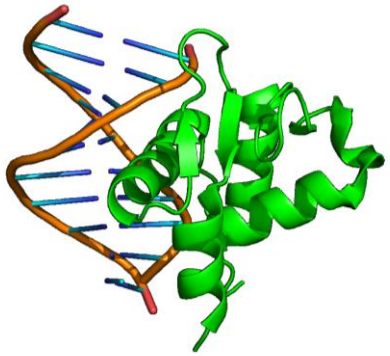

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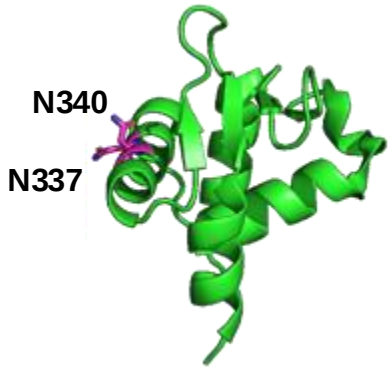

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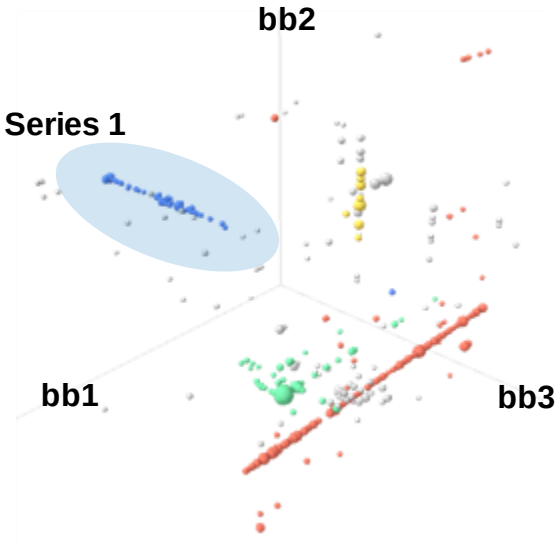
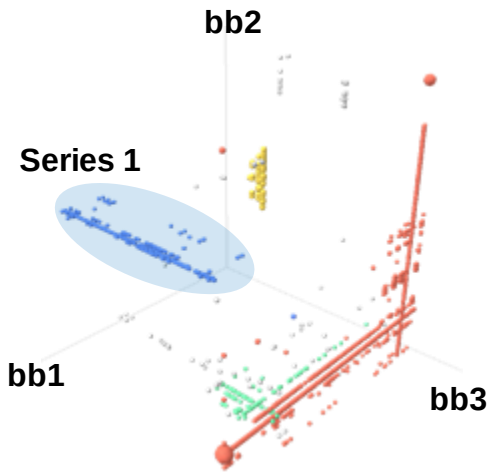
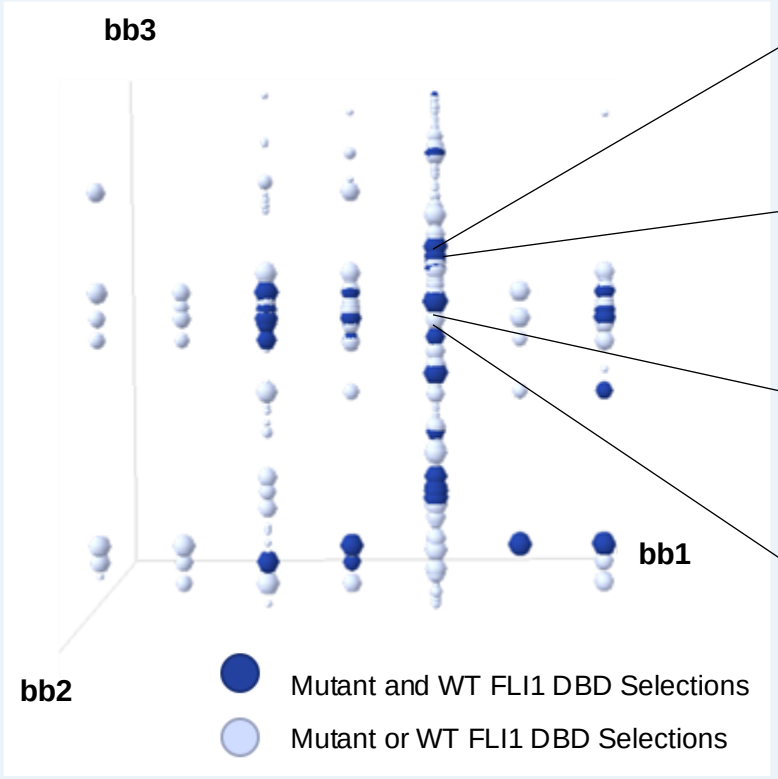


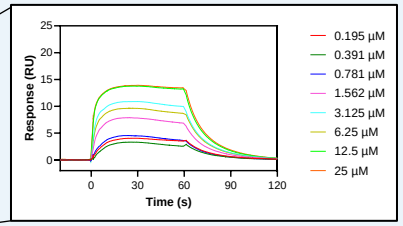


Hou and Tsodikov, Biochemistry (2015)

Multiple DEL hits confirmed to bind EWS-FLI DBD using SPR

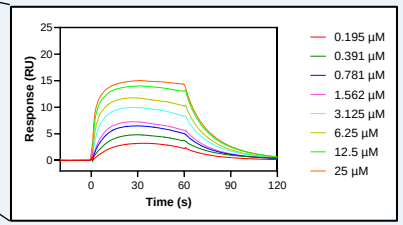


Hit in WT and Mutant Selections



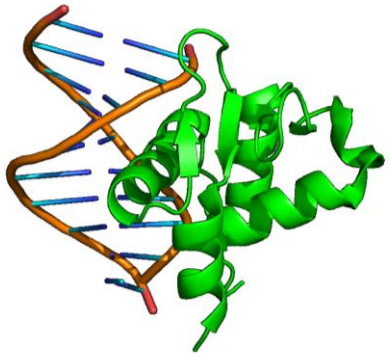
DEL Hit 1
FL1 DBD K_D = 1.4 µM

Hit in Mutant Selection

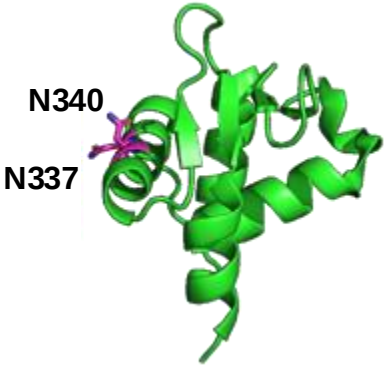


DEL Hit 2
FL1 DBD K_D = 1.8 µM

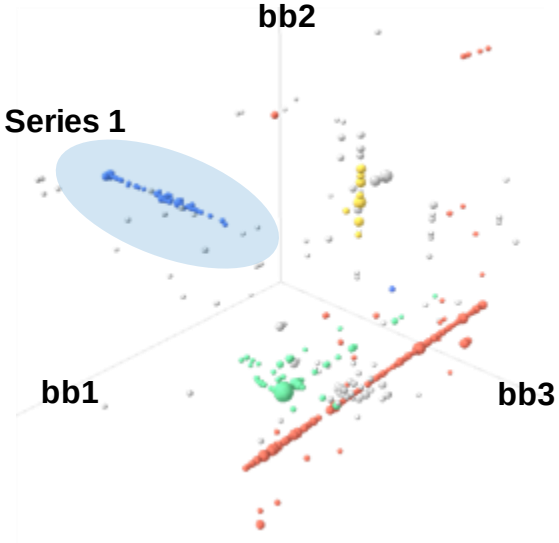
Both hits have FASTA reads containing consensus sequence

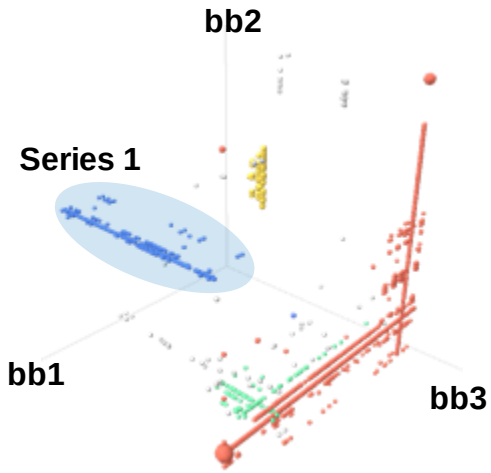


+DNA consensus sequence blocker



DBD$^{R337N/R340N}$ mutant

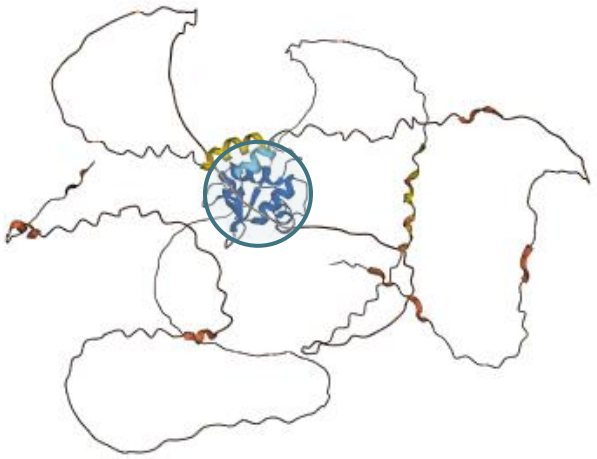


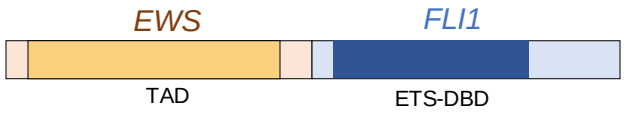


Hou and Tsodikov, Biochemistry (2015)

Leveraging DEL screening methodology to confirm binding to full length EWS-FL1 fusion

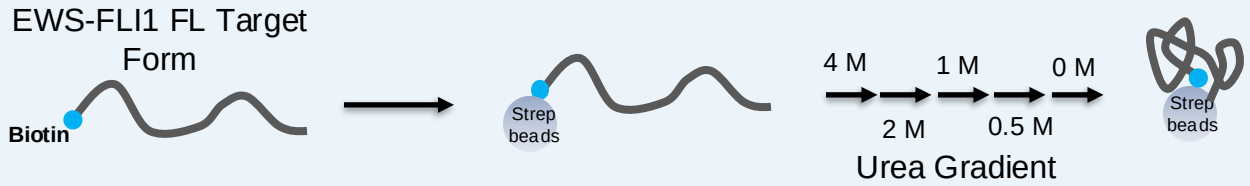
EWS-FLI1 (Alphafold)





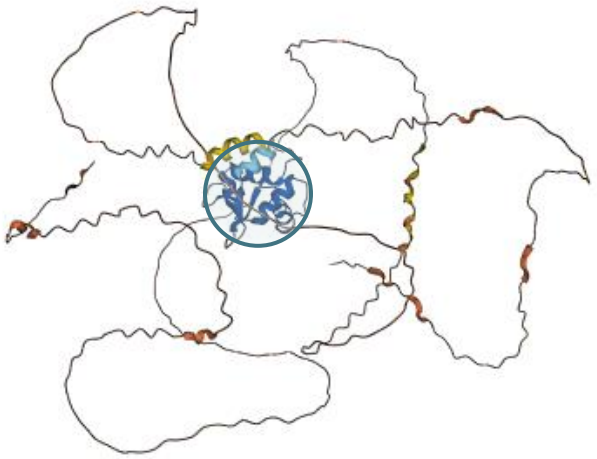
EWS-FLI1 FL

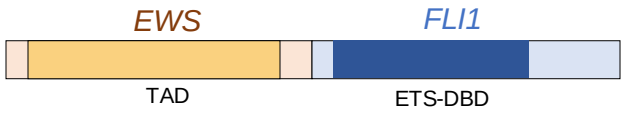
Refolding of EWS-FLI1 FL on resin



Leveraging DEL screening methodology to confirm binding to full length EWS-FL1 fusion

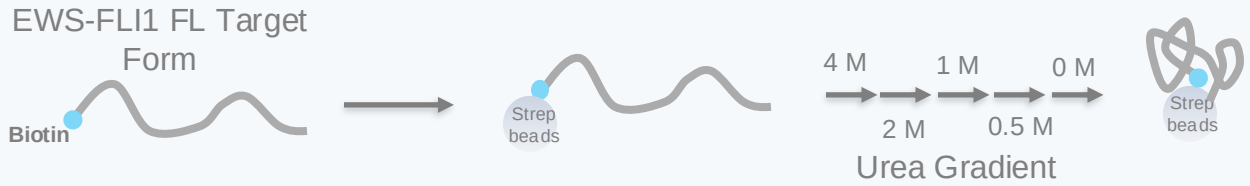
EWS-FLI1 (Alphafold)

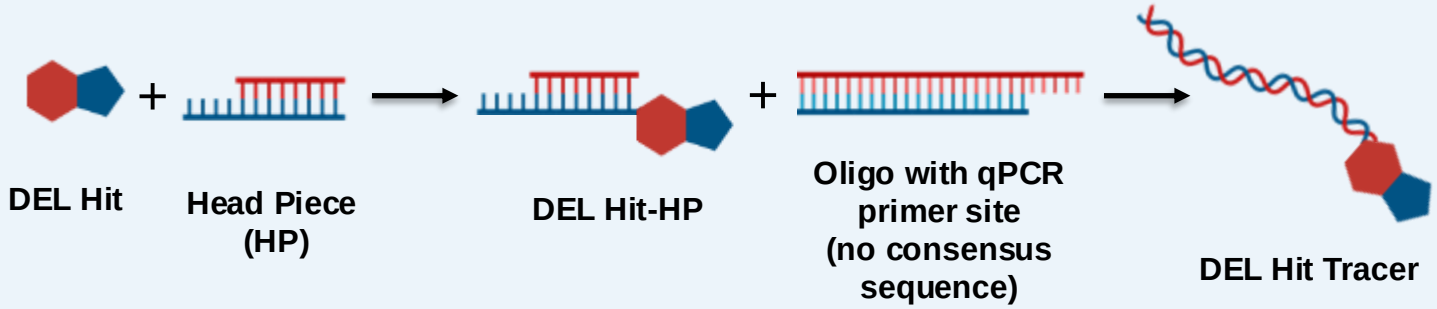




EWS-FLI1 FL

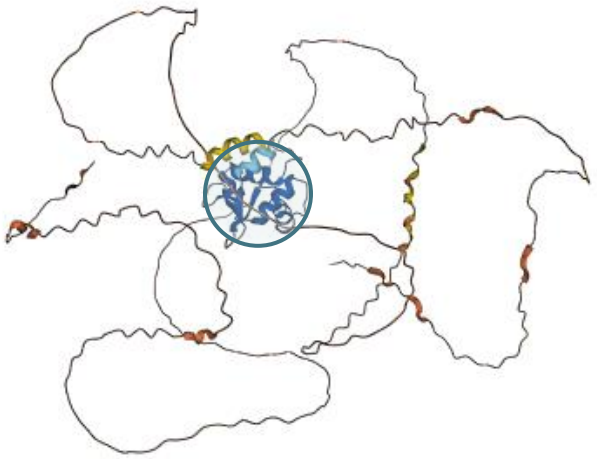
Refolding of EWS-FLI1 FL on resin

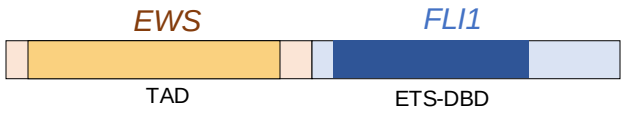




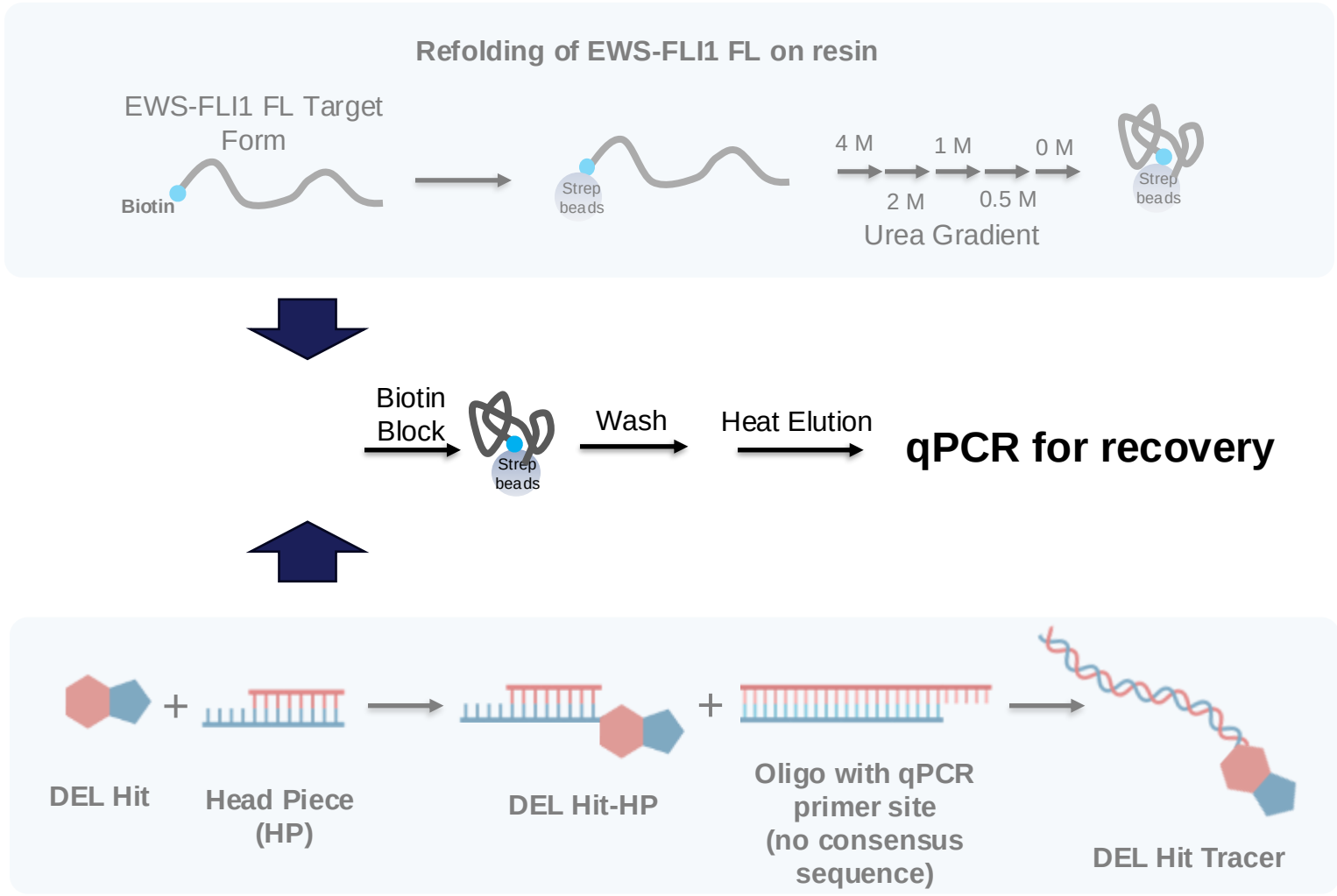
Leveraging DEL screening methodology to confirm binding to full length EWS-FL1 fusion

EWS-FLI1 (Alphafold)





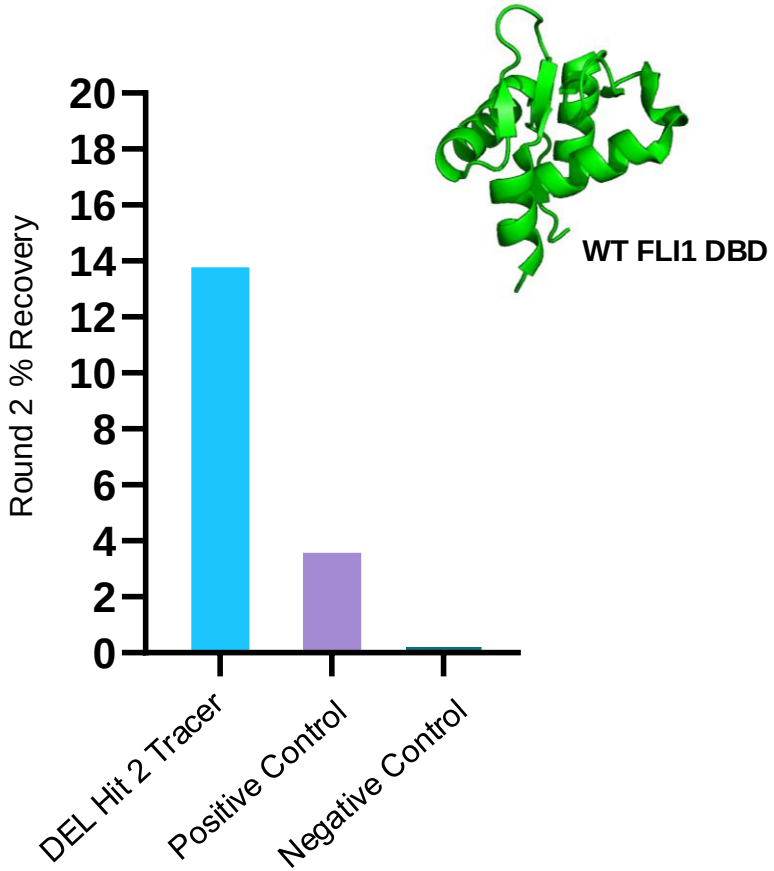
EWS-FLI1 FL



Leveraging DEL screening methodology to confirm binding to full length EWS-FL1 fusion

Binder recovery
~10% per round

Background recovery
~1% per round



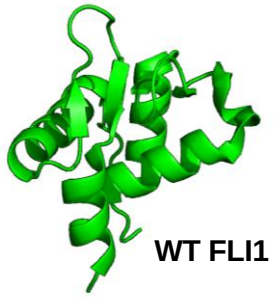
=  =  Positive Control
Consensus Sequence

=  Negative Control
No Consensus Sequence

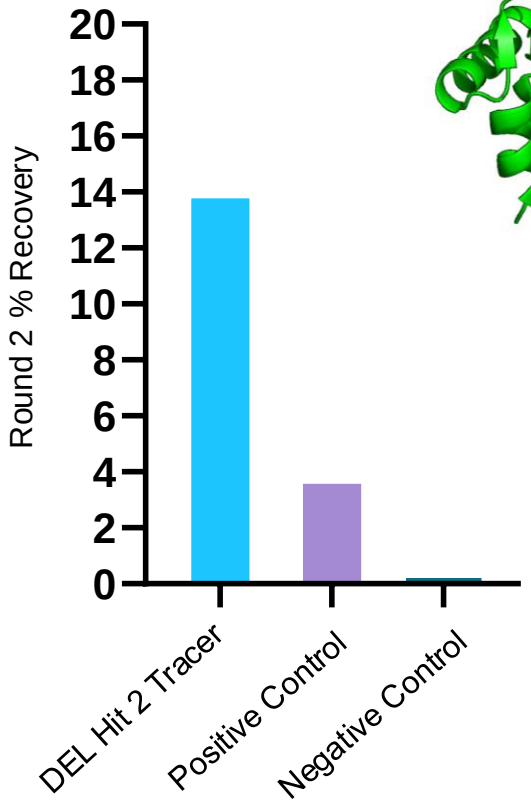
FLI1 binder engages EWS-FLI1 full length fusion

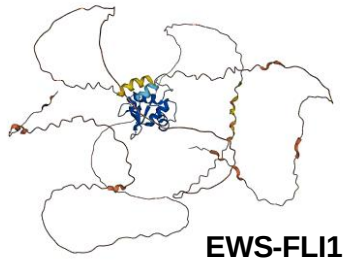
Binder recovery
~10% per round

Background recovery
~1% per round

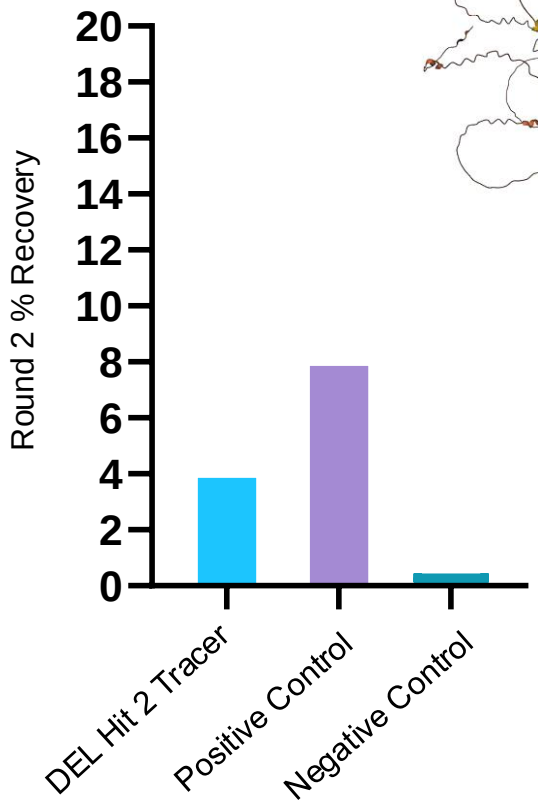


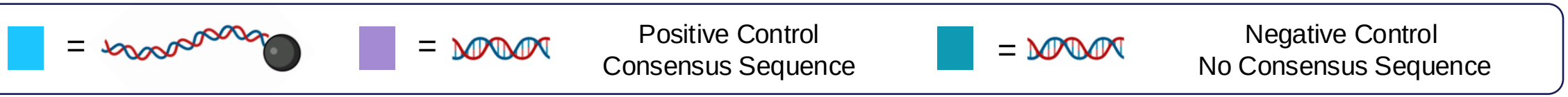
WT FLI1 DBD



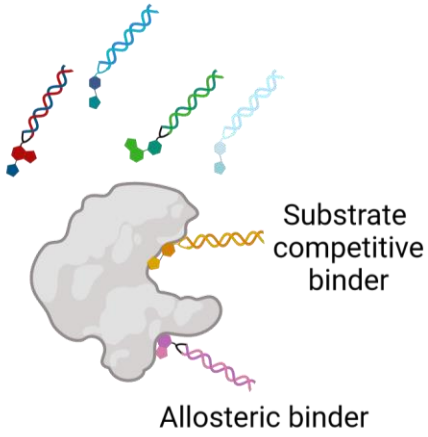


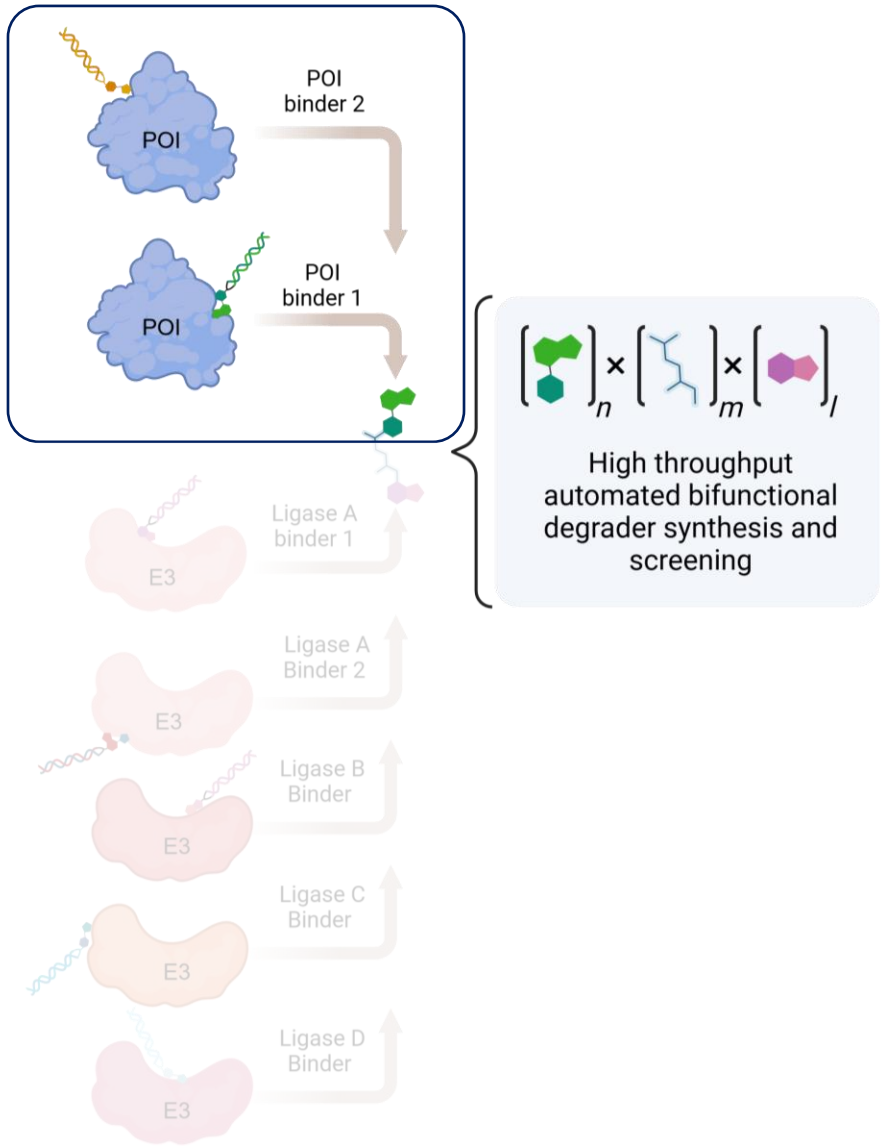
EWS-FLI1 FL





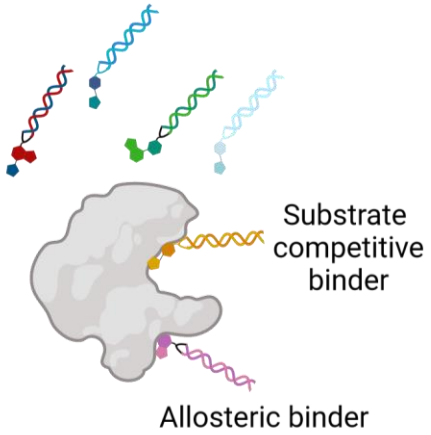
Binders to transcription factor fusion EWS-FL1 identified by DEL and progressed to bifunctional degrader optimization

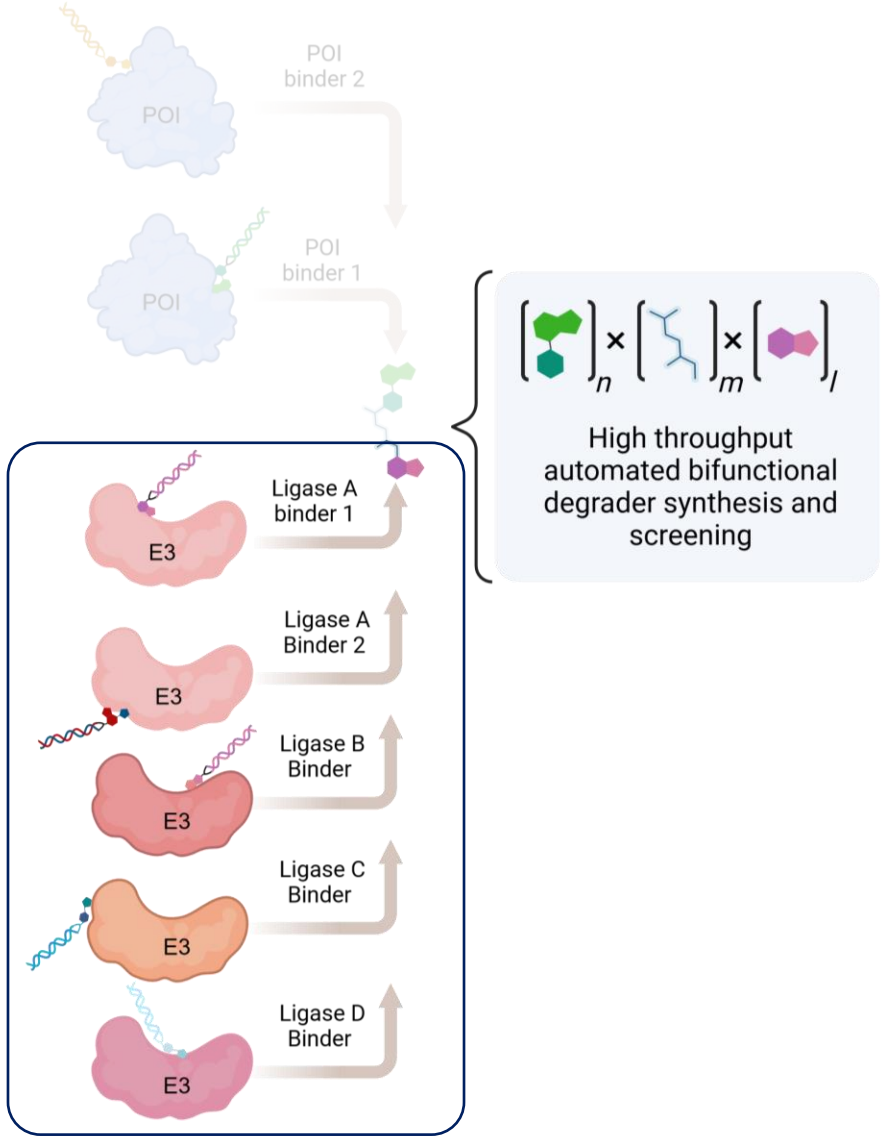




- Affinity-based ligand discovery is the ideal approach to enable Induced Proximity
  - Affinity-based screening of effectors is MoA agnostic
- Low per screen cost allows for a broad exploration of target and ligase chemical space
- DNA attachment provides initial handle for bifunctional molecule synthesis
- **Combinatorial degrader design and synthesis enable rapid hit follow up and optimization**

Leveraging DEL to enable diverse ligases beyond CRBN and VHL for degradation

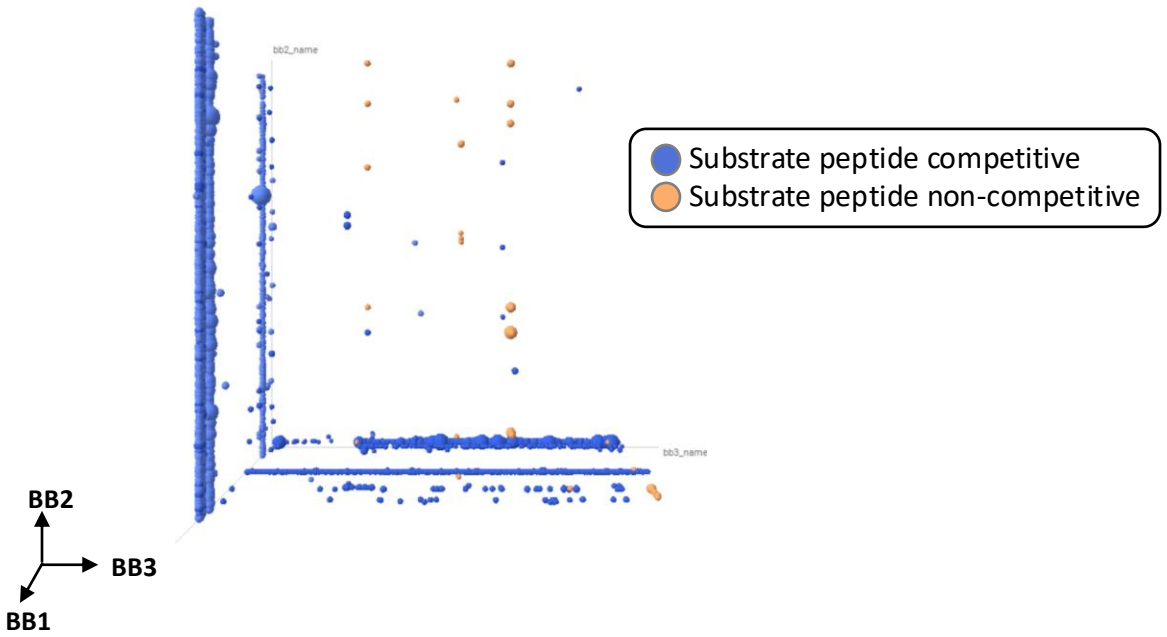




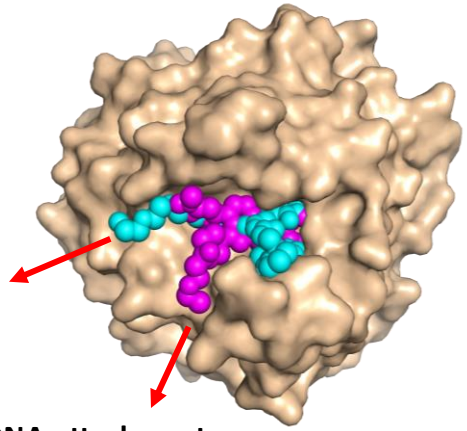
Chemical matter for novel ligases:

- Increases opportunities for complementary ligase/target interfaces (higher cooperativity, ternary complex formation & ubiquitylation)
- Provides opportunities for disease specific, compartment specific, or tissue specific degradation
- Enables alternate vectors & series for degrader optimization
- Potential applications for targeted protein modulation through direct ligase inhibition
- Provides second line degraders if resistance to CRBN or VHL arise

Multiple DEL series identified for KLHDC2, a surveillance ligase expressed in the nucleus, ideally suited to degrade a TF fusion like EWS-FLI1

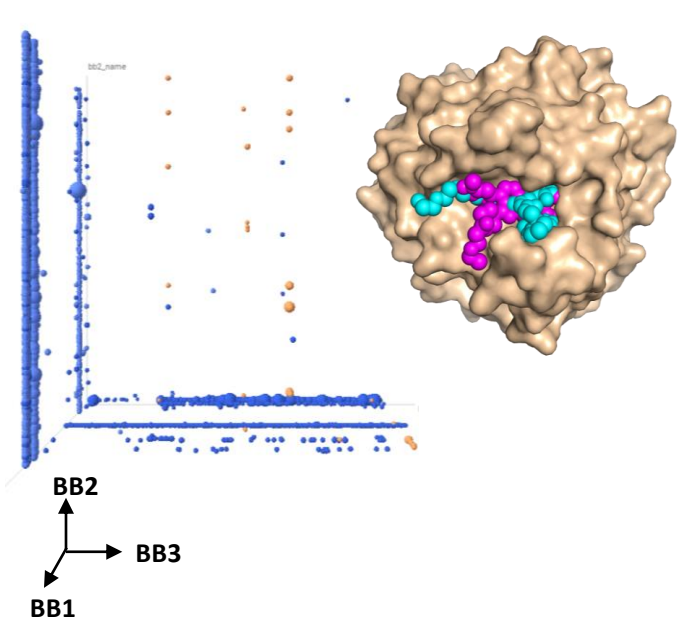


Overlay of Series 1 & 2 bound to KLHDC2



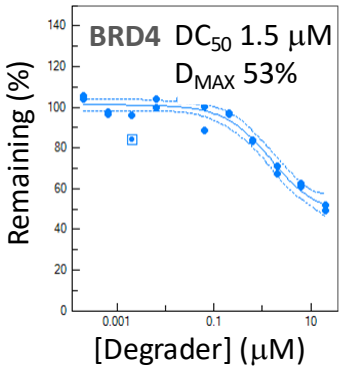
- KLHDC2 screened with native Gly-Gly C-end SelK degron peptide
- 4 peptide-competitive chemical series with IC_{50} < 550 nM identified
- Crystal structures of Series 1 and 2 indicated two distinct DNA-attachment point vectors

Unoptimized KLHDC2 DEL binders competent for TPD



Series 1 Initial Hit

IC_{50} = 4 nM



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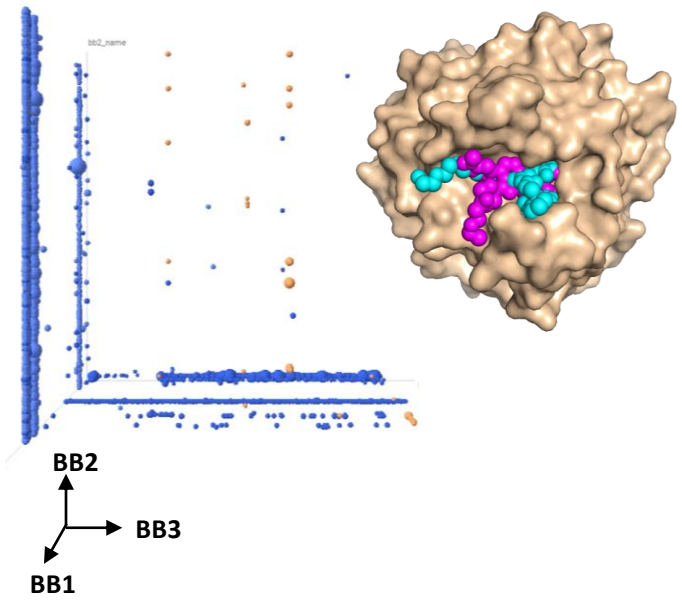
- **Bifunctional degraders containing Series 1 binders show modest degradation of BRD4**

Improvements in potency and exploration of multiple vectors for TPD design broadens the utility of KLHDC2 DEL binders for chemistry automation workflows



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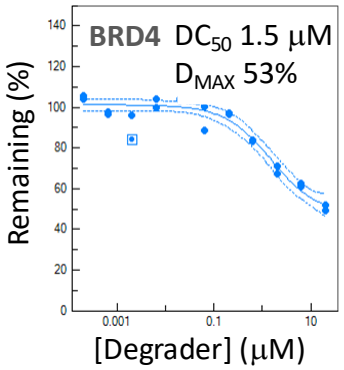
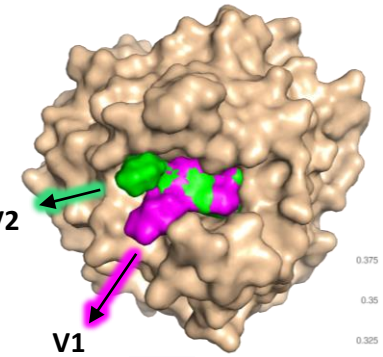
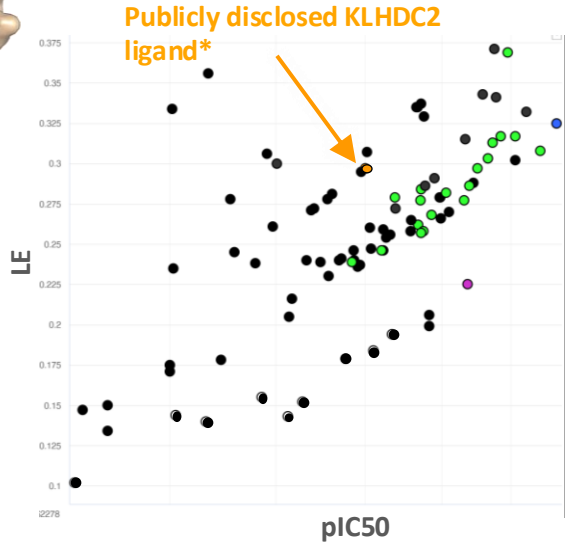


Extensive structure guided Series 1 vector & SAR profiling achieves improved potency



Optimized Hit
IC_{50} = 0.2 nM

Alternate Vector
IC_{50} = 2 nM



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- **Optimization of Series 1 and exploration of alternate vector generated binders with improved potency**

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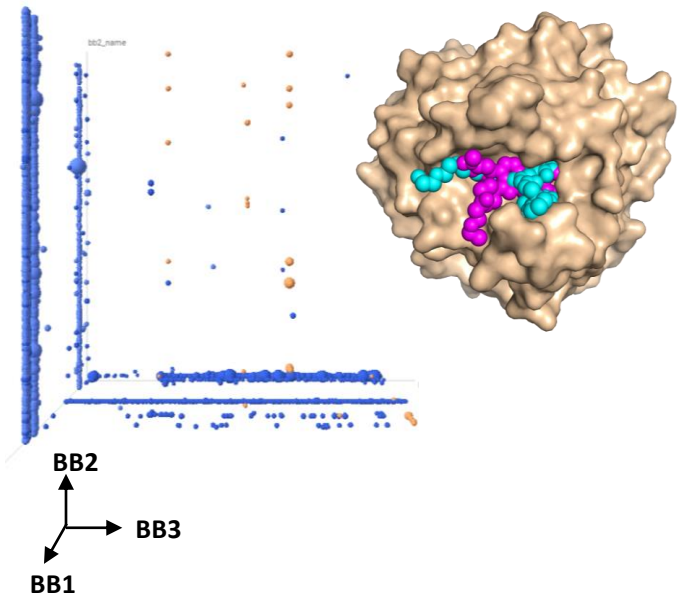
* Hickey, C. M. *et al. Nat Struct Mol Biol.* 31, 311-322 (2024)

Optimized KLHDC2 binders show improved & potent degradation of BRD4



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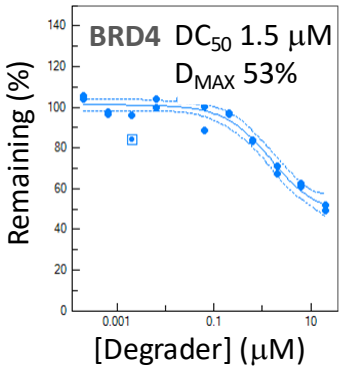

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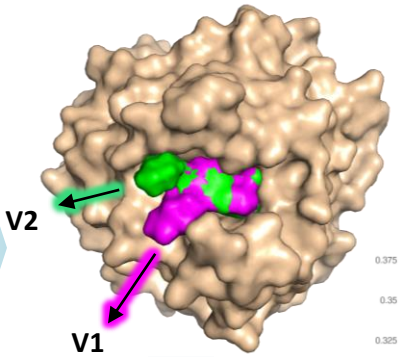

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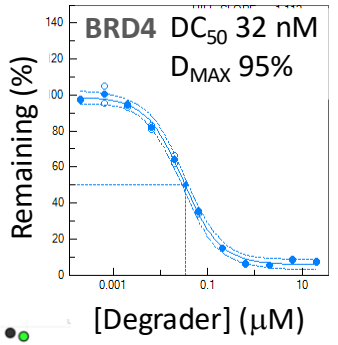
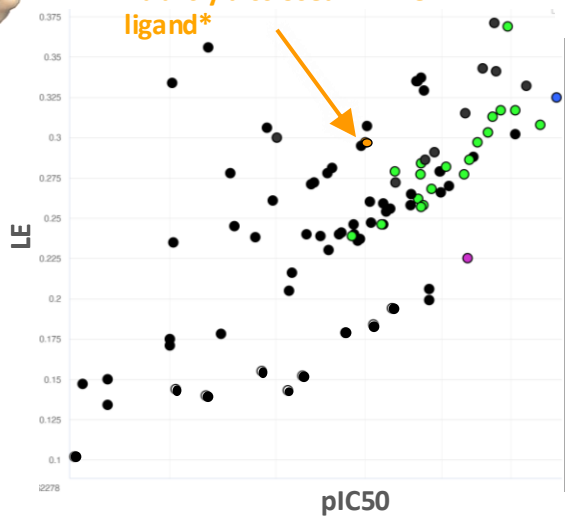


Alternate Vector
IC_{50} = 2 nM



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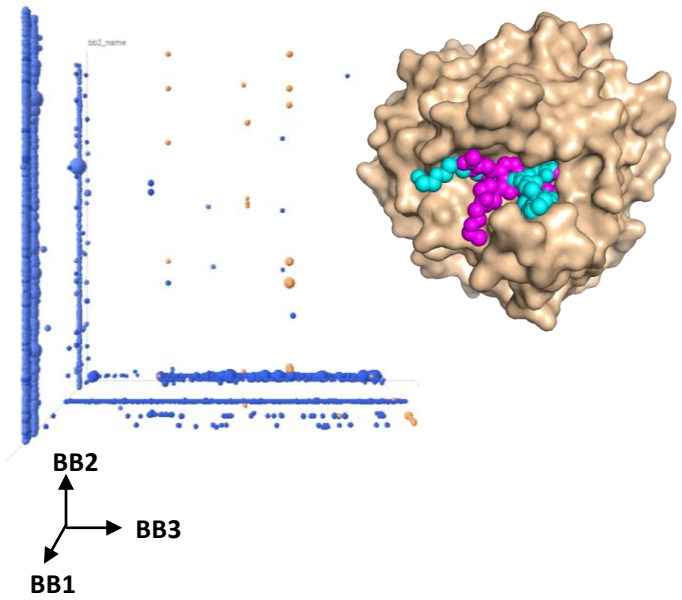
Alternate KLHDC2 vector shows distinct target degradation profile



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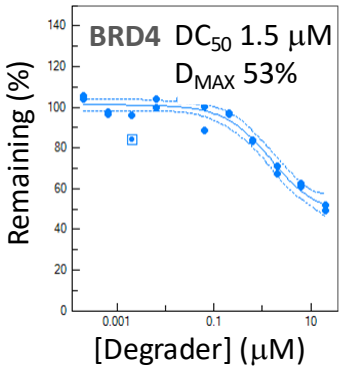

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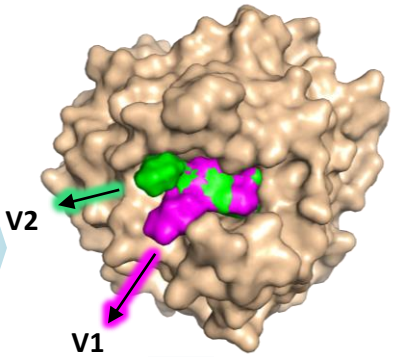

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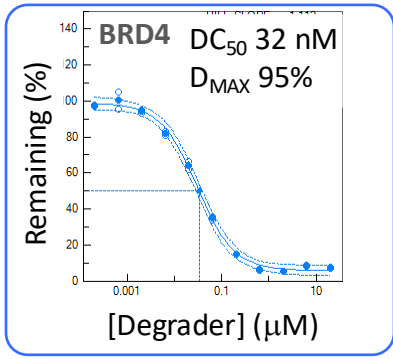
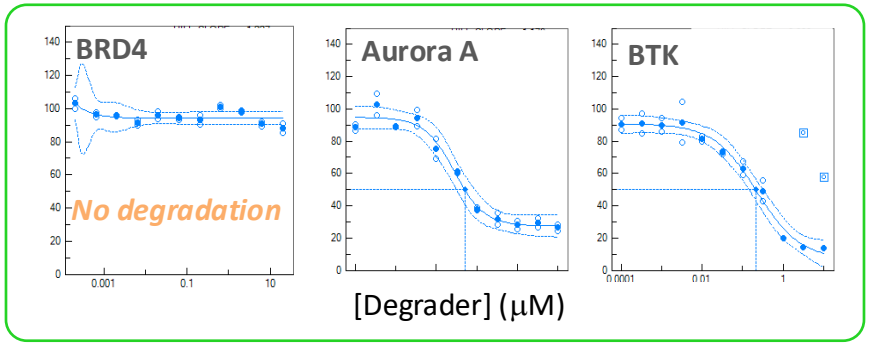


Alternate Vector
IC_{50} = 2 nM



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- **Optimization of Series 1 and exploration of alternate vector generated binders with improved potency translating to improved degradation and distinct target degradation profiles**

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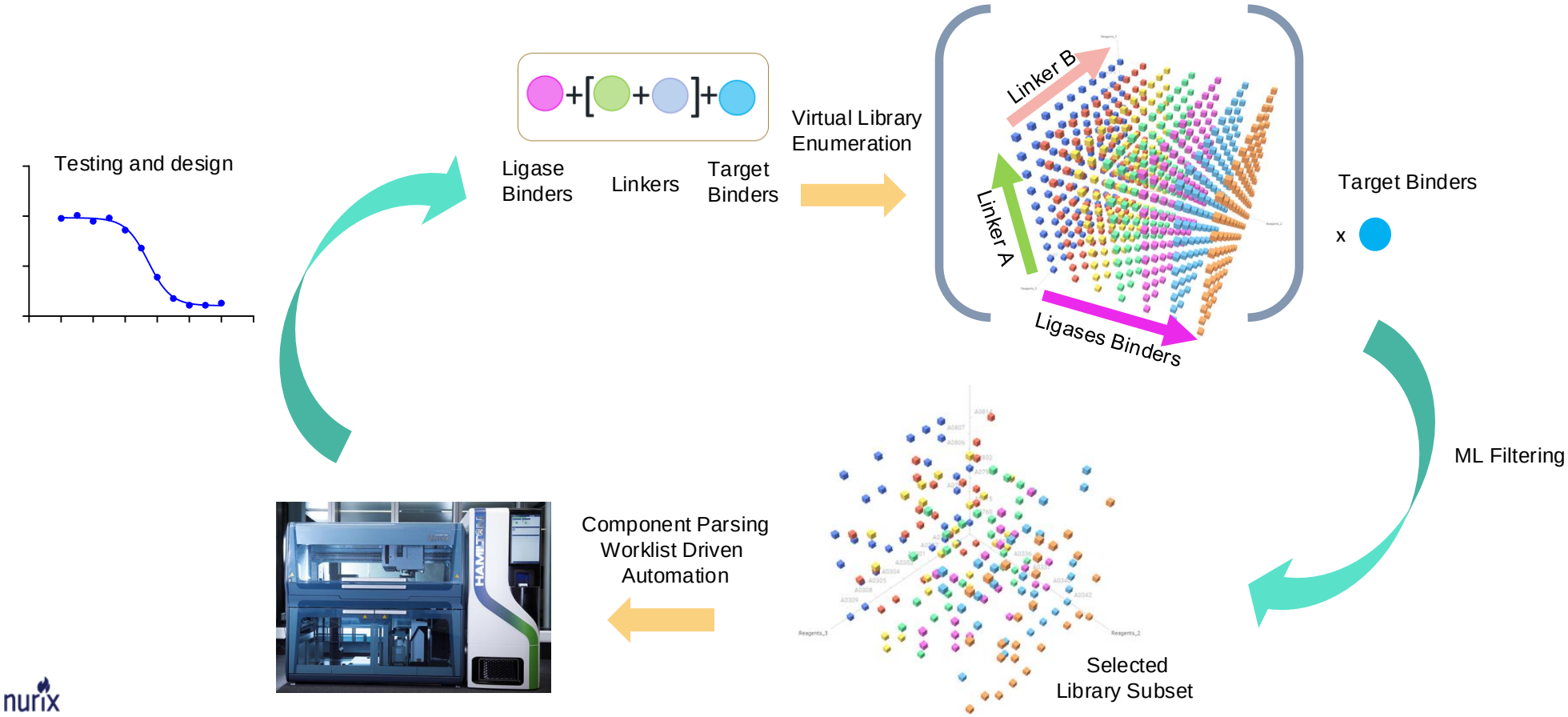
Ultra-High throughput matrix synthesis leverages new ligase binders while maximizing chemical space exploration

- Target degraders rapidly synthesized (100s per library)
- Solution phase and solid phase chemistry
- On Demand linker assembly
- ML driven design enabled

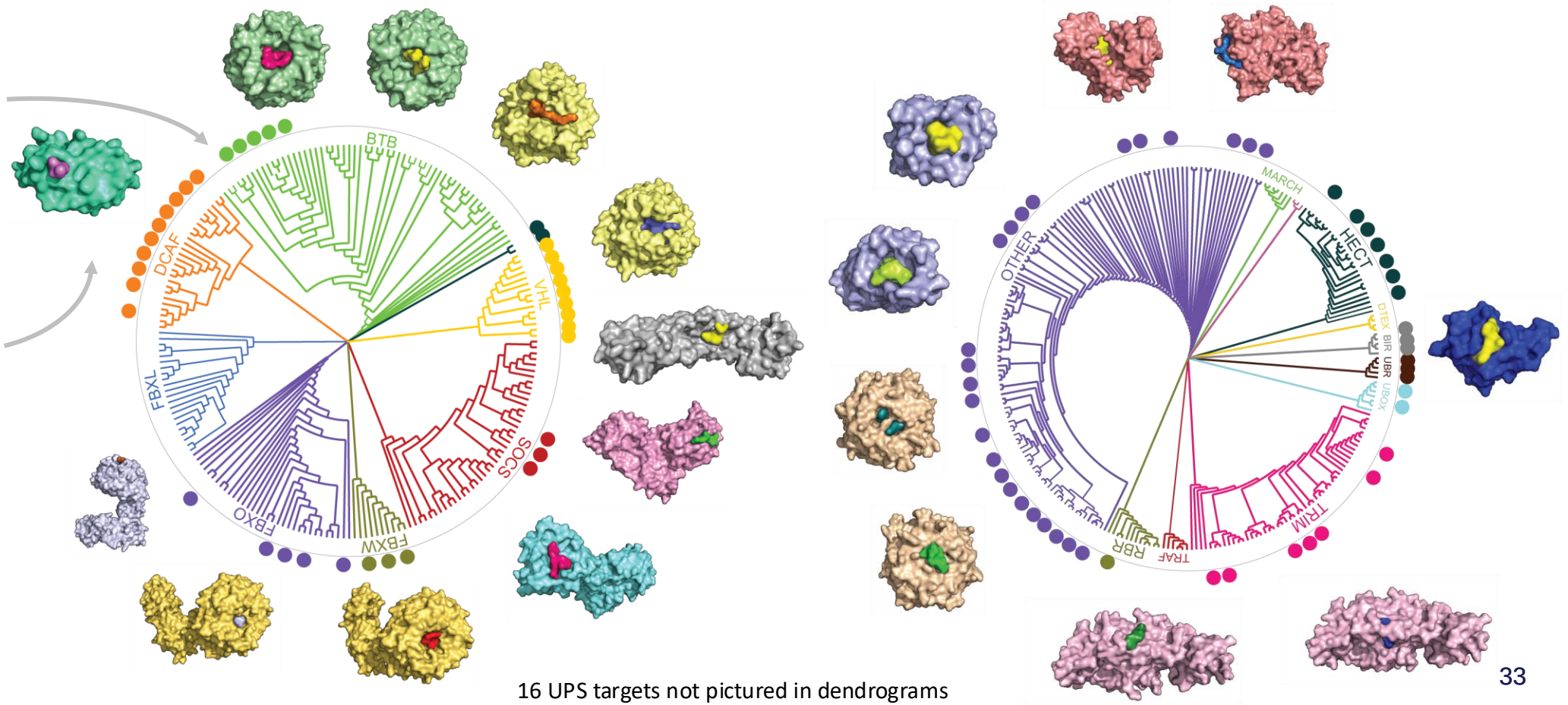


DEL discovery is highly scalable and readily applicable to challenging targets, enabling more comprehensive degrader discovery workflows

- Active pipeline of over 100 Ligase/UPS protein targets

Each Point Represents a UPS Target in Nurix's Pipeline

Examples of Novel Ligand-bound X-ray Structures Solved to Enable SBDD



16 UPS targets not pictured in dendrograms

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