nurix

Leader in Targeted Protein Modulation

Deploying Affinity Selection Mass Spectrometry (ASMS) for Rapid and Flexible Hit Confirmation of DNA-Encoded Library (DEL) Screening in Targeted Protein Modulation (TPM)

Daniel Medina-Cleghorn

3rd Annual Edelris ASMS Symposium

May 31, 2023

Important Notice and Disclaimers

This presentation contains statements that relate to future events and expectations and as such constitute forward-looking statements within the meaning of the Private Securities Litigation Reform Act of 1995. When or if used in this presentation, the words “anticipate,” “believe,” “could,” “estimate,” “expect,” “intend,” “may,” “outlook,” “plan,” “predict,” “should,” “will,” and similar expressions and their variants, as they relate to Nurix Therapeutics, Inc. (“Nurix”, the “Company,” “we,” “us” or “our”), may identify forward-looking statements. All statements that reflect Nurix’s expectations, assumptions or projections about the future, other than statements of historical fact, are forward-looking statements, including, without limitation, statements regarding our future financial or business plans; our future performance, prospects and strategies; future conditions, trends, and other financial and business matters; our current and prospective drug candidates; the planned timing and conduct of the clinical trial programs for our drug candidates; the planned timing for the provision of clinical updates and initial findings from our clinical studies; the potential advantages of our DELigase™ platform and drug candidates; the extent to which our scientific approach and DELigase™ platform may potentially address a broad range of diseases; the extent animal model data predicts human efficacy; and the timing and success of the development and commercialization of our current and anticipated drug candidates. Forward-looking statements reflect Nurix’s current beliefs, expectations, and assumptions. Although Nurix believes the expectations and assumptions reflected in such forward-looking statements are reasonable, Nurix can give no assurance that they will prove to be correct. Forward-looking statements are not guarantees of future performance and are subject to risks, uncertainties and changes in circumstances that are difficult to predict, which could cause Nurix’s actual activities and results to differ materially from those expressed in any forward-looking statement. Such risks and uncertainties include, but are not limited to: (i) risks and uncertainties related to Nurix’s ability to advance its drug candidates, obtain regulatory approval of and ultimately commercialize its drug candidates; (ii) the timing and results of clinical trials; (iii) Nurix’s ability to fund development activities and achieve development goals; (iv) the impact of macroeconomic conditions, including inflation, increasing interest rates and volatile market conditions, and global events, including the COVID-19 pandemic, on Nurix’s clinical trials and operations; (v) Nurix’s ability to protect intellectual property and (vi) other risks and uncertainties described under the heading “Risk Factors” in Nurix’s Quarterly Report on Form 10-Q for the fiscal quarter ended February 28, 2023, and other SEC filings. Accordingly, readers are cautioned not to place undue reliance on these forward-looking statements. The statements in this presentation speak only as of the date of this presentation, even if subsequently made available by Nurix on its website or otherwise. Nurix disclaims any intention or obligation to update publicly any forward-looking statements, whether in response to new information, future events, or otherwise, except as required by applicable law.

Certain information contained in this presentation relates to or is based on studies, publications, surveys and other data obtained from third-party sources and the Company’s own internal estimates and research. While the Company believes these third-party sources to be reliable as of the date of this presentation, it has not independently verified, and makes no representation as to the adequacy, fairness, accuracy or completeness of, any information obtained from third-party sources. In addition, all of the market data included in this presentation involves a number of assumptions and limitations, and there can be no guarantee as to the accuracy or reliability of such assumptions. Finally, while we believe our own internal estimates and research are reliable, such estimates and research have not been verified by any independent source.

Nurix Drugs Engage Ligases for the Treatment of Cancer

Targeted Protein Modulation: TPM = TPD + TPE

A Powerful Cellular System

Targeted Protein Elevation (TPE)

Inhibit ligases to increase specific protein levels

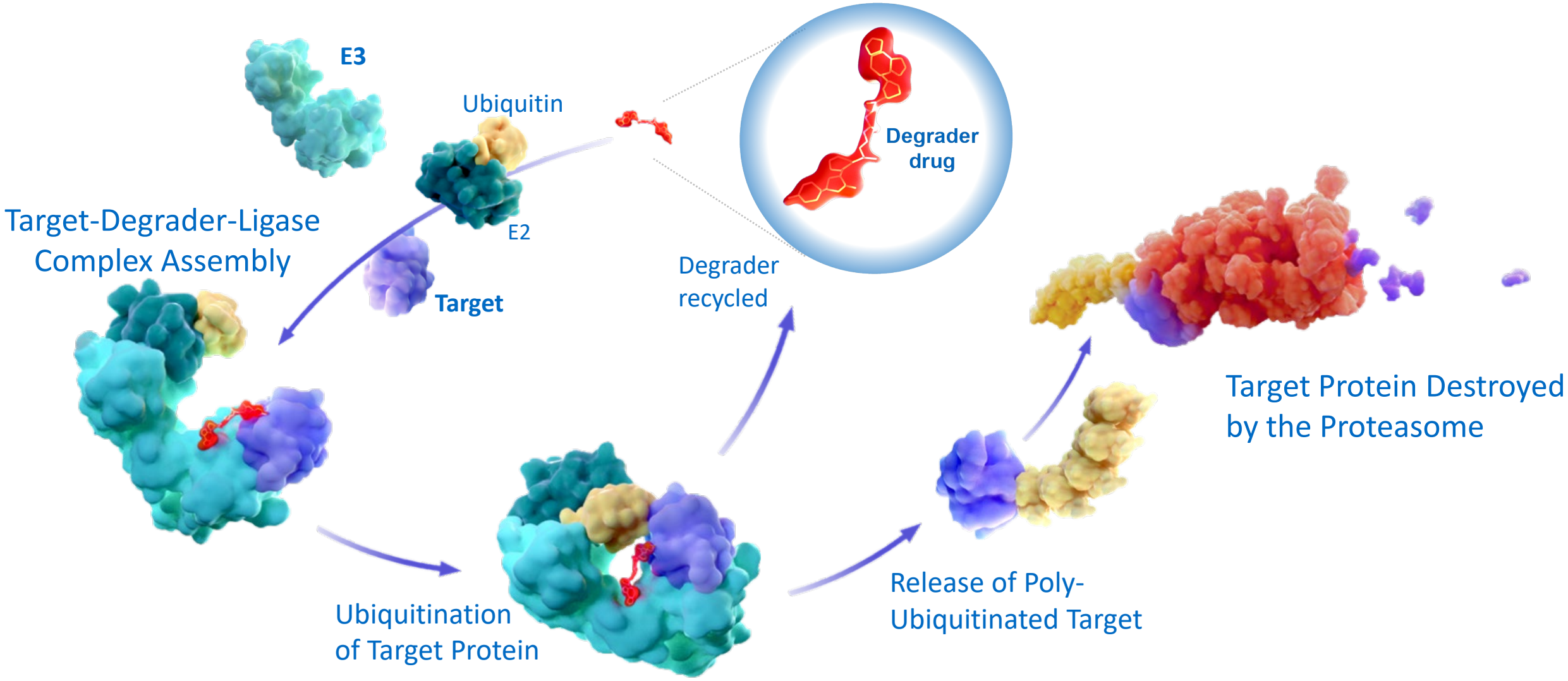
Harness ligases to decrease specific protein levels

Targeted Protein Degradation (TPD)

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

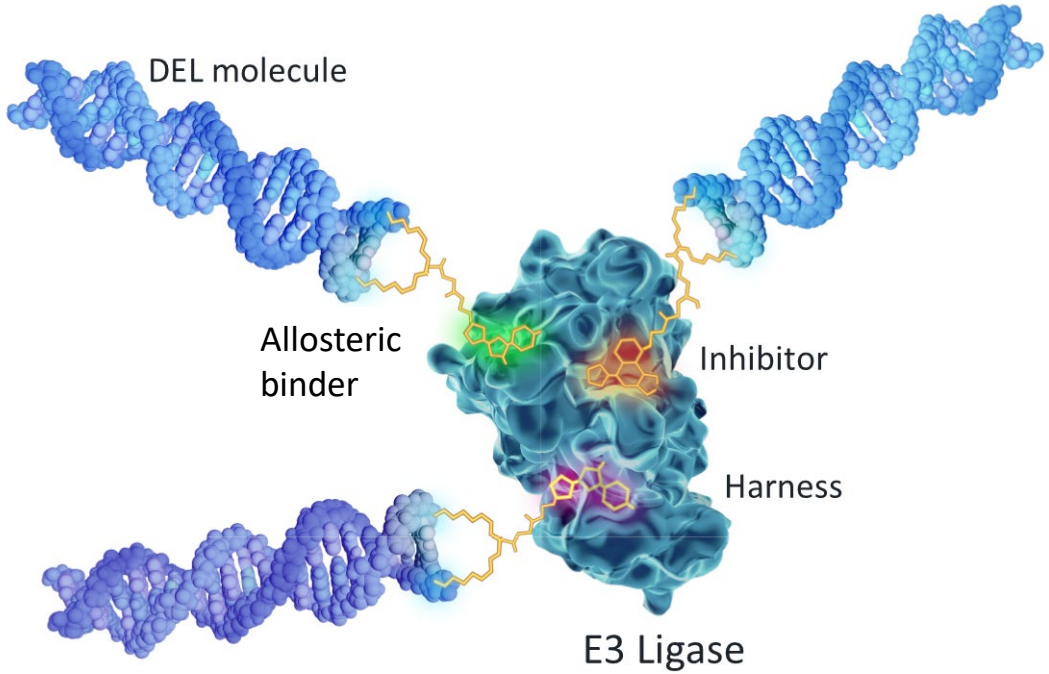
What Is Targeted-Protein Degradation (TPD)?

Harnessing the ubiquitin proteosome system to degrade a protein of interest (POI)



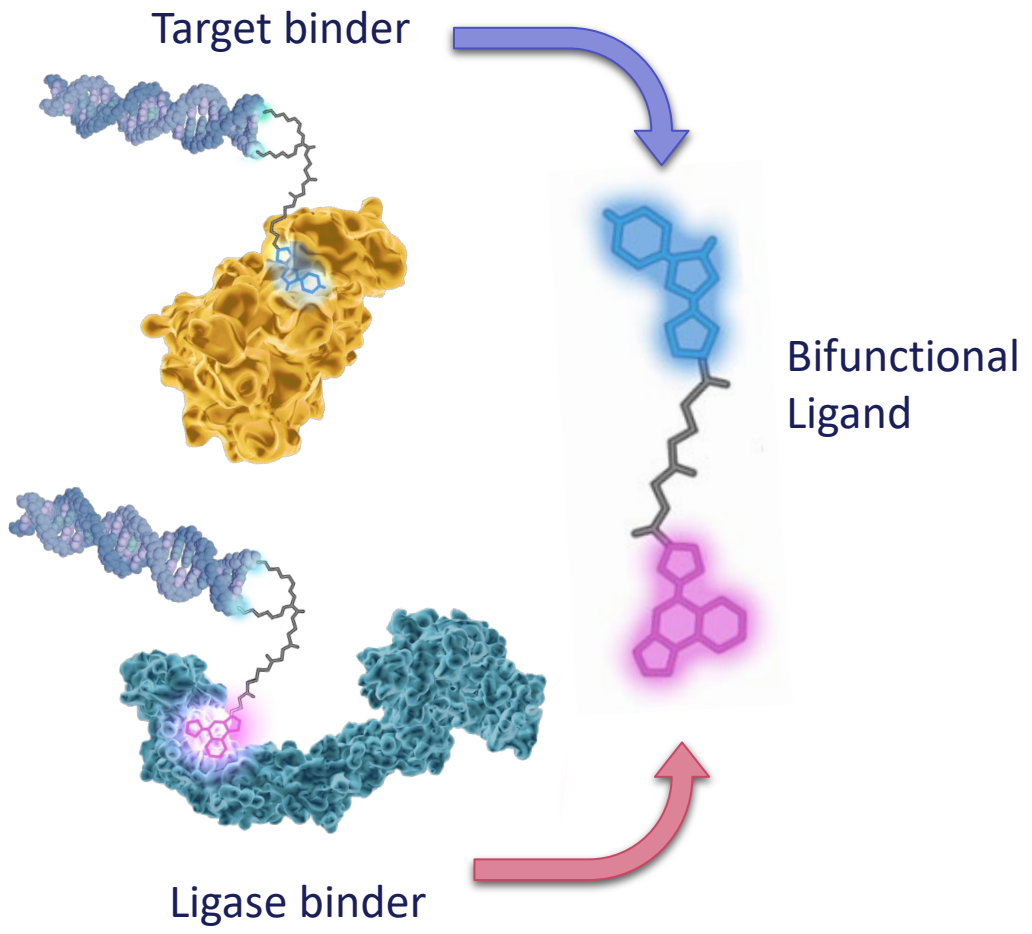
Why DNA Encoded Libraries? – Advantages for TPM

- **Affinity-based ligand discovery is the ideal approach to enable TPD**
  - **Affinity-based screening is MoA agnostic – for E3 ligases we can identify ligands for TPD and inhibitors for TPE from the same screen**
- DNA attachment provides initial handle for bifunctional molecule synthesis
- Combinatorial design enables rapid hit follow up and optimization



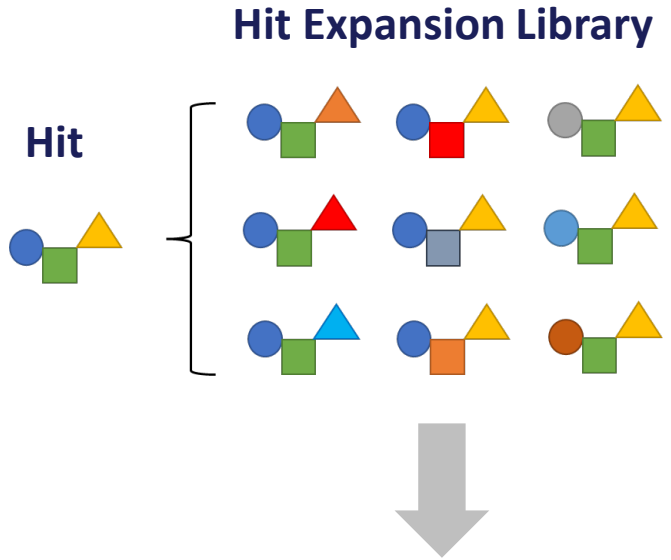
Why DNA Encoded Libraries? – Advantages for TPM

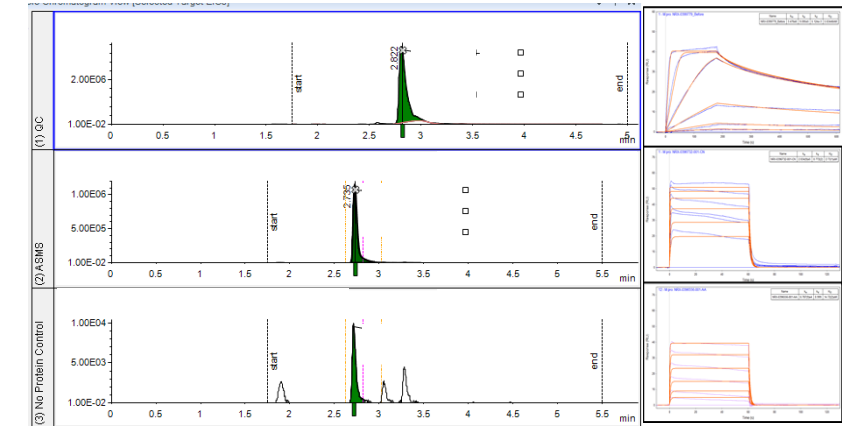
- Affinity-based ligand discovery is the ideal approach to enable TPD
  - Affinity-based screening is MoA agnostic – for E3 ligases we can identify ligands for TPD and inhibitors for TPE from the same screen
- **DNA attachment provides initial handle for bifunctional molecule synthesis**
- Combinatorial design enables rapid hit follow up and optimization



Why DNA Encoded Libraries? – Advantages for TPM

- Affinity-based ligand discovery is the ideal approach to enable TPD
  - Affinity-based screening is MoA agnostic – for E3 ligases we can identify ligands for TPD and inhibitors for TPE from the same screen
- DNA attachment provides initial handle for bifunctional molecule synthesis
- **Combinatorial design enables rapid hit follow up and optimization**



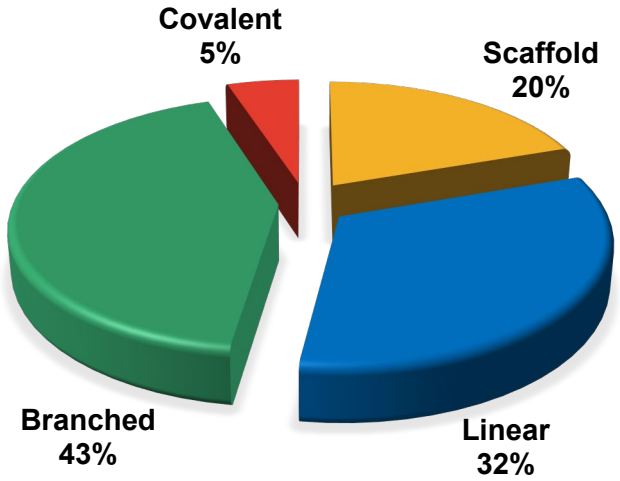


Hit Confirmation

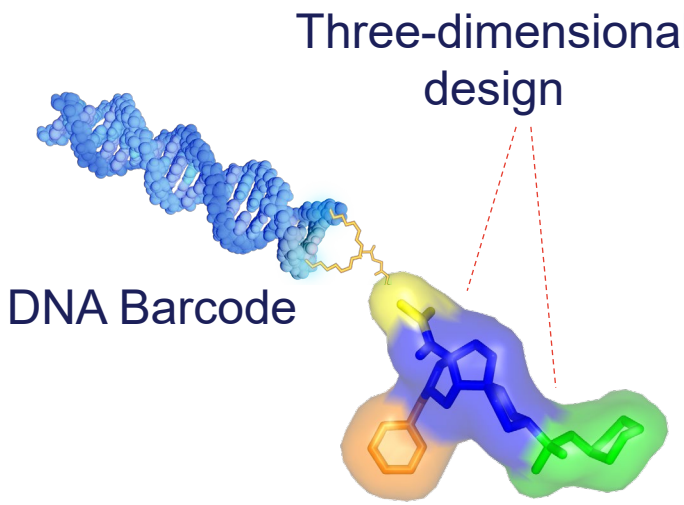
Custom Scaffold-Based DELs Enable Nurix To Identify Binders to Challenging Protein Surfaces

Nurix DEL Collection

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

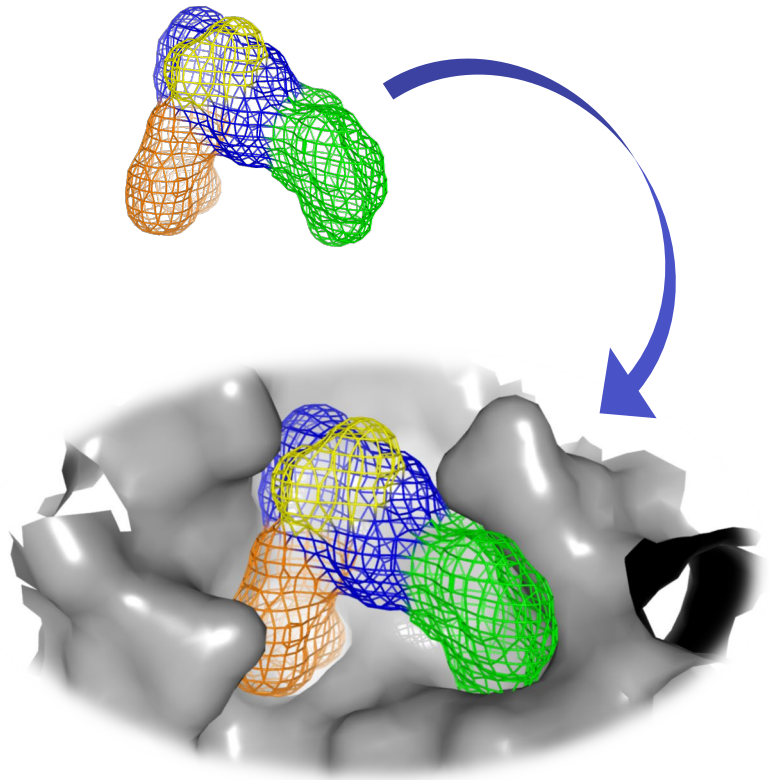


Scaffold Libraries Proving Essential for Delivering Ligands for "Undruggable" Targets (sole source of hits for 75% of these targets)



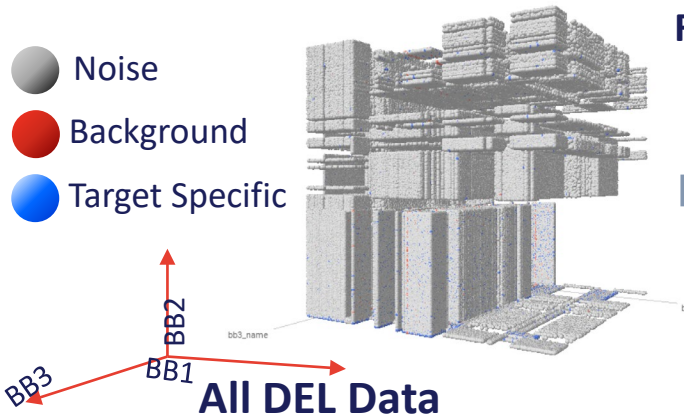
Our proprietary scaffold DELs provide unique geometry and high sp3 character, allowing molecules to achieve optimal pocket fit

Nurix scaffold designs show high pocket complementarity



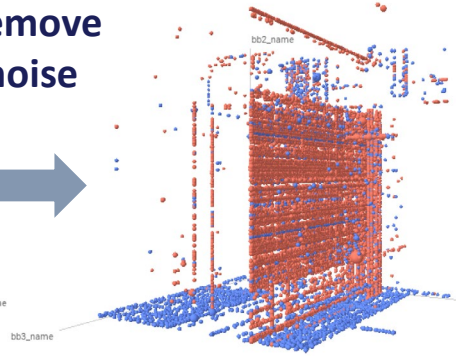
Nurix's Analysis & Follow Up Pipeline Is Designed To Access Broad Chemical Space

Noise
Background
Target Specific



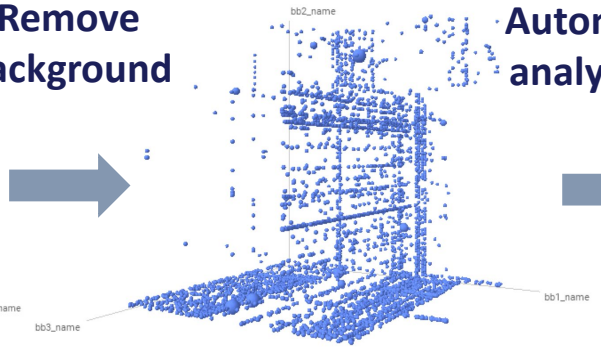
All DEL Data (~10MM)

Remove noise



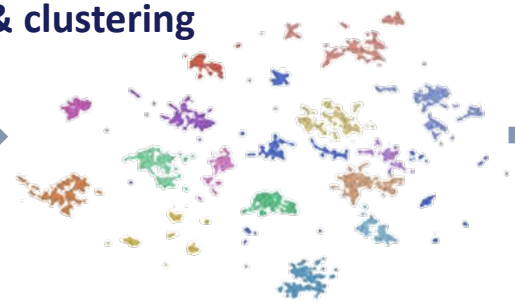
Enriched binders (~500K)

Remove background



Target Specific Binders (~100K)

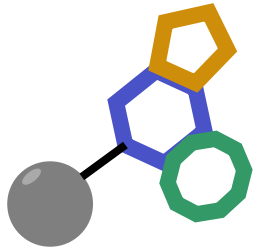
Automated structure analysis & clustering



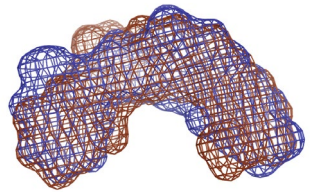
Target binders grouped by chemotype (~100's)

Off-DNA resynthesis (10's)

On-DNA resynthesis (100's)



Solid phase resynthesis (100's)

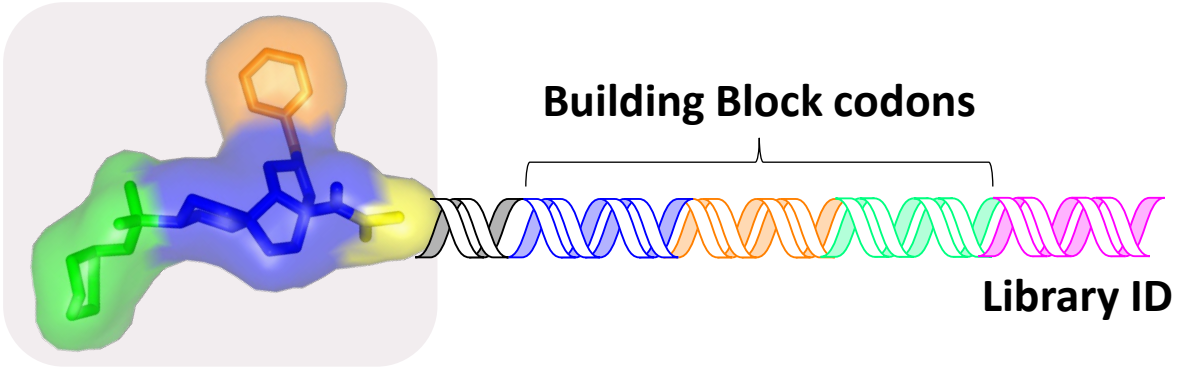


Machine Learning (1000's)

On-DNA Hit Resynthesis Translates DEL Screening Output for Rapid Hit Confirmation

- DEL barcode describes the recipe to build a molecule

  - Cycle 1 BB
  - Cycle 2 BB
  - Cycle 3 BB
  - Library ID

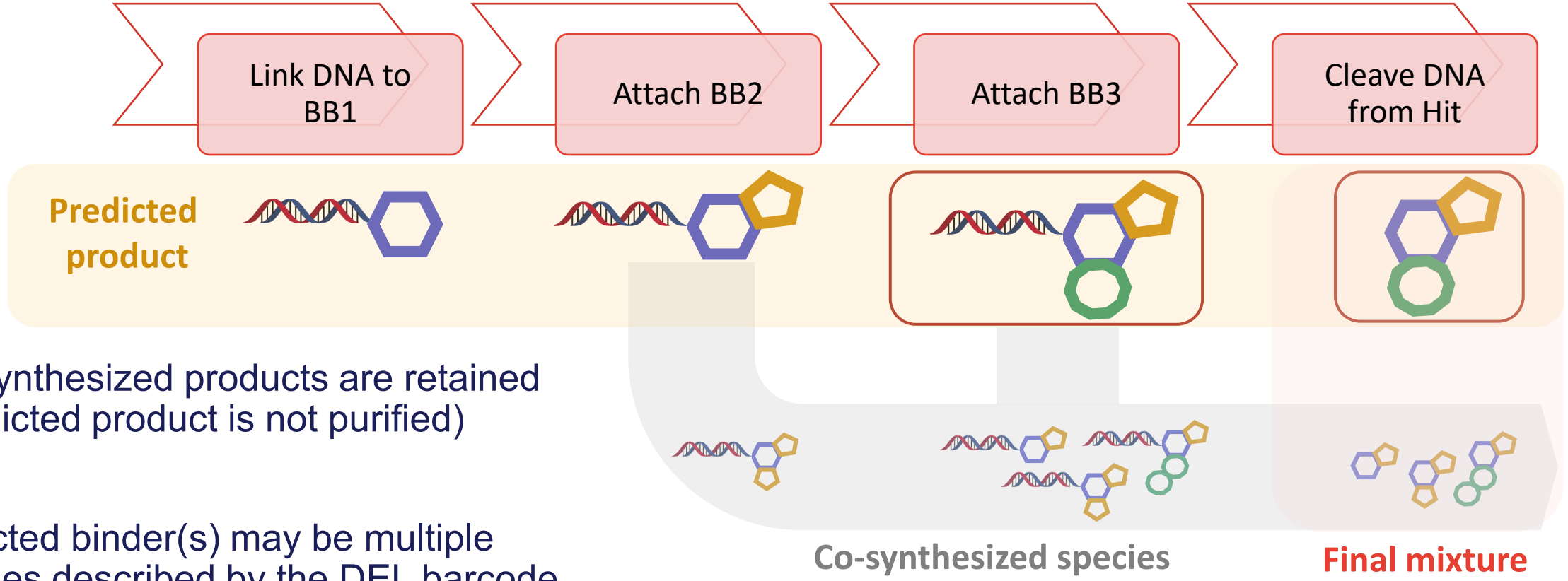


- **On-DNA hit resynthesis (HR)** applies the encoded library chemistry & BBs on a minimal piece of double-stranded DNA

  - Maintains synthetic fidelity to the original selected DEL hit

  - Parallelized process batches resynthesis by chemistry rather than by compound

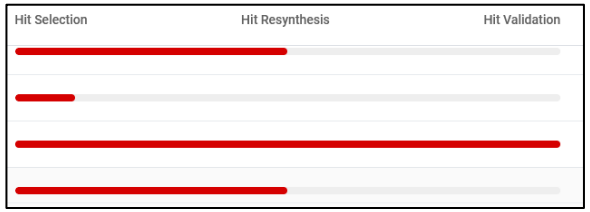
Each On-DNA HR Generates A Pool of Potential Binders



- Co-synthesized products are retained (predicted product is not purified)
- Selected binder(s) may be multiple species described by the DEL barcode
- ASMS can identify the specific binder(s) within the on-DNA HR mixture

DEL Cheminformatics Is Key for on-DNA HR & ASMS Analysis

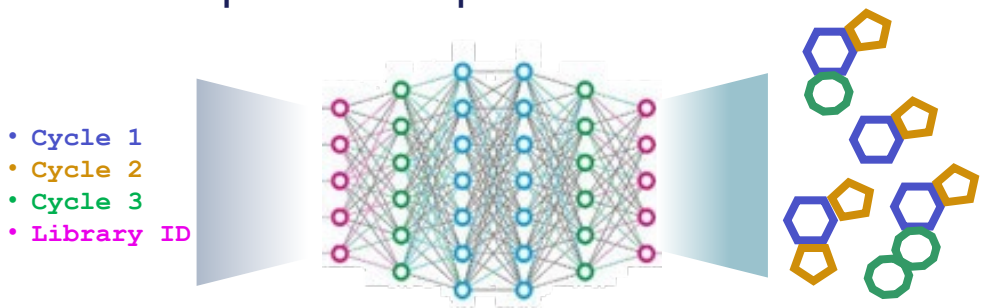
1. Cheminformatic tools plan and track parallelized HR campaign

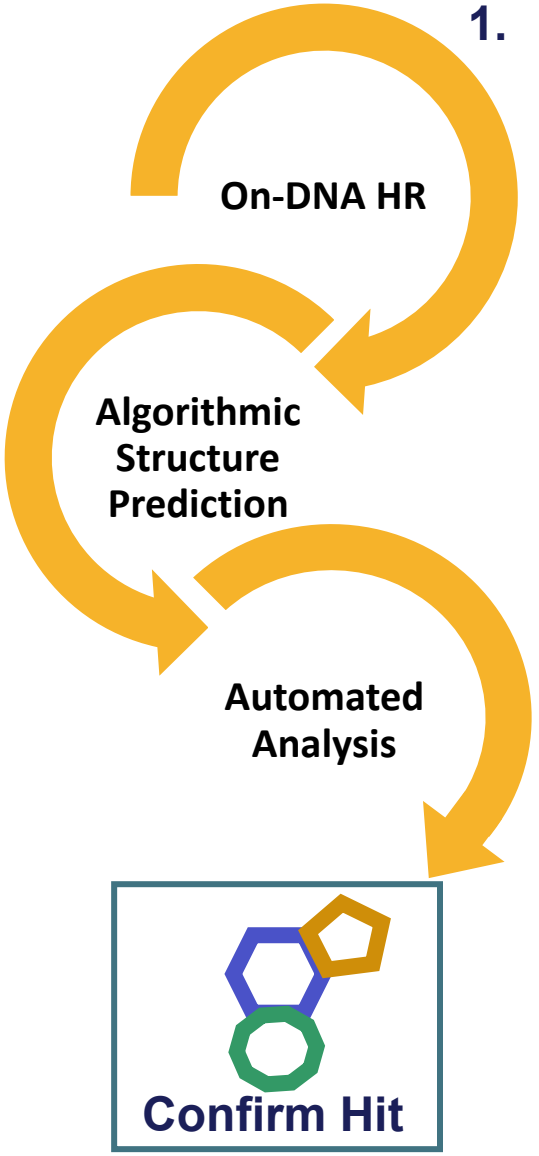


2. Algorithmic Structure Prediction

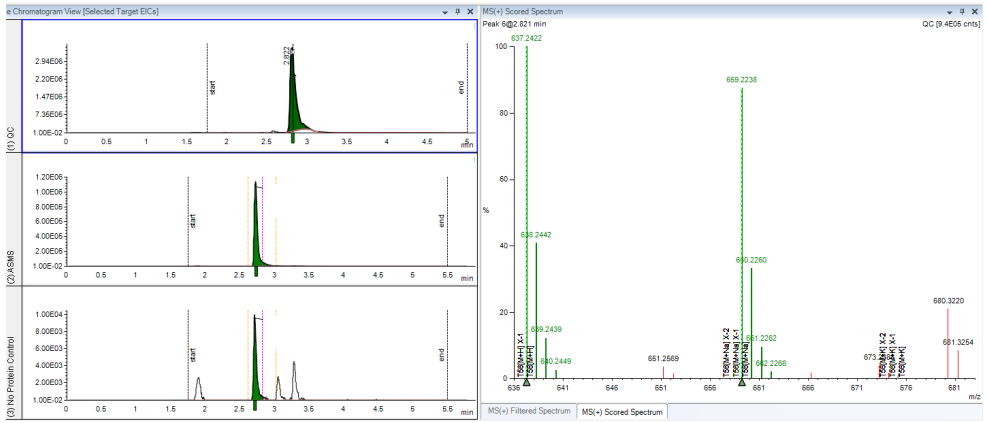
i. In-house algorithm predicts all potential synthesized species using DEL chemistry database

ii. Generates structures for each predicted species





3. Customized Virscidian software automatically processes & IDs hits

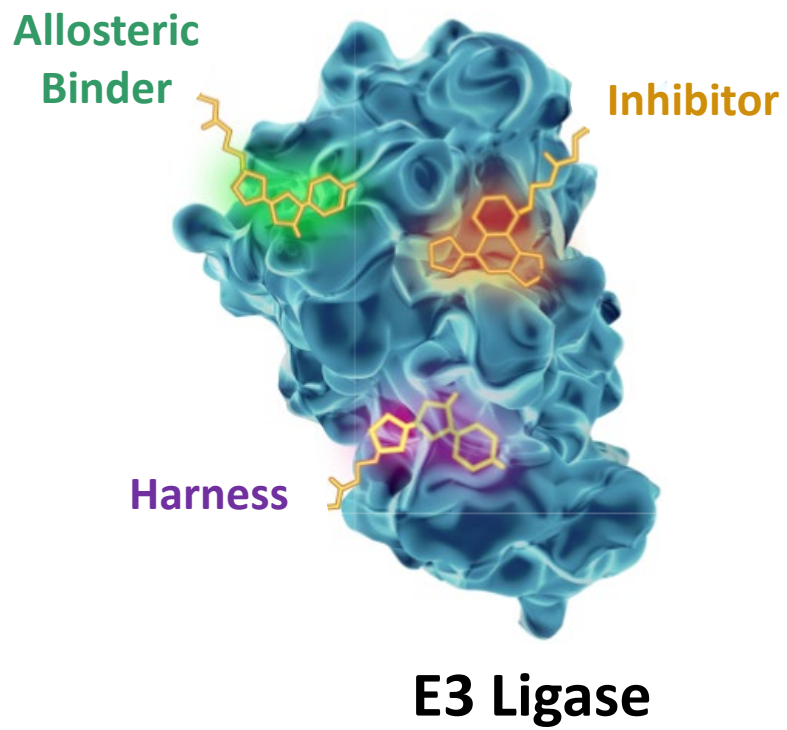


ASMS is Ideal for Confirming DEL-Identified Ligase Binders

- Highly Flexible Assay
  - Not limited by target protein structure/function
  - Identify multiple binding sites simultaneously
  - Modality agnostic
  - Ideal for early hit discovery of poorly characterized targets (e.g. E3 ligases)

- Direct Readout
  - No labeling of compound or protein required
  - Direct detection & ID of binder by LC-MS
  - Ideal for complex pools and mixtures

- Complementary to DEL
  - Affinity-based DEL screening requires a MoA-agnostic assay



Nurix's Hit Validation Approach Utilizes ASMS to Survey Chemical Space Inside and Outside the DEL Collection

a) DEL screening hits encoded in the library are chosen for off-DNA HR of the predicted compound, on-DNA HR of the mixture, or solid support resynthesis of the predicted compound

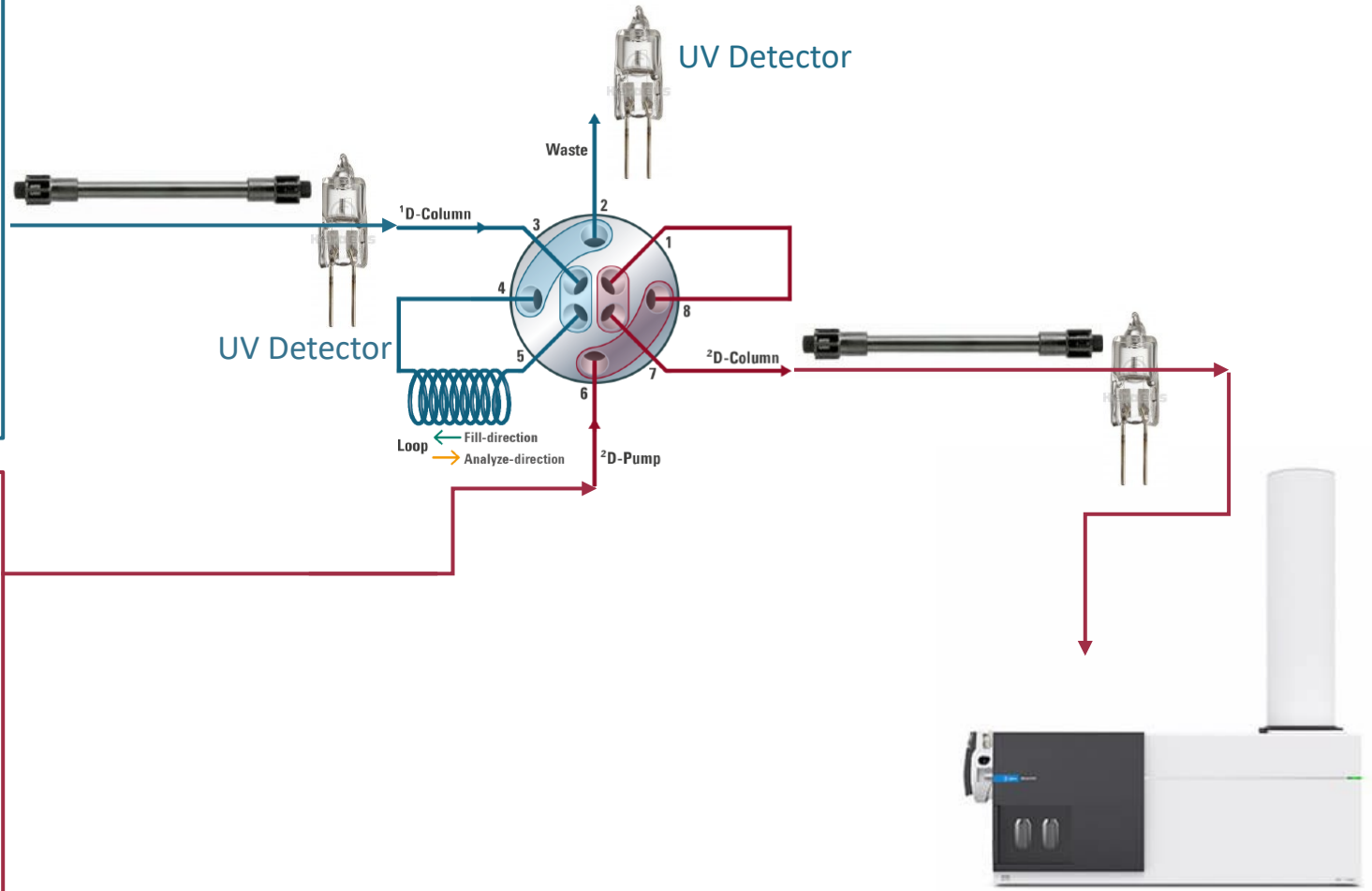
b) DEL data fed into a Machine Learning (ML) model predicts chemotypes of interest and searches commercially available compounds based on the hits selected from the library

Follow up	Source	Quantity	ASMS format	Hit Confirmation
Off-DNA	DEL → Discrete hit synthesis	10's	Pooled	ASMS + biophysics/biochem
On-DNA	DEL → Parallel Single Hit Syntheses	100's	Individual	ASMS
Solid phase	DEL → Solid Phase Syntheses	100's	Individual	ASMS + biophysics/biochem
Machine Learning	DEL → ML → Vendor catalogs	1000's	Pooled	ASMS + biophysics/biochem

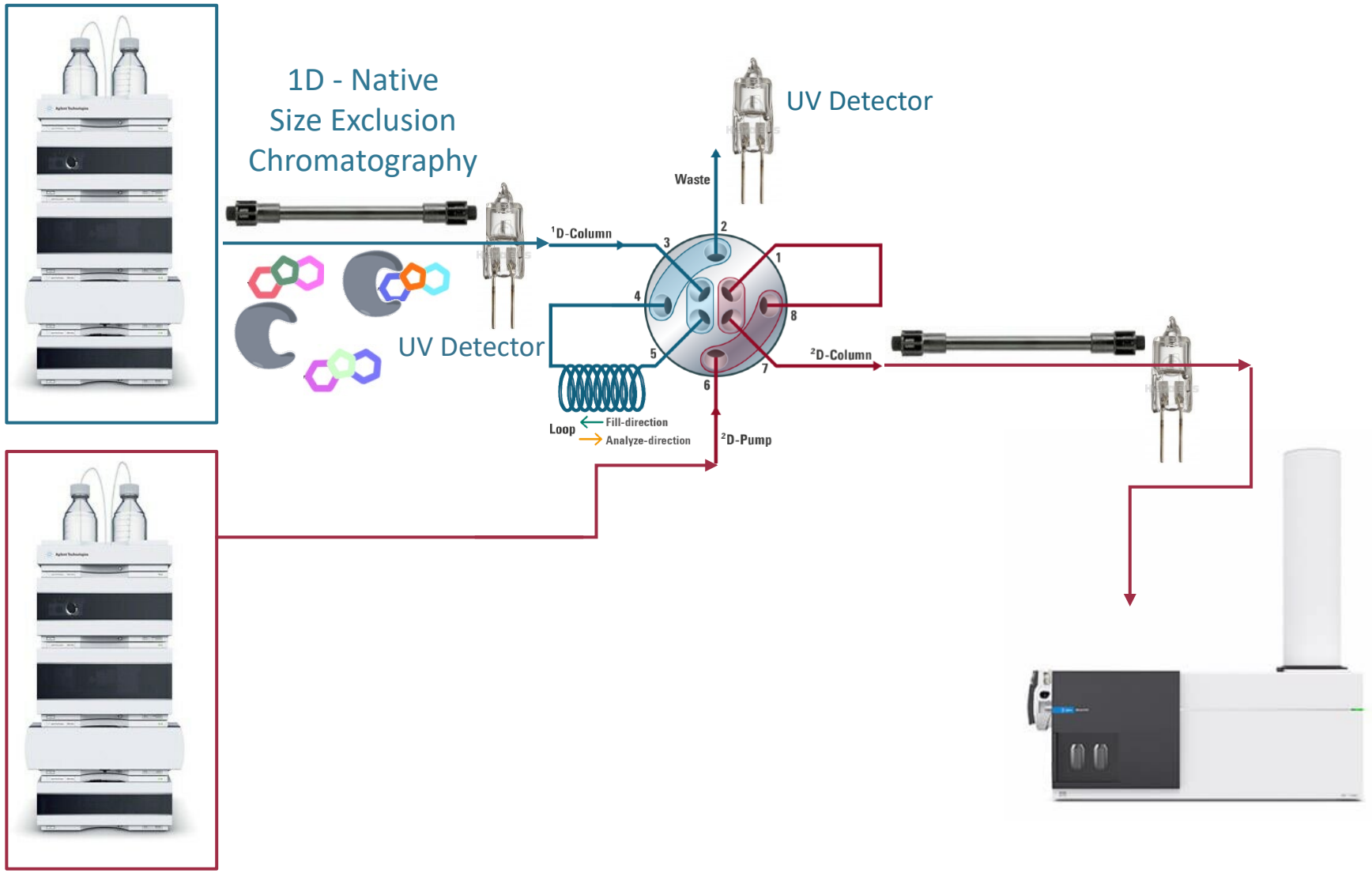
ALIS-ASMS Analytical Workflow

Target

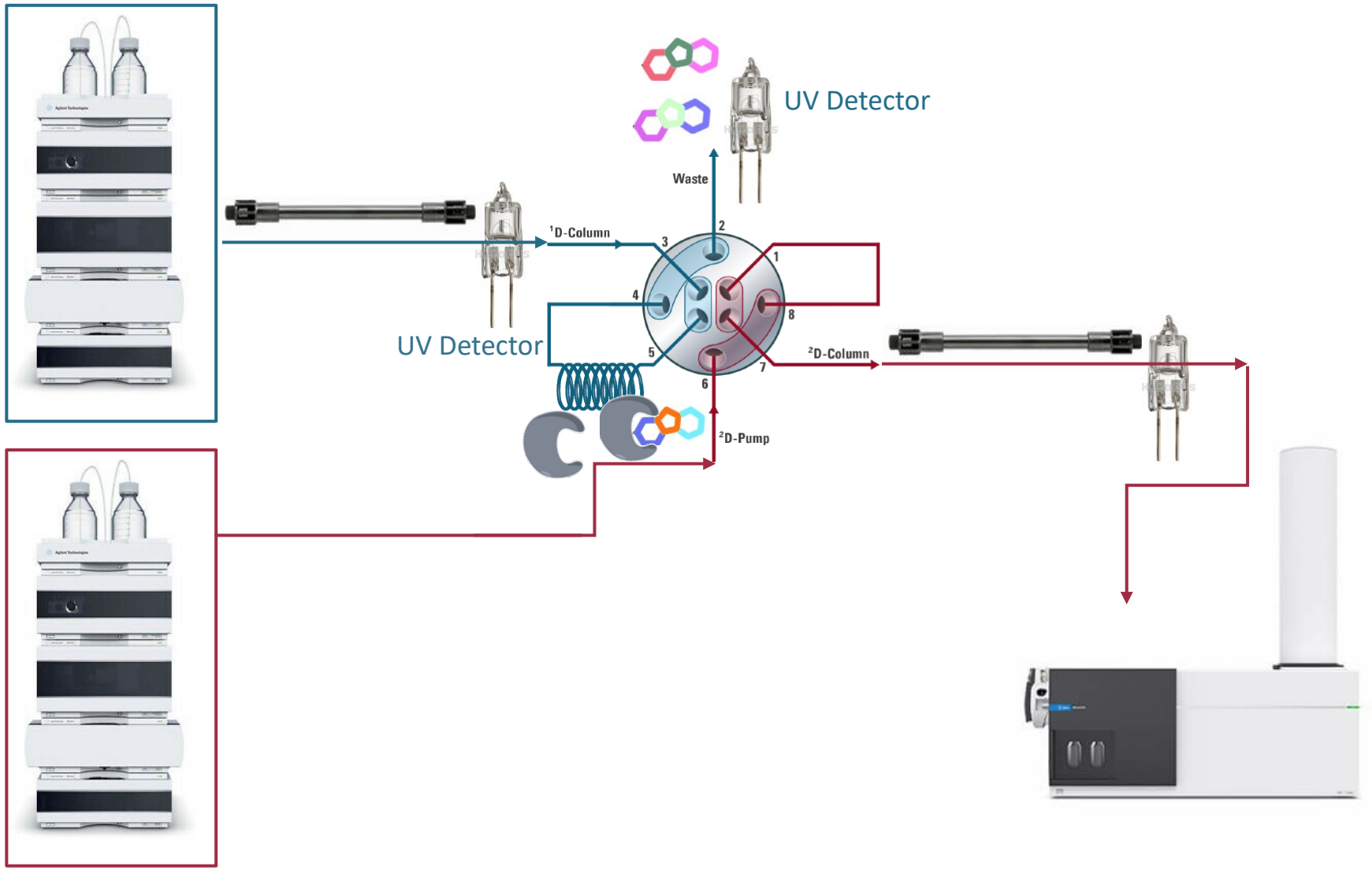
DEL Hits



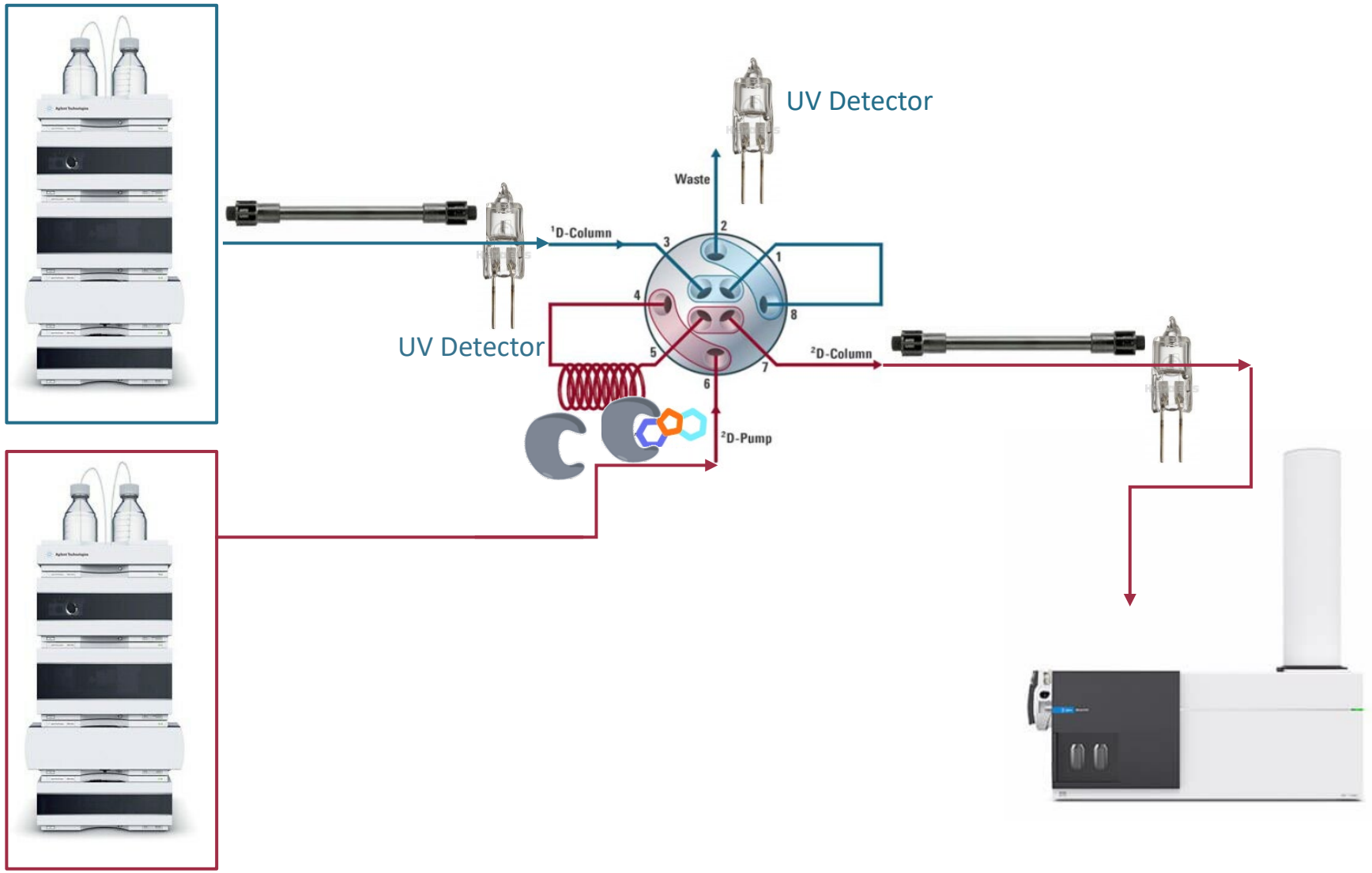
ALIS-ASMS Analytical Workflow



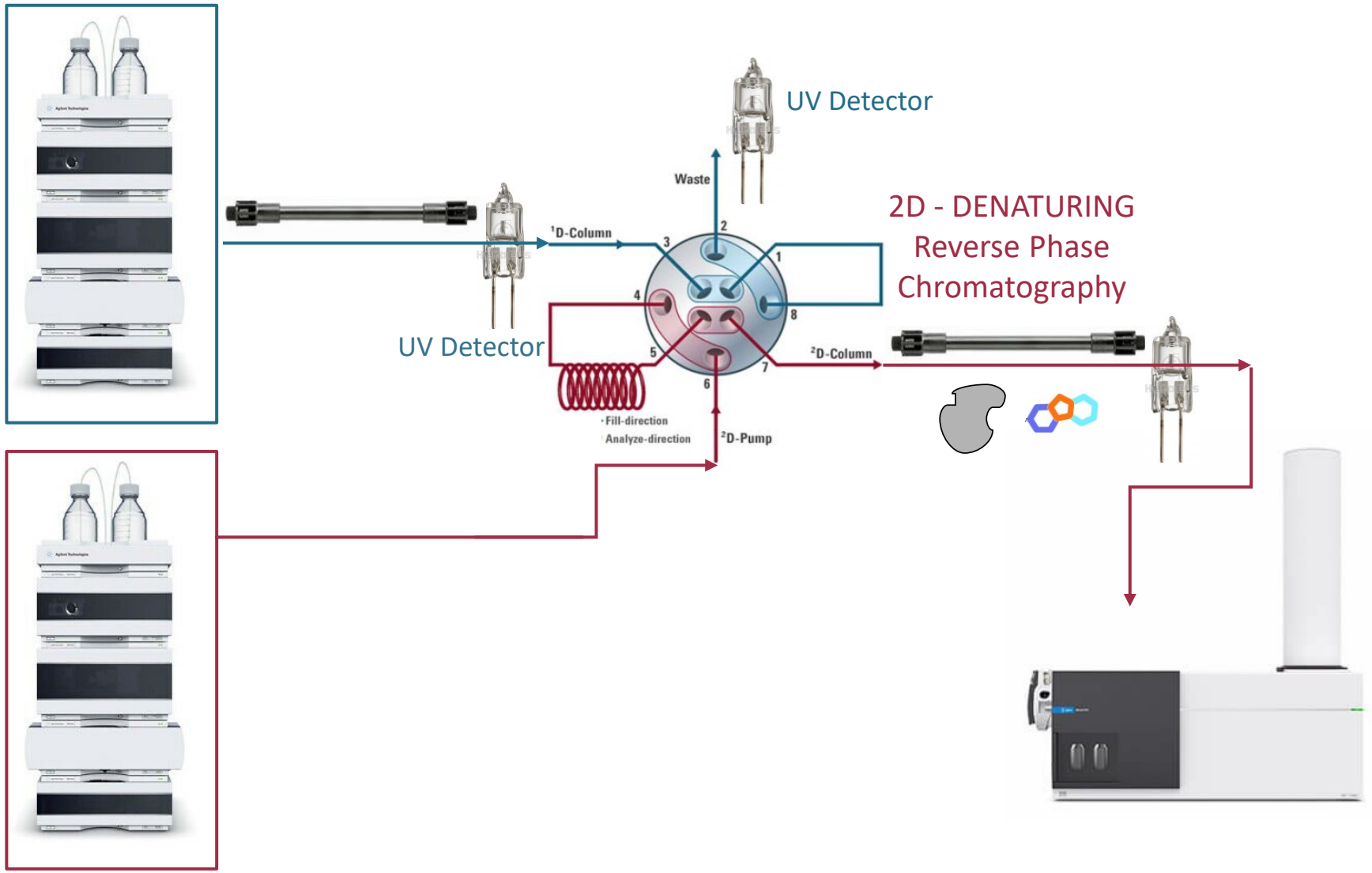
ALIS-ASMS Analytical Workflow



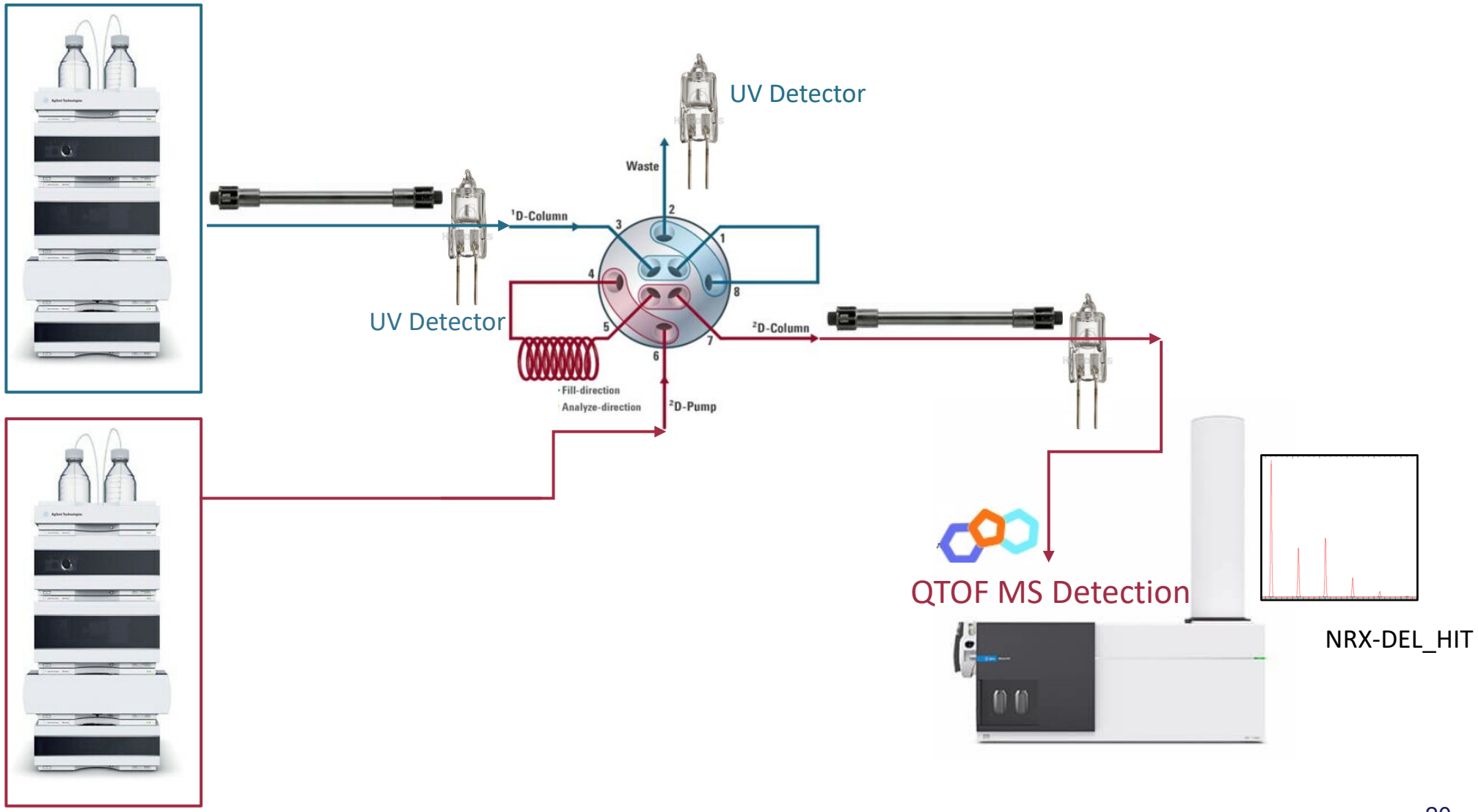
ALIS-ASMS Analytical Workflow



ALIS-ASMS Analytical Workflow



ALIS-ASMS Analytical Workflow

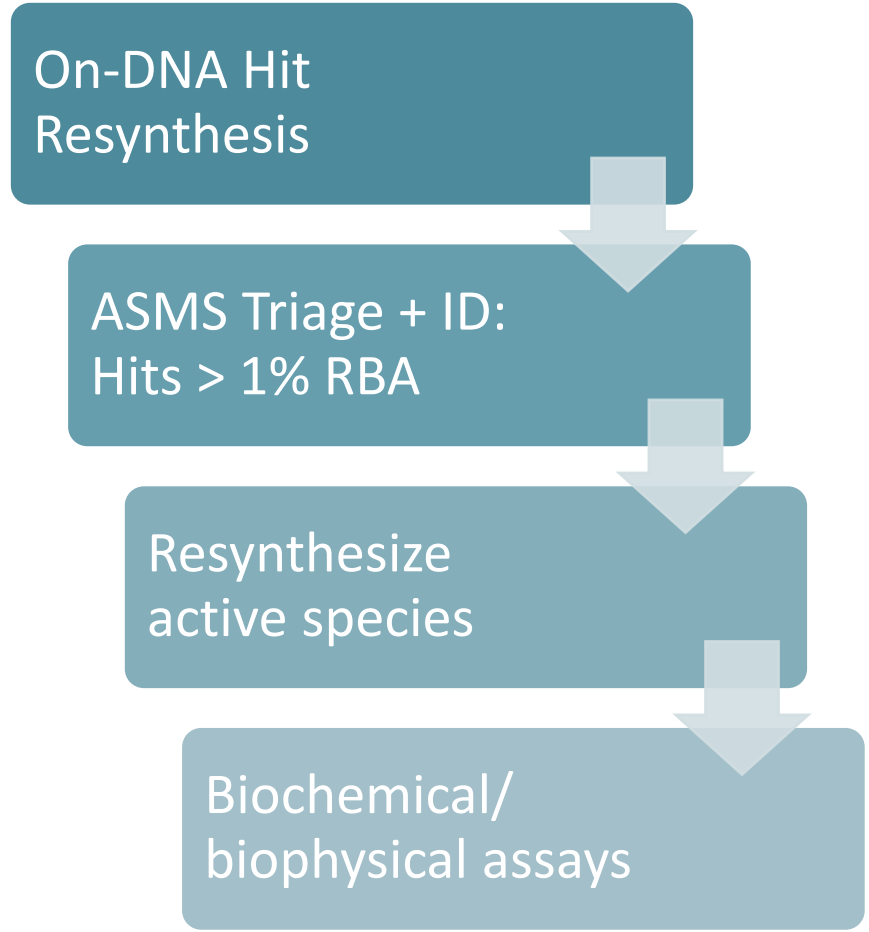


On-DNA ASMS Hit Confirmation & Follow Up

- ASMS hit confirmation scored by % RBA (relative binding affinity)

$$\%\ RBA = \frac{(Protein\ Selected - No\ Protein\ Control)}{Reference\ Injection} \times 100$$

- Hits with RBA score > 1% prioritized for follow up
- **RBA score is a binary hit/no hit metric: RBA ≠ affinity**
  - Useful for triage & rank ordering
- Active species from ASMS-confirmed hits are then resynthesized for testing in more quantitative assays

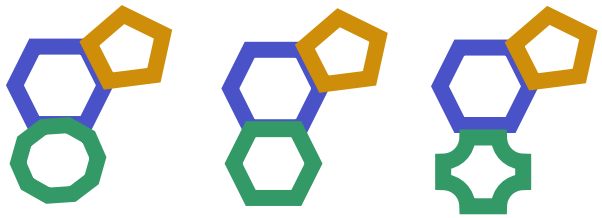


on-DNA ASMS Confirms DEL Hits & Features

- Hits selected from a DEL screen against Ligase 1 were chosen as singletons (individual hits) or from a feature

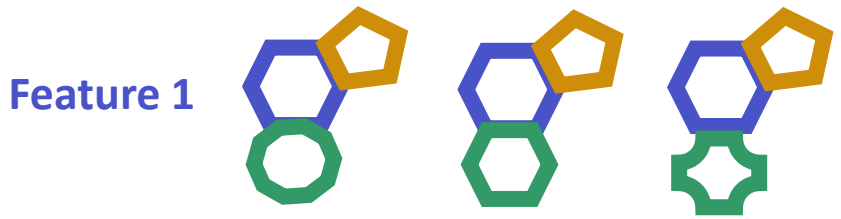
- DEL “features” are hits with similar BBs in the same position
  - Validating multiple hits in a feature supports SAR

Feature 1

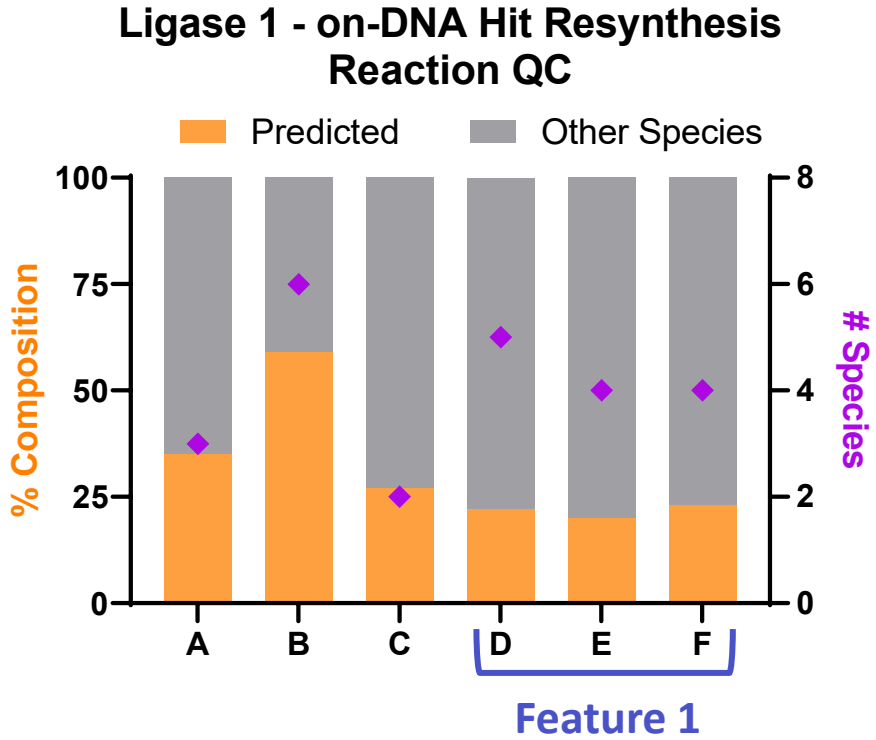


on-DNA ASMS Confirms DEL Hits & Features

- Hits selected from a DEL screen against Ligase 1 were chosen as singletons (individual hits) or from a feature
- DEL “features” are hits with similar BBs in the same position
  - Validating multiple hits in a feature supports SAR

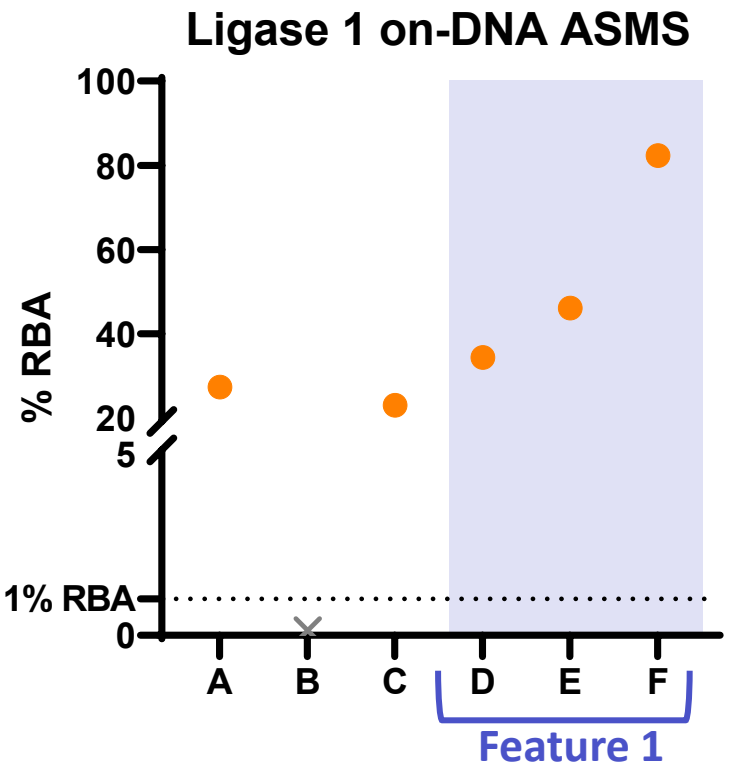


- Each pool contains a variable mixture of species and proportions
  - The DEL-encoded predicted product may not always be the species in greatest proportion



on-DNA ASMS Confirms DEL Hits & Features

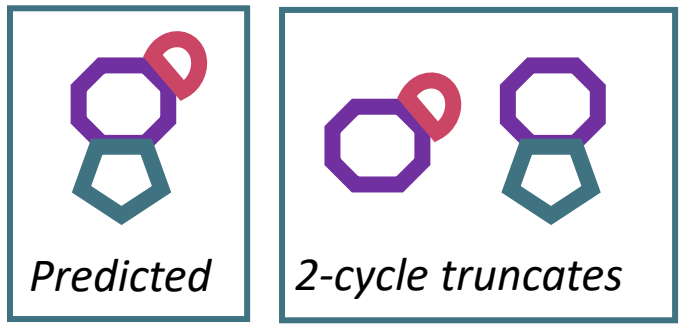
- Predicted 3-cycle products detected & confirmed in ASMS
- Singleton hits A & C do not have overlapping SAR
- Feature 1 hits D-F confirmed, indicating changes at BB3 are tolerated
- Selection of related hits validates the DEL feature and informs SAR for downstream prioritization



ASMS Identifies the Active Species in on-DNA Mixtures

- DEL hits from three structurally distinct features selected against Ligase 2 were chosen for on-DNA HR

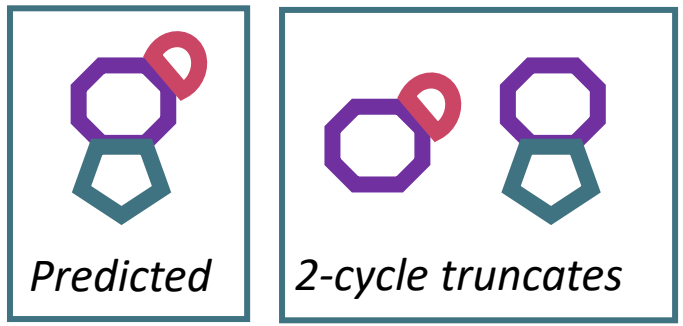
- Each on-DNA HR generates a pool of species, replicating the mixture generated in the DEL screen
  - Predicted 3-cycle compound & 2-cycle truncate species all share the same DEL barcode

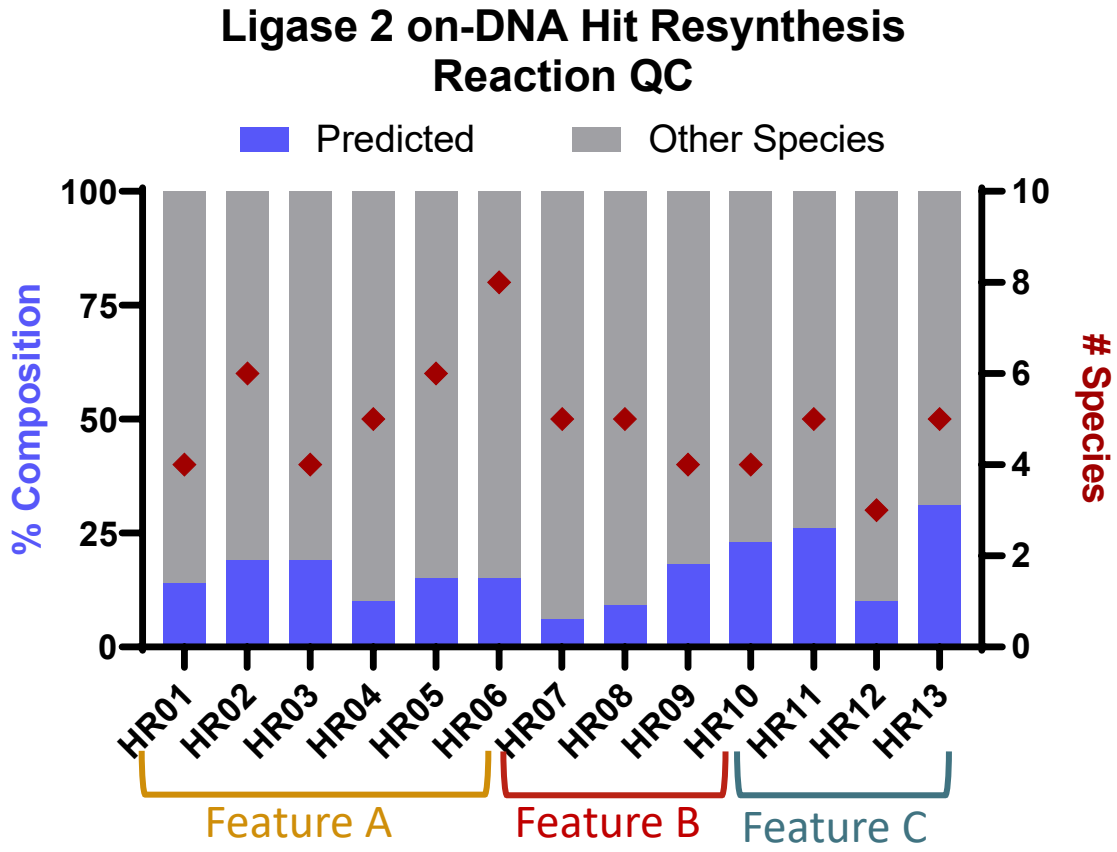


ASMS Identifies the Active Species in on-DNA Mixtures

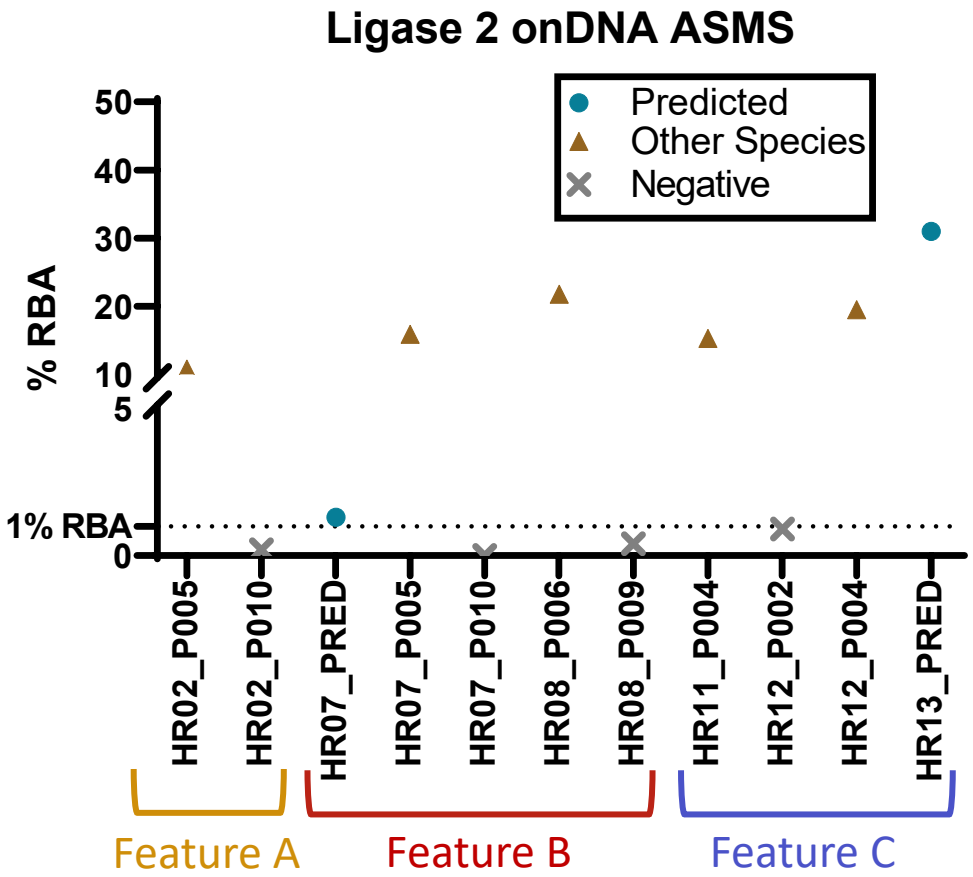
- DEL hits from three structurally distinct features selected against Ligase 2 were chosen for on-DNA HR

- Each on-DNA HR generates a pool of species, replicating the mixture generated in the DEL screen
  - Predicted 3-cycle compound & 2-cycle truncate species all share the same DEL barcode



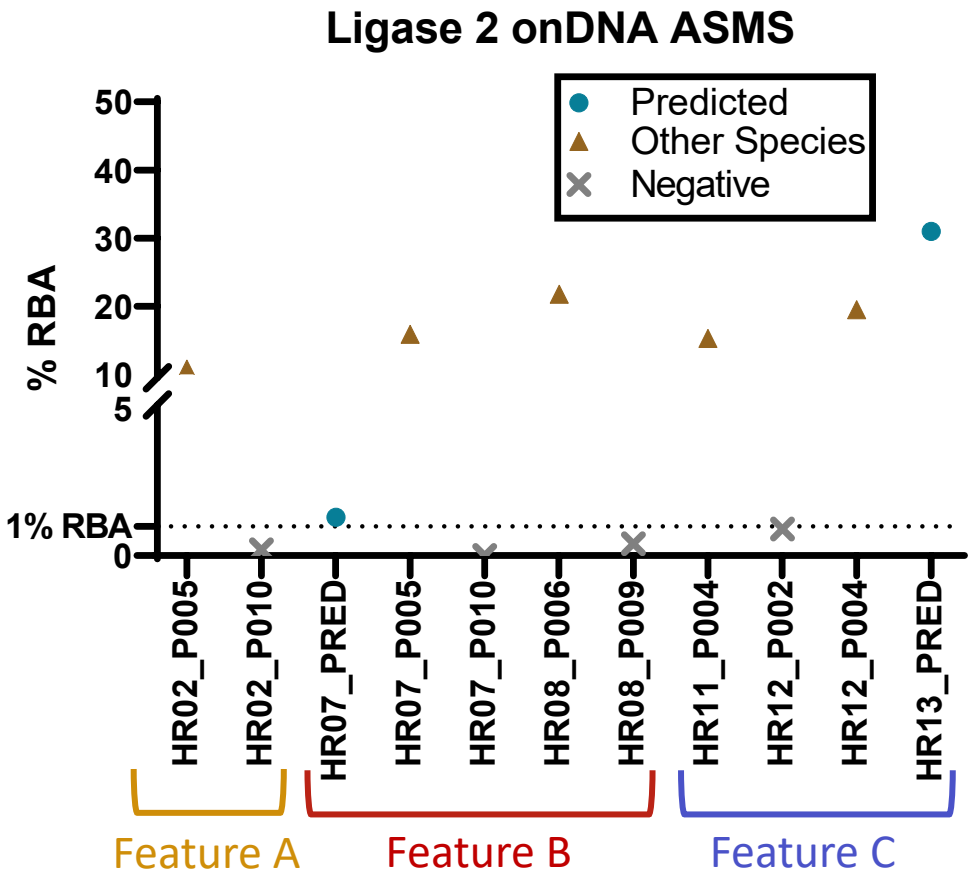


ASMS Identifies the Active Species in on-DNA Mixtures



- In Features A and B only 2-cycle truncates were highly selected, suggesting the truncates bound in the DEL screen
- In Feature C, both predicted and 2-cycle truncates were confirmed, supporting the feature and ID'ing the ligands

ASMS Identifies the Active Species in on-DNA Mixtures

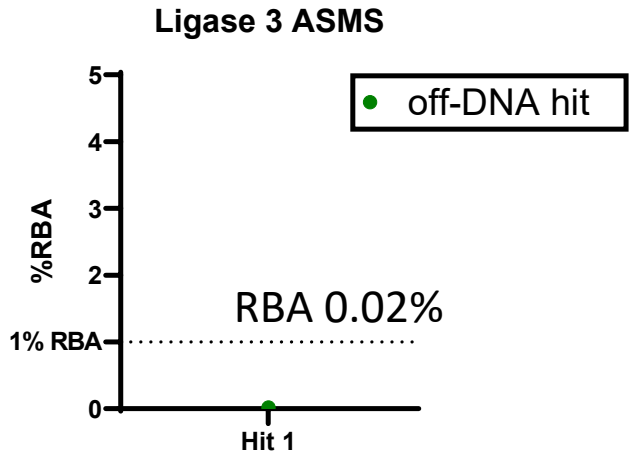


- In Features A and B only 2-cycle truncates were highly selected, suggesting the truncates bound in the DEL screen
- In Feature C, both predicted and 2-cycle truncates were confirmed, supporting the feature and ID'ing the ligands
- Predicted hits from Feature C was discretely resynthesized and confirmed in orthogonal assays

Hit ID	On-DNA ASMS RBA	Off-DNA Biochem. EC_{50}
HR13-PRED	31%	0.5 µM

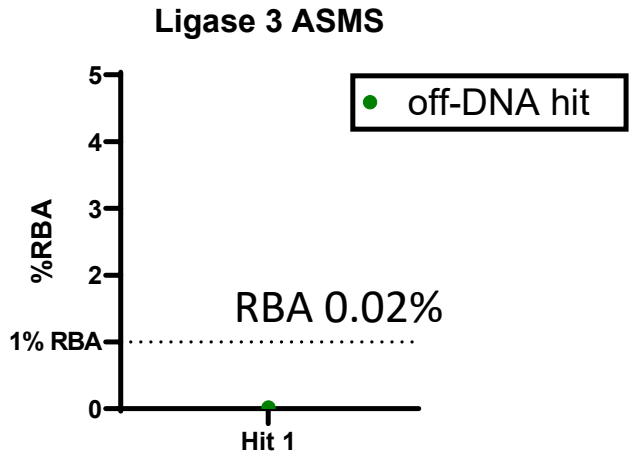
ASMS Reveals the Active Species in A Discretely Resynthesized (Off-DNA) Hit

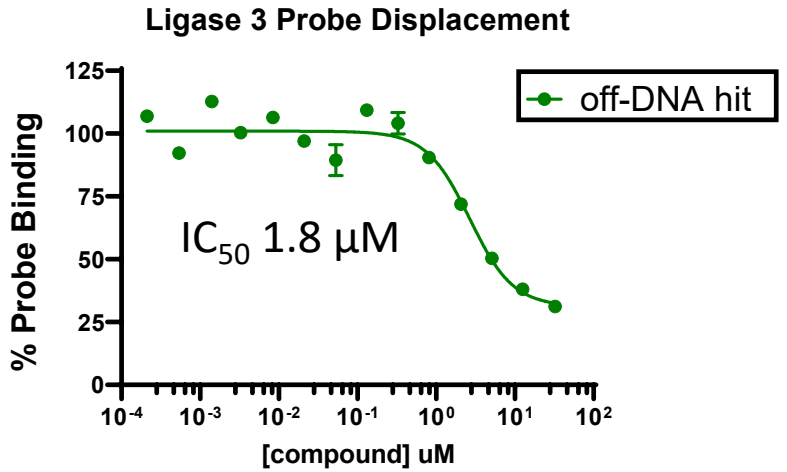
- Ligase 3 DEL hits were discretely resynthesized as purified off-DNA compounds
- ASMS analysis of purified hit showed no enrichment (RBA < 1%)



ASMS Reveals the Active Species in A Discretely Resynthesized (Off-DNA) Hit

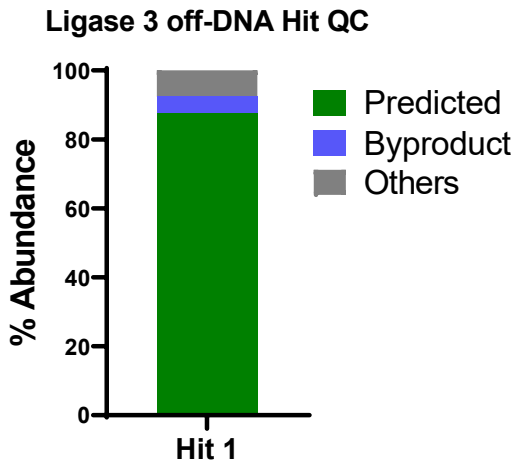
- Ligase 3 DEL hits were discretely resynthesized as purified off-DNA compounds
- ASMS analysis of purified hit showed no enrichment (RBA < 1%)
- Orthogonal probe displacement assay confirmed hit with IC_{50} < 2 µM
- Ligand-bound crystal structure was solved and revealed a byproduct bound to the protein





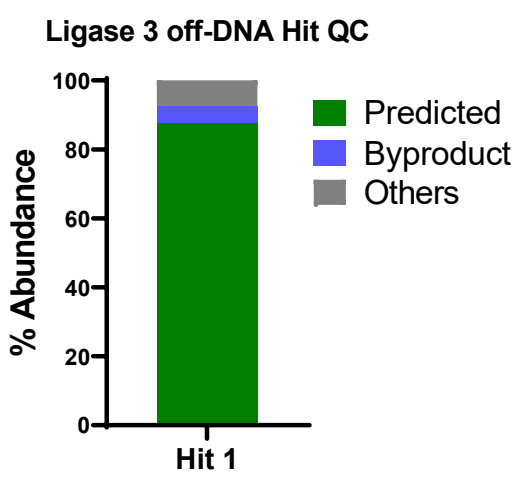
ASMS Reveals the Active Species in A Discretely Resynthesized (Off-DNA) Hit

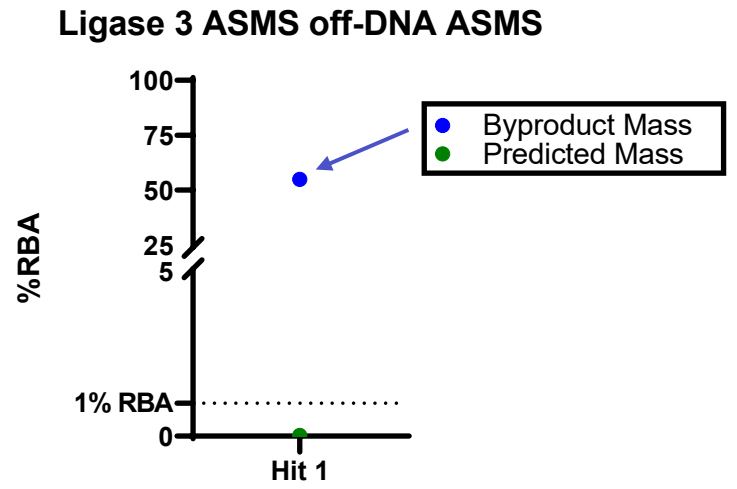
- Chemists identified a minor byproduct in the sample

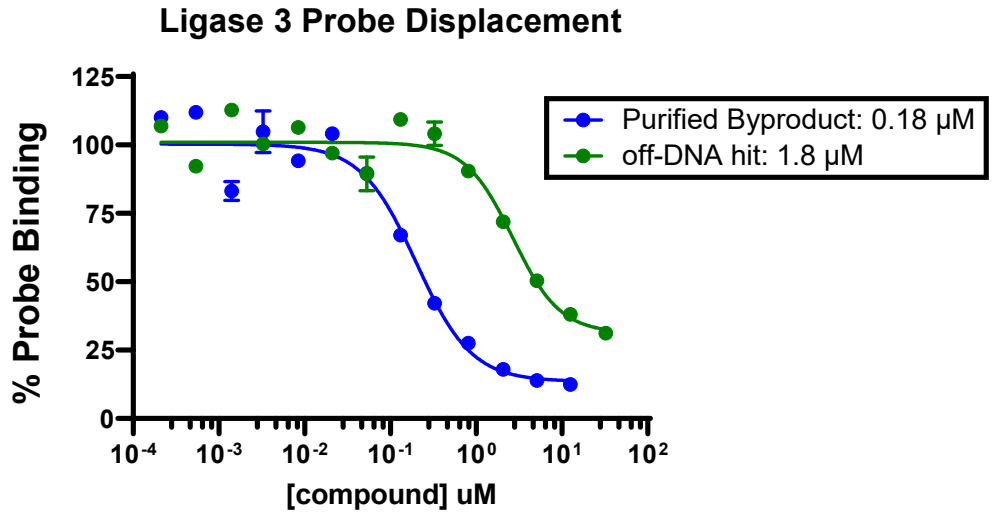


ASMS Reveals the Active Species in A Discretely Resynthesized (Off-DNA) Hit

- Chemists identified a minor byproduct in the sample
- Reanalyzed ASMS data confirmed the byproduct was the active species
  - Resynthesized byproduct reported 0.18 μM IC_{50} by probe displacement
  - DEL hit encoded as predicted compound, yet the byproduct was the best binder in the screen
- Algorithmic Predictor now includes structures for variant species in purified, off-DNA hits







Conclusions

- Nurix's affinity-based DEL screening approach is a powerful hit discovery platform for targeted protein modulation
- ASMS's versatility as an affinity-driven method makes it the ideal next step for confirming novel DEL-derived chemical matter
- Combining on-DNA hit resynthesis and ASMS allows rapid and comprehensive ID & validation of DEL hits at the feature and individual hit levels
- Incorporating ASMS technology into Nurix's DEL discovery platform allows us to generate, ID, and advance a greater number of hits more efficiently

Thank You!