Screening DNA Binding Proteins with DNA Encoded Libraries

Discovery on Target

Boston, MA

October 3, 2024

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Nurix Drugs Engage Ligases for the Treatment of Disease

Targeted Protein Modulation: TPM = TPD + TPE

A Powerful Cellular System

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

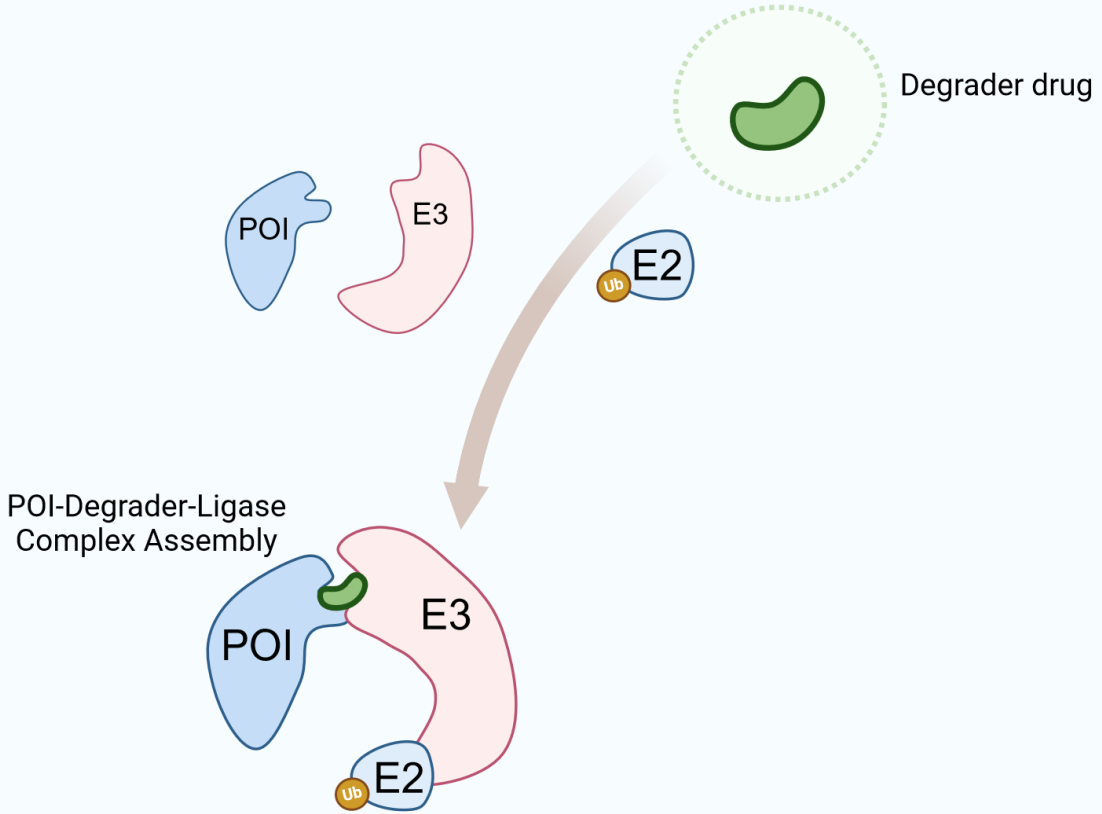
Harness ligases to decrease specific protein levels

Targeted Protein Degradation (TPD)

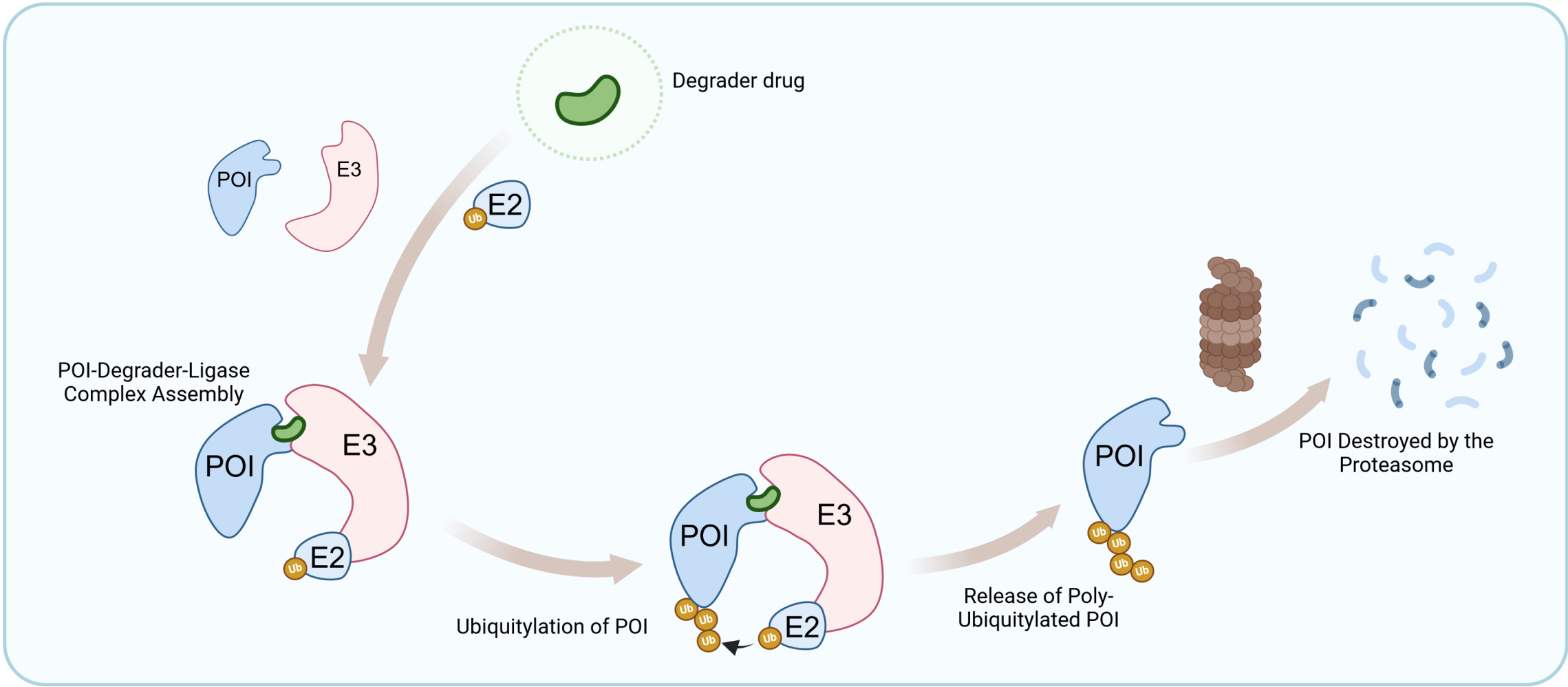
Targeted Protein Elevation (TPE)

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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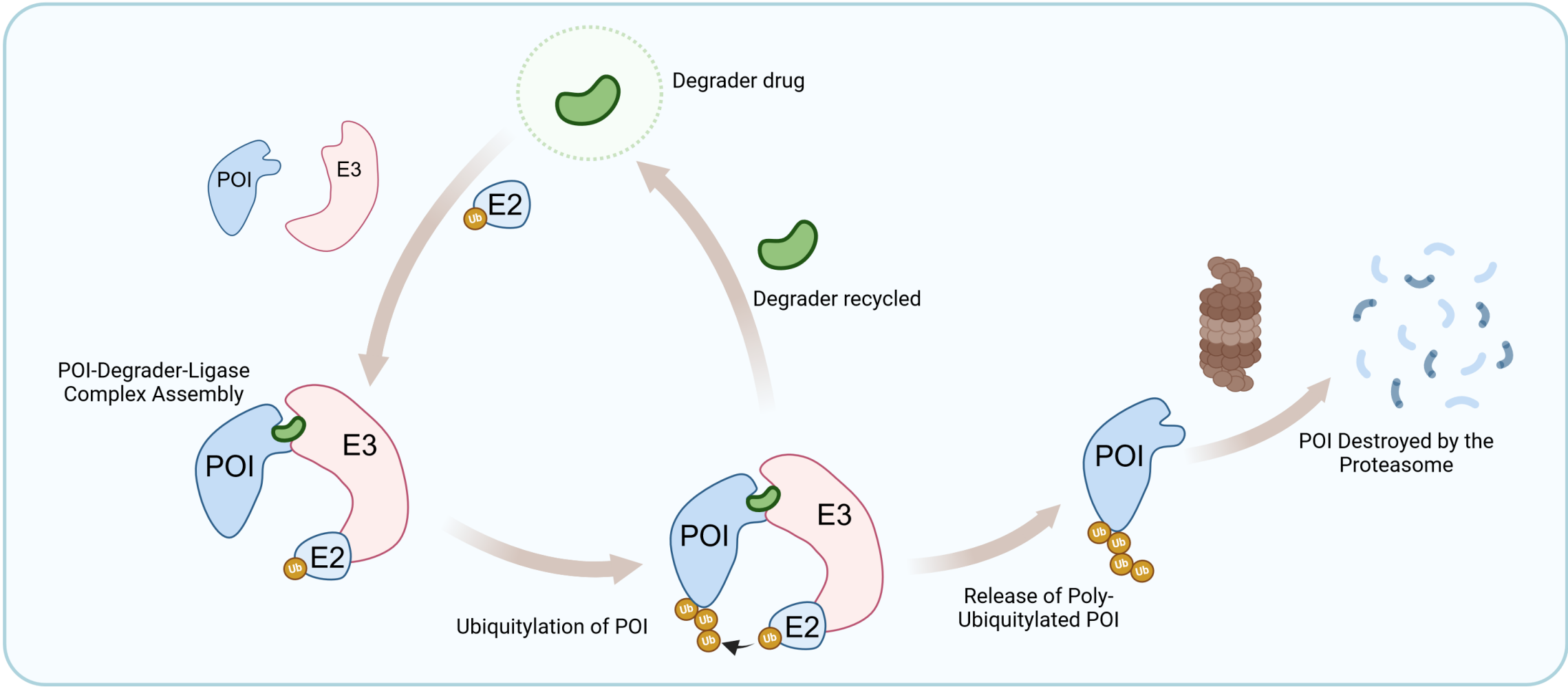
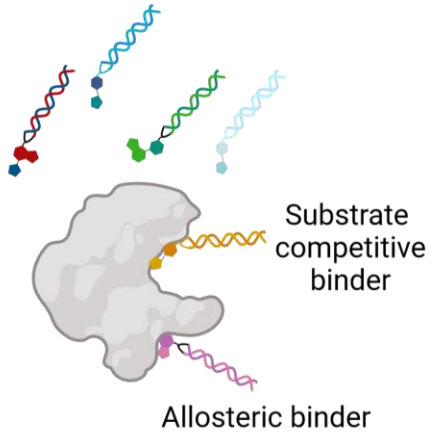
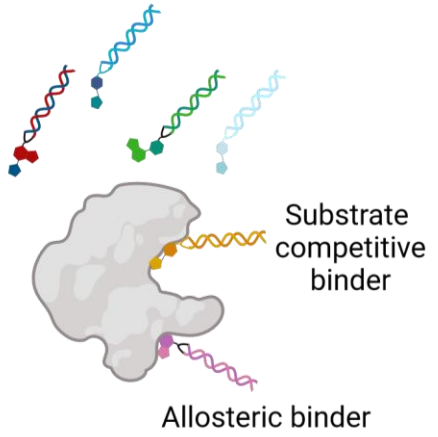


Affinity-based DEL screening is an ideal approach to enable new binder discovery for targeted protein degradation

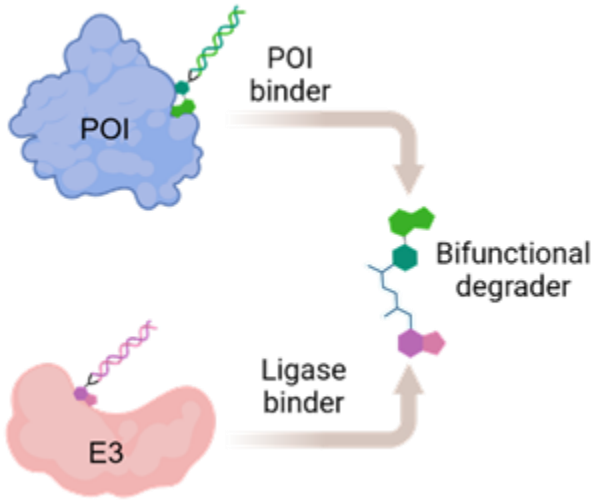


- **Affinity-based ligand discovery is the ideal approach to enable induced proximity**
  - **Affinity-based screening of effectors is MoA agnostic**
- Low per screen cost allows for a broad exploration of target and ligase chemical space

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



Protein of interest (POI)



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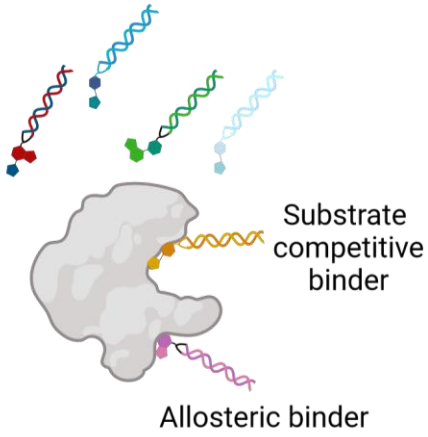

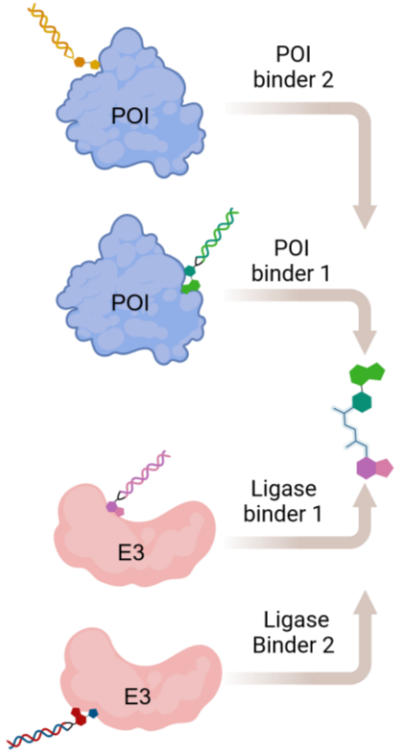

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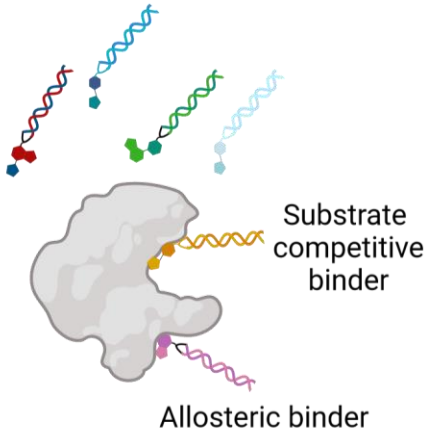

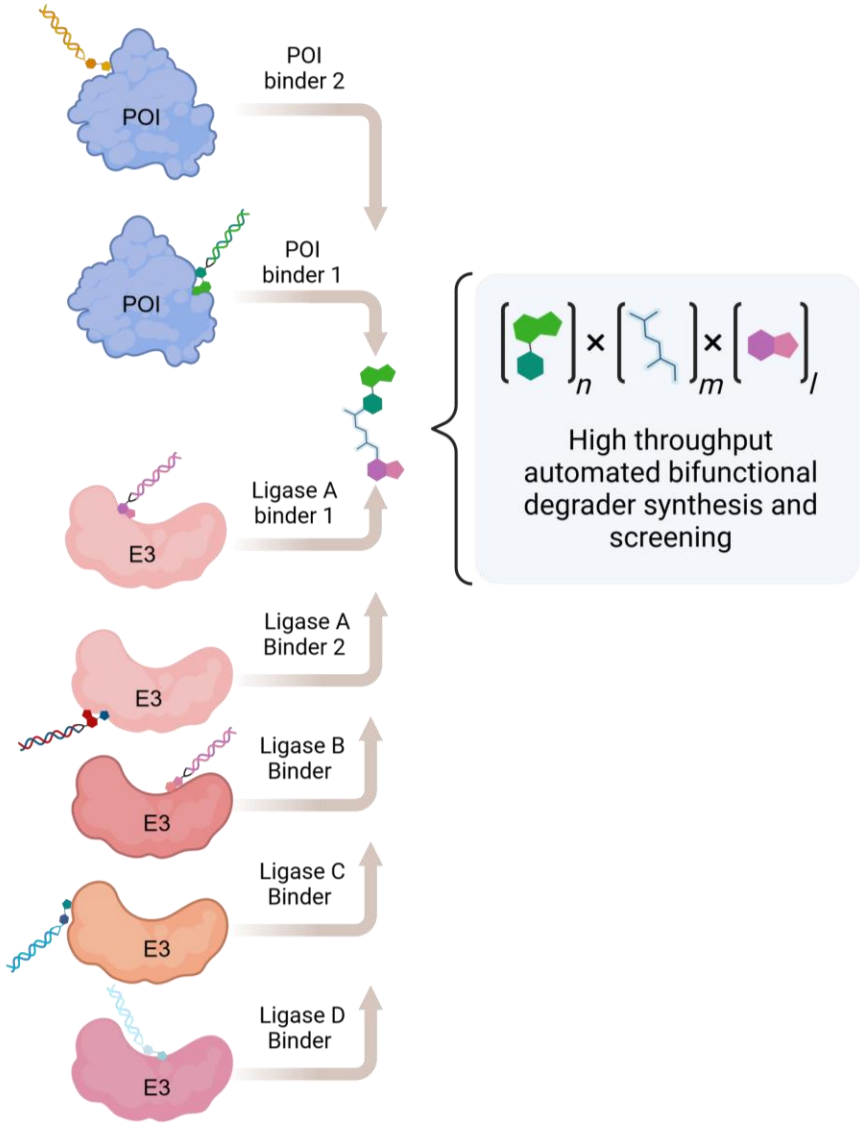

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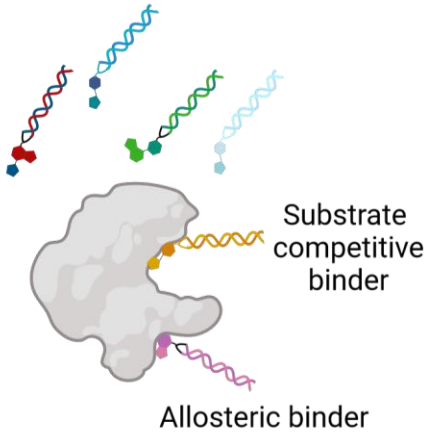
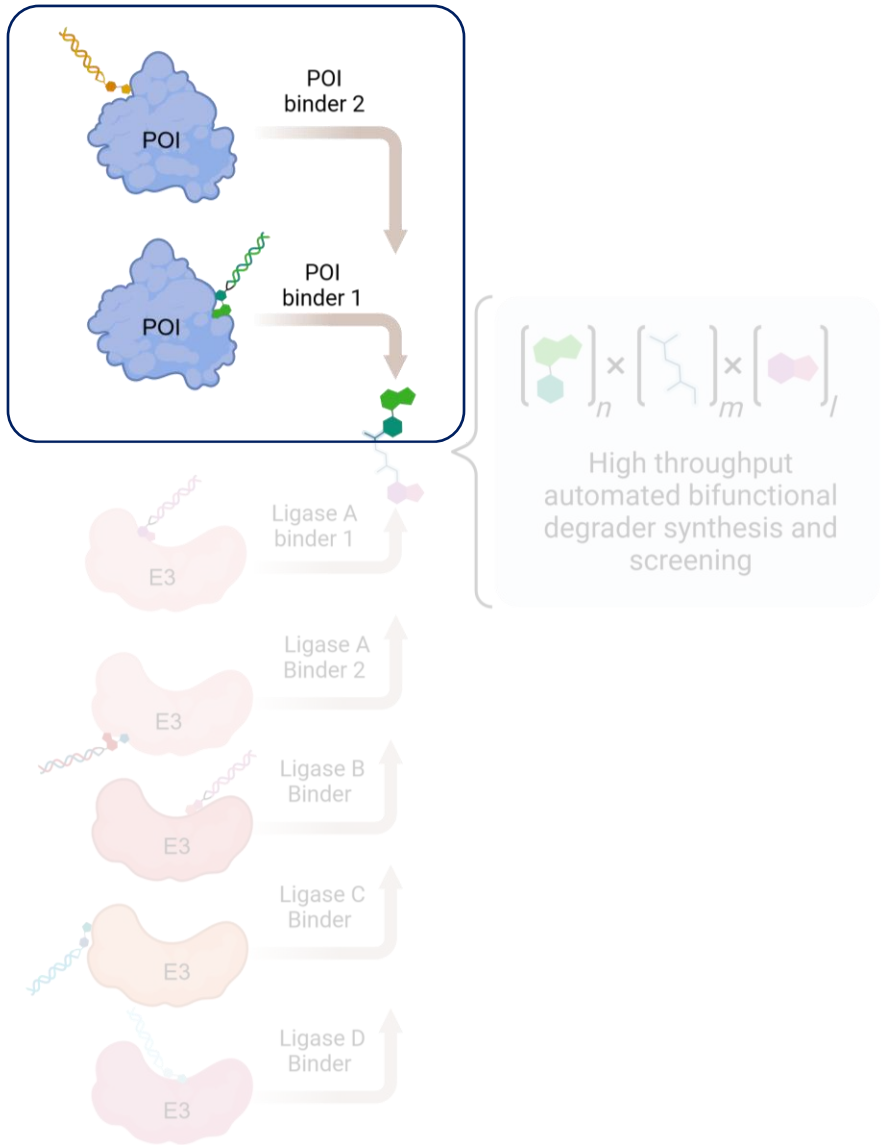
- **Combinatorial degrader design and synthesis enable rapid hit follow up and optimization**

Leveraging DEL to identify binders for challenging targets





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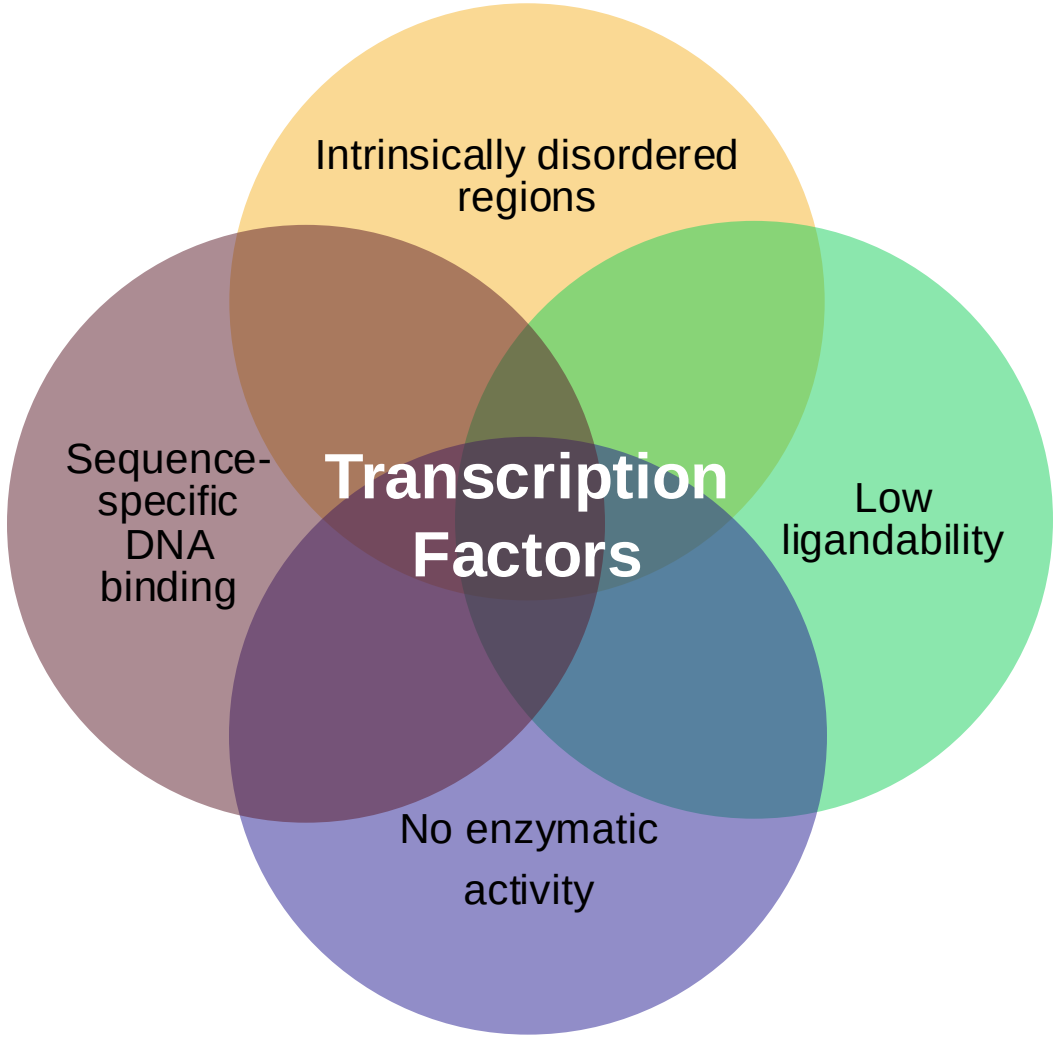
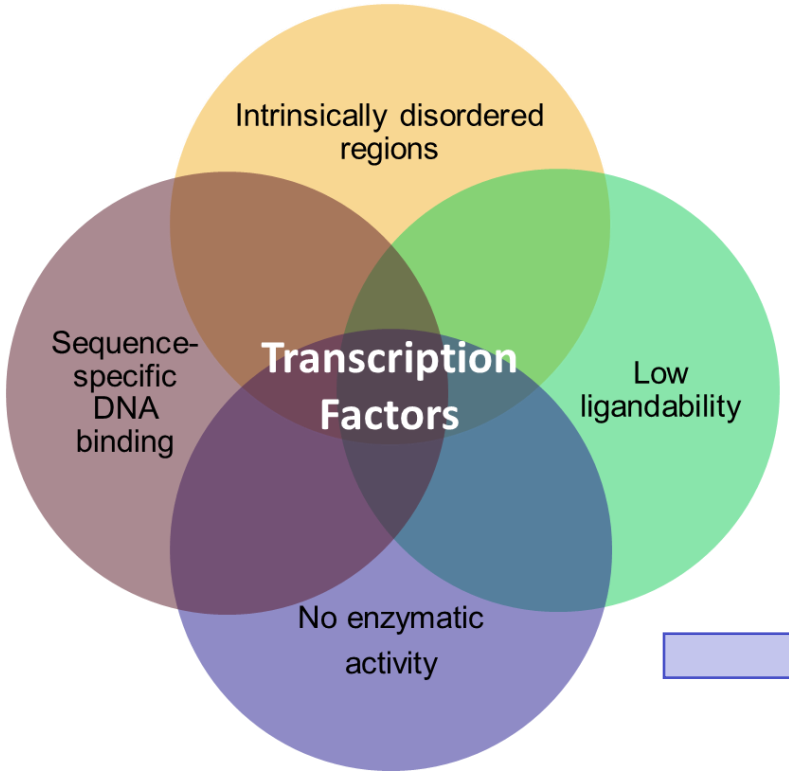
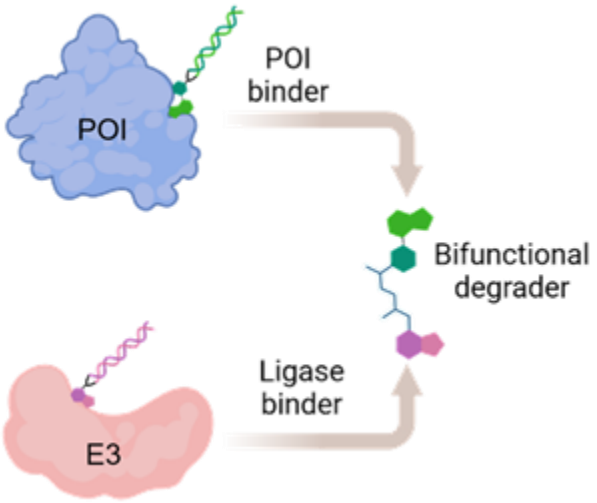
Transcription factors combine multiple challenges in small molecule hit ID



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





TPD only requires binding, not activity
Affinity selections are MOA agnostic

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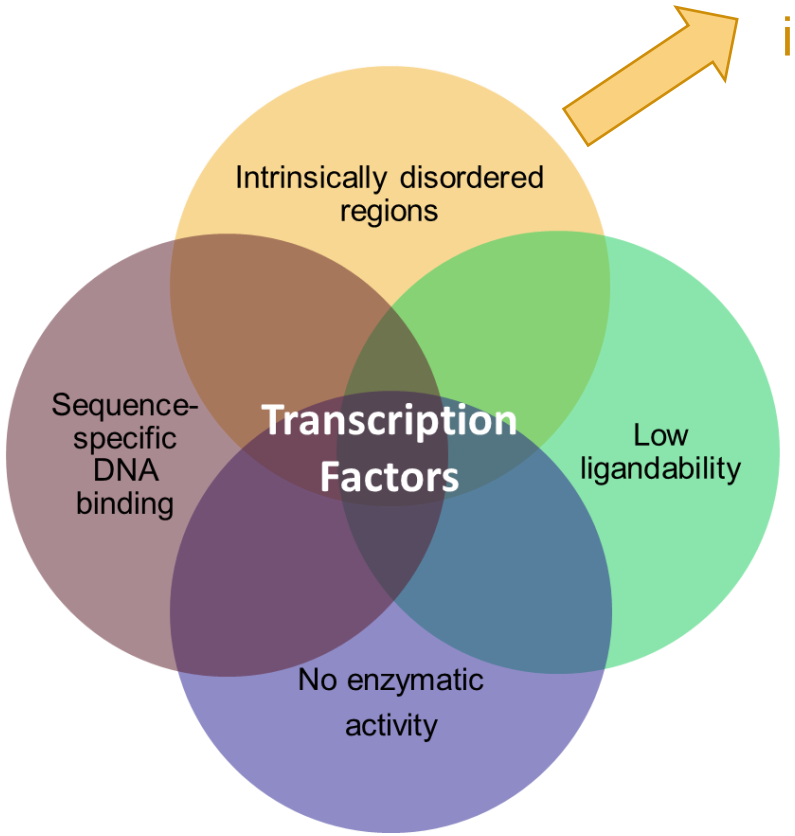
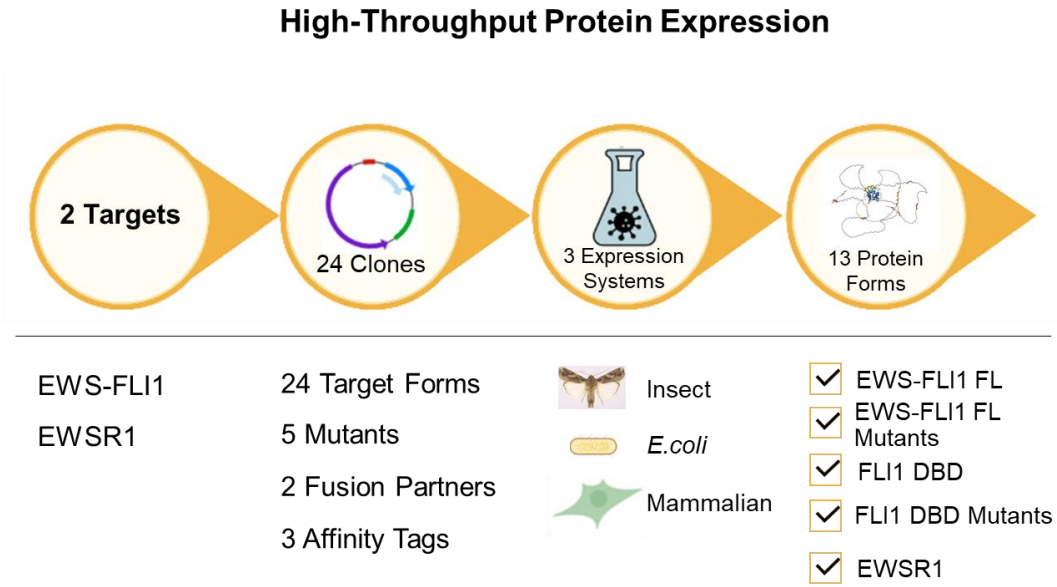
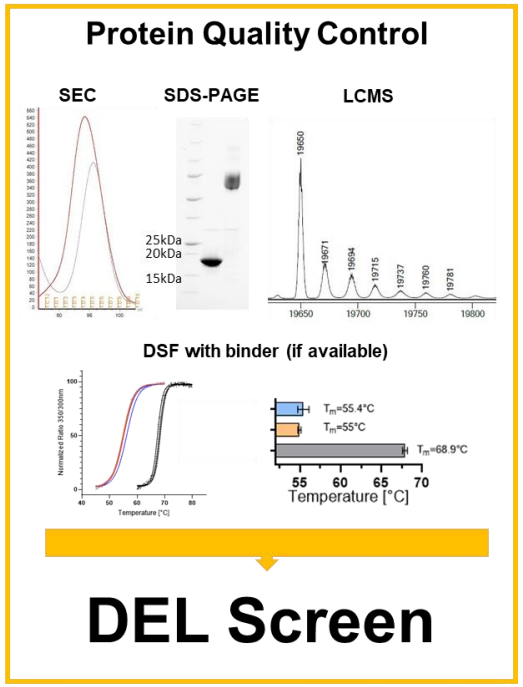


Protein engineering and construct screening to identify tractable domains for DEL screening





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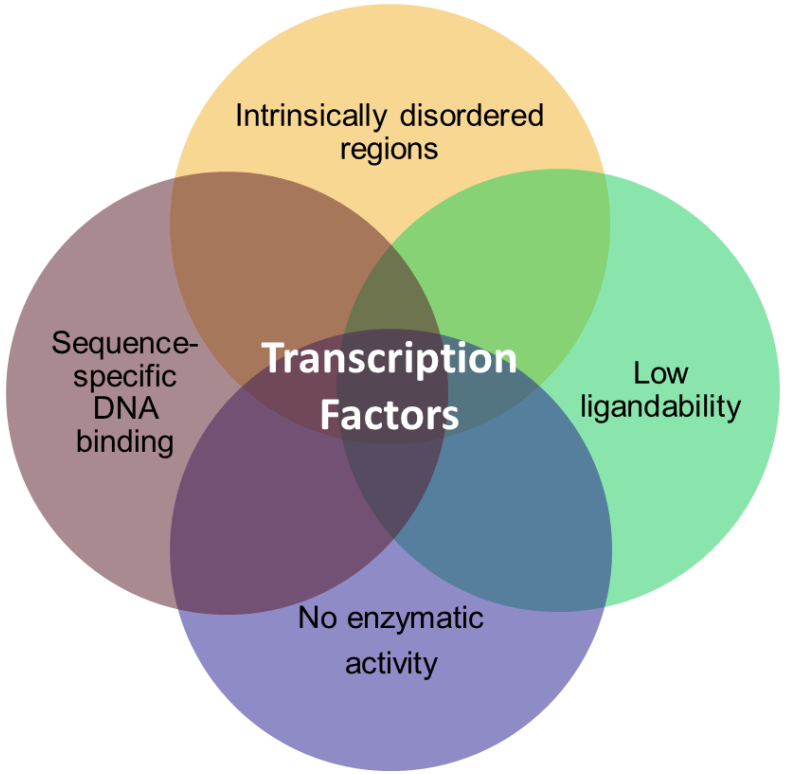
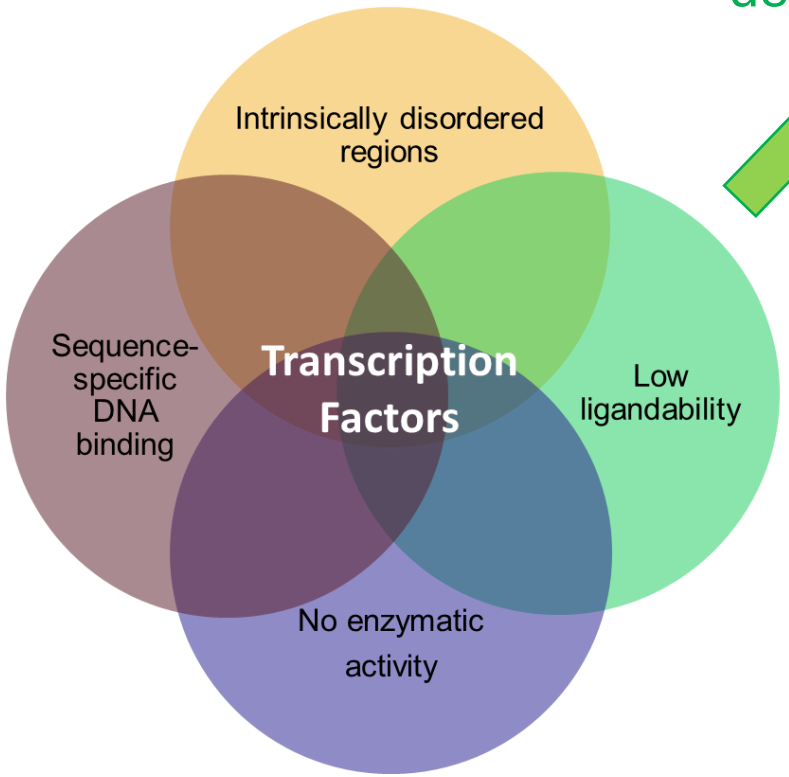


Elimination of tag-driven false positives

- Informatic flagging of enriched tag sequences
- Selection Methods and protein engineering to block sequence specific DNA binding

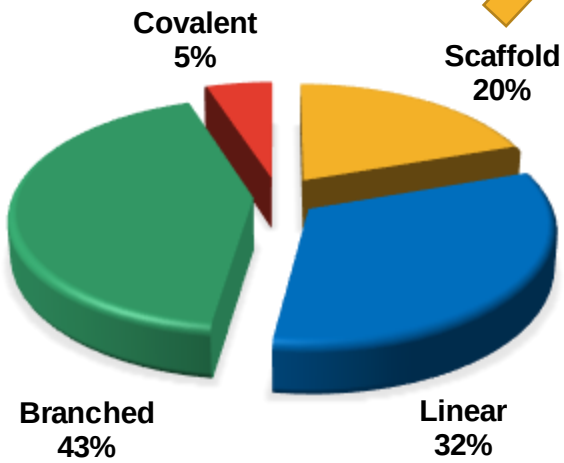
The Nurix DEL screening and analysis platform is designed to unlock challenging targets, including transcription factors



Nurix DEL collection and proprietary custom scaffolds were designed to address hard to ligand targets

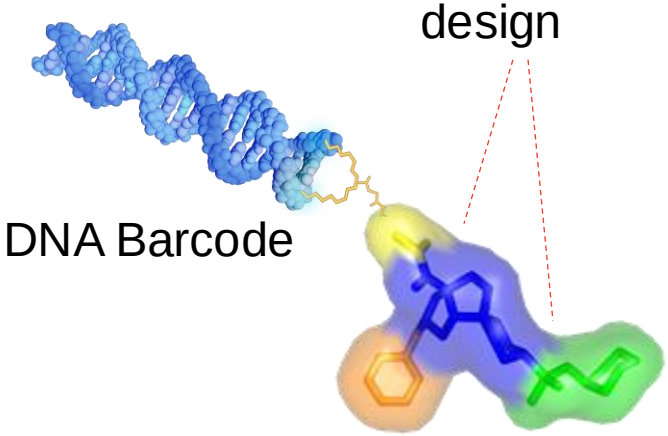
Nurix DEL Collection

- >5 billion unique structures
- Includes proprietary, 3D complex, custom scaffolds



Scaffold Libraries Proving Essential for Delivering Ligands for "Undruggable" Targets (sole source of hits for 75% of these targets)

Three-dimensional design



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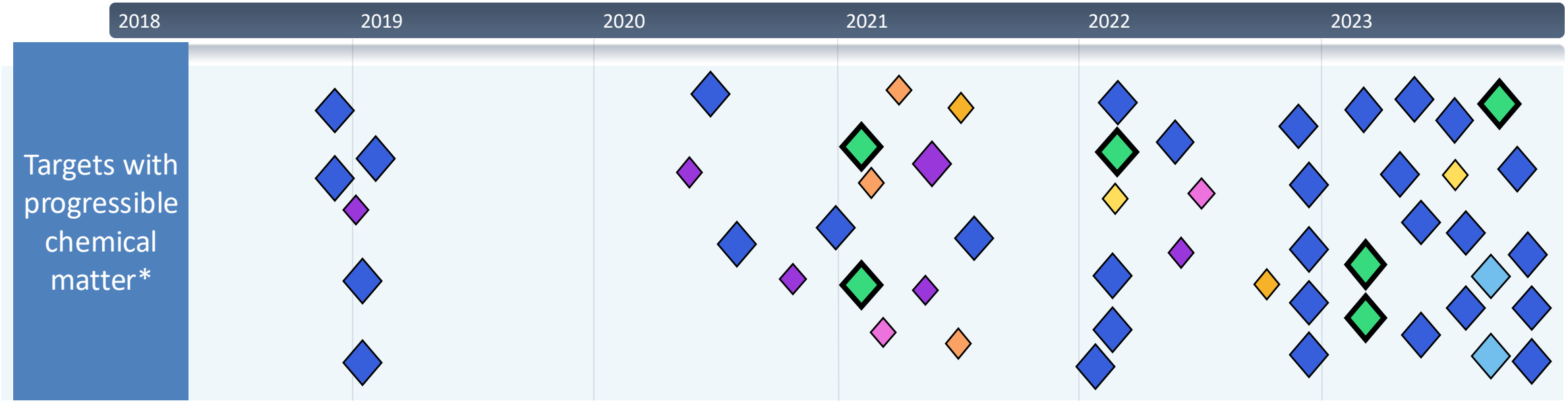


*All series validated by ≥ 2 orthogonal assays

Target Class

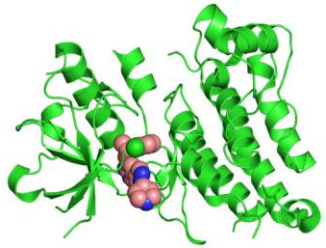
- Ligase
- Kinase
- Transcription Factor
- Epigenetic
- DUB
- Enzyme
- Nuclear Receptor
- Protease

CANCER GRAND CHALLENGES | Team: KOODAC

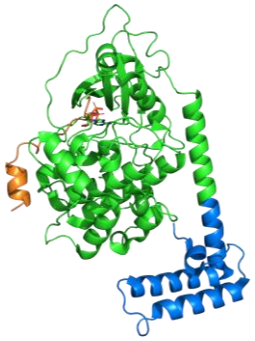
KOODAC3
Knocking Out Oncogenic Drivers And Curing Childhood Cancer

Target five oncoproteins that drive high-risk pediatric cancers with the goal to deliver first-in-child phase 1 clinical candidates

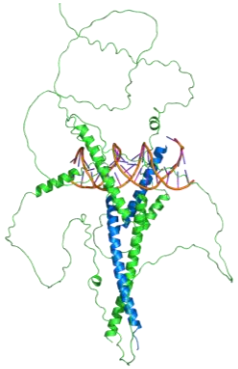
- "KOODAC's long-term vision is to revolutionize the standard of care for children with oncoprotein-driven cancers, causing a paradigm shift in pediatric cancer therapy, by pioneering targeted protein degradation techniques to develop transformative new treatments."



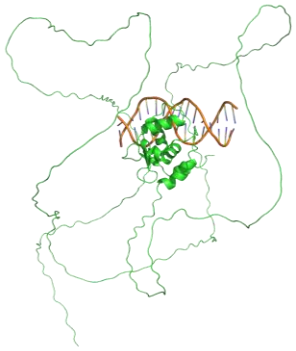
ALK
(Neuroblastoma)



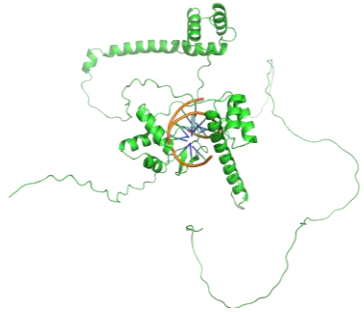
DNAJB1::PRKACA
(FHC)



MYCN
(Meduloblastoma)



EWSR1::FLI1
(Ewing Sarcoma)



PAX3::FOXO1
(Rhadomyosarcoma)

Centre for Targeted Protein Degradation University of Dundee | Julius-Maximilians-Universität Würzburg | CeMM Science is our medicine | institut Curie | UCSF | nurix | Children's Cancer Therapy Development Institute | The Rockefeller University 1901 | Massachusetts Institute of Technology | Children's Hospital of Philadelphia

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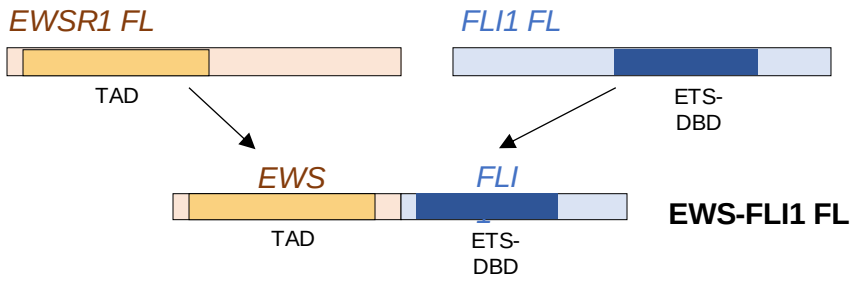
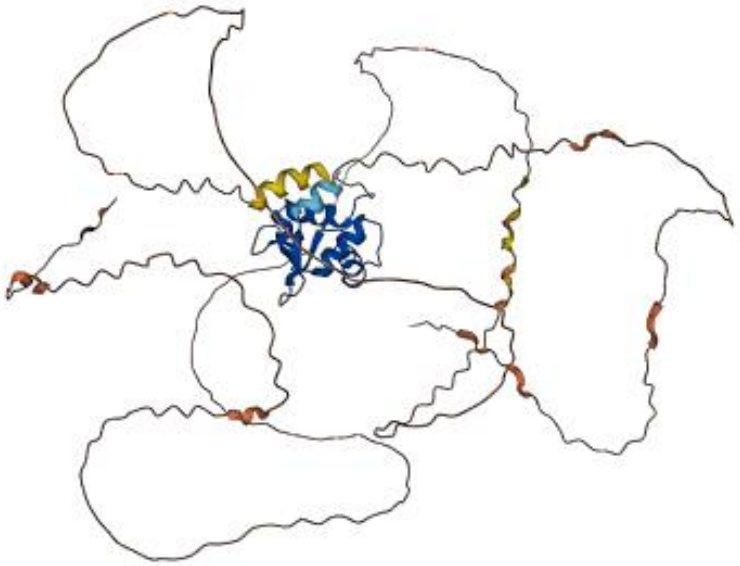
Case Study – finding ligands to the DNA binding domain (DBD) of EWS-FLI1

- **EWS-FLI1** is a fusion protein caused by chromosomal translocation
  - **EWSR1 -** strong transactivation domain (TAD)
  - **FLI1 –** ETS-DNA Binding Domain (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

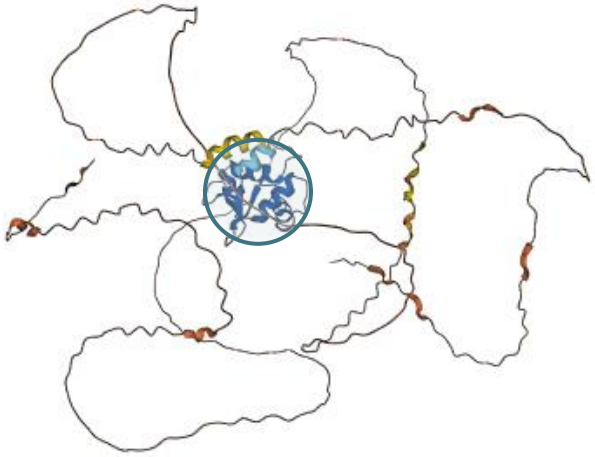


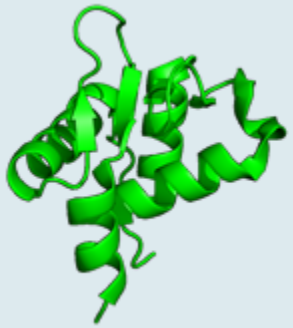
EWS-FLI1 (Alphafold)



EWS-FLI1 DEL screen focused on the DNA-binding domain

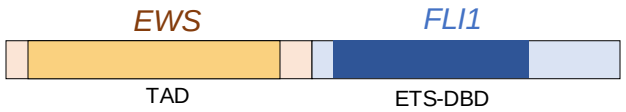
EWS-FLI1 (Alphafold)





WT FLI1 DBD

304-445



EWS-FLI1 FL

TAD = Trans-Activation Domain

DBD = DNA Binding Domain

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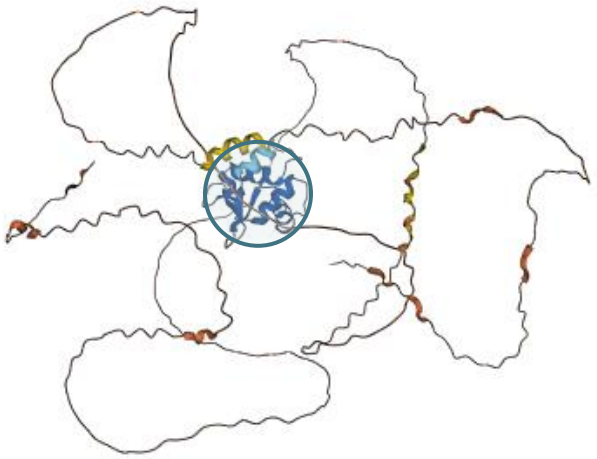
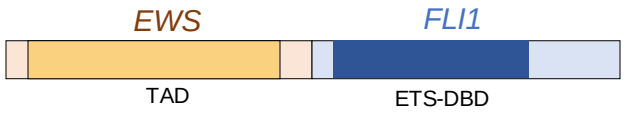
Hou and Tsodikov, *Biochemistry* (2015)

Most enrichment against a DNA binding domain is driven by the tag

EWS-FLI1 (Alphafold)





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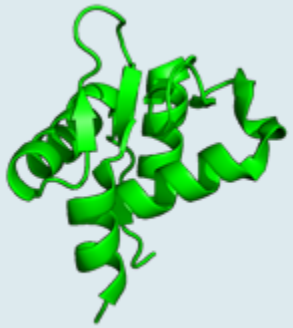
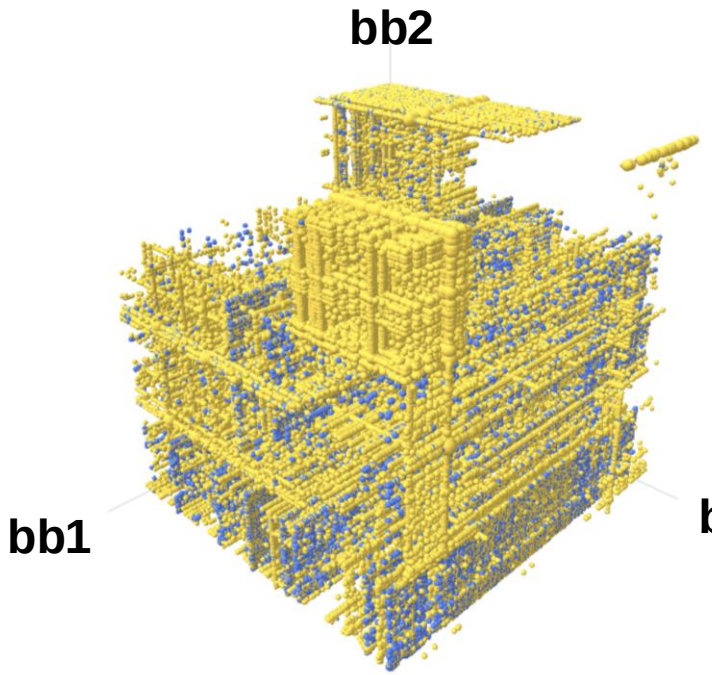


WT FLI1 DBD

304-445

ETS-DBD



26% of all DEL tags in the collection contain the known consensus motif

After FLI1 DBD affinity selection:

72% of all reads contain known DNA consensus sequence

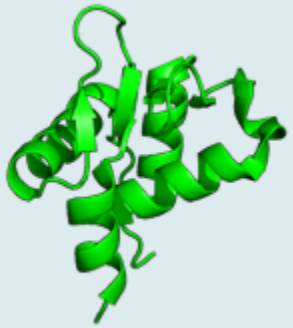
78% of reads for enriched DEL hits contain known DNA consensus sequence

Parallel strategies 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

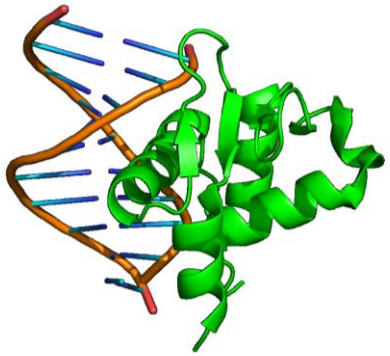
- **DEL selections performed against a mutated DBD that fails to bind DNA**



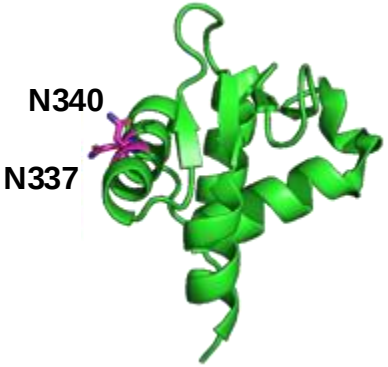
WT FLI1 DBD

304-445

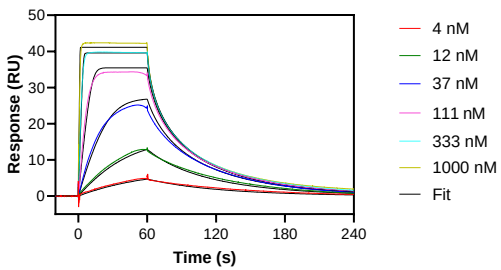
ETS-DBD



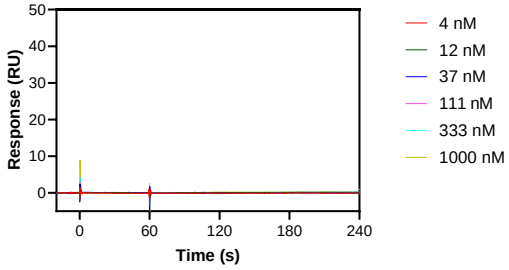
+DNA consensus sequence blocker



DBD$^{R337N/R340N}$ mutant



Consensus sequence DNA
K_D = 20 nM



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

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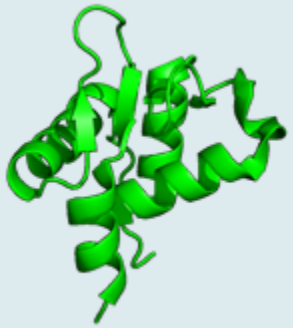
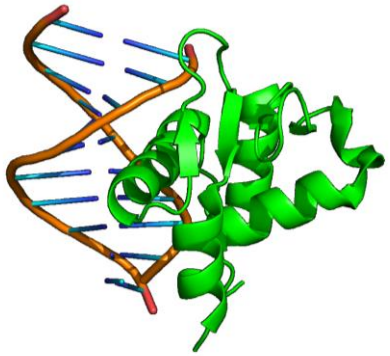
Hou and Tsodikov, Biochemistry (2015)

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?***





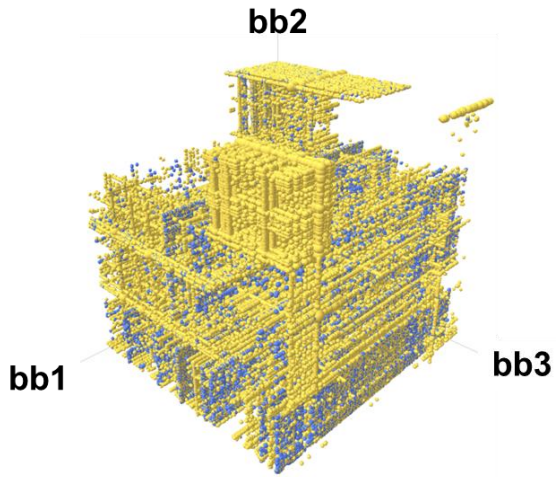
+DNA consensus sequence blocker

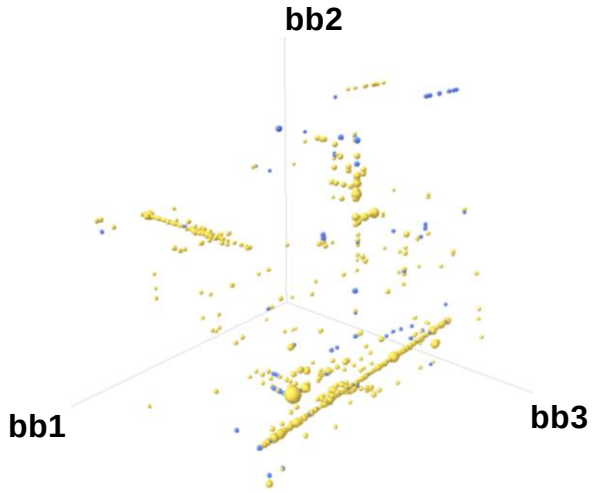
$DBD^{R337N/R340N}$ mutant

≥1 DNA barcode with consensus sequence

All DNA barcodes free of consensus sequence

Consensus: 78% of reads for enriched hits





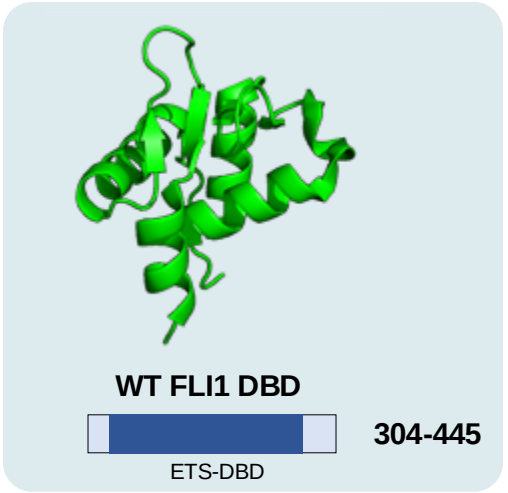
Consensus: 26% of reads for enriched hits (consistent with naïve library)

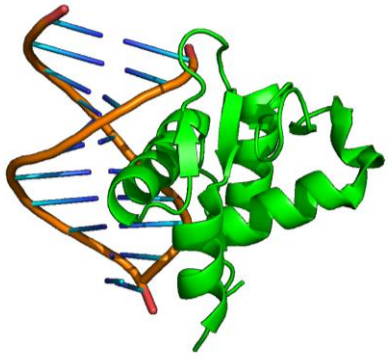
Orthogonal screening conditions show highly consistent DEL output

Strategies to mitigate DNA tag-driven enrichment of consensus sequence

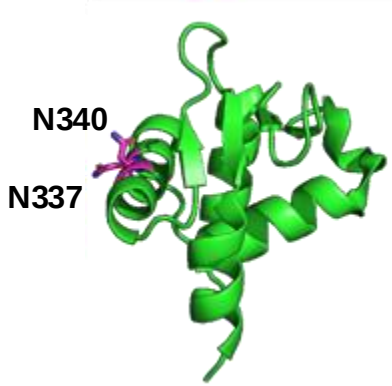
- **DNA blockers**
  - Reduce % FASTA reads containing consensus sequence to levels similar to bead only & Naïve library samples
  - Encoding tags of hits enriched in the presence of consensus sequence blocker contain consensus sequence -> ***how to distinguish between DNA driven vs. true small molecule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

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

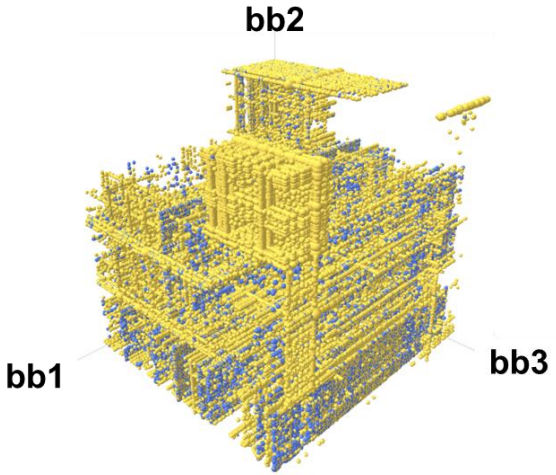


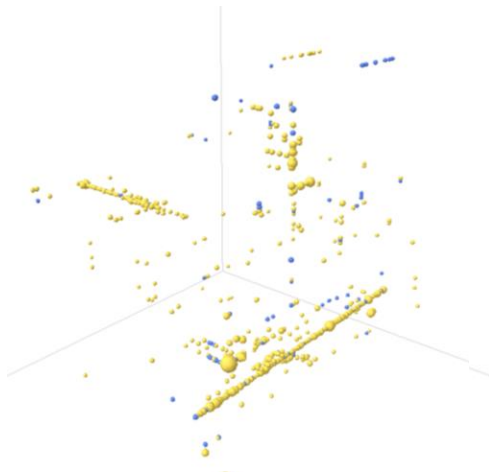


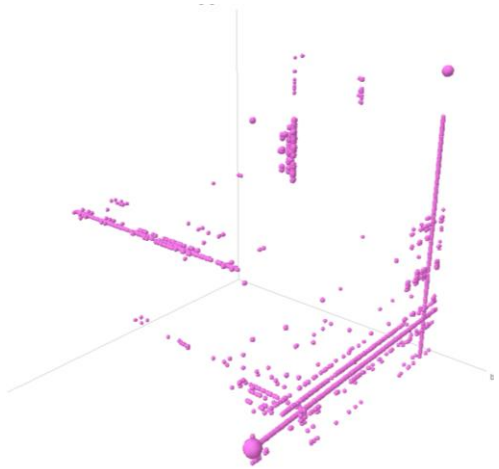
+DNA consensus sequence blocker



DBD$^{R337N/R340N}$ mutant







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Hou and Tsodikov, Biochemistry (2015)

Robust enrichment of four distinct chemical series in orthogonal screening formats

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?***

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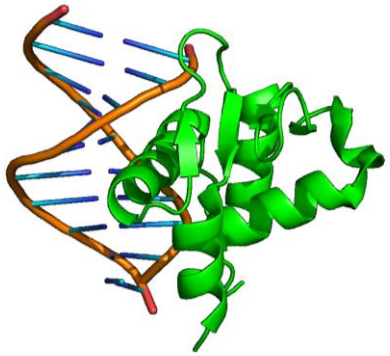
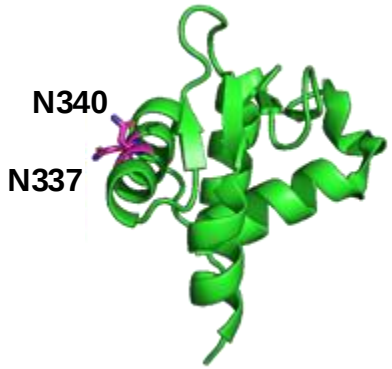
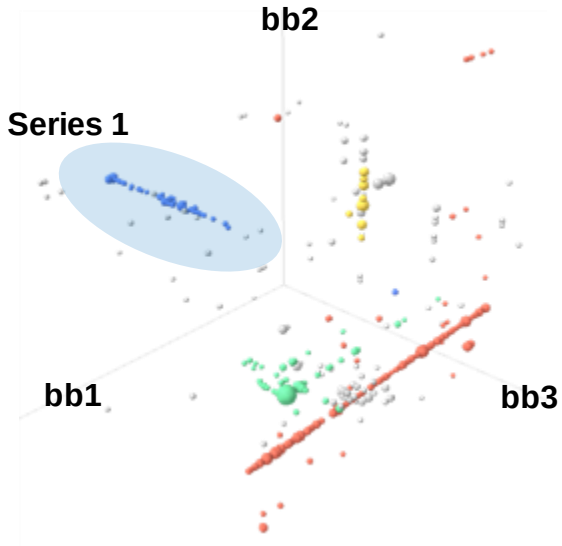
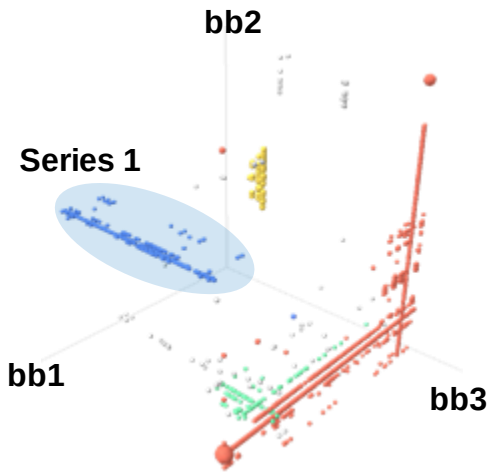


+DNA consensus sequence blocker



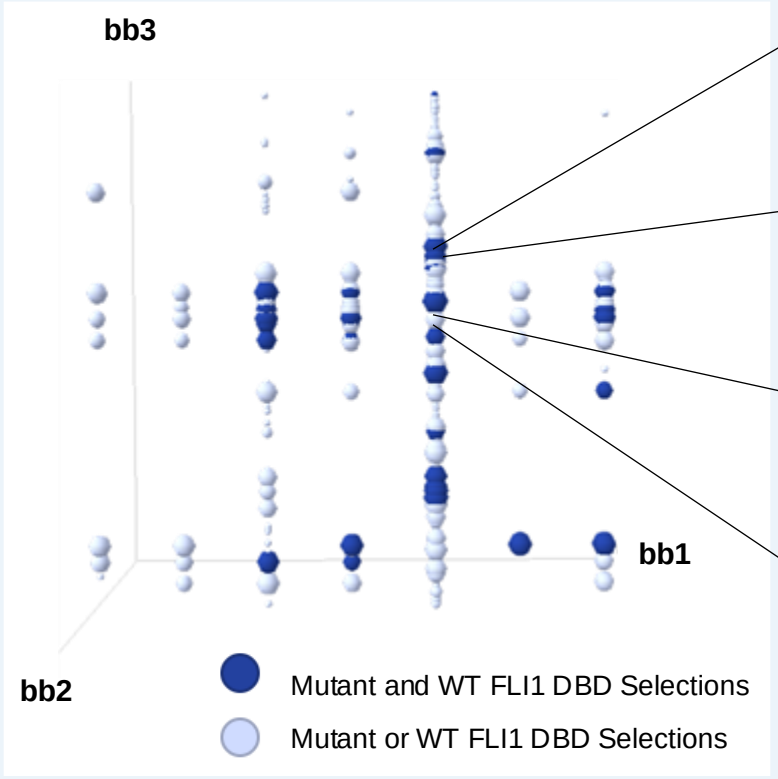
DBD$^{R337N/R340N}$ mutant



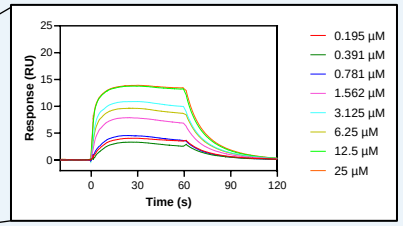


Hou and Tsodikov, Biochemistry (2015)

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

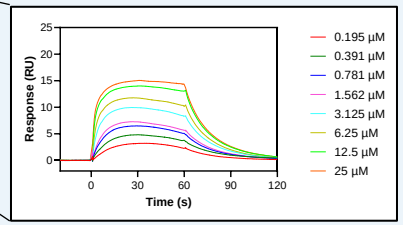


Hit in WT and Mutant Selections



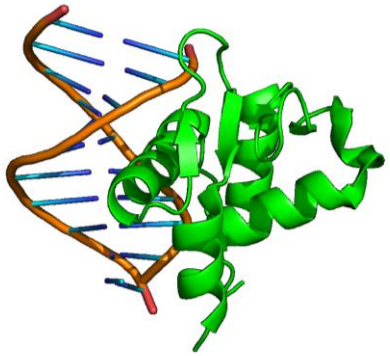
DEL Hit 1
FL1 DBD K_D = 1.4 µM

Hit in Mutant Selection

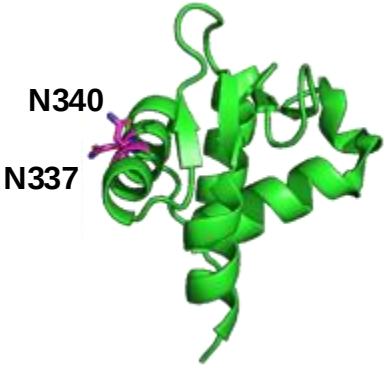


DEL Hit 2
FL1 DBD K_D = 1.8 µM

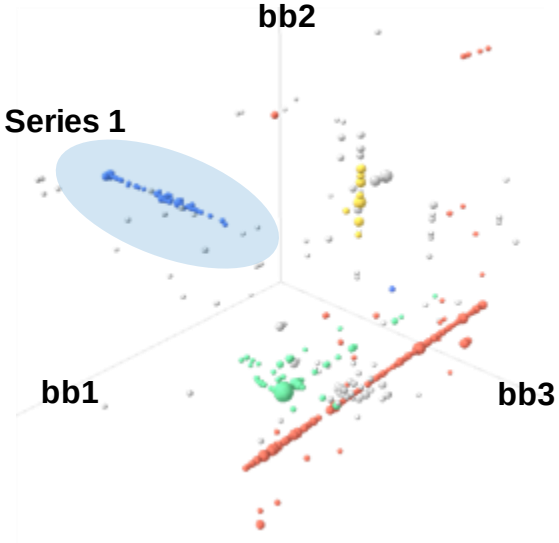
Both hits have FASTA reads containing consensus sequence

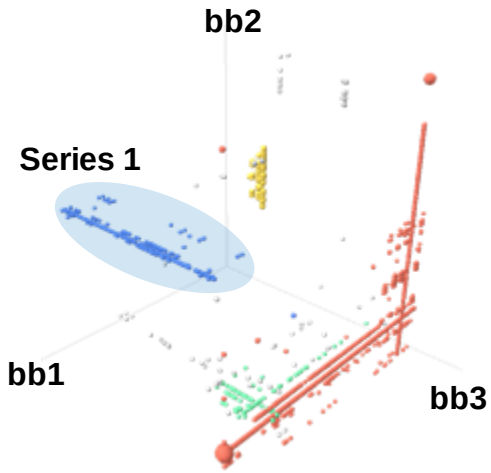


+DNA consensus sequence blocker



DBD$^{R337N/R340N}$ mutant

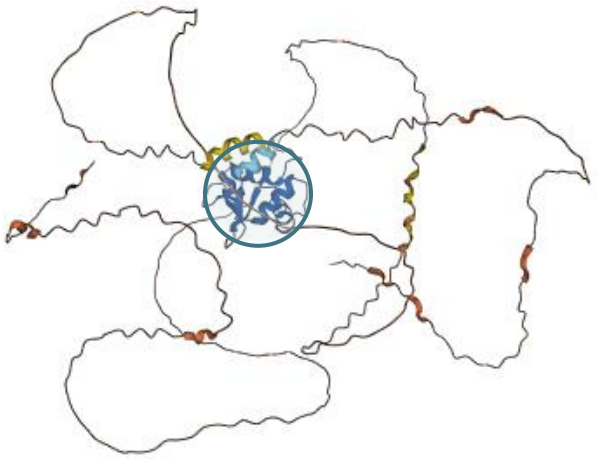


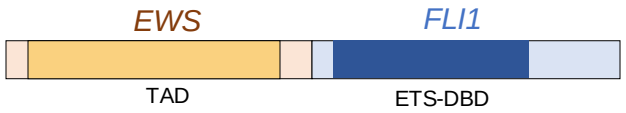


Hou and Tsodikov, Biochemistry (2015)

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

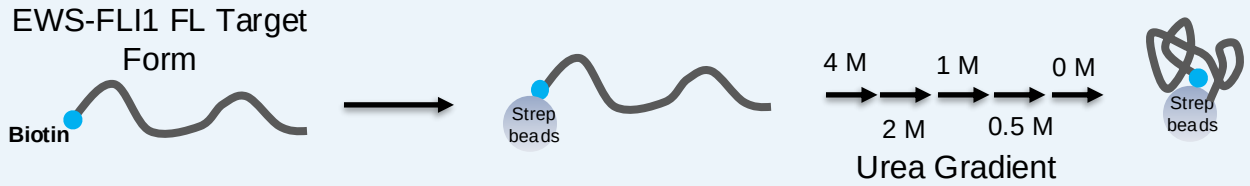
EWS-FLI1 (Alphafold)





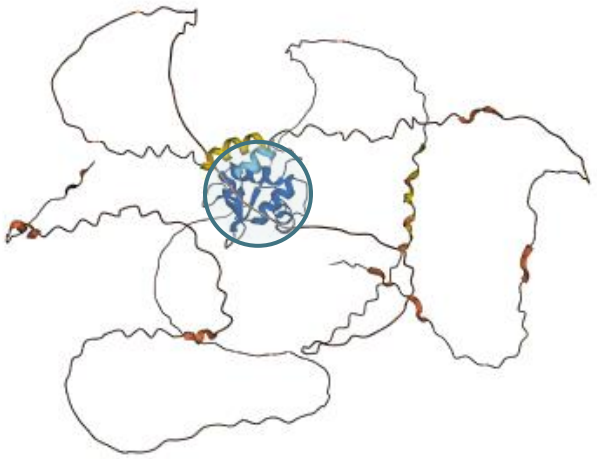
EWS-FLI1 FL

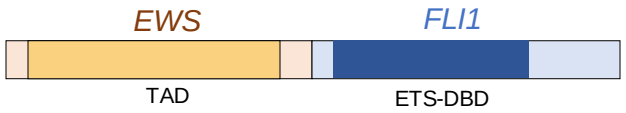
Refolding of EWS-FLI1 FL on resin



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

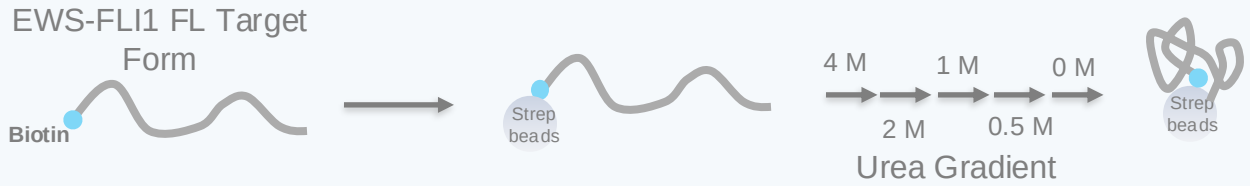
EWS-FLI1 (Alphafold)

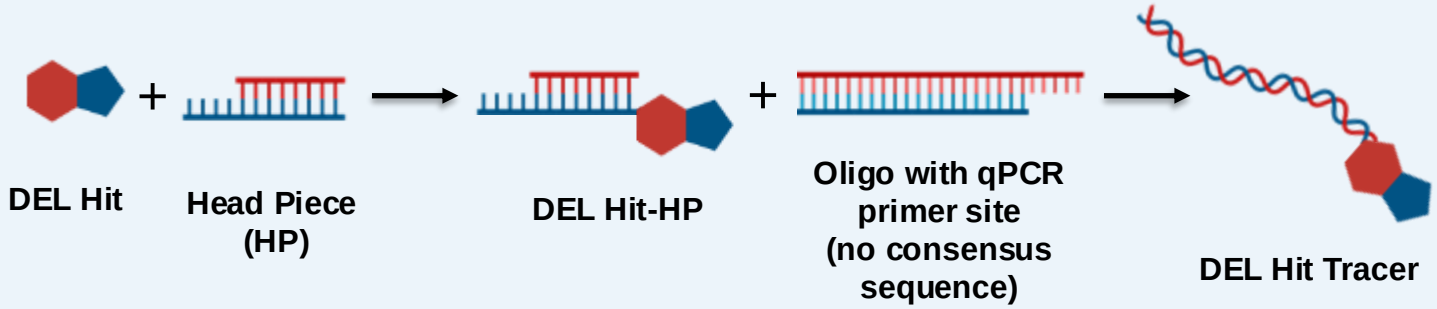




EWS-FLI1 FL

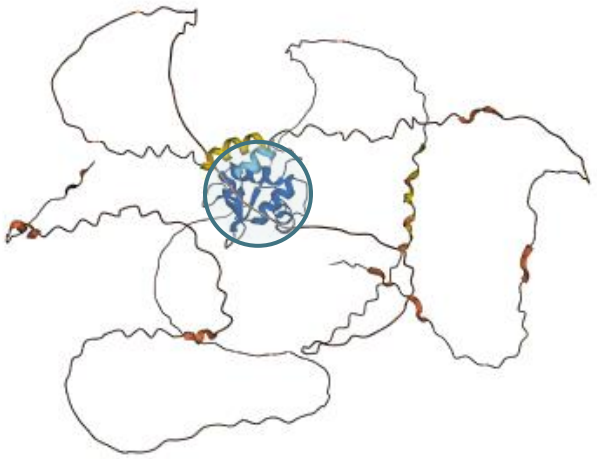
Refolding of EWS-FLI1 FL on resin

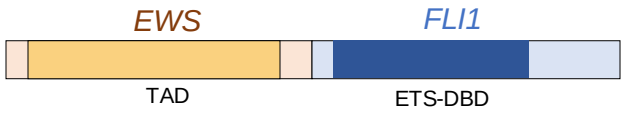




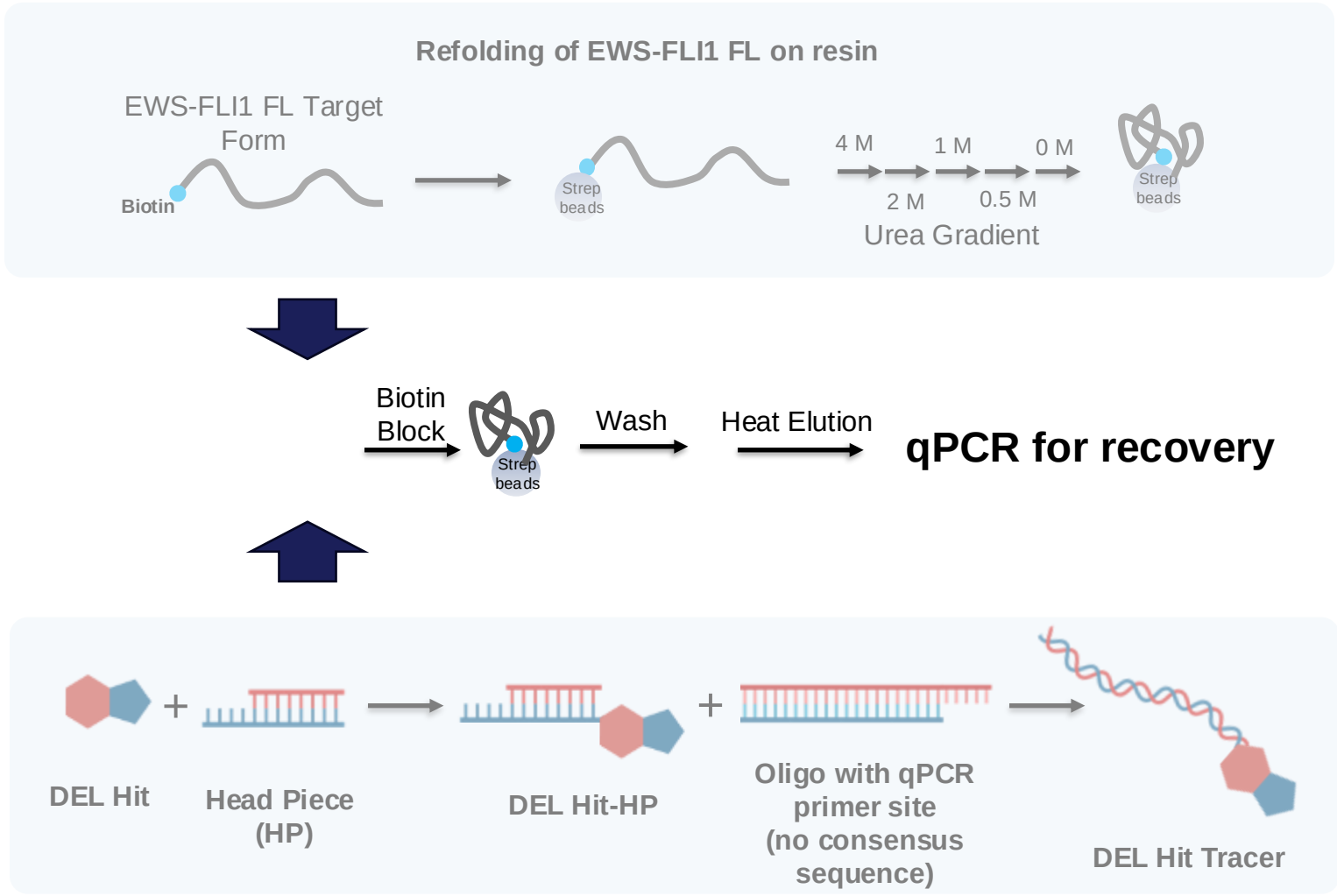
Leveraging DEL screening methodology to confirm binding to full length EWS-FL1 fusion

EWS-FLI1 (Alphafold)





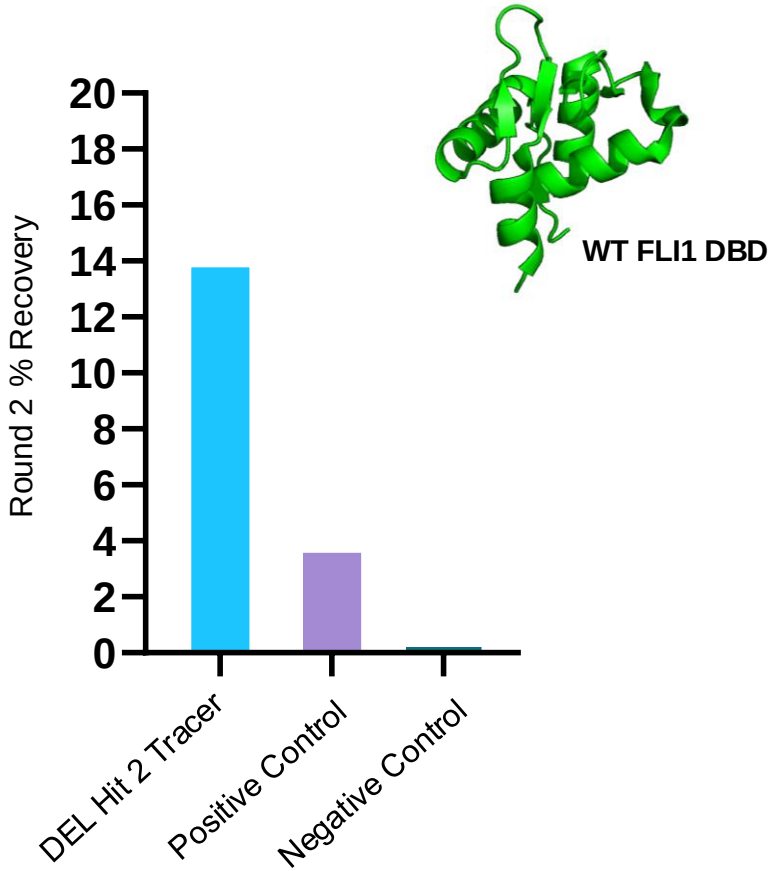
EWS-FLI1 FL



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

Binder recovery
~10% per round

Background recovery
~1% per round

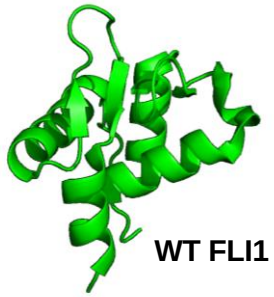


= = Positive Control Consensus Sequence = Negative Control No Consensus Sequence

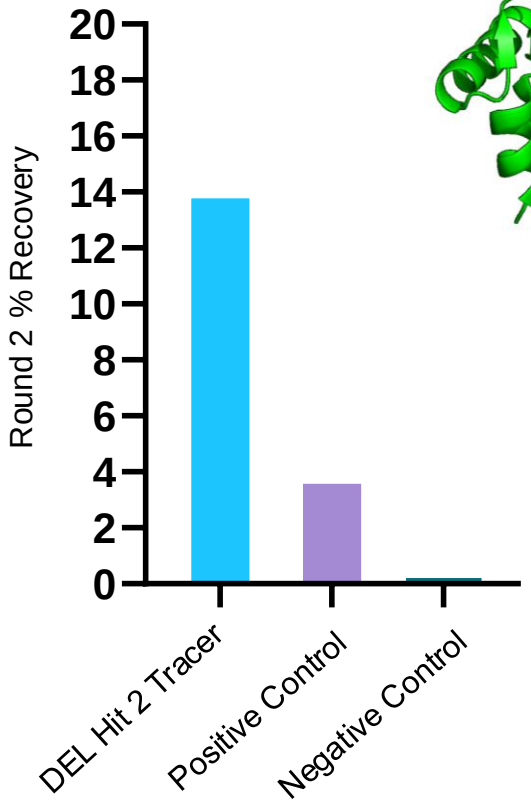
FLI1 binder engages EWS-FLI1 full length fusion

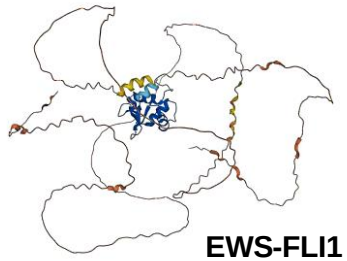
Binder recovery
~10% per round

Background recovery
~1% per round

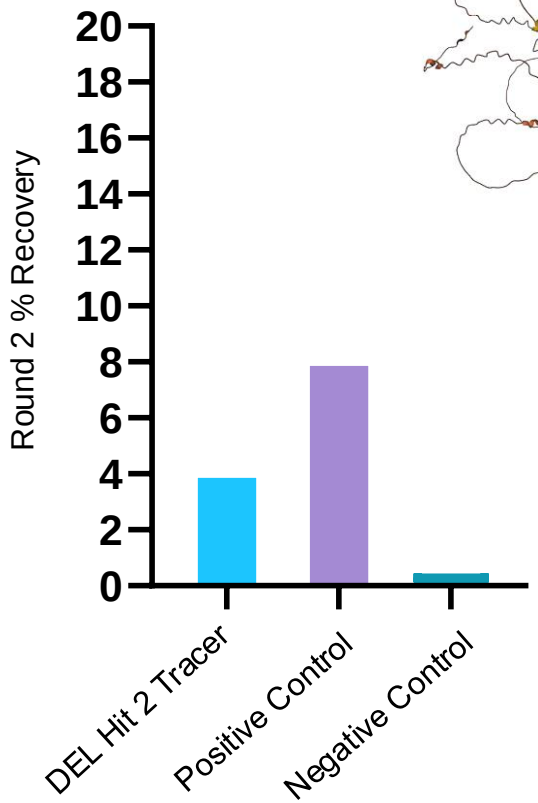


WT FLI1 DBD





EWS-FLI1 FL



Conclusions

- Ligands binding to the full-length EWS-FLI1 fusion protein have been identified by employing a customized workflow for Transcription Factor screening
  - DEL can successfully be applied to sequence-specific DNA binding proteins
  - DEL ligands are ideally suited to applications like TPD discovery

- Successful transcription factor DEL screening requires a combination of experimental approaches
  - Informatic-based approaches can flag the risk of tag driven enrichment, but aren’t sufficient to mitigate the risk
  - Informatic-based approaches that remove known consensus sequences from the output would have eliminated the real hits

Thank You